PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Microbiology Biohazards and Safety

Department of Microbiology

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Table of Contents

1.0 Introduction

Laboratory workers are at high risk for occupational exposure to infectious agents. Infections can be acquired from exposure to contaminated blood, tissue, and other material. The greatest risks for are associated with the processing of specimens and the manipulation of pathogens isolated from these materials. The actual incidence of laboratory of laboratory-acquired infections is probably higher than recognized due to subclinical symptoms and poor compliance in reporting.

Laboratory practices that can reduce the risk of infection include standard precautions, the use of personal protective equipment (PPE), biological safety cabinet (BSC), safety devices, and proper decontamination and disposal of biohazardous material. Standard precautions recognize that all patient specimens should be handled as if they are infectious and capable of transmitting disease. This applies to all body fluids, secretions, excretions, and tissue specimens, regardless of whether they contain visible blood, as well as QC strains and proficiency test samples. All laboratory personnel must follow standard precautions guidelines.

2.0 Routes of Infections and Laboratory Activities

Many different categories of microbiological biohazards are encountered from the time a specimen is collected until it is disposed of permanently. The management of risks associated with working with pathogens is accomplished by the development of standard procedures and practices for handling infectious material and will prevent microbial transmission.

2.1 Inhalation: Activities that Generate Aerosols

- Manipulating needles and syringe – expelling air, withdrawing needles from stoppers, separating needles from syringes
- Manipulating inoculation needles or loops – flaming loops, cooling loops in culture media, subculturing and streaking culture media
- Manipulating pipettes – mixing microbial suspensions, spilling microbial suspensions on hard surfaces

- Manipulating specimens and cultures – centrifugation, mixing, grinding, shaking, and vortexing of specimens or cultures, pouring or decanting fluids, removing caps or swabs from culture containers

2.2 Ingestion: Activities Associated with Oral Transmission

- Pipetting by mouth
- Splashing contaminated material into the mouth
- Placing contaminated material or fingers in the mouth
- Eating, drinking, smoking, or applying cosmetics to the lips

2.3 Inoculation: Activities Related to Direct Intravenous & Subcutaneous Transmission

- Manipulating needles and syringes
- Handling broken glass, scalpels, and other sharp objects

2.4 Inoculation: Activities Related to Contaminated Skin & Mucous Membranes

- Splashing or spilling material into eyes, mouth, and nose and onto skin
- Exposing non-intact skin to contaminated material
- Working on contaminated surfaces
- Inappropriate handling of loops, inoculating needles, or swabs containing specimen or culture material
- Handling contaminated equipment
- Touching contaminated fingers in or near the eyes or nose
- Handling and contaminating personal items such as cell phones, handbags, and eye glasses

3.0 Safe Work Practices

3.1 Specimen Receiving & Accessioning

3.1.1 PPE: Gloves & Lab Coats

- Gloves and lab coats must be worn at all times when handling patient specimens, decontaminating instruments and countertops, and cleaning spills.
- Bandage open cuts and scratches on hands and then wear gloves.
- Laboratory coats must be worn during work and removed when leaving the lab. In event of laboratory accident, the coat must be changed. Lab coats should be routinely changed with a new coat at least once per week.

3.1.2 Specimen Transport

- Do not accept grossly soiled or contaminated specimens. Contact the floor for inpatients or create a CRM case for outpatients to notify the client responsible for submitting such a specimen and follow the laboratory's specimen rejection policy.
- Specimens being sent out from the Microbiology lab should be placed in a plastic bag with a biohazard symbol. Isolates should be designated as category A or B for shipping purposes.

3.1.3 Needles & Syringes

- Do not accept specimens received in syringes with needles attached. Contact the floor for inpatients or create a CRM case for outpatients to notify the client responsible for submitting such a specimen and follow the laboratory's specimen rejection policy.

3.1.4 Hand washing

- Perform frequent hand washing after removing gloves, before leaving the laboratory, and before eating, drinking, or applying cosmetics.
- Use nonirritating soap for routine washing.

