SafetyNet #127 – Biological and Biohazardous Spill Response

safety net
UC DAVIS ENVIRONMENTAL HEALTH AND SAFETY • 2-1493

This Safety Net outlines the steps to take after a spill of any infectious agent or recombinant DNA material has occurred in your laboratory or in nearby areas such as in a corridor. Although any laboratory that uses hazardous materials is required to have an appropriate spill clean-up kit available and to provide spill clean-up training, responding effectively and safely to a spill requires judgment and risk assessment. If you are not comfortable with the situation or are not confident of your abilities (even if you are thoroughly trained), or if you think that clean-up might entail unacceptably elevated risk, discuss the spill with the Biological Safety Office staff at EH&S before going further. No matter what action you decide to take, moderate to high-hazard spills as noted below must be reported to the Biological Safety Office before you attempt to clean them up, and under NIH and UC Davis rules all spills of all biological materials including spills of Risk Group (RG) 1, RG2, or RG3 agents or any recombinant DNA materials must be reported to the Biological Safety Office **(**through the EH&S main number **530 752 1493)** within one business day. You can report the spill by telephone or use the online system at http://safetyservices.ucdavis.edu/programs/biosafety/biohazard-incident-report.

This SafetyNet constitutes the standard UC Davis biohazardous spill response training document, and includes a risk-related spill response matrix and a spill response instruction summary page intended for laboratory posting. Before posting the matrix and instruction sheets please highlight the matrix as appropriate to the types of biological agents handled in your laboratory.

Spill risk assessment: Evaluate the spill to determine the level of risk it represents, so that you can decide whether you or anyone in your group has the training, knowledge, and equipment needed to clean up the spill and to decontaminate all contaminated surfaces so that 100% of the spilled material is removed or inactivated. Your risk assessment should also help you to determine whether an immediate response with absorbent material is necessary to prevent the spill from seeping into places that will be particularly difficult to clean. Consider:

- Biohazard potential of the spilled material (Risk Group (RG) classification, agent infectious route, agent infectious dose)
- Spill volume
- Spill location
- Extent of visible spatter (cryptic spatter is likely to be even more extensive)
- Additional risks (e.g., does the spill include broken glass?)
- Skill, experience, and health status of trained personnel
- Availability of Personal Protective Equipment (PPE)

1. **Moderate to high-hazard spills that must be reported** to the Biological Safety Office ***before*** clean-up but ***after*** necessary personal decontamination include:

- Any spill >500 ml
- Any spill from a fermentor at Biological Safety Level 1--Large Scale (BSL1--LS) or above
- Any spill in a Biological Safety Level (BSL) 3 laboratory
- Any viable cultured RG2 agent of any volume outside a biological safety cabinet
- Any viable cultured RG2 agent ≥10 ml inside a biological safety cabinet
- Any spill of biological or biohazardous materials or agents in a publicly accessible area such as a corridor
- Spills of a RG2 or RG3 agent or rDNA construct inside a centrifuge that occurred during operation, in an unsealed rotor or carrier
- Spills of a RG2 or RG3 agent inside a refrigerator, especially spills discovered when the door is opened
- Any spill for which no person trained to clean up is currently available

Biological Safety Office telephone contact information for immediate assistance:

Spill time and location	**Telephone number to call**	**Request assistance from:**
Normal business hours		
From the Davis or Sacramento campuses	530 752 1493	Biological Safety Office
After hours and on weekends		
From the Davis campus	911 dispatch	EH&S 24/7 on call
From the Sacramento campus	911 dispatch	EH&S 24/7 on call

2. **Spill kit:** a biological or biohazardous spill kit should include the following items:

- Bleach or other approved disinfectant specific to your agents or materials
- Spray bottle
- Appropriate container to dilute disinfectant, if needed
- Gloves (assorted sizes)
- Eye protection/face shield and other appropriate PPE as noted below
- Paper towels (at least one full package)
- Long forceps or egg tongs (or both—egg tongs are better for picking up broken glass, forceps may be better for pushing paper towels into tight corners, and for retrieving disinfectant-soaked paper towels)
- Red biohazard bags or clear autoclave bags, as appropriate for the spilled materials
- Empty, appropriately marked sharps container for disposing broken glass (clear white without biohazard label for RG1 materials, red with a biohazard label for medical waste including human and non-human primate source materials and RG2 and RG3 infectious agents)
- A dust pan and brush for spills of dry RG1 material such as transgenic plants

Store these materials in a container of appropriate size (e.g. Nalgene tub, five-gallon paint bucket) in an easily accessible location, and verify the integrity and completeness of the contents at least

twice per year (ensure that the gloves are not degraded, that the disinfectant is not expired, that the spray bottle, paper towels, sharps container, eye protection, and forceps have not been diverted to other uses, etc). Be sure to label the container and the outside of the storage cabinet prominently.

To clean up a biological or biohazardous spill:

First Priority: Assess yourself and other laboratory occupants for potential personal contamination. If any personal contamination with a RG2 or RG3 agent or contaminated material is found or believed to have occurred:

a. Remove all contaminated clothing, quickly. Place contaminated clothing in a red biohazard/autoclave bag to be autoclaved later. **Do not contaminate public areas with contaminated clothing.** *In anticipation of such emergencies, the PI should provide a fire protection or other blanket that can be used to cover someone who must remove biohazardous spill-contaminated clothing or who must use an emergency shower following a chemical splash.*
b. Flood the skin with flowing water for approximately 15 minutes and wash using soap and water. Do not use hot water and do not scrub so vigorously that you abrade the skin.
c. If aerosol formation is believed to have been associated with the incident leave the contaminated area immediately. Post the contaminated area to prevent entry until it is safe.
d. Seek medical attention promptly: contact Occupational Health Services (530 752 6051) and EH&S (530 752 1493). On weekends and after normal work hours call 911.
e. **For eye splashes**, hold the eyes open and irrigate with plenty of water at an eyewash station for at least 15 minutes. Seek medical attention promptly: contact Occupational Health Services (530 752 6051) and EH&S (530 752 1493). On weekends and after normal work hours call 911.

Second Priority: Clean up the spill:

A. Wear appropriate PPE to clean spills (as detailed in the response matrix that accompanies this SafetyNet).
B. If the spill involved broken glass, pick up the large pieces with the forceps or egg tongs and dispose in a hard-walled sharps container. Handle broken glass with care!
C. Distribute paper towels around the periphery of the spill, then towards the center. Use the forceps or egg tongs to push paper towels into recesses where spilled material may have flowed.
D. Dilute your disinfectant to the appropriate concentration in a spray bottle (if available).
E. When the spill is fully covered with paper towels, spray or very carefully pour 10% bleach or other approved disinfectant on the paper towels. Avoid generating further aerosols or flooding the spill so much that untreated material may flow
F. **Allow at least 30 minutes contact time**.
G. Pick up the paper towels with large forceps or egg tongs and put them in the appropriate waste bag. Change gloves and put used gloves in bag as well. *Avoid direct contact with the contaminated paper towels, even with gloved hands*
H. Spray or carefully pour 10% bleach or other approved disinfectant on the surface residue. Wipe up the residue with paper towels and place in appropriate bag. Small bits and pieces of broken glass should be entrained in the wet paper towels and discarded

into the waste bag. Pieces too large or heavy to entrain must be discarded in a sharps container.

I. Repeat step "H" at least once.
J. Seal and transport the waste collection bag to the appropriate autoclave or medical waste accumulation site.
K. If broken glass was disposed in a sharps container, seal the container permanently, decontaminate the exterior with the sprayed liquid disinfectant, and transport the sealed container to a medical waste accumulation site or request a sharps pickup on the Safety Services website (Davis campus)
L. Clean and disinfect the forceps or egg tongs and any other non-disposable items before returning them to the spill kit. If possible, autoclave the forceps or egg tongs before returning them to the kit.
M. Report the spill to your supervisor and to the Biological Safety Office if you have not already done so.

