



TUBERCULOSIS LABORATORY BIOSAFETY MANUAL



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Executive summary

Following a technical consultation held between the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) in Atlanta, GA, in September 2008 on strategies, approaches and partnerships that could be implemented to improve laboratory biosafety worldwide, an Expert Group meeting was convened at WHO's Headquarters in Geneva, Switzerland, in April 2009 to elaborate guidance on biosafety related to laboratory procedures for diagnosing tuberculosis (TB). Members of the Expert Group submitted Declarations of Interest. These were reviewed by WHO's legal department prior to the meeting. The purpose of the meeting was to reach consensus on the basic principles of laboratory practices and design necessary to establish minimum criteria to ensure biosafety during TB microscopy, culture, drug-susceptibility testing (DST) and molecular testing in different countries and epidemiological settings.

This manual was developed from the Expert Group meeting. The recommendations are based on assessments of the risks associated with different technical procedures performed in different types of TB laboratories; the manual describes the basic requirements for facilities and practices, which can be adapted to follow local or national regulations or as the result of a risk assessment. Risk assessments require careful judgement: on the one hand, underestimating risks may lead to laboratory staff being exposed to biological hazards but, on the other hand, implementing more rigorous risk mitigation measures than are needed may result in an unnecessary burden on laboratory staff and higher costs to establish and maintain the laboratory's infrastructure. Risk assessments should consider the bacterial load of materials (such as specimens and cultures), the viability of the bacilli, whether the material handled is prone to generate aerosols during the activity being assessed, the laboratory's workload, the epidemiology of the disease, and the health of laboratory workers; assessments should also consider other factors that may influence the likelihood or the consequence of exposure to TB.

The intended audience for these recommendations are directors and managers of laboratories and TB programmes as well as the laboratory technicians who test for TB, especially in high-burden, low-resource settings. In this document, the laboratory or section of the laboratory conducting TB testing is referred to as the TB laboratory.

The recommendations are specific to laboratories that follow well defined procedures to test samples potentially containing *Mycobacterium tuberculosis*. For any other combination of pathogen and procedures, a process similar to the one used here could be used to define biosafety precautions.

This manual was approved by WHO's Guidelines Review Committee¹ in May 2012, and explanations are provided throughout the manual where it differs from WHO's *Laboratory biosafety manual*, 3rd edition.² It is intended to inform rather than replace country-level requirements and standards for biosafety. The recommendations do not supersede any local or national rules or regulations.

Review by date: 2017

Participants in the guideline development process

The following contributed to the writing of this manual:

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The following were part of the technical review panel convened at WHO's Headquarters, 22–23 August 2011, Geneva, Switzerland:

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The writers also wish to acknowledge the original contributions of the many professionals who worked on WHO's *Laboratory biosafety manual, 3rd edition*, from which portions of this document have been adapted.

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Abbreviations

ACH	Air exchanges per hour
AFB	Acid-fast bacilli
BSC	Biological safety cabinet
DST	Drug-susceptibility testing
HEPA	High-efficiency particulate air
MDR-TB	Multidrug-resistant tuberculosis
XDR-TB	Extensively drug-resistant tuberculosis

Definitions of terms

Aerosol-generating procedure	High-risk procedures that may increase the potential for generating droplet nuclei as a result of the mechanical force of the procedure (for example, pipetting, vortexing, centrifuging or mixing).
Airborne transmission	The transmission of disease caused by dissemination of droplet nuclei that remain infectious when suspended in air.
Air changes per hour (ACH)	The number of laboratory room volumes of air expelled per hour and replaced with clean air.
Anteroom	A small room leading from one part of a laboratory into another part (for example, leading into a TB-containment laboratory).
Biosafety management plan	The use of a combination of administrative controls, containment principles, laboratory practices and procedures, safety equipment, emergency preparedness, and laboratory facilities to enable laboratory staff to work safely with infectious microorganisms.
Droplet nuclei	Dried-out residue of droplets <5 µm in diameter.
Expelled air (extraction)	Air removed from a laboratory and not recirculated.
Good microbiological technique	Good microbiological technique includes aseptic techniques and other practices that are not uniformly defined but are necessary to prevent contamination of the laboratory with the agents being handled and to prevent contamination of the work with agents from the environment.
Hazard	Anything that has the potential to cause harm, regardless of how likely or unlikely such an occurrence might be.

Hybrid ventilation	A combination of both mechanical and natural ventilation (also called mixed-mode ventilation).
Infectious aerosol	A particulate suspension of infectious agents that has the potential to be inhaled and cause infection.
Laboratory coats	Laboratory coats usually have long sleeves and fasten in the front. These should be worn when working in settings where there is a low risk or moderate risk of tuberculosis (TB).
Laboratory gowns	These gowns should be long sleeved with an elasticised cuff (at least 30mm long) and fasten at the back. Different sized gowns should be available for staff. Gowns should be used when staff work in settings where there is a high risk of TB. When the laboratory technician is standing, the gown must extend below the height of the workbench; the gown should fully cover the technician's lap when he or she is sitting.
Natural ventilation	The use of natural forces to introduce and distribute outdoor air into and out of a laboratory.
Mechanical ventilation system	A mechanical ventilation system uses an exhaust fan to extract air from the laboratory.
Plenum	A plenum is a space in the upper section of a biological safety cabinet where a portion of the air is exhausted from the cabinet and the remainder supplied to the work area.
Risk	A combination of the likelihood and consequences of an event related to a specific hazard.
Risk assessment	The process of evaluating the risk or risks arising from a hazard or hazards, taking into account the adequacy of existing control measures; the process also includes deciding whether the risk is acceptable.
Sterilization	A process that kills all classes of microorganisms and spores.
Ventilation	Ventilation brings outdoor air into a building or a laboratory room and distributes air through the laboratory. For biosafety measures, the purpose of ventilation in buildings is to provide healthy air for breathing by diluting with clean air any potential aerosols generated in the laboratory, and by providing an airflow rate that exchanges air at a given rate.

Introduction

Laboratory biosafety is the process of applying a combination of administrative controls, containment principles, practices and procedures, safety equipment, emergency preparedness, and facilities to enable laboratory staff to work safely with potentially infectious microorganisms; biosafety also aims at preventing unintentional exposure to pathogens or their accidental release. This manual describes the minimum biosafety measures that should be implemented at the different levels of tuberculosis (TB) testing laboratories to reduce the risk of a laboratory-acquired infection.

The recommendations and approaches in this manual should not replace a country's existing biosafety guidance when specific requirements already exist for TB laboratories and procedures. Rather, this manual should be used by laboratory directors, managers, biosafety professionals and programmes to inform and guide the implementation of the minimum requirements for individual laboratories and laboratory networks that perform laboratory testing and procedures associated with TB diagnosis.

Risk assessment is an approach that promotes the consideration of risk and the development of appropriate biosafety practices in laboratories based on the unique combination of test procedures, staff expertise and facilities present in each laboratory. While risk assessment is optimally performed at the level of the individual laboratory, this may not be possible, especially in the tens of thousands of peripheral laboratories that perform relatively lower risk procedures in countries that have a high burden of TB and limited resources for local support and oversight. This manual therefore provides pragmatic recommendations for networks of TB laboratories, and focuses on specific procedures, such as microscopy, culture, drug-susceptibility testing (DST) and molecular testing.

Process of developing the biosafety manual

This manual on biosafety for TB laboratories is adapted from the WHO's *Laboratory biosafety manual*, 3rd edition.² The contents have been shaped by the outcomes of a technical consultation between the World Health Organization (WHO) and the United States Centers for Disease Control and Prevention (CDC) (September 2008), a WHO Expert Group meeting on biosafety as it relates to diagnostic procedures for TB in the laboratory (April 2009) and the consensus achieved by an independent external review (August 2011).

The manual focuses on addressing the specific needs of TB-control programmes, and facilitating the implementation of effective biosafety measures tailored to multi-tiered TB laboratory systems. At the same time, this manual should be read in conjunction with WHO's *Laboratory biosafety manual* since general aspects of laboratory biosafety are covered in that manual, such as the handling of hazardous chemicals not specific to a TB laboratory, fire and other hazards, the transport of infectious substances, and training.

The Expert Group meeting

An Expert Group meeting was convened by WHO in Geneva, Switzerland. Only participants who attended the meeting in person took part in the initial discussion and follow-up discussions where recommendations were made. Individuals were selected to join the Expert Group to represent and balance important perspectives necessary for formulating guidance on laboratory biosafety specifically related to TB. The Expert Group included technical experts, end-users, manufacturers of biological safety cabinets, and biosafety professionals. (Members of the group are listed in Annex 1.)

Declarations of Interest

Members of the Expert Group completed Declarations of Interest. Their responses can be found in Annex 2. These were reviewed by WHO's legal department prior to the meeting, and the statements were summarized by the chair of the Expert Group at the start of the meeting. Representatives from two companies (Peter van't Erve and Scott Kreitlein) were declared to have significant conflicts of interest, and were granted observer status; they did not participate in the development of any recommendations in this manual.

External peer review process

An external technical review of this manual was convened at WHO's Headquarters. Partners'

concerns were addressed where possible and thus informed the manual. A list of people who participated in the peer review process is given in Annex 3.

Rationale and process

The rationale for deviating from previous guidance is explained in the next section. In addition, text boxes with the heading "Expert Group Recommendation" are used to explain where and why current recommendations differ from WHO's *Laboratory biosafety manual*.

The process for synthesizing the evidence and developing these guidelines was reviewed and approved by WHO's Guidelines Review Committee¹ in May 2012. The target date for the next review is 2017.

How this manual differs from WHO's *Laboratory biosafety manual, 3rd edition*

Procedural risk assessment for TB laboratory networks

WHO's *Laboratory biosafety manual*² recommends conducting risk assessments for each individual laboratory to identify appropriate practices, approaches and precautions. This manual differs by providing pragmatic recommendations based on laboratory procedures used specifically to diagnose TB and that are typically conducted by different levels of TB services. These recommendations should guide national TB reference laboratories that manage national or regional networks of TB laboratories towards a better understanding of the risks associated with performing certain procedures; the recommendations should also enable national reference laboratories to implement appropriate biosafety practices within suitable facilities, and ensure that adequately trained staff perform a standard range of diagnostic tests for TB.

In many resource-limited high-burden settings, there is insufficient biosafety expertise available to enable national programmes to conduct individualized risk assessments for all laboratories. To assist these programmes, a consultative, consensus-building process was followed to assess the risks usually found in TB laboratories in these settings, and to develop minimum standards to ensure that laboratory testing for TB is conducted safely.

Standards used to develop the guidelines

In 2008, the European Committee for Standardization published laboratory biorisk management standard CWA 15793,³ which highlighted the key factors that need to be considered to successfully establish and implement a biorisk management system. The standard supports the use of a risk-based approach and does not use risk classifications for biological agents or laboratory safety, or the containment

levels as described in WHO's *Laboratory biosafety manual*. The principles established in CWA 15793 were used to develop this biosafety manual and provide guidance on the minimum requirements for TB facilities conducting diagnostic procedures.

Use of risk-group classifications

The *Laboratory biosafety manual* recommends that countries draw up national or regional classifications of microorganisms by risk group. A risk-group assignment for a pathogen may vary by geography or by strain because of differences in the epidemiological characteristics of the pathogen in a community or the risk of a laboratory-acquired infection.

It is important to recognize that individuals in a laboratory may differ in their susceptibility to developing TB if they become infected, and that only a small fraction of infected individuals develop active disease over their lifetime.⁴ Individuals with reduced immunity, such as that due to HIV infection or pregnancy, may be at a higher risk of developing TB, and additional precautions may be necessary.

Consequently, and in accordance with standard CWA 15793, this manual takes a risk-based approach that does not use risk classification for biological agents or laboratory safety, or the containment levels as described in the *Laboratory biosafety manual*.

Designation of biosafety level

The *Laboratory biosafety manual* describes a four-tier classification system of biosafety. Biosafety levels are based on the composite of the design features, construction, containment facilities, equipment, practices, and operational procedures required for working with agents from various risk groups. It is often erroneously assumed

that a microorganism assigned to a particular risk group (for example, Risk Group 3) requires a laboratory of an analogous biosafety level (that is, Biosafety Level 3) for work to be conducted safely. However, a higher level or lower level of biosafety may be more appropriate based on the specific procedure being performed and other factors (see Chapter 1 in this manual).

