Biosafety Level 2 Guide

2024

Division of Research Safety

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

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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/Chemical Hygiene Plan (LSG) and acts as a training tool in a comprehensive Laboratory Safety Plan.

This document will introduce the concept of a risk assessment for biological agents, guide you through choosing an appropriate biosafety level, cover policies for work at BL- 2, and help you develop Standard Operating Procedures (SOPs) for experiments. This document is not all-encompassing, each Principal investigator (PI) must create lab-specific supplemental training/SOPs and training documentation to ensure that lab personnel are working in a safe environment.

In addition to this BL-2 guide, your Laboratory Safety Plan 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 documents:
  - Emergency procedures and contacts
  - 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.
- Lab-specific practices and techniques with increased risk of occupational exposure (e.g., use of sharps or aerosol producing procedures).
- 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 potentially biohazardous material and examine the experimental manipulations that increase the risk of exposure to that material. It is important to realize that the causes of most laboratory-acquired infections are unknown. Unlike acute injuries such as accidental injections, events like inhalation of infectious aerosols or direct contact with contaminated fomites may go unnoticed at the time of exposure. Risk assessments serve to alert staff to hazards associated with agents used in research.

The PI should carry out an initial risk assessment before 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). A risk assessment for exposure and infection should be based on the potential human host's immune status, potential routes of exposure, infectious dose and virulence of the material, personal protective equipment used, and immunization status (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 recombinant or synthetic nucleic acids expressed? Do these expressed nucleic acids 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 exposure to the agent?
- Are vaccinations or treatments available for the agent?
- Does the agent impose increased risk 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,
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 the absorption of many toxic or pathogenic agents due to their large surface area (3).

Direct contact:

Inadvertent direct contact with the skin, eyes, or other mucous membranes often through contact with a fomite is a frequent mode of exposure/injury in the laboratory. Fomites are inanimate objects that can become carriers of an infectious agent and aid transmission. Examples may include: a pen used to take notes during experiments, contaminated gloves, a doorknob touched with a contaminated glove, a cell phone, etc. Spread of agents by fomites often involves a secondary route of transmission, such as direct contact. Ensure you do not touch your face, "clean" items in the lab (such as doorknobs), or personal items such as cell phones and headphones while wearing gloves. Always ensure work surfaces and associated materials are properly decontaminated.

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. This can lead to systemic toxicity, e.g., T-2 toxin can be absorbed through the skin. Pathogens may also directly infect skin cells, leading to localized infections that 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) may have increased penetration through the skin as the chemical may increase 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 can be absorbed into the bloodstream, leading to systemic infection or toxicity. Fat-soluble toxins are absorbed more rapidly and extensively than water-soluble chemicals but both present significant risks if ingested. Remember, food and drink are not allowed in the laboratory.

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 secondary 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 the 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 diseases 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. However, 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) guide the use of experimentally infected animals housed in indoor research facilities as well as experiments with agricultural species that may be loosely 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.

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 (use or creation)
- 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
- Prions
- Environmental or field collected samples that harbor or may harbor pathogens (e.g. wastewater, soil, wild animal materials)

You can find more information about the IBC and how to create and submit an IBC project to register work with these materials on the Division of Research Safety (DRS) website. All work with materials requiring registration must be approved by the IBC before initiation.

Poliovirus Containment Initiative

Polio remains endemic in two countries resulting in outbreaks around the world. The U.S. National Authority for Containment of Poliovirus (NAC) at the Centers for Disease Control and Prevention (CDC) launched a surveillance program aimed to minimize the risk of poliovirus release from research and diagnostic laboratories. As part of this program, the Institutional Biosafety Committee (IBC) added screening questions to the registration process to identify materials which contain or may contain poliovirus according to the criteria established in the WHO Global Action Plan (GAPIV). For more information, visit the DRS Poliovirus Containment Initiative webpage.

Gene Drive Modified Organism

In April 2024, the NIH Guidelines added a new section III D-8 to cover experiments that involve gene drive modified organisms generated by recombinant or synthetic nucleic acid molecules. These experiments will be conducted at a minimum of Biosafety Level 2 (BL-2), Animal Biosafety Level 2 (ABL-2), Plant Biosafety Level 2 (PBL-2), or Arthropod Containment Level 2 (ACL-2) containment. For additional guidance from the NIH visit; Biosafety Considerations for Contained Research Involving Gene Drive Modified Organisms.

