

## Bio Safety & Risk Precaution

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### Bio Safety and Bio Risk precautions in TB Laboratories

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#### Introduction:

Tuberculosis is an infectious disease caused by species of the *Mycobacterium tuberculosis* complex. This complex includes *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. pinnipedii* and *M. microti*<sup>1,2</sup>. The four first species are human pathogens, *M. microti* infecting voles, guinea-pigs, rabbits and sometimes bovines. *M. pinnipedii* is responsible for tuberculosis in seals <sup>2</sup>*M. bovis* is responsible for pulmonary disease in bovine and sometimes to mammary lesions with passage of tubercle bacilli in milk. Both *M. bovis* and *M. pinnipedii* are responsible for zoonotic diseases. *M. africanum* is responsible for 20 to 80 % of human tuberculosis in sub-Saharan Africa, but also for some tuberculosis cases diagnosed outside this continent.

*M. tuberculosis* is an airborne pathogen included in Risk Group 3 according to the classification of the organisms. <sup>3</sup> It is transmitted via aerosols or less frequently by accidental inoculations. The diagnosis of tuberculosis relies on the isolation and identification of the *Mycobacterium tuberculosis* in clinical specimens. The incidence of the disease among the laboratory personnel is known to be nine times higher as compared to the other Health care workers. <sup>4</sup>

Considering the high risk of Laboratory acquired infection (LAI) for personnel manipulating samples potentially containing *M. tuberculosis* it is mandatory to ensure highest level protection for human health and environment. It should be necessary to specify the bio safety regulatory framework currently in force by providing substantial details about the available containment measures, safety equipment and the various work practices to be applied in diagnostic or research laboratories working / manipulating with mycobacterium.

The TB infection control program should be based on a three-level hierarchy of control measures and include:

1. Administrative measures
2. Environmental controls
3. Use of respiratory protective equipment

**“Laboratory Bio safety”** is the term used to describe the containment principles, technologies, and practices that are implemented to prevent unintentional exposure to pathogens and toxins or their accidental release.

**“Laboratory Bio risk”** generally refers to the risk associated with biological materials and/or infectious agents. An international Laboratory Bio risk Management Standard developed under the auspices of the [European Committee for Standardization](#), defines bio risk as the combination of the probability of occurrence of harm and the severity of that harm where the source of harm is a biological agent or toxin. The source of harm may be an unintentional exposure, accidental release or loss, theft, misuse, diversion, unauthorized access or intentional unauthorized release.

**“Laboratory Bio security”** refers to institutional and personal security measures designed to prevent the loss, theft, misuse, diversion, or intentional release of pathogens and toxins.

#### **Risk assessment for TB laboratories:**

The four-tier classification system of bio safety levels (1–4) described in WHO’s Laboratory bio safety manual provides broad guidance on basic concepts of bio safety for the development of code of practice. Risk assessments require careful discretion: on the one hand, underestimating risks may lead to bio safety hazards but, on the other hand, safeguards that are more rigorous than actually needed may impose unnecessary burdens – both financial and in terms of human resources – on a laboratory’s staff and management.<sup>5</sup>

The risk-assessment approach for a TB laboratory considers:

- the bacterial load of materials (such as sputum specimens and cultures), and the viability of TB bacilli;
- route of transmission of TB;
- whether the material handled and the manipulations required for each procedure are likely to generate infectious aerosols;
- the number of man oeuvres for each technique that may potentially generate aerosols;
- The workload of the laboratory and individual staff members;
- the location of the laboratory;
- the epidemiology of the disease and the patient population served by the laboratory;
- the level of experience and the competence of the laboratory’s technicians;
- The health of the laboratory’s workers (especially HIV-positive technicians).

Methods to minimize the production of aerosols:

When preparing smears, wooden sticks or disposable loops are preferable rather than reusable loops, which need to be heat sterilized.

- If a reusable loop is used, it should be heat sterilized in an enclosed micro incinerator or a Bunsen burner. Reusable loops should be cleaned using a sand-alcohol jar before sterilization.