The *Laboratory biosafety manual* states that the biosafety level assigned to the specific work being done is driven by professional judgement based on an assessment of the risk rather than by automatic assignment of a laboratory biosafety level according to the particular risk group assigned to a pathogenic agent. The approach developed in this manual builds on the guidance in the *Laboratory biosafety manual* and uses a procedural approach to risk assessment. TB is predominantly an airborne infection.⁴ Rather than assigning a particular biosafety level to certain procedures, this manual defines the **minimum requirements** necessary to mitigate risks associated with performing a particular procedure, taking into consideration the risk of aerosolization, the facilities available, and the equipment, practices and procedures required to limit infection.

Mitigating the risks

Using biological safety cabinets

Laboratory-acquired infections often result from the unrecognized production of infectious aerosols containing tubercle bacilli. For laboratories conducting TB testing, the most important hazard (or risk) is the generation of infectious aerosols since infection with *Mycobacterium tuberculosis* occurs primarily through the inhalation of infectious aerosols, although it can also occur through direct inoculation or ingestion. Infectious aerosols may be generated during the manipulation of liquids containing tubercle bacilli. After settling on surfaces, droplet nuclei are not reaerosolized and are considered noninfectious.^{5,6,7} That is, *M. tuberculosis* bacteria are usually transmitted only through air, not by surface contact.⁸

Two important considerations in evaluating the risk of aerosolization are the bacillary load of the materials being manipulated and the likelihood of generating infectious aerosols from the material. For sputum specimens (the most common specimen investigated for TB), the bacillary load ranges from 0 (this is the case for up to 90% of diagnostic samples) to 10^3 – 10^4 /ml in a sputum specimen with a scanty smear grading, to 10^6 /ml in a sample with a 3+ grading.⁹ In a culture grown from sputum specimens, the bacillary load may exceed 10^8 /ml. Due to the viscosity of sputum specimens, the likelihood of generating an infectious aerosol while manipulating such specimens is much lower than the likelihood of generating an infectious aerosol from a liquid culture. Consequently, the risk associated with manipulating a direct sputum sample is significantly less than that associated with handling cultured material.

This manual differs from WHO's *Laboratory biosafety manual* in concluding that biological safety cabinets (BSCs) are not mandatory when performing direct sputum-smear microscopy. The Expert Group recognized that infections with *M. tuberculosis* are a proven risk to laboratory personnel as well as others who may be exposed to infectious aerosols generated by certain procedures. There remains limited evidence on the risks associated with specific procedures in the TB laboratory. A retrospective study in Korea¹⁰ showed that the relative risk of becoming infected with TB for technicians performing direct acid-fast bacilli (AFB) smear microscopy compared with the general population was 1.4 (95% confidence interval [CI], 0.2–10.0); the risk was 21.5 (95% CI 4.5–102.5) for technicians performing drug-susceptibility testing (DST). The Expert Group concluded that BSCs are not mandatory for performing direct sputum-smear microscopy. The Expert Group found that with good microbiological technique, direct sputum-smear microscopy entails a low risk of generating infectious aerosols, and such procedures may therefore be performed on an open bench, provided that adequate ventilation can be assured. This recommendation is consistent with previous guidance.^{11,12}

1. Risk assessment and the classification of TB laboratories

1.1 Risk assessment for TB laboratories: what is it?

The four-tier classification system of biosafety levels (1–4) described in WHO's *Laboratory biosafety manual*² provides broad guidance on basic concepts of biosafety for the development of national and international codes of practice. The challenge for managers of TB programmes and staff at laboratories, particularly in resource-limited settings, has been to interpret the generic risk-group assignments and biosafety levels into specific precautions relevant to a country's activities. As a result, the use of biosafety levels 1–4 when describing the needs of TB laboratories has led to confusion about what precautions are necessary.

Decisions about which are the most appropriate biosafety measures for a specific laboratory should be undertaken using an approach based on risk assessment that considers the different types of procedures performed by the laboratory. Risk assessments require careful judgement: on the one hand, underestimating risks may lead to biosafety 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.

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 manoeuvres 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).

In addition, the ability of laboratory staff to control hazards must be considered. Their ability will depend on the competence, technical proficiency, and microbiological practices of all laboratory technicians; the operational integrity of containment equipment; the facility's safeguards; and the availability and proper use of appropriate standard operating procedures. *Box 1* provides details on conducting a procedural risk assessment. *Table 1* and *Table 2* summarize the considerations used to assess risks for TB laboratories in general, and risks associated with performing different procedures in TB laboratories. These considerations were used by the Expert Group to determine the minimum biosafety requirements necessary for performing different procedures in TB laboratories.

The laboratory manager is responsible for ensuring that the minimum biosafety precautions are implemented as described in this manual, and that appropriate standard operating procedures, equipment and facilities are available to support the work being undertaken. The laboratory's biosafety precautions should be reviewed periodically and revised when necessary, particularly after new procedures or techniques are introduced.

To ensure the safest possible conduct of work, the results of risk assessments should dictate the appropriate laboratory equipment, personal

protective equipment and design features of the facilities which need to be incorporated into the standard operating procedures for each procedure undertaken in the laboratory,

1.2 Hazard identification

A hazard is anything that has the potential to cause harm, regardless of how likely or unlikely it might be to occur. A hazard may be a physical situation (such as a fire or explosion), an activity (such as pipetting) or a material (such as aerosols containing infectious bacilli). Unless hazards are identified effectively, it is not possible to accurately assess the risks associated with the facility and its activities.

1.3 Determining risks

Risk is the combination of the likelihood that a specific hazard will be encountered and the consequences of an event related to that specific hazard. Risks should be identified and categorized, and a determination should be made about which risks need to be controlled or minimized. The analysis of aerosolization risks described in this manual has led to the development of minimum biosafety requirements necessary for performing different procedures in TB laboratories.

Box 1. How to conduct a procedural risk assessment for a tuberculosis (TB) laboratory

The evaluation of risk is a subjective process requiring consideration of the hazardous characteristics of microorganisms and procedures; sometimes, judgements are based on incomplete information. A risk assessment is simply a careful examination of what in your work could cause harm to people; this assessment allows you to weigh up whether you have taken enough precautions or should do more to prevent harm.¹³ Laboratory workers and others have a right to be protected from harm caused by a failure to take reasonable control measures. While there is no standard approach for conducting a risk assessment, the following steps can be used to guide the process.

1. **Identify the inherent hazards.** Different strains of TB carry different levels of individual and community hazards. Drug-resistant strains of TB, in particular multidrug-resistant (MDR)^a and extensively drug-resistant (XDR)^b strains, carry higher risks due to the greater harm that would be caused to an individual who became infected since treatments may be limited or less effective. Laboratories working with strains that are more likely to be drug resistant, whether due to the selection of patients or the prevailing epidemiological situation, should consider establishing higher level precautions.
2. **Decide who might be harmed and how.** The main procedural risks in a TB laboratory are related to the generation of aerosols that could be inhaled by laboratory workers. These aerosols are associated with certain procedures, and may be more likely to be generated depending on the frequency of testing or the workload, the consistency of the material and its predisposition to aerosolize (for example, viscous liquids versus dry solids), the bacillary load of the materials, and the viability of the bacilli. It is also important to recognize that individuals in the laboratory may differ in their susceptibility to TB. Individuals with reduced immunity – caused by certain medications, HIV infection or pregnancy – may be at higher risk of becoming infected with TB. If individuals with impaired immunity work in a TB laboratory, it is important to consult with an occupational physician knowledgeable about TB.
3. **Evaluate the risks and decide on precautions.**
 - a. **Determine the suitability of the physical structure.** The final determination of the appropriate level of TB risk and of any additional precautions that may be necessary requires a comprehensive understanding of the practices, safety equipment and safeguards in the facility. If a risk assessment indicates there is a need to alter the safeguards specified for the selected level of TB risk, a professional with experience in biorisk management should validate this judgement independently and provide the laboratory manager with relevant information and recommendations before the facility's secondary barrier is strengthened.
 - b. **Evaluate the staff's proficiency in following safe practices.** The protection of laboratory workers and others associated with the laboratory will depend ultimately on the laboratory workers themselves. In conducting a risk assessment, the laboratory manager should ensure that workers have acquired technical proficiency in using good microbiological practices and the equipment required for the safe handling of potentially infectious material, and that they have developed habits that sustain excellence while performing these practices. Ensuring that a person is competent, has experience in handling infectious agents, is proficient in using aseptic techniques and biological safety cabinets (BSCs), has the ability

to respond to emergencies, and is willing to accept responsibility for protecting himself or herself and others offers important assurance that a laboratory technician is capable of working safely.

- c. **Evaluate the integrity of safety equipment.** The laboratory manager should ensure that the necessary safety equipment is available, has been certified to be operating properly by a qualified professional, and is routinely checked for integrity. For example, a BSC that has not been certified represents a potentially serious risk to the workers who use it and to others in the laboratory. In addition, laboratory workers should be trained to perform simple daily checks to ensure that equipment in the laboratory is functioning properly. For example, they should check that the caps on the centrifuge buckets are not cracked and that O-rings are in place and undamaged. Simple daily checks should be performed on BSCs to ensure that air is flowing correctly into each cabinet.
4. **Record your findings and implement them.** The findings of the risk assessment and the precautions that need to be taken should be documented as part of all standard operating procedures. The results of the risk assessment will show that a proper check was made, and persons at risk because they perform particular procedures will have been identified. Although hazards such as producing aerosols cannot be completely eliminated in the TB laboratory, reasonable precautions that leave only a low residual risk must be implemented.
5. **Review your assessment and update it if necessary.** Potentially risky procedures and practices should be reviewed periodically; this should become standard protocol in order to promote and ensure safe laboratory practices. Biosafety precautions already in place should be reviewed at least annually; they should be revised when necessary following a risk assessment, and always after the introduction of a new procedure or technique.

^a MDR-TB: This refers to TB caused by strains of *Mycobacterium tuberculosis* that are resistant to at least isoniazid and rifampicin.

^b XDR-TB: This refers to MDR-TB in which the organism is also resistant to a fluoroquinolone and at least one second-line injectable agent (amikacin, kanamycin or capreomycin).

Table 1. Factors to be considered when conducting a procedural risk assessment to determine the precautions needed for laboratories receiving samples for tuberculosis (TB) testing

Factors relevant to all TB laboratories	Considerations
Pathogenicity	Untreated TB has a mortality rate of 30–50%; about 30% of persons with prolonged exposure to an infectious TB case become infected; 5–10% of infected persons develop TB
Primary route of transmission	Inhalation of infectious droplet nuclei
Secondary routes of transmission (uncommon in laboratory)	Ingestion, direct inoculation
Stability	Tubercle bacilli can remain viable for extended periods in the environment
Infectious dose	Estimated to be 10 bacilli by inhalation for humans; ^a in animal studies, infectious doses range from 1 organism to 1000 organisms, depending on the susceptibility of the species
Susceptibility of immunocompetent persons to developing TB	5–10% of infected immunocompetent persons develop TB during their lifetime
Susceptibility of immunocompromised persons to developing TB	5–10% of infected immunocompromised persons develop TB per year
Risk of community-acquired TB in high-burden settings	High
Effective vaccine	No, none available
Effective treatment for strains susceptible to different medicines	Yes
Effective treatment for MDR strains	Yes, but more difficult to treat than susceptible strains
Effective treatment for XDR strains	Few treatment options

MDR, multidrug-resistant; XDR, extensively drug-resistant.

^a Number extrapolated from animal studies.

Table 2. Factors to be considered when conducting a risk assessment to determine the precautions needed for specific procedures performed in different levels of tuberculosis (TB) laboratory

Factors that vary according to procedure or type of laboratory	Procedure		
	Direct sputum-smear microscopy	Processing specimens for culture	Manipulate cultures, DST
Relative risk (95% CI) of laboratory-acquired TB in laboratory staff compared with non-laboratory workers ¹⁰	1.4 (0.2–10.0)	7.8 (1.7–34.9)	22 (4.5–102.5)
TB bacillary load of materials manipulated	Variable	Variable	Uniformly high: >10 ⁸ /ml
Viability of TB bacilli	Uncertain but assumed high	Processing can kill 90% of TB bacilli	High
Likelihood that the manipulations required for each procedure are prone to generating infectious aerosols ^{9,10}	Low	Moderate	High

CI, confidence interval; DST, drug-susceptibility testing.

Based on their assessment of the procedural risks usually encountered in TB laboratories in resource-limited high-burden settings, the Expert Group developed minimum requirements needed to ensure safety for staff conducting different procedures used to diagnose TB. Whenever possible, every laboratory should conduct their own risk assessment to determine which additional measures need to be put in place to provide suitable protection to their laboratory technicians.

The recommendations described in this document are meant to inform national policies and do not supersede or replace any national rules or regulations. The minimum requirements needed to reduce risks in TB laboratories are described in chapters 3, 4 and 5.

