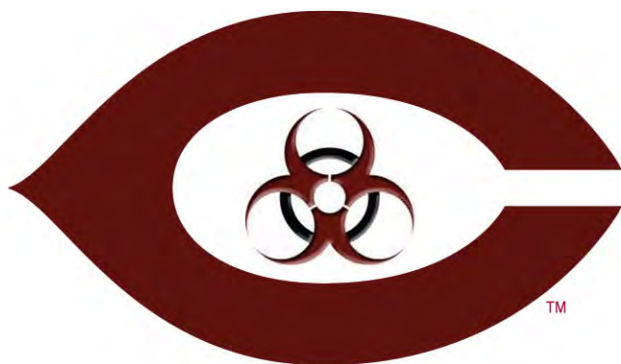


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# **Guidelines for Handling Pathogenic Microorganisms, Other Potentially Infectious Materials, or Recombinant/Synthetic DNA at Biosafety Level 2 (BSL-2)**



## **Biohazard Recognition and Control**

Sixth Edition



August 2017

**Institutional Biosafety Committee  
Office of Research 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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## CHAPTER I: INTRODUCTION

This manual seeks to increase awareness of biological hazards frequently encountered in research, clinical, and teaching laboratories at the University of Chicago (UC), and to provide guidance on recommended practices. Biological hazards include infectious or toxic microorganisms (including viral vectors), infectious or toxic biological materials, 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 or synthetic DNA are required to register their research 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 manual is intended for trained microbiologists as well as individuals handling human clinical materials in other laboratory disciplines, such as biochemistry, biophysics, genetics, oncology, immunology, molecular engineering, and molecular biology. Persons who have little microbiological training might not realize the potential hazard involved with their materials, and should seek additional information.

The safety principles described are based on sound safety practices, common sense, current data, good housekeeping, thorough personal hygiene, and tested accident-response plans. Laboratories that are well organized and procedurally disciplined are not only safe, but also scientifically effective.

## CHAPTER 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 and security protocols.
- Knowledge and awareness of spill and exposure response 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 university training requirements.
- Completion of all lab-specific and other proficiency training requirements.
- Completion of all Occupational Health requirements, including documentation of required physicals, medical clearances, and/or vaccinations, when applicable.
- **Immediate reporting to the Principal Investigator of any situation, including physical or psychological issues, that compromises an individual's ability to perform as required in the laboratory.**
- **Immediate reporting to the Principal Investigator and the UC, where appropriate, of behavior or activities that are inconsistent with safety and security plans.**

The establishment of support systems for the individual scientist is essential to the development of a culture of responsibility at an institution. 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 1-855-775-4357 or seek help online at <http://rsli.acieap.com>. Please contact the Benefits Office (773-702-9634) for log-in 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 for instances of noncompliance 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 on 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 Research 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.



## CHAPTER 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 biological and physical hazard characteristics of the agent,
2. The sources likely to harbor the agent,
3. Host susceptibility,
4. The procedures that may disseminate the agent, and
5. The best method to effectively inactivate the agent.

Globally, numerous government agencies have classified microorganisms pathogenic for humans into risk groups 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, 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. For the purpose of discussion, this manual adopts the Risk Group classification based on the NIH definition. The scheme ascends in order of increasing hazard from Risk Group 1 (RG1) agents, which are nonpathogenic for healthy adults, to Risk Group 4 (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 and can be accessed electronically at:

<https://osp.od.nih.gov/biotechnology/nih-guidelines/>

The American Biological Safety Association also provides a comprehensive risk group listing and references international agencies. This list is accessible at:

<https://my.absa.org/Riskgroups>

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 roughly correspond standard laboratory facilities and microbiological practices, whereas those in RG4 often 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 RG2 and RG3 agents 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 important to assume virulence and handle such agents at the prescribed biosafety level.

## B. ROUTES OF INFECTION

Pathogenic microorganisms are transmitted via several possible routes of infection, depending on the pathogen. The most common routes of infection are inhalation of infectious aerosols, exposure of mucous membranes to infectious droplets, ingestion from contaminated hands or utensils, or percutaneous inoculation (injection, incision, or animal bite). Appropriate precautions should be implemented to reduce the risk of such exposures.

## C. EXPOSURE SOURCES

### 1. CLINICAL AND PATHOLOGICAL SPECIMENS

Any specimen from humans 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 *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 virus (HCV) and other bloodborne pathogens. Such personnel are required by Federal law (OSHA 29 CFR 1910.1030) to undergo bloodborne pathogen (BBP) training. At the University of Chicago, this training requirement can be satisfied either online or by attending an in-person training session. For information on obtaining this training, go to <http://researchsafety.uchicago.edu/page/biological-safety-0>

Some animals, such as non-human primates, may harbor endogenous pathogens that are virulent for humans. For personnel handling these animals or their tissues/body fluids, we recommend an analogous approach to infection control, *universal precaution*, which assumes these animals and their blood and body fluids to be infectious.

## 2. CULTURES

Biosafety level 2 (BSL-2) practices should be used for cell lines of human origin, even well-established lines such as 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 BSL-2. When a cell culture is inoculated with (or known to contain) an etiologic agent of a higher biosafety level, it should be classified and handled at the same biosafety level as the agent.

**When manipulations of these types of cell cultures present a potential to create aerosols, use a biological safety cabinet. Do not use a clean bench as it will not protect you from potential pathogens. Conversely, a fume hood will protect you but will not protect your sample from contaminants in the ambient air. A disambiguation of biological safety cabinets, clean benches, and fume hoods is provided in Chapter IV, C. Engineering Controls.**

Accidental spilling of infectious liquid 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:

### EXAMPLE OF PROCEDURES THAT GENERATE AEROSOLS:

- 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 without a tight seal.
- Vortexing liquid samples without a tight seal.

