
SAFETY IN TB RESEARCH LABORATORY*VK Challu****Abstract**

Prevention of infection among health care professionals, working in tuberculosis research laboratory, requires appropriate safeguards. Laboratory staff, while processing infectious materials has to be educated about the protection from potentially infectious aerosols, which can be prevented. Proper usage of primary and secondary barriers in contagious areas through the regular use of good laboratory practices is important in controlling the risk of laboratory-acquired infection. Based on the activities performed in the laboratory, different levels of containment are essential. Procedures involving manipulation of liquid suspension containing infectious materials needs to be done in the Bio-safety cabinets. For effective and absolute containment of potentially lethal infection, Mycobacteriology research laboratories need to be upgraded as Bio-Safety level III facility which ensures absolute containment of turbulent air currents inside the laboratory besides adhering to the international standards.

Key words : Bio-safety cabinets, Good laboratory practices, infectious aerosols.

Introduction

Tuberculosis (TB) continues to be a significant public health issue, which is further complicated by its association with HIV and increasing problem of multiple drug-resistant strains¹. TB Mycobacteriology laboratories constitute special work environment that needs specific safeguards. There are effective methods for controlling laboratory hazards that may cause

unnoticed exposures to *M.tuberculosis*. Laboratory safety habits, which can protect laboratory workers from the occupational risk, are usually ignored, besides unknowingly compromising the efficacy of engineering controls².

Transmission of tubercle bacilli among health care professionals depends on the type of related activity and effectiveness of preventive measures. Laboratory environment including room volume, ventilation and concentration of droplet nuclei generated from infectious material plays important role in transmission. In this context, risk assessment in laboratories needs to be focussed on prevention of infections³. Cases of laboratory-acquired tuberculosis may be difficult to demonstrate because source of infection is often unclear. Molecular typing techniques like RFLP typing can now distinguish between different strains of *M.tuberculosis* besides identification of laboratory contamination⁴. However, exposure to tubercle bacilli can occur outside the laboratory and the appearance of the symptoms may be delayed⁵. The World Health Organization and Centre for Disease Control (CDC) Atlanta, USA include *M.tuberculosis* among the micro-organisms that require a Biosafety Level III facility, since it is an agent with the potential for transmission by inhalation route that can cause a severe and potentially lethal infection^{6,7}. When processing these agents, using primary and secondary barriers to protect personnel in contiguous areas is important besides protection from potentially infectious aerosols in the laboratory⁸. The activities performed in microbiology laboratories, including receipt and

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disposal of specimens and cultures, no doubt involve risk of infection to the personnel who handle them unless safeguards are followed. The majority of biological hazards in the laboratory can be foreseen and prevented⁹.

Protecting oneself depends on exercising enough self-discipline to maintain proficiency at all times when handling the microbial agent. It is need of the hour that the Mycobacteriology laboratories are upgraded and equipped with the basic facilities that ensure adherence to safety controls.

Basic Laboratory Practices

The regular use of good laboratory practices is of great importance in controlling the risk of laboratory-acquired infection. Simple techniques must be learnt so that they can be executed with ease. Jerky and abrupt movements should be avoided since they can cause hazards for the safety of personnel working in the laboratory. Work must be planned and executed in a disciplined manner. Nothing should be left to chances by following deliberate procedures as a routine, which should be constantly reinforced by the supervisory personnel. The fundamental safety requirements for microbiological laboratory should include:

- (i) Restrict entry to authorized personnel only and use wraparound gown or full sleeve aprons while inside the laboratory.
- (ii) Cover hands with protective gloves. Remove contaminated / soiled gloves and wear a new pair of gloves.
- (iii) Perform all procedures that involve handling of *M.tuberculosis* in properly functioning biological safety cabinet. Avoid splashes and spills to reduce aerosol formation.
- (iv) Never mouth pipette, use mechanical pipetting device.
- (v) No eating, drinking, smoking or handling contact lenses in the laboratory.

- (vi) Decontaminate work surfaces daily with detergent solutions and keep laboratory clean.
- (vii) Discard disposable instruments like used needles in hard container with lid.
- (viii) Use secondary leak-proof containers to transfer cultures and other laboratory materials containing *M.tuberculosis* between areas such as bio-safety cabinets, incubators etc.
- (ix) Steam-sterilize all infected waste materials before disposal.
- (x) Before leaving laboratory always wash hands.
- (xi) On all the laboratory doors and equipments universal biohazard symbol should be pasted.