- When preparing a smear using a stick or loop, move it slowly and smoothly to avoid creating an aerosol.
- Do not move or heat-fix smears until they have been completely air-dried.
- Do not forcibly expel infectious liquids or air from a pipette.
- When using a pipette to add a reagent to a potentially infectious liquid, place the pipette against the inner wall of the container and gently expel the fluid.
- Always avoid disrupting a bubble or film in an open culture tube. This may be avoided by replacing the cap, gently tapping the top of the tube, setting the tube aside and allowing any generated aerosols to settle before reopening.
- When centrifuging a specimen or culture, do so in a sealed safety cup or sealed rotor to avoid releasing an aerosol into the centrifuge and laboratory. Always open safety cups or sealed rotors inside a BSC.
- Following centrifuging, vortexing, or shaking specimens or cultures, place containers inside the BSC and leave them undisturbed for at least 10 minutes to allow aerosols to settle before opening.
- Never vortex an open tube; always ensure that screw caps are securely fastened to tubes before vortexing or shaking. Do not vortex tubes with cotton plugs or rubber stoppers.
- Do not mix or suspend infectious materials by repeatedly filling and fully emptying a pipette.
- Allow vortexed tubes to stand for 10–15 minutes to minimize the spread of aerosols, especially if the tubes contain high concentrations of TB bacilli.
- Ensure that when decanting liquids, tubes are held on an angle so that the liquid runs down the side of the tube or discard container to minimize any splashes.
- Only insert the disposable tip of a micropipette into a tube or container, NEVER insert the barrel of a micropipette.

### **Training:**

All staff should have safety training; this should include reviewing the code of practice and the practices and procedures incorporated into the safety manual. The laboratory manager should ensure that staffs are well trained, and that their technical competence in performing different procedures is evaluated periodically. Training should always include information on safe practices to be followed to avoid or minimize risks of inhalation, ingestion and inoculation. Training should also include information on how to properly decontaminate and dispose of infectious material.

### **Level 3 Laboratory: Design, Construction, and Bio safety/ Bio security rules:**

The basic objectives for establishment of containment laboratories for handling Risk Group-III agents which includes: handling of clinical samples, creating safe environment for detection, identification, propagation and manipulation of such

organisms in the laboratory; and maintaining safety of the community and the environment.

The action plan for constructing a BSL-3 laboratory should be formalized during the planning of the proposed laboratory. A detailed flowchart of the construction work must be chalked out and a schematic drawing be prepared to enable detailed planning in consultation with the bio safety experts, engineers and architects to review whether the proposed design is as per the requirements and also complies with the “General Guidelines of Bio safety and Bio security” Complete Heating Ventilation Air Condition (HVAC) design calculations for maintenance of unidirectional airflow and negative pressure as compared to the ambient within the facility and air flow diagrams must be prepared including the placing of the essential safety equipment.

The Laboratory staff working within the BSL III facility must be well-trained in the concepts and practices of the bio safety and bio security. Laboratory workers should be trained to understand and handle any kind of emergency situations within the containment laboratory without panic while ensuring their own safety first and ensuring that laboratory equipment are put into safe operating mode. Filing the incident report, assessment and management must be ensured for future risk assessment and management.

Bio safety cabinets or the primary containments are unique since they are intended to separate the hands and forearms in direct contact with the personnel from the rest of the body, outside the cabinet, by creating an air-barrier thus preventing the microorganism to escape from the inside of the cabinet into the environment.

Secondary containment consists of devices that prevent or mitigate the presence of pathogens within the Level 3 containment facility and avoiding the pathogen to exit the containment zone in order to protect the outside environment from getting contaminated from what is contained and manipulated within the level 3 facility.

Secondary containment consists of facility design with airtight rooms; air handling and filtration within the laboratory building supply and exhaust air should be passed through high-efficiency particulate air filter (HEPA); air locks; showers; proper waste disposal, sewerage treatment, autoclaves/sterilizers; redundant services and equipment like hermitically sealed refrigerated centrifuges.

Tertiary barriers include the physical operation such as concrete walls, fences, security and animal exclusion zones <sup>678</sup>

An Important aspect in clean room design is controlling air-change per hour (ACH), also known as the air-change rate, or ACR. This refers to the number of times each hour that filtered outside air replaces the existing volume in a building or chamber. Each ACH represents a quantity of airflow per hour relative to the overall room volume. For example, if a room's dimensions are 12 feet wide by 20 feet long by 10 feet in height, its volume would be 2,400 cubic feet. One ACH for that room would then equal a ventilation airflow rate of 2,400 cubic feet per hour. However, since airflow is mostly expressed in terms of cubic feet per minute (cfm), it's necessary to divide cubic feet per hour by 60 (minutes per hour) to find the cfm rate. In this

