Biosafety Level 2 Guide

2023

Division of Research Safety

Office of the Vice Chancellor for Research & Innovation | University of Illinois Urbana-Champaign

102 Environmental Health and Safety Building | 101 S. Gregory St., MC-225 | Urbana, IL 61801

drs@illinois.edu | 217-333-2755

Table of Contents

Introduction

This guide is designed for laboratories working with biological materials requiring biosafety level 2 (BL-2) containment and practices. The BL-2 guide is intended to be used alongside the Laboratory Safety Guide (LSG) and act as training tools in a comprehensive laboratory safety plan. The policies and recommendations outlined in the LSG still apply in addition to the recommendations found here.

This document will introduce the concept of a risk assessment for biological agents and help you complete a Laboratory Safety Plan and develop experimental procedures with safety and compliance in mind. This plan will guide you through choosing an appropriate biosafety level and cover policies for work at biosafety level 2 (BL-2). This document is not all encompassing for your lab, each Principal investigator (PI) will still need to create lab- specific supplemental training and documentation to assure that lab personnel are working in a safe environment.

In addition to this BL-2 guide you should also have:

- A copy of your Institutional Biosafety Committee (IBC) project(s) which outlines:
  - Research description,
  - Risk assessment of materials and methods,
  - Method for decontamination of lab materials and spaces,
  - Mitigation equipment and practices.
- Medical preparedness requirements:
  - Copies of immunization declination statements for each employee when required,
  - Injury and accident reporting requirements.
- Lab specific spill cleanup instructions.
- Procedures for proper use, limitations, care, and maintenance of personal protective equipment specific to your lab.
- Laboratory specific practices and techniques for high-risk procedures and equipment that increases risk of occupational exposure (e.g., use of sharps or aerosol production).
- Permits for possession, transfer, or use of regulated agents from CDC or USDA/APHIS, if required.
- Lab specific training records.

Risk Assessments

Conducting a risk assessment will identify the hazardous properties of a known or potential biohazardous agent and examine the experimental manipulations that may cause exposure to that agent in the laboratory. It is important to realize that the causes of most laboratory acquired infections are unknown. Unlike acute injuries, like accidental injection, inhalation of infectious aerosols or direct contact with contaminated fomites may go unnoticed at the time of exposure. PIs should use risk assessments to alert staff to hazards associated with agents used in research.

The PI should carry out an initial risk assessment prior to beginning research with a hazardous agent. Further instruction and insight on how to conduct a risk assessment as well as descriptions of many hazards can be found in *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) by CDC/NIH (1), *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules* by NIH Office of Science Policy (2), and *Prudent Practices in the Laboratory: Handling and Management of Chemical Hazards* by the National Research Council (3). Not every exposure results in infection. A risk assessment for infection based on the potential host's immune system (the lab worker), mechanism of the exposure, infectious dose of the exposure, virulence of the agent, use of personal protective equipment, and immunization status needs to be performed (5). Briefly, the risk assessment should consider at least the following questions:

- What is the risk group of the parent organism?
- Is there an agent summary described by the CDC, either online or in the BMBL, Section VIII?
- What is the natural route of transmission for the agent?
- What additional routes of transmission should be considered as a part of laboratory methods; e.g., are aerosols generated from centrifugation or vortexing?
- Has the organism been modified in any way?
- Are the transgenes expressed? Do these expressed transgenes increase the risk of the agent(s)? (e.g., oncogenes)?
- What is the working volume and concentration of the agent?
- Will animals be a part of the work?
- Will sharps be a part of the work?
- What is the list of symptoms from an exposure to the agent?
- Are vaccinations or treatments available for the agent?
- Is the agent hazardous to a particular group of people (e.g., children, immunocompromised adults)?

Routes of Exposure

Exposure to biological agents in the laboratory occurs by several routes: 1. inhalation, 2. direct contact with skin or mucous membranes, 3. ingestion, and 4. injection.

Inhalation:

Biological hazards can enter the body via aerosols generated from common lab practices. Common aerosol generators in the lab include pipetting, centrifuging, grinding, blending, shaking, mixing, sonicating, opening containers of infectious materials, and inoculating animals intranasally (1).
Inhalation of toxic or pathogenic agents may be easily absorbed through the mucous membranes of the mouth, throat, and lungs and may seriously damage these tissues after exposure or subsequent infection. Inhaled substances may also pass into the capillaries of the lungs and are carried into the circulatory system, where absorption is rapid. The lungs are the main site for absorption of many toxic or pathogenic agents due to the large surface area of these respiratory tissue (3).

Direct contact:

Inadvertent direct contact with the skin, eyes, or other mucous membranes is a frequent mode of injury in the laboratory.

Skin- In addition to causing local toxic effects, many biotoxins can be absorbed through hair follicles, sebaceous glands, sweat glands, and cuts or abrasions of the outer layer of the skin in sufficient quantity to produce systemic toxicity, e.g., T-2 toxin can be absorbed through skin. Pathogens may also directly infect skin cells and localized infections may evolve into complex or systemic infections. For example, infection with methicillin resistant Staphylococcus aureus (MRSA) may start from skin exposure and become a more complex and difficult to treat infection. When skin is damaged, penetration of agents increases. Additionally, biological agents mixed with lab chemicals such as dimethyl sulfoxide (DMSO) increases the penetration through the skin by increasing its permeability.

