Laboratory Safety: Work Practices for *Mycobacterium tuberculosis*
Agent: *Mycobacterium tuberculosis*

- Infectious dose 1-10 organisms – *No safe level of exposure*
- Airborne droplet nuclei can be spread through normal air currents for long periods of time and spread throughout a room or building
- There is a risk to laboratorians who process specimens in labs
Overview

- Administrative Controls
- Engineering Controls
- Personal Protective Equipment (PPE)
- Standard Operating Procedure (SOP)
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**ADMINISTRATIVE CONTROLS**
Safe Work Practices - Training

• How well are workers trained for the tasks?

• Do workers meet a level of competency before being allowed to work?

• Training should include:
  – Use of safety equipment
  – Decontamination procedures
  – Spill clean-up
  – Use of autoclave
  – Waste disposal
Safety Orientation and Annual Competency Includes…

- Proper and Safe Handling Practices
- Use of the biological safety cabinet (BSC)
- Biohazardous waste handling
- Use of autoclave
- Disease symptoms
- Post exposure management
- Reporting exposures and illnesses
Link to Occupational Health and Safety Program

- Minimum requirements to conduct work in the laboratory:
  - General laboratory safety training
  - Familiarity with safety guidelines
  - Standard precautions
  - Training and experience
Link to Occupational Health and Safety Program

• At a minimum, occupational health program includes:
  – Offering of Interferon Gamma Release Assay (IGRA) or Tuberculin Skin test (TST) who have risk of exposure to TB (two step TST on initial hire)
  – Ongoing evaluation of the respirator program, based on expected work area
Risk based on TB Incidence

- Frequency of *M. tuberculosis* positive specimens encountered
- Concentration of organisms in specimens
- Number of specimens handled by an individual worker
- Safety practices in the laboratory
- Safety equipment available in the laboratory
Chain of Infection

Reservoir of pathogen

Portal of escape

Transmission

Route of entry/infectious dose

Susceptible host

Incubation period

Illness

Proper Work Practices

Protective Equipment

Risk Assessment

Immunization

Treatment

Surveillance
Laboratory Biosafety Plan

Components

• How work is safely performed
• Risk Assessment
• Post Exposure Management
• Protocol driven personal protective equipment (PPE) requirements
• Traffic control / access restriction
• Use of safety equipment
• Sanitation – Cleaning & Disinfection
• Waste Management
• Training
• Medical Emergencies
Risk Assessment – Exposure Prevention

• Employ a combination of methods:
  – Safe work practices
  – Use of containment equipment
  – Specially-designed laboratory facilities

  – What is the incidence of TB in your community?
  – What has been the annual incidence of TB in the last 5 year?
  – Which tasks represent a risk to your laboratorians?
Annual Risk Assessment

• Audit the program (Self audits, internal & external audits)
• Follow up on accidents and incidents
• Revise the program accordingly
• Monitor biosafety practices and perform competency assessment
Specimen Collection: All aerosol producing procedures pose a risk of exposure
Handling the Specimen in the Laboratory

- Aerosol producing procedures pose a risk of exposure
- Where can the work be performed, biosafety level 2 (BSL-2) or biosafety level 3 (BSL-3)?
- What PPE are necessary?
Handling Clinical Specimens

- There are differences in literature regarding recommended safety levels for certain mycobacteriology procedures.
- Specimen receipt and log-in can occur on the open bench.
- All aerosol-generating activities (any actions imparting energy into a fluid specimen) must be conducted in a biological safety cabinet (BSC). For examples:
  - Surface disinfection of contaminated specimen container
  - Preparation of direct smear
  - Primary specimen digestion, decontamination, concentration
  - Concentrated smear preparation
  - Inoculation of culture media
Risky Activities in Other Laboratory Sections:

- Preparing frozen sections of biopsy specimens
  - Wear an N-95 respirator to mitigate the risk
- Cutting or sawing through tissue specimens that have not been fixed
  - Wear an N-95 respirator or powered air-purifying respirator (PAPR) during the procedure.
- Homogenizing tissues for primary culture
  - Use a BSC
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ENGINEERING CONTROLS
What if a BSL-3 is NOT available?