#### **WHAT TO DO TO LIMIT AEROSOLS GENERATION/DISSEMINATION:**

- Manipulate cultures of infectious material carefully to avoid the uncontrolled release of aerosols or the generation of large droplets or spills.
- Centrifuge cultures using gasket-sealable tubes, carriers, and rotors, when available.
- Seal microplate lids with tape or replace them with adhesive-backed Mylar film.
- When vortexing infectious samples, ensure there is a tight seal.
- Load, remove, and open tubes, plates, and rotors within a biological safety cabinet or fume hood. Keep in mind that the fume hood will protect you from your sample but will not protect your sample from potential contamination from room air.

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.

Considerations for shared/core facilities:

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 is often operated at 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.

### **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 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 <https://animalresources.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 be problematic in immunocompromised individuals. Establishment of safety consciousness is integral to the conduct of good science.

### **2. RESEARCH LABORATORIES**

The risk of exposure increases with experiments in research laboratories using high concentrations or large quantities of pathogens. 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 humans or animals 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.

**Regardless of the risk group of the organism you work with, 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 or other metabolism disorders, pregnancy, certain autoimmune diseases, immunodeficiency or immunosuppression, animal-related allergies, chronic skin conditions or respiratory disorders, and steroid therapy, even if only temporary.**

## **CHAPTER 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 (BSL-1 or BL-1) being the least stringent and biosafety level 4 (BSL-4 or BL4) being the most stringent. In general, BSL-1 is recommended for work with nonpathogenic microorganisms, BSL-2 is recommended for disease agents transmitted by direct contact (percutaneous inoculation, ingestion, or mucous membrane exposure) or with human-derived material, BSL-3 is recommended for disease agents with a potential for aerosol transmission, and BSL-4 is recommended when total separation between the infectious agent and investigator is critical. Biosafety levels often, but not always, correlate with risk group designations. For example, deleting the virulence factor of a RG3 pathogen may render it safe to be handled with BSL-2 facility and practices. Conversely, insertion of toxin-producing genes in an RG1 microorganism may require BSL-2 facility and practices. Furthermore, RG2 agents with potential for causing mutagenesis may require additional BSL-3 practices in BSL-2 facility. An Institutional Biosafety Committee (IBC), established under the NIH Guidelines, determines the proper biosafety level. One should always carefully review project-specific, approved IBC protocol prior to starting the research. This manual is designed to focus on BSL-2 management, 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 Tables 1 and 2.

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

<b>Risk Group</b>	<b>Biosafety Level</b>	<b>Laboratory Practices</b>	<b>Safety Equipment</b>	<b>Examples of Laboratories</b>
1	Basic – BSL-1	GMT <sup>a</sup>	None required; open bench work	Basic teaching
2	Basic – BSL-2	GMT plus protective clothing; access control, <i>universal precautions</i> for handling sharps, biohazard sign	Open bench plus BSC <sup>b</sup> for activities with aerosol-potential	<b>Most biomedical research on the Hyde Park campus</b> ; primary level hospital; diagnostic, teaching, and public health
3	Containment – BSL-3	As BSL-2 plus special clothing, respiratory protection considered, restricted access	BSC and/or other primary containment for all activities, directional air flow	Special diagnostic; Regional Biocontainment Laboratory
4	Maximum Containment – BSL-4	As BSL-3 plus airlock entry, decon shower exit, special waste disposal	Class III BSC or positive pressure suits, double-ended autoclave, HEPA-filtered air	National Biocontainment Laboratory; Dangerous pathogen units; (Not at University of Chicago)

<sup>a</sup> GMT, Good Microbiological Technique.

<sup>b</sup> BSC, Biological Safety Cabinet

**Table 2**  
SUMMARY OF BIOSAFETY LEVEL REQUIREMENTS

	Biosafety Level			
	1	2	3	4
<b>Isolation of laboratory</b>	No	No	Desirable	Yes
<b>Room sealable for decontamination</b>	No	No	Desirable	Yes
<b>Inward air flow ventilation</b>	No	Desirable	Yes	Yes
<b>Mechanical ventilation via building system</b>	No	Desirable	Yes	No
<b>Mechanical, independent ventilation</b>	No	No	Desirable	Yes
<b>Filtered air exhaust</b>	No	No	Desirable	Yes
<b>Double-door entry</b>	No	No	Yes	Yes
<b>Airlock</b>	No	No	No	Yes
<b>Airlock with shower</b>	No	No	No	Yes
<b>Effluent treatment system</b>	No	No	Desirable (BSL3-Ag)	Yes
<b>Autoclave on site</b>	Desirable	Desirable	Yes	Yes
<b>Autoclave in laboratory/suite</b>	No	No	Desirable	Yes
<b>Double-ended autoclave</b>	No	No	Desirable	Yes
<b>Class II BSC</b>	No	Desirable	Yes	Yes

<sup>1</sup>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*, 5<sup>th</sup> edition, (2009) <http://www.cdc.gov/biosafety/publications/bmbl5/>.

Experiments involving recombinant or synthetic 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 or Synthetic Nucleic Acid Molecules* (<http://osp.od.nih.gov/office-biotechnology-activities/biosafety/nih-guidelines>).



## **B. PRACTICES AND PROCEDURES**

The following practices, **corresponding to BSL-2**, 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 Research Safety at 773-834-2707 for additional BSL-3 containment information.

### **1. PERSONAL HYGIENE**

- (a) Do not eat, drink, chew gum, use tobacco, apply cosmetics (including lip balm), or handle contact lenses in the laboratory.
- (b) Do not store food for human consumption in laboratory, including 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 remove personal protective equipment (such as lab coats) from the lab.
- (f) First-aid kits should be available and not expired.

