



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...depending on activities performed
- Infection usually results from unrecognized production of infectious aerosols containing tubercle bacilli
- Infection can occur from needle sticks, through broken skin, etc.

What is 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 and dispose of waste appropriately

Containment



- Primary Containment: protect worker and immediate laboratory environment
 - good microbiologic techniques
 - properly maintained safety equipment
- Secondary Containment: protect the environment outside the laboratory
 - facility design
 - waste management

Biosafety Level (BSL)



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- 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

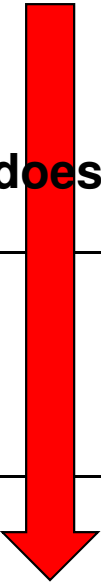
Biosafety Level (BSL)



- **Biosafety level 1 (BSL-1)** is the basic level of protection and is appropriate for agents that are not known to cause disease in normal, healthy humans.
- **Biosafety level 2 (BSL-2)** is appropriate for handling moderate-risk agents that cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure.
- **Biosafety level 3 (BSL-3)** is appropriate for agents with a known potential for aerosol transmission, for agents that may cause serious and potentially lethal infections and that are indigenous or exotic in origin.
- **Biosafety level 4 (BSL-4)** is appropriate to exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available.

Risk Group Classifications and which Biosafety Level (BSL)



RISK GROUP CLASSIFICATION	WORLD HEALTH ORGANIZATION LABORATORY BIOSAFETY MANUAL 3RD EDITION 2004*
Risk Group 1	(No or low individual and community risk) A microorganism that is unlikely to cause human or animal disease. BSL 1
Risk Group 2 <div style="text-align: center;">  <p>Where does TB fit?</p> </div>	(Moderate individual risk; low community risk) A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventive measures are available and the risk of spread of infection is limited. ?BSL 2
Risk Group 3	(High individual risk; low community risk) A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available. ? BSL 3
Risk Group 4	(High individual and community risk) A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available. ? BSL 4

Determining which 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 or staff at greater risk

New approaches are based on Bio-Risk Assessment



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- 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)

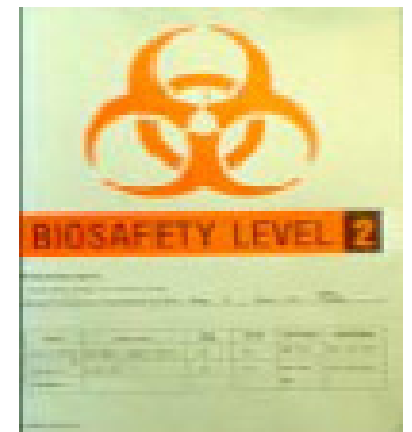


- Suitable for work involving agents of moderate potential hazard to personnel and the environment
 - *Mycobacterium* species other than members of the *M. tuberculosis* complex
 - non-aerosol generating manipulations of clinical specimens from TB patients
 - BSC is to be used for aerosol generating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

BSL2 – Microbiological 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)



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- 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 – Microbiological Practices



BSL2 practices plus:

- Work in a certified biosafety cabinet
 - **certified at least annually**
- Use aerosol-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
- Class I or II Biosafety Cabinet used for all open manipulation of agents
 - **rotors**

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
- *Room penetrations sealed to enable fumigation*
- Walls, floors and ceilings are water resistant for easy cleaning

Are all of the requirements for a BSL-3 Laboratory necessary?



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

Biosafety Guidance



- Consensus recommendations for minimum biosafety requirements for
 - Direct AFB-smear microscopy
 - Processing specimens to concentrate bacilli for smear preparation, culture inoculation or direct DST and 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

Biosafety Guidance - PPE



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

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

Biosafety Guidance:

Processing sputum specimens for culture inoculation and/or direct molecular tests (1)



Moderate 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
 - All work **MUST** be performed within a certified BSC
- Proper disposal of infectious material

Biosafety Guidance:

Manipulating cultures for smear preparation, identification tests, DST, or molecular tests



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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

Biosafety Guidance:

Processing sputum specimens for culture inoculation and/or direct molecular tests (2)



Moderate risk of generating infectious aerosols during centrifugation and specimen manipulation

- 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

Biosafety Guidance: Essential requirements

- Double door airlock
- Separate air inlet
- Venting of BSC via thimble
- Aerosol containment
- Negative pressure monitoring
- Uni-directional airflow
- PPE
- Autoclave for waste disposal

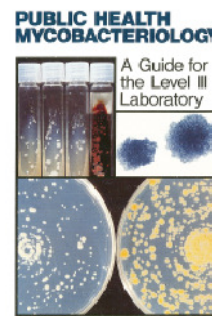
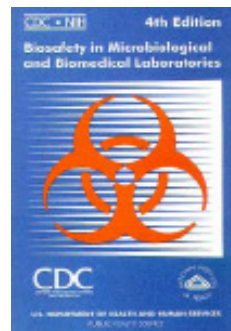
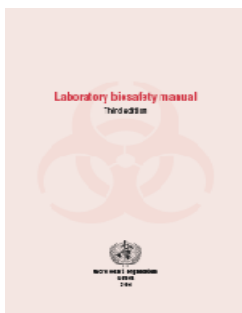


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WHO TB Biosafety Manual 2010 (under review)



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