Mucous Membranes- Because mucous membranes contain many blood vessels, they also are a route for the rapid absorption of chemicals and biological agents. Contact with hazardous agents can be irritating and painful. Exposures to mucous membranes may occur from splashes, sprays or aerosols generated during experimental procedures.

Ingestion:

The gastrointestinal (GI) tract, which consists of the mouth, esophagus, stomach, and small and large intestines, can be thought of as a tube of variable diameter (approximately 5 m long) with a large surface area (approximately 200 m^2) for absorption. Unlike chemicals, infectious agents that enter the GI tract may infect the tissues of the GI tract or may be absorbed into the blood to produce a systemic infection or toxicity. Fat-soluble toxins are absorbed more rapidly and extensively than water-soluble chemicals but both present significant risk if ingested.

Injection:

The injection route of administration is especially dangerous because it introduces the agent beyond the protective skin barrier eliminating the process of absorption. Injections may also introduce a potential pathogen directly into the bloodstream. This is not a typical route of transmission in nature, and it is preventable in the laboratory. Needles should be engineered out of a procedure whenever possible, for example, use a transfer tip instead of a needle when loading a syringe. When needle use is unavoidable, safe handling and disposal procedures should be developed and training must be provided to all personnel handling needles. Non-laboratory personnel, such as custodial workers or waste handlers, must also be protected from potential exposures by placing disposable laboratory sharps in approved sharps disposal containers (SDCs) and reusable sharps in designated waste containers until decontamination. Laboratory sharps including needles or syringes (with or without needles)must never be placed in regular trash receptacles or other regulated waste containers.

Risk Groups and Biosafety Levels

The principal hazardous characteristics of an agent are its capability to infect and cause disease in a susceptible human or animal host, its virulence as measured by the severity of disease, and the availability of preventive measures and effective treatments for the disease (1). Using these agent characteristics, the US Department of Health and Human Services assigns agents to one of 4 classifications, called a risk group (RG) (1, 2). Risk group 1 (RG-1) agents are not associated with disease in healthy adults; examples include lab strain *Escherichia coli, Adeno-Associated Virus,* and opportunistic pathogens like *Bacillus subtilis*. Risk group 2 (RG-2) agents are associated with human disease but are rarely serious and for which preventative or therapeutic interventions are often available; examples include *Staphylococcus aureus* and *Vaccinia virus*. Risk groups 3 and 4 (RG-3, RG-4) are reserved for agents associated with serious or lethal disease that pose a high individual or community risk, e.g., *Human immunodeficiency virus (RG-3)* and *Ebola virus* (RG-4).

Biosafety levels (BL) are a prescribed set of safety precautions that usually, but not always, correlate to RG. For example, RG-1 agents are typically handled using BL-1 precautions. Laboratory methods may expand typical routes of exposure introducing new risk, requiring a change in biosafety level as found in the risk assessment scenarios below. The Urbana-Champaign campus has facilities for BL-1 and BL-2 experiments.

Animal biosafety levels (ABL) provide guidance for the use of experimentally infected animals housed in indoor research facilities as well as experiments with agricultural species that may be loose housed or outdoors. The procedures and containment used for animal biosafety and biosecurity are useful in the maintenance of all animals that may naturally harbor or are experimentally infected with human and/or animal pathogens including many zoonotic infectious agents or biotoxins. The Urbana-Champaign campus has facilities for ABL-1 and ABL-2 experiments.

Risk Assessment Scenarios

Following are some example experiments that show nuance and complexity after considering the concepts of risk assessment and the rules of the Institutional Biosafety Committee (IBC).

Example Experiment #1

A 2L stock of Bacillus cereus will be grown and small volumes will be inoculated into a small rodent.

First glance: B. cereus is an opportunistic pathogen typically found in places with poor food handling. While the bacteria will not live long in a healthy adult, they produce enteric and emetic toxins that result in foodborne illness. The traditional route of transmission is through ingestion which is unlikely to occur in the lab. This pathogen is not listed by the NIH to be RG-2 and may be manipulated using BL-1 containment and practices.

Closer look: 2L of culture solution creates a splash/spill hazard and the use of a needle to inoculate an animal introduces a parenteral risk not found in nature. The inoculation risk provides the toxin producing agent a direct path into the bloodstream. Additionally, our IBC requires review of all pathogens, even those not explicitly listed as RG-2 by the NIH, and this work requires registration with the IBC.

Conclusion: Completing a risk assessment through an IBC registration may show this work should be carried out using BL-2 and ABL-2 containment and practices.

Example Experiment 2:

A 12L stock of B. subtilis with a recombinant gene insert will be grown.

First glance: B. subtilis is unlikely to cause disease in healthy adults and is a common soil bacterium. This RG-1 organism can be worked with using BL-1 containment and practices.

Closer look: 12L can be hard to handle and these recombinant bacteria may pose an environmental hazard. Also, the NIH Guidelines mandates special practices be in place whenever recombinant organisms are handled at volumes >10L.

Conclusion: BL-1 is not acceptable, additional BL-1-Good Large Scale Practices must be followed as they are laid out Appendix K of the NIH Guidelines. Moreover, experiments using >10L of bacteria containing recombinant or synthetic nucleic acids requires an IBC project to be submitted and approved before beginning these experiments.