### **2. LABORATORY PROCEDURES FOR HANDLING INFECTIOUS MICROORGANISMS**

- (a) A laboratory biosafety 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 BSL-2 Biosafety Manual, will provide you with the necessary information to work safely.
- (b) If you are working with recombinant or synthetic DNA and/or working with agents at BSL-2 or higher, you must obtain approval from the IBC before work begins.  
The IBC can be reached at 773-834-5850 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 and equipment before starting work.
- (e) Keep laboratory doors closed; limit access to lab personnel when needed.
- (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. Templates of these biohazard signs will be generated by the Office of Research Safety based upon the information provided in your lab's IBC protocol.

- (g) BSL-2 laboratories should have a sink for hand washing, an eyewash station in which the eyewash is tested/flushed weekly, be relatively clutter-free, and be easy to clean.
- (h) 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. Wear eye protection when working in the BSL-2 laboratory when necessary.
- (i) Remove and leave all protective clothing, including gloves, within the laboratory before exiting. If transport of research materials through public spaces is required, one glove may be removed and ungloved hand used to handle public equipment (door handles, elevator buttons, etc.) and lab coats may be carried.
- (j) Never mouth-pipette; use mechanical pipetting devices.
- (k) When practical, perform all aerosol-producing procedures such as shaking, grinding, sonicating, mixing, and blending in a properly operating biological safety cabinet (BSC). Note that placement of certain equipment within the BSC may compromise cabinet function by disturbing the air curtain. BSC certification and annual re-certification should be performed with regularly used equipment inside the BSC during certification.
- (l) When centrifuging materials containing infectious agents use durable, shatter-resistant, closable tubes. Use a centrifuge with sealed heads or screw-capped safety cups. After centrifugation, open the tubes within a BSC to capture aerosols.
- (m) Minimize the use of needles, syringes, razor blades, and other sharps when possible. After use, syringe-needle units must be disposed in a dedicated sharps container without removing or recapping the needles.
- (n) Cover countertops where hazardous materials are used with plastic-backed disposable paper to absorb spills and dispose of them daily or following a spill.
- (o) Wipe work surfaces with an appropriate disinfectant according to corresponding IBC protocol after experiments and immediately after spills.
- (p) Decontaminate all contaminated or potentially contaminated materials by appropriate methods before disposal (See Chapter V of this Manual).
- (q) Report all accidents and spills to the laboratory supervisor. All laboratory personnel should be familiar with the emergency spill protocol and the location of cleanup equipment. Step-by-step spill response protocols should be posted in the laboratory and can be provided by Office of Research Safety.
- (r) Good housekeeping practices are essential in laboratories engaged in work with infectious microorganisms. Do not forget to routinely decontaminate all shared equipment and equipment in common areas.

- (s) Be sure to advise custodial staff of hazardous areas and places they are not to enter. Use appropriate biohazard signs.
- (t) Equipment used with biohazards must be decontaminated prior to repair.

## 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 Research Safety 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 ensures that air will enter the laboratory and not egress to the hallway. While negative air pressure is recommended at BSL-2, it is required at BSL-3. **If you wish to maintain negative room pressure, laboratory doors should be kept closed while biohazardous work is taking place.**

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 BSL-3 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. When functioning correctly and used in conjunction with good microbiological techniques, BSCs 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. The Office of Research Safety provides training for proper BSC usage in the “Recombinant DNA at BSL-2” training module:

#### (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.

**CLASS I** - cabinets are manufactured on a limited basis and have largely been replaced by Class II cabinets. A Class I cabinet is essentially a HEPA-filtered chemical fume hood in which all of the air entering the cabinet is exhausted into the room or ducted to the outside.

**CLASS II** - The most utilized class of BSC on campus. Two varieties of Class II BSCs are used and 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. **Volatile chemical or radioactive material should NOT be used in this cabinet.**  
Some older BSCs may require additional modifications due to changes in National Sanitation Foundation (NSF) regulations. Specifically, “Effective April 15, 2016, NSF Accredited field certifiers shall no longer certify either direct-connected Type A cabinets or canopy connected Type A cabinets without alarms”
- **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 II TYPE C1**—capable of being configured and operate in either Type A or Type B mode
- Since 2002, NSF has adopted a new classification system. A table comparing the current and pre-2002 BSC classification is shown below:

New NSF BSC Classification	Pre-2002 BSC classification
A1	Class II, Type A
A2	Class II, Type A/B3
A2	Class II, Type B3
B1	Class II, Type B1
B2	Class II, Type B2

**CLASS III** - cabinets are totally enclosed glove boxes and are used only for the most hazardous biological operations. Class III BSCs have dedicated, independent 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.

Many BSCs have ultraviolet lamps inside them. These lamps 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 should not be the sole source of decontamination of BSC surfaces.**

**(b) BSC OPERATION**

• **START UP**

- Turn on blower and fluorescent light.
- Wait at least two minutes before loading equipment. This is to purge the BSC of contaminated air.
- Check grilles for obstructions
- Disinfect all interior work surfaces with a disinfectant appropriate for the agent in use.
- Adjust the sash to proper position; NEVER use above the 8-inch mark.
- RESTRICT traffic in the BSC vicinity. To ensure proper functioning of a BSC, it is best to locate them away from high-traffic areas and doorways to common areas.

• **LOADING MATERIALS AND EQUIPMENT**

- Load only items needed for the procedure.
- Do not block the rear or front exhaust grilles.
- Disinfect the exterior of all containers prior to placing them in the BSC.
- Arrange materials to minimize movement within the cabinet.
- Arrange materials within the cabinet from CLEAN to DIRTY (or STERILE to CONTAMINATED).
- Materials should be placed at least six inches from the front BSC grille.
- Never place non-sterile items upstream of sterile items.
- Maintain the BSC sash at proper operating height, approximately level with your armpits.

• **RECOMMENDED WORK TECHNIQUE**

- Wash hands thoroughly with soap and water before and after any procedure.
- Wear gloves and lab coat/gown; use aseptic technique.

