



University of Hawaii Safe handling of Biohazardous Laboratory Spills

A. SCOPE

This document presents guidelines for decontamination and handling of biological material spills that may occur in laboratories.

University of Hawaii Contact Information	
UH Biosafety Officer	(808) 956-3197
UH Manoa Campus Security	(808) 956 6911

B. DEFINITIONS

Biohazardous Materials. Biohazardous materials are materials that may pose a risk to human, animal or environment. This includes recombinant DNA, synthetic nucleic acids, microorganisms, blood and blood products, biological active material (toxin, venom, allergens) that may cause disease in another living organism.

Major Spill. Spill that spreads rapidly greater than 200ml in volume and poses a danger to human, or environment.

Minor Spill. Spill that has relatively low potential of causing risk to human health, low potential of aerosolization or inhalation hazard.

C. Responsibilities of lab personnel

1. Before working with biological materials;
 - Read and understand the procedures described in this document for the safe handling of spills
 - Read the Safety Data Sheets (SDS) (if available), literature on the specific microorganisms and lab specific SOPs to better understand the nature and hazards of working with the microorganism
 - Familiarize yourself with the location of the nearest eye wash station, safety shower, spill kits and first-aid kits
2. Notify the appropriate personnel (coworkers, supervisor)
3. For major spills (greater than 200ml volume, notify the Manager of Animal Welfare and Biosafety Program). An incident report must be completed.

D. Basic Spill Kit

The Principal Investigator (or appropriate designee) should ensure that spill kits are prepared and maintained according to their specific laboratory agents. All laboratory personnel should be aware of the location of this spill kit. Commercial spill kits are

available through various scientific vendors or the items can be purchased separately.

The spill kit should be clearly marked, dated and easily accessed.

A basic Biological Spill Kit should include:

- Spill Cleanup Protocol or instructions (specific to laboratory agents used)
- Disinfectant (Bleach or other appropriate disinfectant for specific biological agents)
- A spray bottle or other container for fresh disinfectant
- Forceps, dust pan, plastic scoop, or other device for handling sharps or broken glass
- Absorbent material, such as paper towels or pads
- Autoclavable red biohazard bags for the collection of contaminated spill clean-up items
- Disposable nitrile gloves or compatible for the spill and durable utility gloves
- Appropriate PPE (lab coat or protective outerwear, safety goggles, shoe covers, face mask). Lab personnel who are required to use N95 respirators must have a medical evaluation from a registered health care professional prior to being trained, fit tested and wear a respirator. (Respirator fit testing is provided at your respective EHSO)

E. Spill Prevention

Use secondary containment such as break and puncture resistant bottles or leak proof containers when transporting liquid biological material

1. Use the minimal amounts; it is easier to clean up a small spill than a large one
2. If possible, use plastic-backed liner to absorb spills

F. Spill Cleanup and Decontamination Procedures

Outside of Biosafety Cabinet (BSC) <i>BSL-1 Spills, or Small Scale (<200mL) BSL-2 Spills</i>	
Notify	Notify others in the immediate area that a spill has occurred.
Treat Human Injury	Tend to any injured personnel. Call 911 if necessary
Treat personal contamination	Place contaminated clothing in an autoclavable biohazard waste bag. Wash skin thoroughly with soap and water.
Wear PPE	Don lab coat, gloves, face protection.
Decontaminate spill	Cover spill with paper towel or other absorbent. Saturate the paper towel with the appropriate disinfectant (e.g. 1:10 bleach solution), starting with the edges of the spill and working toward the center. Allow contact time of at least 20 minutes.
Clean up	After the 20-minute contact time, pick up absorbent material and place in an autoclavable biohazard waste bags. Use appropriate equipment (scoop, dustpan, or forceps) to clear any broken glass. Place shard in sharps container.
Disinfect surrounding area	Spray contact area and surrounding areas of the spill with 10% bleach or appropriate disinfectant and allow to air dry. Repeat if necessary
Decontaminate material and PPE	Autoclave contaminated material and PPE. Decontaminate any reusable items with disinfectant.
Wash	Wash hands and exposed skin areas with soap and water
Document and reporting	Notify PI or supervisor of large spills PI shall submit incident report to Biosafety Program.

