Nuts and Bolts and Biosafety in the TB Lab

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Agent: *Mycobacterium tuberculosis*

- Infectious dose 1-10 organisms – *No safe level of exposure*
  - All workers must receive safety training
  - Adherence to biosafety practices must be monitored and annual competency assessment completed
  - Workers must be familiar with engineering components of the lab: number of air exchanges per hour, negative pressure monitoring, etc.
Background information on the risk of TB to laboratorians

• While the incidence rate of TB in the U.S. has declined over the past decade, the risk to the laboratorian continues.

• Tuberculosis among persons who work with *M. tuberculosis* in the laboratory is 3-5X greater than among those who do not

• Frequency of infection for persons who manipulate *M. tuberculosis* is 100 X greater than for the general population.

  – BMBL 5th ed., work with human lung tissue is a risk to laboratorians
Reports of conversions


• Lab-acquired infections are under reported
• Kubica estimated that 8-30% of laboratories may experience tuberculin conversions
• Kubica described 15 separate incidents in which 80 of 291 (27%) exposed lab staff developed positive TST:
  – 8 involved poor directional airflow
  – 5 associated with BSC failures
  – 1 linked to an autoclave failure
  – 1 due to equipment failure.
Recent Findings

- Overall HCW TST conversion 2.3 per 10,000 FTEs in non hospital settings
- TST reactivity claims highest for physician offices 3.7 / 10,000 FTEs
- Medical labs 2.6 / 10,000 FTEs were second

The worker is key to preventing exposures

- Host factors placing staff at increased risk
  - Known immunosuppression
  - Chronic diseases such as, asthma, emphysema or severe respiratory conditions
  - Use of medications known to reduce dexterity or reaction time
  - Pregnancy

Worker is pivotal in controlling the safe outcome of any operation!

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
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
Safe Work Practices - Training

• How well are workers trained for the tasks?
  – Do workers meet a level of competency before being allowed to work?

• Has a risk assessment been performed?

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

• Proper and Safe Handling Practices
• Use of the BSC
• Biohazardous waste handling
• Use of autoclave
• Disease symptoms
• Post exposure management
• Reporting exposures and illnesses
Personal Precautions and Work Practices

• General laboratory safety training and familiarity with safety guidelines, universal precautions, training and experience are required to conduct work in the laboratory.

• Link to Occupational Health and Safety Program:
  – Offering of Interferon Gamma Release Assay (IGRA) or TST who have risk of exposure to TB (two step TST on initial hire)
  – Ongoing evaluation if in the respirator program, based on expected work area
Specimen Collection: All aerosol producing procedures pose a risk of exposure

- Suspect or confirmed TB patients should be in a negative pressure room
- Anyone in the room during specimen collection must wear a particulate respirator type N 95 and be part of the respirator protection plan
- All mycobacteria specimens are collected into a sealed leak proof container
Initial Processing of Specimen

BSL-2 – when potential for aerosols is low

– Receipt and log-in
  • Consider all sputum containers as coming from patient with tuberculosis or pneumonia, reject those that leaked
  – Work on specimen inside a BSC
  – Examine container for external contamination and disinfect surface with 10% bleach

– Can prepare direct smear for AFB not culture
– Can inoculate routine cultures with no aerosol generating procedures
Specimen handling in main lab

• Use PPE (lab coat and gloves)
• Work inside the BSC
• Disinfect the outside before opening
• Move to TB lab (BSL-3) for further work
Suspect XDR TB?

BSL-2 with full BSL-3 practices are highly recommended for manipulations of the clinical specimens, including additional personal protective equipment (PPE) and autoclaving of waste before leaving the laboratory (see 5th edition BMBL for full description of BSL-3 practices).

http://www.cdc.gov/tb/topic/laboratory/BiosafetyGuidance_xdrtb.htm
Propagation and manipulation of cultures

“BSL-3 practices, containment equipment and facilities required”

– Should include the use of respiratory protection
– Implementation of specific procedures
– Use of specialized equipment to prevent and contain aerosols
– Only use disinfectants proven to be tuberculocidal
Specimen Processing for TB

- Aerosol generating procedures involving a TB specimen must be performed in a BSL-3 lab.
  - In the Guidelines, BSL-2 plus negative air flow and respiratory practices can be substituted.
- TB specimen decontamination, concentration, culture and concentrated smear preparation.
- Smears from cultures.
- Manipulating growth of cultures.
Design Features of BSL-3

- Access through 2 self closing doors with an air space between
- Single pass ventilation system, exhausting all room air to the outside
- Handwashing sink with “hands-free” operation
- Seams, floors, walls, and ceiling surfaces should be sealed. Spaces around doors and ventilation openings should be capable of being sealed to facilitate space decontamination.
BSL-3 lab design

