US Environmental Protection Agency
Office of Pesticide Programs

Office of Pesticide Programs
Microbiology Laboratory
Environmental Science Center, Ft. Meade, MD

Standard Operating Procedure for Handling Spills of Biohazardous Materials

SOP Number: MB-13-04

Date Revised: 09-05-14

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Title	Handling Spills of Biohazardous Materials
Scope	The protocol presents guidelines for decontamination and cleanup of biohazardous spills.
Application	This SOP distinguishes between large spills vs. small spills, and spills inside vs. outside of the biological safety cabinet. Procedures for responding to a spill may vary, depending upon the degree and location of the spill of biohazardous material.

	Approval Date
SOP Developer:	_____ Print Name: _____
SOP Reviewer	_____ Print Name: _____
Quality Assurance Unit	_____ Print Name: _____
Branch Chief	_____ Print Name: _____

Date SOP issued:	
Controlled copy number:	
Date SOP withdrawn:	

TABLE OF CONTENTS

1. Definitions	1. Appropriate disinfectant = EPA-registered hospital disinfectant (efficacious against *S. aureus, P. aeruginosa,* and *S. choleraesuis*), hospital disinfectant with tuberculocidal claims (efficacious against *S. aureus, P. aeruginosa, S. choleraesuis,* and *M. bovis* (BCG)), or EPA-registered disinfectant towelette efficacious against spore-former *Clostridium difficile*. All disinfectants must be used according to the directions (e.g., use dilution, contact time, etc.) specified on the labeling. 2. Spill = A spill is defined as a *biohazardous material out of control*. The quantity of the biohazardous material spilled is not the sole determining factor in deciding whether or not an event is classified as a spill. Rather, the essential issue is whether the biological agent, the location, and the quantity collectively cause the situation to be beyond the control of the laboratory worker. A major spill is one that cannot be handled safely by laboratory employees in the immediate area. A minor spill is one which can be handled by the laboratory workers in the immediate area without posing a serious threat to their health and safety, and that can be cleaned up with available absorbents and disinfectants Additional abbreviations/definitions are provided in the text.
2. Health and Safety	Follow procedures specified in SOP MB-01, Laboratory Biosafety. The Study Director and/or lead analyst should consult the Material Safety Data Sheet for specific hazards associated with any disinfectants.
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Not applicable
5. Sample Handling and Storage	Refer to SOP MB-22, Disinfectant Sample Preparation.
6. Quality Control	Not applicable
7. Interferences	Failure to become familiar with and to put into practice the procedures set forth in this SOP will result in analysts who are a danger to themselves, others, and the environment.
8. Non-conforming Data	Strict adherence to the biosafety practices is required. Nonconformance will result in notification, retraining, or disciplinary action of laboratory employees.

9. Data Management	The Branch Chief is responsible for documenting spills and accidents.
10. Cautions	1. Lack of use or understanding of this SOP may negatively impact the decontamination efforts of laboratory staff and hence, cause unnecessary exposure of employees to biohazardous microorganisms. 2. Failure to clean the ultraviolet lamps in the BSCs will reduce the lamps' effectiveness. Periodically clean the ultraviolet lamps in the biological safety cabinets (BSCs) with a lint-free cloth dampened with alcohol. 3. If a liquid bleach solution is used to decontaminate stainless steel surfaces (e.g., BSC) following a spill, be sure to wash the surface with water, 70% ethanol, or an EPA-registered disinfectant to remove excess sodium hypochlorite.
11. Special Apparatus and Materials	1. *Autoclave*. 2. *Trash bags* (clear in color, autoclavable) or containers inside and outside of the biological safety cabinets for collection and storage of biohazardous waste. 3. *Personal protective equipment (PPE)* such as gloves, safety glasses, lab coats, disposable laboratory garments, shoe covers, and temporary clothing (i.e., scrubs). 4. *Biosafety Spill Kit* containing items such as gloves and tongs for handling broken glass, dustpan/brush, shoe covers, disposable lab coat, and safety glasses. 5. Signage to identify biohazardous materials and to limit access to laboratories. 6. Appropriate EPA-registered hospital disinfectant/tuberculocide. 7. Bleach solutions made fresh as needed. Discard solution at the end of the day. The container of bleach will be discarded six months from the date of receipt or designated as use for cleaning only. a. 1:10 diluted bleach solution at **neutral pH** for decontamination purposes (spore-forming microorganisms). Using an EPA registered sodium hypochlorite product containing at least 6% sodium hypochlorite, dilute as follows: 1 part bleach, 8.4 parts water, and 0.6 parts 5% white vinegar or 5% lab grade acetic acid. 8. Key card readers are used to limit access to testing laboratories. Only authorized personnel are permitted to enter.