- Avoid blocking front and back grilles. Work only on a solid, flat surface; ensure chair is adjusted 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.
  - Two people working together in one BSC is discouraged, however in the event it is necessary ensure that both workers are following the correct precautions.
- **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 BSC.
    - Decontaminate interior work surfaces of the BSC with an appropriate disinfectant.
- **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 entire work surface. 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 towels and placing it into the biohazard bag. Any splatter onto items within the cabinet, as well as the walls of the cabinet interior, should be immediately wiped with a towel dampened with disinfectant. 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. Drain pan should be wiped down with 70% alcohol to prevent corrosion. 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 properly calibrated and the HEPA filters must be certified and leak tested. The University of Chicago requires that all BSCs be certified annually by a certified professional. **This is imperative for BSCs intended for work at BSL-2 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 Research 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 by the Office of Research Safety as part of the Recombinant DNA at BSL-2 training module. Contact us to arrange this training.

## CHAPTER V: DISPOSAL OF WASTES CONTAMINATED WITH INFECTIOUS AGENTS

These biohazard waste disposal guidelines are designed to not only protect 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 biohazard waste in the laboratory must ensure 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 REGULATED BIOHAZARD WASTE

The following items are usually considered to be regulated biohazard waste.

1. Microbiological laboratory waste (cultures derived from clinical specimens and pathogenic microorganisms, disposable laboratory supplies or consumables that have come into contact with the cultures, etc.).
2. Samples containing recombinant or synthetic DNA
3. Tissues, bulk blood, or body fluids from humans.
4. Tissues, bulk blood, or body fluids from animals that have the potential to carry an infectious agent that can be transmitted to humans.
5. Sharps (needles, scalpels, etc) and broken glass that may be contaminated.

Organisms carrying regulated recombinant DNA 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 are mixed with infectious waste, they must be managed as though they are infectious. For this reason, you should **segregate regulated biohazard 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 an 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.

There are several ways this is dealt with at UC:

1. Many labs and buildings collect biohazard waste in red bags and/or red bins with the biohazard symbol. This waste is picked up by people from Environmental Services or Environmental Health and Safety and is ultimately carried away by a certified contractor (e.g., Stericycle) where it is managed as regulated medical waste and decontaminated off-site before it is disposed in the environment.
2. As an added precaution, some labs choose to autoclave their biohazard waste before it gets picked up as in (1) above. For labs that choose to autoclave their own biohazard waste and discard in the general waste afterward, it is necessary (and legally required by the Illinois Environmental Protection Agency) to periodically test your autoclaves with bio-indicators or an equivalent. Please contact the Office of Research Safety if this is an option that your lab wants to use.

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.

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 until the scheduled pick-up by the off-site contractor. Biohazard 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.

Usually a standard autoclave cycle of 121 °C, 15 psi for 45 minutes to an hour is sufficient, 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, a longer cycle may be necessary. 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 the Office of Research Safety for more information.

### **2. SEWAGE TREATMENT**

Most fluid waste, including human blood or infectious cultures that have been decontaminated by the appropriate method, can be discarded by pouring into the sanitary sewer (laboratory sink), followed by flushing with water. Care should be taken to avoid the 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 (e.g., bleach) 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 that is 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 chemical

compatibility, 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 non-target organic substances, and corrosiveness, even in dilute forms. Household bleach is typically diluted 1:10 to 1:100 such that the available halogen is approximately 0.5%-0.05% (chlorine concentration of 5000 ppm-500 ppm). A 1:10 dilution of household bleach is generally effective for most biohazardous agents (the exceptions are prions and certain biological toxins). If a hypochlorite compound is used as a disinfectant for wiping a surface, it is recommended that the decontamination step is followed by a wipe-down using 70% alcohol or water to mechanically remove corrosive residue. 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) is effective against vegetative forms of bacteria, fungi, and enveloped viruses, but will not readily inactivate bacterial spores or non-enveloped viruses. The most effective alcohol concentration for decontamination is 70%. 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 Research Safety.

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, chlorine dioxide, 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.**

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

**Table 3a.**

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

+++; good; ++, fair; +, slight; -, nil; v, depends on virus.

<sup>a</sup> above 40 °C.

<sup>b</sup> above 20 °C.

**Table 3b.**

	Inactivated by			Toxicity			Stable? <sup>a</sup>	Corrosive?	Flammable?
	Protein	Hard water	Detergent	Skin	Eyes	Lung s	>1 week		
<b>Phenolic Compounds</b>	+	+	C	Y	Y	N	Y	Y	N
<b>Hypochlorites</b>	+++	+	C	Y	Y	Y	N	Y	N
<b>Alcohols</b>	+	+	-	N	Y	N	Y	N	Y
<b>Formaldehyde</b>	-	+	-	Y	Y	Y	Y	N	Y/N
<b>Glutaraldehyde</b>	-	+	-	Y	Y	Y	Y	N	N
<b>Iodophores</b>	+++	+	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.

<sup>a</sup>Stability may be effected by exposure to light and/or air.

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).

## CHAPTER VI: EMERGENCY PLANS AND REPORTING

No matter how carefully one works, laboratory accidents occur and may 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. INFECTIOUS AGENT SPILL RESPONSE

It is the policy of the University of Chicago (UC) that spills of potentially infectious materials shall immediately be contained and cleaned up by employees **properly trained and equipped** to work with potentially infectious materials. Ultimately, the goal of cleaning up any spill of infectious agent or potentially infectious agent is to ensure the safety of the researcher/clinician and those around him/her. When cleaning up a spill, there are several important points that all researchers/clinicians should keep in mind:

- Many, but not all, pathogenic agents carry a risk of exposure by inhalation. Droplets are relatively large and settle with gravity and can be easily cleaned. Aerosols are small (less than 10  $\mu\text{m}$ ) and must be removed by the building's ventilation system. If the pathogen involved in the spill carries a risk of exposure via the aerosol route, *immediately* leave the area for 30 minutes to allow droplets to settle and aerosols to be removed.
- In order to ensure the safety of the researchers and anyone in the vicinity, it is important to contain the spill. If possible, paper towels should be used to cover the spill and contain the agent *prior* to leaving the room.
- A solution of 10% household bleach (1:10 dilution) is recommended for cleaning up any spill regardless of the otherwise approved chemical disinfectant.
- *The goal of any spill clean up is the safety of the researcher and those in the vicinity.* With that in mind, below is the recommended protocol for cleaning up a known or potentially infectious agent.