example, 2,400 cubic feet per hour divided by 60 yields an airflow rate of 40 cfm. Therefore, to ventilate a room of 2400 cubic feet at the rate of 8 ACH would require an airflow rate of  $40 \text{ cfm} \times 8 \text{ cfm}$  which equals 320 cfm. In a normal home, an air-conditioner changes room air 0.5 to 2 times per hour. The minimum ventilation flow recommends to about 9.1 air change per hour for a 9-foot ceiling or an 8.2 ACH rate for a 10-foot ceiling space. Based on the risk assessment air change are often set to a single value between 6 and 12 ACH for most of the Level 3 laboratories. However, the ventilation rates in animal facilities usually range between 10 to 15 ACH. The Laboratory quality stepwise implementation tool (WHO, 2015) recommends a minimum of six ACH for Level 3 facilities. This minimum air flow should be maintained regularly whether the laboratory is used for experiments / any manipulations or not. These air changes are established not only for efficient prevention of airborne contamination but also for odour control within the laboratory ambience. The direction of airflow should be designed to move from “clean” areas toward the bio containment space. The laboratory quality stepwise implementation tool recommends to maintain a negative pressure differential of 12.5 pa must be maintained between each pressure zone (WHO, 2015).<sup>9</sup> The level 3 laboratories which are having multiple zones, greater negative pressure must be established in high risk rooms. In order to ensure the pressure difference in all containment rooms control devices and specific monitoring devices shall be installed with alarm devices at the entrance of the containment room.

Another most important feature of Level 3 laboratory includes proper procedures for disposal of biomedical waste. The bio hazardous waste and other liquid waste should be decontaminated with validated chemical treatments and should be autoclaved before letting out the laboratory.

### **Material and technical considerations:**

Control of airborne particulates is mandatory to protect employees from contact with hazardous materials. Bio safety cabinets and Level 3 Laboratory structures are equipped with High Efficiency Particulate Air (HEPA) filters that trap hazardous microorganisms for personnel and collective protection. The risk of infection is linked with the number of particles inhaled and the duration of exposure.<sup>10</sup>

High efficiency particulate air filters are composed of randomly arranged fibreglass with diameters between 0.5 and 2.0  $\mu\text{m}$  and essential factors affecting the filtration are fibre diameter, fibre thickness, and face velocity, if air space between filter fibres is much greater than 0.3  $\mu\text{m}$  and unlike membrane filters, HEPA filters are designed to target much smaller contaminants and the particles. These particles of the same size or bigger are trapped as they get to stick to the fibre through a series of mechanisms viz. interception, impaction, diffusion and sieving.

Bio-Safety cabinets (BSC) should be equipped with H14 HEPA filters having efficiency of 99.995 % at its most penetrating particle size (MPPS) and selected primarily in accordance with type of protection needed. Bio-Safety cabinets should be certified during its installation and regularly according to national / International norms and manufacturer’s instructions. Certification or servicing should include HEPA filter

leaks, down flow velocity profile, face velocity, negative pressure / ventilation rate, air flow pattern, alarm and interlocks. The HVAC HEPA filter should be certified annually <sup>6</sup>. The Laboratory staff working under the Bio safety cabinets should know how to operate and check all the parameters are working satisfactory before starting of the work. Trainings need to be imparted to each technical staff before using the bio safety cabinets. Care should be taken not to disrupt the air curtain while working in the BSC. Prepare a checklist of the requisite items and ensure they are neatly arranged inside the cabinets before starting the work.

### **PPE in Level 3 Laboratories:**

Direct protection against pathogens consists of Personnel Protective Equipments (PPE). The purpose of PPE is to protect him from direct contact with the infectious agent's right from the collection of samples to the manipulation in the laboratory. PPE consist of gloves, gowns, masks, positive pressure ventilation suits.

Personnel protective Equipment is the primary barrier worn by the laboratory personnel and intend to protect from direct contact with the infectious agents manipulated in the laboratory. PPE consist of gloves, gowns, masks, respiratory protection and positive pressure ventilation suits as well as implementation of good laboratory practices. The PPE must be selected based on the risk assessment and technical practices.

#### **Gloves:**

Gloves act as barriers protecting persons by reducing the risk of exposure to infectious materials and also prevent pathogen dissemination through contaminated hands. Double gloving practice is good practice since it allows for removal and replacement of the outer glove without exposing the bare skin. They must be worn over the wrists of the gown. Nitrile gloves are preferred to latex gloves as it provides better microbiological and chemical protection. However, in certain circumstances powder-free latex gloves are preferred as it provides better dexterity and high degree of sensitivity. <sup>11</sup> Gloves should be last piece of PPE to be donned.