Example Experiment 3:

The B. subtilis genome will be edited using CRISPR/Cas9 technology.

First glance: B. subtilis is unlikely to cause disease in healthy adults and is a common soil bacteria. This RG-1 organism can be worked with using BL-1 containment and practices.

Closer look: The Cas9 gene originates from *Streptococcus pyogenes,* an RG-2 organism. Experiments in which DNA from an RG-2 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes require BL-2 containment, as outlined in Section III-D-2 of the NIH guidelines. However, after reviewing the PI's risk assessment, the IBC may approve lowering containment for a specific experiment to BL-1 requirements.

Conclusion: Introduction of an RG-2 gene (Cas9) into *B. subtilis* requires BL-2 containment and practices unless specific lowering of containment is approved by the IBC. The IBC must also review the manipulations proposed to determine the appropriate biosafety level for experiments.

IBC Project Registration

The Institutional Biosafety Committee (IBC) advises on matters relating to the safe handling, transport, use, and disposal of biological materials, including recombinant DNA and synthetic nucleic acid molecules, on the Urbana campus. The committee reports to the Vice Chancellor for Research and Innovation.

The following materials require registration with the IBC:

- Recombinant or synthetic nucleic acid molecules (even work that is exempted from the NIH guidelines must be registered*)
- Transgenic animals (use or creation)
- Transgenic plants
- Pathogens (human, animal, or plant)
- Human materials *(cell lines; blood, blood products, tissues, any bodily fluid)
- Nonhuman primate *(NHP) materials (cell lines, blood, blood products, tissues, any bodily fluid)
- Biotoxins
- Environmental samples that harbor or may harbor pathogens.

You can find more information about the IBC and how to create an IBC project with these materials at the DRS website. All work with materials requiring registration must be approved by the IBC prior to initiation.

Laboratory Audits

Laboratory audits help promote the culture of safety in the laboratory by engaging the partnerships between DRS, PIs, and laboratory personnel. The audit process facilitates a collaborative review of research methods and practices to ensure that laboratory personnel understand and follow standard microbiological practices common to all laboratories. DRS informs and confirms that all special practices designed to mitigate additional risks associated with handling agents requiring BL-2 containment (1). More information regarding when a BL-2 laboratory audit is required, who should attend, and what is covered, can be found at the DRS BL-2 and ABL-2 audit webpage.

Training requirements

Biosafety is a discipline that uses safe practices, administrative procedures, protective equipment, and facility design to eliminate or reduce exposure to biohazardous organisms, and their products, and is guided by two main principles: containment and risk assessment. The fundamentals of containment include the

microbiological practices, safety equipment, and facility safeguards to protect laboratory workers, the environment, and the public from exposure to infectious microorganisms that are handled and stored in the laboratory. Risk assessment is the process that enables the appropriate selection of microbiological practices, safety equipment, and facility safeguards that can mitigate exposure to biohazards and can only by carried out with trained personnel who are aware of the hazards. It is the PI's responsibility to conduct a risk assessment and train all personnel on the hazards, mitigation strategies, and policies of their laboratory. Below are trainings associated with biological material use on campus:

Introduction to Biosafety

Training is an effective tool for understanding key concepts and methods, and DRS has established the online training, *Understanding Biosafety* to introduce basic topics such as risk assessment, containment, biosafety levels, waste disposal, and emergency preparedness within BL-2 containment. This training is required for everyone working at BL-2.

NIH Guidelines Overview

The online training, *NIH Guidelines Overview,* provides information regarding the NIH Guidelines and is required training for everyone working with recombinant or synthetic nucleic acids; including transgenic animals and plants at the University of Illinois. Topics include NIH requirements, responsibilities, classification of experiments, and incident reporting.

OHSA Required Bloodborne Pathogen Training

All lab personnel working with human cell lines and other human-origin materials are required to take annual training, titled *Safe Handling of Human Cell Lines/Materials in a Research Laboratory* to comply with the OSHA standard. The initial training is a live training session that is registered through the OVCRI's training portal; if you are unable attend a scheduled session, contact DRS at drs-bbp@illinois.edu as soon as possible to arrange an additional session. An online refresher course must be completed annually as long as lab personnel work with human materials.

Biological Material Transport

Transport of biological materials, on and off campus, requires precautions to limit exposure. Requirements to transport material between buildings and off campus can be found on the DRS biological material transport page. Be aware, even transport through hallways and between labs requires decontaminated secondary containment that may be handled without personal protective equipment like gloves without the risk of exposure. If shipping infectious agents off campus, two online training are required which are valid for 2 years; 1. *Awareness Training for the Transport of Hazardous Materials* and 2. *Transportation of Infectious Substances, Category B.*

Lab Specific Training

The NIH, CDC, and DRS require that all lab members be trained on the specific biohazards that exist in their lab and the procedures, equipment, and resources available in their lab for working safely with these biohazards. Minimally, resources that should be utilized in training include this document, all relevant IBC projects, and

section 4 of the BMBL (1). Lab-specific training must be provided by the PI or their designee with the following requirements: All training is 1. Documented to include the training processes including SOP review and hands-on training, which is signed and dated by the trainer and trainee once the training process is completed, and 2. personnel must receive ongoing training at least annually and when IBC projects are submitted or updated to include new hazards, procedural changes, and when laboratory policy changes.