Any investigator working with microorganisms known to be infectious, or potentially infectious, to humans, animals or plants should be trained and equipped to deal with spills.

Examples of infectious/potentially infectious materials include:

1. Microbiological cultures derived from clinical specimens or pathogenic microorganisms and laboratory equipment that have come into contact with such cultures.

2. Tissues, bulk blood, and body fluids from humans and non-human primates.
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.

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.

Well-prepared staff can appropriately manage the majority of spills. One exception to this general rule is a spill of a significant volume outside of a biological safety cabinet (significance varies depending on the nature of the biohazard, but for purposes of this discussion, we define this to include cultures in excess of one liter in volume). For spills of this nature, please follow the **Incident Notification** procedure described at the end of this response protocol.

## **B. BIOHAZARDOUS SPILL PROTOCOLS**

<b>INSIDE A BIOLOGICAL SAFETY CABINET (BSC)</b>
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1. Immediately stop all work but leave the BSC blower fan on during cleanup.
2. Remove and replace contaminated gloves
3. The operator should be wearing gloves and a lab coat throughout the cleanup procedure. Cover spill with paper towels and carefully pour appropriate disinfectant\* solution on to the spill-soaked paper towels.
4. With paper towels and the disinfectant, wipe down the walls and work-surface of the BSC and any equipment within the BSC that may have been contaminated.
5. Spray down the work surface with disinfectant. Examine the drain pan and flood the drain pan with disinfectant solution if the spill has contaminated the drain pan. Allow the disinfectant to stand at least 10 minutes.
6. If bleach or other chlorine-based disinfectant was used, wipe up excess disinfectant and spray work surface and BSC walls with 70% alcohol to remove residual disinfectant as bleach can be corrosive.
7. Dispose all waste, including PPE, as biohazard waste.
8. Wash hands with soap and water.

\*For most spills, the best disinfectant is a 1:10 solution of household bleach, freshly made. Please consult the Office of Research Safety if you have questions about the best disinfectant for your agent.

<p><b>BIOHAZARDOUS SPILL (UP TO ONE LITER IN VOLUME)</b> <b>OUTSIDE A BIOLOGICAL SAFETY CABINET (BSC)</b></p>
---

1. If necessary, remove contaminated clothing and place into a biohazard bag, wash all contaminated body parts, and flush exposed mucous membranes with water.

**Optional steps: If the spilled material has potential to be infectious via the aerosol route or if there are exceptionally large amounts of aerosols generated:**

1. Immediately following the spill, alert co-workers, cover spill with paper towels and leave the lab area immediately.
  2. Close lab door and post a “**DO NOT ENTER**” sign.
  3. Wait at least 30 minutes prior to re-entry (allowing droplets to settle and aerosols to dissipate).
  4. Upon re-entry, don appropriate personal protective equipment (PPE), i.e. lab coat, gloves and mucous membrane protection (safety glasses and/or face mask, gloves) and proceed with clean-up as described above.
2. Put on appropriate PPE.
  3. Place absorbent material over the spill (such as paper towels) to prevent the spill from spreading.
  4. Notify supervisor. If necessary, contact the Office of Research Safety for additional guidance or assistance, although this is not a requirement.
  5. Carefully pour an appropriate disinfectant solution\* onto the towel-soaked spill; care should be taken to minimize splashing. **Allow disinfectant to interact with the spill FOR AT LEAST 10 MINUTES.**
  6. If broken glass or sharp objects are present, handle with tongs, forceps, brush and dustpan, or other mechanical means. Place broken glass in sharps container. Do not

use your hands!

7. Wipe up spill/excess disinfectant working from the outside of the spill toward the center and place paper towels and other contaminated waste into biohazard bag. Spray the area with 70% alcohol and wipe up to remove residual disinfectant.
8. If necessary, wash and mop the entire area around the spill using an appropriate disinfectant.
9. Disposable PPE should be placed into a biohazard bag. Reusable PPE (e.g., lab coats) should be decontaminated and laundered if contaminated with infectious material.
10. Wash hands with soap and water.

\*For most spills, the best disinfectant is a 1:10 solution of freshly made household bleach. Please consult the Office of Research Safety if you have questions about the best disinfectant for your agent.

#### **SMALL LABORATORY EQUIPMENT**

Liquid spills on small laboratory equipment shall be contained as follows:

1. Don appropriate PPE (lab coat, gloves, mucous membrane protection)
2. Drain excess liquid with paper towels
3. Immerse the contaminated equipment in a 10% bleach solution (made fresh weekly) and allow 10 minutes contact time
4. Remove equipment from the decontaminant, blot off excess liquid with paper towels
5. Spray with a 70% alcohol solution, wipe clean to remove potentially corrosive bleach residue
6. Dispose of paper towels and gloves as biohazard waste; and
7. Wash hands with soap and water.

#### **LARGE LABORATORY EQUIPMENT**

Liquid spills on large laboratory equipment (e.g., centrifuge, incubator, autoclave) shall be contained as follows:



1. Drain excess liquid with paper towels
2. Spray the contaminated equipment in a 10% bleach solution (made fresh weekly) including area surrounding the spill
3. Allow to 10 minutes contact time
4. Wipe with paper towels
5. Spray with a 70% ethanol/isopropyl alcohol solution, wipe clean
6. Dispose of paper towels and gloves as biohazard waste; and
7. Wash hands with soap and water.

