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**
  - Expert consultation in Sept. 2009
  - 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**

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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**

Processing Cultures for Smear, ID, Subculture, DST, Molecular Tests – 1

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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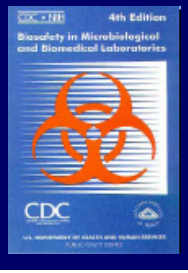

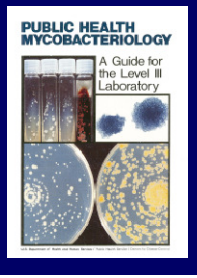

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Interim Guidance for XDR TB

- **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.

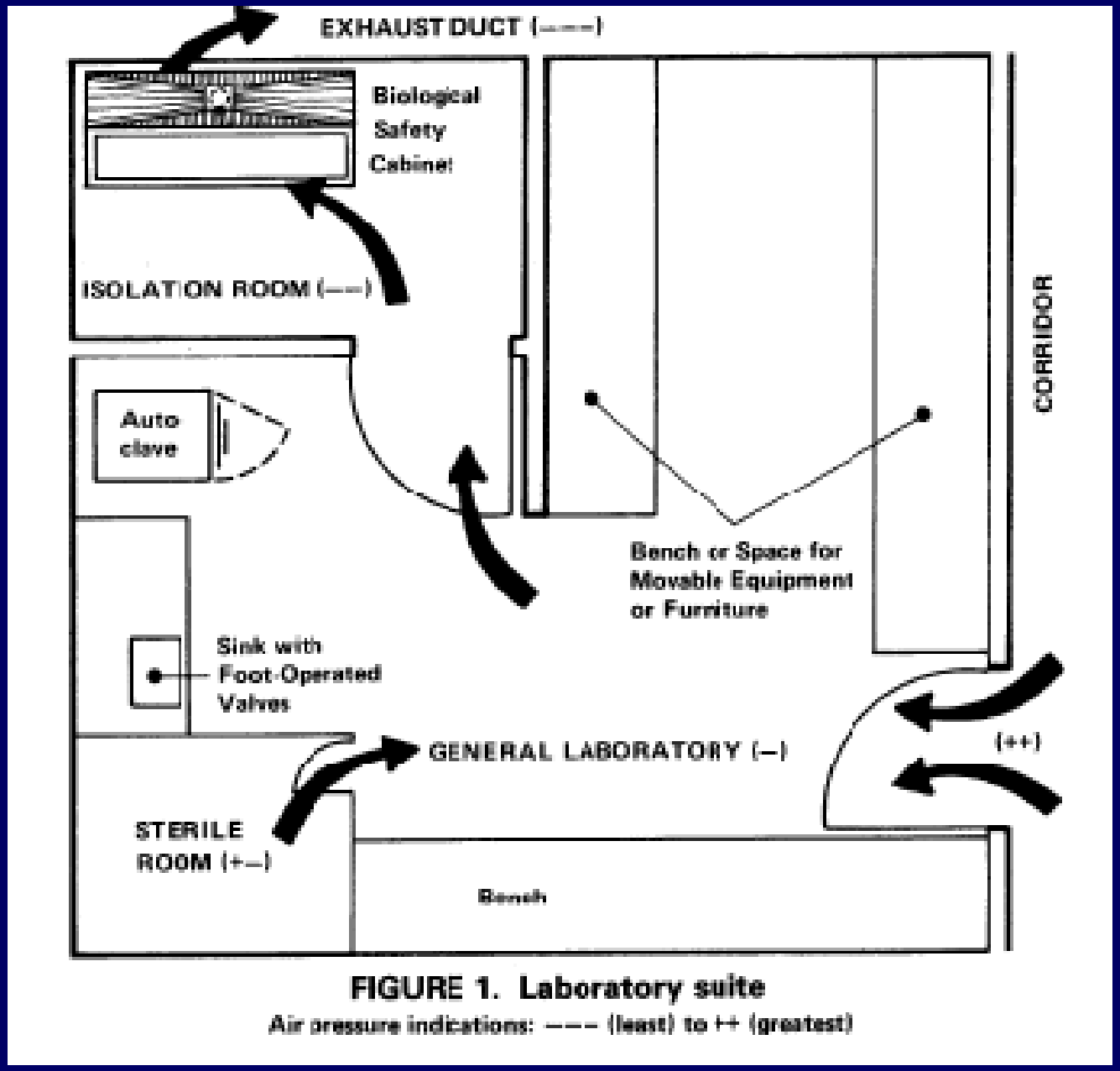

FIGURE 1. Laboratory suite

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