

**SUMMARY REPORT**  
**GUIDANCE ON BIO-SAFETY RELATED TO**  
**TB LABORATORY DIAGNOSTIC PROCEDURES**  
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Contributors: Véronique Vincent, CN Paramasivan, Chris Gilpin, Daniela Cirillo, Jean Joly, Jenny Allen, John Ridderhof, Jon Crane, Knut Feldmann, Moses Joloba, Paul Jensen, Peter van't Erve, Sang Jae Kim, Shanna Nesby, Thomas Shinnick, Andrew Ramsay, Karin Weyer, May Chu, Nicoletta Previsani, Sebastien Cognat.

While TB laboratories have suffered years of neglect, biosafety has even more so. Because of the increased incidence of MDR-TB and the emergence of XDR-TB, laboratories have to handle many dangerous strains. Biosafety conditions should therefore be optimal in laboratories to prevent occupationally acquired TB among laboratory staff. Laboratory acquired TB infections are poorly documented. A retrospective study led in Korea (IJTLD 2007, 11, 138-142) showed that the relative risk of TB compared to the general population was found to be 1.4 in microscopists and 21.5 among technicians carrying out drug-susceptibility testing.

Biosafety in laboratories includes three components: 1) facility layout; 2) adequate equipment regarding specifications and maintenance, and 3) work practice. It is generally admitted that good laboratory practice in laboratories associated with good microbiological techniques are essential in minimizing the generation of infectious aerosols. The WHO laboratory safety manual provides guidance on the basic concepts prevailing in biosafety for the development of national codes of practice. However, recommendations should be detailed and tailored to specific laboratory activities for a more efficient support to countries for the implementation or renovation of laboratories adequate for TB activities. As an example, most BSL3 have been implemented in countries (Northern America, Europe) where norms are well beyond the WHO minimal standards. The present situation contributes to create confusion on the really needed requirements for the design of BSL2 or BSL3. Some experts refer to BSL2 plus or BSL3 plus, whereas these terms are not documented; consultant recommendations may widely differ for a same setting.

Following a CDC/WHO technical consultation held in Atlanta in September 2008 on strategies, approaches, and partnerships for improving global laboratory biosafety, an expert meeting convened in WHO, Geneva in April 2009 to elaborate guidance on biosafety related to TB laboratory diagnostic procedures. The purpose of the meeting was to define consensus on interim guidance on basic principles of laboratory design necessary to assure minimum standards for biosafety capability for TB microscopy, culture, drug susceptibility testing (DST), and molecular testing in different countries and epidemiological settings.

The document is based on risk assessment of each technical procedure and describes the minimum requirements for facilities which can be upgraded according to the risk assessment needs. Risk assessment requires careful judgment as on the one hand underestimation of risks may lead to biosafety hazards and on the other more rigorous safeguards than actually needed may result in unnecessary burden for the laboratory and as a consequence in circumvention of required safeguards. Risk assessment should consider the bacilli load of materials (specimens, cultures), the viability of bacilli, whether the material handled is prone to generate aerosols, the number of maneuvers generating infectious aerosols of each technique, the workload of the laboratory, the epidemiological characteristics of patients, the medical fitness of the laboratory workers.

Experts decided to specify the minimum standards for TB facilities without assigning a biosafety level 1, 2 or 3, according to the risk-based approach recommended in the CWA 15793:2008 laboratory biorisk management standard. The objective is to define the requirements for laboratories ensuring aerosol protection for each technique used for TB diagnosis.

**Recommendations for the minimum laboratory design features for:**

➤ **direct AFB microscopy**

Because sputa are viscous and not prone to generate aerosols, smears performed directly on sputa can be carried out on open bench in an adequately ventilated laboratory, i.e. with 6 to

12 air changes per hour (ACH). Ventilation can be ensured by opening windows if local climatic conditions allow. A fan can be used to ensure the 6 to 12 ACH. When climatic conditions prevent window opening, consideration should be given to mechanical ventilation systems that provide an inward flow of air without recirculation in the room. Natural ventilation does not provide controlled direction of airflow. Unidirectional airflow is desirable. Air conditioners should be correctly placed considering the direction of airflow.

The laboratory should be separated from other areas and access to the laboratory restricted to authorized persons. The bench for smear-preparation should be separated from other benches in the laboratory.

Infectious material may be decontaminated in a pressure cooker or incinerated then to be buried.

➤ **processing specimens for concentrated AFB microscopy and/or culture purpose and/or line-probe assays (LPA)**

The access to the laboratory should be restricted, with administrative and physical separation. Floors, walls, ceilings and benches and furniture should have impervious surfaces.

Work with specimens must be carried out in biological safety cabinets (BSC), class I (EN12469/NSF49) or ClassIIA2 (NSF49) or ClassII (EN12469) equipped with HEPA filters H14. Class IIA1 (NSF49) should be discouraged because they may have positive pressure contaminated ducts and plenums positive to the room. Because class IIB1 and B2 (NSF49) have to be hard-ducted to the outside, the building exhaust system must be precisely matched to the airflow requirements specified by the manufacturer for both volume and static pressure, making certification of these BSCs more difficult.