Outside of Biosafety Cabinet (BSC) <i>Large Scale (>200mL) BSL-2 Spills or BSL-3 Spills outside of BSC</i>	
Evacuate area	Avoid inhaling airborne material, while quickly leaving room. Notify others to evacuate the area immediately.
Notify	Notify others in the immediate area that a spill has occurred and not to enter until spill up is complete. Close laboratory door and post a warning sign. Notify supervisor and contact Biosafety Program.
Treat Human Injury	Tend to any injured personnel. Call 911 if necessary
Treat personal contamination	Decontaminate yourself. Place contaminated clothing in an autoclavable biohazard waste bag. Wash exposed skin thoroughly with soap and water
Wear PPE	Don lab coat, gloves, face protection, and booties. If necessary for particular biological agent, wear respirator instead of surgical mask.
Wait before reentering	Allow aerosols to disperse for at least 30 minutes before reentering laboratory. Seal and sign room- "DO NOT ENTER" with time and date. Depending on biological agent and exhaust features of the lab, this time period may be longer. PI should confirm when it is safe to reenter the laboratory.
Decontaminate spill	Cover spill with paper towel or other appropriate absorbent. Saturate the paper towel with the appropriate disinfectant (e.g. 1:10 bleach solution), starting with the edges of the spill and working towards the center. Pour slowly to avoid splashing. Allow contact time of at least 20 minutes. If bleach is used on stainless steel, wipe down with water after decontamination.
Clean up	Pick up absorbent material and place in an autoclavable biohazard waste bags. Use appropriate equipment (scoop, dustpan, or forceps) to clear any broken glass. Place in sharps container.
Disinfect surrounding area	Spray contact area and surrounding areas of the spill with 10% bleach or appropriate disinfectant and allow to air dry. Repeat if necessary
Decontaminate material and PPE	Autoclave contaminated cleanup material and PPE. Decontaminate any reusable items with disinfectant.
Wash	Wash hands and exposed skin areas with soap and water
Document and reporting	Document spill response procedures. If exposure occurred, submit exposure incident report to Supervisor. PI shall submit exposure incident report to Biosafety Program.

Inside BSC <i>BSL-1, BSL-2, BSL-3 spills</i>	
Notify	Notify others in the immediate area that a spill has occurred.
Maintain BSC Air Flow	Leave BSC blower fan on
Treat Human Injury	Tend to any injured personnel. Call 911 if necessary
Treat personal contamination	Place contaminated clothing in an autoclavable biohazard waste bag. Wash exposed skin thoroughly with soap and water
Wear PPE	Don lab coat, gloves, face protection, and booties. If necessary for particular biological agent, wear respirator instead of surgical mask.
Decontaminate spill	Cover spill with paper towel or other appropriate absorbent. Saturate the paper towel with the appropriate disinfectant (e.g. 1:10 bleach solution), starting with the edges of the spill and working towards the center. Pour slowly to avoid splashing. Allow contact time of at least 20 minutes. If bleach is used on stainless steel, wipe down with water after decontamination.
Decontaminate BSC	Wipe cabinet walls, work surfaces, and inside view screen with disinfectant
Decontaminate BSC catch basin, if necessary	If a large spill resulted in liquid flowing into the catch basin, it is necessary to extensively decontaminate BSC. Ensure drain valve is closed. Pour disinfectant onto work surface and through front and rear grilles into the drain pan. Allow 20 minutes of contact time. Absorb disinfectant with paper towels from work surface. Empty drain pan by using tubing to drain disinfectant into a container.
Clean up	Pick up absorbent material and place in an autoclavable biohazard waste bag. Use appropriate equipment (scoop, dustpan, or forceps) to clear any broken glass. Place in sharps container.
Decontaminate material and PPE	Autoclave contaminated cleanup material and PPE. Decontaminate any reusable items with disinfectant.
Wash	Wash hands and exposed skin areas with soap and water
Document and reporting	Notify Supervisor and Document spill response procedures for large spills or BSL3 spills. If exposure occurred, submit exposure incident report to Supervisor. PI shall submit exposure incident report to Biosafety Program.

