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Office of Pesticide Programs

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

Standard Operating Procedure for
Biosafety in the Laboratory

SOP Number: MB-01-10

Date Revised: 01-22-24

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Title	Biosafety in the Laboratory
Revisions Made	• Editorial changes for clarification purposes. • Created “Attachment 2: Disinfection of Laboratory Equipment” and removed disinfection of equipment from SOP. • Incorporated materials from page second page throughout document and removed that page. • Clarified sonication when manipulating cultures. • Removed language that appears in other specific SOPs that was duplicated in this SOP.

SOP Number	MB-01-10
Title	Biosafety in the Laboratory
Scope	This procedure encompasses the safety requirements for working with Biosafety Level 1 and 2 microorganisms in the Microbiology Laboratory Branch (MLB) laboratories.
Application	This SOP is based largely on the guidance provided in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) publication "Biosafety in Microbiological and Biomedical Laboratories" (BMBL). This protocol does not include working with BSL-3 microorganisms or select agents in the laboratory. For work with BSL-3 microbes, consult the EPA/OCSPP High Containment Laboratories' Policy, Practices, and Procedures for Working with Biosafety Level 3 Microorganisms. For select agents, consult the Biosafety Plan for Bacillus anthracis.

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TABLE OF CONTENTS

1. Definitions	1. Microorganism = includes bacteria in vegetative and spore form, fungi, bacteria in biofilms, and viruses. Refer to Attachment 1 for a list of organisms used by MLB. 2. Biosafety Level (BSL) = The BMBL manual presents recommended guidelines for working with microorganisms assigned to Biosafety Levels 1 through 4. 3. Manipulation of culture = handling of open vessels containing microorganism. Activities involving manipulation of culture including culture transfers, virus harvest, plating, inoculation of carriers, and recording results from tubes and agar plates, must be performed in the biological safety cabinet (BSC). Vessels with loose closures (e.g., glass tubes with Morton closures) should also be manipulated in the BSC. 3. Appropriate antimicrobial product = EPA-registered antimicrobial product with a label claim for the class of microorganisms (e.g., vegetative bacteria, spore formers, viruses, fungi, mycobacteria) being decontaminated. Use antimicrobial product according to the directions (e.g., use dilution, contact time, etc.) specified on the label. 4. Additional abbreviations/definitions are provided in the text.
2. Health and Safety	1. To protect the laboratory worker from possible infection by microorganisms, follow the safety guidelines provided in this procedure and in the BMBL. All laboratory personnel are required to read and familiarize themselves with this protocol and sections on Biosafety Level 2 in the BMBL. 2. Laboratory workers must familiarize themselves with the laboratory's biosafety spill clean-up procedures (see SOP MB-13) and the facility's Chemical Hygiene Plan (CHP) prior to performing any laboratory work. Biosafety spill clean-up procedures are posted in the laboratories. 3. Laboratory workers are required to participate in the Agency's Occupational Medical Surveillance Program as established by EPA Order 1460.1. 4. Medical emergencies are handled according to procedures outlined in the ESC Occupant Emergency Plan (OEP). 5. Spills and accidents are handled according to the practices outlined in this SOP and SOP MB-13, as well as procedures referenced in the OEP and the CHP. 6. To promote the health of exposed individuals, the Branch Chief will

	encourage individuals to seek follow up, if necessary, depending upon recommendations of the SHEM manager. 7. All laboratory workers must meet the requirements of the Hazard Communication Program's Employee Training Program, as described in the CHP. 8. In accordance with the CDC/NIH guidelines, the Branch Chief may restrict access to the laboratory as specified under "special practices".
3. Personnel Qualifications and Training	1. SOP ADM-04, OPP Microbiology Laboratory Training. 2. Strict adherence to these biosafety practices is required. Non-conformance will result in notification, retraining, or possible disciplinary action of the laboratory employees.
4. Instrument Calibration	None
5. Sample Handling and Storage	None
6. Quality Control	None
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. 2. BSCs that are not operating properly (e.g., no power, airflow issues, alarming, etc.) cannot be used until fixed per SOP EQ-11, Use and Maintenance of Biological Safety Cabinets. 3. Inspect gloves prior to use. Do not use gloves that have holes, rips, or are otherwise degraded.
8. Non-conforming Data	None
9. Data Management	None
10. Cautions	1. Lack of following or understanding of this SOP may negatively impact the quality of the microbiological practices used in the laboratory. 2. Failure to use the "STOP/DO NOT ENTER" signs (section 12.2) to control access to the laboratory while cultures are being manipulated may result