**Do NOT attempt to clean up a spill if any of the following conditions apply:**

- If the spill is an unknown agent;
- The quantity spilled is greater than one liter.

**If you are UNABLE to deal with the spill OR if the spill is greater than 1 liter adhere to the following steps.**

□

### **Incident Notification**

1. Notify the University of Chicago Police Department at extension **123** from a campus phone or **773-702-8181** to report the incident in campus buildings or Public Safety at **773-702-6262** for the Medical Center.
2. Evacuate the area and post lab with “**DO NOT ENTER**” sign.
3. The University Police shall immediately notify the “On-Call” Safety Officer.

## **B. EXPOSURE PROTOCOLS**

In the event of an exposure to a biologically hazardous agent:

- Let someone know immediately (get a “buddy”).
- Perform first aid.
- Contact the infectious disease exposure (aka “needlestick”) hotline/pager: Dial 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. This hotline is available 24 hr/day and 7 days/week.
- Let your supervisor know immediately.

- Contact the Office of Research Safety as soon as reasonably possible.

## **C. REPORTING**

The importance of reporting accidental spills (when necessary) 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 or synthetic 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://humanresources.uchicago.edu/fpg/policies/100/p103.shtml>  
In addition, reports can be provided to UC at the Whistleblower hotline: 1-800-971-4317.

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

## **CHAPTER VII: SHIPPING HAZARDOUS BIOLOGICAL MATERIALS**

Hazardous materials capable of posing an unreasonable risk to health, safety, and property, are common 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 individuals involved in shipping certain hazardous materials to be trained and certified in proper handling of these materials.

Activities for which training is required include:

- Identify hazard material

- Preparing shipping papers in compliance with national/international regulations

- Marking and labeling packages

- Properly pack hazardous materials

- Supervising these activities

Required training for shipping of hazardous biological materials is available on University learning management system. Contact Office of Research Safety for information on obtaining this training and prior to shipping any biohazardous material.

## CHAPTER 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. Acute Respirator Distress Syndrome (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, 1:10 dilution of household bleach (made fresh weekly), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate.

## **B. ADENO-ASSOCIATED VIRUS (AAV)**

**Virology:** Adeno-associated virus 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 the presence of wild type adenovirus or herpesvirus; in the absence of helper virus, AAV will stably integrate into the host cell genome. Co-infection 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. 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, 1:10 dilution of household bleach (made fresh weekly), 2% glutaraldehyde, 0.25% sodium dodecyl sulfate.

## **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-lymphotropic 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.

**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, 1:10 dilution of household bleach (made fresh weekly), 70% ethanol, 2% glutaraldehyde/formaldehyde.

## **D. LENTIVIRUS**

**Virology:** The genus of the family Retroviridae consists of nononcogenic retroviruses that produce multiorgan 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. The 2<sup>nd</sup> generation packaging system combine all the important packaging components: *gag*, *pol*, *rev*, and *tat* in one single plasmid. The 3<sup>rd</sup> generation packaging system eliminated the Tat protein and expresses *rev* on an independent plasmid. Even though it is more cumbersome to use, this design provide maximum biosafety by further reducing the probability of replication-competent virus.

**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.

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

**Please note that if the lentivirus is carrying an oncogene or potential oncogene, an exposure could result in the oncogene integrating into your genome. A lentivirus harboring an oncogenic transgene is likely one of the most hazardous viral vector constructs on this campus, particularly if it has been pseudotyped with VSV-G.**

**Use of lentivirus at the University of Chicago must be approved by the IBC prior to initiation of the work and requires laboratories operating at Biosafety Level 2 with Biosafety Level 3 practices. Please contact the Office of Research Safety for more information.**

**Susceptibility to disinfectants:** Susceptible to many disinfectants – Clidox, 1:10 dilution of household bleach (made fresh weekly), 70% ethanol, 2% glutaraldehyde/formaldehyde.

## **E. RETROVIRUS (OTHER THAN LENTIVIRUS)**

Infectious viruses that integrate into transduced cells with high frequency and may have oncogenic potential in their natural hosts. Retrovirus vectors 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 (including human) and non-mammalian cells.

**Clinical features:** None to date.

**Epidemiology:** MoMuLV infects only actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germ-line 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, 1:10 dilution of household bleach (made fresh weekly), 70% ethanol, 2% glutaraldehyde/formaldehyde.

## **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 [e.g., modified Ankara strain (MVA)]. Poxviruses are highly stable, and vaccinia virus can cause severe infections in immunocompromised 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; autoinoculation 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, 1:10 dilution of household bleach (made fresh weekly), 2% glutaraldehyde/formaldehyde.

## **G. BACULOVIRUS**

Non-mammalian viruses that usually infect 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 BSL-1.

*Note: Even though this vector is nonpathogenic 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 for replication competent virus that is mandated by the UC IBC, please go to this link:

[http://ibc.uchicago.edu/docs/ibc\\_Testing\\_Requirements\\_Viral\\_Vectors.pdf](http://ibc.uchicago.edu/docs/ibc_Testing_Requirements_Viral_Vectors.pdf)

## CHAPTER 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 bacteria, highly toxic (i.e., LD<sub>50</sub> on the order of µg/kg) and are relatively unstable (destroyed rapidly when heated to  $\geq 60^{\circ}\text{C}$ ). Bacterial endotoxins are lipopolysaccharide complexes derived from the cell membrane of Gram-negative bacteria that are released upon bacterial death. Endotoxins are relatively stable (can withstand heating at  $60^{\circ}\text{C}$  for hours without losing activity) and moderately toxic (i.e., LD<sub>50</sub> on the order of tens to hundreds of µg/kg).