3.2 Processing Specimens

3.2.1 PPE: Gloves, Lab Coats, and Respirators

- Gloves and lab coats must be worn at all times when processing patient specimens.
- Bandage open cuts and scratches on hands and then wear gloves.
- Wash hands immediately after gloves are removed, after a task that involves heavily contaminated matter, and before leaving the laboratory.
- Laboratory coats must be worn during work and removed when leaving the lab. In event of laboratory accident, the coat must be changed. Lab coats should be routinely changed with a new coat at least once per week.
- An N95 respirator must be worn when working in the Mycobacteriology and Mycology biosafety cabinets in the BSL-3 lab.

3.2.2 Biosafety Cabinet (BSC)

- Process all specimens in a BSC. It is critical that all staff understand when and how to work in a BSC (refer to Biosafety Cabinet Operation and Maintenance).
- Do not sweep your arms into or out of the cabinet. Move arms in and out slowly, perpendicular to the face opening.
- Limit air movement in area surrounding BSC. Excessive air movement from opening the specimen receiving windows can compromise safe air flow in the BSC.
- Do not block the front grill where down flow of air is conducted, or the rear grill where air is removed from the cabinet.
- At the beginning and end of the day, with the blower running, disinfect all surfaces with a 1:10 dilution of household bleach, and remove residual bleach with 70% alcohol.
- Do not use open flames inside the cabinet.
- Open sealed rotors or safety cups from high-speed and ultracentrifuges in a BSC.
- Cytocentrifuge devices should be loaded and capped in a BSC. After centrifugation, return the devices to the BSC before opening.
- Collect medical waste generated inside the BSC in bags or sharps containers. Seal these before removal and place in medical waste containers outside the BSC.

3.2.3 Centrifuges

- Centrifuge tubes must be intact and properly balanced when centrifuged.
- Centrifuge tubes used in mycobacteriologic areas must be enclosed in sealed safety cups.
- Centrifuge safety cups must be opened in a BSC after centrifugation.
- A maximum of 1 mL should be used in the cytocentirfuge devices. Overfilling the reservoir may lead to aerosolization and rotor contamination during centrifugation.

3.2.4 Needles & Syringes

- Use mechanical devices or one-handed techniques when handling sharp objects.
- Never bend, break, or recap needles.
- Discard any needles used to perform laboratory testing into a puncture-resistant container designated for biohazard sharp disposal.
- Use blunt needles whenever possible.

3.2.5 Pipetting, Mixing, and Inoculation

- Mix or transfer liquids by using disposable plastic pipettes or mechanical pipetting devices. Pipetting of clinical specimens should be performed in a BSC.
- Use caps or Parafilm to cover tubes when mixing or vortexing.
- Follow standard precautions. This also applies when performing nonculture techniques (e.g., antibody, antigen, and molecular methods).
- Sterilize bacteriological wire needles and loops to avoid spattering of material on heating. Cool needle and loop tips enough to avoid searing the surface of the medium.

3.2.6 Hand washing

- Perform frequent hand washing after removing gloves, before leaving the laboratory, and before eating, drinking, or applying cosmetics.
- Use nonirritating soap for routine washing.

3.3 Handling Cultures & Manipulating Isolates

3.3.1 PPE: Gloves, Lab Coats, and Respirators

- In the general microbiology laboratory, masks and disposable gloves are not required in the open laboratory but may be voluntarily used. If gloves are used, they can easily become contaminated during routine use; therefore, gloves are not to be washed and reused. Discard gloves, and don a new pair when leaving the workstation.
- Gloves should be worn for manipulating AFB and fungus cultures.
- Bandage open cuts and scratches on hands and then wear gloves.
- Wash hands immediately after gloves are removed, after a task that involves heavily contaminated matter, and before leaving the laboratory.
- Laboratory coats must be worn during work and removed when leaving the lab. In event of laboratory accident, the coat must be changed. Lab coats should be routinely changed with a new coat at least once per week.
- An N95 respirator must be worn when working in the Mycobacteriology and Mycology biosafety cabinets in the BSL-3 lab.

3.3.2 Biosafety Cabinet (BSC)

- Any work performed on AFB & fungus cultures must be performed in the BSC located in the Mycobacteriology/Mycology room.
- All organisms submitted for identification should be Gram stained and subcultured inside a BSC. Subsequent subcultures of gram-negative or gram-variable bacilli should be manipulated and tested inside a BSC until high-risk agents, such as *Brucella* species, *Francisella tularensis,* and *Yersinia pestis* have been ruled out.

3.3.3 Centrifuges

- Centrifuge tubes must be intact and properly balanced when centrifuged.
- Centrifuge tubes used for AFB isolates must be enclosed in sealed safety cups.
- Centrifuge safety cups must be opened in a BSC after centrifugation.

3.3.4 Needles & Syringes

- Use blunt needles to access culture bottles.
- Secure culture bottles before inserting needles into the bottles (e.g., place bottles in support racks).

3.3.5 Culture Work-up: Pipetting, Mixing, Sampling, Subculture, Inoculation, etc.

Any procedure that imparts energy to a microbial suspension can produce infectious aerosols. Procedures and equipment frequently associated with aerosol production include pipetting, pouring, mixing or vortexing. These procedures and equipment generate particles that remain airborne for protracted periods. When inhaled, these tiny particles can be retained in the lungs. These procedures and equipment also generate larger droplets that can contain larger quantities of infectious agents. The larger droplets settle out of the air rapidly, contaminating work surfaces as well as the hands and possibly the mucous membranes of persons performing the procedure.

- Gram stain and subculture all positive blood cultures in a BSC.
- As soon as a high-risk agent is suspected, perform all work in a BSC. Bacterial agents that pose the greatest risk for aerosolization include: *Bacillus anthracis, Brucella* species, *Burkholderia pseudomallei, Francisella tularensis, Neisseria meningitidis,* and *Yersinia pestis.*
- Sterilize bacteriological wire needles and loops to avoid spattering of material on heating.

    - Cool needle and loop tips enough to avoid searing the surface of the medium.
    - Do not place a loop full of microorganisms directly into the flame of a Bunsen burner causing it to sear or pop. Instead, slowly heat the loop starting with the wire shaft and gradually work up to the loop.
- Discard contaminated applicator sticks, pipettes, swabs, etc. into biohazard containers on workbenches. The containers should be emptied at the end of each shift or when full.
- Discard used glass slides and coverslips into the disposable sharps containers on the workbenches. When they are full, the containers should be securely closed and placed into the biohazard waste.
- Scotch tape preparations of mold isolates should be mounted on a slide with lactophenol cotton blue stain. A second application of stain should then be placed on the top side of the tape and then a cover slip applied. This reduces the risk of release of fungal spores during microscopy.

3.3.6 Hand washing

- Perform frequent hand washing after removing gloves, before leaving the laboratory, and before eating, drinking, or applying cosmetics.
- Use nonirritating soap for routine washing.

3.4 Housekeeping & Miscellaneous Safe Practices

3.4.1 General

- Observe clean and contaminated work areas.
- Clean and disinfect all surfaces after spills and at the end of each work shift.
- Keep all work areas neat and uncluttered.
- ***Do not*** use or store personal items in the work areas. Personal items can easily become contaminated from being handled while working in the laboratory. Do not lay personal articles, such as handbags, cell phones, or eyeglasses on your work area.
- Long hair should be tied back to prevent contact with specimens and cultures.
- Turn Bunsen burners off when not in use. When in use, keep flame away from combustible materials.
- Dispose of all contaminated material in containers for autoclaving.

3.4.2 Compressed Gasses

- Secure cylinders in an upright position with wall mounts.
- Store cylinders away from open flames and sources of heat.
- Verify the contents of the cylinder before gas is used.
- Transport cylinders in secured hand trucks or carts.

3.4.3 Chemicals

- Wear appropriate PPE when handling hazardous chemicals.
- Label all reagents with their chemical names and appropriate hazards warnings provided from the MSDS information.
- Refer to MSDS located on the intranet for specific hazard information. When new chemicals are brought into the lab, notify the Lab Safety department.
- Store flammable and combustible liquids in fire-rated storage cabinets.
- Hazardous chemicals should not be stored above eye level.
- Place chemical waste in fume hood until final disposal.
- In the event of a chemical spill follow instructions in the laboratory safety manual, under Lab Chemical Hygiene Plan – Spills and Accidents & Chemical Waste Removal/Disposal. Copies of MSDS for all hazardous chemicals to which employees of the laboratory may be exposed can be found electronically in the MAXCOM system on the PSHMC intranet.

