

4 LABORATORY BIOSAFETY AND INFECTION CONTROL

Purpose

Transmission of tuberculosis, including drug-resistant TB, is a recognized occupational risk for laboratory personnel, as well as a hazard to others who may be exposed to infectious aerosols in the laboratory. Tubercle bacilli may be present in sputum, gastric lavage fluids, cerebrospinal fluid, urine, and in a variety of tissues. MTB bacilli may survive in heat-fixed smears and may be aerosolized during the processing of specimens and inoculation of culture media. Although clinical specimens from TB cases contain a low infective dose of MTB, all specimens from suspected or known TB cases must be considered potentially infectious and handled with appropriate precautions. Furthermore, MTB cultures and drug susceptibility testing procedures generate high concentrations of organisms that pose an increased risk of aerosol production. Thus, all aforementioned procedures necessitate special biosafety containment and practices.

Principle

Regardless of the level of risk for spread of TB infection, standards are recommended for all laboratories handling specimens from suspected or known TB cases. These standards are endorsed by WHO, US CDC, and US National Institute of Health (NIH). In addition, Good Clinical Laboratory Practice (GCLP) standards, a set of regulations that guide laboratories conducting clinical trials, require a laboratory design that ensures the safety of personnel and the quality of work. In summary, these standards require:

- Administrative controls, including good lab practices, SOPs, and accident management plans
- Engineering controls, such as a controlled ventilation system
- Use of personal protective apparel/equipment appropriate for the task
- Waste management procedures
- General lab safety procedures – including physical, biohazard, fire, chemical, and electrical safety

Materials/Equipment for all TB Laboratory Procedures

- Class II Biosafety Cabinet (BSC)
- N95, FFP2, or equivalent respirators
- Lab gowns
- Disposable gloves
- Biohazard bags
- Tuberculocidal disinfectant
- Waste receptacles
- Centrifuge with biosafety canisters/lids
- Autoclave
- Autoclave tape
- Spore test for autoclave

4.1 *Recommended Facilities and Equipment*

- The lab must be contained, i.e., physically separated from other labs.
- Access to the lab must be restricted, preferably through an anteroom.
- Controlled ventilation should be installed which maintains a directional airflow into the lab, supported by a visual monitoring device showing that proper directional airflow is maintained at all times.
- Air from the containment lab should not be re-circulated to other areas in the building – this environment can be achieved through high-efficiency particulate air (HEPA) filtration within the ventilation system.
- Class II biosafety cabinets (BSC; vertical, laminar-flow which blows HEPA-filtered air over work area; UV lamp optional) must be used for all manipulations of clinical specimens and positive cultures.
- BSCs must be certified at least annually by personnel trained in the certification process.
- Clinical centrifuges must be equipped with biosafety canisters (buckets with aerosol-containment lids).
- Microcentrifuges should be operated inside BSC using tubes with caps having O-rings.

4.2 *Recommended Biosafety Practices*

- Take caution when performing aerosol-generating procedures such as centrifugation, vortexing, mixing, pipetting, pouring, and inoculation of media. For example:
 - Delay opening caps until aerosols have settled
 - Open centrifuge canisters only inside the BSC
 - Use pipettes that are easy to control
 - Eject micropipette tips down inside discard bucket
- Heat-fix slides on a warmer in the BSC, heating them at 65-75° C for at least two hours before handling outside the BSC. Heat-fixed smears may contain viable tubercle bacilli, but they are not easily aerosolized if dried on a slide warmer for two hours.
- Inside the BSC, keep arms parallel to the work surface, work in the center, and minimize arm movements; once beginning work, do not move hands out of the hood until work is completed. These precautions will minimize interruption of airflow inside the BSC.
- Keep the amount of equipment inside the BSC to a minimum so as to not interfere with the airflow pattern.
- Disinfect the BSC and all work surfaces with a tuberculocidal disinfectant (capable of killing MTB), before and after every procedure.
 - The most common tuberculocidal disinfectants are bleach (hypochlorite) and phenol-based disinfectants. Diluted (working) bleach solutions must be prepared daily and stay at or above 0.5 % chlorine – undiluted commercial bleach is usually 4-5 %. Similarly, phenol-based disinfectants must be diluted daily, preferably with deionized (not “hard”) water, to 2-5 %. Both types of disinfectants are only effective if left in contact with the contaminated material for at least 15 minutes. Check the manufacturer’s recommendations for the specific disinfectant to be used.
- Place all wastes containing MTB in a leak-proof container or autoclavable plastic bag that contains disinfectant solution which can be sealed before being removed from the BSC and autoclaved.

- The autoclave should be monitored with a spore test at least monthly to ensure that sterility is achieved.
- Avoid practices that can result in spills, e.g., hand-carrying tubes, vials, and bottles, or improperly stacking racks or baskets. All tubes, plates, and other containers should be transported on carts in protected racks or baskets.
- Write a procedure for the appropriate handling of a spill, both inside and outside the BSC. The procedure should include:
 - Inside the BSC: the BSC should continue to run and the appropriate disinfectant should be applied to the spill for at least 20 minutes, after which the items can be transported to the autoclave. If a liquid culture is spilled, all personnel should exit the containment room, and allow the BSC to run for at least 30 minutes before entering the room.
 - Outside the BSC (but within the lab): all staff should leave the room for at least 30 minutes to allow the aerosols to settle. The person cleaning the spill should wear a respirator when disinfecting the area. If the lab is a biosafety level 3 (BSL3) laboratory, all staff should leave the room, and the person disinfecting the spill can enter immediately (wearing a respirator), without waiting for the aerosols to settle.

4.3 Recommended Personal Protective Equipment

- Staff working in the containment lab must wear protective laboratory clothing such as a solid-front or wrap-around gown. If scrub suits are worn, protective gowns should be worn on top. The scrub suits should be changed daily. The protective gown must have long sleeves with snug (knit) cuffs.
- Gloves must be worn and must be long enough to overlap the sleeves of the gown.
- Hair covers (caps) and shoe covers are recommended.
- All outer protective clothing must be removed when leaving the containment laboratory.
- Respiratory protective devices are highly recommended while working in the containment lab. Any respirator conforming to the National Institute for Occupational Safety and Health (NIOSH) N-95 rating, European Committee for Standardization 'FFP2' rating, or equivalent is acceptable.
- Respirator protection is more than just wearing a mask! Attention must be given to:
 - Selecting the appropriate respirator for the individual
 - Conducting fit-testing
 - Training personnel on the use, fit checking, and storage of the respirator

NOTE: Laboratory infections are nearly always caused by poorly monitored BSCs or a BSC in which normal aerosol containment capability is compromised, thereby permitting escape of droplet nuclei. A respirator can serve as an additional barrier to reduce the likelihood that tubercle bacilli will enter the lung.

4.4 Personnel Protection - Training and Monitoring

- All personnel working in the containment lab must have proper training on biosafety procedures, use of personal protective equipment, and how to monitor all equipment (especially the BSC) for proper operation. Documentation of this training must be kept with personnel training records.
- Staff should receive frequent re-training and be monitored to ensure compliance.
- Staff should be participating in a TB screening program, per the laboratory's national guidelines for preventing TB infection.