	in the inadvertent exposure of personnel to biohazardous microorganisms. 3. Autoclaving flammable and caustic liquids (e.g., alcohols or highly acidic antimicrobial products) can present an explosion or exposure hazard. Seek advice from the SHEM Manager for appropriate method of decontamination and disposal.
11. Special Apparatus and Materials	1. *Biological Safety Cabinet (BSC):* All work is to be performed in a BSC, not on the open bench. This is due to the availability of BSCs within the laboratory, the ease and practicality of working within the BSC, and the ease of containing spills of chemical or biohazardous materials that may occur within the BSC. 2. *Autoclave:* used for sterilization of media, reagents, materials, and waste. 3. *Biohazard waste bags:* (clear in color, autoclavable) inside and outside of the biological safety cabinets for collection and storage of biohazardous waste. 4. *Personal protective equipment (PPE)* such as gloves, safety glasses, lab coats, disposable laboratory garments, shoe covers, and temporary clothing (i.e., scrubs). 5. *Signs:* to identify biohazardous materials and to limit access to laboratories. 6. *Appropriate antimicrobial product*: see section 1. 7. *Sporicide*: 5or microorganisms in spore form: prepare 1:10 diluted bleach solution **at neutral pH** (see Appendix M of the Biosafety Plan for *Bacillus anthracis*). 8. *Key card readers:* used to limit access to testing laboratories. Only authorized personnel are permitted to enter. 9. *Attachment I:* a list of microorganisms maintained by the laboratory. 10. *Secondary containment* (e.g., durable, autoclavable trays and bins; containment cart). 11. *Lab equipment (e.g., chillers, water baths, sonicators, etc.):* See Attachment 2.
12. Procedure and	

Analysis	
12.1 General Laboratory Practices	a. Eating, drinking, smoking, handling contact lenses, chewing gum, and applying cosmetics (including lip balm) are not permitted in the laboratory. b. Do not store food for human consumption in laboratory areas. c. Do not wash or reuse disposable gloves. d. Once work is complete, wash hands prior to leaving the laboratory. e. Do not mouth pipette. Only mechanical pipetting devices will be used. f. Manipulate cultures inside a BSC to minimize risk of exposure and risk of contamination of lab surfaces. g. Perform all procedures carefully to minimize the creation of aerosols. h. Animals and plants not related to work being conducted are not permitted in the laboratory. i. Do not place any material suspected or known to be contaminated with biohazardous material (e.g., gloves, pipet wrappers, paper towels, etc.) in the trash cans. Place these items in an appropriate biohazardous waste bag. j. Protect vacuum lines with traps and HEPA filters.
12.2 Access to Laboratories and Placement of Signage	a. Key card readers are used to limit access to laboratories. Only authorized personnel are permitted to enter. b. When manipulating cultures, post the magnetic “STOP/DO NOT ENTER” sign on the outside (i.e., side facing corridor) of the external laboratory door. c. Only MLB staff are authorized to enter the laboratory while the “STOP/DO NOT ENTER” sign is posted. Non-MLB personnel must be escorted into a laboratory while the sign is posted. d. Remove the “STOP/DO NOT ENTER” sign once work is complete.
12.3 Checking Airflow to Laboratories Equipped with	a. Airflow monitors are located above the B202 and B207 laboratory doors. The laboratories have two monitors each: one above the door leading from the corridor to the anteroom, and one above the door leading from the anteroom to the lab. The orange ball in the tube

