



US Environmental Protection Agency Office of Pesticide Programs

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

Standard Operating Procedure for Biosafety in the Laboratory

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SOP Number	MB-01-08
Title	Biosafety in the Laboratory
Scope	This protocol encompasses the safety requirements for working with the microorganisms in the Microbiology Lab Branch 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”. This protocol does not include working with select agents in the laboratory. For safety measures associated with working with select agents, consult the Biosafety Plan for <i>Bacillus anthracis</i> .

	Approval	Date
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Date SOP issued:	
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The Laboratory recognizes the biosafety levels set forth in the BMBL, and the need to provide different degrees of protection (i.e., ascending biosafety levels) depending upon the danger of the microbe to the worker, community, and the environment. The laboratory follows BMBL Biosafety Level (BSL) 2 procedures for work with organisms identified on Attachment 1. The current inventory contains BSL 1 and 2 microorganisms. If a project requires the use of a BSL 3 organism, this SOP will be revised to reflect BSL 3 practices and procedures before the work begins.

This SOP is structured so all work involving manipulation of culture of all microorganisms, regardless of the biosafety level, is performed in the BSC and 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.

For new microorganisms, laboratory staff must first determine the biosafety level of that microorganism by consulting the BMBL or vendor.

Although these guidelines are not currently legally enforceable guidelines, they are considered to be international standards of practice. Should an exposure event occur, the CDC/NIH guidelines could take on the force of law in that the laboratory management could be held legally responsible for not following accepted standards of practice. Consequently, the laboratory will comply with the CDC/NIH guidelines.

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1. Definitions	<ol style="list-style-type: none">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 = The BMBL (see ref. 15.2) 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, sonication of inoculated carriers, recording results from tubes and plates, must be performed in the BSC. The following are examples of activities involving manipulation of culture: culture transfers, virus harvest, plating, inoculation of carriers, sonication of inoculated carriers, recording results from tubes and plates, etc.3. 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.4. Water = reagent-grade water.
2. Health and Safety	<ol style="list-style-type: none">1. To protect the laboratory worker from possible infection by microorganisms, the safety guidelines provided in this protocol and in the BMBL manual must be followed. 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.

	<p>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.</p> <p>7. All laboratory workers must meet the requirements of the Hazard Communication Program’s Employee Training Program, as described in the CHP.</p> <p>8. In accordance with the CDC/NIH guidelines, the Branch Chief may restrict access to the laboratory as specified under “special practices”.</p>
3. Personnel Qualifications and Training	Refer to SOP ADM-04, OPP Microbiology Laboratory Training.
4. Instrument Calibration	Performing maintenance and repairs on the BSCs and maintaining annual certification of the BSCs are the responsibilities of the Facility Manager or his designees (e.g., operations, maintenance and repair contractors). See SOP EQ-11, Use and Maintenance of Biological Safety Cabinets.
5. Sample Handling and Storage	Not Applicable
6. Quality Control	<p>1. See QC-13, Performance Verification of Autoclaves, for verification of autoclave performance and corrective actions.</p> <p>a. If an autoclave undergoes repair, do not use the autoclave until its performance is verified using the monthly verification procedure for a kill cycle (see QC-13, Section 6).</p>
7. Interferences	<p>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.</p> <p>2. Improper maintenance and/or sudden power failures may result in failure of the BSCs to operate properly. Refer to proper use and maintenance procedures in SOP QC-06.</p> <p>3. Inspect gloves prior to use. Do not use gloves that have holes, rips, or are otherwise degraded.</p>
8. Non-conforming Data	Strict adherence to these biosafety practices are required. Nonconformance will result in notification, retraining, or possible disciplinary action of laboratory employees.
9. Data Management	Completed forms are archived in notebooks in D217.

<p>10. Cautions</p>	<ol style="list-style-type: none"> 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 to control access to the laboratory while cultures are being manipulated (section 12.2) may result in the inadvertent exposure of personnel to biohazardous microorganisms. 3. Failure to clean the ultraviolet lamps in the BSCs will reduce the lamps’ effectiveness. Periodically clean the ultraviolet lamps in the BSCs with a lint-free cloth dampened with alcohol (200 proof ethanol), and record the cleaning on the BSC Monitoring Record Form. 4. Autoclaving flammable and caustic liquids (e.g., alcohols or highly acidic disinfectants) can present an explosion or exposure hazard. Seek advice from the SHEM Manager for appropriate method of decontamination and disposal. 5. Halogenated materials are not recommended for routine use on stainless steel surfaces of the BSC. 6. This protocol does not include working with select agents in the laboratory. For biosafety measures associated with working with select agents, consult the Biosafety Plan for <i>Bacillus anthracis</i>.
<p>11. Special Apparatus and Materials</p>	<ol style="list-style-type: none"> 1. Biological Safety Cabinet (BSC) 2. Autoclave 3. Biohazard bags or containers inside and outside of the biological safety cabinets for biohazardous waste. 4. Personal protective equipment (PPE) such as gloves, safety glasses, and lab coats. 5. Signs to identify biohazardous materials and to limit access to laboratories. 6. Appropriate disinfectant (see section 1). 7. An additional option for microorganisms in spore form: prepare 1:10 diluted bleach solution at neutral pH. 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). Contact time is 60 minutes. 8. Key card readers are used to limit access to testing laboratories. 9. Microorganisms maintained by the laboratory are specified in Attachment 1.