BSCs must be designed by certified manufacturers, properly installed, regularly maintained and re-certified at least annually on site. In settings with irregular power supply, BSCs must be connected to a suitable UPS system. The use of pre-filters will contribute to lengthen the timelife of the BSC HEPA filters.

Class I BSC:

Option#1: Hard ducted to the outside with an external exhaust fan. Air flow rate is critical to ensure proper operation. A variable-speed fan would be ideal to account/correct for loading of the BSC HEPA filters.

Option #2: Hard ducted to the outside with an internal exhaust fan (i.e., fan is integral to the BSC). Care must be exercised to ensure the internal fan can handle the additional resistance of the duct. This option is possible only if the duct is very short (BSC close to a window and exhaust outside that window) and assumes that the ductwork is not leaking.

Option #3: "thimble fitting" with internal and external extractor fans.

Option #4: Recirculation into the room. Assumes good performance, operation, and maintenance.

For options 1 and 2, an anti blow-back-valve is needed.

For options 1 and 3, the external exhaust fan should be preferably located at the very end of the exhaust pipes and interconnected with the BSC fan so that the BSC cannot be switched on unless the external extractor fan is on. A slightly negative pressure maintained in the duct will prevent leakage of contaminated air into the laboratory.

ClassIIA2 (NSF49) or ClassII (EN12469):

Option #1: "thimble" fitting should be used as described in option 3 above.

Option #2: Recirculation into the room. Assumes good performance, operation, and maintenance.

Windows must not be opened. Air supply is either passive or mechanical without recirculation.

Air conditioners (split level type) have to be correctly placed without air recirculation to other areas within the building.

Centrifuge with aerosol tight buckets must be used.

➤ **manipulation of cultures for identification, drug-susceptibility testing (DST) or LPAs**

The laboratory should be a containment laboratory with double door entry, i.e. with two doors from the general traffic. As above, the access to the laboratory should be restricted, with administrative and physical separation. Floors, walls, ceilings and benches and furniture should have impervious surfaces. However, the room has not to be sealable as there is no need for fumigating the room.

Work with cultures must be carried out in a BSC, class I or ClassIIA2 (NSF49) or ClassII (EN12469), regularly maintained and re-certified at least annually on site, as stated above for work with specimens. However, because of the high load of TB bacilli handled with cultures, options of installation of BSCs vented to the outside are preferred.

There must be a controlled ventilation system that maintains a directional airflow in the laboratory from functionally clean to dirty areas, with a minimum of 6 up to 12 air changes per hour (windows must be hermetically closed). Control of ventilation may be ensured with a supply grill adjustable (preferably with 70-80% efficient pre-filter). It is not essential that the BSC exhaust is filtered through an additional HEPA filter providing the exhaust outlet is away from people and air inlets. Extraction of BSC exhaust air to outside may be used to create pressure differential and air dilution, as long as the BSC is working properly and if the room is designed properly. Air conditioners (split level type) have to be correctly placed without air recirculation to other areas within the building.

Depending on options, energy needs for air supply and control of temperature in the laboratory will widely vary. Local conditions of electrical power supply should be considered as well as budget estimations.

Autoclave must be available on site, in the vicinity of the laboratory for the elimination of tubes/vials with cultures of TB bacilli.

**Other recommendations**

➤ **Work practice**

Workers must adhere rigorously to good laboratory practice and good microbiological technique, and strictly adhere to standard operational procedures.

➤ **Personal protective equipment (PPE)**

Gowns should be worn in the laboratory. Laundering services should be provided at/near the facility. Gloves should be worn while handling specimens and while working in the BSC. Reusing single-use gloves is *not* advised. Proper hand-washing with (liquid) soap and adequate care in the handling of contaminated materials are critical elements of safe laboratory practice.

Other PPE are not usually required. However, risk assessment should be carried out, regularly reviewed and revised when necessary to define the safest possible conditions for work with additional protection barriers. Respirators must not be used in lieu of not maintaining and certifying BSC performance regularly. Use of respirators may be considered if the risk of accidental spillage increases in case of unusual workload, understaffing, .. or to meet specific requirements related to the medical status of the worker. Even if not worn regularly, respirators must be available in laboratories in case of accidental biohazard.

➤ **Medical surveillance**

In accordance with national laws and practices, health surveillance of TB laboratory workers should be performed:

- before enrolment in the TB laboratory;

- at regular intervals thereafter, annually or bi-annually;
- after any biohazard incident;
- at the onset of TB symptoms.

Medical questionnaire should be specific to settings and rely on CXR (plus TST and IGRAs in low prevalence countries).

Workers should be educated about the symptoms of TB and provided with ready access to free medical care if symptoms arise. Confidential HIV counselling and testing should be offered. Options for reassignment of HIV-positive or immuno-suppressed individuals away from the high-risk areas of the TB laboratory should be considered.