Guidelines and rules to help prevent spills:

- Practice manipulations involving biohazardous materials and agents by handling similar volumes of non-hazardous materials with the same tools and containers in the same working environment (e.g., biological safety cabinet) until you are adept and comfortable with the entire procedure.
- Always transport biohazardous materials outside of a biological safety cabinet in secure secondary containment.
- Always use sealed rotors or carriers to spin biohazardous materials in a centrifuge.
- Always store biohazardous liquids in refrigerators in a manner that prevents spillage if the container is tipped (secondary containment is important).
- Always ensure that the bottom drain is closed before working at a biological safety cabinet.
- Always transport biohazardous materials in publicly accessible areas in secondary leakproof containment, with sufficient absorbent material to absorb the entire liquid contents of the primary container. Label secondary containers with the universal biohazard symbol.

Tips to help handle spills:

- Study the attached Spill Response Matrix in advance so that you know how to handle location-specific spills.
- Mark the dilution container in the spill kit in advance to show how much disinfectant to add and how much diluent to add in addition, to avoid delays when the time comes to handle a spill.
- Keep a pair of shoes at the lab just for use in the lab. If you routinely change shoes when you arrive at the lab and change back when you leave for the day you won't track everyday contaminants to your automobile or home, and if you need to remove your "lab" shoes because of spill contamination you will still have shoes available to leave the lab.
- Conduct periodic hands-on drills with volumes of spilled water similar to fluid volumes in use in the laboratory to ensure that all laboratory staff members are well-experienced in the location of the spill kit and in spill handling. Practice clean-up in typical and atypical spill situations.

Created 06/08, IBC reviewed and updated 10/10 (nld, sjb)

Biohazard Spill Response Matrix

University of California, Davis, Biological Safety Office, EH&S
530 752 1493

Highlight the rows that include the types of biohazardous materials your laboratory handles

Risk Group/Biological Safety Level of laboratory	Spilled Material	Spill Location, Spill Volume Where Applicable	Appropriate PPE	Preliminary actions	Waste disposal and follow-up
RG1/BSL1	Microbial agents with no infectious or pathogenic potential to humans or other mammals; recombinant constructs, cloning hosts, and non-infectious vectors, waste materials such as spent culture media that have been in contact with RG1 agents	All	Lab coat, gloves, eye protection	Conduct risk assessment[1]	Deposit waste in clear autoclave bags or sharps containers, autoclave the bags and dispose to landfill, request a sharps pickup from Safety Services, notify Biological Safety Office of incident and clean-up results
RG2/BSL2	Human or non-human primate source materials such as established cell lines, primary cell cultures, tissues, blood, and body fluids, infectious or pathogenic agents that cause disease in humans which is usually not serious and for which treatments are often available, viral vectors derived from agents capable of infecting humans; plasmids that include coding sequences for oncogenes, toxins, or virulence factors, and other recombinant constructs normally used at BSL2; waste materials such as spent culture media that have been in contact with RG2 agents or materials	Biological safety cabinet, <10ml	Lab coat or Tyvek gown, double gloves, goggles or face shield	Conduct risk assessment[1]	Deposit waste in red medical waste bags and biohazard sharps containers, transport closed bags to a medical waste accumulation site, permanently close sharps containers and disinfect exterior surfaces, request a sharps pickup from Safety Services, notify Biological Safety Office of clean-up results
		Biological safety cabinet, >10ml	Lab coat or Tyvek gown, double gloves, goggles or face shield, and a surgical mask or professionally fit-tested N95 respirator to entrain droplets	Conduct risk assessment,[1] notify Biological Safety Office	
		In laboratory, outside of BSC		Evacuate the laboratory, notify the Biological Safety Office, conduct risk assessment,[1] wait 30 minutes before clean-up	
		Discovered in centrifuge or refrigerator			Handle waste as described above for other RG2 agents, **decontaminate the entire interior of the unit**, notify Biological Safety Office of clean-up results, seek medical follow-up
		In public area		Evacuate the area, divert foot traffic, notify the Biological Safety Office, conduct risk assessment,[1] wait 30 minutes before clean-up	Deposit waste in red medical waste bags and biohazard sharps containers, transport closed bags to a medical waste accumulation site, permanently close sharps containers and disinfect exterior surfaces, request a sharps pickup from Safety Services, notify Biological Safety Office of clean-up results
RG2/BSL2 aerosol transmissible pathogens	Enteric and viral RG2 agents designated in Cal-OSHA Standard 5199 App. D as potentially aerosol transmissible pathogens;[2] viral vector preparations incorporating oncogene, toxin, or virulence factor coding sequences; waste materials that have been in contact with these agents	Any	Solid front lab coat or Tyvek gown, double gloves, face shield or goggles, and a professionally fit-tested respirator (at least N95).	Evacuate the laboratory or other area, divert foot traffic, notify the Biological Safety Office, conduct risk assessment,[1] wait 30 minutes before clean-up	Handle waste as described above for other RG2 agents, notify Biological Safety Office of clean-up results, seek medical follow-up
RG3/BSL3	All	Any	As determined and pre-approved by the Institutional Biosafety Committee		

