Biosafety in the Mycobacteriology Laboratory

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Why is Biosafety Needed in the Mycobacteriology Laboratory?

• Risk of infection with *Mycobacterium tuberculosis* is 3-9x higher for TB lab workers than for other lab workers

• Infection usually results from unrecognized production of infectious aerosols containing tubercle bacilli

• Infection can occur from needle sticks, through broken skin, etc.
Biosafety

The application of a combination of administrative controls, containment principles, laboratory practices and procedures, safety equipment, and laboratory facilities to enable laboratorians to work safely with potentially infectious microorganisms.
Administrative Controls

• Supervision by an experienced scientist
• All personnel are well trained, proficient, aware of hazards, follow rules
• Routine medical surveillance
• Biosafety and operations manuals
• Emergency plans for spills, accidents, etc.
• Appropriate facilities and safety equipment
Good Laboratory Practices

- Restrict or limit access when working
- Biohazard warning signs
- Prohibit eating, drinking and smoking
- Prohibit mouth pipetting
- Minimize splashes and aerosols
- Decontaminate work surfaces daily
- Decontaminate wastes
Containment

• **Primary Containment**: protect worker and immediate laboratory environment
  • good microbiologic techniques
  • safety equipment

• **Secondary Containment**: protect the environment outside the laboratory
  • facility design
  • waste management
Biosafety Level (BSL)

• Conditions under which an infectious agent can ordinarily be safely handled.

• Conditions are a combination of:
  • laboratory practices and techniques
  • safety equipment
  • laboratory facilities

• Usually agent and procedure specific
Determining the BSL to Use

• The laboratory director assesses potential risks for work with a specific agent and assigns a BSL
• Recommended BSLs for many of the infectious agents have been developed
• Lab directors may specify more or less stringent practices when information is available to suggest altered risk
  • e.g., increased BSL for XDR TB cultures
  • e.g., decreased BSL for the BCG vaccine
Risk Assessment

- Pathogenicity of the infectious agent
- Route of transmission
- Agent stability and infectious dose
- Concentration of agent
- Type of laboratory procedures to be done
- Availability of effective prophylaxis or therapy
- Skill level and vulnerability of at-risk personnel
Biosafety Level 2 (BSL2)

- Suitable for work involving agents of moderate potential hazard to personnel and the environment
  - *Mycobacterium* species other than members of the *M. tuberculosis* complex
  - non-aerosol generating manipulations of clinical specimens from TB patients
  - BSC is to be used for aerosol generating procedures
BSL2 – Microbiologic Practices

• Restrict or limit access when working
• Biohazard warning signs
• Prohibit eating, drinking and smoking
• Prohibit mouth pipetting
• Minimize splashes and aerosols
• Decontaminate work surfaces daily
• Decontaminate wastes
BSL2 – Primary Containment

• Protective clothing - lab coat, gloves, eye protection
• Class I or II Biosafety Cabinet used for manipulations that generate splashes or aerosols
BSL2 – Secondary Containment

- Laboratories have lockable doors and are separated from public areas
- Air flows into lab without re-circulation to non-lab areas
- Sink for hand washing
- Impermeable, easily cleaned work surfaces
- Eyewash readily available
- Autoclave available
- Windows fitted with flyscreens
Biosafety Level 3 (BSL3)

• Suitable for work with infectious agents which may cause serious or potentially lethal disease as a result of exposure by the inhalation route.
  • members of the *M. tuberculosis* complex
BSL3 – Microbiologic Practices

BSL2 practices plus:
• Work in a certified biosafety cabinet
  • certified at least annually
• Use bioaerosol-containing equipment
  • aerosol-containment centrifuge rotors
• Decontaminate spills promptly
BSL3 – Primary Containment

- Protective clothing, gloves, eye protection
- Respiratory protection as needed
  - N95 respirator or equivalent
  - Powered Air-Purifying Respirator (PAPR)
- Class I or II Biosafety Cabinet used for all open manipulation of agents
BSL3 – Secondary Containment

BSL2 secondary containment plus:
- Controlled access to a separate area
- Double door entry
- Directional inward airflow
- Single-pass air; 6-12 air changes/hour
- Enclosures for aerosol generating equipment
- Room penetrations sealed
- Walls, floors and ceilings are water resistant for easy cleaning
If a facility does not have all required BSL3 features (e.g. sealed penetrations, solid ceiling), an acceptable level of safety for conducting routine procedures, including culture, may be achieved in a BSL2 facility providing:

- Directional inward airflow is maintained and exhaust air is discharged to the outside
- Access to the laboratory is restricted when work is being performed
- The recommendations for BSL3 practices, procedures, and safety equipment are rigorously followed
GLI Biosafety Projects

- Biosafety guidance for TB lab procedures
  - technical consultation in Sept. 2008
  - expert meeting in April 2009
  - WHO and CDC were the lead agencies

- Specifications for a ventilated work station suitable for direct AFB-smear microscopy
  - CDC and APHL were the lead agencies

- WHO TB Laboratory Biosafety Manual
  - Laboratory design and layout
  - Risk assessment and procedures
Biosafety Guidance

• Consensus recommendations for minimum biosafety requirements for
  • Direct AFB-smear microscopy
  • Processing specimens to concentrate bacilli for smear, culture, DST, molecular tests
  • Manipulating cultures for smear, subculture, identification, DST, molecular tests

• Based on a risk assessment for each TB diagnostic procedure
  • generation of infectious aerosols
  • concentration of bacilli
Personal Protective Equipment

- Gowns should be worn in the laboratory
- Gloves should be worn while manipulating specimens and working in a BSC
  - Proper hand washing os a cornerstone of biosafety
- Conduct a risk assessment to determine the need for other PPEs such as N95 respirators
Administrative Control
Medical Surveillance

• Done in accordance with national rules
• Surveillance conducted
  • at start of work in the TB laboratory
  • at regular intervals – at least annually
  • after any biohazard incident
Direct AFB Smear Microscopy

Limited risk of generating infectious aerosols

- Work can be done on an open bench
  - restricted access to the laboratory
  - separate bench for smear-preparation

- Adequately ventilated laboratory
  - 6-12 ACH, directional airflow
  - Natural or mechanical ventilation

- Proper disposal of infectious material
Processing Sputum Specimens for Smear, Culture, Molecular Tests – 1

Risk of generating infectious aerosols during centrifugation and specimen manipulation

- Laboratories must have restricted access and be separated from public areas
- Impermeable surfaces for easy cleaning
- Air flows into lab without re-circulation to non-lab areas (directional airflow)
  - 6-12 ACH, passive or mechanical ventilation
  - closed windows
- Proper disposal of infectious material
Processing Sputum Specimens for Smear, Culture, Molecular Tests – 2

- Class I or II Biosafety Cabinet used for all open manipulation of agents
  - BSCs must be properly installed and certified at least annually
  - BSC exhaust may be
    - ducted to outside using a hard duct or thimble fitting
    - recirculated into the room if assured that the BSC is functioning properly
- Use aerosol-containment centrifuge rotors
High risk of generating infectious aerosols during manipulation of liquid suspensions

- Work done in a containment lab which has restricted access and a double door entry
- Impermeable surfaces for easy cleaning
  - sealing room for fumigation is not required
- Air flows into lab without re-circulation to non-lab areas (directional airflow)
  - 6-12 ACH, mechanical ventilation, sealed windows
- Autoclave available on site
Processing Cultures for Smear, ID, Subculture, DST, Molecular Tests – 2

• Class I or II Biosafety Cabinet used for all open manipulation of agents
  • BSCs must be properly installed and certified at least annually
• BSC exhaust may be
  • ducted to outside using a hard duct or thimble fitting (preferred)
  • recirculated into the room if assured that the BSC is functioning properly
• Use aerosol-containment centrifuge rotors
References

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  Biosafety7.pdf
- Biosafety recommendations for the contained use of *M. tuberculosis* complex isolates in industrialised countries.
  http://www.biosecurite.be/CU/PDF/Mtub_Final_DL.pdf
- Interim Laboratory Biosafety Guidance for Extensively Drug-Resistant (XDR) *M. tuberculosis* strains.
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Biosafety Recommendations

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Interim Guidance for XDR TB

• Clinical specimens from known or highly suspected XDR TB patients
  • BSL2 with full BSL3 practices are highly recommended
• Manipulation of cultures of XDR TB strains
  • BSL3 practices, containment equipment, and facilities are required. BSL3 practices must include the use of respiratory protection and the implementation of specific procedures and use of specialized equipment to prevent and contain aerosols.
FIGURE 1. Laboratory suite

Air pressure indications: --- (least) to ++ (greatest)
Biosafety Cabinets (BSCs)

- **Class I**
  - inward airflow protects worker
  - exhaust to outside (w/wo HEPA filter)
- **Class II**
  - Protects worker, product, environment
  - used with aerosol-transmissible microorganisms, tissue culture
- **Class III**
  - totally enclosed, ventilated, air-tight
  - suitable for work with BSL3/4 agents