



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**

SOP Number: MB-01-05

Date Revised: 10-14-08

EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

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for
Biosafety in the Laboratory

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1.0 SCOPE AND APPLICATION:

- 1.1 This protocol outlines the required safety measures for working with the microorganisms received and maintained by the OPP Microbiology Laboratory.
- 1.2 The scope of 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 *Bacillus anthracis* (2007).
- 1.3 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,” 5th ed. (BMBL; see ref. 15.2).
 - 1.3.1 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 majority of the microorganisms contained in-house fall within Biosafety Level 2.
 - 1.3.2 This SOP is structured so all work involving manipulation of culture (section 2.4) 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.
 - 1.3.3 This SOP provides additional practices and procedures to be followed when working with Biosafety Level 3 microorganisms in order to provide analysts added protection from disease.
 - 1.3.4 Recommendations set forth in Section D (Laboratory Facilities) of the BMBL’s Biosafety Level 3 criteria apply only to Biosafety Level 3 microorganisms.
 - 1.3.4.1 Manipulation of Biosafety Level 1 and 2 microorganisms may occur in either a Biosafety Level 2 (B201, B203, B204, B205, B206, or D122) or a Biosafety Level 3 laboratory (B202, B207).
 - 1.3.4.2 Manipulation of Biosafety Level 3 microorganisms may only occur in a Biosafety Level 3 laboratory (B202, B207).

- 1.4 Prior to manipulating (section 2.4) cultures of a microorganism other than those listed in Attachment A, the laboratory staff must first determine the biosafety level of that microorganism (see Attachment A).
- 1.5 The BMBL (see ref. 15.2) manual presents recommended guidelines for working with microorganisms assigned to Biosafety Levels 1 through 4. 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.

2.0 DEFINITIONS:

- 2.1 Microorganism = includes bacteria in vegetative and spore form, bacteria in biofilms, and viruses.
- 2.2 *Mycobacterium bovis* (BCG) is a live attenuated vaccine strain organism.
- 2.3 BSC = biological safety cabinet
- 2.4 Manipulation of culture = handling of open vessels containing microorganism. Activities involving manipulation of culture must be performed in the BSC. The following are examples (not an exhaustive list) of activities involving manipulation of culture: culture transfers, inoculum or virus harvest, plating, inoculation of carriers, sonication of inoculated carriers, recording results from tubes and plates.
- 2.5 PPE = Personal Protective Equipment
- 2.6 CDC = Centers for Disease Control and Prevention
- 2.7 NIH = National Institutes of Health
- 2.8 “Appropriate” disinfectant = EPA-registered hospital disinfectant (have label claims for *S. aureus*, *P. aeruginosa*, and *S. choleraesuis*) or hospital disinfectant with virucidal or tuberculocidal claims (efficacious against *M. bovis* (BCG)). All disinfectants must be used according to the directions (e.g., use dilution, contact time, etc.) specified on the labeling.

- 2.9 ATCC = American Type Culture Collection
 - 2.10 OEP = Occupant Emergency Plan
 - 2.11 CHP = Chemical Hygiene Plan
 - 2.12 P100 HEPA = Oil proof High-Efficiency Particulate Air Filter, 99.97% efficient in removing particles 0.3 microns or larger
 - 2.13 References to water mean reagent-grade water
- 3.0 HEALTH AND SAFETY:
- 3.1 To protect the laboratory worker from possible infection by microorganisms, the health and safety guidelines provided in this protocol and in the BMBL (see ref. 15.2) manual must be followed. All laboratory personnel are required to read and familiarize themselves with this protocol and sections on Biosafety Levels 2 and 3 in the BMBL.
 - 3.2 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.
 - 3.3 Laboratory workers are required to participate in the Agency's Occupational Medical Surveillance Program as established by EPA Order 1460.1. The Branch Chief evaluates the duties and responsibilities of the team and identifies the employees that are subject to exposure to chemical and biological agents in the laboratory. The names are forwarded to the ESC Safety, Health and Environmental Management (SHEM) manager who has responsibility for coordinating the medical monitoring program. The program is administered through the Department of Health and Human Services/U.S. Public Health Service.
 - 3.4 Medical emergencies are handled according to procedures outlined in the ESC Occupant Emergency Plan (OEP). All emergencies are reported to the Branch Chief and SHEM manager. In the event that analysts are unable to reach the SHEM manager, they are to call security at extension 2800 and report the emergency. Security will locate the SHEM manager or his designee. The Branch Chief is responsible for documenting medical emergencies or accidents.

