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-13-07

Date Revised: 11-03-23

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Title	Handling Spills of Biohazardous Materials
Revisions Made	• Minor editorial changes for clarification purposes. • Changed terminology from “small” and “large” spills to “minor” and “major” spills to be in line with the facility Chemical Hygiene Plan. • Added definitions for “biohazardous materials”, “minor spill”, and “major spill”. • Clarified reporting requirements for accidents and spills. The analyst is responsible for appropriately reporting incidents to the Branch Chief. Initial reporting may be via telephone but an email detailing the event and clean-up process will be required after spill clean-up is complete. • Created two separate sets of instructions for responding to spills: one section for minor spills and one set for major spills.

SOP Number	MB-13-07
Title	Handling Spills of Biohazardous Materials
Scope	The protocol presents guidelines for decontamination and cleanup of biohazardous materials spills in Biosafety Level 1 and 2 laboratories.
Application	This SOP distinguishes between minor vs. major biohazardous materials spills. Procedures for responding to a spill may vary, depending upon the degree and location of the spill of biohazardous materials. Refer to the facility's Chemical Hygiene Plan for spills of substances other than biohazardous materials.

	Approval	Date
SOP Developer	MICHELE COTTRILL Digitally signed by MICHELE COTTRILL Date: 2023.11.06 10:06:55 -05'00'	11/06/23
	Print Name: Michele Cottrill	
SOP Reviewer	MICHAEL DUNCAN Digitally signed by MICHAEL DUNCAN Date: 2023.11.06 11:16:09 -05'00'	11/06/23
	Print Name: Jason Duncan	
Quality Assurance Unit	Kiran Verma Digitally signed by Kiran Verma Date: 2023.11.06 11:56:02 -05'00'	11/06/23
	Print Name: Kiran Verma	
Branch Chief	REBECCA PINES Digitally signed by REBECCA PINES Date: 2023.11.06 12:12:48 -05'00'	11/06/23
	Print Name: Rebecca Pines	

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1. Definitions	1. Appropriate disinfectant = EPA-registered hospital disinfectant with a label claim for the class of microorganisms (e.g., vegetative bacteria, spore formers, viruses, fungi, mycobacteria) being disinfected. All disinfectants must be used according to the directions (e.g., use dilution, contact time, etc.) specified on the label. 2. Biohazardous materials = Any material containing infectious or potentially infectious biological agents, such as bacteria and viruses. 3. Spill of biohazardous materials = A biohazardous material out of control. a. Minor spill (e.g., less than approximately 10 mL) is one which can be cleaned up 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. b. A major spill is one that cannot be cleaned up safely by laboratory employees (e.g., a large quantity spill). The spill will be cleaned up under the direction of the SHEM Manager and Branch Chief. 4. Additional abbreviations/definitions are provided in the text.
2. Health and Safety	1. Follow procedures specified in SOP MB-01, Laboratory Biosafety. 2. The Study Director and/or lead analyst should consult the Safety Data Sheets for specific hazards associated with any disinfectants.
3. Personnel Qualifications and Training	1. Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Not applicable
5. Sample Handling and Storage	1. Refer to SOP MB-22, Preparation and Sampling Procedures for Antimicrobial Test Substances.
6. Quality Control	Not applicable

7. Interferences	1. 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	1. Strict adherence to the biosafety practices is required. Nonconformance will result in notification, retraining, or disciplinary action of laboratory employees.
9. Data Management	1. The Branch Chief is responsible for documenting accidents and exposures associated with spills.
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 human pathogenic microorganisms. 2. Failure to clean the ultraviolet lamps in the biological safety cabinets (BSCs) will reduce the lamps' effectiveness. Periodically clean the ultraviolet lamps in the BSCs with a lint-free cloth dampened with alcohol. 3. If a liquid sodium hypochlorite (bleach) solution is used to decontaminate stainless steel surfaces (e.g., BSC) following a spill, wash the surface with water, 70% ethanol, or an EPA-registered disinfectant to remove excess sodium hypochlorite.
11. Special Apparatus and Materials	1. *Autoclave*: for sterilization. 2. *Biohazardous waste 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 or a 1:10 bleach

	solution. a. See Appendix M of the Biosafety Plan for *Bacillus anthracis* for a bleach preparation sheet for a 1:10 diluted bleach solution at **neutral pH** for decontamination of spore-forming microorganisms. b. Prepare bleach solutions for daily use, with an EPA registered sodium hypochlorite product containing at least 6% sodium hypochlorite. Discard solution at the end of the day. 7. Key card readers are used to limit access to testing laboratories. Only authorized personnel are permitted to enter.
12. Procedure and Analysis	1. Follow procedures listed below.
12.1 Guidance for Spills of Biohazardous Material - Reporting Instructions	a. Handle accidents and spills 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. The analyst is responsible for appropriately reporting incidents to the Branch Chief. Initial reporting may be via telephone but an email detailing the event and clean-up process will be required after spill clean-up is complete. c. The Branch Chief and SHEM manager have authority to 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 or solid media. b. During an assay, use secondary containment to store biohazardous waste not appropriate for placement into biohazardous waste bags (e.g., closed containers of liquid biohazardous waste, contaminated glassware, and small equipment such as forceps, etc.). Transport waste to the autoclave at the completion of testing. c. Prepare the least amount of culture necessary for an assay. d. Maintain a clean, well-organized work environment.

12.3 Guidance for Minor Spills of Biohazardous Materials Requiring Biosafety Level 1 and 2 Containment – Spills Outside and Inside the BSC	a. Alert workers in the laboratory that a spill has occurred and treat any injury (call x52800 if necessary). b. Maintain BSC airflow: do not raise the sash or turn BSC off. c. Stop all work, upright any overturned vessels, and close all open materials. Unplug any small, contaminated equipment (i.e., vortex). d. Cover the spill with a paper towel (not plastic-backed material) or other appropriate absorbent material (e.g., Isosorb 13000). e. Remove any contaminated gloves, lab coat, shoes, 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 every lab). If desired, proceed to the restroom to shower for 10 minutes. Discard temporary clothing in autoclavable container or bag. i. Don scrubs located in the restroom. ii. Return to the laboratory to resume decontamination. f. For small spills only on the skin, wash thoroughly with soap and water. g. For spills outside of the BSC and/or on analyst, contact the SHEM Manager (or call security desk at x52800) and Branch Chief. h. Decontaminate surfaces. i. 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 an EPA-registered liquid disinfectant and let stand for 20 to 30 minutes. ii. To decontaminate spores: Treat surface with an EPA-registered sporicidal product or a bleach solution at a neutral pH (see section 10.3). Turn on UV light overnight. i. 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. j. Steam-sterilize all contaminated materials using a 3-hour liquid cycle. k. Treat contaminated gloves (for handling broken glass), tongs, scoop, and brush with disinfectant or place under the UV light overnight. l. Treat contaminated PPE and any contaminated street clothing with disinfectant or steam-sterilize as described in section 12.5. m. Discard steam-sterilized broken glass in a broken glass container.
12.4 Guidance for Major Spills of Biohazardous Materials Requiring Biosafety Level 1 and 2 Containment – Spills Outside and Inside the BSC	a. Alert workers in the laboratory that a spill has occurred and treat any injury (call x52800 if necessary to dispatch emergency personnel). b. Maintain BSC airflow: do not raise the sash or turn BSC off. c. Stop all work, upright any overturned vessels, and close all open materials. Unplug any small, contaminated equipment (i.e., vortex). d. Contact the SHEM Manager (or call security desk at x52800) and Branch Chief. e. If practical, cover the spill with a paper towel (not plastic-backed material) or other appropriate absorbent material (e.g., Isosorb 13000). f. Remove any contaminated gloves, lab coat, shoes, 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 every laboratory). g. If desired, proceed to the restroom to shower for 10 minutes. Discard temporary clothing in autoclavable container or bag. i. Don scrubs located in the restroom. h. The spill will be cleaned up under the direction of the ESC SHEM Manager and Branch Chief. For example, the SHEM Manager may

	consult with the ESC's emergency environmental cleanup contractor for assistance or contact the Fort Meade fire department HAZMAT team. i. Decontamination procedures for major spills of biohazardous materials vary according to the quantity, location of the spill, and biosafety level of the organism. j. Await further instructions from the SHEM Manager or Branch Chief.
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. Steam-sterilize clothing potentially contaminated with microorganisms in spore form. Use a 3-hour liquid cycle as per SOP MB-01. d. It is less harmful to clothing to steam-sterilize 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 dry, then set aside to be sent out with the 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. Chemical Hygiene Plan, USEPA Environmental Science Center, Fort Meade, Maryland. Current version.