	10. Secondary containment (e.g., durable, autoclavable trays and bins; containment cart).
12. Procedure and Analysis	
12.1 General Laboratory Practices	<ul style="list-style-type: none"> a. Eating, drinking, smoking, handling contact lenses, chewing gum, and applying cosmetics (including lip balm) are not permitted in the laboratory. b. Storing food for human consumption in laboratory areas is prohibited. c. Laboratory workers must not wash or reuse disposable gloves. d. Once work is complete, wash hands prior to leaving the laboratory. e. Mouth pipetting is prohibited. Only mechanical pipetting devices will be used. f. Organisms will be manipulated inside a BSC to minimize risk of exposure and risk of contamination of lab surfaces. g. All procedures are performed carefully to minimize the creation of aerosols. h. Animals and plants not related to work being conducted are not permitted in the laboratory. i. No material suspected or known to be contaminated with biohazardous material (e.g., latex gloves, pipet wrappers, paper towels, etc.) is to be placed in the trash cans. These items are to be placed in an appropriate biohazardous waste bag. j. Protect vacuum lines with disinfectant traps and HEPA filters.
12.2 Access to Laboratories and Placement of Signage	<ul style="list-style-type: none"> a. Key readers are used to limit access to laboratories. Only authorized personnel are permitted to enter. b. When manipulating infectious microorganisms, post the magnetic “STOP/DO NOT ENTER” sign on the outside (i.e., side facing corridor) of the external laboratory door. c. Only Microbiology Laboratory Branch (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	<ul style="list-style-type: none"> a. Airflow monitors are located above the B202 and B207 laboratory

<p>Airflow to Laboratories Equipped with Monitoring Devices</p>	<p>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 rolls in the direction of the airflow. For negative airflow, the orange ball will roll from the corridor into the laboratory or anteroom, or from the anteroom into the lab. Monitors must indicate negative airflow for entry.</p> <ul style="list-style-type: none"> b. Prior to entering laboratories visually verify that the laboratories with monitoring devices are under negative pressure (airflow is into the room from the corridor). c. Do not enter the laboratory if airflow is positive for either the laboratory or anteroom. Report positive airflow status to the Branch Chief and facilities hotline (x54357). d. In the event that airflow becomes positive during work, cease work immediately and close all open materials. If working inside a BSC, back out and pull down the sash. Report the problem to the Branch Chief and facilities hotline (x54357). Work should only proceed when proper airflow has been restored.
<p>12.4 Using the BSC</p>	<ul style="list-style-type: none"> a. Turn off ultraviolet light. b. Turn on the blowers, lights, and outlets, and allow to operate for a minimum of 15 minutes prior to use. c. Record that the downflow and exhaust rates are within the acceptable range on the BSC Monitoring Record Form immediately prior to use. d. Spray the surface of the BSC with the use dilution of an appropriate disinfectant or with 70% ethanol. Allow the surface to remain wet for at least 10 seconds for 70% ethanol or the label-specified contact time for a disinfectant. e. At the conclusion of activities involving bacteria in spore form (e.g., <i>Bacillus subtilis</i> spore suspensions, spore strips), turn on the ultraviolet light and leave it on overnight. f. In the event that the BSC alarms during work, cease work immediately, pull down the sash, and call the facilities hotline (x54357) to report the problem.
<p>12.5 PPE Requirements</p>	<ul style="list-style-type: none"> a. A lab coat and safety glasses must be worn at all times in any MLB laboratory. b. Safety Glasses <ul style="list-style-type: none"> i. Safety glasses must be worn while working in the laboratory.

	<ul style="list-style-type: none"> c. Lab Coats <ul style="list-style-type: none"> 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 <ul style="list-style-type: none"> 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 contaminated 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
<p>12.6 Conducting Staining of Micro organisms</p>	<ul style="list-style-type: none"> a. While staining and viewing slides, wear gloves and a lab coat, and conduct any steps involving manipulation of the organism (e.g., smear preparation) in the BSC. b. After microscopically viewing organisms, remove slides from the 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. c. 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.
<p>12.7 Transport of Cultures</p>	<ul style="list-style-type: none"> a. Use of secondary containment is required for transporting cultures within the laboratory to reduce potential for generating a spill. b. For transporting live cultures between laboratories, place the cultures in secondary containment; use a cart to facilitate transport of larger volumes between laboratories. c. Autoclave bags containing biohazardous waste should be taped shut prior to transport. d. Cap cuvettes containing microbial suspensions prior to removal from the BSC. Use a cuvette rack for transporting capped cuvettes within the laboratory. For transporting microbial suspensions in capped