- 3.5 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. All spills and accidents are reported to the Branch Chief and the SHEM manager. The Branch Chief is responsible for documenting spills and accidents.
 - 3.6 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.
 - 3.7 All laboratory workers must meet the requirements of the Hazard Communication Program's Employee Training Program, as described in the CHP.
 - 3.8 In accordance with the CDC/NIH guidelines (see ref. 15.2), the Branch Chief may restrict access to the laboratory as specified under "special practices".
 - 3.9 All employees required to use respirators are participants in the Agency Occupational Medical Surveillance Program and the ESC Respiratory Protection Program. They have been medically cleared, fit tested for the specific respirator, and attended initial respirator use training. They receive annual respirator fit testing, annual use training, have documentation of training placed in the training file, and ensure that their respirators are inspected before and after each use, or at least monthly, (see 16.1) as specified in the CHP.
- 4.0 CAUTIONS:
- 4.1 Lack of following or understanding of this SOP may negatively impact the quality of the microbiological practices used in the laboratory and, hence, the laboratory's mission.
 - 4.2 Failure to use the "STOP/DO NOT ENTER" signs to control access to the laboratory while cultures are being manipulated (section 2.4) may result in the inadvertent exposure of personnel to biohazardous microorganisms.
 - 4.3 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.
 - 4.4 Autoclaving flasks containing diluted bleach may result in pitting of the autoclave's stainless steel interior.

4.5 Autoclaving flammable liquids (e.g., alcohols) is an explosion hazard. See 10.9 for guidance on autoclaving stain rinsate. Stain rinsate, although it contains low levels of ethanol and isopropanol, is sufficiently diluted in water (>20% water) and is safe to autoclave (David Knower/Steris Corporation, personal communication to M. Cottrill, 5/23/03).

5.0 INTERFERENCES:

5.1 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.

5.2 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, Use and Maintenance of Biological Safety Cabinets.

5.3 Proper certification of the BSCs must be maintained by Facilities management.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable of the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

6.2 Each new analyst will complete twenty-four hours of initial safety training before entering the laboratory to work. All analysts will complete safety re-certification training (eight hours of training) on at least an annual basis. The facility SHEM manager is responsible for coordinating the training program.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Biological Safety Cabinet (BSC)

7.2 Autoclave

7.3 Biohazard bags (clear in color, autoclavable) or containers inside and outside of the biological safety cabinets for collection and storage of biohazardous waste.

7.4 When specified, personal protective equipment (PPE) such as gloves, safety glasses, lab coats, disposable laboratory garments, booties, and respiratory protection (respirators with P100 HEPA filter cartridges).

- 7.5 Appropriate signs to identify biohazardous materials and to limit access to laboratories.
- 7.6 Appropriate disinfectants for microorganisms listed in Attachment A.
- 7.6.1 For microorganisms in vegetative form, use a 1:10 bleach solution (1 part EPA-registered sodium hypochlorite product, 9 parts water) or EPA- registered hospital disinfectant/tuberculocide/virucide.
- 7.6.2 For microorganisms in spore form, prepare 1:10 diluted bleach solution **at neutral pH** (1 part EPA-registered sodium hypochlorite product, 8.4 parts water, and 0.6 parts 5% white vinegar or 5% lab grade acetic acid).
- 7.7 Key card readers are used to limit access to testing laboratories. Only authorized personnel are permitted to enter.
- 7.8 Microorganisms maintained by the laboratory are specified in Attachment A.
- 7.9 Secondary containment (e.g., durable, autoclavable trays and bins; containment cart).
- 8.0 INSTRUMENT OR METHOD CALIBRATION:
- 8.1 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 QC-06, Use and Maintenance of Biological Safety Cabinets, for additional information.
- 9.0 SAMPLE HANDLING AND STORAGE: Not applicable.
- 10.0 PROCEDURE AND ANALYSIS:
- Organization of Section 10.0
(Information for quick finder)
- 10.1: General Laboratory Practices
 - 10.2: Access to Laboratories and Placement of Signage
 - 10.3: Checking Airflow to Laboratories Equipped with Monitoring Devices
 - 10.4: Using the BSC
 - 10.5: Personal Protective Equipment (PPE) Requirements
 - Safety glasses