1.4 Monitoring risks and mitigation measures

The laboratory manager should conduct regular audits to monitor risks and control measures. These can be done by reviewing reports of corrective actions taken after problems were identified

earlier, thoroughly investigating incidents or accidents and implementing preventive measures, and ensuring that adequate resources are provided to maintain the necessary level of precautions. Documenting the risk assessment process and identifying mitigation measures are integral and important steps to ensure that biosafety measures selected and implemented are constantly improved.

The following events should trigger either a new procedural risk assessment or review of an existing one:

- commencement of new work or changes to the programme of work, or alterations to workflow or volume;
- new construction of or modifications to laboratories, or the introduction of new equipment;
- altered staffing arrangements (including the use of contractors and other non-core personnel, or the need to accommodate visitors);

- alterations to standard operating procedures or working practices (for example, changes in disinfection or waste management protocols, provision of personal protective equipment and its use, changes to entry or exit protocols);
- an incident in the laboratory (for example, a major spill);
- evidence of or suspicion of a laboratory-acquired infection;
- consideration of emergency responses and contingency planning requirements;
- the existing management system review process (for example, annually or at other appropriate and predetermined frequencies).

1.5 Employee occupational health programme

Employee occupational health programmes should promote a safe and healthy workplace. This is accomplished by minimizing any exposures, promptly detecting and treating exposures, and using information gained from laboratory incidents and accidents to enhance safety precautions. A baseline medical check-up and provision for regular follow-up should be considered for all staff prior to commencing work in the TB laboratory. The medical personnel providing occupational health services should be knowledgeable about the nature of potential health risks in TB laboratories and have access to experts for consultation. Medical services should be readily available to allow timely and appropriate evaluation and treatment.

1.6 Classification of TB laboratories

TB laboratory facilities can be classified into three main levels of procedural risk, based on the activities being performed and their associated risks:

- low TB risk
- moderate TB risk
- high TB risk (such as a TB-containment laboratory).

The probability of aerosols being generated is a key factor to consider in determining the level of risk and the necessary mitigation or control measures. Direct sputum-smear microscopy, when performed using good microbiological techniques, entails a low risk of generating infectious aerosols, and this procedure may therefore be performed on an open bench, provided that adequate ventilation can be assured. Guidance and recommendations for safe practices to be followed when performing direct-smear microscopy have been described in WHO's guidelines on laboratory services for TB control.^{11,12}

Procedures that liquefy specimens – such as those used during specimen digestion and processing for culture inoculation, direct DST or direct line-probe assays – have an increased risk of generating aerosols when compared with other techniques even when good microbiological technique is used; thus, these procedures should be performed in a BSC. Manipulation of cultures for indirect DST or line-probe assays involves procedures where a high concentration of bacilli are present and a high risk of aerosol generation exists; such activities must be performed in a BSC within a TB-containment laboratory. The appropriate activities, the assessment of procedural risk, and the minimum level of precautions required for the different levels of TB laboratories are presented in *Table 3*.

EXPERT GROUP RECOMMENDATION

The Expert Group noted that WHO's Laboratory biosafety manual² recommends that a biological safety cabinet be used whenever infectious samples are handled. The Expert Group found that with good microbiological technique, direct sputum-smear microscopy entails a low risk of generating infectious aerosols, and this procedure may therefore be performed on an open bench, provided that adequate ventilation can be assured. This recommendation is consistent with previous guidance.^{11,12}

Collecting sputum specimens from patients is potentially hazardous and should not be performed in the laboratory. A well ventilated area that is separate from the laboratory should be identified for sputum collection. This area should preferably be outdoors.

Table 3. Risk precaution levels, associated laboratory activities and risk assessment for tuberculosis (TB) laboratories

Risk level of TB laboratory ^a	Laboratory activities	Assessment of risk
Low risk	Direct sputum-smear microscopy; preparation of specimens for use in an automated nucleic acid amplification test cartridge (such as the Xpert MTB/RIF assay)	Low risk of generating infectious aerosols from specimens; low concentration of infectious particles
Moderate risk	Processing and concentration of specimens for inoculation on primary culture media; direct DST (for example, line-probe assays on processed sputum)	Moderate risk of generating infectious aerosols from specimens; low concentration of infectious particles
High risk (TB-containment laboratory)	Culture manipulation for identification; DST or line-probe assays on cultured isolates	High risk of generating infectious aerosols from specimens; high concentration of infectious particles

DST, drug-susceptibility testing.

^aThe risk level refers to how likely it is that someone in the laboratory will become infected with TB as a result of procedures performed in the laboratory.

2. Essential biosafety measures for TB laboratories

All TB laboratories, regardless of the procedures being undertaken, should enact a set of essential biosafety measures to minimize risks. These measures affect:

1. codes of practice
2. equipment
3. laboratory design and facilities
4. health surveillance
5. training
6. waste handling.

Depending on the specific tests conducted by the laboratory and the results of a procedural risk assessment, additions and modifications to the measures described below may be made to accommodate different levels of risk. (See Chapters 3, 4 and 5 for more details.).

2.1 Codes of practice

A code of practice describes the laboratory practices and procedures essential for implementing good (that is, safe) microbiological technique. The laboratory manager should use the code of practice to develop written descriptions of procedures that should be followed to perform work safely. This safety or operations manual should also identify known and potential hazards, and specify practices and procedures to minimize the risks associated with such hazards.

Specialized laboratory equipment should always be accompanied by, but can never replace, appropriate procedures and good microbiological technique.

The most important concepts to be included in codes of practice are outlined below.

2.1.1 Laboratory access

- The international biohazard warning symbol and sign must be displayed on the laboratory door.

- Only authorized persons should be allowed to enter the laboratory's working areas.
- Children should not be authorized or allowed to enter the laboratory's working areas.

2.1.2 Responsibilities of the laboratory manager

- It is the responsibility of the laboratory manager to ensure that a biosafety management system is developed and adopted, as well as a safety or operations manual and a set of standard operating procedures.
- The manager should ensure that staff are trained and their technical competence evaluated for performing different procedures.
- Personnel should be advised of special hazards and be required to read the safety (or operations) manual as well as follow standard practices and procedures. The manager should make sure that all personnel have read the appropriate manuals and have signed a statement declaring that they have understood them. A copy of the most recent safety or operations manual, with its date of issue, should be available in the laboratory.
- Systems for heating, ventilation, air and containment (directional airflow) must have a permanent maintenance plan to ensure they always function properly.

2.1.3 Personal protective equipment

- Protective laboratory clothing must be worn at all times while staff are working in the laboratory. Protective clothing must not be worn outside the laboratory area (for example, in canteens, coffee rooms, offices, libraries, staff rooms and toilets). Laboratory coats and gowns must be stored separately from personal clothing. Clean

gowns and used gowns must be stored in different areas of the laboratory. Laboratory coats and gowns should be changed at least weekly, but laundering should not occur at home.

- Laboratory gowns should have long sleeves and elasticized cuffs (at least 30 mm long); they should fasten at the back. Different sizes of gowns should be available for staff. Gowns must be worn when working in a laboratory where there is a high risk of TB infection.
- Laboratory coats usually have long sleeves and fasten in the front. Different sizes of laboratory coats should be available for staff.
- Gloves must be worn for all procedures that involve direct contact, or may involve accidental contact, with sputum, blood, body fluids and other potentially infectious materials. After use, gloves should be removed aseptically and hands washed.
- Personnel must wash their hands after any overt contamination, after completing work during which infectious materials were handled, and always before they leave the laboratory's working areas. Personnel should thoroughly lather their hands with soap, using friction, for at least 15 seconds; rinse them in clean water; and dry them using a clean paper towel. Automated or hands-free taps (faucets) are preferable. However, where these are not available, a paper towel should be used to turn off the tap to avoid recontaminating clean hands.
- Eating, drinking, smoking, applying cosmetics and handling contact lenses are prohibited in the laboratory.
- Storing food or drink anywhere in the laboratory's working areas is prohibited.
- Open-toed footwear must not be worn in the laboratory.
- Mobile telephones should not be used in the laboratory.

2.1.4 Procedures

- All procedures must be performed in such a way as to minimize or prevent the formation of aerosols and droplets (see Box 2).
- Mouth pipetting must be strictly prohibited.
- No materials should be placed in the mouth. All labels used in the laboratory must be self-adhesive.
- The use of needles and syringes should be limited, and they should never be used as a substitute for pipetting.
- Written documentation that may be removed from the laboratory must be protected from contamination.
- All contaminated materials, specimens and cultures must be decontaminated appropriately before disposal or cleaning for reuse.
- All accidents, spills and potential exposures to infectious materials must be reported to the laboratory manager. Records of such incidents and corrective actions taken need to be maintained for future prevention.
- Standard operating procedure for handling accidents and spills must be developed and be available in the laboratory. Practical training must be provided at least annually to ensure the procedure is adopted and becomes an automatic response.
- Packing and transportation of samples must follow applicable national or international regulations.
- Standard operating procedures must be developed and staff trained to be competent in their use. Manuals explaining the procedures must be readily available in different parts of the laboratory. Procedures should be reviewed annually. Standard operating procedures should include details of risk assessments, and the mitigation and control measures identified and implemented.

Box 2. How to minimize the production of aerosols

The use of engineering controls (for example, biological safety cabinets [BSCs] and room ventilation) and personal respiratory protection (such as respirators) can help prevent laboratory-acquired tuberculosis (TB) infections associated with the inhalation of infectious aerosols. However, the most important consideration in reducing the risk of infection in the laboratory is to minimize the production of aerosols. **Some of the practical steps for reducing the creation of aerosols are applicable to all TB laboratories, while others are applicable only to laboratories considered to be moderate risk or high risk.**

For all laboratories

- 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 microincinerator 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.

For moderate-risk and high-risk TB laboratories

- Do not forcibly expel infectious liquids from a pipette.
- Do not forcibly expel air from a pipette into potentially infectious liquids.
- 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.

2.1.5 Work areas

- The laboratory should be divided into “functionally clean” and “potentially contaminated” areas, with the clean areas reserved for administrative and preparatory work. Access to the clean areas and the contaminated areas must be controlled and enforced by the laboratory’s manager.
- The laboratory should be kept neat, clean and free of materials and equipment not used for performing routine work. Equipment and materials that are not being used or that do not work should be removed from work areas.
- Work surfaces must be decontaminated after any spill of potentially infectious material and at the end of each work session. (See the section on spills in Chapter 8 for additional information.)

2.2 Equipment

Equipment should be selected to take certain general principles into account – that is equipment should be:

- designed to prevent or limit contact between the operator and the infectious material;
- constructed of materials that are impermeable to liquids and resistant to corrosion;
- fabricated to be smooth and without sharp edges and unguarded moving parts;
- designed, constructed and installed to facilitate simple operation, and provide for easy maintenance, cleaning, decontamination and certification testing; glassware and other breakable materials should be avoided, whenever possible.

In addition to the specific equipment needed for laboratories with different risk levels (described in Chapters 3, 4 and 5) more information on BSCs is given in Chapter 6, and information on other safety equipment is given in Chapter

7. In laboratories where the risk of infection is considered to be moderate or high, the BSC provides the primary containment of infectious aerosols generated by certain procedures.

2.3 Design and facilities

The proper design and construction of laboratory facilities contributes to the protection of all laboratory workers and provides a barrier that protects the community from TB aerosols that may be created with the laboratory. Specific features of the laboratory, including separated laboratory areas and a ventilation system, are secondary containment measures. The secondary barriers that are recommended for a laboratory depend on the procedures conducted and their associated risk of transmission.

In a low-risk TB laboratory, secondary barriers include separating the laboratory’s work area from the public, ensuring proper waste disposal, and providing hand washing facilities. In a high-risk TB laboratory, the presence of an anteroom separating the laboratory from public areas serves as an additional secondary barrier.

Laboratory managers are responsible for providing facilities commensurate with the laboratory’s functions and risk level.

When designing a TB laboratory, special attention should be paid to common issues that are known to pose safety problems, including the use of permeable surfaces, overcrowding in work areas, the ability of unauthorized people to enter the laboratory, the flow of personnel and patients near or inside the laboratory, and poorly designed workflow.

The following list identifies the basic recommended design features of a TB laboratory.

- Adequate ventilation and directional airflow are required.
- Ample space must be provided for the safe conduct of laboratory work, and for cleaning and maintenance.

- Walls, ceilings and floors should be smooth and easy to clean. Floors should be slip-resistant.
- Bench tops should be impervious to water, and resistant to the chemicals and disinfectants normally used in the laboratory; they should also be impervious to moderate heat.
- Illumination should be adequate for all activities. Undesirable reflections and glare should be avoided. Curtains must not be used.
- Laboratory furniture should be sturdy. Furniture should be made of impervious materials and able to be decontaminated easily. No cloth-covered furniture should be used.
- Open spaces between and under benches, cabinets and equipment should be accessible for cleaning.
- Storage space must be adequate to hold supplies for immediate use and prevent clutter on bench tops and in corridors outside the laboratory. Additional space for long-term storage should be provided and located conveniently outside work areas.
- An area for the safe preparation, handling and storage of acids, stains and solvents should be established.
- Facilities for storing outer garments and personal items should be provided outside work areas.
- Facilities for eating and drinking, and for rest, should be provided outside work areas.
- A sink for handwashing and soap should be provided in each room in the laboratory, preferably near the exit. Automated or hands-free taps are recommended. A dispenser for paper towels should be near the sink.
- Laboratory doors should have a glass window panel and appropriate fire ratings; they should be self-closing.
- There should be a reliable and adequate electricity supply.

2.4 Training

Human error and poor technique can compromise the best safeguards put in place to protect laboratory workers. Well informed, competent and safety-conscious staff are essential for preventing laboratory-acquired infections, incidents and accidents.

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 staff are trained, and that their technical competence in performing different procedures is evaluated. 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.

2.5 Waste handling

Waste-management procedures must comply with all pertinent local or national requirements and regulations. Waste is anything that is to be discarded. The overriding principle in minimizing risks from waste is that all infectious materials should be decontaminated, incinerated, prepared to be buried or autoclaved. Discard bags should be used to segregate waste. Most glassware, instruments and laboratory clothing will be reused or recycled.

The principal questions to be asked before any objects or materials are removed from a laboratory are:

- Have the objects or materials been effectively decontaminated or disinfected using proper procedures?
- If not, have they been packaged in a closed container or bag for immediate on-site incineration or autoclaving?

- Does the disposal of the decontaminated material involve any additional potential hazards or risks, biological or otherwise, to those who carry out the disposal procedures or who might come into contact with the items outside the facility?

Incineration is useful for disposing of laboratory waste, regardless of whether it has been decontaminated. Incinerating infectious materials is an alternative to autoclaving only if the laboratory manager can ensure that proper incineration procedures are followed.

2.5.1 Incineration

To incinerate hazardous waste properly requires an efficient means of controlling the temperature, and a secondary burning chamber. Many incinerators, especially those with a single combustion chamber, are unsatisfactory for dealing with infectious materials or plastics. If this type is used, such materials may not be completely destroyed, and the effluent from the chimney may pollute the atmosphere with microorganisms, toxic chemicals and smoke. However, there are many satisfactory configurations for combustion chambers. Ideally the temperature in the primary chamber should be at least 800°C, and in the secondary chamber at least 1000°C. In order to achieve the required temperatures, the incinerators must be properly designed, operated and maintained.

Materials for incineration, even if they have been decontaminated, should be transported to the incinerator in bags, preferably plastic. Attendants should receive proper instruction in loading the incinerator and controlling the temperature. The efficient operation of an incinerator depends on having the right mix of materials in the waste being incinerated.

There are concerns about the possible negative environmental effects of incinerators, and efforts continue to make incinerators more environmentally friendly and energy efficient. Autoclaves provide an alternative to incineration.

2.5.2 Autoclaving

Separate autoclaves should be used to sterilize solutions or glassware (clean materials), and to decontaminate infectious materials.

The following materials are suitable for autoclaving:

- instruments, glassware, media or solutions for sterile use in the general diagnostic TB laboratory;
- mycobacterial cultures for waste disposal;
- all infectious materials from TB-containment laboratories where mycobacterial culture is performed.

The time, temperature and pressure should be recorded each time the autoclave is run to monitor whether it is functioning properly. Biological indicators should be used regularly to validate the ability of the autoclave to achieve sterilization.

2.5.3 Disinfection

The killing action of disinfectants depends on the population of organisms to be killed, the concentration used, the duration of contact, and the presence of organic debris.

Proprietary disinfectants recommended as suitable for use in TB laboratories are those containing phenols, chlorine or alcohol. These are usually selected depending on the material to be disinfected.

Phenol

Phenol should be used at a concentration of 5% in water. However, inhalation and dermal exposure to phenol is highly irritating to the skin, eyes and mucous membranes. Ingestion of phenol is considered to be toxic. Because of its toxicity and odour, phenol derivatives are generally used in place of phenol.

Phenol solutions are used for decontaminating equipment and single-use items prior to disposal.

Chlorine

Chlorine is widely available. Sodium hypochlorite solutions (domestic bleach) contain 50 g/l available chlorine, and should therefore be diluted to 1:50 or 1:10 in water to obtain final

concentrations of 1 g/l or 5 g/l. Bleach, either in stock or in solution, must be stored in a well ventilated, dark area. In good storage conditions, the 50g/l solution may last as long as 3 months; diluted solutions should be prepared daily.

Bleach can be used as a general purpose disinfectant and for soaking contaminated metal-free materials; because it is highly alkaline, it can corrode metal.

Alcohol

Alcohols, ethanol (denatured ethanol, methylated spirits) or isopropyl alcohol are used at a 70% solution. Alcohols are volatile and flammable, and must not be used near open flames. Solutions should be stored in proper containers to avoid evaporation. Bottles with alcohol-containing solutions must be clearly labelled so they are not autoclaved.

A solution of 70% alcohol can be used on laboratory benches and BSCs for routine decontamination. A major advantage of aqueous solutions of alcohols is that they do not leave any residue on treated items. When hands become contaminated, a rinse with 70% ethanol or isopropyl alcohol followed by thorough washing with soap and water is effective.

Peracetic acid

Peracetic acid is characterized by rapid action against all microorganisms. Special advantages of peracetic acid are that it lacks harmful decomposition products, enhances removal of organic material, and leaves no residue. Working solutions (2% concentration) are stable for 48 hours after preparation.

2.6 Disposal procedures for contaminated materials

A system for identifying and separating infectious materials and their containers should be adopted. Categories may include:

- uncontaminated (non-infectious) waste that can be reused, recycled or disposed of in the same way as general household waste;

- contaminated (infectious) sharps, such as broken glass, syringes and slides;
- contaminated infectious material to be disposed of by burying, incinerating or autoclaving.

2.6.1 Broken glass and glass slides

Broken slides and used slides must be disposed of in a sharps container. Containers for sharps disposal must be puncture-proof, have a fitted lid, and must not be filled to capacity. When they are three quarters full, they should be placed in containers for infectious waste and incinerated. Containers for sharps disposal must not be discarded in a landfill unless they have been incinerated or autoclaved. Used slides must not be reused.

2.6.2 Contaminated or potentially infectious materials for disposal

All positive TB cultures must be autoclaved before disposal. An autoclave should be available close to or in the laboratory where TB culture is performed.

All contaminated (that is, potentially infectious) materials except sharps should be placed in disposable plastic bags before being transported for incineration. If possible, materials from TB laboratories should not be discarded in a landfill even after decontamination.

Discard containers, or pans or jars that are unbreakable (for example, plastic), should be placed at every work station. Appropriate disinfectants effective against *M. tuberculosis* must be used; waste materials must remain in contact with the disinfectant (that is, they must not be protected by air bubbles) for the appropriate time, depending on the disinfectant used. Discard containers should be decontaminated and washed before reuse.

In laboratories where the risk of infection with TB is low, plastic sputum containers, cartridges used for molecular analysis (e.g. Xpert MTB/RIF cartridges), and wooden applicator sticks should be removed from the laboratory in sealed disposal bags and incinerated.

3. Low-risk TB laboratories

The recommendations in this chapter are the **minimum** requirements needed to limit or reduce risks of infection in laboratories carrying out specific procedures that are considered to have a low risk of spreading TB. Additional measures may be deemed necessary following a site-specific risk assessment.

Low-risk laboratories that follow the minimum biosafety requirements described in this chapter can safely perform certain procedures with sputum specimens, given that the viscous nature of sputum is not prone to generating aerosols when good microbiological techniques are followed. Low risk laboratories can:

- manipulate sputum specimens for direct sputum-smear microscopy;
- manipulate sputum specimens for the Xpert MTB/RIF[®] assay (Cepheid, Sunnyvale Ca., USA).

While opening sputum containers and making a direct sputum smear may produce aerosols, the risk of transmission from such procedures is negligible in comparison with aerosols produced by a single unprotected cough. There is little epidemiological evidence that preparing a direct smear is associated with a measurable excess risk of acquiring TB infection.^{14,15}

Note: The collection of sputum specimens from patients should not occur in the laboratory.

3.1 Factors that increase the risk of infection

In addition to the general risks that are addressed by the biosafety measures described in Chapter 2, the low-risk TB laboratory may also face the following challenges, all of which increase risks:

- bench spaces may be used improperly;
- specimen containers may leak;

- specimens manipulated carelessly may lead to subsequent aerosolization;
- specimens may be shaken vigorously;
- ventilation or illumination may be poor.

3.2 Specific features and essential minimum biosafety measures

To address specific potential risks, the following biosafety requirements^{14,15} should be established in a low-risk TB laboratory.

- 1. Use of bench spaces:** The bench used to process specimens for direct sputum-smear microscopy or the Xpert MTB/RIF assay should be separate from areas used to receive specimens and from administrative areas used for paperwork and telephones.
- 2. Ventilation:** Smears performed directly on sputum samples, and processing specimens for the Xpert MTB/RIF assay, may both be carried out on an open bench in an adequately ventilated area when appropriate microbiological techniques are used.

Adequate ventilation for TB laboratories is typically described as directional airflow with 6–12 air exchanges per hour (ACH) (see Boxes 3 and 4). Directional airflow refers to air flowing from clean areas towards areas where aerosols may be generated; this air should be safely discharged from the room. “Air exchanges per hour” refers to the number of room volumes of air expelled per hour and replaced with clean air. When mechanical ventilation is used, air exchanges per hour can be readily calculated.

For low-risk procedures, natural ventilation should be sufficient providing that air flows away from the technician and across the work area along with potentially infectious materials, then away from occupied areas of the room and outside the

laboratory; this flow should provide protection from aerosols that might be generated in the work area. In order to have directional control of contaminants in the air, air should move at least 0.5 m/s.¹⁶

Ventilation can be ensured by opening windows if the local climate allows. When the climate prevents windows from being opened, consideration should be given to using mechanical ventilation systems that provide an inward flow of air without

recirculation in the room. Air conditioners should be placed only after the direction of airflow has been considered. It is important to ensure that air in the laboratory flows away from the technicians.

Ventilated work stations are an optional solution for aerosol containment for direct sputum-smear microscopy or the Xpert MTB/RIF assay in environments where natural or mechanical ventilation is not practical. Guidance and specifications for ventilated work stations are available.¹⁷

EXPERT GROUP RECOMMENDATION

Standards for adequate ventilation in laboratories have not been defined internationally. The Expert Group recommended as adequate ventilation for TB laboratories a pragmatic definition of directional airflow to include 6–12 air exchanges per hour. The Expert Group noted that there is no evidence to suggest that a greater number of air exchanges per hour would reduce the risk of a laboratory-acquired infection, and recognized that the costs of ventilation systems with higher capacity are considerable.

Box 3. Determining ventilation requirements

Ventilation moves outdoor air into a laboratory room and distributes the air within the room. The purpose of ventilation in a laboratory is to provide clean air to dilute any potentially contaminated air and to remove it from the laboratory. Laboratory ventilation has three basic elements:

ventilation rate – the amount of outdoor air that flows into the laboratory;

airflow direction – the overall direction of air flowing through the laboratory should be from functionally clean areas to dirty areas;

airflow pattern – external air should be delivered to each area of the laboratory and be removed efficiently.

There are three methods that may be used to ventilate a laboratory: natural, mechanical and hybrid (mixed mode).

Natural ventilation

Natural forces drive outdoor air through open laboratory windows and doors. Natural ventilation can generally provide a high rate of ventilation more economically due to the use of natural forces and large openings, which together can achieve high rates of air exchange. Whether natural ventilation is suitable for a particular laboratory depends on the climate, the design of the laboratory and the work practices of the laboratory staff.

Mechanical ventilation

Mechanical fans can be installed in windows or on walls, or installed in ducts that expel air from the laboratory. The type of mechanical ventilation used depends on the climate. Mechanical ventilation systems are considered to be reliable in delivering the desired rate of airflow regardless of the impact of variable winds and ambient temperature. Mechanical ventilation can be used with an air-conditioning system to control temperature and humidity. Mechanical ventilation can also be achieved by using a ventilated work station.