#### **Protective clothing:**

Protective clothing is required to protect the wearer against potential contact with infectious substances and avoid germs contamination. In level 3 Facilities, suits with over taped seams are recommended, since virus, bacteria and spores are small enough to penetrate through the openings of sewn seams. These protective suits should be specified for "single use only". However, in practice and after specific training, Level 3 procedures might specify that individual were responsible to their own individual protection and have to specify the periodic control allowing the conditions for reuse of each personal protection.

**Respiratory protection against Bio aerosol:**

The use of respiratory protective equipment (RPE) or class III microbiological safety cabinet is highly recommended based on the risk assessment when manipulating pathogens having drug resistant tuberculosis. Bio aerosol particle size, the airborne agent concentration, and the type of biological agent are the main decision when choosing RPE and every user should be fit tested, trained and certified in the correct use of the respirator. Based upon the risk assessment, powered air-purifying respirators (PAPRs) and valved / unvalved disposable respirators were the type of RPE that could be used in the Laboratory.

Powered air purifying respirators or Positive pressure mask ensures eye protection and protection against airborne pathogen in level 3 laboratory. PAPRs should be stored and as for protective clothing, Level 3 procedures have to define the periodic control allowing the conditions for their reuse.

**Biological waste Management****Autoclave – Maintenance and inspection:**

Bio hazardous waste requires inactivation and steam autoclaving as per the standard methods for all decontamination processes. <sup>6</sup> The autoclaves should have two doors and has to be located or connected exclusively to the containment area. The autoclave should be a steam sterilization one with external steam supply and any item or reagents / solution was considered to be sterile when it was completely free of all microorganisms. The sterilization process depends on the four factors such as size/ type load, time and throughput constraints. The aim is reach a potential surviving of microorganism less than in one million ( $10^{-6}$ ) at the end of the sterilization process. The use of Biological indicator (BI) is considered as “Gold Standard” for to access the efficiency of the sterilization process. *Geobacillus stearothermophilus* is considered as the most resistant microorganism and is used commonly to validate the sterilization process. It is imperative that pathogens do not escape through the autoclave doors – either via cracked or poorly designed gaskets or inadvertently opening both the doors simultaneously. Auto clavable bags and materials should be packed specifically in the chamber to allow easy steam penetration and air removal. They should have proper safety door gasket and safety mechanisms. Autoclaves should be equipped with a biological sealing flange that provide separation and a positive seal between the hazardous side i.e. contained side and safe side of non-contained side meant for the safety of the personnel.

**Disinfection and Decontamination:**

The disinfection process and its efficacy depend on the product used and the targeted pathogen. It depends upon the product concentration; its formulation, water solubility and pH are the critical factors as well as the type of surface, soil, temperature and contact time, humidity and mode of product application. <sup>12</sup> Validated methods for surface disinfection and laboratory decontamination are based on the risk assessment and testing the efficacy of the product. The mechanism of action of these disinfectants are that contain multiple active substances that

inactivated microorganism from reversible processes either through disruption of trans membrane proton motive force or irreversible changes like lysis of cell wall.

### **Surface disinfectant:**

After each active work surfaces and materials need to be decontaminated. Spray the disinfectant and wait for a defined time duration defined by the manufacturer. The efficacy of the surface disinfectant is based on the mechanisms of action of the active disinfectant with the microorganism and also depends upon the microorganism population, concentration of the disinfectant, the presence of interfering substances i.e. the organic material and duration of the contact.

### **Airborne Surface Disinfection:**

Airborne surface disinfection is used in situation such as

1. Decontamination before any periodic maintenance of the laboratory
2. Before entering or exiting large equipment handling
3. Before in situ maintenance of a contaminated device or system
4. After accidental spill over of infectious material.

The different methods of airborne surface disinfection are:

- a) Nebulisation: droplet size is less than 5 µm
- b) Spraying: droplet size ranges from 10 to 50 µm
- c) Flash evaporation: any heated bio cidal product for example hydrogen peroxide vaporizers and is drawn by an airstream into the room

In general practice the room to be disinfected should be made safe, sealed and prepared with a pre cleaning step. The air handling unit should be stopped or bypass configuration before the decontaminating process and also during biocide dispersion and room aeration.

### **Transportation of Risk group 3 Pathogen:**

According to the WHO guidance infectious pathogens including infected biological product / animal / medical clinical waste belonged to category A substance and regulate for transport.

Category A substance were defined as biological material for which can lead to permanent disability, life threatening, or fatal disease in otherwise healthy humans / animal.

Category A infectious substance have to be transported following the United Nations 6.2 specifications and Packing instruction P620. It must be used for all infectious substances and consisted of three layers. The WHO reference document refers to "Packages are marked to provide information about the content of the package, the nature of the hazard, and the packaging standards applied." <sup>13</sup> All markings on packages or over packs shall be placed such that they are easily visible and properly labelled.



Packaging shall display the following information on the outer packing or the over pack:

1. The shipper or senders' consignors name and address.
2. The telephone number of a responsible person, knowledgeable about the shipment.
3. The receivers or consignee's name and address.
4. The United Nations followed by the proper shipping name (UN 2814 "Infectious Substance, affecting Humans" or UN 2900 "Infectious Substance, affecting Animals only")
5. Temperature storage requirements
6. When dry ice or liquid Nitrogen is used: the technical name of the refrigerant, the appropriate United Nations and the net quality.

Two types of labels were needed: Hazard labels and specific handling one for infectious substance, non-infectious miscellaneous dangerous substance, dry ice, liquid nitrogen, cryogenic liquid or orientation label

The following shipping documents were requirements and signed by the shipper:

- For air: the shipper's Declaration of Dangerous Goods
- An invoice including the receivers address plus the number of packages and its contents as well as the weight and value.
- An import and / or export permit and /or declaration if required.
- An airway bill for air transport or equivalent documents for road, rail, and sea shipments.

## **SPILL Response**

### **a. Infectious spills** (outside a biological safety cabinet)

A spill of infectious material outside a BSC is considered a major event. Spills of infectious liquid will generate infectious aerosols. Everyone should immediately vacate the affected laboratory area. The laboratory manager should be informed of the incident immediately, and staff must be prevented from re-entering the laboratory for at least 1 hour to allow aerosols to be removed through the laboratory's ventilation system and allow time for heavier particles to settle.

### **b. Infectious spills** (contained within a biological safety cabinet)

When a spill of infectious material occurs within a BSC, a clean-up procedure should begin immediately, and the cabinet should continue to operate.

1. Place absorbent tissue over the spill area, and apply disinfectant solution liberally.
2. If the walls of the BSC have been splashed, clean with a layer of absorbent paper towel liberally soaked in disinfectant solution.
3. Leave affected areas covered with disinfectant for 30 minutes to 1 hour.
4. Carefully collect contaminated sharps material, and place in a puncture-resistant container for disposal.

5. Any equipment or reusable material (for example, centrifuge buckets) that has been splashed should be cleaned with the same disinfectant.
6. Electrical equipment should be checked carefully before it is used; check the integrity of circuit breakers and earth-fault interrupters.
7. Collect other contaminated material in a sealed bag for appropriate disposal.

### **Laboratory Bio Risk Management:**

The management of bio safety and bio security is based on the application of legal obligations and standard Operational procedures. In practice, Laboratory supervisors are responsible for the implementation of Bio Safety and Bio Security to ensure workers, population, or environment protection. As per the WHO Bio safety and Bio security guidelines 2011 update specifies the requirements for establishing a robust bio risk management system. It provides a comprehensive risk based approach that takes into account the legal requirements and current knowledge on bio safety and bio security.<sup>14</sup>

The operating budget for a high containment facility should be the total cost associated with day to day operations and maintenance. The cost may be high due to many considerations that are driven by the need for bio safety / bio security. The cost for maintaining a high containment laboratory runs more than of a non-containment facility even if great variation is possible depends on the procedure performed, type of experiment or method of decontamination process used.<sup>15</sup> The following are the component should be kept in mind while budgeting.

- HEPA Filter (both Testing and Replacement of Filters)
- BSCs (annual Testing and recertification)
- Biomedical Waste Handling including Autoclaving
- High Energy cost for HVAC due to high air changes rates.
- Annual inspection and testing to confirm the integrity of the facility relative to internal bio safety operating standards.
- High tech systems and equipment for security requirement.

### **Conclusion:**

Stringent regulation in the norms of Bio safety and Bio security have increased in the last few decades and clear decrease in Laboratory Acquired Infection had demonstrated an improvement in the situation. However, such regulation has become more complex for people for people designing or managing Level 3 laboratory. The complexity of the situation to integrate and abide by these new regulations is not only time but cost consuming too. But it can also be counterproductive face to international concurrence.

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