[1] Consider all risks that the uncontained agents or materials entail, determine whether your training is adequate to ensure complete clean-up of the spill and decontamination of all surfaces, determine whether an immediate response such as immediate application of absorbent material is needed to prevent escalation of the spill hazard

[2] e.g., Salmonella sp., Shigella sp., E. coli O157: H7, HIV in clinical samples, consult the Biological Safety website for the complete list

Biohazardous Spill Clean-up

1. **If this is a moderate to high hazard spill reportable to the Biological Safety Office *before* clean-up (**through the EH&S main number **530 752 1493), have you reported it?**
2. **Have you confirmed that appropriate PPE is available?**
3. **Have you checked yourself and others nearby the spill for spatter or shoe contamination?**
4. **Have you alerted the lab personnel and passersby (for spills in corridors) and evacuated the lab if appropriate?**
5. **Have you located the spill kit and verified that you have everything you need?**
6. **For spills outside of the biological safety cabinet, have you allowed 30 minutes settling time?**
7. **Are you trained in biohazardous spill clean-up?**

If you answered "yes" to questions 1-7 and it is appropriate for you to clean up the spill, you may proceed as outlined below:

A. Wear appropriate PPE to clean spills.
B. If the spill involved broken glass, pick up the large pieces with the forceps or egg tongs and dispose in a hard-walled sharps container. Handle with care!
C. Distribute paper towels around the periphery of the spill, then towards the center. Use the forceps or egg tongs to push paper towels into recesses where spilled material may have flowed.
D. Dilute your disinfectant to the appropriate concentration in a spray bottle (if available).
E. When the spill is fully covered with paper towels, spray or very carefully pour 10% bleach or other approved disinfectant on the paper towels. Avoid generating further aerosols or flooding the spill so much that untreated material may flow.
F. **Allow at least 30 minutes contact time**.
G. Pick up the paper towels with large forceps or egg tongs and put them in the appropriate waste bag. Change gloves and put used gloves in bag as well. *Avoid direct contact with the contaminated paper towels, even with gloved hands*.
H. Spray or carefully pour 10% bleach or other approved disinfectant on the surface residue. Wipe up the residue with paper towels and place in appropriate bag. Small bits and pieces of broken glass should be entrained in the wet paper towels and discarded into the waste bag. Pieces too large or heavy to entrain must be discarded in a sharps container.
I. Repeat step "H" at least once.
J. Seal and transport the waste collection bag to the appropriate autoclave or medical waste accumulation site.
K. If broken glass was disposed in a sharps container, seal the container permanently, decontaminate the exterior with the sprayed liquid disinfectant, and transport the sealed container to a medical waste accumulation site or request a sharps pickup on the Safety Services website (Davis campus)
L. Clean and disinfect the forceps or egg tongs and any other non-disposable items before returning them to the spill kit. If possible, autoclave the forceps or egg tongs before returning them to the kit.
M. Report the spill to your supervisor and to the Biological Safety Office