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 BSL-2 setting. In some cases (e.g., large scale production, manipulation of large quantities of powder form of toxin) management at BSL-3 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, BSL-2 management is usually sufficient for work with most biological toxins. 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 Research 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 <sub>50</sub> (µg/kg) *
<b>Abrin</b>	<b>0.7</b>
Aerolysin	7
<b>Botulinum toxin A</b>	<b>0.0012</b>
<b>Botulinum toxin B</b>	<b>0.0012</b>
<b>Botulinum toxin C1</b>	<b>0.0011</b>
<b>Botulinum toxin C2</b>	<b>0.0012</b>
<b>Botulinum toxin D</b>	<b>0.0004</b>
<b>Botulinum toxin E</b>	<b>0.0011</b>
<b>Botulinum toxin F</b>	<b>0.0025</b>
b-bungarotoxin	14
<i>Clostridium difficile</i> enterotoxin A	0.5
<i>Clostridium perfringens</i> lecithinase	3
<i>Clostridium perfringens</i> perfringolysin O	13-16
<i>Clostridium perfringens</i> delta toxin	5
<i>Clostridium perfringens</i> epsilon toxin	0.1
<b>Conotoxin (Only short, paralytic alpha conotoxins with specific sequences are considered Select Agents)</b>	<b>12-30</b>
<b>Diacetoxyscirpenol</b>	<b>1000-10,000</b>
Diphtheria toxin	0.1
Listeriolysin	3-12
Modeccin	1-10
Pertussis toxin	15
Pneumolysin	1.5
<i>Pseudomonas aeruginosa</i> toxin A	3
<b>Ricin</b>	<b>2.7</b>
<b>Saxitoxin</b>	<b>8</b>
Shiga toxin	0.25
<i>Shigella dysenteriae</i> neurotoxin	1.3
<b><i>Staphylococcus</i> enterotoxin B</b>	<b>25</b>
<b><i>Staphylococcus</i> enterotoxin F</b>	<b>2-10</b>
<b><i>Staphylococcus</i> enterotoxins A, C, D, and E</b>	<b>20(A); &lt;50(C)</b>
Streptolysin O	8
Streptolysin S	25
<b>T-2 toxin</b>	<b>5,000-10,000</b>
Taipoxin	2
Tetanus toxin	0.001
<b>Tetrodotoxin</b>	<b>8</b>
Volkensin	1.4
<i>Yersinia pestis</i> murine toxin	10

\* Note that the LD<sub>50</sub> 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 noted in RED are considered Select Agents if being stored in large enough quantities (see Chapter X below). For more information please consult:  
<https://www.selectagents.gov/PermissibleToxinAmounts.html>

#### REFERENCES:

1. Gill, D. Michael; 1982; Bacterial toxins: a table of lethal amounts; Microbiological Reviews; 46: 86-94.
2. Stirpe, F.; Luigi Barbieri; Maria Giulia Battelli, Marco Soria and Douglas A. Lappi; 1992; Ribosome-inactivating proteins from plants: present status and future prospects; Biotechnology; 10: 405-412.
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 cabinets, freezers, and/or 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 on-site, the date and amount removed from storage, the person removing the aliquot from storage, the purpose of use, and the quantity remaining. *Toxin Inventory* forms are available from the Office of Research Safety upon request.

### DECONTAMINATION METHODS

The majority of biological toxins can be inactivated or decontaminated with household bleach or autoclaving. Tables 5 and 6 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**  
**HYPOCHLORITE (NaOCl) +/- SODIUM HYDROXIDE (NaOH)**

<b>Toxin</b>	<b>2.5% NaOCl<sup>a</sup> + 0.25 N NaOH</b>	<b>2.5% NaOCl<sup>a</sup></b>	<b>1.0% NaOCl<sup>b</sup></b>	<b>0.1% NaOCl<sup>c</sup></b>
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)

<sup>a</sup>2.5% NaOCl is approximately equal to 50% household bleach (1:2 dilution)

<sup>b</sup>1.0% NaOCl is approximately equal to 20% household bleach (1:5 dilution)

<sup>c</sup>0.1% NaOCl is approximately equal to 2% household bleach (1:50 dilution)

**Table 6**  
**COMPLETE INACTIVATION OF TOXINS BY AUTOCLAVING OR 10-MINUTE**  
**EXPOSURE TO VARYING TEMPERATURES OF DRY HEAT**

<b>Toxin</b>	<b>Autoclaving</b>	<b>Dry Heat<sup>o</sup>F</b>			
		<b>200</b>	<b>500</b>	<b>1000</b>	<b>1500</b>
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.

## **CHAPTER X: SELECT AGENTS AND TOXINS**

The federal government has published a list of infectious microorganisms 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>

Certain toxins on the Select Agent list may be used with the caveat that an investigator does not possess more than a certain amount of a given toxin. These “permissible limits” can be found here:

<https://www.selectagents.gov/PermissibleToxinAmounts.html>

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



## CHAPTER 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 or synthetic nucleic acid 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 Science Policy (NIH-OSP), 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-OSP website, which can be accessed here: <https://www.nih.gov/news-events/videos/dual-use-research-dialogue>

In general, experiments that aim to produce, or are *reasonably* anticipated to produce one of the effects below have the potential to be DURC:

- Enhance the harmful consequences of the agent;
- Disrupt immunity or the effectiveness of an immunization against the agent without clinical and/or agricultural justification;
- Confer to the agent resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that agent or facilitates their ability to evade detection methodologies;
- Increase the stability, transmissibility, or the ability to disseminate the agent;
- Alter the host range or tropism of the agent;
- Enhance the susceptibility of a host population to the agent; and
- Generate or reconstitutes an eradicated or extinct listed agent.