Biological Safety Cabinets & Containment

Procedures involving the manipulation of liquid suspensions containing *M.tuberculosis* must be carried out inside Vertical Laminar Flow Cabinet (Class-II Biological safety cabinet). This equipment is designed to prevent the worker from aerosol infection generated inside the cabinet besides checking outside contamination. These cabinets and engineering control associated with the laboratory facility provide primary and secondary levels of containment against accidental release of *M.tuberculosis*. Primary containment protects the laboratory worker from exposure and facility associated engineering controls, provides additional protection to prevent escape of *M.tuberculosis* to other areas especially in Biosafety Level III Laboratory. In biological safety cabinets, containment of aerosols is provided by room air that is drawn into the cabinet through its front opening, generally referred as intake airflow. It captures aerosols released during experiments within the cabinet and transported to a High-

Efficiency Particulate Air (HEPA) filters. Descriptions of different biological safety cabinets are available in several review articles^{10,11}. Only Class II cabinets can protect experimental material from the airborne contamination. When using biological safety cabinets, the following additional practices are important:

- Advance planning of the experiment.
- A disinfectant –soaked towel placed on work surface reduces contamination due to minor spills or droplets.
- Biosafety cabinet's UV light to be switched on before and after completion of work.
- Before beginning the work all materials needed for the experiment to be placed inside the cabinet. A metal cart or bench adjacent to the cabinet can be used for excess clean material required during the experiment.
- During the course of experiment, while bringing items into or out of the cabinet, slow and deliberate motion to be used.
- Bacilli concentrating methods such as centrifugation need to be carried out in air-sealed centrifugation buckets.
- Avoid opening and closing of doors during experimentation and place a sign that experiment is in progress.
- Disinfect the work surface in the cabinet after completing experiment, preferably with 5% phenol.
- Periodic inspection of bio-safety cabinets especially HEPA filters.

For controlling the risks of laboratory acquired tuberculosis, training, technique and containment are the essential elements. Selecting appropriate practices and the skill to handle *M.tuberculosis* should be learnt through careful

guidance of experienced and responsible supervisors. The safety training must precede the first time handling of *M.tuberculosis* in the laboratory. Until the laboratory worker can demonstrate proficiency in handling less serious pathogen, no experimental procedure involving *M.tuberculosis* should be performed. Using BCG or other low-risk organisms can be relevant strategy for acquiring necessary laboratory skills. The laboratory worker must be trained in emergency procedures in addition to learning safe techniques before being allowed to work unsupervised in the laboratory. Good laboratory practices eventually become a habit and sharing information through laboratory meetings must be a dynamic process.

Bio-Safety level III facility safeguards

Although the design and containment of BSL III laboratory is complex, the directional airflow and access controls are its two important features. The safety function of these features is to prevent exposure of persons inside and outside the laboratory. Directional airflow minimizes the chance of aerosols generated during the course of experiments to other areas of the building. Provision of access control prevents unauthorized people from entering the laboratory. The safety of the laboratory worker gets compromised in case, design and operation of the airflow system is poor. The basic principle of BSL III facility ensures that air supplied inside the laboratory moves from areas free from *M.tuberculosis* to the work place where experimental procedures are being performed. To make it happen, the laboratory will be at a relative air pressure lower than the adjacent areas. The air pressure differential has to be small so that turbulent air currents are not created when the doors are opened or closed¹². One has to be conscious of the fact that class I and II Bio-safety cabinets are not absolute containment devices since airflow alone cannot prevent the escape of aerosols from the front opening of the cabinet.

To reduce the risk of inhalation exposure during accidental biological spills outside the biosafety cabinet, one should hold the breath and leave the laboratory immediately. Decontaminating and cleaning of the infected spills from the surface must be ensured before reentering the laboratory besides functional exhaust air ventilation system, which will cleanup the aerosols. Appropriate protective equipments like the respirator with full face shield, disposable gloves, shoe covers and wraparound laboratory gowns or alternative protective body coveralls should be used in a BSL III facility especially during course of experimentation that could generate aerosols outside the Biosafety cabinets. While decontaminating the affected area, one must put on protective equipment. Absorbent material or paper towel should be used to cover the spill before pouring appropriate disinfectant solution for a 20 minute contact period. Fresh towel soaked in disinfectant should be used to clean the spill area. The used towel can be placed in plastic bag and decontaminated in the autoclave. The facility safeguards, which prevent accidental entry of microbes into the laboratory space, include both administrative instructions and engineering design of the laboratory.

Based on need of the country, Mycobacteriology laboratory facilities should be upgraded to the international standards to promote TB research especially in the field of immuno-diagnostics, vaccines development and other related fields of Biotechnology. In a experimental TB laboratory all workers should have confidence in everyone's ability to maintain proficiency at all times when handling the infectious organisms.

The purpose of this chapter is to provide scientists and technicians, who conduct research that involve handling of *M.tuberculosis*, a basic orientation to health and safety practices that are appropriate for the control of hazards associated with the handling of *M.tuberculosis* in the research laboratory.

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