Centrifuge Precautions and Spill Clean Up	
Prevent Spills and Aerosolized Particles	Always use sealed safety buckets and sealed rotors with O-rings. Check rotor and O-Rings periodically. Examine and replace O-ring, tubes, and bottles for cracks and deformities. Wait 5 minutes after end of centrifuge run before opening lid to allow for aerosols to settle. Centrifuge lids should be opened slowly, preferable in a BSC.
Evacuate area	If a spill (i.e. from a broken tube) is identified after centrifuge lid is opened, slowly close lid and evacuate laboratory.
Notify	Notify others in the immediate area that a spill has occurred and not to enter until spill up is complete. Close laboratory door and post a warning sign. Notify supervisor and contact Biosafety Program.
Treat personal contamination	Place contaminated clothing in an autoclavable biohazard waste bag. Wash exposed skin thoroughly with soap and water
Wear PPE	Don lab coat, gloves, face protection, and booties. If necessary for particular biological agent, wear respirator instead of surgical mask.
Wait before reentering	Allow aerosols to disperse for at least 30 minutes before reentering laboratory. Depending on biological agent and exhaust features of the lab, this time period may be longer. PI should confirm when it is safe to reenter the laboratory.
Decontaminate buckets and rotors	Transfer rotors and buckets to BSC, Immerse rotors/buckets in 70% ethanol or non-corrosive disinfectant. Allow at least 1 hour contact time. Use forceps to remove any broken glass and discard in sharps container. After soaking wash with soap and water, air dry.
Decontaminate centrifuge	Spray inside of centrifuge bowl with appropriate disinfectant. Place paper towels over the entire spill area. Allow 20 minutes contact time.
Clean up	Pick up absorbent material and place in an autoclavable biohazard waste bag.
Decontaminate material and PPE	Autoclave contaminated cleanup material and PPE. Decontaminate any reusable items with disinfectant.
Wash	Wash hands and exposed skin areas with soap and water
Document and reporting	Notify Supervisor and document spill and response procedures for large spills or BSL3 spills. If exposure occurred, submit exposure incident report to Supervisor. PI shall submit exposure incident report to Biosafety Program/.

Spill of Biohazardous Radioactive Material	
<p>Emergency responses to radioactive and biohazardous spills are different (mixed spill). Use caution to prevent the radioactive material from spreading when trying to decontaminate the biohazardous spill. Considerations: type of radioactive material, characteristics of the microorganisms and the volume of spill. Contact Radiation Safety for assistance 956-6475 first, then Biosafety Program.</p>	
Evacuate area	<p>If a spill (i.e. from a broken tube) is identified after centrifuge lid is opened, slowly close lid and evacuate laboratory. Avoid inhaling any airborne material. Post a Warning sign on the door.</p>
Treat personal contamination	<p>Remove all contaminated clothing and place into a autoclavable biohazard waste bag. Label bag as radioactive and biohazardous. Wash exposed skin thoroughly with soap and water.</p>
Notify	<p>Notify others in the immediate area that a spill has occurred and not to enter until spill up is complete. Close laboratory door and post a warning sign. Contact Radiation Safety 956-6475. Notify supervisor and Biosafety Program.</p>
Wear PPE	<p>Don lab coat, gloves, face protection, and booties. If necessary for particular biological agent, wear respirator instead of surgical mask.</p>
Wait before reentering	<p>Allow aerosols to disperse for at least 30 minutes before entering the laboratory again. Confirm with Radiation Safety that it is safe to enter lab.</p>
Decontaminate spill	<p>Special precautions should be used when decontaminating a biohazardous radioactive material. <u>10% bleach</u>. Bleach solutions should not be used with iodinated material because radioiodine gas could be released. <u>Autoclave</u>. Radioactive gas may be released. Contact Radiation Safety for approval before attempting to decontaminate.</p>
Wash	<p>Wash hands and exposed skin areas with soap and water</p>
Document and reporting	<p>Contact Radiation Safety. Notify Supervisor and Biosafety Program. Document spill and response procedures for large spills or BSL3 spills. If exposure has occurred, submit exposure incident report to Supervisor, Radiation Safety and Biosafety Program.</p>

G. Exposure, Incidents and Reporting

1. If laboratory workers are contaminated with a biological material, tending to them generally takes priority over implementing the spill control measures. It is important to seek medical attention for minor burns, cuts and abrasions, serious accidents, body, eye, mucous member exposure. The SDS of the agents should be consulted to determine if any delayed effects should be expected.
2. Spills on small areas of skin: Immediately flush with flowing water for at least 15 minutes. If there is no visible burn, wash with warm water and soap.
3. Spills on clothes: Do not wipe clothes. Quickly and carefully remove contaminated clothing, shoes, and jewelry, taking care not to spread the material on the skin. Immediately flood affected body area with warm water for at least 15 minutes.
4. Splashes into the eye: Flush affected area for 15 minutes using eyewash.
5. Principal Investigator, lab manager or supervisor must be notified of all incidents. The PI or lab manager will initiate an accident or exposure incident report.
6. Animal Welfare and Biosafety Program, Manager must be notified within 24 hours of any of the following adverse event or incidents;
 - i. Large biological spills (including recombinant DNA and synthetic nucleic acid material) over 200 mL (all Biosafety levels)
 - ii. BSL-3 incidents
 - iii. Select agent incidents
 - iv. personal injury or illness
7. Minor spills (< 200ml and not involving BSL3 or Select agents)