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 Office of Research Safety by phone (773-834-2707) or email at [researchsafety@uchicago.edu](mailto:researchsafety@uchicago.edu) . 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 Research Safety (773-834-2707) or email [researchsafety@uchicago.edu](mailto:researchsafety@uchicago.edu) .**

Additional copies of this booklet can be downloaded from the Office of Research Safety’ website: <http://researchsafety.uchicago.edu/>

# Appendix 1

## Safety Information and Assistance

### Contact

### Reason

#### UC Police

123 (campus phone)  
773-702-8181

**Biological Exposure Hotline:**  
1-773-753-1880 enter pager: 9990#

*-Emergency Situations*  
*-Biological Exposure*  
*-Large biological spills*

#### Office of Research Safety

773-834-2707 (general)  
773-702-6299 (radiation)

[researchsafety@uchicago.edu](mailto:researchsafety@uchicago.edu)  
<http://researchsafety.uchicago.edu>

*-Laboratory Spills*  
*-Accidents involving biological agents*  
*-Biological Safety Cabinets*  
*-Recombinant DNA guidelines and rules*  
*-Shipping Requirements*  
*-Shipping Permits*  
*-Bloodborne Pathogen Information*  
*-Chemical Safety Training*  
*-Biological Safety Training*  
*-Radioactive Materials Training*  
*-Radioactive Spills*  
*-Radioactive Material Disposal*

#### Environmental Health and Safety

773-702-9999  
[safety@uchicago.edu](mailto:safety@uchicago.edu)  
<http://safety.uchicago.edu>

*-Hazardous waste disposal*  
*-Fire Safety Training*  
*-Environmental Health*  
*-Odor compliant*

#### Animal Resource Center

773-702-6756 (general)  
773-702-1342 (emergency)

*-All research involving animals*  
*-Animal-related accidents*

#### IBC & IACUC

773-834-5850  
[ibc@uchicago.edu](mailto:ibc@uchicago.edu)  
<https://ibc.uchicago.edu>

*-IBC-related Questions*  
*-ACUP-related Questions*  
*-AURA Help*  
*-AIMS Help*

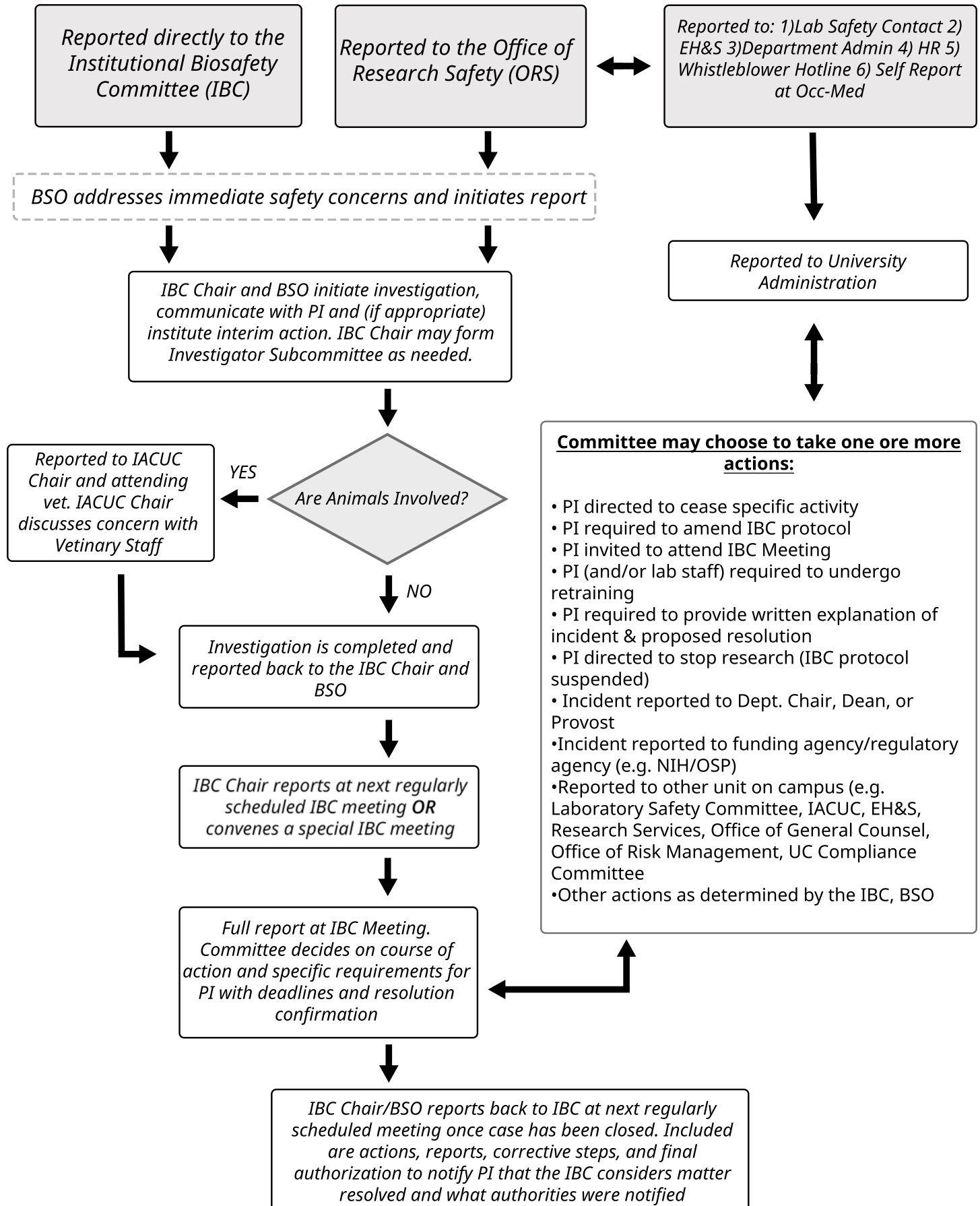
#### Occupational Medicine

773-702-6757

*-Non-emergency medical advice and treatment*

## Appendix 2

# IBC PROCEDURES FOR DEALING WITH BIOSAFETY/BIOSECURITY CONCERNS



## **NOTES**

