Biosafety Level 2 Guide

2022

Division of Research Safety

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Introduction

This guide is designed for users working with biological materials and is intended to be used alongside the Laboratory Safety Guide (LSG) as part of your 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 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 restricted 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 there any transgenes expressed; do they increase risk (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 of 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 produces poisoning by absorption through the mucous membranes of the mouth, throat, and lungs and damages these tissues seriously by local action. Inhaled substances pass into the capillaries of the lungs and are carried into the circulatory system, where absorption is extremely rapid. Because of the large surface area of the lungs in humans they are the main site for absorption of many toxic or pathogenic agents (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. When skin is damaged, penetration of agents increases. Additionally, coupling biological agents with lab chemicals such as dimethyl sulfoxide increases the penetration of other chemicals through the skin by increasing its permeability.
>
> *Mucous Membranes*- Contact of hazardous agents with the mucous membranes is of concern because they are sensitive to irritants, few substances are innocuous; most are painful and irritating. Because the membranes typically contain many blood vessels, they also are a route for the rapid absorption of chemicals and biological agents.

Ingestion:

The gastrointestinal 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 gastrointestinal tract do not have to be absorbed into the blood to produce a systemic injury through infection. Fat-soluble toxins are absorbed more rapidly and extensively than water-soluble chemicals.

Injection:

The intravenous route of administration is especially dangerous because it introduces a potential pathogen directly into the bloodstream; eliminating the process of absorption. As this is not a typical route of transmission in nature, it is very 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 should be considered. Non-laboratory personnel, such as custodial workers or waste handlers, must be protected from exposure by placing disposable laboratory sharps in approved sharps disposal containers (SDCs) and reusable sharps in designated waste containers until decontamination; not in lab trash 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 principle 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* and *Ebola virus*, respectively.

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. Sometimes our laboratory methods expand typical routes of exposure introducing new risk, requiring a change in biosafety level as found in the risk assessment scenarios below. The Urbana campus has facilities for BL-1 and BL-2 experiments.

Likewise, Animal biosafety levels (ABL) provide guidance for the use of experimentally infected animals housed in indoor research facilities and are also useful in the maintenance of laboratory animals that may naturally harbor zoonotic infectious agents. The Urbana 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 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 worked with at BL-1.

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 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 at BL-2.

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 bacteria. This RG-1 organism can be worked with at BL-1.

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, this work requires an IBC registration.

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 at BL-1.

Closer look: The Cas9 gene originates from *Streptococcus pyogenes,* a RG-2 organism. Experiments in which DNA from a RG-2 agent is transferred into nonpathogenic prokaryotes or lower eukaryotes require BL-2 containment, as outlined in Section III-D2 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.

Conclusion: Introduction of a RG-2 gene (Cas9) into *B. subtilis* requires BL-2 containment 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 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. You can find more information about the IBC and materials to register at the DRS website.

Laboratory Audits

Laboratory audits help to ensure that PIs and laboratory personnel know and follow standard microbiological practices common to all laboratories as well as special practices addressing additional risks associated with handling agents requiring increased levels of 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 website.

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 staff; 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. The initial training is a live training session titled *Safe Handling of Human Cell Lines/Materials in a Research Laboratory*. Register for live training at the OVCRI's training portal; if you are unable to make a scheduled session please contact DRS as soon as possible to create a new session. An online refresher course must be completed annually as long as lab personnel work with human materials and can be found at the OVCRI's training portal.

Biological Material Transport

Transport of biological materials, on and off campus, requires a few precautions to limit exposure. Requirements to transport material between buildings and off campus can be found on the DRSbiological material transport page. Be aware, even transport through hallways and between labs requires decontaminated secondary equipment that can be handled without personal protective equipment like gloves. 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: 1. documented by the PI or their designee, which is signed and dated by the trainee, and 2. personnel must receive annual training and additional training when procedural or policy changes occur.

Standard Microbiological Practices and Equipment

BL-2 Personal Protective Equipment Requirements

Personal protective equipment selection will vary based on the risk assessment you have conducted. 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) Remove gloves and wash hands when work with hazardous materials is complete 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! Do not spray your gloves with ethanol. 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. 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 garment before leaving for non-laboratory areas (e.g., cafeteria, library, or administrative offices).*

Decontamination

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 material, such as cultures, stocks, and other potentially infectious material. 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. The effective ingredient in bleach is sodium hypochlorite, which is typically around 5% of household bleach. When made daily; fresh, 10%, 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. However, 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, Envorionmental 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 70% isopropanol or 3% hydrogen peroxide. DRS should be consulted for appropriate uses of alternate decontamination procedures.

Autoclave

Autoclaves use high pressure and high temperature steam 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, hazardous chemicals, bleach, radioactive materials, pathological waste, 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 the entire biological load 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 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.

Regulated Waste Storage

Biohazardous waste to be autoclaved, often referred to as “red bag” waste, should be collected in an autoclavable bag that is stored in a leak-proof container with a lid and displays the international biohazard symbol. 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 if 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

Oftentimes labs 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. 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.

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 needles, syringes, and sharps; manipulating 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 their use as a primary containment is an effective way to limit the spread of aerosols. More information about BSCs can be found at the BSC website.

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 sealed rotors and then opening them inside a BSC greatly reduces the chances of exposure. If safety cups are not available, sealed O-ring 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 to minimize exposure to the aerosols generated.

House and Local Vacuum

Improperly setup vacuum systems are sources of aerosol generation. A properly setup vacuum system utilizes an in-line HEPA filter 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 vortexers 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 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 causing a leak and an 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). 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.

Shipping and 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. 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.

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, 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 symbolthat are posted on doors to laboratories where RG-2 materials are being manipulated or stored. 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.

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 a BL-2 door sign:

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) BSCs must be installed 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.
5) 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 the DRS page about protecting vacuum lines from biohazards.
6) An eyewash station must be readily available.
7) Class II BSCs must be tested and certified at least annually. Find more information on the DRS BSC page.
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.

Reporting

Spills, Accidents, and Exposures

Spills or accidents occurring in BL-2 laboratories resulting in an overt exposure must be immediately reported to the PI, DRS, and possibly the NIH. Lab worker's names do not appear in reports as the reports are for surveillance purposes only. The NIH Guidelines states that "...any significant problems, violations of the NIH Guidelines, or any significant research-related accidents and illnesses" must be reported to NIH. Contact DRS if you have questions or need to report an exposure.

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

For information regarding Workers Compensation, please see the University Office of Risk Management's website.

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.

A Public Injury Report, also found at the University Office or Risk Management website, should be completed if a person suffers an injury during activities for which they are not paid.

Exposure to Blood or Other Potentially Infectious Materials

The "Exposure to Blood or Other Potentially Infectious Materials" form should be completed in addition to the appropriate injury report listed above if there has been an occupational exposure to blood borne pathogens.

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