Hybrid (mixed-mode) ventilation

Hybrid (mixed-mode) ventilation relies on natural forces to provide the desired rate of airflow. It uses mechanical ventilation when the flow of natural ventilation is too low. When natural ventilation alone is not suitable, exhaust fans can be installed to increase ventilation in laboratories performing acid-fast bacilli microscopy. However fans need to be installed so that the air in the room can be expelled directly outdoors, either through a wall or the roof. The size and number of exhaust fans needed depends on the target ventilation rate, and this rate should be calculated before this method is used (see Box 4).

3. Minimizing the generation of aerosols:

Preparing sputum samples for direct sputum-smear microscopy or for the Xpert MTB/RIF assay theoretically has the potential to generate aerosols. However, because sputum specimens are usually viscous, aerosol generation can be minimized by using good microbiological techniques. Care should be taken when opening specimen containers, which may have been shaken during transportation to the laboratory. The risk of infectious material spattering in an open Bunsen burner flame should be avoided when drying smears. It is preferable to air-dry smears, and use a flame to fix the smears only when they are completely dry. Disposable wooden applicator sticks or transfer loops are preferred for making smears.

4. Handling leaking specimen containers:

The integrity of specimen containers delivered to the laboratory needs to be checked upon arrival to the laboratory. Leaking containers may need to be discarded and a fresh sample requested.

If an adequate specimen remains in a leaking container, the container may be decontaminated with a suitable disinfectant before processing. Samples should be transported to the laboratory in an upright position to minimize leakage.

- 5. Personal protective equipment:** Each country and facility must evaluate its risks and decide on the level of personal protection that is appropriate for the different procedures being undertaken. Protective laboratory coats should be worn at all times in the laboratory. Gloves must be worn for all procedures that involve direct contact, or may involve accidental contact, with sputum, blood, body fluids and other potentially infectious material. Gloves must be changed regularly and should not be reused. Staff should always wash their hands before leaving the laboratory.

Respirators are not required for use during the preparation of sputum smears.

Box 4. How to determine adequate ventilation in a tuberculosis (TB) laboratory that uses mechanical ventilation

Adequate ventilation in TB laboratories is typically described as directional airflow with 6–12 air exchanges per hour (ACH). Directional airflow refers to air that flows from clean areas of the laboratory towards areas where aerosols may be generated, and is then expelled safely from the room. ACH refers to the number of room volumes of air expelled per hour and replaced with clean air. When mechanical ventilation is used, one method of measuring ACH is to:

1. identify the air exhaust vent or vents.
2. cover the vent with a piece of cardboard that has an opening of 10 cm x 10 cm;
3. measure the outflowing air velocity with a vaneometer or anemometer;
4. calculate the volumetric airflow rate for each air exhaust port

$$Q = V \times A \times 3600$$

Q = Volumetric airflow rate in m³/h

V = Velocity of air in m/s

A = Area of opening in m² (for example, 10 cm [0.1 m] x 10 cm = 0.01 m²)

3600 = conversion of seconds to hours;

5. sum up all the exhausts for the room;
6. measure the volume of the room
Vol = Length x Width x Height = m³ (measure in metres);
7. Calculate the ACH

$$ACH = Q/\text{Vol.}$$

Measurements of ACHs when natural ventilation is used are too variable to provide a reliable measure of ventilation. Rather, it is better to use directional airflow to provide safe working conditions. Ensuring that air flows past the worker, across the work area where there are potentially infectious materials, and away from occupied areas of the room should provide protection from aerosols generated in the work area.

4. Moderate-risk TB laboratories

The recommendations in this chapter are the **minimum** requirements needed to limit or reduce risks of infection in laboratories carrying out specific procedures that are considered to have a moderate risk of spreading TB. Additional measures may be deemed necessary following a site-specific risk assessment.

Moderate-risk laboratories that follow the minimum biosafety requirements described in this chapter can safely perform certain procedures that entail a moderate risk of specimen aerosolization with a relatively low concentration of infectious particles. Moderate-risk laboratories can:

- process specimens for inoculation on primary solid-culture media;
- perform direct DST (for example, direct line-probe assays, Microscopic observation drug susceptibility [MODS], Nitrate reductase assay [NRA] on processed sputum).

4.1 Factors that increase the risk of infection

In addition to the general risks that are addressed by the biosafety measures described in Chapter 2 (such as unauthorized persons in the laboratory, mouth pipetting, cluttered work stations, improper waste disposal), the TB laboratory classified as moderate risk also faces the following challenges, all of which increase risks:

- staff may work in areas with poor ventilation;
- they may work with poor illumination;
- BSCs may be poorly maintained and not certified;
- BSCs may not be properly ducted;
- the work environment may be dusty, and high-efficiency particulate air (HEPA) filters in BSCs may become blocked;

- careless manipulation of specimens may lead to aerosolization;
- precautions for using the vortex may not be followed properly (for example, it may be used outside the BSC);
- specimen containers may break or leak during centrifuging;
- problems may be associated with opening centrifuge buckets outside the BSC;
- adequate warnings of biohazards may be lacking, and information on who should be contacted during an emergency may be inadequate;
- cooling or heating systems may not work properly.

Good microbiological techniques are essential to minimize the risk of aerosolization.

4.2 Specific features and essential minimum biosafety measures

In a laboratory where there is a moderate risk of infection, there are two levels of containment: the BSC (primary containment) and the laboratory itself (secondary containment). To address the specific risks associated with a moderate-risk laboratory, the following mitigation and control measures should be established.

- 1. Biological safety cabinets:** All processing and digestion of sputum samples and manipulation of liquefied sputum specimens must be conducted in a BSC. The cabinet is the primary form of containment while specimens are processed for culture inoculation or for performing direct DST. Hence, good microbiological techniques and proper use of the cabinet are critical to allow work to be conducted safely.

Improper use of the cabinet allows aerosols to be released into the laboratory. (See Chapter 6 for more information on BSCs.)

BSCs should be sited away from thoroughfares and out of cross-currents from doorways and air-inlet systems. Air expelled from properly maintained cabinets will have passed through HEPA filters at the top of the cabinet and so can be expelled either into the room or ducted to the outside, depending on the degree of sophistication of the ventilation system installed.

Adequate space is needed between the cabinet and the ceiling to ensure that the air flowing from the cabinet is not impeded.

Class I or Class II cabinets are recommended; however they must be designed by a certified manufacturer and regularly maintained. They must be certified to be functioning properly on site at least annually. Class II type A2 cabinets are preferable because they offer protection both for personnel and the media being inoculated (product protection).

Class II type B cabinets are suitable but are not recommended for new TB laboratories because they require hard-ducting. Additionally, it is more difficult to balance and maintain them, and ensure they are functioning properly. Hard-ducting requires that the building's exhaust system be precisely matched to the airflow requirements of the manufacturer.

An uninterrupted power supply for the cabinet and the exhaust fan is necessary in settings where power is unreliable; this supply gives laboratory staff time to safely complete any hazardous work, and for contaminated air remaining in the cabinet to be vented outside. Devices to prevent the backflow of air should be installed in the ducting of cabinets to stop potentially contaminated air from flowing back into the laboratory in the event of a power failure.

It is helpful to have a stand-by generator for the cabinet and other essential equipment, such as incubators and freezers.

1. **Ventilation:** In addition to the BSC (the primary barrier), the secondary barrier (provided by the laboratory itself) is achieved by maintaining a unidirectional airflow into the laboratory, and by ensuring there are a minimum of 6–12 ACHs.

A simple means of creating unidirectional airflow is to place a vent that allows air to flow into the clean area of the laboratory and to operate continuously one or more thimble-fitted BSCs to draw air towards the dirty area, remove the air from the laboratory, and expel it outside the building. A visual monitoring device with or without an alarm should be installed so that staff can ensure at all times that proper directional airflow is maintained in the laboratory (see Box 5).

Ducting the BSC to the outside using a thimble connection helps create unidirectional airflow into the laboratory, and any contaminated air in the BSC is expelled from the laboratory through the HEPA filters in the BSC. When the cabinet is turned on, the external fan extracts air from both the cabinet and the room. When the cabinet is switched off, the expelled air will be extracted only from the room. An external fan can be installed with or without a link to the status (operating or stand-by) of the cabinet. It is best if the external fan has a separate switch from the BSC, or alternatively it can be coupled with a relay circuit so that the external fan continues operating for a given time after the BSC has been turned off to ensure that all of the air expelled from the BSC is vented outside. The major advantage of a thimble connected BSC is that no adjustments need to be made to the cabinet and the direction of air flowing from the laboratory to the outside will be maintained.

Alternatively, air expelled through the HEPA filters within the BSC can be released into the laboratory. However, in such cases, there must be a separate exhaust system for the building that ensures a minimum of 6–12 ACH in the laboratory. The building's ventilation system must be constructed in such a way that air from the moderate-risk laboratory is not recirculated to other areas within the building.

When air expelled from the laboratory is discharged to the outside of the building, it must be dispersed away from occupied buildings and air intakes.

Windows must be kept closed at all times in moderate-risk and high-risk TB laboratories.

- **Personal protective equipment:** Each laboratory must evaluate its risks (for example, by assessing the laboratory's activities and workload, the prevalence of TB and the prevalence of drug-resistant strains) and decide on the level of personal protection that is appropriate for staff. Protective laboratory gowns and gloves must be worn at all times in laboratories where there is a moderate risk of infection.

During specimen processing, samples are liquefied; this makes it more likely that aerosols will be generated so measures to minimize the production of aerosols are essential.

Gloves should be changed regularly. Staff must always wash their hands before leaving the laboratory.

Respirators are not required, provided that specimens are processed within a properly maintained BSC using good

microbiological techniques. Respirators should not be seen as an alternative to a BSC.

- **Laboratory design:** The laboratory must be separate from the areas that are open to unrestricted traffic flow within the building. A station for hand washing should be provided near the laboratory's exit.
- **Decontamination and waste disposal:** All infectious waste must be removed from moderate-risk laboratories for proper disposal. Waste must be transported in sealed plastic bags or containers, following appropriate local regulations. Any materials that are reused must be decontaminated with a suitable disinfectant or autoclaved before being removed from the laboratory.
- **Minimizing the generation of aerosols:** Staff training should always include information on the safest methods to use for culture procedures to prevent inhalation of aerosols generated when using loops, pipetting, opening specimen containers, handling damaged or leaking containers, centrifuging and vortexing. The possibility of infectious material spattering when an open Bunsen burner flame is used should be avoided by using an enclosed electric microincinerator to sterilize reusable loops. The use of sterile disposable transfer loops and transfer pipettes is recommended.

Centrifuges require safety buckets or containment rotors. Infectious materials may be centrifuged in the open laboratory provided that sealed centrifuge safety cups are used and buckets are loaded and unloaded within a BSC.

Box 5. How to calculate the number of air changes per hour (ACH) in a laboratory that uses a biological safety cabinet (BSC) ducted with a thimble connection

- Determine the volume of the room in the laboratory (floor area x room height).
- Determine the volume of ACH required (multiply the room volume by 6 for the minimum number of air exchanges and 12 for the maximum number).
- Determine the number of BSCs, and the air expelled from each cabinet. The expelled air of one BSC with a width 150 cm will be about 500 m³/h, (that is, air inflow area 1.50 m x 0.2 m x air velocity 0.38 or 0.5 m/s x 3600 s = 410–540 m³/h). Calculate this for each type of cabinet used.
- Determine the power of the external extraction fan installed at the end of the ducting; this should exceed the volumetric flow rate of each BSC by 30–50%, and should be controllable and connected to an uninterrupted power supply. The air from the BSC should be ducted with ventilation pipes that have a diameter exceed 20 cm.

For example: A laboratory floor of 5 m x 10 m with a 2.5 m high ceiling would require between 750 m³ and 1500 m³ of air to be expelled every hour to allow for the required 6–12 exchanges of the room volume. So two thimble-connected BSCs could expel 1300–1500 m³ of air from the laboratory every hour.

- The ventilation system for the laboratory should be planned with a qualified specialist engineer.

5. High-risk TB laboratories (TB-containment laboratories)

The term **TB-containment laboratory** refers to a facility that has the minimum design features necessary to safely manipulate TB cultures. This type of facility may or may not meet all of the requirements of a Biosafety Level 3 laboratory as described in WHO's Laboratory biosafety manual.² All laboratory facilities must comply with local and national regulations.

The recommendations in this manual are the **minimum** requirements needed to limit or reduce risks of infection in laboratories carrying out specific procedures that are considered to have a high risk of spreading TB. Additional measures may be deemed necessary following a site-specific risk assessment.

High-risk laboratories (also known as TB-containment laboratories) that follow the minimum biosafety requirements described in this chapter, are designed to work with high volumes and concentrations of *M. tuberculosis* organisms and to engage in procedures that pose an increased risk of aerosol spread. High-risk TB laboratories can:

- manipulate cultures to identify *M. tuberculosis*;
- manipulate cultures or suspensions of tubercle bacilli for all indirect DST methods and molecular assays.