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 given a Risk Group designation by the NIH.

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

Conclusion: Completing a risk assessment through an IBC project registration may show that 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. The NIH has given this organism an RG-1 designation. This suggests it can be worked with using BL-1 containment and practices.

A closer look: 12L can be hard to handle and these recombinant bacteria may pose an environmental hazard. Also, the NIH Guidelines mandate special practices to 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 laid out in Appendix K of the NIH Guidelines. All experiments with recombinant organisms require IBC registration, and the required large-scale practices would be assessed by the IBC for use of >10L of bacteria containing recombinant or synthetic nucleic acids.

Example Experiment 3:

The Escherichia coli strain BL21 genome will be transfected to express Cas9 protein.

First glance: E. coli BL21 is unlikely to cause disease in healthy adults and is a commonly used bacteria for protein expression. This RG-1 organism can be worked with using BL-1 containment and practices.

A closer look: The Cas9 gene originates from *Streptococcus pyogenes,* a RG-2 organism. The lab will transfect a plasmid into *E. coli* BL21 for expression and purification of Cas9 protein. Experiments in which DNA from a RG-2 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes defaults to 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 containment.

Conclusion: Introduction of an RG-2 gene (Cas9) into *E. Coli* B strain requires BL-2 containment and practices. The PI may request a downgrade to BL-1 containment and practices since the host is non-pathogenic and the gene does not increase pathogenicity. The IBC routinely reviews experiments using Cas9 protein expression including the manipulations proposed. These experiments are frequently approved by the IBC to be carried out using BL-1 containment and practices.

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 are 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 on the DRS BL-2 and ABL-2 audit webpage.

Training requirements

It is the PI's responsibility to conduct a risk assessment and train all personnel on their laboratory's hazards, mitigation strategies, and policies. Below are trainings associated with biological material use on campus:

Introduction to Biosafety

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 Bloodborne Pathogen standard. The initial training is a live training session that is registered through the OVCRI's training portal; if you are unable to 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, that even transport through hallways and between labs requires decontaminated secondary containment that may be handled without personal protective equipment 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.

BL-2 Containment and Practices

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. It is guided by two main principles: risk assessment (discussed above) and containment. The fundamentals of containment include 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. This section discusses common practices used for BL-2 containment.

BL-2 Personal Protective Equipment Requirements

Personal protective equipment (PPE) selection will vary based on the completed risk assessment but it builds on basic PPE outlined in the Laboratory Safety Guide/Chemical Hygiene Plan which are lab coats, gloves, and safety glasses. At BL-2 you must, at a minimum, protect your street clothes, skin, and mucous membranes. This includes wearing lab coats/gowns, gloves, and full-face protection (i.e. full-face shields or surgical/dust masks and safety glasses/goggles) for any work occurring on the bench. It may also include additional PPE such as shoe covers/boots or respirators (if required by the IBC based on a specific experiment) or use an engineering control such as a Biological Safety Cabinet (BSC) that is designed to protect users, products, and the environment when working with infectious agents.

Protective clothing: Coats, gowns, smocks, coveralls, or uniforms designated for laboratory, animal, or plant facility use must be worn while working with biological materials and provide a protective barrier for your skin and street clothes so that agents are not taken home. Protective clothing is not worn in public spaces.

Gloves: Disposable single-use gloves must be worn to protect hands from exposure to biological materials. Glove selection should be based on an appropriate risk assessment. Nitrile gloves are commonly used with biological materials over latex gloves due to potential latex allergies. 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) Gloves must not be worn outside the laboratory in public spaces.
5) 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, wear two pairs of gloves so only the outer pair of gloves is removed when compromised by chemicals. Another option is to sterilize gloves in an autoclave prior to use.

Full-face protection: Full-face protection must cover all mucous membranes (i.e. eyes, nose, and mouth) and is worn when splashes or sprays of biological materials may occur when working outside of a BSC or containment device. This could be a full-face shield or a combination of separate eye and nose/mouth protection (e.g., safety glasses and surgical mask). Disposable eye and face protection must be discarded with other contaminated laboratory waste. Reusable safety glasses or full-face shields must be decontaminated before reuse. Prescriptive eyeglasses cannot be substituted for safety glasses and contact lens users must wear safety glasses in the laboratory.