3.5 Facilities

- Separate fire safety instructions and building evacuation routes are posted in each laboratory. Each employee has the responsibility of becoming familiar with these instructions.
- Eye-washing fountains are available in all areas of Microbiology and marked with signage. Employees should familiarize themselves with the location of the eyewash stations in their respective work areas. In the event of accidental eye or body contamination with a caustic agent, immediately flush eyes for 15 min. Notify Supervisor and get assistance from the ED.

4.0 Decontamination

Routine decontamination and cleaning of work environment are the responsibility of all workers.

4.1 Preparation of Disinfectant

Prepare a working solution of 10% household bleach fresh each day. Bleach dilution sprayers can be used for on demand disinfectant. Ensure that the water bottle and bleach bottle contain sufficient volume for use. The water bottle may be refilled with tap water. When the bleach bottle is empty, replace the entire bottle with a new, pre-filled bottle.

4.2 Decontamination of Work Surfaces

- Spray disinfectant directly onto work surfaces or soak a paper towel or soft cloth.
- Wipe and distribute disinfectant over the entire work surface.
- Allow bleach solution to air dry (minimum contact time should be 10 min).
- Decontaminate work surfaces at least once per shift, at the completion of a procedure, or when surfaces become overtly contaminated. Decontamination of the Microbiology benches must be documented in the computer once per day.

4.3 Decontamination of Equipment (vortex, centrifuge, telephone, etc.)

- Use a soft cloth moistened with disinfectant to wipe the surfaces of the equipment. Use caution to avoid saturating any instrument parts that may become faulty due to corrosion.
- Remove bleach residue from equipment with a soft cloth moistened with tap water or alcohol.

4.4 Decontamination of Reusable Biohazard Pails & Barrels

- Remove biohazard lining bags from benchtop pails and dispose of the entire bag into a larger biohazard barrel. If the inside of the pail appears soiled, wipe the entire inside surface with 10% bleach.
- All contaminated materials should be placed in special orange biohazard bags, which are used to line the hazardous material barrels. Do not overfill these bags. Carefully secure bags prior to transport to Central Services for autoclaving

5.0 Biohazardous Spills

Management of biological spills in clinical laboratories must account for the specific infectious agent (if known), the volume of infectious material spilled, and the presence of aerosols. Aerosols may readily transmit organisms in spills involving BSL-3 agents.

5.1 BSL Level 3 Lab Spill (possible aerosolization of positive AFB culture or fungal spores)

- Alert other personnel in the lab and evacuate the room, taking care not to breathe in aerosolized material.
- Ensure that the door to the affected area is closed and post a sign so that no other personnel enter.
- Alert laboratory supervisor.
- Do not re-enter the area for 2 h.

- PPE necessary to reenter the room to clean spills includes puncture-resistant gloves for sharps, N95 respirator, fluid impenetrable shoe covers, coats or gowns, and facial protection.
- Remove any broken glass in a spill area, and discard without contact with the hands (use broom, forceps, tongs, etc.).
- Spills should be covered with paper towels and then flooded with 10% bleach (minimum contact time should be 20 min).
- Discard towels in biohazard autoclave bags.
- Decontaminate any residual spill in work area with 10% bleach and allow to air dry.
- Rinse spill site with water and allow site to dry.
- Place all disposable contaminated cleanup material in the biohazard bag and treat as infectious waste.
- Remove gloves and wash hands with soap and water.

5.2 Biohazardous Spill Due to Breakage in a Centrifuge

- When breakage occurs in a centrifuge (which inherently would produce aerosols), keep the centrifuge tightly closed for 30 min before decontamination commences.
- Do not pick up broken glass with gloved hands. Use forceps or cotton held in forceps, or tongs or hemostats, and dispose into a biosafety sharps container.
- Use a soft cloth moistened with disinfectant to wipe the surfaces of the equipment. Use caution to avoid saturating any instrument parts that may become faulty due to corrosion.
- If a specimen tube breaks within the plastic screw-capped canister in a centrifuge:
    - Turn the motor off.
    - Remove the canister immediately and place in a BSC.
    - Notify supervisor and other personnel working in the area.
    - While wearing protective clothing, open the canister under the BSC.
    - Pour a 1:10 dilution of bleach into the canister to decontaminate all surfaces. Let the canister soak in bleach or disinfectant solution for 10 min. Clean canister thoroughly.
    - Do not pick up broken glass with gloved hands. Use forceps or cotton held in forceps, or tongs or hemostats, and dispose into a biosafety sharps container.
    - Discard all non sharp contaminated materials from canister into a red biohazard bag for biohazard waste disposal.
    - Swab unbroken capped tubes with the same disinfectant; then swab again, wash with water and dry.

5.3 Biohazardous Spills within a BSC

- PPE should include gloves, lab coat. Wear facial protection if there is enough contaminated material to splash.
- If a spill occurs in a BSC, do not turn off the cabinet fan. Minor spills in a BSC can be absorbed with paper towels. If infectious material flows into the grille, wipe all items in the cabinet with disinfectant and remove them. Pour disinfectant onto the surface and through the grille into the drain pan. Allow appropriate contact time, and then wipe up disinfectant with paper towels. Remove the primary work surface and clean the area below.
- Remove gloves and wash hands with soap and water.
- Allow the cabinet to run for 10 min before resuming activity.

5.4 Minor Spill Cleanup

- PPE should include gloves and lab coat. Wear facial protection if there is enough contaminated material to splash.
- Spills should be covered with a paper towel and then flooded with 10% bleach (minimum contact time should be 10 min). Discard towels in biohazard waste container.
- Remove gloves and wash hands with soap and water.

6.0 Limitations

- Do not use alcohol solutions near open flames.
- Use caution when cleaning some items since isopropyl alcohol will attack some forms of plastic and rubber.
- Do not use bleach solutions on metal parts. It may cause rusting and pitting. If bleach must be used, do not leave bleach in contact with metal for more than 5 min and then rinse thoroughly with tap water.

7.0 Incident Reports

- In case of injury or unusual incident, notify the person in charge immediately.
- Prepare a spill/incident report, identify cause, and determine remedial action.
- All employees must complete a "Sacred Heart Medical Center Employee Incident Report Form" anytime they have an injury, occupational illness, or exposure to a communicable disease.
- The employee should fill out the Employee Section as completely as possible, explaining detailed description of incident, contributing factors, current symptoms, source patient information if applicable, vaccination and allergy status, use of safer sharps devices and employee's input for preventing reoccurrence.
- Employee Health Service and Epidemiology will follow-up as necessary for treatment and for evaluation of any infectious disease to which the employee may have been exposed. See the Exposure Control Plan for Post-Exposure procedures.

8.0 References

- Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories: Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel.
- Clinical Microbiology Procedures Handbook, 3rd ed. and 2007 update, Vol. 2. Garcia, L.S., editor in chief. ASM Press, Washington, D.C.
- NCCLS M29-A2, Protection of Laboratory Workers from Occupationally-Acquired Infections.

9.0 Document Control

- Medical Director Approval: Reviewed by Dr. Schappert 3/10/2010
- Microbiology Director Approval: Dr. Ann Robinson 4/5/2002, Updates reviewed 1/15/13
- Microbiology Supervisor Approval: Jerry Claridge 4/5/2002
- Reviews by Jerry Claridge: 4/5/2002, 4/4/2003, 3/18/2004, 5/5/2004, 7/28/2005, 6/2006, 11/2006, 11/2007, 11/2008, 11/2009, 3/2011, 4/1/20011
- Revisions & Updates: 10/24/2011 Updated content for new laboratory. Added instruction for pipetting specimens in BSC. 2/22/12 Added weekly lab coat change. 1/14/2013 Updated to PPM format and added information from 2012 CDC Guidelines.