12. Procedure and Analysis	
12.1 Guidance for Spills of Biohazardous Material - Reporting Instructions	a. Accidents are handled according to the practices outlined in this subpart, as well as procedures referenced in the Occupant Emergency Plan (OEP) and the ESC Chemical Hygiene Plan (CHP). b. *All spills and accidents* involving a Biosafety Level 2 or 3 microorganism, regardless of how minor a spill, are reported to the Branch Chief and the SHEM manager (or call security desk at extension 5-2800). c. The Branch Chief is responsible for documenting spills and accidents. d. The Branch Chief and SHEM manager will determine if additional written documentation or follow-up is warranted.
12.2 Recommendations for Reducing Potential for Spills of Biohazardous Material	a. Use secondary containment (e.g., autoclave bin) when transporting live cultures in liquid media or solid media. b. Use secondary containment to store biohazardous waste that is generated during the course of an assay. c. Prepare the least amount of culture necessary for an assay. d. Maintain a clean, well-organized work environment.
12.3 Biohazardous Organisms Requiring Biosafety Level 1 and 2 Containment	a. For guidance on spills outside and inside the BSC requiring Biosafety Level 1 and 2 containment, refer to Attachment 1: Guidance for Spills of Biohazardous Organisms Requiring Biosafety Level 1 and 2 Containment – Spills Outside and Inside the BSC.
12.4 Guidance for Spills of *Mycobacterium bovis* (BCG)	a. For guidance on spills of *Mycobacterium bovis,* refer to Attachment 2: Guidance for Spills of Mycobacterium bovis.
12.5 Decontamination of Cloth Lab Coats, Street Clothing, and Footwear	a. Decontaminate clothing with an appropriate disinfectant or by autoclaving. b. If using disinfection as a means of decontamination, treat area of contamination and surrounding area with an EPA- approved disinfectant, following the label-specified dilution and contact time.

	c. Clothing potentially contaminated with microorganisms in spore form must be autoclaved according to MB-01. d. It is less harmful to clothing to autoclave it in a tray than it is to bag it. Do not put water in the tray with the lab coat. Rather, put a second tray into the autoclave and add water to this tray. e. After clothing is decontaminated (by disinfection or autoclaving), immerse it in water containing detergent to aid physical removal of decontaminated biohazardous material. f. Rinse lab coat and set aside to be sent out with the weekly lab coat laundry service. g. Take street clothing and footwear home and launder.
13. Data Analysis/ Calculations	None
14. Forms and Data Sheets	None
15. References	1. Centers for Disease Control and Prevention and National Institutes of Health, 2007. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. U.S. Department of Health and Human Services. U.S. Government Printing Office, Washington, D.C.

Guidance for Spills of Biohazardous Organisms Requiring Biosafety Level 1 and 2 Containment – Spills Outside and Inside the BSC

- Alert workers in the laboratory that a spill has occurred and treat any injury (call 911 if necessary).
- Maintain BSC airflow: do not raise the sash or turn BSC off.
- Stand the spilled container upright, and unplug any small contaminated equipment (i.e., vortex, timer).
- Cover the spill with a paper towel (not plastic-backed material) or other appropriate absorbent material (e.g., Isosorb 13000).
- Remove any contaminated gloves, lab coat, and street clothing and place in an autoclavable container or bag. Wash contaminated skin thoroughly with soap and water. Replace any contaminated street clothing with temporary clothing such as scrubs (located in B202 and B207), or jumpsuit from the shower area of the restrooms.
- For small spills only on the skin, wash thoroughly with soap and water.
- For large spills to a significant portion of the body and clothing, disrobe and put on temporary clothing and shoe covers. Wash hands with soap and water. Proceed to the restroom to shower for 10 minutes. Discard temporary clothing in autoclavable container or bag.
- Contact the SHEM Manager (or call security desk at 5-2800) and Branch Chief.

  To decontaminate vegetative cells: Saturate the paper towel or other absorbent material, starting with the edges of the spill and working towards the center, with a liquid disinfectant (e.g. an EPA registered hospital disinfectant) and let stand for 20 to 30 minutes.

  To decontaminate spore-forming bacteria: Wipe surface with a registered sodium hypochlorite-containing towelette or a bleach solution at a neutral pH. After the contact time wash any treated stainless steel with water, 70% ethanol, or an EPA-registered disinfectant to remove excess sodium hypochlorite. Turn on UV light overnight.

- Pick up absorbent materials and place in autoclavable container or bag. Use thick gloves, tongs, scoop, and brush found in biosafety spill kit to clean up any broken glass and place in autoclavable container. Carefully check the entire affected area and beyond for remaining spill or cleanup residue.

  Autoclave all contaminated materials using a 3 hour liquid cycle. Treat contaminated gloves (for handling broken glass), tongs, scoop, and brush with disinfectant or place under the UV light overnight. Treat contaminated PPE and any contaminated street

clothing with disinfectant or autoclave as described in section 12.5. Discard autoclaved broken glass in a broken glass container.

Attachment 2: Guidance for Spills of *Mycobacterium bovis*

Follow Attachment 1 for spills inside the BSC; for spills outside of BSC proceed as follows:

- Alert workers in the laboratory that a spill has occurred and treat any injury (call 911 if necessary).
- Maintain BSC airflow: do not raise the sash or turn BSC off.
- Stop all work and close all open materials.
- Remove labcoat, gloves and shoe covers and discard them in a laboratory biohazard bin. Exit the laboratory to the anteroom.
- In the anteroom, remove any contaminated street clothing and place in an autoclavable container. Replace removed street clothing with temporary clothing located in anteroom. Wash hands.

  If exposure to skin and/or clothing has occurred, proceed to the restroom to fully shower for 10 minutes. Discard all temporary clothing in an autoclavable container. Don a new set of temporary clothing.
- Contact the SHEM Manager (or call security desk at 5-2800) and Branch Chief.

  Direct everyone (via telephone, note, etc.) to stay out of the laboratory until spill cleanup is complete.
- Wait at least 30 minutes before re-entering the lab.
- Re-enter the laboratory with appropriate level of PPE (including respirator for spills outside of BSC).
- Stand the spilled container upright, and unplug any small contaminated equipment (i.e., incubator shaker, spectrophotometer). Cover the spill with a paper towel (not plastic-backed material) or other appropriate absorbent material (e.g., Isosorb 13000).

  To decontaminate: Saturate the paper towel or other absorbent material, starting with the edges of the spill and working towards the center, with a liquid disinfectant (e.g. an EPA registered tuberculocide and let stand for 20 to 30 minutes).
- Wearing appropriate PPE, pick up absorbent materials and place in autoclavable container or bag. Use thick gloves, tongs, scoop, and brush found in biosafety spill kit to clean up any broken glass and place in autoclavable container. Carefully check the entire affected area and beyond for remaining spill or cleanup residue.

- Autoclave all contaminated materials using a 3 hour liquid cycle. Treat contaminated gloves (for handling broken glass), tongs, scoop, and brush with disinfectant or place under the UV light overnight. Treat contaminated PPE and any contaminated street clothing with disinfectant or autoclave as described in section 12.5. Discard autoclaved broken glass in a broken glass container.