Safe Operating Procedure

Revised 5/24

SPILL AND EXPOSURE RESPONSE FOR BIOHAZARDOUS MATERIALS

Scope

This SOP applies to work at UNL that is subject to the UNL Biosafety Guidelines, with the exception of 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, 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 to 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 spill kit is required in all labs that use or store biohazardous materials. Recommendations for assembly of a general-purpose biohazard spill kit is provided in Appendix 1 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 safety glasses. 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**

A freshly prepared 10% solution of household bleach with a contact time of 20 minutes is an appropriate disinfectant for most biohazardous materials. Alternatively, disinfectants (and contact times) as listed in the IBC protocol for the biological agent can also be used for spill clean-up and decontamination. When applying disinfectants to absorbed spills, gently pour rather than spray solutions as spraying creates aerosols. 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)**

1. Retrieve disinfectant and absorbent materials (e.g. paper towels).
2. Apply absorbent materials over the spill.
3. Slowly pour disinfectant on absorbent to wet thoroughly.
4. If spill spread to drain pan, flood the drain pan with disinfectant.
5. Allow appropriate contact time. Place disinfectant-soaked absorbents in the biohazard waste container.
6. Wipe down the interior of the BSC with fresh disinfectant and wipes. Place wipes in biohazard waste container.
7. If flooded with disinfectant, empty the drain pan into a collection container via the drain port. Lift the front exhaust grill and tray and wipe all surfaces.

8. If bleach is used, the BSC will also need to be wiped down with water or ethanol because bleach is corrosive and will damage metal surfaces.

**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. Retrieve supply of disinfectant and place in 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. 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 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 before opening the centrifuge.

2. Post the attached “Biohazard Spill” sign on the centrifuge to alert others of the spill.

3. Retrieve stock of disinfectant and paper towels.

4. Don PPE.

5. Place paper towels soaked in disinfectant over all accessible surfaces of the interior of the centrifuge and allow proper contact time.

6. Remove paper towels and manage as biological waste.

7. Use mechanical means (tongs, forceps) to remove broken tubes and other fragments. Place them in a sharps container for autoclaving and disposal.
8. Remove buckets, trunnions and rotor, and place in disinfectant bath. If too large for immersion in a bath, surface wiping with disinfectant will be necessary.

9. Surface disinfect newly exposed areas of the centrifuge that were previously inaccessible.

10. Notify the Biosafety Officer and Principal Investigator. Consult the Biosafety Officer regarding the need for replacement of vacuum lines, HEPA filters, etc.

**Spills Outside of a Primary Containment Device**

1. Notify other people in the lab that there has been an unconfined spill.

2. If the spill involved human 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.

3. Don appropriate PPE.

4. Retrieve biohazard spill kit.

5. Cover the spill with disinfectant-soaked paper towels and allow proper contact time.

6. Using mechanical device, remove paper towels to biohazard waste receptacle and retrieve broken glass, sharps, containers, etc. Place in the biohazard waste receptacle.

7. Disinfect the spill area a second time, allowing proper contact time.

8. Clean the affected area with soap and water.

9. 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 other decontaminant proven to be effective against a specific toxin.

**Procedures**

- Create a berm or dike with absorbents.
- Follow procedures as outlined for spills of microorganisms above but replacing disinfectant with 2N sodium hydroxide solution. Allow one hour contact time.
- Clean up contaminated absorbent material and place in a bag or container that is disposable.
• Remove personal protective equipment and thoroughly wash hands, arms, face, and any other exposed body parts. Place PPE in same container as spill materials.
• Tag spill materials and residues for collection by EHS. Clean PPE or containerize and tag for collection by EHS. **DO NOT autoclave these materials, damage to the autoclave may result!**
• Clean area with soap and water.
• If you have not already done so, notify your supervisor of the spill.

### Exposure Response for Biohazardous Materials

**Skin, Mucous Membrane, or 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 with a contaminated instrument:
   a. Thoroughly wash area with soap and water.
   b. Avoid use of abrasive chemical soaps or disinfectant washes as they can cause skin abrasions and a possible additional route of entry for the agent.
   c. Cover the wound with a sterile dressing.
   d. For mucous membranes (e.g., eyes, mouth), flush thoroughly.

2. Report the incident to your supervisor immediately. Refer to the EHS SOP, *On-the-job and Student Injuries* for instructions about incident reporting forms and seeking medical attention.

3. Contact the Biosafety Officer immediately if the injury involved any of the following:
   a. Contact with mucous membranes.
   b. Contact with non-intact skin.
   c. Percutaneous exposure
   d. Ingestion
   e. Any type of exposure that involves concentrated cultures.

4. If the exposure involves r/sNA, the BSO will notify the UNL IBC at the next meeting and the NIH Office of Science Policy (OSP) in writing within 30 days of the incident, as applicable. The BSO will also perform a follow-up investigation to determine if additional training or changes in procedures are required to prevent similar incidents. See the EHS SOP, *Incident Reporting – National Institute of Health (NIH) Guidance* for details about reporting requirements.
Biohazard Spill Kit Guidance

All labs conducting experiments involving the use of biological materials should have a properly stocked biohazard spill kit available and accessible at all times. These kits are especially important for labs designated as biosafety level 2 or labs conducting experiments with large volumes of biological materials.

General-Purpose Biohazard Spill Kit Contents
1. Nitrile or latex gloves (multiple pairs)
2. Disposable gown or lab coat
3. Safety glasses
4. Surgical mask (this can be a combination eye shield and face mask.)
5. Red/orange biohazard bags
6. Disinfectant suitable for the biologically hazardous materials found in the lab. (Commonly, this is a container of household bleach (< 1 year old) (Disinfectant should be labeled with expiration date.)
7. Absorbent materials (i.e., 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 2)
10. Copy of spill cleanup procedures (i.e., this SOP)

Optional items for larger spills
- Disposable shoe covers
- 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.

5. All of these items can be stored in a five (5) gallon bucket with a lid or a plastic tub. The bucket or tub 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., bleach is <1 yr old and PPE is intact).