Biosafety cabinets (BSC): When working in a BSC, lab coats and gloves are worn, however, when the BSC sash is in its proper position, full-face protection is not required. In situations where it is impractical to work in BSCs (e.g. equipment needed is too large or the activity cannot be properly performed) standard PPE for working on

the bench is required (i.e. lab coats, gloves, and full-face protection).

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) in 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).*

Laundry and Reusable PPE

Protective laboratory coats, gowns, smocks, or uniforms designated for laboratory use must be worn while working with biological 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. Disposable protective clothing is placed into biohazardous waste containers. Reusable PPE is decontaminated by autoclaving, soaking in a fresh 10% bleach solution, laundering in facilities in your building (if applicable), or deposit to a professional cleaning service 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 before 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.

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 materials, 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. 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), the solution should be mixed with a sufficient volume of bleach to make a final mixture of 10% bleach (v/v). For surface decontamination, a minimum contact time of 10 minutes is required. 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 DRS-waste@illinois.edu or 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 an effective, Environmental Protection Agency (EPA) approved, alternative to bleach. You can find lists of EPA-registered disinfectants, titled by the agents they are effective against, on the EPA website. DRS recommends lists, B and S for most of your decontamination needs. Common EPA-registered bleach alternatives include commercially 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 and note the required contact time 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 Sharps Disposal Container, hazardous chemicals, bleached materials, radioactive materials, animal carcasses, large tissues/organs, 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 before 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 validation fails can be found on 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 before 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. DRS provides free biohazard stickers that can be requested via email.

More information about biohazard bags and containers can be found on the Biosafety Lab Supplies page.

Laboratory Glass and Plastic

Substitute plastic for glass whenever possible in the lab. Lab waste from experiments with biological material must be decontaminated/treated by the lab before 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 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

Where applicable, plant pathogens and pests along with infested plant materials and soils must be decontaminated according to APHIS permit instructions. Plant pathogens, pests, infested plant materials and soils 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 pick up as chemical waste. Questions about chemical disposal can be sent to DRS-waste@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, all procedures must incorporate practices that minimize the creation of splashes and aerosols.

Biological Safety Cabinets

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 on 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.

Culture Shaking

Bacterial cultures often require containers be shaken during incubation to allow proper growth, which is an aerosol generating procedure. To mitigate exposure, aerosol tight lids or HEPA-filtered tops must be used when shaking BL-2 cultures. Alternatively, sealed shakers may be used and a settling time (e.g. 10 minutes) employed before opening.

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 set up 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 for 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 and generating a leak or aerosol when put under pressure.

Sharps Alternatives

Users should investigate alternative methods and equipment that will remove sharps from the procedure whenever possible. 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 a syringe is required (e.g., a 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 is 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 being placed 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 removing 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, NeedleSafe II, 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 an 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 materials must be 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 at BL-2

Storage Units

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.

Samples Stored in Liquid Nitrogen

Hazards associated with Liquid Nitrogen include extreme cold, asphyxiation, and explosion; resulting in personal injury and sample loss . Always use proper PPE when handling cryogenic vials. Fill dewars/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 grey background for BL-1 containment and an orange background for BL-2 containment. Hallway doors will list all hazards within lab even if there are multiple inner rooms. Inner doors, such as room within the main lab, will the hazard in the room you are about to enter. Both signs will include contact information in case of emergency. Animal Biosafety Level 2 (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. The sign should only be displayed when active work is ongoing. 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) An eyewash station must be readily available in laboratories designated for BL-2 containment or when corrosive chemicals are used.

4) 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.

5) 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.

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

7) 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.

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. However, any incident may result in 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. An incident report form can also be found on the DRS website.

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. Wear proper PPE, contain the spill with absorbent material, apply a disinfectant that is effective against the agent, allow it to sit for the manufacturer's recommended contact time, and discard it into a regular waste receptacle. A detailed procedure for a Biological Material Spill Response is available.

Injuries and Exposures

Lab workers who sustain an exposure from biological material to the skin/body should immediately wash the affected area thoroughly with soap and water. 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 information page is available on the DRS website.

Medical Treatment Options: Employees

Employees, including students who 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) 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 of Risk Management has reporting requirements for Worker's Compensation and public injury.

Find more information about Incident Reporting and Investigation on the DRS website.

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