

SPILL AND EXPOSURE RESPONSE - BIOHAZARDOUS MATERIALS (INCLUDING RECOMBINANT AND SYNTHETIC NUCLEIC ACIDS)

Scope

This SOP applies to all work at UNL that is subject to the **UNL Biosafety Guidelines, including but not limited to recombinant/synthetic nucleic acid (r/sNA) experiments**. 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

Definition: *Biohazardous materials are infectious agents or biologically-derived infectious materials that present a risk or potential risk to the health of humans, animals, plants, or the environment. The risk can be direct through infection or indirect through damage to the environment.* Biohazardous materials may include r/sNA; organisms and viruses infectious to humans, animals or plants (e.g., parasites, viruses, bacteria, fungi, prions, rickettsia); and biologically active agents (i.e., toxins, allergens, venoms) that may cause disease in other living organisms or cause significant impact to the environment or community.

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

Spills involving biohazardous materials create exposure hazards. Exposure routes generally include absorption through the skin, especially if the skin is broken or irritated; inhalation if the spill created aerosols that may have dispersed in the air; contact with mucous membranes from aerosols or splashes; and accidental ingestion through hand-to-mouth contact. Use of appropriate personal protective equipment is particularly important in decontaminating spills involving human pathogens.

Recommendations for assembly of a basic Biohazard spill kit is provided as an addendum to this SOP.

Spills Involving Microorganisms, Infectious Materials, and r/sNA (Except Toxins)

Personal Protective Equipment (PPE) & Supplies

- Impervious outer garment, such as tyvek coveralls or lab coats (depending on spill volume)
- Impervious boot covers
- Full face shield or goggles and mask
- Disposable gloves
- Respiratory protection
- Disposable paper towels or other suitable absorbent (e.g., diatomaceous earth, kitty litter, commercially-available spill material, etc.)
- Freshly-prepared 10% household chlorine bleach solution (1 part bleach and 9 parts water; or add ½ cup of bleach to 1 quart of water) or a disinfectant approved for the specific agent

The appropriate ensemble of PPE to be used when cleaning up a spill depends on the severity of the spill and the microorganism. Risk Group 2 agents are recognized human pathogens. Risk Group 3 agents are severe human pathogens and are infective by the inhalation route.

If the spill is small and involves a Risk Group 1 or 2 agent, a lab coat is usually sufficient as an outer garment so long as it is unlikely that the lab coat can become saturated with the spilled material. Impervious coveralls are appropriate if the spill is large and it is likely that the outer garment can become significantly saturated or if the spill involves a Risk Group 3 agent.

Likewise, the appropriate eye protection depends on the severity of the spill. If splashing is likely, as with large spills, goggles and a face shield are appropriate to protect mucous membranes (e.g., eyes, nose and mouth). If the spill is small and splashes are unlikely, safety glasses with side shields are appropriate.

Other protective gear may be appropriate depending on the circumstances. For example, rubber boots are appropriate if you must step into or traverse areas where the spill occurred. Respiratory protection is required if the spill involves an inhalation hazard or Risk Group 3 agent.

Procedures

- Alert others to the spill. If someone is available to provide assistance, have them provide surveillance so that people don't wander into the spill area.

- Follow laboratory-specific procedures for exiting the laboratory, including removal of contaminated clothing..
- Wash all exposed skin.
- If assistance is needed because the spill is unusually large, it involves additional hazards or a Risk Group 3 agent, or clean-up materials are not available, contact EHS at 402-472-4925 or the campus operator at '0' to mobilize additional qualified persons to provide assistance.
- Gather necessary supplies and don appropriate PPE. If aerosols are of concern, allow 30 minutes settling time before attempting to clean-up the spill.
- As necessary, create a berm around the spill to prevent additional spreading.
- Remove contaminated sharps (e.g., broken glass) from the spilled material and place in a rigid, water-tight container. Use a mechanical device such as tongs to pick up sharps. **Do not use your hands.**
- Spread the absorbent material (e.g., paper towels, diatomaceous earth, kitty litter, commercially-available spill material, etc.) over the area of the spill, working from the outside edges toward the middle.
- Allow the absorbent to soak up the liquid and carefully place it into a biohazardous waste receptacle.
- Treat the spill area with a **freshly-prepared** 10% solution of household bleach or other agent-specific disinfectant. It is best to gently pour the bleach solution on the spill area, as opposed to spraying, since spraying could create aerosols.
- Allow the bleach/disinfectant to stay in contact with the surface for at least 20 minutes. After 20 minutes, absorb any remaining bleach/disinfectant solution on paper towels or other absorbent and place into a biohazardous waste container.
- Clean the affected area with soap and water.
- Remove personal protective equipment and thoroughly wash hands, arms, face, and any other exposed body parts. Disinfect non-disposable PPE.
- If you haven't already done so, notify your supervisor of the spill.

Spills Inside a Biological Safety Cabinet

- Immediately stop all work. Leave BSC blower fan on during cleanup.
- Cover spill with paper towels and carefully pour an appropriate disinfectant solution around spill. Cover spill with disinfectant soaked paper towels. The operator should be wearing gloves during this procedure.
- With paper towels & appropriate disinfectant detergent, wipe down BSC walls, work surfaces, and equipment.
- Flood work surface & drain pan (Type II BSC) with disinfectant. Allow to stand at least 20 minutes.
- Wipe up all excess disinfectant.
- Autoclave all contaminated materials.

- BSC must run for at least 10 minutes after cleanup before being used for experiments.