5.1 Factors that increase the risk of infection

In addition to the hazards described in Chapter 4 for TB laboratories classified as moderate risk, and the general risks that are addressed by the biosafety measures described in Chapter 2, laboratories classified as high-risk (or containment) also face the following challenges, all of which increase risks:

- staff must open positive culture vials;
- staff must prepare smears from positive cultures;

- DNA extraction must be performed on a positive culture;
- manipulation of cultures for identification and indirect DST;
- broken culture containers must be disposed of;
- cultures or areas where spills occurred must be decontaminated.

5.2 Specific features and required biosafety measures

Similar to the moderate-risk laboratory, there are two levels of containment in a high-risk laboratory: the BSC (primary containment) and the laboratory itself (secondary containment).

In TB laboratories classified as high risk, all procedures for handling viable *M. tuberculosis* cultures and aqueous suspensions of TB bacilli for identification, indirect DST and molecular assays must be conducted within a BSC in a TB-containment laboratory.

In addition to the safety elements required for a moderate-risk laboratory, a high-risk (or TB-containment) laboratory requires the following additional enhancements.

- 1. Laboratory design:** Two sets of entry doors are essential to create an anteroom to the containment laboratory. This design provides a physical barrier between the containment section of the laboratory and the outer laboratory areas. It also permits a unidirectional flow of air into the laboratory. The anteroom should have facilities for separating clean clothing from dirty clothing. Doors to the anteroom may be self-closing and interlocking so that only one door can be open at a time. A break-through panel may be provided for emergency exit. Air can flow into the TB-containment laboratory

through the anteroom; and grills fitted with pre-filters can be placed in the lower panels of the anteroom's doors to ensure that only clean air flows into the TB-containment laboratory.

A glass panel should be installed to give a view from the outer laboratory areas into the containment laboratory.

- 2. Personal protective equipment:** Each facility must evaluate its risks and decide on the level of personal protection that is appropriate for staff.

Protective laboratory gowns must be worn. Gowns should have solid front panels and must be impermeable to liquids. Laboratory gowns should have long sleeves and an elasticized cuff (at least 30 mm long) and fasten at the back.

Gloves must be worn. Staff must always wash their hands before leaving the laboratory

The use of hair coverings, shoe covers or dedicated shoes are optional; they may be adopted as additional protective measures. However, any protective clothing used in

the TB-containment laboratory must not be worn in the other areas of the laboratory.

Respiratory equipment provides additional protection during high-risk procedures – such as the manipulation of liquid cultures for identification and DST– that generate aerosols with high concentrations of infectious particles. Protective respiratory equipment should not be considered a substitute for a poorly functioning BSC or a BSC lacking certification. In all cases, good microbiological techniques are essential to minimize the risk of laboratory-acquired infections.

- 3. Decontamination and waste disposal:** An autoclave must be available on site in the vicinity of the TB-containment laboratory to allow tubes and vials with cultures of TB bacilli to be sterilized prior to being removed for disposal. All other infectious waste needs to be removed from the TB-containment laboratory for proper disposal. It must be transported in sealed plastic bags or containers, following appropriate local regulations. Any materials that are reused must be decontaminated with a suitable disinfectant or autoclaved before being removed from the laboratory.

EXPERT GROUP RECOMMENDATION

WHO's Laboratory biosafety manual² recommends that containment laboratories be able to be sealed off so that they can be decontaminated by fumigation. The Expert Group found that it is not essential that a TB-containment laboratory has the capability of being sealed for decontamination given that infectious particles from bacteria that have dried onto surfaces are unlikely to become aerosolized. The Expert Group therefore concluded that surface decontamination procedures are sufficient for TB-containment laboratories, and that the ability to fumigate the containment laboratory is not mandatory.

6. Safety equipment

Safety equipment may be used to eliminate or reduce certain risks in TB laboratories (Table 4). Such equipment offers no assurance of protection unless the operator is competent and uses proper techniques. Equipment should also be tested regularly to ensure that it continues to perform safely.

6.1 Biological safety cabinets

Owing to their small size, droplet nuclei aerosols may be generated by certain laboratory procedures without the laboratory worker's knowledge; this may result in the inhalation of infectious agents or cross-contamination of work surfaces or materials. BSCs are designed to protect people and the environment from infectious agents and, depending on their classification, offer varying degrees of protection from contamination of specimens and cultures.

The HEPA filter in the exhaust system of a BSC effectively traps infectious organisms and ensures that only microbe-free exhaust air is discharged from the cabinet. A HEPA filter mounted in the BSC above the BSC work surface protects the surface and its materials from contamination. This is often referred to as product protection.

There are three classes of BSCs: I, II and III (corresponding to standards AS/NZS 2252.1:1994, AS/NZS 2252.2:1994, NSF/ANSI 49 – 2008 and EN 12469).^{18,19,20,21} According to the NSF/ANSI 49 – 2008 standard, class II BSCs have a variety of types (known as A1, A2, B1, B2); these are used to classify variations in airflow patterns, velocities, the position of the HEPA filter inside the cabinet, ventilation rates and exhaust methods.

6.1.1 Selecting a biological safety cabinet for a TB laboratory

The two types of BSCs described below are best suited for use in moderate-risk laboratories and in high-risk laboratories (TB-containment laboratories).

Class I

- This type of BSC provides personal and environmental protection but does not offer product protection. This lack of product protection may contribute to increased contamination rates, especially when preparing and inoculating liquid cultures (see Figure 1).

Class II

- A Class II BSC offers personal, environmental and product protection, and, in type A2 models all biologically contaminated ducts are under negative pressure or are surrounded by negative-pressure ducts (see Figure 2). **(This is the PREFERRED type of BSC.)**
- Class II type A1 BSCs are not a good choice because the ducts may become contaminated, and plenums have positive pressure relative to the room
- Class II type B1 and type B2 BSCs must be hard-ducted to the outside; this means that the building's exhaust system must precisely match the airflow requirements specified by the manufacturer for both volume and static pressure. Certification, operation and maintenance of these types are therefore more difficult, so these BSCs are not recommended for any new TB laboratory facilities.

BSCs should be equipped with HEPA filters that meet applicable international standards (for example, European norm standards EN12469 or United States NSF/ANSI Standard 49 – 2008).^{20,21}

For all newly procured BSCs, Class II type A2 cabinets with a moveable sash are recommended.

A BSC should be selected primarily according to the type of protection needed: product protection or protection for personnel against the risk of infection. Selecting the correct type of BSC, installing it, using it properly, and annually certifying its operation are complex processes. It is highly recommended that these processes are performed by well trained and experienced professionals familiar with all aspects of BSCs.

BSCs should be connected to an uninterrupted power supply to ensure that staff have adequate time to complete a procedure in the event of a power outage.

Biosafety cabinets must undergo certification at the time of installation, whenever they are moved, and following any repairs or filter changes; they also require regular (annual) maintenance to ensure proper functioning. Delaying maintenance or using under-qualified personnel to conduct maintenance can put laboratory workers at risk. (See section 6.1.5.)

6.1.2 Class I biological safety cabinets

Class I BSCs work by drawing unfiltered room air in through a front opening, passing it over the work surface, and then expelling it through an exhaust duct.

Class I BSCs protect workers but do not protect work products (such as specimens or cultures) against contamination because unsterilized room air is drawn over the work surface.

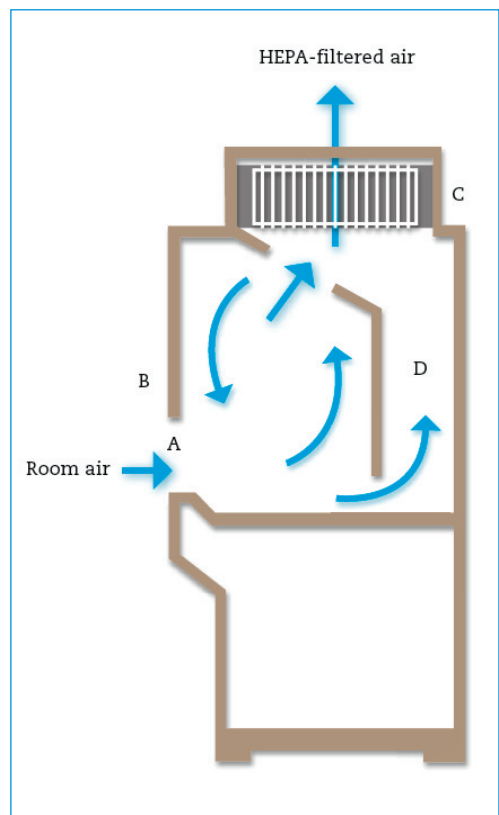
Figure 1 is a schematic diagram of a Class I BSC. Room air is drawn in through the front opening at a minimum velocity of 0.38 m/s (NSF/ANSI).²⁰ It then passes over the work surface and is expelled from the cabinet through the exhaust duct. The directional flow of air carries aerosol particles that may be generated on the work surface away from technicians and into the exhaust duct. The front opening allows the technician's arms to

reach the work surface inside the cabinet while he or she observes the surface through a glass window. The window can also be fully raised to provide access to the work surface for cleaning or other purposes.

The air from the cabinet is expelled through a HEPA filter: (a) into the laboratory and then to the outside of the building through the building's exhaust system; or (b) to the outside through the building's exhaust system; or (c) directly to the outside.

Figure 1. Schematic diagram of a Class I biological safety cabinet A.

Front opening; B Sash; C Exhaust HEPA filter; D Exhaust plenum.



6.1.3 Class II type A2 biological safety cabinets

Class II BSCs differ from Class I cabinets in that they allow only air from a HEPA-filtered (sterile) supply to flow over the work surface.

A Class II type A2 BSC is shown in *Figure 2*. An internal fan draws room air (supply air) into the cabinet through the front opening and then into the front intake grill. After passing through the grill, the supply air is drawn upwards and through a HEPA filter before flowing downwards over the work surface.

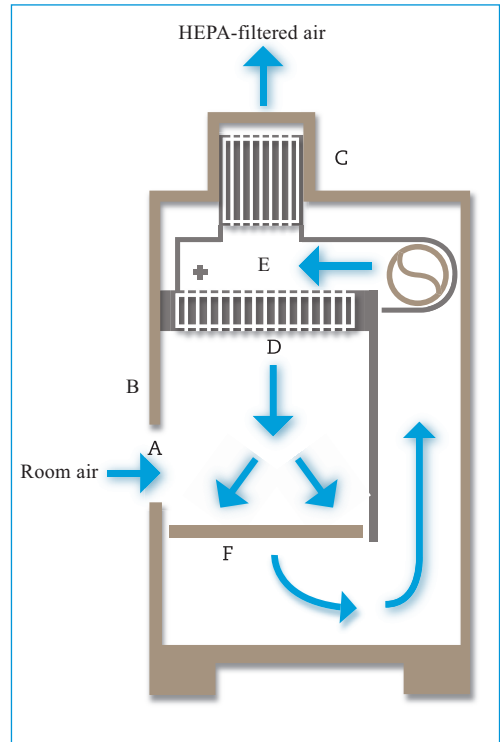
As the air flows downwards at about 6–18 cm above the work surface it splits so that approximately one half of the volume of the air passes through the front exhaust grill and the other half passes through the rear exhaust grill. Any aerosol particles generated at the work surface are immediately captured in this downward airflow and passed through the front or rear exhaust grills, thereby providing the highest level of product protection. The air is then discharged through the rear plenum and exhaust filter located at the top of the cabinet. Owing to the relative size of these filters, 60–70% of the air recirculates through the supply HEPA filter back into the work zone; the remaining 30–40% passes through the exhaust filter into the room or outdoors.

Air from a Class II type A2 exhaust can be recirculated to the room or discharged to the outside of the building through a thimble connected to a dedicated duct; it must NOT be discharged through the building's exhaust system.

In a containment laboratory where air from the Class II BSC is recirculated to the room, a separate dedicated ventilation system is needed to ensure unidirectional flow of air into the laboratory with 6–12 ACH. Recirculating the exhaust air to the room has the advantage of lowering the energy costs of the building because heated or cooled air is not being passed to the outside environment.

Figure 2. Schematic diagram of a Class II type A2 biological safety cabinet.

A Front opening; B Sash; C Exhaust HEPA filter; D Supply HEPA filter; E Positive-pressure plenum; F Negative-pressure plenum.