Standard Microbiological Practices and Equipment

BL-2 Personal Protective Equipment Requirements

Personal protective equipment selection will vary based on the completed risk assessment. At BL-2 you must, at a minimum, protect your street clothes, skin, and mucous membranes. This may include gloves, coats/gowns, shoe covers/boots, respirators, face shields, surgical or dust masks, and safety glasses/goggles. Personal protective equipment is often used in combination with engineering controls, for instance a Biological Safety Cabinet (BSC), and other containment devices. In situations where it is impractical to work in BSCs; personal protective equipment may form the primary barrier between personnel and the infectious materials; example situations may include: some animal studies, animal necropsy, agent production activities, and laboratory facility and equipment maintenance (1).

Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials.

Full face protection, covering eyes, nose, and mouth, is used for anticipated splashes or sprays of hazardous materials when handled outside the BSC or containment device. This could be a full-face shield or the combination of separate eye and nose/mouth protection (e.g., safety glasses and surgical mask). Eye and face protection must be discarded with other contaminated laboratory waste or decontaminated before reuse. Those who wear corrective lenses like eyeglasses or contact lenses in laboratories must still wear eye protection.

Gloves must be worn to protect hands from exposure to hazardous materials. Glove selection should be based on an appropriate risk assessment. Alternatives to latex gloves should be available. Gloves must not be worn outside the laboratory. Keep the following in mind when choosing and wearing disposable gloves:

1) Change gloves when contaminated or when glove integrity is compromised.
2) Wash hands after removing gloves when work with hazardous materials is complete, whenever gloves are changed, and before leaving the laboratory.
3) Do not wash or reuse disposable gloves. Dispose of used gloves with other contaminated laboratory waste.
4) Latex and nitrile gloves are not chemically resistant to ethanol or isopropyl alcohol! Do not spray your gloves with alcohol or other disinfectants as this degrades glove integrity. If sterility is an issue, double glove so only the outer pair of gloves is compromised by chemicals or sterilize gloves in an autoclave.

Eye, face, and respiratory protection should be used in rooms containing infected animals as determined by the risk assessment.

Laundry and Reusable PPE

Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with hazardous materials but may not be removed from BL-2 space. It is important to remove protective clothing before leaving BL-2 space to prevent the accidental spread of microscopic infectious material. Dispose of protective clothing appropriately into biohazardous waste, decontaminate by autoclaving or soaking in a fresh 10% bleach solution, or deposit it for laundering. If a professional cleaning/uniform service will be used to clean lab coats, then the service provider must be capable of working with biohazard contaminated laundry like those used for hospitals. Inform the professional service that biohazard contaminated coats may be submitted for cleaning. If the professional cleaning service is unable to handle and decontaminate the biohazards from lab coats such as standard dry cleaners, then laboratory personnel must decontaminate the coats prior to submitting them for laundry service Please find more information on how to launder lab coats and reusable PPE in the Campus Exposure Control Plan. And remember, never take a lab coat home for laundering.

Case Study (7):

Outbreak Summary: Between August 20, 2010, and June 29, 2011, a total of 109 individuals infected with strain X of Salmonella typhimurium were reported from 38 states. Infected individuals ranged in age from less than 1 year to 91 years old, and the median age was 21 years. Twelve percent of patients were hospitalized. One death was reported.

Investigation: Analysis of this study suggested that exposure to clinical and teaching microbiology laboratories was a possible source of illness. Illnesses were identified among students in microbiology teaching laboratories and employees in clinical microbiology laboratories. Ill persons (60%) were significantly more likely than control persons (2%) to report exposure to a microbiology laboratory in the week before illness. Additionally, several children who live in households with a person who worked or studied in a microbiology laboratory became ill with the outbreak strain. Staff working at laboratories that were associated with illness were less likely to have knowledge of biosafety training materials. In comparison, staff working in laboratories that were not associated with illness were more likely to train students and staff on the signs and symptoms of infection with Salmonella when conducting safety training. Similar safety policies were in place across the different laboratories. However, some policies appeared to be more difficult to monitor and enforce, such as not allowing the use of handheld devices (e.g., cell phones) at the laboratory workspace.

Personal protective equipment (PPE) lessons learned:

- *Be aware that bacteria used in microbiology laboratories can make you or others who live in your household sick, especially young children, even if they have never visited the laboratory.*
    - *If you work in a laboratory, it is possible for you to bring bacteria home through contaminated lab coats, pens, notebooks, and other items that you use in the microbiology laboratory.*
    - *Avoid taking laboratory supplies outside of the laboratory to limit contamination.*
- *Wear a lab coat or other protective garment over personal clothing when working. Remove protective garments before leaving for non-laboratory areas (e.g., cafeteria, library, or administrative offices).*

Decontamination and Waste Treatments

This section describes basic strategies for decontaminating surfaces, items, areas, and waste in laboratories to eliminate the possibility of transmission of infectious agents to laboratory workers, the public, and the environment. When working with biohazardous material, all labs must have an effective method for decontaminating materials, such as cultures, stocks, and other potentially infectious materials. In addition, lab surfaces require daily decontamination. Determining which disinfectant is effective against a biological agent is a necessary part of the risk assessment process.