Inside a Centrifuge

*The potential for multiple infections from a single centrifuge accident is great. Aerosols are created when fluid escapes from the rotor or cup while the centrifuge is operating at high speed. **All opening of centrifuges must be performed slowly.***

Unsealed buckets:

1. If a centrifuge tube breaks while the centrifuge is running, turn off motor. Allow the machine to be at rest for 30 minutes before opening. If breakage is discovered after the machine has stopped, re-close the lid immediately & allow the unit to be at rest for 30 minutes.
2. Post the sign attached to this document on the door to the room where the centrifuge is located.
3. Unplug centrifuge before initiating clean up.
4. Don lab coat, gloves & other appropriate PPE before proceeding with clean up. Use of a respirator is recommended and double gloving is advisable if glass tubes were used and broken.
5. Cover the contaminated inner surfaces of the centrifuge with paper towels and soak with disinfectant.
6. Allow appropriate and sufficient contact time (usually at least 20 minutes).
7. Use mechanical means (such as forceps or tongs) to remove broken tubes & glass fragments. Place them in a sharps container for autoclaving & disposal as infectious waste.
8. Carefully retrieve unbroken tubes, wipe outside with disinfectant, and place them into another empty container.
9. Autoclave all removable centrifuge parts or wipe their surfaces with disinfectant soaked towels. After proper decontamination, carriers, rotors etc. can be washed with a mild detergent according to the manufacturer's instructions.
10. Use mechanical means to remove remaining disinfectant soaked materials from centrifuge bowl & discard in a leak-proof biohazard bag.
11. Wipe down all surfaces inside the centrifuge once more with disinfectant soaked towels. Discard disinfectant soaked materials in a leak-proof biohazard bag.
12. Remove protective clothing used during cleanup & place in a biohazard bag for autoclaving.
13. Wash hands thoroughly with soap and water after gloves are removed.
14. Report the spill incident to your lab supervisor or PI.
15. Report the spill incident to the Biosafety Officer (402-472-9554 or 402-472-4925) for further investigation

Sealed buckets (safety cups):

1. Follow steps 1-6 above.
2. If breakage is suspected, remove the sealed bucket to a biological safety cabinet before opening.
3. Obtain and place into the BSC containers suitable for holding tubes, broken glass or other containers while cleaning centrifuge components.
4. Open the rotor or safety cup carefully.
5. Use mechanical means (such as forceps or tongs) to remove broken tubes & glass fragments. Place them in a sharps container for autoclaving & disposal as infectious waste.
6. Carefully retrieve unbroken tubes, wipe outside with disinfectant, and place them into the other empty container in the BSC, out of the way.
7. Autoclave all removable centrifuge parts or wipe their surfaces with disinfectant soaked towels. After proper decontamination, carriers, rotors etc., can be washed with a mild detergent according to the manufacturer's instructions.
8. Follow steps 9-15 above.

Spills Involving Biological Toxins

Personal Protective Equipment

- Lab coat
- Full face shield or goggles and mask
- Disposable gloves
- Disposable paper towels or other suitable absorbent (e.g., diatomaceous earth, kitty litter, commercially-available spill material, etc.)
- Use 2N NaOH or other decontaminant proven to be effective against a specific toxin.

Procedures

- Create a berm or dike with absorbents.
- Treat the spill area with the 2N sodium hydroxide. Allow a contact time of one hour.
- Remove personal protective equipment and thoroughly wash hands, arms, face, and any other exposed body parts.
- Tag spill residues for collection by EHS. Clean PPE or containerize and tag for collection by EHS.
- Clean area with soap and water.
- If you haven't already done so, notify your supervisor of the spill.

Skin, Mucous Membrane, or Injury Exposure to Etiologic 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 the case of skin contact or injury with a contaminated instrument:
 - a. Thoroughly wash area with soap and water (wash for at least 5 min). Do not squeeze the wound to induce bleeding.
 - 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 for a minimum of 15 minutes.
2. Report incident to your supervisor immediately and refer to the EHS SOP, ***On-the-job and Student Injuries*** for instructions about forms to complete and where to seek 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; or
 - e. Any type of exposure that involves concentrated cultures.

UNL Biosafety Officer 402-472-9554 or 402-472-4925
4. If the exposure involves r/sNA, the BSO will notify the UNL IBC at the next meeting and the NIH Office of Biotechnology Activities (OBA) in writing within 30 days of the incident. 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.



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.

Basic Biohazard Spill Kit Contents

1. Nitrile or latex gloves (several pairs can be used for double gloving)
2. Lab coat or disposable gown
3. Goggles, face shield, and face mask to prevent splashing of disinfectant or agent into mucous membranes
4. Disposable shoe covers
5. Small disposable broom with dust pan, tongs, or forceps
6. Red medical waste/biohazard bags
7. Disinfectant suitable for the biologically hazardous materials found in the lab. (Most often this is a container of household bleach (< 1 year old) and a spray bottle to make up a fresh 10% solution.)
8. Absorbent materials (i.e., paper towels)
9. Diking material or spill pillows for large spills (stops the spread of a spill)
10. Signage to post at lab entrance for controlling access (provided on next page)
11. Copy of this SOP

NOTE: This information is for a basic 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 information regarding this please contact the UNL Biosafety Officer at 402-472-9554 or 402-472-4925.

All of these items can be stored in a five (5) gallon bucket with a lid. The bucket can also serve as a container for mixing disinfectant used in the clean up. The bucket should be labeled indicating this is a biological spill kit. The contents of this kit should be checked on 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).



BIOHAZARD
SPILL



Do Not Enter!