Monitoring Devices	rolls in the direction of the airflow. For negative airflow, the orange ball will roll from the corridor into anteroom, or from the anteroom into the lab. Monitors must indicate negative airflow for entry. b. Prior to entering laboratories visually verify that the laboratories with monitoring devices are under negative pressure (airflow is into the room from the anteroom/corridor). c. Do not enter the laboratory to perform laboratory work if airflow is positive for either the laboratory or anteroom. Report positive airflow status to the Branch Chief and facilities hotline (x54357). d. If airflow becomes positive during work, cease work immediately and close all open materials. Report the problem to the Branch Chief and facilities hotline (x54357). Work should only proceed when proper airflow has been restored.
12.4 PPE Requirements	a. Wear a lab coat and safety glasses while work is being performed in any MLB laboratory. b. Safety Glasses i. Wear safety glasses while work is being performed in the laboratory. c. Lab Coats i. Don cloth or disposable lab coats. Remove lab coats before going to non-laboratory areas such as the office areas, restrooms, library, etc. d. Gloves i. Wear gloves (latex or nitrile) when manipulating culture and when handling any vessel (e.g., tube, rack, plate, biohazard bag), closed or open, containing live organism. ii. Replace gloves immediately in the event of overt contamination (e.g., visible drops of liquid) with infectious material. Dispose of all gloves in the biohazard bin only. iii. To minimize risk of contamination of the test system while working in the BSC, analysts may periodically apply a solution of 70% ethanol over the exterior surface of the gloves, change gloves frequently, or use sterile gloves.
12.5 Staining of	a. After microscopically viewing organisms, remove slides from the

	Microorganisms	microscope stage and discard them in a biohazard bin. If it is necessary to keep a prepared slide, store it in a sealed petri dish or a microscope slide case to which a biohazard label has been affixed. b. To decontaminate stain rinsate, collect the rinsate and add an EPA-registered sodium hypochlorite product full strength to the rinsate in an approximate 1:10 ratio (one-part household bleach to nine parts rinsate) for a minimum of 60 minute before disposal.
12.6	Transport of Cultures	a. Use of secondary containment is required for transporting cultures within and between laboratories to reduce potential for generating a spill. Use a cart for larger volumes. b. Tape autoclave bags containing biohazardous waste closed prior to transport.
12.7	Managing Biohazardous Waste	a. After manipulating culture, analysts must bag biohazardous waste and place it in a closed container (e.g., biohazard bin with lid, closed biohazard bag). b. Storage of items awaiting sterilization i. No biohazardous waste may be removed from the second floor B-wing prior to sterilization. ii. Place all contaminated articles in autoclavable bins. Place full bins in the autoclave to await sterilization. iii. Place contaminated cuvettes, homogenizers, and other small equipment into a container covered with aluminum foil prior to placing the items in the autoclavable bin. iv. Keep all biohazardous waste-containing articles (e.g., autoclave bags, containers, tubes, flasks, homogenizers, cuvettes, etc.) closed, covered, or in the BSC while awaiting sterilization to prevent the generation and release of infectious aerosols into the laboratory environment. v. Cap all test tubes/flasks containing liquid waste (including used micropipette tips) or cover with aluminum foil. vi. Tape full autoclave bags closed but do not completely seal. vii. It is recommended that used pipettes be collected in a waste container (e.g., bag, bin, stainless steel beaker) inside the BSC rather than discarded in the autoclave bag outside of the BSC. Transport the waste container to the autoclave

	for sterilization and discard autoclaved pipettes with other biohazardous waste. viii. Autoclave biohazardous waste as soon as possible. ix. Use a **three-hour** (180 minute) **liquid cycle** to sterilize both liquid and solid biohazardous waste.
12.8 Resource Management	a. Laboratory personnel should be mindful of water consumption, and whenever possible, employ practices that minimize water use. b. Laboratory personnel should run full autoclave loads whenever possible.
13. Data Analysis/ Calculations	None
14. Forms and Data Sheets	1. Attachments are stored separately from the SOP under the following file names: Attachment 1: Microorganisms Used by the OPP Microbiology Laboratory — MB-01-10_A1_XXXXXX.docx Note: XXXXXX references date Attachment is updated. Attachment 2: Decontamination of Laboratory Equipment — MB-01-10_A2.docx
15. References	1. Centers for Disease Control and Prevention and National Institutes of Health, 2020. Biosafety in Microbiological and Biomedical Laboratories, 6th edition. U.S. Department of Health and Human Services. U.S. Government Printing Office, Washington, D.C. 2. EPA/OCSPP High Containment Laboratories' Policy, Practices and Procedures for Working with Biosafety Level 3 Microorganisms.