- Lab coats
- Gloves
- Shoe covers
- Face protection
- Respiratory protection
- 10.6: Disinfection of Laboratory Equipment
- 10.7: Conducting Staining of Microorganisms
- 10.8: Transport of Cultures
- 10.9: Managing Biohazardous Waste
- 10.10: Resource Management

10.1 General Laboratory Practices

- 10.1.1 Eating, drinking, smoking, handling contact lenses, and applying cosmetics (including lip balm) are not permitted in the laboratory.
- 10.1.2 Wash hands prior to leaving the laboratory.
- 10.1.3 Mouth pipetting is prohibited. Only mechanical pipetting devices will be used.
- 10.1.4 Organism will be manipulated inside a BSC to minimize risk of exposure and risk of contamination of lab surfaces.
- 10.1.5 All procedures are performed carefully to minimize the creation of aerosols.
- 10.1.6 Animals and plants not related to work being conducted are not permitted in the laboratory.
- 10.1.7 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.
- 10.1.8 Vacuum lines are protected with disinfectant traps and HEPA filters.

10.2 Access to Laboratories and Placement of Signage

- 10.2.1 Key readers are used to limit access to testing laboratories. Only authorized personnel (e.g., laboratory staff, maintenance staff, etc.) are permitted to enter.

- 10.2.2 Further limit access to the laboratory when manipulating infectious microorganisms by posting the magnetic “STOP/DO NOT ENTER” sign on the outside (i.e., side facing corridor) of the external laboratory door.
- 10.2.3 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.
- 10.2.4 Remove the “STOP/DO NOT ENTER” sign once work is complete.

10.3 Checking Airflow to Laboratories Equipped with Monitoring Devices.

- 10.3.1 Prior to entering laboratories equipped with airflow monitoring devices, visually verify that the laboratories are under negative pressure (airflow is into the room from the corridor).
- 10.3.2 Airflow monitors are located above the laboratory doors. The laboratories have two monitors: 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.
- 10.3.3 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 (x4357).
- 10.3.4 In the event that airflow becomes positive during work, cease work immediately. If working inside a BSC, back out and pull down the sash. Report the problem to the Branch Chief and facilities hotline (x4357). Work should only proceed when proper airflow has been restored.

10.4 Using the BSC.

- 10.4.1 If ultraviolet light was left on overnight for decontamination purposes, turn it off.

- 10.4.2 Turn on the blowers, lights, and outlets, and allow to operate for a minimum of 15 minutes prior to use.
 - 10.4.3 Record the Downflow (FPM) and Exhaust (CPM) rates on the BSC Monitoring Record Form immediately prior to use (See SOP QC-06, Use and Maintenance of Biological Safety Cabinets).
 - 10.4.4 BSC surfaces must be disinfected prior to and after working with infectious material and immediately after any spill of infectious material. 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 the label-specified contact time. Halogenated materials are not recommended for routine use on stainless steel surfaces of the BSC.
 - 10.4.5 As an additional step, at the conclusion of activities involving bacteria in spore form (e.g., *Bacillus subtilis* spore suspensions, spore strips), turn on the ultraviolet light and leave it on overnight. Visually verify that UV bulb is clean. If not, wipe with alcohol and record in BSC Monitoring Record Book.
 - 10.4.6 See SOP QC-06, Use and Maintenance of Biological Safety Cabinets, for more details.
 - 10.4.7 In the event that the BSC alarms during work, cease work immediately, pull down the sash, and call the facilities hotline (x4357) to report the problem.
- 10.5 PPE Requirements.
- 10.5.1 Safety glasses. Safety glasses must be worn while working in the laboratory. Safety glasses do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.4) or other laboratory work is in progress.* However, safety glasses should be immediately available in the work area.
 - 10.5.2 Lab coats.
 - 10.5.2.1 Lab coats do not have to be worn while doing paperwork in the laboratory or when entering the laboratory solely to

retrieve an item such as a document, *provided that no manipulation of cultures (see section 2.4) or other laboratory work is in progress.*

10.5.2.2 For Biosafety Levels 1 and 2: Wear cloth or disposable lab coats.

10.5.2.2.1 Remove lab coats before going to non-laboratory areas such as the office areas, restrooms, cafeteria, library, etc.