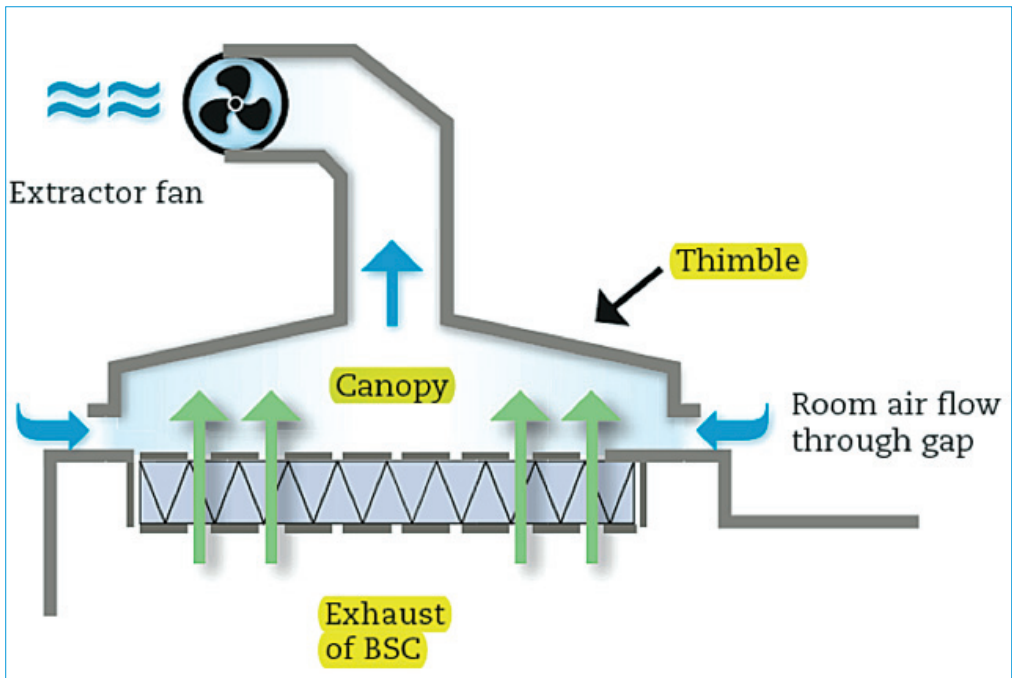
6.1.4 Thimble connections

A thimble connection (see *Figure 3*) is used with Class II type A2 BSC that is ducted to the outside. The thimble fits over the cabinet's exhaust housing, sucking the air expelled from the cabinet into ducts that lead outside. A small opening (usually 5 cm wide) is maintained between the thimble and the cabinet's exhaust housing. This opening enables room air to be drawn into the exhaust ducting system. The capacity of the exhaust system must be sufficient to capture both room air and the cabinet's exhaust. The thimble must be removable or be designed to allow for operational testing of the cabinet. Generally, the performance of a thimble-connected BSC is not affected much by fluctuations in the building's airflow.

One advantage to using a thimble connection is that the BSC does not need any adjustments and the pressure in the room will remain nearly constant. To keep a controlled, constant, lowered pressure within the containment room, a damper control for the exhaust system is usually needed to enable the air flow through the thimble to be balanced with the exhaust capacity of the extractor fan placed at the end of the ducting.

Another advantage of using a thimble connection is that in case of power outages, the air flowing back into the room where there is lowered pressure will pass nearly exclusively through the thimble air intake, and not wash off bacteria from the HEPA filter. Installing a valve to prevent backflow in the duct ensures that air flowing in will travel through the clean-air intake.

Figure 3. Schematic diagram of a thimble connection for a Class II type A2 biological safety cabinets ducted directly outside the laboratory.



6.1.5 Using biological safety cabinets in the laboratory

Location

The integrity of the directional air inflow is fragile and can be easily disrupted by air currents generated by people walking close to the BSC, by open windows or air-supply registers, and by the opening and shutting of doors. Ideally, BSCs should be situated as recommended by the manufacturer in a location away from traffic and

from potentially disruptive air currents. Whenever possible, a clearance of 30 cm should be provided behind and on each side of the cabinet to allow easy access for maintenance. A clearance of 30–35 cm above the cabinet may be required to accurately measure air velocity across the exhaust filter, and to change exhaust filters.

Operators

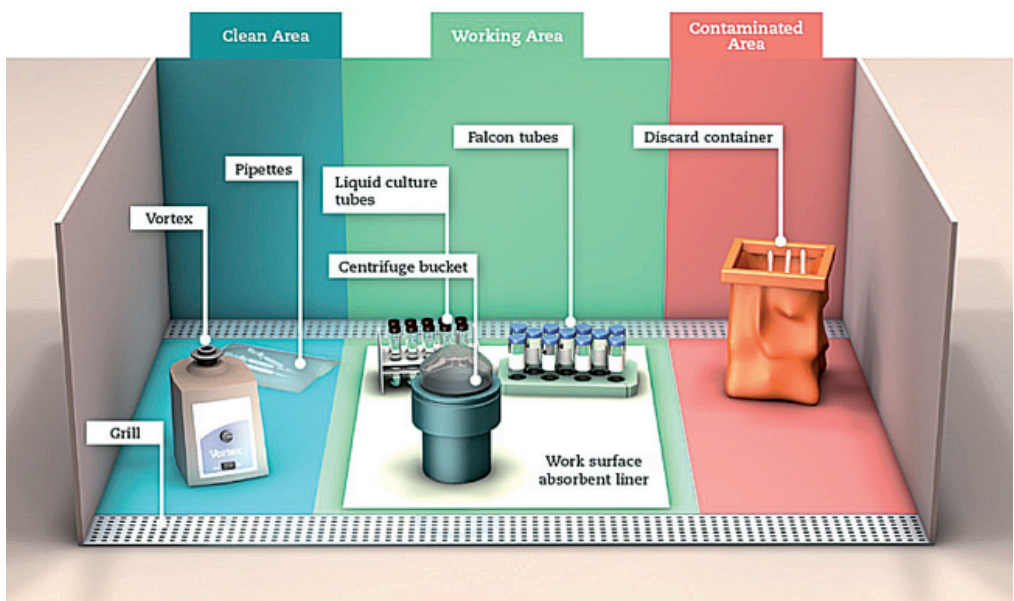
If BSCs are not used properly, their protective benefits may be greatly diminished; in some

instances improper use can even result in increased risk to the laboratory worker. Written protocols, as well as a biosafety manual, should be issued to laboratory staff and they should sign a form to confirm that they have read and understood the required protocols. All individuals working in BSCs should be observed to ensure they follow correct working practices before they routinely perform testing in the BSC. Operators need to maintain the integrity of air flowing through the front opening when moving their arms into and out of cabinets. They should move their arms slowly and ensure they are perpendicular to the front opening. Staff should wait about 2 minutes after placing their hands and arms inside the BSC before they begin manipulating materials; this will allow the airflow within the cabinet to adjust and the air to sweep the surface of their hands and arms. The number of movements made across the front opening should be minimized by placing all necessary items into the cabinet before beginning manipulations.

Material placement

The front intake grill of Class II BSCs must not be blocked with paper, equipment or other items. It is recommended that all work be performed on disinfectant-soaked absorbent towels arranged to capture splatters and splashes. All materials should be placed as far back in the cabinet as practical – that is, towards the rear of the work surface – without blocking the rear grill. Aerosol-generating equipment (such as vortexes and centrifuges) should be placed towards the rear of the cabinet. Bulky items (such as biohazard bags and discard containers) should be placed to one side of the interior of the cabinet. Active work should flow from clean areas to contaminated areas across the work surface. Paperwork should never be placed inside BSCs. The cabinet must not be overloaded because overloading may affect the efficiency of the airflow (see *Figure 4*).

Figure 4. Typical layout for working from clean areas to dirty areas within a Class II biological safety cabinet. Clean materials are placed to the left of the cabinet; samples are inoculated in the centre of the cabinet; and contaminated pipettes and other materials are placed into waste containers on the right side of the cabinet. This arrangement can be reversed for people who are left-handed



Ultraviolet lights

Ultraviolet lights are not recommended in BSCs used in TB laboratories.

Open flames

Open flames must be avoided in BSCs because heat disrupts the patterns of airflow within the cabinets. To sterilize bacteriological loops, microincinerators or electric furnaces are available, and their use is preferable to open flames. The use of disposable loops and disposable transfer pipettes is preferred.

Spills

A copy of the laboratory's protocol for handling spills should be posted, read and understood by all laboratory staff. When a spill occurs inside a BSC, clean up should begin immediately and the cabinet should continue to operate. An effective disinfectant should be used and applied in a manner that minimizes the generation of aerosols. All materials that come into contact with the spilled agent should be disinfected and disposed of properly.

Certification

The functional operation and integrity of each BSC should be certified to national or international performance standards at the time it is installed, following any relocation with the laboratory, and regularly thereafter (at least annually) by qualified service technicians, according to the manufacturer's specifications. An evaluation of the effectiveness of the cabinet's containment capability should include tests of the cabinet's integrity; tests for HEPA filter leaks; assessments of the down flow velocity profile, face velocity, negative pressure and ventilation rate, airflow smoke pattern, and alarms and interlocks.

The velocity of air flowing through the front opening into a BSC should meet the manufacturer's specifications. Optional tests may also be conducted for electrical leakage, lighting intensity, ultraviolet light intensity, and noise level and vibration. Special training, skills and equipment are required to perform these tests, and it is highly recommended that they are

undertaken by an experienced professional. The professional should be familiar with and trained in all aspects of BSCs.

Cleaning and disinfecting the work area

When work is completed, all items within a BSC, including equipment, should have surfaces decontaminated and be removed from the cabinet.

The interior surfaces of BSCs should be decontaminated before and after each use. Work surfaces and interior walls should be wiped with a disinfectant that will kill any microorganisms that might be found inside the cabinet. At the end of the workday, the final surface decontamination should include wiping down the work surface, and the sides, back and interior of the glass. A second wiping with sterile water is needed when a corrosive disinfectant, such as bleach, is used.

Before it is switched off, the BSC should be left to run for 15 minutes after work is completed in order to purge the atmosphere inside.

Decontamination

BSCs must be thoroughly decontaminated before filters are changed and before the cabinet is moved; decontamination must include plenums and filters. See standard NSF/ANSI 49 – 2008 for procedures and details of decontamination.²⁰ Decontamination should be performed by a qualified professional.

Alarms

BSCs can be equipped with one of two audible alarms. Sash alarms are found only on cabinets with sliding sashes. The alarm sounds when the laboratory worker has moved the sash to an improper position. When this alarm sounds, the sash must be returned to the proper position. Airflow alarms indicate a disruption in the cabinet's normal airflow pattern. This alarm represents an immediate danger to the worker or product. When an airflow alarm sounds, work should cease immediately and the laboratory manager should be notified. Manufacturers' instruction manuals should provide further details about how to address this type of alarm. Training

in the use of BSCs should include information on how to respond to this type of alarm.

6.2 Centrifuges with safety buckets

During the centrifugation process, aerosols may be produced. Consequently, safety measures must be strictly followed when operating the centrifuge.

During centrifuge operation, the lid must be fully sealed. The use of a wide nonporous seal will ensure that the lid is closed tightly. The lid must not be opened until the rotor has stopped completely. Appropriate rotors have safety caps for each slot. The caps for individual buckets and tubes must be closed properly before the centrifuge is operated. To contain aerosols, individual sealed centrifuge buckets should be loaded and unloaded in a BSC. For processing TB cultures, refrigerated centrifuges with swinging buckets are recommended.

When using a micro-centrifuge for DNA extraction, a safety rotor is needed with a sealed lid; the micro-centrifuge should be loaded and unloaded inside a BSC.

Centrifuges must be inspected periodically for wear and tear; and maintenance must follow the manufacturer's specifications.

6.3 Autoclaves

In general TB laboratories that perform diagnostic tests, an autoclave that uses saturated steam under pressure is the most efficient means of sterilizing instruments, glassware and media solutions; it is also used for decontaminating biological material (such as mycobacterial cultures). Two factors are essential for an autoclave to function optimally: (1) all of the air in the chamber should be replaced by steam; and (2) the temperature must be 121 °C.

Autoclaves should be located away from the main laboratory working area because they may be noisy, hot and release steam. An autoclave used to decontaminate infectious material, should have an exhaust air valve equipped with a bacterial filter. The autoclavable sterile filter should consist of a filter cartridge with a membrane (pore size 0.2 µm) incorporated into a pressure-resistant housing; the filter should be easy to replace. The filter is automatically sterilized during each sterilization process. An autoclave **MUST** be available in each facility where TB cultures are performed and should ideally be placed within the TB-containment laboratory.

The manufacturer's instructions for operating and cleaning the autoclave must be followed at all times.