	<p>cuvettes between laboratories, place the cuvette rack in secondary containment.</p>
<p>12.8 Managing Biohazardous Waste</p>	<ul style="list-style-type: none"> a. After manipulating culture, analysts must bag biohazardous waste and place it in a closed container (e.g., biohazard bin with lid, biohazard bag taped shut). b. Storage of items awaiting sterilization <ul style="list-style-type: none"> 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 beaker 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 in order to prevent the generation and release of infectious aerosols into the laboratory environment. v. All test tubes/flasks containing liquid waste (including used micropipette tips) must be capped or covered with aluminum foil. vi. Tape full autoclave bags closed. 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. If using a waste bin or beaker to collect pipettes, place about an inch of liquid disinfectant or bleach in the container prior to collecting the pipettes. Transport the waste container to the autoclave for sterilization and discard autoclaved pipettes with other biohazardous waste. viii. Biohazardous waste should be autoclaved as soon as possible (i.e., within one day). c. Preparation of Autoclave Bags <ul style="list-style-type: none"> i. Place one bag in an autoclave bin. ii. If an autoclave bag contains only dry material, open the bag and pour approximately 250 mL of water into the bag and 250

	<p>mL of water into the bin.</p> <p>d. Preparation of containers of liquid waste and small items.</p> <p>i. To prepare containers of liquid waste and materials such as contaminated micropipette tips, homogenizers, racks, cuvettes, and glassware for autoclaving, place the items in an autoclavable bin.</p> <p>ii. Use a three hour (180 minute) liquid cycle to sterilize both liquid and solid biohazardous waste.</p>
<p>12.9 Disinfection of Laboratory Equipment</p>	<p>a. Chillers</p> <p>i. On a weekly basis, when in use, disinfect the water in the recirculating chiller and remote water bath prior to draining.</p> <p>ii. Disinfect the water in the chiller by adding the appropriate amount (i.e., to achieve the product's use dilution in the chiller water) of a disinfectant labeled for use against the test organisms to the recirculating chiller and remote water bath. Follow label directions for use. Record information on the Recirculating Chiller Cleaning and Disinfection Log (see Section 14).</p> <p>iii. During use, the recirculating chiller/remote water bath system circulates approximately 23.5 L of water (20.5 L in chiller reservoir + 3 L in remote water bath). Use this value as the volume of diluent in calculations to determine the amount of disinfectant to add to the chiller water.</p> <p>iv. After the disinfectant is added, turn on the unit and run it thoroughly to circulate the disinfectant throughout the chiller, tubing, and remote water bath. Turn off the unit and allow the disinfectant to remain in the unit for the specified contact time (e.g., 10 minutes).</p> <p>v. Each recirculating chiller is equipped with a drain valve and a drain hose located on the back of the unit. With the unit off, open the valve and allow the reservoir contents to drain into an appropriately sized container or directly into a sink.</p> <p>vi. Rinse the unit with tap water and drain as above. Refill the reservoir with fresh tap water on the day of testing.</p> <p>b. Sonicator</p> <p>i. Disinfect the tap water in the sonicator bath at the end of the testing week by adding appropriate disinfectant to the tap</p>

	<p>water in the bath to achieve the disinfectant product's use dilution. Let the disinfectant remain in the sonicator bath for the contact time stated on the disinfectant labeling. Once the contact time is achieved, discharge the treated water appropriately, rinse the unit with tap water, and dry the sonicator bath with paper towels. Record cleaning and disinfection of sonicator on the Sonicator Disinfection Log (see Section 14).</p> <p>c. Spectrophotometer</p> <p>i. Periodically and prior to shipping the unit out for calibration, remove the cell holder from the instrument and disinfect it with an appropriate disinfectant. Allow the surface to remain wet for the label-specified contact time. Thoroughly rinse with tap water, allow to dry, and replace cell holder in the spectrophotometer.</p> <p>ii. Do not spray or wipe disinfectant on the inner surfaces of the spectrophotometer because disinfectant residue may remain on the optics, negatively impacting instrument operation.</p>
<p>12.10 Resource Management</p>	<p>a. Laboratory personnel should be mindful of water consumption, and whenever possible, employ practices that minimize water use.</p> <p>b. Laboratory personnel should run full autoclave loads whenever possible.</p>
<p>13. Data Analysis/ Calculations</p>	<p>None</p>
<p>14. Forms and Data Sheets</p>	<p>1. Data Sheets and Attachments are stored separately from the SOP under the following file names:</p> <p>Attachment 1: Microorganisms Used by the OPP Microbiology Laboratory Recirculating Chiller Cleaning and Disinfection Log. MB-01-08_F1.docx Sonicator Disinfection Log MB-01-08_F2.docx</p>
<p>15. References</p>	<p>1. Fleming, D.O. and Hunt, D.L. eds. 2000. Biological Safety: Principles and Practices. ASM Press, Washington, D.C.</p> <p>2. Centers for Disease Control and Prevention and National Institutes of Health, 2009. Biosafety in Microbiological and Biomedical Laboratories, 5th edition. U.S. Department of Health and Human Services. U.S. Government Printing Office, Washington, D.C.</p>