Bleach

Bleach is a common and intermediate disinfectant that is cheap and widely available. Sodium hypochlorite (~ 5-7% in stock solution) is the effective ingredient in household bleach is, . When made fresh, a 10% solution of household bleach (~0.5% sodium hypochlorite) can kill vegetative microorganisms, including *Mycobacterium tuberculosis*, all fungi, and inactivates most viruses. A fresh 10% bleach solution for at least 60 minutes contact time is adequate to disinfect most liquid waste. Bleach solutions should be made fresh when decontaminating spills and weekly for routine decontamination of work surfaces. When mixing bleach with aqueous solutions (broth, culture medium, others), then the solution should be mixed with a sufficient volume of bleach to make a final mixture of 10% bleach (v/v).
Some biohazards are resistant to, or even immune to, the effects of bleach; for example: prion protein and biofilm forming microbes. After chemical disinfection, some solutions may be disposed of via sanitary sewer (sink drains). Procedures for the disposal of non-hazardous chemicals in sewer must be cleared with DRS, which can be reached at cws@illinois.edu or at 217-333-2755.

Bleach Alternatives: EPA Registered Disinfectants

Although bleach is effective in most cases there are some instances when it is not recommended. For example, immersion in sodium hypochlorite may damage some instruments, particularly those that are stainless steel. In these cases, it is important to find a useful, Environmental Protection Agency (EPA) approved, alternative to bleach. You can find lists of EPA registered disinfectants, titled by the agents they are effective against, at the EPA website. DRS recommends lists, B, D, and E for most of your decontamination needs. Common EPA-registered chemical disinfectants used as bleach alternatives include commercial available products containing quaternary ammonium based disinfectants (e.g., Steris Coverage Spray TB, Formula 409 Cleaner Degreaser Disinfectant) or accelerated hydrogen peroxide products (Oxivir Tb, Rescue). Laboratories may also make solutions of 70% isopropanol or 3% hydrogen peroxide for disinfecting surfaces. DRS should be consulted for appropriate uses of alternate decontamination procedures. IBC projects should be approved to use EPA approved disinfection products when these options are used in the laboratory.

Autoclave

Autoclaves use high temperature steam under pressure (e.g., 121° C @ 15 PSI) to kill microorganisms and render biohazardous material inactive. Onsite training on how to use the autoclave properly and safely is essential for all new employees to prevent injury. Items such as sharps deposited in a Disposable Sharps Container, hazardous chemicals, bleached materials, radioactive materials, animal carcasses, large tissues/organs, and bedding from infected animals, low molecular weight biotoxins, and prions should never be autoclaved. Information about operating autoclaves safety can be found on the DRS autoclave safety page.

Waste validations- To ensure that biohazardous waste has been effectively treated prior to disposal into the regular waste stream, monthly validations using biological indicators must be performed for BL- 2 waste. Biological indicators are composed of a standardized population of heat-resistant bacterial spores such as *Geobacillus stearothermophilus*, most commonly in the form of spore vials. They are used to determine if the sterilization cycle parameters were sufficient to kill the test microorganisms in a typical waste load from your laboratory. More information on the validation procedure, reporting results and what to do if a validation fails can be found at the DRS autoclave waste and validation page.

Biohazardous Waste Containers, Treatment and Disposal

Biohazardous waste, often referred to as "red bag" waste, must be treated by autoclaving prior to disposal into the regular trash. Biohazardous waste is collected in an autoclavable bag that is stored in a leak-proof container with a lid and displays the international biohazard symbol. Autoclavable biohazard waste bags must be purchased by the laboratory and always display the international biohazard symbol until the autoclave process is completed. After autoclaving is complete, the biohazard waste bag must be over bagged in an opaque bag to fully obscure the biohazard symbol from view before discarding into the regular trash. Any leak-proof plastic waste container with a lid can easily be converted to a biohazard container by placing biohazard stickers on the sides and lid. More information about biohazard containers can be found at the Biosafety Lab Supplies page. DRS provides free biohazard stickers that can be requested via email.

Laboratory Glass and Plastic

Substitute plastic for glass whenever possible in the lab. Lab waste from experiments with biological material should be decontaminated/treated by the lab prior to disposal. For example, cell culture, disposable labware, and recombinant DNA can be decontaminated with a method such as autoclaving or chemical disinfection. Sharps disposal containers and biological waste requiring incineration are collected by DRS.

Biological Materials Requiring Incineration

The State of Illinois considers animal carcasses, tissues, organs, and bedding from infected animals to be pathological waste. University policy requires that the following items be incinerated:

- Any animal inoculated with infectious agents.
- Transgenic animals, potentially transgenic animals, "no-takes" in the production of transgenic animals, and offspring of transgenic animals.
- All sheep and goats.
- Small research animals (e.g., cats, dogs, rabbits, rats, mice, birds).
- Central nervous systems of adult cattle over 30 months old.
- Human tissues and organs.
- Bedding from animals inoculated with infectious agents.

There are no exceptions to this policy without prior notification and approval by the Division of Research Safety (DRS). Other animals, tissues, or organs not listed may still qualify for incineration; contact DRS with specific questions.

Please find more information on the DRS website regarding the packaging of materials to be incinerated.

Plant Pathogens and Pests

Plant pathogens and pests must be decontaminated according to APHIS permit instructions where applicable. Plant pathogens and pests without APHIS permits must be disposed of by the user via autoclaving or chemical disinfection to protect the environment from a breach of containment.

Biotoxins

The disposal method depends on the chemical composition of the biotoxin. Most proteinaceous biotoxins, such as staphylococcus enterotoxin, ricin, and cholera toxin, can be effectively inactivated by exposure to fresh 10% bleach for at least one hour or by autoclaving at 121°C and 15 psi for one hour. See the DRS website for information on autoclaving.

Inactivating non-proteinaceous biotoxins is less straightforward. Examples of non-proteinaceous biotoxins are T-2 toxin, conotoxins, and tetrodotoxin. There is conflicting evidence as to which methods are most effective. Instructions have been developed to ensure that the manner of disposal of all the non-proteinaceous biotoxin wastes is consistent and safe for all personnel involved.

Please find more information in our safety library regarding biotoxin treatment and disposal.

Mixed Waste

Experimental procedures may generate waste that contains more than one hazard. Radiological or chemical waste mixed with biohazards makes the method for proper disposal more complex. The best practice is to rank the hazards based on how readily they are decontaminated, and in many cases, it may be more practical to first decontaminate the biological hazard. For example, if a waste contains both chemical and biological hazards, consider whether the chemical hazard is compatible with bleach or another EPA approved disinfectant. If so, then start by inactivating the biological hazard. After disinfection of the biological hazard, the remaining chemical hazard may then need to be submitted for pickup as chemical waste. Questions about chemical disposal can be sent to cws@illinois.edu or at 217-333-2755.

Aerosol Minimization

Not all laboratory-acquired infections (LAI) are as overt as puncturing the skin with an infected needle or splashes to the eye or mouth. Aerosols of infectious material can also be a source of LAI (1). For instance, Brucellosis accounts for 24% of LAIs and 11% of deaths due to these infections, however, the major route of infection was through inhalation of aerosols. Therefore, it is important that all procedures incorporate practices that minimize the creation of splashes and aerosols. Whenever aerosol generating procedures are used, such as: manipulating biohazardous materials with needles, syringes, and sharps; manipulating materials with inoculation needles, loops, and pipettes; or manipulating specimens and cultures, the use of a Biological Safety Cabinet (BSC) or other engineering controls greatly reduces exposure to aerosols. BSCs are an effective primary barrier against biohazards and proper use of a BSC is an effective way to limit the spread of aerosols. The most commonly used BSC in BL-2 labs is Class II, Type A2. More information about BSCs can be found in the DRS Library page, Biological Safety Cabinets.

Centrifugation

Centrifuging biological material generates aerosols, however, there are practices and equipment that should be used to mitigate exposure to these aerosols. Using O-ring sealed safety cups or aerosol tight rotors and then

opening them inside a BSC greatly reduces the chances of exposure. If safety cups are not available, sealed O-ring or aerosol tight tubes can be used in place of safety cups.

Pipetting

Pipetting is another common technique used in biological research, but beware; pipetting can create aerosols. Therefore, pipetting potentially infectious material should be done in a BSC whenever possible to minimize exposure to the aerosols generated.

House and Local Vacuum

Improperly configured vacuum systems are sources of aerosol generation. A properly configured vacuum system uses an in-line HEPA filter (0.3 µM pore size) to capture aerosols generated by the vacuum. For more information on in- line HEPA filters and how to properly setup vacuum lines please read the DRS page about protecting vacuum lines from biohazards.

Cell or Tissue Disruption

Blenders, sonicators, grinders, mortar and pestle, homogenizers, and vortex mixers are all devices that release considerable aerosols during their operation. For maximum protection to the operator during the use of these devices, the following practices should be observed: 1) Operate and open the equipment inside a BSC whenever possible or 2) if disruption is not possible inside a BSC, use an airtight or sealed container which is opened and manipulated inside a BSC.

Sharps Use at BL-2

Safety Lock Systems

When working at BL-2, safety lock systems, in which the needle is secured to the syringe (e.g., luer-lok and tru-lok), or fixed needle syringes, are required to mitigate the risk of the needle separating from the syringe generating a leak or aerosol when put under pressure.

Sharps Alternatives

Whenever possible, users should investigate alternative methods and equipment that will remove sharps from the procedure. A needle is only necessary if you must transfer materials through a septum, etc., or puncture the skin of an animal. If a needle is not necessary but syringe is required (e.g., syringe paired with a filter disk); then use a blunt end catheter or a dispenser tip to draw up your solution.

Sharps Handling

Tracking injury data on campus has shown that recapping and removing needles from syringes are the most common causes of sharps injuries. Careful management of needles and other sharps are of primary importance; according to the OSHA Bloodborne Pathogens Standard, “needles must not be: bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal when used with infectious or potentially infectious materials.”

Needles should not be recapped before placing them in a sharps container for disposal. Recapping needles should be avoided to prevent accidental injury. However, there are circumstances where recapping or removing the needle from a syringe is unavoidable. If your work necessitates that you recap needles or remove them

from a syringe, never touch the needle with your hand or hold the cap in one hand while placing it over the needle.

If there is no viable alternative to recapping a needle or remove a needle or scalpel blade, it is required that you develop a plan for a safe procedure and incorporate this method in your lab-specific training.

Below are a few safer alternatives to recapping and removing sharps by hand:

- Use a recapping device (e.g., Point-Lok, NeedleSafeII, or a simple microcentrifuge tube rack) to hold the cap and direct the needle into it with one hand, pressing firmly to recap the needle.
- Hold the cap with tongs, forceps, or pliers, and place it over the needle. This is also a good method for removing a needle from a reusable syringe and the best method for mounting and removing a disposable scalpel blade from its handle.
- One-handed "scoop" technique: Use the needle itself to pick up the cap, and then push the cap against a hard surface to ensure a tight fit onto the device.

Sharps Storage

Material that qualifies as regulated sharps must be properly disposed of in approved Sharps Disposal Containers (SDC). Reusable sharps (razor blades, scalpels, microtome/cryostat blades, others) must be stored in a secondary containment device that prevents accidental injury that results from inadvertently contacting the sharp. More information on what qualifies as a sharp, how to order SDCs, and how to request a SDC pickup can be found on the DRS Laboratory Sharps website.

Transport

Transport of BL-2 materials all over campus and abroad is very common but it is important that materials are moved in a way to limit exposure to the public and to the environment. For intra-campus transport, biological samples must be placed in a primary container or vessel that is a securely closed, leak-proof (or o-ring) tube, vial, or ampoule, which is then placed in an unbreakable, lidded, watertight, secondary container (e.g., Rubbermaid tote or Playmate-type cooler) labeled with a biohazard sticker. The outside of the secondary container must be free of any biohazardous material so that personnel can carry the package safely between buildings without wearing gloves or lab coats outside. You can learn more about transporting biological materials throughout campus (e.g. between labs or buildings), and shipping off campus at the DRS Biological Material Transport page.

Storage

Storage of materials at BL-2 must be in a secure location so that access is limited. Storage units like freezers, refrigerators or liquid nitrogen containers must be dedicated for research purposes only and labeled with a biohazard symbol. Storage containers holding primary samples must be labeled and inventoried. Containers/tubes/vials must be intact, leak-proof, and closed to avoid spills or cross-contamination. Find more information in the DRS library, Storage of Risk Group 2 Biological Materials.

Hazards associated with Liquid Nitrogen include extreme cold, asphyxiation, and explosion; resulting in sample loss and personal injury. Always use proper PPE when handling cryogenic vials. Fill Dewar/vessels to an appropriate level to maintain samples in the vapor phase while avoiding submersion of samples. Never store Dewars in an unventilated space such as a cold room. Find more information in the DRS library, Biological Samples Stored in Liquid Nitrogen

Signage

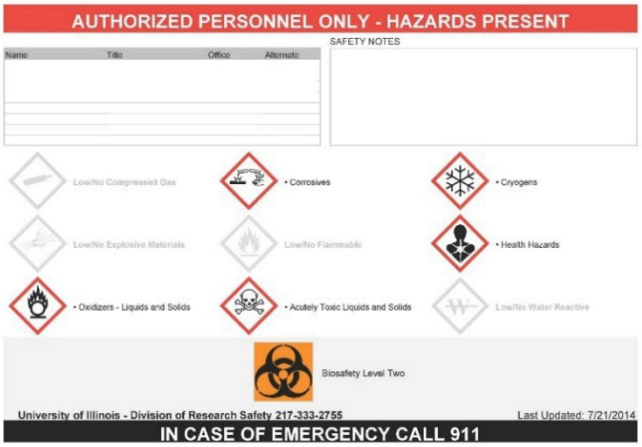
The International Biohazard Symbol is used to alert personnel to the presence of biohazards. A biohazard is something that poses a danger to living organisms, this may be a human health hazard or an environmental hazard; for example, influenza and soybean rust will have the same door signs but carry very different sets of hazards. When you see the biohazard symbol it is important to identify the hazard present.

The biohazard symbol should be posted on anything where biohazards are used, stored, or discarded, such as autoclave bags, biohazard containers, incubators, freezers, refrigerators, or other equipment. Stickers to label equipment and containers are freely available from DRS upon request.

Door Signs

DRS issues lab door signs that include the biohazard symbol for rooms that use biological materials requiring IBC registration. Signs are posted on doors to laboratories where materials are being manipulated or stored. The international biohazard symbol is displayed with a white background for BL-1 containment and an orange background for BL-2 containment. Doors facing halls will have all hazards and an overall white background to warn emergency personnel. Inner doors, such as nested labs, will identify the nature of the hazard in the room you are about to enter. Both signs will include contact information in case of emergency. ABL-2 signs are generated by DRS and are specific to the materials and location described in the IBC.

Example of a BL-2 door sign:



When working with biotoxins in the laboratory, all entry doors into the lab must clearly display a “Biotoxins in Use” sign on the outside of the door. All “Biotoxins in Use” signs are provided to labs by DRS.

Example of “Biotoxins in Use” sign:

Additional Facility Requirements

Additional facility requirements are needed when working in a BL-2 laboratory. BL-2 laboratory facilities should have the following:

1) Laboratory doors should be self-closing and have locks in accordance with the institutional policies.

2) Laboratories must have a sink for hand washing. The faucet may be manual, hands-free, or automatically operated. Ideally, the sink should be located near the exit door.

3) Laboratory windows that open to the exterior are not recommended. However, if a laboratory does have windows that open to the exterior, they must be fitted with screens. If windows are not fitted with screens they must be sealed shut.

4) Biosafety Cabinets (BSCs) must be placed in a room in such a way so that fluctuations of the room air supply and exhaust do not interfere with proper operation. BSCs should be located away from doors, windows that can be opened, heavily traveled laboratory areas, and other possible airflow disruptions. Find more information about BSC placement on the DRS Biological Safety Cabinets webpage.

5) BSCs must be tested and certified at least annually by an accredited BSC field certifier. Contact DRS for a certifier list.

6) When working with biological material, vacuum lines should be protected with liquid disinfectant traps and an in-line HEPA filter. More information about the setup can be found on the DRS page about protecting vacuum lines from biohazards.

7) An eyewash station must be readily available in laboratories designated for BL-2 containment or when corrosive chemicals are used.

8) A method for decontaminating all laboratory wastes should be available in the facility (e.g., autoclave, chemical disinfection, incineration, or other validated decontamination method). Waste cannot be transferred to other buildings for decontamination without prior DRS approval.

Incidents, Medical Treatment, and Reporting

Incidents-Spills, Injuries and Exposures

Incidents occurring in BL-2 laboratories can vary from spills, splashes or needle-sticks to more severe lacerations from razor/scalpel blades. But any incident may result in an exposure to biological material. If an accident happens resulting in an exposure/injury that requires immediate medical attention, call 911. Accidents in BL-2 labs will require varying levels of first aid and medical follow-up. All incidents will require reporting to the PI and DRS. Contact DRS if you have questions or need to report a spill, accident or exposure.

Spills

Most biological spills can be decontaminated by the user. Spills outside of a BSC are considered a breach of containment even when no exposure occurs. Wearing proper PPE, contain the spill with absorbent material, apply a disinfectant that is effective against the agent and allow to sit for the manufacturer's recommended contact time, discard into regular waste receptacle. A detailed procedure for a Biological Material Spill Response is available.

Injuries and Exposures

Lab workers who sustain an exposure to biological material to skin/body should immediately wash the affected area thoroughly with soap and water. If the area is bleeding from a sharps injury such as a needle-stick, dry the area after washing and apply disinfectant such as 70% alcohol or 3% hydrogen peroxide and apply band aid. For exposures to a mucous membrane (eyes, nose, or mouth) flush the area with water using an eyewash station located in the room. Flush for 15 minutes or as long as tolerable. A detailed Emergency Response is available.

Medical Treatment Options- Employees

Employees, including students that are compensated for their work, should seek treatment at the Occupational Medicine Departments identified by the Workers' Compensation program:

Weekdays from 8 a.m. to 5 p.m.

1) Carle Occupational Medicine (Carle), 810 W. Anthony Drive, Urbana, IL. 61801, 217-383-3077
2) OSF Occupational Health, 501 N. Dunlap St., Savoy, IL. 61874, 217-560-6320
3) Safeworks Illinois, 1806 N. Market Street, Champaign, IL. 61820, 217-356-6150 After

hours and weekends

1) Carle Hospital Emergency Department, 602 W. University Avenue, Urbana, IL 61801, 217-383- 3313 or Convenient Care locations
2) OSF HealthCare Heart of Mary Medical Center Emergency Department (OSF), 1400 W. Park Street, Urbana, IL 61801, 217-337-2131 or Urgent Care locations

Medical Treatment Options- Students and Volunteers

Students may seek basic medical care at the McKinley Health Center or with their personal physician. Non-employees should seek treatment at the emergency room of either Carle or OSF. Costs associated with most injuries incurred during unpaid activities are the responsibility of the individual and their health insurance.

Reporting

Depending on the accident and the materials involved, who needs to be informed will vary. Always report a spill, accident, or exposure to your PI or supervisor and DRS who will help you determine what needs to be done for reporting. For example, when an accident involves recombinant or synthetic nucleic acids, the NIH Office of Science Policy may require a report to be submitted. The NIH Guidelines state that "...any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses" must be reported to NIH. At the campus level, the Office Risk Management has reporting requirements for Worker's Compensation and public injury.

Find more information about Incident Reporting and Investigation at DRS.

References

1. Meechan P, Potts J, editors. *Biosafety in Microbiological and Biomedical Laboratories, 6th ed.* HHS Publication No. (CDC) 300859: U.S. Department of Health and Human Services; June 2020.
2. *NIH guidelines for research involving recombinant DNA molecules.* Bethesda: The National Institutes of Health (US), Office of Biotechnology Activities; 2019, April.
3. Heiden PG. *Prudent practices in the laboratory: Handling and management of chemical hazards.* Choice (Conari Press) 2011 12;49(4):710
4. Miller, J. M., Astles, R., Baszler, T., Chapin, K., Carey, R., Garcia, L., Wiedbrauk, D. (2012). *Guidelines for safe work practices in human and animal medical diagnostic laboratories. Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. MMWR Supplements*, *61*(1), 1–102.
5. Weinstein, R. A., & Singh, K. (2009). *Laboratory-Acquired Infections*. Clinical Infectious Diseases, *49* (1), 142–147. doi:10.1086/599104
6. Harding, A. L., & Byers, K. B. (2006). *Biological Safety*. (D. O. Fleming & D. L. Hunt, Eds.). American Society of Microbiology. doi:10.1128/9781555815899
7. *Human Salmonella Typhimurium Infections Associated with Exposure to Clinical and Teaching Microbiology Laboratories (Final Update).* Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 11 Jan. 2012, www.cdc.gov/salmonella/2011/lab-exposure- 1-17-2012.html