10.5.2.3 For Biosafety Level 3: Wear disposable lab coats.

10.5.2.3.1 Cloth lab coats may not be worn in the Biosafety Level 3 laboratory during manipulation of Biosafety Level 3 microorganisms.

10.5.2.3.2 Replace disposable lab coat immediately in the event of suspected or known contamination with infectious material. Dispose of contaminated lab coat in a biohazard bin.

10.5.2.3.3 All disposable lab coats worn in the laboratory when cultures are being manipulated may **not** be worn out of the Biosafety Level 3 laboratory. This includes lab coats worn by personnel who are present in the laboratory but not directly involved in culture manipulation. Prior to exiting the laboratory and entering the double door access zone, remove and discard lab coats (in biohazard bin) according to the descriptions of activities provided in sections 10.5.2.3.4 and 10.5.2.3.5.

10.5.2.3.4 Disposable lab coats worn while harvesting or homogenizing cultures of a Biosafety Level 3 microorganism must be discarded in a biohazard bin before the analyst leaves the laboratory and enters the double-door access

zone. The lab coat may not be stored in the laboratory for re-use later in the day.

- 10.5.2.3.5 Disposable lab coats worn for purposes other than harvesting or homogenizing cultures of a Biosafety Level 3 microorganism during the day may be stored in a designated area in the laboratory in the event that the analyst leaves the laboratory (e.g., to retrieve an item or take a break). Upon return, the analyst may re-use the lab coat to resume laboratory activities. At the end of each day, all used disposable lab coats must be disposed of in the biohazard bin.

10.5.3 Gloves.

- 10.5.3.1 Wear gloves (latex or nitrile when manipulating culture (section 2.4) and when handling any vessel (e.g., tube, rack, plate, biohazard bag), closed or open, containing live organism.
- 10.5.3.2 Prior to beginning work, inspect gloves. Do not use gloves that have holes, rips, or are otherwise degraded.
- 10.5.3.3 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.
- 10.5.3.4 Under certain circumstances (e.g., testing of towelette products), to minimize risk of contamination while working in the BSC, analysts may opt to periodically apply a solution of 70% ethanol over the exterior surface of the gloves, change gloves frequently, or use sterile gloves.
- 10.5.3.5 Additional guidance for Biosafety Level 3 work: Wear a single pair of gloves (latex or nitrile) when manipulating culture (see section 2.4). Prior to leaving the BSC to conduct other activities (e.g., open the incubator, record data, retrieve supplies, etc.), discard the gloves in the

biohazard bin. Put on a new pair of gloves upon returning to the BSC or before handling any vessel containing live organism.

OR

Wear a double layer of gloves (latex or nitrile) when manipulating culture (see section 2.4). Prior to leaving the BSC to conduct other activities (e.g., open the incubator, record data, retrieve supplies, etc.), the outer pair of gloves must be discarded in the biohazard bin. Replace the outer gloves upon returning to the BSC.

10.5.4 Shoe covers/booties.

10.5.4.1 Biosafety Levels 1 and 2: Not required.

10.5.4.2 Biosafety Level 3: Wear shoe covers or tyvek booties when manipulating culture in the Biosafety Level 3 laboratory (section 2.4).

10.5.4.2.1 Remove shoe covers or booties prior to exiting the laboratory and discard in the biohazard bin. Do not wear shoe covers or booties into the double door access room.

10.5.5 Face protection.

10.5.5.1 Biosafety Levels 1 and 2: If a certain procedure involving manipulation of the organism is impossible or impractical to conduct within the BSC, face protection (e.g., a face splash shield) must be used for protection from anticipated or unanticipated splashes or sprays of infectious materials to the face.

10.5.6 Respiratory protection.

10.5.6.1 Biosafety Levels 1 and 2: Respirators are not required for manipulations of Biosafety 1 and 2 microorganisms.

10.5.6.2 Biosafety Level 3: If a certain procedure involving manipulation of the organism is impossible or impractical

to conduct within the BSC (e.g., reading the percent transmittance or optical density of a culture using an uncapped cuvette), respiratory protection (respirators with P100 HEPA filter cartridges) must be worn while working with the organism outside of the BSC. Once work is complete, remove filter cartridges from respirator and place in a biohazardous waste bag. Respirators may be washed with mild soap and water if desired. Allow to dry and return to storage.

