ENVIRONMENTAL HEALTH AND SAFETY
University of Nebraska-Lincoln

Safe Operating Procedure

(Revised 5/25)

SPILL AND EXPOSURE RESPONSE FOR BIOHAZARDOUS MATERIALS

Scope

This SOP applies to work at UNL that is subject to the **UNL Biosafety Guidelines, apart from research conducted at Biosafety Level 3 containment** (which have unique specific procedural plans). The content of this SOP is based on requirements established by the following standards:

- *NIH Guidelines for Research Involving Recombinant and Synthetic Nucleic Acid Molecules (NIH Guidelines)*, National Institutes of Health
- *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, Centers for Disease Control and National Institutes of Health
- *Bloodborne Pathogens Standard, 29 CFR 1910.1030*, Occupational Safety and Health Administration

Biohazardous materials may include recombinant or synthetic nucleic acids; microorganisms infectious to humans, animals (including insects), or plants (e.g., parasites, viruses, bacteria, fungi, prions); and biologically active agents (i.e., toxins, allergens, venoms) that may cause disease or have significant impact on the environment or community.

> Bloodborne pathogen source materials, such as human blood and certain body fluids as well as human or non-human primate cell cultures, are also considered biohazardous materials. For spills involving bloodborne pathogens, please refer to the EHS SOP, ***Cleaning up Spills of Bloodborne Pathogens.***

A biological spill kit is required in all labs that use or store biohazardous materials. Recommendations for assembly of a general-purpose biohazard spill kit are provided in **Appendix A** of this SOP.

Personal Protective Equipment

The appropriate ensemble of PPE to be used when cleaning a spill depends on the severity of the spill and the characteristics of the biohazardous agent and any co-mingled chemical agents. Minimum PPE includes an outer garment (e.g. lab coat), gloves, and eye protection. Impervious coveralls (e.g. Tyvek) are needed if it is likely that the outer garment can become saturated. If a splash hazard exists, goggles and a face shield are appropriate to protect mucous membranes (e.g., eyes, nose and mouth). Gloves should be changed frequently and when known to be contaminated. Hands should be washed thoroughly after removing gloves and before donning a new pair.

When working within a Biological Safety Cabinet (BSC), gloves must be removed and placed in the biowaste container within the BSC every time that you remove your hands from the BSC during spill clean-up and hands must be washed thoroughly after removing gloves and exiting the BSC.

Other personal protective equipment may be appropriate depending on the circumstances of the spill. For example, fluid resistant shoe covers are appropriate if you must step into or traverse areas where the spill occurred. Respiratory protection is required if the spill presents an inhalation hazard.

Disinfectants

Disinfectants (and contact times) as listed on product labels and in the IBC protocol for the biological agent should be used for spill clean-up and decontamination. Cover the spill area with absorbent material like thick paper towels (do not use the bathroom, hand drying towels). Gently pour rather than spray (spraying creates aerosols) the disinfectant around the spill area, allowing the disinfectant to absorb into and soak the spill area. Organic and microbial load can interfere with disinfectant efficacy; for additional information about selecting a proper disinfectant see EHS SOP, ***Chemical Disinfectants for Biohazardous Materials***.

Small Spills Confined Within a Biological Safety Cabinet (BSC) Procedure

In unique situations, decisions may need to be made in the laboratory that are not specifically stated in this document. A spill that is confined to the interior of the BSC should present little hazard to personnel in the area. However, agent-specific disinfection procedures should be initiated at once while the cabinet ventilation system continues to operate to prevent escape of contaminants from the cabinet. If a biological spill exits the BSC, Initiate cleanup of the biological spill outside the BSC first.

Try to keep hands inside of the BSC until the spill has been contained.

1. If the spill is **contained on an absorbent liner and the absorbent liner has plastic/liquid- resistant backing**:
    a. Carefully and slowly remove materials away from the spill to the "dirty" side.
        - Tube racks/ autoclavable plasticware/ glassware that are contaminated, need to be placed in a liquid waste container or a biohazard autoclave bag with disinfectant. The materials can be retrieved once autoclaved and sterilized.
    b. Slowly fold up the spill in the absorbent liner and place gently into the autoclave bag inside the BSC.
    c. Remove gloves and put on a new pair or if double gloved, remove the outer layer of gloves and put on a new outer layer of gloves. Place all clean up material into the biohazard autoclave bag(s), seal the bag and wipe the bag down with agent-specific disinfectant.
    d. Replace soiled materials/ replace autoclave bag with a new one and resume working in the BSC.