- 6-12 air changes per hour (removes 99% of the airborne particulate matter)
- Ducted ventilation system, airflow should be from “clean” to “less clean” areas
- The lab should be kept under negative pressure at all times regardless of BSC power
BSL-3 lab design

- Interior surface of walls, floors and ceiling sealed and utility penetrations
- Chairs should be covered in a non-porous material
- Decontaminate trash within the lab - Autoclave
- Biosafety sign posted on the door
- All work must be performed in a BSC
What’s ahead?
ANSI Z9.14 Draft

The standard will provide methodologies to identify recommended testing and verification procedures for ventilation systems for BSL-3 laboratories based on their design, use, and a risk assessment of the agents used. The standard will exist to assist managers and owners to certify the safe operation of their facility with respect to ventilation.
Containment Equipment - BSC

- All work performed within the BSC
- The BSC should be certified at least annually
- Staff need to be trained on appropriate use
  - Working within the BSC
  - Movements that could disturb the air flow
  - Storage of items in the BSC
Evaluating BSC Work

- Minimize traffic
- Do not impede air flow
- Allow room for operation
- To conveniently perform the work without entering and exiting the BSC

If a BSC worker becomes infected with TB:
- Have the BSC checked and recertified
- Evaluate workers technique (reeducated if needed)
- Evaluate other workers who use the BSC for infection
BSC Maintenance

• Daily cleaning just the start...

Read the manual! Be familiar with the performance characteristics of the model in use

Review the report and understand what was measured.

BSC Maintenance

Annual Preventive Maintenance by whom? Are they certified?
The NSF Biosafety Cabinetry Program was initiated over 25 years ago at the request of the regulatory community, including the Centers for Disease Control (CDC), National Institutes of Health (NIH), and the National Cancer Institute (NCI).

The first phase of the program was the development of NSF/ANSI Standard 49 for the evaluation of Class II laminar flow biological safety cabinets. The standard was completed in 1976, followed by the implementation of a testing and certification program to that standard, titled the Biosafety Cabinetry Certification Program.

The third and final stage was completed in 1993, titled the Biosafety Cabinet Field Certifier Accreditation Program.
TB Specimen Processing: decontamination and concentration

Aerosol generating activities:

• 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
Containment Equipment - Centrifugation

- All culture tubes sealed tightly and placed in centrifuge safety cups inside the BSC
- After centrifugation, open safety cups in BSC
Personal Protective Equipment - PPE

- Solid front disposable gown with snug (knit) cuffs.
- Gloves long enough to overlap the sleeves of the gown.
- Remove all outer protective clothing when leaving the BSL-3 laboratory and place the clothing into bags for autoclaving.
Containment Equipment – Respiratory Protection

• No BSC is 100%

• Respirators provide greater protection
  – 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 lab 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 (BMBL 5th ed. p330)

**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 Lab

- Sterilization: complete elimination of microbes
- High-level disinfection: destroys all microbes except bacterial spores
- Intermediate-level disinfection: inactivates *M. tuberculosis*, nonspore forming bacteria, most viruses and most fungi
  - Phenolics
    - NacPhene (http://www.nclonline.com/products/view/nac_phene_256)
  - Iodophors
  - Chlorine compounds
  - Alcohols
- 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”)
Spill Clean Up Procedures

MINIMAL aerosols produced

- Cover the spill with paper towels
- Saturate with disinfectant
- Leave the lab until 99% of the airborne particles have been removed
- Wear PPE to clean up
- Autoclave material
- Disinfect floors and countertops
How long after a spill can you enter the lab?

• Because of the variability of air exchanges per hour in the lab, no set time can be given
  – “It would take 23 minutes to clear the air of *M. tuberculosis* from a spill at 99% removal efficiency if the room has 12 air exchanges per hour, and 35 min for this removal at 99.9% efficiency”
Spill Clean Up

**MAJOR** aerosols produced

- Evacuate immediately
- Do not reenter for at least 4 hours or until 99.9% of droplet nuclei are removed
- May decontaminate with formaldehyde gas or other agent
- Reenter using appropriate respirator protection and PPE
- Do not pick up broken glass with hands
CAP Inspection

- **MIC.33050 Specimen Collection Phase II** All specimens for mycobacterial culture are collected and/or received in sealed leak-proof containers.

- **MIC.33100 Centrifuge Safety Phase II** In centrifuging specimens, sealed screw-capped tubes are enclosed in sealed safety centrifuge carriers (*i.e.* a double closure system) used to minimize aerosol.

- **MIC.33300 Biological Safety Cabinet Phase II** The biological safety cabinet meets minimum requirements for mycobacterial work.
Additional valuable reference:
