Nuts and Bolts and Biosafety in the TB Lab

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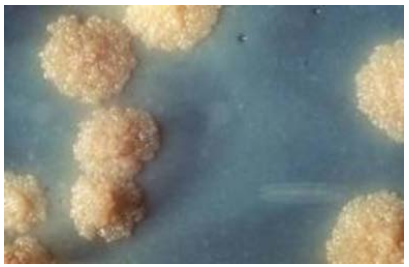
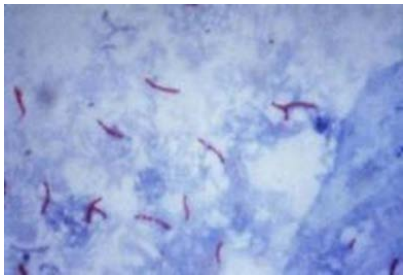
Agent: *Mycobacterium tuberculosis*

- 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.
  - Reid DP. Incidence of tuberculosis among workers in medical laboratories. Br Med J 1957;2:10-14.
  - BMBL 5th ed., work with human lung tissue is a risk to laboratorians

Reports of conversions

Kubica GP. Your Tuberculosis Laboratory: Are You really Safe from Infection ? Clinical Microbiology Newsletter 1990; 12: 85-87.

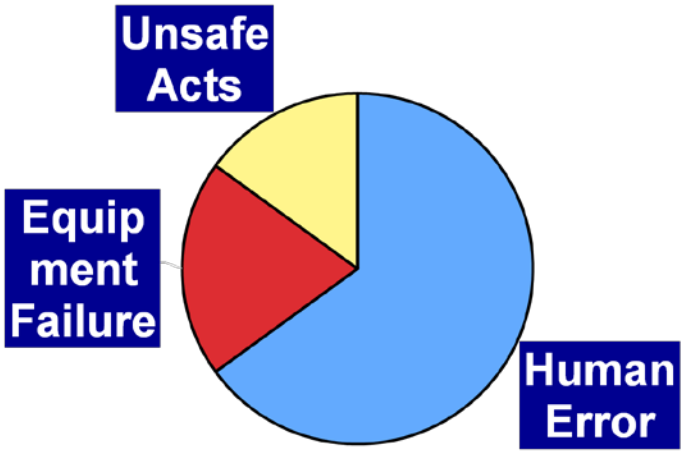
- Lab-acquired infections are under reported
- Kubica estimated that 8-30% of laboratories may experience tuberculin conversions
- Kubica described 15 separate incidents in which 80 of 291 (27%) exposed lab staff developed positive TST:
  - 8 involved poor directional airflow
  - 5 associated with BSC 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

Shah et.al.Am J Infect Control 2006 34:338-342.

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!

Phillips, G.B. In Lab Safety: Principles and Practices. 1st Edition

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

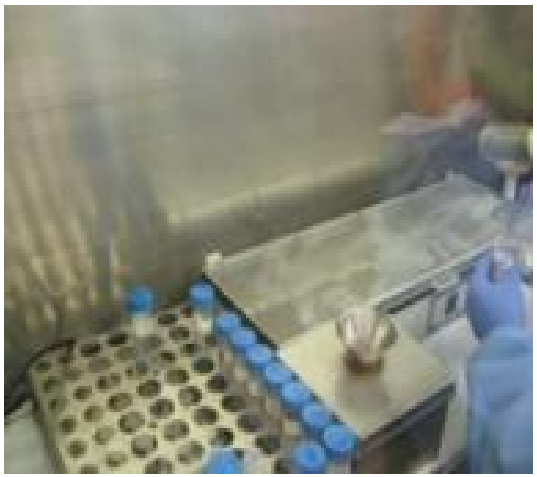
- "**BSL-3 practices, containment equipment and facilities required**"
  - Should include the use of respiratory protection
  - Implementation of specific procedures
  - Use of specialized equipment to prevent and contain aerosols
  - Only use disinfectants proven to be tuberculocidal

Specimen Processing for TB

- Aerosol generating procedures involving a TB specimen must be performed in a BSL-3 lab

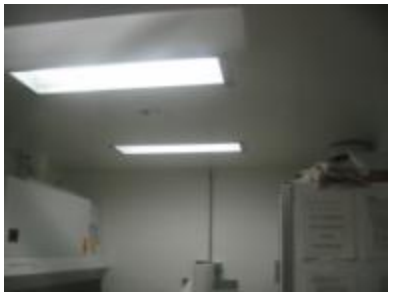
In the Guidelines, BSL-2 plus negative air flow and respiratory practices can be substituted.

- TB specimen decontamination, concentration, culture and concentrated smear preparation
- Smears from cultures
- Manipulating growth of cultures



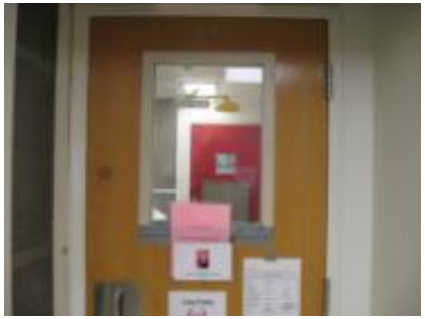
Design Features of BSL-3

- 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

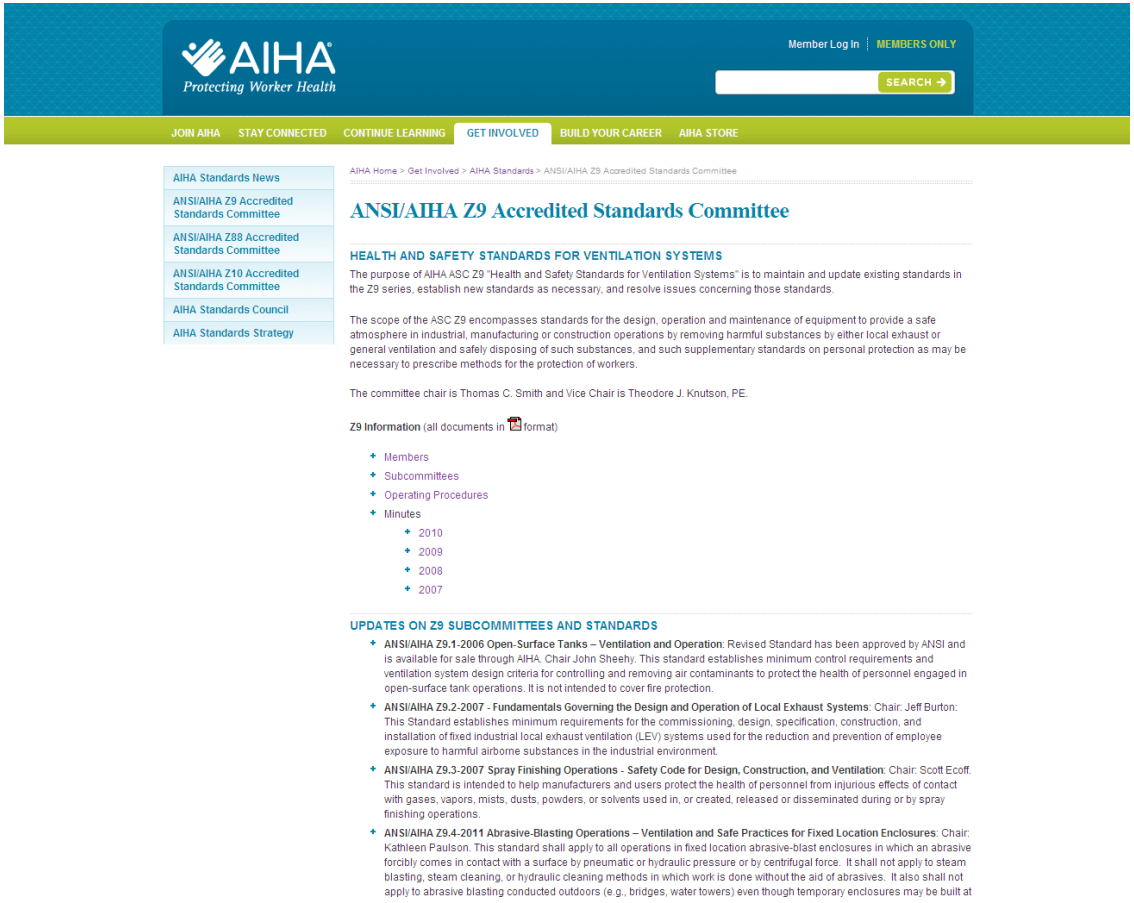
- 6-12 air changes per hour (removes 99% of the airborne particulate matter)
- Ducted ventilation system, airflow should be from “clean” to “less clean” areas
- The lab should be kept under negative pressure at all times regardless of BSC power



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



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HEALTH AND SAFETY STANDARDS FOR VENTILATION SYSTEMS

The purpose of AIHA ASC Z9 "Health and Safety Standards for Ventilation Systems" is to maintain and update existing standards in the Z9 series, establish new standards as necessary, and resolve issues concerning those standards.

The scope of the ASC Z9 encompasses standards for the design, operation and maintenance of equipment to provide a safe atmosphere in industrial, manufacturing or construction operations by removing harmful substances by either local exhaust or general ventilation and safely disposing of such substances, and such supplementary standards on personal protection as may be necessary to prescribe methods for the protection of workers.

The committee chair is Thomas C. Smith and Vice Chair is Theodore J. Knutson, PE.

Z9 Information (all documents in format)

- Members
- Subcommittees
- Operating Procedures
- Minutes
  - 2010
  - 2009
  - 2008
  - 2007

UPDATES ON Z9 SUBCOMMITTEES AND STANDARDS

- **ANSI/AIHA Z9.1-2006 Open-Surface Tanks – Ventilation and Operation**: Revised Standard has been approved by ANSI and is available for sale through AIHA. Chair John Sheehy. This standard establishes minimum control requirements and ventilation system design criteria for controlling and removing air contaminants to protect the health of personnel engaged in open-surface tank operations. It is not intended to cover fire protection.
- **ANSI/AIHA Z9.2-2007 - Fundamentals Governing the Design and Operation of Local Exhaust Systems**: Chair: Jeff Burton: This Standard establishes minimum requirements for the commissioning, design, specification, construction, and installation of fixed industrial local exhaust ventilation (LEV) systems used for the reduction and prevention of employee exposure to harmful airborne substances in the industrial environment.
- **ANSI/AIHA Z9.3-2007 Spray Finishing Operations - Safety Code for Design, Construction, and Ventilation**: Chair: Scott Ecoff. This standard is intended to help manufacturers and users protect the health of personnel from injurious effects of contact with gases, vapors, mists, dusts, powders, or solvents used in, or created, released or disseminated during or by spray finishing operations.
- **ANSI/AIHA Z9.4-2011 Abrasive-Blasting Operations – Ventilation and Safe Practices for Fixed Location Enclosures**: Chair: Kathleen Paulson. This standard shall apply to all operations in fixed location abrasive-blast enclosures in which an abrasive forcibly comes in contact with a surface by pneumatic or hydraulic pressure or by centrifugal force. It shall not apply to steam blasting, steam cleaning, or hydraulic cleaning methods in which work is done without the aid of abrasives. It also shall not apply to abrasive blasting conducted outdoors (e.g., bridges, water towers) even though temporary enclosures may be built at

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

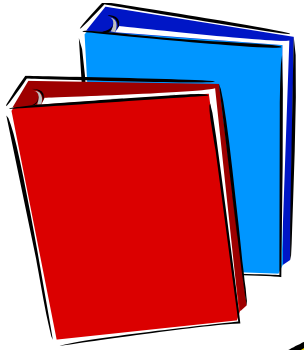
If a BSC worker becomes infected with TB:

- Have the BSC checked and recertified
- Evaluate workers technique (reeducated if needed)
- Evaluate other workers who use the BSC for infection

BSC Maintenance

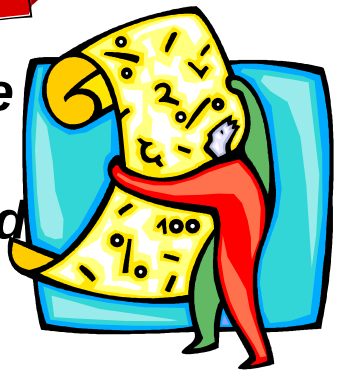
- Daily cleaning just the start…

Annual Preventive Maintenance by whom? Are they certified?



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

Review the report and understand what was measured.



CLINICAL MICROBIOLOGY REVIEWS, Apr. 1991, p. 207–241
0893-8512/91/020207-35$02.00/0

Vol. 4, No. 2

Biological Safety Cabinetry

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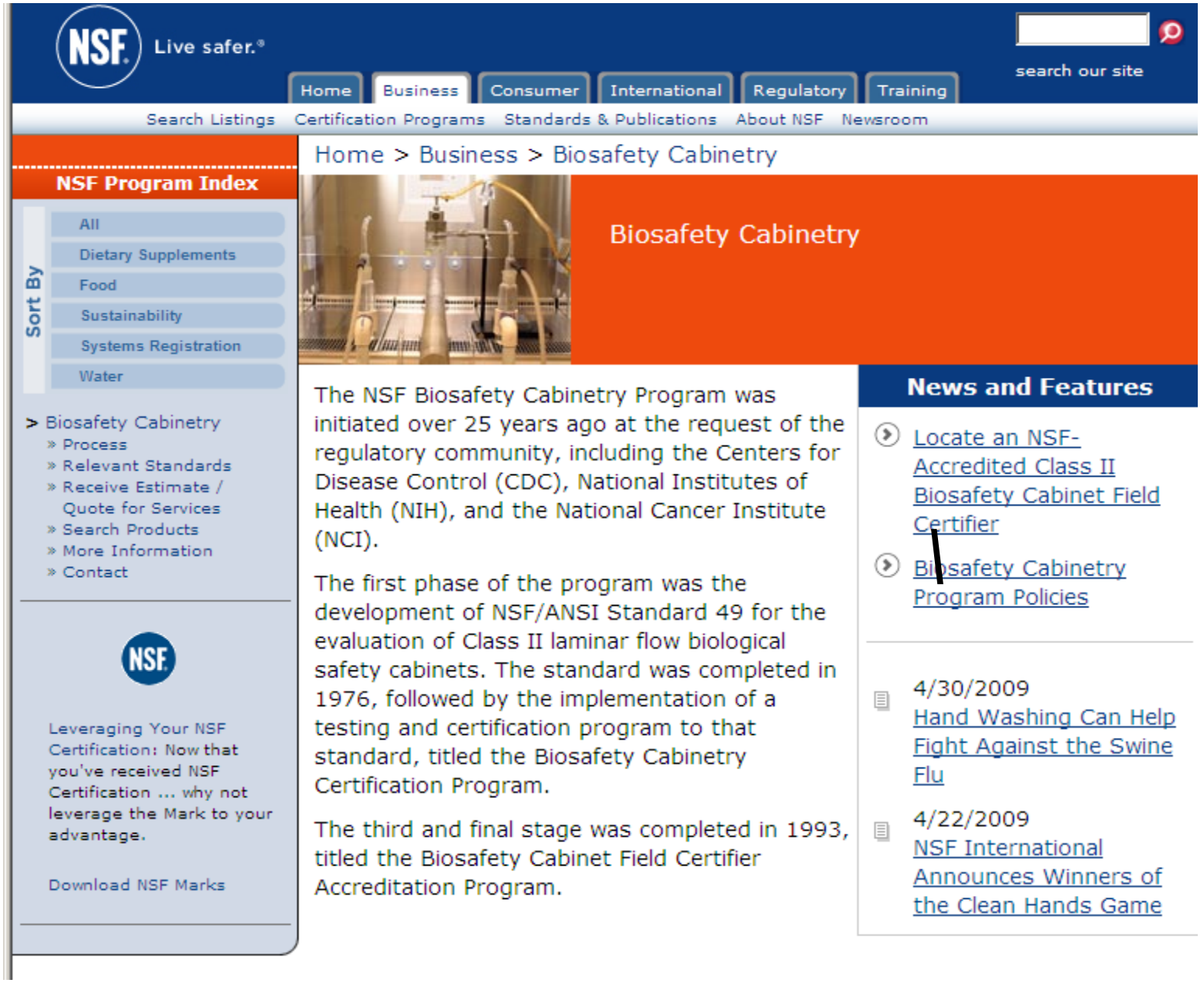
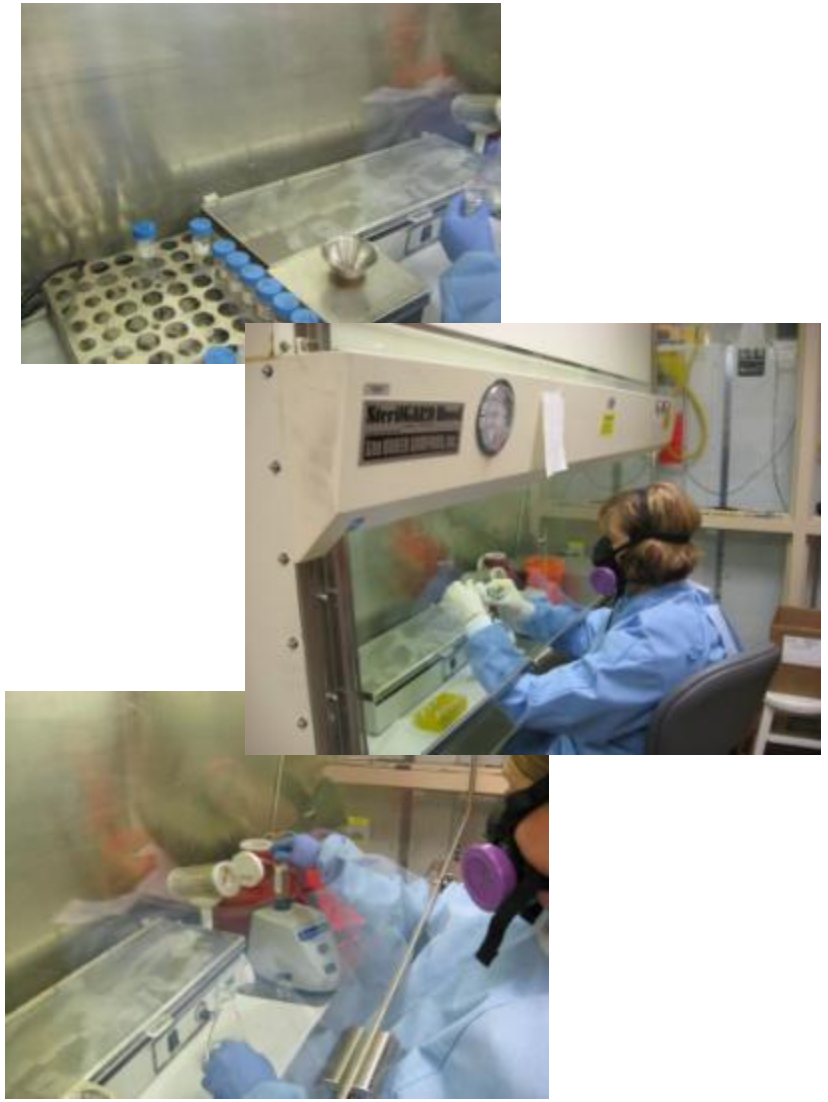


http://www.nsf.org/business/biosafety_cabinetry/index.asp?program=BiosafetyCab

TB Specimen Processing: decontamination and concentration

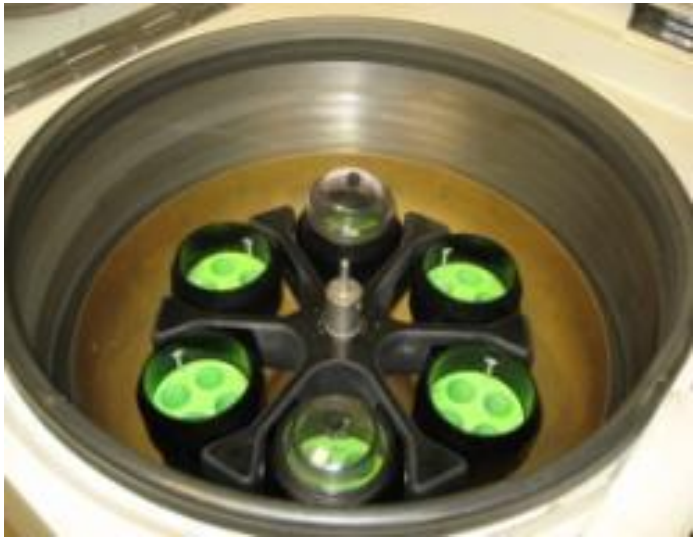
Aerosol generating activities:

- 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)

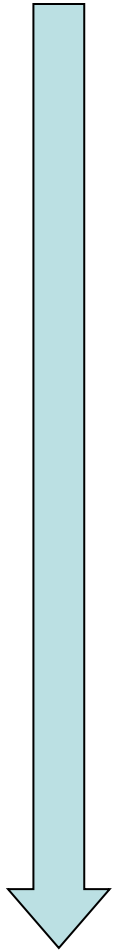
***Most* Resistant**

- 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

Most Resistant



Most Sensitive

- 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"

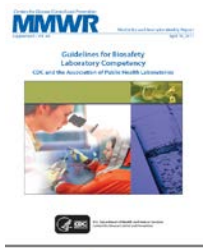
Spill Clean Up
MAJOR aerosols produced

- 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

- **<u>MIC.33050 Specimen Collection Phase II</u> All specimens for mycobacterial culture are collected and/or received in sealed leak-proof containers.**
- **<u>MIC.33100 Centrifuge Safety Phase II</u> In centrifuging specimens, sealed screw-capped tubes are enclosed in sealed safety centrifuge carriers (*i.e.* a double closure system) used to minimize aerosol**
- **<u>MIC.33300Biological Safety Cabinet Phase II</u> The biological safety cabinet meets minimum requirements for mycobacterial work.**

Additional valuable reference:



- Delany,J., J. Rodriguez, D. Holmes, M. Pentella, K. Baxley, and K. Shah. CDC/APHL Laboratory Biosafety Competencies for the BSL-2, BSL-3, and BSL-4 Laboratories. MMWR, Supplement, April 15, 2011. http://www.cdc.gov/mmwr/pdf/other/su6002.pdf

- Miller, M.J., R. Astles, T. Baszler, K. Chapin, R. Carey, L. Garcia, L. Gray, D. Larone, M. Pentella, A. Pollock, D.S. Shapiro, E. Weirich, and D. Wiedbrauk. Guidelines for safe work practices in Human and Animal medical diagnostic laboratories. MMWR, Supplement, January 6, 2012. http://www.cdc.gov/mmwr/pdf/other/su6101.pdf