2. If the spill is **NOT contained on the absorbent liner/ the absorbent liner does NOT have plastic/liquid-resistant backing**:
    a. Cover the spill with paper towel(s).
    b. Replace gloves or replace the outer layer of gloves with a new pair if double gloved.
    c. Apply the agent-specific disinfectant to paper towel to disinfect the spill starting from the outer edges of the spill and moving inward in a circular fashion to keep the spill from spreading.
    d. Allow sufficient amount of contact time (Every disinfectant has an amount of time needed before it is able to kill or prevent the growth of microorganisms- see the disinfectant label/ technical report).
    e. Place materials that may have come in contact with the spill in the biohazard autoclave bag or liquid catching container/pipette boat containing disinfectant.
        i. Replace (outer) gloves accordingly
    f. Wipe down equipment with a disinfectant saturated towel and let sit for the contact time of the disinfectant if it was involved with the spill area.
        i. Replace (outer) gloves accordingly.
    g. Thoroughly wipe down the internal surfaces of the cabinet with agent-specific disinfectant.
    h. Place all clean up material into an autoclave bag(s), seal the bag and wipe

the bag down with agent-specific disinfectant.

i. Replace soiled/ used materials and resume working in the BSC.

3. If the spill has **entered the vent/ grill area** (**KEEP THE CABINET RUNNING**):

NOTE: Take care of yourself first!

- Know the transmission route of the biological agent being used.
- If you are unsure of your potential for exposure/safety, treat the spill like a spill outside of the BSC.

If safe, proceed to:

a. Unscrew the lid of agent-specific disinfectant and flood the BSC front grille/ vent (or back grill/vent if the spill began there) area with the agent-specific disinfectant.

b. Clean up spill on top (work area) according to Procedure 2. (above).

c. Summon an individual (a lab mate or biosafety staff) to help with the cleanup process.

d. Wipe down the countertop where you will be placing the disinfected BSC parts.

e. Place a new autoclave bag in a secondary container/ bag holder, for the Biohazardous waste.

i. This will allow you to keep the bag in the BSC but will not be in the way when it is time to remove the stainless-steel work area

ii. Do not block the front or back grills/ vents.

f. As a team:

i. Take apart the BSC moving the arm rest, then the front grille/ air vent to the inner part of the cabinet and wipe each piece with agent-specific disinfectant allowing the correct contact time before removing the piece from the cabinet.

ii. For the work area, (this will take both people) both individuals will hold the work area up and work together to clean the stainless steel of the underside of the work area.

g. Using a Swiffer® sweeper, or equivalent, clean the undercarriage of the BSC

h. If sharps are involved, use forceps or the Swiffer® to place them in a disinfectant-filled sharps container.

DO NOT USE YOUR HANDS as sharps ARE present!

i. Have a person not in the BSC assist with providing new paper towels, as needed.

ii. Replace (outer) gloves.

i. Wipe down the biohazard bag with agent-specific disinfectant, seal it, and remove it from the BSC.

j. Wipe down the BSC parts again with agent-specific disinfectant and place the parts back into the BSC accordingly.

Small Spills Confined Within a Portable Primary Containment Device (e.g., Sealed Safety Cup, Sealed Rotor, etc.)

1. Turn on the BSC and allow time for the BSC to equilibrate if the cabinet is not already on and running. Retrieve disinfectant (making sure it is not expired), towels, biohazard autoclave bag and any other supplies you may need for spill cleanup and place these items in the BSC.
2. Move the sealed primary containment device to the BSC.
3. Open the device in the BSC and examine seal/O-ring for damage. If a seal or O-ring is damaged, follow the procedure for an unconfined spill outside of the BSC. If seals and O-rings are intact, continue with step 4.
4. Flood the device with disinfectant and allow proper contact time.
5. Disinfect the exterior of the device before removing it from the BSC.
6. Rinse components with water, dry and reassemble.
7. Replace O-rings/filters as necessary.
8. Disinfect the BSC.

Spill Due to Centrifuge Failure

1. Unplug the centrifuge. If it is a bench model, and will fit in the BSC, move it into the BSC. If it is a floor model, wait a minimum of 60 minutes and add other PPE as needed before opening the centrifuge.
    a. Post the attached “Biohazard Spill” sign on the centrifuge to alert others of the spill.
    b. Respiratory protection may be needed. If this is the case, have everyone exit the laboratory or equipment room with the centrifuge and contact biosafety (472-4925) for assistance.
2. Retrieve disinfectant and paper towels.
3. Place paper towels soaked in disinfectant over all accessible surfaces of the interior and exterior of the centrifuge and allow proper contact time.
4. Remove paper towels and manage as biological waste.
5. Use mechanical means (tongs, forceps) to remove broken tubes and other fragments. Place them in a sharps container for immediate autoclaving and disposal.
6. Remove buckets, trunnions and rotor, and immerse them in a disinfectant bath.

   a. Use the biological spill kit bucket as needed for this process.
7. Surface disinfect newly exposed areas of the centrifuge that were previously inaccessible while waiting for the contact time of the immersed equipment parts.
8. Notify the Biosafety Officer and Principal Investigator.
   a. Consult the Biosafety Officer regarding the need for replacement of vacuum lines, HEPA filters, etc.

Spills Outside of a Biosafety Cabinet/Primary Containment Device

1. Notify other people in the lab that there has been a spill.
   a. If the spill involved human/ zoonotic pathogens, remove PPE and wash hands and exposed skin thoroughly. Vacate the area for 30 minutes and notify the Biosafety Officer and the PI. This will allow aerosols to settle. Post the attached "Biohazard Spill" sign on the door to alert others of the spill.
   b. Respiratory protection may be needed. If this is the case, have everyone exit the laboratory/ room with the spill and contact biosafety (472-4925) for assistance.
2. Retrieve biohazard spill kit, don appropriate additional PPE and make proper dilution of disinfectant, as applicable.
   a. Make sure the disinfectant is not expired!
3. Cover the spill with disinfectant-soaked paper towels and allow proper contact time.
   a. You may need to add more disinfectant as needed during the contact time period if you notice the towels drying out
4. Using tongs or forceps, remove paper towels to an appropriate biohazard waste receptacle and retrieve any broken glass, sharps, containers, etc. Place the towels that may have sharps material on them and the sharps material in the appropriate sharps waste receptacle.
   a. You may need to use the biohazard spill kit bucket/ container for this purpose.
5. Repeat step 4 and disinfect the spill area a second time, allowing proper contact time.
6. Clean the affected area with soap and water.
7. Thoroughly wash hands and exposed skin each time after removing PPE.

Spills Involving Biological Toxins

Toxins require specific disinfectants to ensure the toxin is inactivated. Use 2N NaOH or another decontaminant proven to be effective against a specific toxin.

Procedures

1. Create a berm or dike with absorbents, as needed.
2. Follow procedures as outlined for spills of microorganisms above but replacing disinfectants with 2N sodium hydroxide solution. Allow one-hour contact time.
3. Clean up contaminated absorbent material and place it in a black bag or container that is disposable.
    - The biological spill kit bucket may be used for secondary containment.
4. Clean area with soap and water.
5. Remove personal protective equipment and thoroughly wash hands, arms, face, and any other exposed body parts. Place PPE in the same container as the spill materials.
6. Tag spill materials and residues for collection by EHS including disposable PPE. **DO NOT autoclave these materials, damage to the autoclave may result!**
7. If you have not already done so, notify your supervisor and the biosafety officer of the spill.

Exposure Response for Biohazardous Materials

1.1 Skin, Mucous Membrane, Injury Exposure to Infectious Agents, Biological toxins, or r/sNA

If you are exposed to infectious agents or materials containing r/sNA while working in the lab, follow these steps:

1. In case of skin contact or injury:
    a. Thoroughly wash the exposed area with soap and water.
        i. Do not use disinfectants or other chemical solutions as they can cause skin abrasions and a possible additional route of entry for the agent!
    b. Using antiseptic and a bandage; cover open wounds.
    c. For mucous membranes (e.g., eyes, nose), flush thoroughly using the eyewash, drench hose, sink and/ or emergency shower.
2. Report the incident to your supervisor and biosafety officer immediately.
    a. Refer to the EHS SOP, ***On-the-job and Student Injuries*** for instructions about incident reporting forms and seeking medical attention for employees and students.
3. The BSO will perform a follow-up review with the lab to find ways to try to identify how the incident occurred and ways to minimize the incident from happening in the future.
    a. See the EHS SOP, **NIH Incident Reporting for r/sNA Molecule Work** for details about reporting incidents involving r/sNA.

Appendix A
Biohazard Spill Kit Guidance

All labs conducting experiments involving the use of biological materials need to always have a properly stocked biohazard spill kit available and accessible.

General-Purpose Biohazard Spill Kit Contents

1. Nitrile or latex gloves (multiple pairs)
2. lab coat or disposable gown
3. Eye protection
4. Surgical mask (or N95 Respirator as appliable to the risk of the work performed in the lab)
5. Red/orange biohazard bags
6. Disinfectant suitable for the biologically hazardous materials found in the lab. (The *disinfectant needs to be labeled with expiration date.)*
    a. This can be stored next to or on the biological spill kit
7. Absorbent materials (i.e., thick/ durable paper towels)
8. Tongs or forceps (used to grab disinfectant soaked towels or sharp objects)
9. Signage to post at lab entrance for controlling access (provided in Appendix B)
10. Copy of spill cleanup procedures (i.e., this SOP)

Optional items for larger spills

- Disposable shoe covers
- N95 Respirator
- Face Shield
- Diking material or spill pillows for large spills (stops the spread of a spill)

NOTE: This information is for a general-purpose kit only and may serve your purpose. However, a careful risk analysis of the biological hazards found in your particular laboratory may require additional items not found on this list. For additional guidance, contact the UNL Biosafety Officer at 402.472.4925.

All of these items can be stored in a five (5) gallon bucket with a lid or a Rubbermaid™/ plastic bin with a lid. The bucket or bin should be labeled indicating it is a **Biohazard Spill Kit**. The contents of this kit should be verified **at least** annually to make sure the kit is complete, and the components are in usable condition (i.e., Disinfectant is not expired, and PPE is not degrading).

Appendix B

Biohazard Spill signage to print and post