10.5.6.2.1 Transporting closed petri dishes (containing seeded carriers) to the incubator for drying, counting colonies on plates which are closed and wrapped with parafilm, and using a capped cuvette to transport culture to the spectrophotometer and read percent transmittance or optical density are not considered to be aerosol-producing manipulations of culture. Wearing respiratory protection is not mandatory for these activities.

10.5.6.2.2 To reduce the volume of organism during a potential spill, use semimicro cuvettes (4 mm inside width that limits capacity to 1.5 mL) with 1.0 mL sample volume. Place the capped cuvettes in a cuvette rack for transport to the spectrophotometer.

10.6 Disinfection of Laboratory Equipment

10.6.1 Chillers. On a weekly basis, following testing, disinfect the water (chillers are filled with tap water) in the recirculating chiller and remote water bath prior to draining.

10.6.1.1 Disinfect the tap 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 16.0).

- 10.6.1.2 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.
 - 10.6.1.3 After the disinfectant is added, the unit should be turned on and run thoroughly. Turn off the unit and allow the disinfectant to remain in the unit for the specified contact time (e.g., 10 minutes).
 - 10.6.1.4 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.
 - 10.6.1.5 Rinse the unit by filling it with tap water. Allow it to run for approximately 10 minutes. Turn off the unit and drain as above. Refill the reservoir with fresh tap water on the day of testing.
- 10.6.2 Sonicator. If the sonicator has been used in a given week, disinfect the tap water in the sonicator bath, at the end of the testing week, by adding appropriate disinfectant to the tap 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.
- 10.6.3 Spectrophotometer. After each use of the spectrophotometer, 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.
- 10.6.3.1 Disinfectant is not to be sprayed or wiped on the inner surfaces of the spectrophotometer as disinfectant residue may remain on the optics, negatively impacting instrument operation.

10.6.4 Administrative supplies (Biosafety Level 3). Ink pens and clip boards used to record data while manipulating cultures will be identified for use in the Biosafety Level 3 laboratories. These items are not to leave the BSL 3 labs.

10.6.4.1 Discard all old or broken items in a biohazard bag.

10.7 Conducting Staining of Microorganisms

10.7.1 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.

10.7.2 Decontamination of rinsate.

10.7.2.1 Collect the rinsate and add EPA-registered sodium hypochlorite product full strength to the rinsate in an approximate 1:10 ratio (one part household bleach to nine parts rinsate) (see ref. 15.1, pg. 390) for a minimum of 60 minutes (in the event that the rinsate contains spores) before disposal. Or,

10.7.2.2 Collect rinsate and autoclave as specified in section 10.9, Managing Biohazardous Waste.

10.7.3 After microscopically viewing organisms, remove slides from the microscope stage and discard them in a biohazard bin. If it is necessary or desirable 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.

10.8 Transport of Cultures.

10.8.1 Intra-laboratory transport of liquid cultures. Use of secondary containment is recommended. To reduce potential for generating large spills (i.e., spill that cannot be handled safely by employees in the laboratory, see SOP MB-13), use secondary containment for transport of large quantities (e.g., ≥ 500 mL) across the laboratory.

10.8.2 Inter-laboratory transport. When removing live cultures (e.g., agar plates, racks of tubes, biohazard bags containing biohazardous waste)

from the immediate laboratory for incubation in other laboratories or for decontamination purposes, place the cultures in secondary containment and use a cart to transport from the laboratory.

- 10.8.3 Biosafety Level 3 work: Remove and discard disposable lab coat and gloves (latex or nitrile), and shoe covers/booties prior to exiting the Biosafety Level 3 laboratory and replace them with a clean disposable coat and a new set of gloves (latex or nitrile) to be worn during the transport process.
- 10.8.4 Autoclave bags containing biohazardous waste should be taped shut prior to transport.
- 10.8.5 Do not transport live cultures outside of the microbiology laboratory wing.

10.9 Managing Biohazardous Waste

- 10.9.1 After manipulating culture (see section 2.4), analysts must bag biohazardous waste and place it in a closed container (e.g., biohazard bin with lid, biohazard bag taped shut).
- 10.9.2 Storage of items awaiting sterilization.
 - 10.9.2.1 No biohazardous waste may be removed from the second floor B-wing prior to sterilization.
 - 10.9.2.2 Use autoclave bins. Some procedures result in the constant generation of contaminated articles that must be removed from the BSC work area and stock-piled on a countertop or table for autoclaving at the completion of the procedure or the next morning. In this case, place all contaminated articles in autoclavable bins. **Place full bins in the autoclave to await sterilization.**
 - 10.9.2.3 Small items. Place contaminated cuvettes, homogenizers, and other small equipment into a beaker covered with aluminum foil prior to placing the items in the autoclavable bin.
 - 10.9.2.4 Close containers. 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.

- 10.9.2.5 Cover liquid waste with foil. All test tubes/flasks containing liquid waste (including used micropipette tips) must be capped or covered with aluminum foil.
- 10.9.2.6 Autoclave bags. Tape full autoclave bags closed.
- 10.9.2.7 Pipettes. 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.
- 10.9.2.8 Biohazardous waste should be processed as soon as possible and not be allowed to sit around for days at a time.
- 10.9.3 Preparation of autoclave bags.
 - 10.9.3.1 To prepare autoclave bags of biohazardous waste for sterilization, place one bag in an autoclave bin.
 - 10.9.3.2 Open the bag and pour approximately 250 mL of water into the bag and 250 mL of water into the bin.
 - 10.9.3.3 Make sure that the bag is opened wide prior to placing the bin into the autoclave.
- 10.9.4 Preparation of containers of liquid waste and small items.
 - 10.9.4.1 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.
 - 10.9.4.2 Add approximately 250 mL of water to the bin.

10.9.4.3 Place the bin into the autoclave.

10.9.4.4 No liquid waste containing bleach (will damage stainless steel interior of autoclave) or flammable liquid (explosion hazard) is to be autoclaved. Up to a liter of stain rinsate may be safely autoclaved. Rinsate contains low levels of isopropanol and ethanol. However, these flammable components are sufficiently diluted in water (>20% water), rendering the rinsate safe for autoclaving (David Knower/Steris Corporation, personal communication to M. Cottrill, 5/23/03).

10.9.5 Use a **three hour** (180 minute) **liquid cycle** to sterilize both liquid and solid biohazardous waste.

10.9.6 See QC-13, Performance Verification of Autoclaves, for verification of autoclave performance and corrective actions.

10.10 Resource Management.

10.10.1 Water conservation. Laboratory personnel should be mindful of water consumption, and whenever possible, employ practices that minimize water use.

10.10.2 Specifically, laboratory personnel should run full autoclave loads whenever possible.

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Results of the monthly respirator check will be recorded legibly and in indelible ink on the Respirator Inspection Checklist form (see 16.0). The forms will be kept in the Respirator Inspection Notebook.

12.2 The Respirator Inspection Notebook is kept in laboratory space rather than the file room, D217. In addition to monthly inspections, respirators must be inspected prior to each use. Therefore, it is crucial that the Respirator Inspection Notebook be readily available rather than retained in a file room. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and

Archives. Copies of the Respirator Inspection Checklist forms are presented to the SHEM manager during annual respirator fit-testing.

- 12.3 Data will be recorded promptly, legibly, and in indelible ink on the BSC Monitoring Record Form (see SOP QC-06, Use and Maintenance of Biological Safety Cabinets) and the Sonicator Disinfection Log. Completed forms are archived in notebooks kept in secure file cabinets in file room D217. Only authorized personnel have access to the secure files. Archived data are subject to OPP's official retention schedule contained in SOP ADM-03 (Records and Archives).
- 12.4 The Branch Chief is responsible for documenting medical emergencies, accidents, and spills.
- 13.0 QUALITY CONTROL: None
- 14.0 NONCONFORMANCE AND CORRECTIVE ACTION:
 - 14.1 Strict adherence to the biosafety practices is required. Nonconformance will result in notification, retraining, or possible disciplinary action of laboratory employees.
- 15.0 REFERENCES:
 - 15.1 Fleming, D.O. and Hunt, D.L. eds. 2000. Biological Safety: Principles and Practices. ASM Press, Washington, D.C.
 - 15.2 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.
- 16.0 FORMS AND DATA SHEETS:
 - 16.1 Respirator Inspection Checklist.
 - 16.2 Recirculating Chiller Cleaning and Disinfection Log.
 - 16.3 Sonicator Disinfection Log.

Attachment A: Microorganisms Maintained by the OPP Microbiology Laboratory.

Respirator Inspection Checklist

OPP Microbiology Laboratory

Respirators must be inspected before and after each use, and monthly. A respirator kept ready for emergency use must be inspected before and after each use and monthly to assure that it is maintained in satisfactory condition. Use this sheet to document the inspections.

| | |
|--------------------|--|
| Name | |
| Type of Respirator | |
| Storage Location | |

| | | | | | | | | | | |
|-------------|--|--|--|--|--|--|--|--|--|--|
| Date | | | | | | | | | | |
| Initials | | | | | | | | | | |
| Facepiece | | | | | | | | | | |
| Connections | | | | | | | | | | |
| Headbands | | | | | | | | | | |
| Valves | | | | | | | | | | |
| Cartridges | | | | | | | | | | |
| Other | | | | | | | | | | |
| Comments | | | | | | | | | | |

Safety Office Review: _____

Date: _____

Attachment A:

Microorganisms Maintained by the OPP Microbiology Laboratory

| Organism | ATCC # | Biosafety Level |
|--|----------------|-----------------|
| <i>Mycobacterium bovis</i> (BCG)* | Not applicable | 3 |
| <i>Pseudomonas aeruginosa</i> * | 15442 | 2 |
| <i>Pseudomonas aeruginosa</i> *** | 700888 | 2 |
| <i>Staphylococcus aureus</i> * | 6538 | 2 |
| <i>Salmonella enterica</i> * | 10708 | 2 |
| <i>Bacillus subtilis</i> * | 19659 | 1 |
| <i>Streptococcus equi</i> subsp. <i>Zooepidemicus</i> ** | 43079 | 2 |
| <i>Enterococcus casseliflavus</i> ** | 700327 | 1 |
| <i>Kocuria kristinae</i> ** | BAA-752 | 1 |
| <i>Listeria monocytogenes</i> ** | BAA-751 | 2 |
| <i>Staphylococcus aureus</i> subsp. <i>aureus</i> ** | 29213 | 2 |
| <i>Staphylococcus saprophyticus</i> ** | BAA-750 | 1 |
| <i>Staphylococcus sciuri</i> subsp. <i>sciuri</i> ** | 29061 | 1 |
| <i>Streptococcus thermophilus</i> ** | 19258 | 1 |
| <i>Proteus vulgaris</i> ** | 6380 | 2 |
| <i>Shigella sonnei</i> ** | 25931 | 2 |
| <i>Stenotrophomonas maltophilia</i> ** | 17666 | 1 |
| <i>Klebsiella oxytoca</i> ** | 700324 | 2 |
| <i>Acinetobacter baumannii</i> ** | BAA-747 | 2 |
| <i>Enterobacter cloacae</i> ** | 700323 | 1 |
| <i>Ochrobactrum anthropi</i> ** | BAA-749 | 2 |
| <i>Aneurinibacillus aneurinoliticus</i> ** | 11376 | 1 |
| <i>Bacillus circulans</i> ** | 61 | 1 |
| <i>Brevibacillus agri</i> ** | 51663 | 1 |
| <i>Brevibacillus laterosporus</i> ** | 64 | 1 |
| <i>Geobacillus stearothermophilus</i> ** | 12978 | 1 |
| <i>Paenibacillus macerans</i> ** | 8509 | 1 |
| <i>Paenibacillus polymyxa</i> ** | 7070 | 1 |
| <i>Virgibacillus pantothenicus</i> ** | 14576 | 1 |
| <i>Bacillus licheniformis</i> ** | 12759 | 1 |
| <i>Clostridium sporogenes</i> * | 3584 | 1 |
| <i>Clostridium difficile</i> *** | 700057 | 2 |
| <i>Mycobacterium terrae</i> *** | 15755 | 1 |
| Feline Calicivirus*** | VR2057 | 2 |

* Used as a Test Microbe in the Antimicrobial Testing Program
 ** Used in Quality Control of the VITEK 2 Compact
 *** For use in research