Table 4. Safety equipment used to process specimens in TB laboratories, potential hazards and associated safety features

Equipment	Potential hazard or risk	Safety features
Biological safety cabinet		
Class I	Aerosol and spatter	<ul style="list-style-type: none"> • Minimum inward airflow (face velocity) at work access opening as per manufacturer's recommendations; exhaust air passes through HEPA filter • Provides protection to personnel and the environment
Class II	Aerosol and spatter	<ul style="list-style-type: none"> • Minimum inward airflow (face velocity) at work access opening as per manufacturer's recommendations; exhaust air passes through HEPA filter • Provides protection to personnel, products and the environment
Ventilated workstation	Aerosol and spatter	<ul style="list-style-type: none"> • Not a replacement for a BSC • Minimum inward airflow (face velocity) at work access opening as per specifications; • No HEPA filter • Provides limited protection for personnel
Centrifuges with safety buckets or sealed rotors	Aerosols and spillage	<ul style="list-style-type: none"> • Contains aerosols
Pipetting aids	Hazards from pipetting by mouth (for example, ingestion of pathogens, inhalation of aerosols produced by mouth suction on pipette, blowing out of liquid or dripping from pipette, contamination of suction end of pipette)	<ul style="list-style-type: none"> • Easy to use • Controls contamination of suction end of pipette, protecting pipetting aid, user and vacuum line • Can be sterilized • Controls leakage from pipette tip
Microincinerators for loops, disposable loops	Spatter from transfer loops	<ul style="list-style-type: none"> • Microincinerators shield loops in open-ended glass or ceramic tubes; they are heated by gas or electricity • Disposable loops removes the need for micro-incinerators.
Leak-proof vessels for collecting and transporting infectious materials for sterilization within a facility	Aerosols, spills and leaks	<ul style="list-style-type: none"> • Leak-proof construction with lid or cover • Durable • Autoclavable

Equipment	Potential hazard or risk	Safety features
Sharps disposal containers	Puncture wounds	<ul style="list-style-type: none">• Autoclavable• Robust, puncture-proof
Containers used for transport between laboratories or institutions	Release of microorganisms	<ul style="list-style-type: none">• Robust• Watertight primary and secondary storage contains spills• Absorbent materials contain spills
Autoclaves (manual or automatic)	Positive cultures sterilized before being removed from the laboratory	<ul style="list-style-type: none">• Approved design• Effective heat sterilization

HEPA, high-efficiency particulate air; BSC, biological safety cabinet.

7. Personal protective equipment and clothing

Personal protective equipment and clothing may act as barriers to minimize the risk of exposure to aerosols, splashes and accidental inoculation. The choice of clothing and equipment depends on the nature of the work. Protective clothing should be worn whenever staff work in the laboratory (see

Box 6). Before leaving the laboratory, staff should remove their protective clothing, and wash their hands. *Table 5* summarizes the types of personal protective equipment used in laboratories and the protection each type offers.

Table 5. Personal protective clothing and equipment that may be used in by staff in tuberculosis (TB) laboratories

Equipment	Potential hazard	Safety features
Laboratory coats	Contamination of street clothing	<ul style="list-style-type: none"> Laboratory coats usually have long sleeves and fasten in the front to cover street clothes Coats should be used for activities where there is a low-risk of becoming infected with TB
Laboratory gowns	Contamination of street clothing	<ul style="list-style-type: none"> Laboratory gowns should have long sleeves and an elasticized cuff (at least 30 mm long) Gowns should open in the back Gowns should cover street clothing
Respirators	Inhalation of aerosols	<ul style="list-style-type: none"> Designs available include the N95 (United States standard) and FFP2 (European standard); full-face or half-face air purifying models;
Gloves	Direct contact with microorganisms	<ul style="list-style-type: none"> Disposable microbiologically approved latex, vinyl or nitrile

7.1 Laboratory gowns

Laboratory gowns should have long sleeves and open in the back. When the laboratory technician is standing, the gown must extend below the height of the workbench; the gown should fully cover the technician's lap when he or she is sitting. Reusable gowns should be autoclaved before being washed. Gowns must not be taken home for washing; laundering services should be provided at or near the facility. Laboratory gowns

should be changed at least once a week and immediately after being overtly contaminated.

Laboratory gowns should not be worn outside the laboratory. A changing area should be available where gowns can be stored. All laboratory staff, as well as all others entering the laboratory, must wear a gown. Protective laboratory clothing should not be stored in the same lockers or cupboards as street clothing. Extra gowns should be available in case of contamination.

7.2 Respirators

Respirators are not normally required for work in a TB laboratory. However, they may be recommended after a risk assessment if cultures are being manipulated within a TB-containment laboratory. Even if not worn regularly, respirators must be available in laboratories where culture manipulations are performed in case an accidental biohazard (such as a spill) occurs outside the BSC. Respirators should be included as part of a laboratory's spill clean-up kit.

Respirators should never be used as a substitute for a properly maintained and functioning BSC.

N95 (United States Standard NIOSH N95) or FFP2 (European Standard EN149:2001) respirators should be worn if indicated by a risk assessment. Such respirators are lightweight, disposable devices that cover the nose and mouth and filter 94–95% of particles that are ≥ 0.3 – $0.4 \mu\text{m}$.

If respirators are used in a laboratory, all staff should be instructed and trained in their proper use and fitting, and in their limitations. Ideally, staff should undergo a fit test to ensure leakage does not occur. Respirators should not be used by people with facial hair. Respirators must be stored in a convenient, clean, dry and sanitary location, and must not be worn outside of the laboratory. Once a respirator has been put on, under no circumstances should the wearer touch the front of it. Staff should not place the respirator under their chin or on their head when answering the phone or talking.

Respirators must be inspected before every use to ensure that there are no holes other than the punctures around the staples, and to ensure that no damage has occurred. (Enlarged holes resulting from ripped or torn filter material around staple punctures are considered to be damage.) Straps and valves must also be checked. A damaged respirator must be discarded and replaced immediately.

Surgical masks are not respirators, are not certified as such and do not offer significant protection to personnel performing aerosol-producing

diagnostic tests for TB. They are not designed to protect the wearer from inhaling small infectious aerosols and therefore should not be used.

7.2.1 Fitting a respirator

Staff who use respirators must be trained. They should be taught to:

- cup the respirator in one hand, with the nosepiece at the fingertips; they should allow the headbands to hang freely;
- position the respirator under the chin with the nosepiece upwards; pull the top strap over their head and place it high at the back of the head; pull the bottom strap over the head and position it around the neck below the ears;
- place the fingertips of both hands at the top of the metal nosepiece; using two hands, mould the nose area to the shape of their nose by pushing inward while moving their fingertips down both sides of the nosepiece; pinching the nosepiece with only one hand may result in an improper fit and less effective respirator performance; they should always use two hands.

7.2.2 Removing a respirator

- Staff should remove their gloves and thoroughly wash their hands before removing respirators. Only the straps should be handled; staff should avoid touching the front of the respirator.

7.3 Gloves

Gloves must be worn for all procedures that involve direct contact, or may involve accidental contact, with sputum, blood, body fluids and other potentially infectious materials. After use, gloves should be removed aseptically and hands washed.

Contaminated gloves (and unwashed hands) may be a source of infection for other staff members if they are used to handle or operate equipment in the laboratory (such as a centrifuge or telephone).

Regular hand washing is essential to prevent many types of laboratory-acquired infections, including those caused by bloodborne pathogens.

Disposable latex, latex-free vinyl (clear) or nitrile gloves can be used, and the correct size (small, medium or large) should be available for all individuals. Gloves should fit as comfortably as possible and should cover the wrists.

Disposable gloves must never be reused, and once they have been used they should be discarded with infectious laboratory waste. There must be a reliable supply of gloves. Gloves should not be worn outside the laboratory.

Staff should remove gloves and wash their hands thoroughly with water and soap after handling infectious materials, working in a BSC, and before leaving the laboratory.

7.3.1 Removing gloves

Laboratory staff should be trained to remove their gloves by following these steps:

1. peel one glove off by grasping it under the cuff and rolling the glove off the hand so that it comes off inside out. This keeps most of the contamination inside;
2. hold the used glove in the opposite still-gloved hand. Carefully slip exposed fingers under the cuff of the gloved hand, being careful not to touch the surface of the contaminated glove. Peel the glove off, inside out, rolling it over the other used glove to form a bag of used gloves with contamination inside.
3. Dispose of the gloves properly and safely.

Box 6. Guidelines for the use of gloves and respirators according to the risk level of the tuberculosis (TB) laboratory

This guidance summarizes the minimum requirements for the use of this equipment at the different biosafety levels in TB laboratories.

Respirators

Respirators are normally not required for work in TB laboratories but whether they are used depends on a risk assessment undertaken at the local level or national level. Such an assessment might recommend their use for laboratories manipulating cultures or performing drug-susceptibility testing (DST) within a containment laboratory. These aids must not be considered a replacement for working inside a biological safety cabinet (BSC).

Gloves

Gloves must be worn when handling any potentially infectious specimens or manipulating cultures containing tubercle bacilli.

Personal protective equipment	Low-risk TB laboratory	Moderate-risk TB laboratory	High-risk TB laboratory (containment laboratory)
Respirators	Not required	Not required	May be required following a risk assessment
Surgical masks	Not designed to protect the user from inhaling infectious aerosols and therefore should not be used for respiratory protection		
Gloves	Required	Required	Required

8. Plans for emergency preparedness and response

A written emergency preparedness plan for dealing with laboratory incidents and accidents is a necessity in any facility that works with or stores *M. tuberculosis* isolates.

8.1 Emergency preparedness plan

The plan should provide operational procedures for:

- responses to natural disasters, such as fires, floods, earthquakes or explosions
- risk assessments associated with any new or revised procedure
- managing exposures and decontamination
- emergency evacuation of people from the premises
- emergency medical treatment of exposed and injured persons
- medical surveillance of persons exposed to an incident
- clinical management of persons exposed to an incident
- epidemiological investigation
- continuing operations after an incident.

In developing this plan the following items should be considered for inclusion:

1. location of high-risk areas, such as laboratories and storage areas
2. identification of at-risk personnel and populations
3. identification of procedures according to the level of risk
4. identification of responsible personnel and their duties, such as the biosafety officer, safety personnel, local health authority, clinicians, microbiologists, veterinarians, epidemiologists, fire services and police services

5. treatment and follow-up facilities that can receive exposed or infected persons
6. transport for exposed or infected persons
7. how emergency equipment will be provided, such as protective clothing, disinfectants, chemical and biological spill kits, decontamination equipment and supplies.

8.2 Emergency response procedures for TB laboratories

8.2.1 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.

Signs should be posted indicating that entry is forbidden during the clean-up procedure. Appropriate protective clothing and respiratory protection **MUST** be worn.

The following spill clean-up procedure should be used.

1. Put on gloves, a protective laboratory gown and respirator.
2. Re-enter the affected area.
3. Cover the spill with cloth or paper towels to contain it.
4. Pour an appropriate disinfectant over the paper towels and the immediate surrounding area (generally, 5% bleach solutions are appropriate).
5. Apply disinfectant concentrically beginning at the outer margin of the spill and working towards the centre.

6. Allow sufficient time for the disinfectant to act before clearing away any material for disposal. If broken glass or other sharps are involved, use a dustpan or a piece of stiff cardboard to collect the material and place it in a puncture-resistant container for disposal.
7. Place other contaminated material in a sealed bag for appropriate disposal.
8. Clean and disinfect the area of the spill.

Anyone who was exposed to the spill should be referred for medical advice; a record should be kept of the incident.

8.2.2 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.

8.2.3 Breakage of tubes inside sealed buckets (safety cups)

Always use sealed centrifuge buckets, and load and unload them in a BSC. If breakage occurs during centrifuging, broken tubes must be

discarded in a puncture-resistant container and disposed of immediately.

Decontaminate centrifuge buckets by soaking them in a suitable disinfectant. Do not use bleach to disinfect metal parts because it causes corrosion. Alternatively, buckets may be decontaminated by autoclaving.

8.3 Spill clean-up kit

The laboratory manager is responsible for maintaining spill response kits. Two spill response kits should be prepared: one placed outside the containment laboratory and one placed inside the laboratory. The kits should include the items listed below.

Spill response kit:

- Hypochlorite solution stored in an opaque bottle^a (or another suitable disinfectant)
- Respirators (1 box)
- Gloves (1 box)
- Laboratory gowns (4-6 disposable gowns)
- Dustpan and brush (for disposal if necessary)
- Chloramine tablets (10 tablets)
- Paper towels
- Soap
- Sharps container
- Biohazard bags
- Goggles (2 pairs)

^a Hypochlorite in solution has a limited shelf life. For a large spill, it may be better to prepare the disinfectant solution at the time of clean up.

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None declared

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