- BSL-2 with BSL-3 practices?
- A risk assessment is essential for determining if work with *M. tuberculosis* can be conducted safely in a separate, closed BSL-2 laboratory using BSL-3 practices and procedures
Common Design of BSL-3 within a BSL-2 Laboratory

- BSL-2 laboratory facility with separate BSL-3 section
- Access through 2 doors
- Arrows indicate negative pressure air flow
Common Design of BSL-3 Laboratory

- BSL-3 laboratory facility
- Access through two-door air-lock (anteroom)
- Pass-through autoclave
BSL-3 Laboratory Design

- Dedicated, single pass ventilation system exhausts all room air to outside
  - 6-12 air changes per hour (ACH) removes 99% of the airborne particulate matter in 23-46 minutes. Time depends on ACH.
  - Create negative pressure, airflow should be from “clean” to “less clean” areas
  - The laboratory should be kept under negative pressure at all times (alarm for failure of air handling system)
  - BSL-3 facility operation should be re-verified at least annually
BSL-3 Laboratory Design

- Interior surface of walls, floors, ceiling, and utility penetrations sealed
- Bench tops resistant to acids, alkalis, organic solvents, and moderate heat
- Foot-operated hand washing
- Automatic door closures
- Autoclave
Safe Work Practices - Design

- Keep TB lab door closed and access limited while working with infectious agents
- Biosafety sign posted on the door
- All work must be performed in a BSC
Biological Safety Cabinets
Class I BSC
Class II BSC
Class III BSC

Fume Hoods

Glove Box
Clean Bench

Ventilation Equipment

Class II Type A1 BSCs
Class II Type A2 BSCs
Class II Type B1 BSCs
Class Type B2 BSCs
Containment Equipment - BSC

• Perform all work within the BSC
• The BSC should be certified at least annually
• Train staff on appropriate use:
  – BSC installed away from walking traffic and doors
  – No storage of items in BSC
  – Work 4-6” from front grill
  – Clean with 10% bleach followed by 70% alcohol

Remember: Don’t block the front or back grill
BSC Maintenance

• Daily cleaning just the start...
• Read the manual! Be familiar with the performance characteristics of the model in use
• Read your annual recertification report and understand what was measured.

Annual Preventive Maintenance by whom? Are they certified?
TB Specimen Processing: Decontamination and Concentration

• Risky activities that can generate droplets & droplet nuclei
  • Vortexing
  • Pouring liquid cultures and supernatant fluids
  • Using fixed-volume automatic pipettors
  • Mixing liquid cultures with a pipette
  • Preparing specimen and culture smears
  • Dropping tubes or flasks containing cultures
  • Spilling suspensions of bacilli
  • Breaking tubes during centrifugation
  • Heat fixing smears
Containment Equipment - Centrifugation

- All culture tubes sealed tightly and placed in centrifuge safety cups inside the BSC
- After centrifugation, open safety cups in BSC
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**PERSONAL PROTECTIVE EQUIPMENT (PPE)**
Personal Protective Equipment - PPE

• Solid front disposable gown with snug (knit) cuffs.
• Gloves long enough to overlap the sleeves of the gown.
• N-95 or N100 respirator (must be fit tested)
• Remove all outer protective clothing when leaving the BSL-3 laboratory and place into bags for autoclaving.
# Sequence for Donning PPE

**Donning Personal Protective Equipment (PPE)**

1. **Perform Hand Hygiene**
   - Images courtesy of [justcleanyourhands.ca](http://www.justcleanyourhands.ca)

2. **Put on Gown**
   - Select appropriate size and type
   - Opening to the back
   - Secure neck and waist
   - If gown is too small, use two gowns:
     1. Gown #1 ties in front
     2. Gown #2 ties in back

3. **Put on Mask**
   - Use a fluid resistant procedure mask or surgical mask or one step mask with attached eye protection
   - Place over nose, mouth and chin
   - Fit flexible nose piece over nose bridge
   - Secure on head with ties or ear loops
   - Adjust fit
   - **Or N95 Particulate Respirator**
     - Select respirator according to fit testing
     - Place over nose, mouth and chin
     - Fit flexible nose piece over nose bridge
     - Secure on head with top elastic followed by bottom elastic
     - Adjust to fit
     - Perform a fit check:
       1. Inhale - respirator should collapse
       2. Exhale - check for leakage around face

4. **Put on Eye Protection**
   - Position goggles over eyes and secure to the head using the ear pieces or headband
   - Position face shield over face and secure brow with head band
   - Adjust to fit comfortably

5. **Put on Gloves**
   - Don gloves last
   - Select correct type and size
   - Insert hands into gloves
   - Extend gloves over isolation gown and cuffs

### Sequence for Removing PPE

**SEQUENCE FOR REMOVING PERSONAL PROTECTIVE EQUIPMENT (PPE)**

Except for respirator, remove PPE at doorway or in anteroom. Remove respirator after leaving patient room and closing door.

