



SPILL AND EXPOSURE RESPONSE FOR BIOHAZARDOUS MATERIALS

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 and/or recombinant or synthetic nucleic acid molecules that present a known 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 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***.

Spills involving biohazardous materials create personnel exposure hazards. Exposure routes can 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 personal protective equipment to clean up spills mitigates the risk of exposure to biohazardous materials and is particularly important in decontaminating spills involving human 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.

Biohazardous Spill Procedures

The appropriate ensemble of PPE to be used when cleaning up a spill depends on the severity of the spill and the characteristics of the microorganism, including mode of transmission and susceptibility to disinfectants. Human pathogens (including zoonotic agents) are grouped into risk groups (RG1 to RG4, 1 is lowest risk, 4 is highest) by these characteristics. Animal and plant-specific pathogens are not grouped into risk categories and with few exceptions are considered to not cause disease in healthy adult humans.

Knowing the risk group of an agent helps determine the PPE and precautions necessary to safely clean up the spill. The basic clean-up procedures will be the same for all types of biohazardous materials, but human pathogens in risk group 2 or higher may require enhancements to the basic procedures. Those enhancements are identified below.

Personal Protective Equipment (PPE)

Minimum PPE for all types of spills includes an outer garment (e.g. lab coat), gloves and eye protection. Fluid-resistant garments are always recommended but not required. Impervious coveralls (e.g. Tyvek) are recommended if the spill is large (e.g. > 1L) **and** it is likely that the outer garment can become significantly saturated **or** if the spill involves a human pathogen that is contagious via an airborne or aerosol route (i.e. 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 (< 1L) and splashes are unlikely, safety glasses with side shields are appropriate.

Other protective gear may be appropriate depending on the circumstances. 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 involves an inhalation hazard or Risk Group 3 agent.

General Procedures for ALL Spills

1. Wash hands with soap and water before and after cleanup.
2. Put on fresh pair of disposable gloves, a lab coat and eye protection before starting cleanup.
3. Prepare a fresh 10% solution of household bleach or another IBC approved disinfectant for the biohazardous material.
4. Spread 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.
5. Allow the absorbent to soak up the liquid and carefully place the solidified spill or paper products into a biohazardous waste receptacle.
6. Treat the spill area with a **freshly prepared** 10% solution of household bleach or other agent-specific disinfectant. Gently pour the disinfectant solution on the spill area, as opposed to spraying, since spraying could create aerosols. For bleach allow at least 20 minutes contact time or follow manufacturer's recommendation for other disinfectants.
7. After appropriate contact time, absorb any remaining disinfectant solution on paper towels or other absorbent and place into a biohazardous waste container.
8. Repeat steps 6 and 7 as necessary until confident the area is decontaminated.
9. Pick up any broken glass with forceps and place in a sharps container.
10. Dispose of clean up materials in a biohazard container
11. Report the spill to your supervisor. If the spill involved r/sNA molecules, it must be reported to EHS as well.

Procedures when BSL-2 containment required

Spills outside a Biological Safety Cabinet

1. Alert others to the spill. If someone is available to provide assistance, have him or her provide surveillance so that people do not wander into the spill area.
2. Follow laboratory-specific procedures for exiting the laboratory, including removal of contaminated clothing. For risk group 2 or higher human pathogens this means evacuating the lab for at least 30 minutes to allow any aerosols to settle.
3. 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.

4. Retrieve lab spill kit, gather other necessary supplies and don appropriate PPE.
5. Prepare a biohazard waste receptacle for disposal of spill clean up materials.
6. As necessary, create a berm around the spill to prevent additional spreading.
7. Remove contaminated sharps (e.g., broken glass) from the spilled material and place in a rigid, watertight container. Use a mechanical device such as tongs to pick up sharps.
Do not use your hands.
8. Spread 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.
9. Allow the absorbent to soak up the liquid and carefully place the solidified spill or paper products into a biohazardous waste receptacle.
10. Treat the spill area with a **freshly prepared** 10% solution of household bleach or other agent-specific disinfectant. Gently pour the disinfectant solution on the spill area, as opposed to spraying, since spraying could create aerosols.
11. If using a bleach solution, allow a contact time of at least 20 minutes. After 20 minutes, absorb any remaining disinfectant solution on paper towels or other absorbent and place into a biohazardous waste container. If using another approved disinfectant, observe the manufacturer's recommended contact time.
12. Repeat steps 11 and 12 as necessary until confident the area is decontaminated.
13. Clean the affected area with soap and water or similar routine cleaning agents.
14. Remove personal protective equipment and thoroughly wash hands, arms, face, and any other exposed body parts. Disinfect non-disposable PPE.
15. Place the biohazard bag in a second bag and secure the outer bag. Dispose of the waste as you would other biohazard waste in the lab.
16. If you have not already done so, notify your supervisor of the spill.

Spills inside a Biological Safety Cabinet

1. Immediately stop all work and secure containers of infectious agents. Leave BSC blower fan on during cleanup. Obtain spill supplies if not available in the BSC. Spray disinfectant on gloved hands prior to removing from BSC to retrieve spill supplies.
2. 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.

3. Wait at least 20 minutes for bleach or follow manufacturer's recommended contact time before continuing to Step 4.
4. With disinfectant soaked paper towels & appropriate disinfectant, wipe down BSC walls, work surfaces, and equipment.
5. Flood work surface & drain pan (Class II BSC) with disinfectant (e.g. fresh 10% bleach or other IBC approved disinfectant). Allow to stand at least 20 minutes for bleach or follow manufacturer's recommended contact time.
6. Wipe up all excess disinfectant.
7. Repeat step 3 if necessary.
8. Place all cleanup materials in a biohazard bag.
9. Finish with water or ethanol wipe down of surfaces, especially if bleach or other corrosive disinfectant was used.
10. Remove personal protective equipment and thoroughly wash hands, arms, face, and any other exposed body parts. Disinfect non-disposable PPE.
11. Autoclave all contaminated materials.
12. Let the BSC run for at least 10 minutes after cleanup before resuming normal operations.

Inside a Centrifuge

Aerosols can be formed when fluid escapes from the rotor or cup while the centrifuge is operating at high speed.

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. **For RG-2 or higher human pathogens, use of a particulate respirator should be considered and double gloving is advisable.**
5. Cover the contaminated inner surfaces of the centrifuge with paper towels and soak with disinfectant.
6. Allow appropriate and sufficient contact time (at least 20 minutes for bleach or follow manufacturer's recommended contact time).

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

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 (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 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; or

- e. Any type of exposure that involves concentrated cultures.



UNL Biosafety Officer 402-472-9554 or 402-472-4925
Email: ehs@unl.edu

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 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) and a spray bottle to make up a fresh 10% solution.) (*Disinfectant should be labeled with expiration date.*)
7. Absorbent materials (i.e., paper towels, granular absorbent material, etc.)
8. Tongs or forceps (used to grab disinfectant soaked towels or sharp objects)
9. Small broom with dust pan or a manila file folder (tear in half and use to scoop broken glassware or granular absorbent)
10. Signage to post at lab entrance for controlling access (provided in Appendix 2)
11. 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 402-472-9554 or 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 can also serve as a container for mixing disinfectant used in the clean-up. 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).

BIOHAZARD



DO NOT ENTER!

For Access Contact: _____

Questions or Concerns?

Contact UNL Environmental Health and Safety · 402.472.4925 · <http://ehs.unl.edu>