1. **GLOVES**
   - Outside of gloves is contaminated!
   - Grasp outside of glove with opposite gloved hand; peel off
   - Hold removed glove in gloved hand
   - Slide fingers of ungloved hand under remaining glove at wrist
   - Peel glove off over first glovet
   - Discard gloves in waste container

2. **GOGGLES OR FACE SHIELD**
   - Outside of goggles or face shield is contaminated!
   - To remove, handle by head band or ear pieces
   - Place in designated receptacle for reprocessing or in waste container

3. **GOWN**
   - Gown front and sleeves are contaminated!
   - Unfasten ties
   - Pull away from neck and shoulders, touching inside of gown only
   - Turn gown inside out
   - Fold or roll into a bundle and discard

4. **MASK OR RESPIRATOR**
   - Front of mask/respirator is contaminated — DO NOT TOUCH!
   - Grasp bottom, then top ties or elastics and remove
   - Discard in waste container

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**PERFORM HAND HYGIENE IMMEDIATELY AFTER REMOVING ALL PPE**

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Respiratory Protection

• No BSC is 100%
• The National Institute for Occupational Safety and Health (NIOSH) states that respirators provide greater protection
  – Particulate filters are more efficient
  – Can be fit-tested
  – Can be fit-checked by the user to ensure a tight seal to the face
• Respiratory protection program requires: SOP, training, storage, inspection, medical review, program evaluation
Disinfect the TB Laboratory Environment

• TB is very resistant to drying and can survive for long periods on solid surfaces
• Surfaces should be disinfected daily
• All work in the BSC should be performed over a gauze pad or paper towel soaked in disinfectant
• Decant fluids into a splash-proof container with disinfectant
Descending Order of Resistance to Disinfectants

**Most Resistant**

- Bacterial Spores - Clostridium sp. and Bacillus sp.
- Mycobacteria - *M. tuberculosis*
- Nonlipid or small viruses - polioviruses, coxsackie virus, rhinovirus
- Fungi - Trichophyton sp., Cryptococcus sp., Candida
- Vegetative Bacteria - Pseudomonas, S. aureus, Salmonella
- Lipid or medium sized viruses - Herpes simplex, Cytomegalovirus, RSV, Hepatitis B, HIV

**Most Sensitive**
Select the Right Level of Disinfectant for the TB Laboratory

- Sterilization: complete elimination of all forms of microbial life
- High-level disinfection: destroys all microorganisms except bacterial spores
- **Intermediate-level disinfection:** inactivates *M. tuberculosis*, nonspore forming bacteria, most viruses and most fungi
- Low-level disinfection: kills most bacteria (not TB), some viruses, and some fungi
  - Hospital-type germicides used primarily for housekeeping such as quaternary ammonium compounds ("quats")
Intermediate-Level Disinfection

• Kills *M. tuberculosis* and all other vegetative bacteria, all fungi, and most viruses
  – Tuberculocidal chemicals
    • Phenolics
    • Iodophors
    • Chlorine compounds
    • Alcohols
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STANDARD OPERATING PROCEDURE (SOP)
MINIMAL aerosols produced

• Cover the spill with paper towels
• Saturate with disinfectant
• Leave the laboratory until 99% of the airborne particles have been removed.
  – The time to reenter will depend on the air changes per hour (ACH)
• Wear PPE to clean up
• Autoclave material
• Disinfect floors and countertops
Spill Clean Up Procedures

**MAJOR** aerosols produced
• Evacuate immediately
• Do not reenter until **99.9%** of droplet nuclei are removed.
  – The time to reenter will depend on the air changes per hour in the laboratory
• May decontaminate with formaldehyde gas or other agent
• Reenter using appropriate respirator protection and PPE
• Do not pick up broken glass with hands
Post Exposure Management

Infectious Agent Exposure – Laboratory Occupational Health Protocol

- Laboratory staff should be trained to recognize symptoms of TB disease
- Report exposure events and illnesses
- Respond to potential exposure events
- Respond to respiratory illness in laboratory workers post exposure
- Initiate diagnostic testing for exposed worker
What if There is a IGRA or TST Conversion?

• If a staff member converts from IGRA or TST negative to positive, an evaluation is performed:
  • Evaluate the BSC, repair and recertify
  • Evaluate procedures and techniques
  • Retrain and re-educate as indicated
Additional References:
