laboratory safety

The handbook
Global edition

A publication of the Global Laboratory Initiative a Working Group of the StopTB Partnership
laboratory safety

Richard Lumb
Lisa Shephard
Ivan Bastian
Mark Fitz-Gerald

The handbook
Global edition

TUBERCULOSIS WORK SAFE
The Laboratory Safety handbook was developed as a product of the Global Laboratory Initiative (GLI) Core Group. The GLI is a Working Group of the Stop TB Partnership. Development was led by Richard Lumb (Independent Laboratory Consultant), Ivan Bastian and Lisa Shephard (Adelaide-Supranational Reference Laboratory (SRL), and Mark Fitz-Gerald (SA Pathology).

The writing team wish to thank the following current and former members of the GLI Core Group and the WHO GLI Secretariat for their invaluable and critical reviews of the draft documents: Maka Akhalaia, Heidi Albert, Heather Alexander, Uladzimir Antonenka, Martina Casenghi, Petra de Haas, Kathleen England, Lucilaine Ferrazoli, Marguerite Massinga Loembe, Alaine Umubeyi Nyaruhirira, Daniel Orozco, Kaiser Shen, Thomas Shinnick, Alena Skrahina, Khairunisa Suleiman, Sabira Tahseen, Elisa Tagliani, Abiola Olajumoke Tubi, and Hung Van Nguyen. Lucilaine Ferrazoli, and Marguerite Massinga Loembe reviewed the final version of the document, on behalf of the GLI Core Group.

The highly professional inputs from illustrator Kerry Reid and Sue Dyer Design are most appreciated for their longstanding support and contributions to educational materials developed for laboratory diagnosis of TB, and especially for their enthusiasm and commitment to the Laboratory Safety handbook.

In particular, thanks and appreciation are expressed to Lice González-Angulo, Alexei Korobitsyn and Chris Gilpin (World Health Organization (WHO) Headquarters), Heidi Albert (FIND), Heather Alexander (US Centers for Disease Control and Prevention) and Cornelia Hennig (retired; formerly at the WHO Regional Office for the Western Pacific) for their enthusiastic support for the development of the Laboratory Safety handbook.

Development and publication of this document were made possible with financial support from:

The United States Agency for International Development (USAID).

Australian Respiratory Council (ARC), in collaboration with Dr Ral Antic (President) and the Executive Committee of The Union Asia Pacific Region.

- To the Council members, and especially to Ms Amanda Christensen (Executive Director)

Department of Thoracic Medicine Research within the Royal Adelaide Hospital.

- To Professor Paul Reynolds, Head of the Department of Thoracic Medicine at the Royal Adelaide Hospital, and to Dr Richard Stapledon (Consultant Medical Officer)

The contents of the Laboratory Safety handbook are the responsibility of the writers and do not necessarily reflect the views of USAID, ARC or the Department of Thoracic Medicine, Royal Adelaide Hospital.
Approximately 1.7 billion people (23%) of the world’s population are estimated to have a latent TB infection and therefore are at risk of developing active TB disease during their lifetime. TB is one of the ‘top 10’ causes of death and the leading cause from a single infectious agent. Globally, some 10 million people developed active TB disease in 2017, across all countries and age groups.

Drug-resistant TB continues to be a public health crisis. In 2017, there were an estimated 3.5% of new cases and 18% of previously treated cases with multidrug-resistant TB (MDR-TB) or rifampicin-resistant TB (RR-TB). The WHO estimated that there were 558,000 incident cases of RR-TB. Among RR-TB cases, an estimated 82% had MDR-TB.

The international rollout of new molecular diagnostic tools (line probe assays and GeneXpert) now enables rapid identification of people with active TB disease, and concomitantly, resistance to rifampicin, a key proxy for MDR-TB. Culture and drug susceptibility testing (DST) are required, especially for patients at risk of having drug-resistant TB, and for monitoring their response to treatment.

Although culture and DST is increasingly available, the laboratory infrastructure to contain culture and DST is more complex, and it requires more specialised and expensive equipment. A high level of technical competency is vital to ensure that laboratory staff perform the work correctly and safely.

These laboratories now face the reality of an increasing proportion of their workload coming from patients with MDR/RR-TB. Working safely to protect the individual, their colleagues, and the wider community is even more important than ever before.

Unfortunately, in many laboratories, training in safe working practice is not optimal.

The Laboratory Safety handbook is a practical guide for laboratory staff; it draws on decades of experience working in culture and DST laboratories, and references best practice documents released by WHO, the Global Laboratory Initiative, and The Union. The Handbook uses simple text and clear illustrations to assist laboratory staff in understanding the important safety issues involved in performing culture and DST.

The TB Laboratory Safety handbook should be used with the Tuberculosis laboratory biosafety manual.
The purpose of The Handbook is to teach staff working in a TB culture and/or a drug susceptibility testing laboratory how to work safely in order to reduce the risk of infection or injury for you, your co-workers, and the community.

**General principles**
Biosafety has three key parts, all of which are needed to handle TB bacilli safely:

1 **Primary**
   - Safe working practices to minimise creation of infectious aerosols and prevent spills
   - Equipment that is ‘fit for purpose’, correctly used and maintained

2 **Secondary**
   - Infrastructure and layout to support the primary activities

3 **Tertiary**
   - Buildings to contain the laboratory and its activities

**Working safely**
Personal protective equipment (PPE), fit-for-purpose equipment, and management of infectious waste all support good aseptic technique but do not replace it.

These items help contain aerosol formation but may not prevent aerosols being formed due to unsafe work practices.

**Aerosols and cross-contamination**
Good aseptic technique to minimise aerosol formation is your best protection.

The major risk of TB infection in the laboratory is associated with inhalation of aerosols generated by laboratory processes. Minimising their production is arguably the most effective means of staying safe. All aerosols should be considered as potentially infectious.

Less than 20% of laboratory acquired infections can be traced back to a recognised accident. The rest have no identifiable cause but aerosol formation is the most likely.

Aerosols once settled onto a surface, do not re-aerosolise. However, they may contaminate specimens or cultures, consumables, reagents, equipment, or PPE creating a cross-contamination risk.

**Protecting the patient**
Laboratory results will have a profound impact upon the patient. Minimising aerosol formation and cross-contamination will reduce the risk of the patient receiving a false-positive result.

Being incorrectly diagnosed as having TB disease, or drug-resistant TB may be catastrophic for the patient and their family.
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACH</td>
<td>(Room) Air Changes per Hour</td>
</tr>
<tr>
<td>AFB</td>
<td>Acid-Fast Bacilli</td>
</tr>
<tr>
<td>BSC</td>
<td>Biological Safety Cabinet</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribose Nucleic Acid</td>
</tr>
<tr>
<td>DST</td>
<td>Drug Susceptibility Testing</td>
</tr>
<tr>
<td>GLI</td>
<td>Global Laboratory Initiative</td>
</tr>
<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Air</td>
</tr>
<tr>
<td>INH (H)</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>LPA</td>
<td>Line Probe Assay (diagnostic molecular assay)</td>
</tr>
<tr>
<td>LRN</td>
<td>Laboratory Register Number</td>
</tr>
<tr>
<td>MDR-TB</td>
<td>Multidrug-Resistant TB</td>
</tr>
<tr>
<td>MGIT960</td>
<td>Semi-automated liquid culture system</td>
</tr>
<tr>
<td>μm</td>
<td>Micrometre</td>
</tr>
<tr>
<td>μl</td>
<td>Microlitre</td>
</tr>
<tr>
<td>ml</td>
<td>Millilitre</td>
</tr>
<tr>
<td>MPT64</td>
<td>Rapid test for identifying <em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>MTB</td>
<td><em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td>MTBC</td>
<td><em>Mycobacterium tuberculosis</em> complex</td>
</tr>
<tr>
<td>NTM</td>
<td>Non-tuberculous mycobacterial</td>
</tr>
<tr>
<td>NTRL</td>
<td>National TB Reference Laboratory</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate Buffered Saline</td>
</tr>
<tr>
<td>PPE</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>PMDT</td>
<td>Programmatic Management of Drug Resistant TB</td>
</tr>
<tr>
<td>RCF</td>
<td>Relative Centrifugal Force (equivalent to xg)</td>
</tr>
<tr>
<td>RPM</td>
<td>Revolutions Per Minute</td>
</tr>
<tr>
<td>RIF (R)</td>
<td>Rifampicin</td>
</tr>
<tr>
<td>RR-TB</td>
<td>Rifampicin-Resistant TB</td>
</tr>
<tr>
<td>RRV</td>
<td>Relative Risk Value</td>
</tr>
<tr>
<td>SRL</td>
<td>Supranational TB Reference Laboratory</td>
</tr>
<tr>
<td>TAT</td>
<td>Turn Around Time</td>
</tr>
<tr>
<td>TB</td>
<td>Tuberculosis</td>
</tr>
<tr>
<td>UPS</td>
<td>Uninterruptable Power Supply</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-Violet light</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume for Volume</td>
</tr>
<tr>
<td>USAID</td>
<td>United States Agency for International Development</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>w/w</td>
<td>Weight for Weight</td>
</tr>
<tr>
<td>XDR-TB</td>
<td>Extensively Drug Resistant TB</td>
</tr>
<tr>
<td>Xpert MTB/RIF assay</td>
<td>Diagnostic molecular assay</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Airlock</td>
<td>A small room separating a laboratory area from a corridor or another space (equivalent to an anteroom)</td>
</tr>
<tr>
<td>Aerosol (infectious)</td>
<td>A particulate suspension (of an infectious agent) that has the potential to be inhaled and to cause infection</td>
</tr>
<tr>
<td>Aerosol generating procedures</td>
<td>Procedures that increase the risk of aerosols because of the mechanical force of the procedure</td>
</tr>
<tr>
<td>Anteroom</td>
<td>See airlock</td>
</tr>
<tr>
<td>‘Clean’</td>
<td>An area or item that is less likely to contain, or to be contaminated with TB bacilli or other infectious agents</td>
</tr>
<tr>
<td>Cross-contamination</td>
<td>An unplanned event that transfers material from one sample/culture/reagent to another sample/culture/reagent</td>
</tr>
<tr>
<td>Bacillary load</td>
<td>The number of tubercle bacilli/unit volume in the solid or liquid being handled</td>
</tr>
<tr>
<td>‘Dirty’</td>
<td>An area or item that is more likely to contain, or be contaminated with TB bacilli or other infectious agents</td>
</tr>
<tr>
<td>High risk</td>
<td>The risk of generating infectious aerosols during manipulation of cultures; high concentration of infectious particles</td>
</tr>
<tr>
<td>Low risk</td>
<td>The risk of generating infectious aerosols from specimens; low concentration of infectious particles</td>
</tr>
<tr>
<td>Laboratory Manager</td>
<td>This position has ultimate responsibility for the function, operation and performance of the entire laboratory</td>
</tr>
<tr>
<td>McCartney bottle</td>
<td>Reusable, clear glass container (= 30ml volume) with screw-cap closure and either a metal or plastic lid with rubber insert; used for solid media</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>The risk of generating infectious aerosols from specimens; low concentration of infectious particles</td>
</tr>
<tr>
<td>Respirator</td>
<td>An FFP2 or N95 type respirator</td>
</tr>
<tr>
<td>Risk</td>
<td>A combination of the likelihood and consequences of an incident related to a specific hazard(s)</td>
</tr>
<tr>
<td>Specimen tracking</td>
<td>A laboratory process used to ensure that the correct results are matched to the correct patient</td>
</tr>
<tr>
<td>Staff</td>
<td>Persons, regardless of qualifications or gender, that undertake work within a TB culture/DST laboratory</td>
</tr>
<tr>
<td>Supervisor</td>
<td>This position has responsibility for the day to day function, operation and performance of one or more sections within a TB laboratory</td>
</tr>
<tr>
<td>Weather vestibule</td>
<td>Outer laboratory area that is adjacent to the anteroom entry. It may, or may not, connect public space to the anteroom</td>
</tr>
<tr>
<td>Warning</td>
<td>Failure to follow these instructions may harm your health or cause immediate damage to equipment</td>
</tr>
<tr>
<td>---------</td>
<td>--------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Correct</td>
<td>The preferred way to do something</td>
</tr>
<tr>
<td>Warning</td>
<td>Failure to follow these instructions may affect test results, or cause equipment damage over time</td>
</tr>
<tr>
<td>Do not do this</td>
<td></td>
</tr>
<tr>
<td>Wear a laboratory gown for this procedure</td>
<td></td>
</tr>
<tr>
<td>Wear gloves for this procedure</td>
<td></td>
</tr>
<tr>
<td>Covered shoes must be worn in the laboratory at all times</td>
<td></td>
</tr>
<tr>
<td>Wear glasses for this procedure</td>
<td></td>
</tr>
<tr>
<td>Wash your hands</td>
<td></td>
</tr>
<tr>
<td>This substance is corrosive</td>
<td></td>
</tr>
<tr>
<td>This substance is flammable</td>
<td></td>
</tr>
<tr>
<td>This substance is toxic</td>
<td></td>
</tr>
<tr>
<td>CHAPTER</td>
<td>PAGE</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>1 Safe working practices</td>
<td>11</td>
</tr>
<tr>
<td>2 Laboratory infrastructure and layout</td>
<td>17</td>
</tr>
<tr>
<td>3 Personal protective equipment</td>
<td>41</td>
</tr>
<tr>
<td>4 Using the biological safety cabinet</td>
<td>59</td>
</tr>
<tr>
<td>5 Aerosol generation and prevention</td>
<td>77</td>
</tr>
<tr>
<td>6 Contamination causes and prevention</td>
<td>85</td>
</tr>
<tr>
<td>7 Containers and reagents</td>
<td>103</td>
</tr>
<tr>
<td>8 Using equipment safely</td>
<td>111</td>
</tr>
<tr>
<td>9 Managing laboratory waste</td>
<td>131</td>
</tr>
<tr>
<td>10 Managing infectious spills</td>
<td>141</td>
</tr>
</tbody>
</table>
SAFE WORKING PRACTICES

Laboratories present numerous hazards to staff, and not all are immediately obvious. Safe working practices are designed to

• Reduce the risk of infection or injury to you, co-workers, and the community

• Protect the patient from incorrect results

Less than 20% of laboratory acquired infections can be traced back to a recognised accident. The rest have no identifiable cause but aerosol generation is the most likely.

This chapter introduces the concepts and definitions that will be used throughout the handbook.
How infection occurs

TB is an infectious disease. Transmission occurs when small aerosols containing acid fast bacilli (AFB) become airborne and are inhaled. When a person coughs, sneezes, sings or vigorously exhales, they produce aerosols that could be infectious if the person has pulmonary TB.

Natural history of TB infection

If 100 healthy people became infected with TB today, the natural course of progression to disease may occur in two ways:

- 2–5% will develop TB disease within 2 years
- Another 2–5% will develop TB disease in their lifetime

People who are immunocompromised have a much higher risk of progression from infection to disease. Risk factors include, but are not limited to, co-infection with HIV, diabetes, chemotherapy, malnutrition, smoking, and chronic illness.

A laboratory worker who is immunocompromised is at increased risk of TB infection progressing to disease. A medical clearance must be obtained before working in a TB culture/DST laboratory.

As few as 10 AFB is enough to establish infection. Working with large numbers of AFB and using aerosol generating procedures greatly increases the risk of TB infection.
Pillars of biosafety

Biosafety has three key parts, all of which are needed to handle TB bacilli safely.

1 Primary
   Safe working practices to minimize spills and creation of infectious aerosols
   Equipment that is ‘fit for purpose’, correctly used and maintained

2 Secondary
   Infrastructure and layout to support the primary activities

3 Tertiary
   Buildings to contain the laboratory and its activities

The combination of all three is required to handle TB bacilli safely. However, safe working practice is essential.

Definitions

Aerosol
Airborne droplets containing infectious agents capable of
   • Being inhaled and establishing infection
   • Contaminating consumables, reagents, and equipment

Droplet nuclei
   • Dried-out residues of aerosols <5 μm in diameter
   • Capable of floating in the air for an extended period of time
   • Small and light enough to reach deep into the lungs
**Aerosol generating activities**
- Activities that increase the risk of aerosol production due to mechanical force
- Aerosols are produced more easily from less viscous fluids
  - Sputum is usually viscous and more difficult to create aerosols
  - Liquid cultures are fluid and hence create aerosols more easily
- Examples include vortexing, shaking, centrifuging, mixing, or pipetting

**Bacillary load**
Bacillary load is the number of AFB in a specimen or culture, typically classified as variable, low, medium or high.

Very small volumes of positive cultures may hold very large numbers of AFB.

**Cross-contamination**
Any unplanned event that transfers AFB from one item to another.

For example
- AFB from a specimen or culture is transferred into a reagent
- Aerosols from a strong positive specimen or culture are transferred to another
- AFB on contaminated gloves transferred to a mobile phone

**Specimen tracking**
A process used to ensure that laboratory results are matched to the correct patient. Specimen tracking helps protect patients from false results.

1 Specimen
2 Request form
3 Laboratory processes
4 Results

Specimen tracking is required at every step from specimen collection to reporting results.
TB infection risk and work activity

A retrospective study undertaken in South Korea provided objective evidence linking work activity with risk of developing TB. Administrative staff without direct laboratory contact were compared with laboratory staff performing microscopy, culture, culture/DST, and DST.

Administrative staff had a relative risk value (RRV), similar to the general community that is a RRV of 1.0. Due to the relatively small number of staff by activity, the confidence intervals are necessarily wide.

<table>
<thead>
<tr>
<th>Activity</th>
<th>Relative risk value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Administration</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Microscopy only</td>
<td>1.4</td>
<td>0.2 – 10.0</td>
</tr>
<tr>
<td>Culture only</td>
<td>2.0</td>
<td>0.2 – 13.3</td>
</tr>
<tr>
<td>Combined culture and DST*</td>
<td>7.8</td>
<td>1.7 – 34.9</td>
</tr>
<tr>
<td>DST only</td>
<td>21.5</td>
<td>4.5 – 102.5</td>
</tr>
</tbody>
</table>

* In most laboratories, staff performing TB culture undertake DST also. Laboratories where staff perform culture only or DST only are those with very large workloads.

Key findings included

- Sputum microscopy is a low-risk activity; there is no need for BSCs in laboratories performing smear microscopy only
- Processing specimens for TB culture only marginally increases the RRV
- Manipulating positive cultures for DST had the highest RRV

Neither GeneXpert nor the Line Probe Assay (LPA) were available during the study timeline. The WHO *Tuberculosis Laboratory Biosafety Manual* provides guidance on the relative risks with these activities.
SAFE WORKING PRACTICES

<table>
<thead>
<tr>
<th>Activity</th>
<th>Relative risk value</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneXpert</td>
<td>≤1.4</td>
<td>Equivalent to sputum smear microscopy. Lysis buffer reduces AFB viability by $10^6$ within 15 minutes.</td>
</tr>
<tr>
<td>LPA (on specimens)</td>
<td>≤2.0</td>
<td>Equivalent to culture. Specimen processing as for culture. DNA extraction step inactivates AFB.</td>
</tr>
<tr>
<td>LPA (on culture)</td>
<td>≤21.5</td>
<td>May be as high as for DST. Requires manipulation of a positive culture. DNA extraction step inactivates AFB.</td>
</tr>
</tbody>
</table>

Summary

The major risk of TB infection in the laboratory is associated with inhalation of aerosols generated by laboratory processes. Minimising their production is the most effective means of staying safe.
LABORATORY INFRASTRUCTURE AND LAYOUT

Laboratory infrastructure and layout is the second pillar of biosafety.

The aim of this chapter is to

• Define risk by laboratory activity
• Explain how laboratory design can minimise risk by
  - Engineering controls
  - Location of activities within a laboratory area
  - Location of equipment
• Introduce topics for discussion between the laboratory users, architects, engineers, and builders responsible for design and construction.

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activity and risk</td>
</tr>
<tr>
<td>Infrastructure – guiding principles</td>
</tr>
<tr>
<td>Structure</td>
</tr>
<tr>
<td>Topics for the engineer and architect</td>
</tr>
<tr>
<td>Scale modelling</td>
</tr>
<tr>
<td>Infrastructure maintenance</td>
</tr>
<tr>
<td>Laboratory layout</td>
</tr>
<tr>
<td>Triangulation</td>
</tr>
<tr>
<td>Summary</td>
</tr>
</tbody>
</table>
There has been an evolution in biosafety guideline development over the last decade that aligns risk with activity.

Previously, an organism was assigned a Risk Group based on virulence, transmissibility, and the availability of treatments. The containment level for a Risk Group took no account of the actual procedures being performed or their inherent risks.

The WHO *Tuberculosis Laboratory Biosafety Manual* (2012) incorporated a risk assessment approach that considered the procedures being performed.

A safe and efficient laboratory layout complements infrastructure by separating low-risk (‘clean’) from high-risk (‘dirty’) activities, and optimising movement within the laboratory.

**Activity and risk**

TB laboratory activities are assessed according to the risk of generating aerosols, and the bacillary load. For further information on conducting risk assessments, refer to the WHO *TB Laboratory Biosafety Manual*.

<table>
<thead>
<tr>
<th>Risk level</th>
<th>Laboratory activities</th>
<th>Assessment of risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>Direct sputum microscopy; preparation of specimens for the Xpert MTB/RIF assay</td>
<td>Low risk of generating infectious aerosols from specimens; low concentration of infectious particles</td>
</tr>
<tr>
<td>Moderate risk</td>
<td>Processing and concentration of specimens for inoculation on primary culture media; direct molecular testing on processed sputum by a line probe assay</td>
<td>Moderate risk of generating infectious aerosols from specimens; low concentration of infectious particles</td>
</tr>
<tr>
<td>High risk</td>
<td>Culture manipulation for identification, phenotypic DST, or a line probe assay on cultures</td>
<td>High risk of generating infectious aerosols from cultures; high concentration of infectious particles</td>
</tr>
</tbody>
</table>
Infrastructure – guiding principles

TB culture and DST require a dedicated laboratory space and equipment. It must not be used for other routine diagnostic services.

In laboratories where low-risk activities only are conducted (microscopy, GeneXpert), equipment may be shared.

‘Clean’ versus ‘dirty’ activities
‘Clean’ and ‘dirty’ are relative terms within a TB culture or DST laboratory. All equipment, surfaces, plus consumables and reagents are potentially contaminated.

- ‘Clean’ (low-risk) means the area or item is less likely to contain, or to be contaminated with AFB or other infectious agents
- ‘Dirty’ (high-risk) means it is more likely to contain, or be contaminated with AFB or other infectious agents

Risk level and laboratory areas
The entry area should be reserved for ‘clean’ activities. ‘Dirty’ activities should be furthest away from the entry.

Low-risk activities include
- Administration, hand-washing station, microscopy, GeneXpert, consumables and reagent storage, staining

Moderate-risk activities include
- Culture processing and media inoculation

High-risk activities include
- Handling positive cultures, identification of MTB, DST, preparing DNA extracts from positive cultures

Air movement
Directional airflow helps reduce risk. It is created using a negative pressure gradient. Air should move from the entry, where low risk activities take place, to the end of the laboratory where the highest risk activities occur.

The air-handling system must also be able to exchange the volume of air in a laboratory 6-12 times per hour (room air changes/hour = ACH). The WHO Tuberculosis Laboratory Biosafety Manual provides guidance on determining ACH in a laboratory that uses mechanical ventilation.

Multi-room laboratories
For laboratories with separate rooms for specific activities, the same principles apply. The entry point has the lowest level of risk increasing as you move further into the room. In a multi-room laboratory, air flows from ‘clean’ to ‘dirty’ areas within each room.
Single room laboratory
Air flows from ‘clean to dirty’ areas

Multi-room laboratory
Air flows from ‘clean to dirty’ within each individual laboratory area

Note: for simplicity, the airlocks in the culture and DST laboratories have not been shown.
Structure

The laboratory structure, how we assign the functional spaces, and their relationship to each other, has a fundamental impact on both workflow and safety.

For TB culture-only laboratories, some structural requirements are optional. However, the role of a TB culture-only laboratory is changing from a diagnostic to a monitoring role for patients with drug resistant TB. Laboratories should anticipate an increasing proportion of specimens from patients with MDR/XDR-TB. Accordingly, these options are strongly recommended for TB culture-only laboratories.

Weather vestibule

This area is the first entry point into the laboratory.

Key considerations
- May connect public space to the airlock
- At positive pressure to the airlock
- May include a handwashing station

Additional considerations
- A space for storing larger amounts of consumables, reagents and PPE
- Toilets and showers may adjoin the weather vestibule
- Access to building services

Weather vestibule relative to the airlock and adjacent areas
**Airlock**

Airlocks are highly recommended for TB culture-only laboratories and mandatory for TB culture/DST laboratories.

Airlocks should not be used for hand-washing and storage of personal protective equipment, consumables and reagents, or equipment.

Safety items such as fire extinguishers and a spills kit may be stored in the airlock.

**Key considerations**

- Provides a physical barrier between the weather vestibule and laboratory
- At negative pressure to the weather vestibule but at positive pressure to the laboratory
- Must be large enough to accommodate, and manoeuvre, the largest item of equipment used within the TB laboratory
- Airlock doors must have windows allowing a view of the laboratory
- Pressure gauges for airlocks/laboratory/weather vestibule
- Security system for access
- Interlocking doors: one door only may be opened at a time plus an emergency button to override interlocks
- Seamless floors with coving

**Additional considerations**

- Can be fumigated independently of main laboratory area
- Outer/inner doors should be aligned

**AIRLOCKS ARE MANDATORY FOR TB LABORATORIES PERFORMING DST**
Laboratory – TB culture
The TB culture laboratory monitors an increasing number of patients with drug-resistant TB. Accordingly, the risks within the laboratory have increased and should be reassessed. Culture laboratory infrastructure may need to be aligned with DST requirements.

Consider including
- An airlock
- Pass-through box
- Steam pressure sterilizer
Laboratory design has three parts; (i) architecture, (ii) engineering, and (iii) fit out.

**Architecture**
Floors, walls, and structural joints must be smooth, easy to clean, non-porous to liquids, and resistant to chemicals and disinfectants used in the laboratory.
- Floors are slip-resistant and coved to walls
- Structural joints should be minimised
- Suitable materials include, but not limited to, vinyl sheeting and epoxy paint

Benches must be strong enough to support equipment, smooth, easy to clean, non-porous to liquids, and be resistant to chemicals and disinfectants used in the laboratory.
- Strong support frame
- Preferred bench materials include
  - Resin based materials
  - Fibreglass/epoxy
  - Wood core sealed and covered with a non-porous laminate
- Painted wooden surfaces not recommended
- Bench height should vary according to work activity
  - General work bench ≈ 900mm
  - Administration, microscopy ≈ 720mm
- Under bench areas accessible for ease of cleaning
  - Storage under bench should be minimised

**Engineering**
Laboratories require a stable, reliable, and adequate supply of electricity and water (utilities). Designing a new laboratory or renovating an existing laboratory with additional equipment will place pressure on existing utilities, particularly for electricity. Bringing additional utilities into a laboratory is often expensive. Working with relevant utility suppliers requires planning well ahead of construction.

Is the electricity supply stable and sufficient for current and projected future needs?
- Requires surge protection to protect electrical circuits and equipment
- Air-handling systems and additional equipment may substantially increase electricity needs
- Include emergency power needs to maintain air-handling systems (directional airflow, air-conditioning), lighting, key equipment items (BSC, GeneXpert, refrigerators) when electricity supply fails
  - Is emergency electricity to the laboratory a dedicated system or is it part of a facility system?
  - Is sufficient fuel available to operate the emergency electricity system?
• Do key equipment items have UPS support for short-term power loss (e.g. BSC, GeneXpert)

Sufficient water supply to meet current and future needs
• Sinks and handwash stations within the laboratory
• Toilets and showers in the weather vestibule area
• Emergency water supply via an overhead tank (5,000 litres) plus pump

A combination of natural and artificial lighting is recommended. In particular, windows onto corridors or other laboratory spaces are required so that the safety of workers within the laboratory can be observed. Windows must be sealed shut and cannot be opened.

Gas is not required in the laboratory.

Fit-out

1 Soap dispenser
2 Back-up hand-wash (e.g. alcohol-based hand gel)
3 Hands-free tap
4 Paper towel dispenser
5 ‘How to wash your hands’ poster
6 Hooks for holding gowns being used within the laboratory; near to the hand wash station
7 Waste bin

Note: An eye wash station is not shown due to the wide variety of options available.
A handwashing station must be located at the laboratory entry
- Hands-free tap
- Soap dispenser
- Secondary hand-wash available as back-up
- Paper towel dispenser
- Handwashing poster (optional)
- Waste bin
- Eye wash station – this may be as simple as a bag of sterile saline or having dedicated plumbing, with adjustable pressure outlet
- Hooks for holding gowns being used within the laboratory and near to the hand wash station

Dedicated sinks for laboratory activities
- Made from a material resistant to stains, solvents, and chemicals
- Ceramic sinks must not be used

Laboratory furniture must be strong, made from non-pervious materials able to be decontaminated easily
- Laboratory chairs
  - Adjustable height
  - Castors must lock into place when weight is applied to the seat
  - A 5-leg base is preferred to increase stability

Area for small amounts of acids, solvents, and stains
- Preparation of stains and reagents done outside of the laboratory

Storage area within the laboratory
- Cupboard or shelving separate from work benches
- In ‘clean’ area of the laboratory
- Capacity to hold at least one working week of PPE, plus consumables and reagents
- Storage area outside of the laboratory
  - Secure area to prevent theft
  - Weather vestibule or near-by area
  - Longer-term storage for larger amounts of PPE, plus consumables and reagents
  - Small supply of less frequently used items
Good posture
Supporting your feet straightens your back

Poor posture
Feet unsupported

Good posture
Raise the microscope to help straighten your back and keep your feet flat on the floor

Poor posture
Seat too high or bench too low, feet not flat
Laboratory – Culture/DST
Additional infrastructure is mandatory for a laboratory performing DST.

Laboratory space
- Airlock including interlocks to ensure that only one airlock door may be opened at any time
  - Emergency over-ride required for both doors
- Security system by code pad or key card for access
- Pass-through box for specimens and other items
- Two independent communications systems
  - Telephone
  - 2-way intercom system
- Steam pressure sterilizer located at ‘dirty’ end of the laboratory
  - Stand-alone steam pressure sterilizer should be used
    - Pass-through steam pressure sterilizers are not recommended due to cost and complexity
  - Directional airflow such as thimble exhaust to remove steam and heat
- Alarm system to indicate that negative pressure within the laboratory is out of range
  - Excessive high or low pressure is a health risk and systems to turn off air handling system must be in place
Topics for the engineer and architect

Expert input from both staff and qualified TB laboratory experts must inform the design brief. The design requirements and final laboratory size will depend on many factors.

Workload
- Test types presently performed (microscopy, GeneXpert, LPA, culture, DST, other)
- Estimated future workload
- Implementation of additional tests, space requirements?

Staff numbers
- Current staff numbers
- The estimated number of tests performed per technician in an 8-hour work day is in the table below

Supporting services
- Will a laboratory information system be installed during construction or within the next 10 years?
A scaled floor plan with equipment and benches cut out to scale provides a simple and effective means of exploring structural options, laboratory workflow, and equipment placement.

### Scale modelling

<table>
<thead>
<tr>
<th>Measurement (mm)</th>
<th>Scaling</th>
<th>Dimension to scale (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>1:100</td>
<td>10</td>
</tr>
<tr>
<td>1,000</td>
<td>1:50</td>
<td>20</td>
</tr>
</tbody>
</table>

### Estimate of maximum workload by test procedure that can be performed by one competent technician

<table>
<thead>
<tr>
<th>Test procedure</th>
<th>Maximum number of tests/technician(^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day</td>
</tr>
<tr>
<td>AFB light microscopy</td>
<td>25</td>
</tr>
<tr>
<td>AFB fluorescence microscopy</td>
<td>50</td>
</tr>
<tr>
<td>Culture (liquid/solid) specimen processing</td>
<td>40</td>
</tr>
<tr>
<td>DST (liquid)</td>
<td>20</td>
</tr>
<tr>
<td>DST (solid)</td>
<td>20</td>
</tr>
<tr>
<td>Xpert (4-module)</td>
<td>16</td>
</tr>
<tr>
<td>LPA – First line (manual)</td>
<td>24</td>
</tr>
</tbody>
</table>

1 The maximum number is indicative* only and depends on
- Human resources, staff experience and expertise,
- Laboratory infrastructure and layout,
- Equipment, and
- Reliability of utilities such as electricity and water

2 Year is based on 230 working days
- Equipment needs by test
  - Amount of equipment (BSCs, incubators, MGIT960, centrifuges) determined by workload, test type, liquid and/or solid media, and staffing
  - Restrictions on equipment placement, especially BSCs, due to doors, people movement, airflow (refer to Using the BSC chapter)
- Electrical
  - Loads from: Equipment, Lighting, Ventilation, Air-conditioning
  - Placement of equipment and electrical loads required by area
  - Number and placement of general power outlets
  - Emergency power circuit outlets when power outages occur
- Water
  - Supply to: Handwash stations, Sinks, Toilets and showers (if included as part of the laboratory facility)

Infrastructure maintenance

Imagine buying a brand-new car and then doing no maintenance and repairs for the life of the vehicle. Maintenance and repairs is always required for continuing good performance.

In many countries, infrastructure maintenance is often ignored. Annual funding must be available for infrastructure maintenance to ensure the laboratory performs to specifications. Priorities for maintenance are provided in the table below.

<table>
<thead>
<tr>
<th>Item</th>
<th>Action</th>
<th>Timeline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handover of laboratory</td>
<td>Confirmation that the structure meets specifications and is ‘fit-for-use’ Laboratory should be operational Sign-off should include representatives from relevant Ministry, from donors, and partners</td>
<td>Completion of commissioning</td>
</tr>
<tr>
<td>post-completion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First year of operation</td>
<td>Any defects or breakdowns/repairs addressed under warranty</td>
<td>During the 12 months from date of commissioning</td>
</tr>
<tr>
<td>Air-handling system</td>
<td>Maintenance of system including HEPA performance</td>
<td>Annual</td>
</tr>
<tr>
<td>Electrical</td>
<td>System check of all power outlets</td>
<td>Every 2 years</td>
</tr>
<tr>
<td>Breakdown</td>
<td>Any breakdown that stops work</td>
<td>On demand &lt;24hrs response time</td>
</tr>
<tr>
<td>BSC</td>
<td>Maintenance</td>
<td>At installation and before use, annually, if the BSC is moved, or has a filter replaced</td>
</tr>
<tr>
<td>All other equipment items</td>
<td>Maintenance</td>
<td>Annual or according to manufacturer’s instructions</td>
</tr>
</tbody>
</table>
Laboratory layout – Low-risk area

There should be only one entry door into the laboratory. The handwashing/eyewash station should be located immediately adjacent to the door.

Other items include:

- Administration
- Bench space, laboratory registers, computer, printer/scanner/fax, telephone
- Microscope(s)
- Ready-use consumables & reagents storage area
- Pass through box
- GeneXpert
- Gown hooks
- Refrigerator (consumables and reagents only)

From a biohazard context, preparation of working reagents and the staining area are ‘clean’. However, both activities require a wet area and should be further away from the entry.
Activities generate low concentration of infectious particles. Specific equipment is required and all specimen processing must be performed within a BSC.

Equipment includes:

- BSC
- Centrifuge
- MGIT960
- Incubator
- Infectious waste
- Refrigerator for non-infectious material
- Refrigerator for infectious material
- Vortex (inside BSC)
- Access to an autoclave
Handling positive cultures creates a high risk of generating infectious aerosols containing a high concentration of infectious particles. Activities include preparing smears from positive cultures, identification, DST, and preparing cultures for LPA. All activities must be performed within a BSC using safe working practices.

Equipment includes:

- BSC
- MGIT960
- Incubator
- Refrigerator for non-infectious material
- Refrigerator for infectious material
- Ultra-cold freezer
- Autoclave
**Triangulation**

To optimize a work area, place key items of equipment together in a ‘triangular shape’ around the work station where an activity is performed.

Resolve these questions as part of planning the equipment location

- What specific activity will be performed?
- What equipment is required to perform the activity?
- How often is the equipment used when performing the activity?

**Triangulation for Microscopy**

Microscopy

This activity includes

- Administration (registration, recording and reporting)
- Smear preparation (open bench or in a BSC)
- Staining (sink with drainboard, water supply)
- Microscopy (microscope and bench space)

Frequency of movement

- **Frequent**
- **Less frequent**
Triangulation for GeneXpert

GeneXpert includes:
- Administration (registration, recording and reporting)
- Sample preparation (open bench or in a BSC)
- Loading/unloading the GeneXpert machine

Frequency of movement:
- Frequent
- Less frequent
Specimen processing for culture
Includes
Administration
(labelling, recording and reporting)
Centrifugation
Specimen decontamination
Inoculating media
Infectious waste management

Since most time is spent using a BSC, it needs to be the center around which other activities occur and equipment is placed.
Positive cultures
Includes

- Administration (labelling, recording and reporting)
- Preparing smears
- Conducting rapid test (MPT64, LPA)
- Inoculating media
- Infectious waste management

Since most time is spent using a BSC, it needs to be the center around which other activities occur and equipment is placed.
Drug susceptibility testing
Includes
- Administration (labelling, recording and reporting)
- Adding drug solution to media (liquid)
- Inoculating media
- Preparation of inoculum
- Infectious waste management

Frequency of movement

Since most time is spent using a BSC, it needs to be the center around which other activities occur and equipment is placed.
Summary

A functional TB culture-only or TB culture/DST laboratory rarely occurs by chance. It requires collaboration by people bringing specialist skills to help identify the problems, and to develop a strategy for solutions through design, construction, and layout. Inputs from laboratory staff and TB laboratory specialists are vital to help create a functional laboratory and with a safe working environment.
PERSONAL PROTECTIVE EQUIPMENT

This chapter provides advice regarding the applicability, use, and availability of personal protective equipment (PPE) in the TB laboratory.

<table>
<thead>
<tr>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gowns and coats</td>
<td>42</td>
</tr>
<tr>
<td>Gloves</td>
<td>45</td>
</tr>
<tr>
<td>Respirators</td>
<td>49</td>
</tr>
<tr>
<td>Surgical masks</td>
<td>55</td>
</tr>
<tr>
<td>Eye and face protection</td>
<td>56</td>
</tr>
<tr>
<td>Footwear</td>
<td>58</td>
</tr>
<tr>
<td>Summary</td>
<td>58</td>
</tr>
</tbody>
</table>
Personal protective equipment provides a physical barrier to minimise the risk of exposure to aerosols, splashes and accidental inoculation. PPE must be worn at all times within the laboratory.

Poorly fitting, inappropriate, or incorrectly worn PPE will reduce its effectiveness and may create a false sense of safety. The choice of PPE depends upon the type of work being performed and the risk to be reduced.

All PPE should be supplied by the laboratory. PPE items should be disposable and not reusable.

Staff must not take home any PPE for washing, disposal, or for use outside of the laboratory.

**Gowns and Coats**

**Gowns**
Laboratory gowns fasten at the back, providing protection across the front.

Gowns may be disposable or reusable
• Reusable gowns must be autoclaved (121°C for 15 minutes) before being taken away for cleaning
• For re-usable gowns, there should be at least three gowns available per staff member
  - In-use
  - Being cleaned
  - Ready for use
• Gowns should be changed weekly or after an obvious spill occurs
• Gowns must be available in small, medium, and large sizes

Qualities
• Ties at the neck and waist
• Full cover to the front
• Elasticised cuffs at least 30mm
• Long enough such that the gown fully covers the lap when sitting down
• Non-absorbent material

USE OF DISPOSABLE GOWNS IS STRONGLY RECOMMENDED

Gowns must not be worn outside of the laboratory.

Gowns must not be stored in the same place as street clothing.

Do not use gowns with an attached mask.
Coats
Laboratory coats open at the front and may have short or long sleeves.

Laboratory coats
1. Open at the front and do not provide protection for TB culture or DST work
2. Have short or long sleeves
3. Do not have elasticised wrist bands

COATS MUST NOT BE USED IN LABORATORIES PERFORMING TB CULTURE OR DST
Gloves

Disposable gloves only are to be worn in a TB laboratory.

DO NOT RE-WEAR USED GLOVES

Several pairs of gloves will be used each day, a sufficient supply must be readily available.

Gloves must be worn for all procedures that involve contact with specimens or laboratory items used in handling specimens or cultures.

Allergic reactions such as skin rash (dermatitis) and hypersensitivity reactions may occur in staff wearing latex gloves (powdered and non-powdered). Alternative glove materials include vinyl and nitrile which rarely cause allergic reactions.

Do not take gloves outside of the laboratory.

Wearing gloves
Different sizes of gloves must be available (small, medium, large). Poorly fitting gloves reduce the dexterity of the fingers and increase the risk of glove contamination and accidents.

• Too small and they are easy to tear
• Too large and fine motor skills are lost

Correctly fitting gloves

Poorly fitting gloves
Gloves must cover cuffs by at least 30mm
Gloves always cover cuffs

Fine motor skills are compromised by gloves that are too large
PERSONAL PROTECTIVE EQUIPMENT

Never use your mobile phone wearing gloves or while working in the laboratory.

Do not tape gloves to cuffs.

Replace torn gloves immediately.
Removing gloves
Used gloves must be discarded into a laboratory infectious waste bin.

1 Hold the cuff of the glove on the other hand and slowly peel the glove from the hand
2 Gather the used glove into the palm of the other hand and close fingers
3 Carefully slip ungloved fingers under the cuff of the gloved hand; be careful not to touch the outer surface of the glove
4 Peel the glove off such that the held glove is now inside of the glove being removed
5 Dispose of gloves into an infectious waste bin

Once gloves are removed, wash your hands immediately.
Respirators

Respirators and surgical masks are not the same. Surgical masks provide no effective respiratory protection from aerosols and must not be used.

Respirators must filter >95% of infectious particles greater than 0.2μm in size. N95 and FFP2 respirators meet the requirements and are lightweight, disposable devices that cover the nose and mouth.

Both FFP2 and N95 respirators may be ‘valved’ or ‘unvalved’
- ‘Valved’ respirators allow expired air to move easily from the lungs to the environment but closes when breathing in occurs
- ‘Unvalved’ respirators do not have a valve

Respirators are not usually required for work in a TB culture laboratory. However, they must be worn when setting up DST.

They can be reused provided that they are properly worn, stored and cared for.

RESPIRATORS ARE NOT A SUBSTITUTE FOR A PROPERLY MAINTAINED AND FUNCTIONING BSC

If respirators are used, staff must be
- Instructed in correct use
- Taught how to care for a respirator
How to wear a respirator correctly

1. Hold the respirator in the palm of your hand, straps underneath and aligned appropriately.
2. Position the respirator under the chin, place the respirator gently over the face.
3. Place the upper headband over the head, locate it high at the back of the head, and over the ears.
4. Pull the lower headband over the head and locate under the ears.
5. Place the fingertips of both hands on the metal nose-piece and mould the metal strap to the nose. Exhale and inhale to confirm pressure and detect possible leakage.

ALWAYS USE FINGERS OF BOTH HANDS TOGETHER – USING ONE HAND MAY RESULT IN AN INCORRECT FIT

THICK BEARDS MAY PREVENT AN EFFECTIVE SEAL BETWEEN FACE AND RESPIRATOR
Correct removal of a respirator
Remove gloves and wash hands properly.

1. Locate the lower headband strap and pull over the head; release strap tension and hold the strap in one hand
2. Locate the upper headband strap and pull over the head; release strap tension and hold the strap in one hand
3. Remove the respirator away from the face and hold both straps in one hand

Store the respirator in a clean, dry area (see caring for a respirator)

Wash your hands immediately

Common mistakes
Straps not placed correctly.

- Both straps above the ears
- Both straps below the ears
- Straps crossed
Common mistakes

- Placing respirator around the neck will cause damage
- Placing respirator on the forehead will cause damage
- Nosepiece not correctly shaped – gap at the nosepiece
Caring for a respirator
With care, a respirator may be reused multiple times over two-three weeks. Before use, always carefully check that
- There are no holes
- Strap connection is not damaged
- Surface of respirator is clean and no loose fibres
- Straps have not overstretched

Store in a paper bag with air holes to allow moisture to evaporate.

**Do not** disinfect, clean, or repair damaged respirators; discard into a biohazard waste bin and obtain a new respirator.

**Do not** use a plastic bag to store a respirator as the moisture cannot evaporate.
PERSONAL PROTECTIVE EQUIPMENT

A respirator should be stored in a well ventilated place when not in use so the respirator can dry out.

**DON'T STORE A RESPIRATOR IN A POCKET
IT WILL CRUSH THE PRE-FORMED SHAPE**

**Fit testing a respirator**
Fit testing requires specialized equipment and trained personnel not commonly available in many settings. Additionally, different facial features, shapes and sizes will require a range of different respirators to comply with the various standards.
Surgical Masks

Surgical masks provide no effective respiratory protection from aerosols and must not be used in a laboratory setting.

SURGICAL MASKS PROVIDE NO EFFECTIVE RESPIRATORY PROTECTION
### Eye and face protection

Equipment selection to protect the eyes and face from splashes is determined by the type of laboratory activity.

The equipment must be available within the laboratory at all times.

#### Equipment options

Protective glasses are made from shatterproof plastic and curved around the sides. Some protective shields are designed to fit over standard prescription glasses.

Face shields are made from shatterproof plastic, cover the front and sides of the face, and are held in place by a head strap.

Prescription glasses and contact lenses do not provide protection.

**DO NOT USE PRESCRIPTION GLASSES AND CONTACT LENSES AS EYE PROTECTION**

#### Equipment selection

<table>
<thead>
<tr>
<th>Type</th>
<th>Activity</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Safety glasses or protective shields</strong></td>
<td>Dilution of strong acids for decolourization reagent in staining</td>
<td>Always add strong acids to water. Heat will be generated so add acid slowly and mix</td>
</tr>
<tr>
<td></td>
<td>Preparation of strong alkaline solutions (4% NaOH) for specimen decontamination</td>
<td>Heat will be generated so add NaOH pellets slowly and mix</td>
</tr>
<tr>
<td></td>
<td>Spill kit</td>
<td></td>
</tr>
<tr>
<td><strong>Face shield (see page 137)</strong></td>
<td>Unpacking an autoclave</td>
<td>Post-autoclaving, large liquid volumes may boil over if moved before cooling</td>
</tr>
</tbody>
</table>
Safety glasses are made from shatterproof plastic and are curved around the sides.

Protective shield covers prescription glasses, are made from shatterproof plastic and cover the front and sides of the face.
Footwear

Shoes must cover the toes, the upper part of the feet, and have a closure to the back of the heel such that footwear cannot be removed easily.

Footwear such as thongs and sandals do not provide protection from physical injury due to hitting toes on solid structures, dropping heavy items onto the feet, cuts from sharp objects and splashes of infectious material/liquids.

Summary

Wearing PPE correctly provides an important barrier to aerosols and contamination. However, wearing PPE does not protect the user from unsafe work practices.
4 USING THE BIOLOGICAL SAFETY CABINET

This chapter provides practical advice on the placement, use and maintenance of Biological Safety Cabinets.
Myths of safety

One of the most persistent myths held by laboratory staff the world over is that a BSC provides complete protection from the infectious material it contains. This is not true.

Good safe working practice is your best protection

Poor technique when using a BSC will expose you to potential infection.
- A BSC can maintain the level of sterility you create, it cannot produce it by itself
- Your actions must always complement the operation of the BSC
- You prevent cross-contamination by using safe working practice

Alarms and BSC operation

Most BSCs are equipped with both visual and audible alarms. BSC alarms warn you that it is no longer safe to operate, and that its protective functions may not be working properly.

Before using a BSC you must familiarize yourself with the alarms. Check the manufacturer’s instructions to ensure you fully understand the operation of the BSC before using it.

The laboratory must have a copy of the equipment instructions available at all times.

Alarms are instructions – you must act when you hear an alarm.

How a BSC protects

BSCs are categorized as Class I, Class II, or Class III.

Class I
Class I BSCs draw unfiltered room air through the front opening, passing it over the work surface, and expelling it through an exhaust duct and through a HEPA filter.

Class I BSCs protect the worker but do not protect the work area against contamination because unfiltered room air is drawn into the cabinet and over the work surface.

Class II
Class 2 BSCs draw around 70% of purified air from the HEPA filter above the work area and around 30% air through the front grille.
Class II provides protection for the user, environment and the work area. There are four types of BSC Class II: A1, A2, B1 and B2. The most suitable for all TB work is the type A2.

**TYPE A2 CLASS II BSCS ARE RECOMMENDED FOR ALL TB WORK**

Class III
Also known as glove boxes, generally they are installed only in maximum containment laboratories.

**DO NOT USE CLASS III BSCS IN TB LABORATORIES**

BSC should be connected to a stabilized power supply, ideally through a dedicated UPS with enough capacity to ensure that the BSC can operate for at least 15 minutes. When a power failure occurs, work should stop immediately and the 15 minutes be used to clear the cabinet of aerosols.

**Class 2 BSCs are recommended for all TB work because they protect both the technician and the work area**

**Class 1 BSCs protect the technician but do not protect the work area against contamination because unfiltered room air is drawn over the work surface**
BSC placement

When considering where to place a BSC within a laboratory, you must consider the potential for all air movements to compromise its effectiveness.

The airflow curtain that helps provide protection when working in a BSC is fragile. Its ability to protect is easily damaged by other air movement. The large volumes of air moved by air conditioners and fans are an obvious risk, however simply opening and closing doors or walking too close to a BSC may breach the fragile protective air curtain. A BSC must not be placed near to high traffic areas.

Locating a BSC in your laboratory
BSC placement

The figure opposite illustrates some of the common challenges you will face in making your decision.

1. **Air movement**
   Air conditioners force air through the room, potentially compromising the air curtain from over 3 metres away.

2. **People movement**
   Walking creates air movement
   - Allow 1.5 metres between a BSC and laboratory traffic areas
   - In small laboratories restrict people movement within the laboratory when the BSC is being used

3. **Doors and windows**
   Opening and closing doors can create sufficient air movement to disrupt the air curtain
   - Open windows allow disruptive airflow in and out of the laboratory
   - Always close and lock windows in moderate or high risk TB laboratories

4. **Location of other equipment**
   Exhaust from another BSC or other equipment may disrupt the air curtain
   - Carefully consider the influence of all other equipment and its effect on air movement when locating a BSC

5. **Clearance**
   Placing a BSC is too close to a wall or ceiling may create back pressure compromising BSC function
   - Allow a minimum distance of 35cm each side and above the BSC

**BSC support**
- A BSC should be placed on a dedicated stand or bench which can safely hold several hundred kilograms
- A stand may include lockable wheels for easier movement of the BSC within the laboratory
- Ensure that the BSC is level
Ergonomics

You may spend several hours a day working at a BSC. Good ergonomics is essential to enable you to focus on the work you are doing, and to do it safely. To reduce stretching set the BSC close to the edge of the bench.

1. Is there enough space under the bench to sit comfortably and move your legs? Be aware of cupboards and bench supports that restrict movement.

2. Can you put your feet flat on the floor? If not, use a foot rest.

3. Is the chair height adjustable so that your forearms rest horizontally on the front of the BSC?

4. Are services within the BSC (like electrical outlets) within easy reach so you don’t have to twist or bend to reach them?

5. Is the BSC light shielded to protect you from light and heat?

6. Can you sit, keeping your back straight and your neck, shoulders, and arms relaxed?

7. Reduce the amount of head and body twisting to less than 30º for all common activities.
Center your work area within the BSC to limit stretching or twisting.

A Front grille
B Back grille

Safe working area
Working in a BSC

Only allow one worker to operate a BSC; more than one person will damage the front air curtain and allow aerosols to be released.

Where the BSC is small, there will not be enough space to keep all necessary materials within the cabinet. Instead, store clean items on a trolley, so they will be easily accessible without work interruption.

Grille areas
- The front grille (A) helps to create and maintain the protective air curtain
- The back grille (B) helps to maintain laminar flow within the BSC and collects air to exhaust

If grilles are blocked, even partially, their efficiency is reduced and BSC performance is compromised.
**Open flames**

Open (naked) flames must not be used within a BSC.
- Hot air movement can adversely affect airflows within the BSC
- Hot air from Bunsen burners may damage the fragile HEPA filter

---

**THE USE OF STERILE DISPOSABLE LOOPS AND PIPETTES IS STRONGLY RECOMMENDED**
Separation of work areas

The task being performed determines what items you need in the BSC. Storing additional items in the BSC increases the risk of cross-contamination.

A BSC IS NOT A STORAGE CABINET – REMOVE ITEMS NOT NEEDED FOR THE TASK

The space within the BSC is quite small, it is important to separate relatively ‘clean’ from ‘dirty’ activities.

Suggested layout for right-handed staff working from left (clean) to right (dirty) – reverse for left handed staff. Typical activities include

<table>
<thead>
<tr>
<th>Left hand side ‘Clean’</th>
<th>Central area (main work area)</th>
<th>Right hand side ‘Dirty’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vortex</td>
<td>Rack holding specimens (towards the back)</td>
<td>Infectious waste discard container</td>
</tr>
<tr>
<td>Container holding bag(s) of sterile disposable loops and disposable pipettes</td>
<td>Rack holding centrifuge tubes (towards the back)</td>
<td>Sharps container</td>
</tr>
<tr>
<td>Timer (towards the front)</td>
<td>Rack holding media (towards the back)</td>
<td>Slide rack</td>
</tr>
<tr>
<td></td>
<td>Decontamination reagents (towards the front)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Centrifuge buckets (to the front)</td>
<td></td>
</tr>
</tbody>
</table>
Body movement within a BSC

The air curtain is very fragile and easily damaged.

Reducing in and out, and side to side arm movements in the BSC will help maintain the air curtain.

Keep all unnecessary arm movements to a minimum – when you move, move slowly to allow the air curtain to wrap your arms in protective filtered air.

Once your arms are in the BSC – keep still to allow the air curtain to re-establish and for your sleeves/gloves to be swept with filtered air.

- Take 2-3 seconds to complete the movement
- Minimise sideways arm movements
BSC layout

Processing specimens
Processing specimens for culture has three stages.
1 Specimen decontamination
2 Centrifugation
3 Media inoculation

Each stage of the workflow requires specific items, remove all items not required for the specific processing stage you are performing.

For example
- The vortex is required only during specimen decontamination and post centrifugation
  - Wipe down with 70% v/v alcohol and remove from the BSC once decontamination is complete
- Decontamination reagents are required only during specimen decontamination phase
- Solid or liquid media is only required in the BSC once centrifugation has been completed

Specimen decontamination
**Centrifugation**

Centrifuge buckets must always be loaded, and unloaded within a BSC.

![Image of a BSC with items numbered 1 to 6]

1. Vortex
2. Centrifuge tube rack
3. Central work area with centrifuge buckets
4. Diluting reagent (PBS or sterile water)
5. Discard bucket
6. Sharps container

![Warning icon] A BSC IS NOT A STORAGE CABINET - REMOVE ITEMS NOT NEEDED FOR THE TASK
### Media inoculation

1. **Micropipettes**
2. **Centrifuge tube rack**
3. **Sterile disposable loops and pipettes**
4. **Rack holding MGIT tubes**
5. **Central work area**
6. **Small bottle of PBS or sterile water**
7. **Discard bucket**
8. **Sharps container**
9. **Slide rack if smears prepared from concentrate**

<table>
<thead>
<tr>
<th>Item</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Micropipettes</td>
</tr>
<tr>
<td>2</td>
<td>Centrifuge tube rack</td>
</tr>
<tr>
<td>3</td>
<td>Sterile disposable loops and pipettes</td>
</tr>
<tr>
<td>4</td>
<td>Rack holding MGIT tubes</td>
</tr>
<tr>
<td>5</td>
<td>Central work area</td>
</tr>
<tr>
<td>6</td>
<td>Small bottle of PBS or sterile water</td>
</tr>
<tr>
<td>7</td>
<td>Discard bucket</td>
</tr>
<tr>
<td>8</td>
<td>Sharps container</td>
</tr>
<tr>
<td>9</td>
<td>Slide rack if smears prepared from concentrate</td>
</tr>
</tbody>
</table>

#### DST

**Using culture as an example, follow a similar approach for the various steps of DST.**

1. **Adding anti-TB drug solutions into the MGIT tubes**
2. **Inoculum preparation**
3. **Inoculating DST media**

For each stage, specific items are required within the BSC – remove all items not required for the specific task you are doing.
After working in a BSC

YOU MUST CLEAN AND DECONTAMINATE THE BSC AFTER EVERY USE

When you have finished working
- Leave the BSC running for 15 minutes to remove aerosols
- Do not use the BSC or remove any items during this time
- After 15 minutes decontaminate all items in the BSC
- Then remove the items leaving an empty BSC
- Wipe down the work surface, internal walls and interior of the glass front of the BSC (70% v/v alcohol)
- Use a long-handled wiper to reach the difficult parts

Empty BSC ready for cleaning

Do not put any part of your body into a BSC
**Chlorine based disinfectants**
Chlorine-based solutions like domestic bleach are highly corrosive. If used, then rinse with sterile water or 70% v/v ethanol.

**Ultraviolet light**
UV lights are not recommended for BSCs used in TB laboratories.
- UV radiation does not penetrate solid surfaces and is ineffective on dry organisms
- Human exposure to UV radiation may cause eye damage and acute burns to skin
- UV radiation breaks down plastics and other materials used in a BSC
- Radiation intensity from the UV lamp falls over time reducing effectiveness

**Fumigation**
Fumigation involves decontamination of the BSC and must be performed only by a qualified service professional.

Fumigation is required
- After a major biohazardous spill
- Before replacement of HEPA filters
- When access to the sealed plenum is necessary
- For service or replacement of components
- Before movement of the BSC cabinet to another laboratory
- When changing work activities in the BSC, e.g. from TB to routine microbiology
- Before release of the BSC for sale or salvage
Certification

The purpose of certification is to protect you by ensuring that the BSC is working properly.

To check its performance your BSC must be certified at least annually.

A qualified engineer must assess the BSC using an accepted national or international standard. It is the responsibility of the engineer to decontaminate the BSC before inspection.

It is the responsibility of the laboratory manager to organize certification and to advise staff that the BSC may be safely used.

BSC certification is required

- Before first use of a newly-installed BSC
- Annually
- When a BSC is moved within the laboratory
- Whenever a HEPA filter is replaced
- Whenever components within the plenum are replaced

Certification must be displayed on the BSC.
Summary

A correctly functioning BSC is vital for TB culture and DST. However, the level of protection provided depends on the competency of laboratory staff. Unsafe techniques when using a BSC will expose you to potential infection.
AEROSOL GENERATION AND PREVENTION

The aim of this chapter is to understand the risks associated with aerosols, how they are created, and how to minimise their production.
In the TB laboratory, all aerosols should be considered as potentially infectious. Aerosols are capable of being inhaled and establishing infection. Once they settle onto a surface, they are not re-aerosolised, and are no longer infective. However, they may contaminate specimens, equipment, consumables, and reagents creating a cross-contamination risk.

Aerosols may be formed during procedures such as pipetting, vortexing, centrifugation, or shaking of specimens or cultures.

Key factors in the infectivity of aerosols are

- Size
- Bacillary load
- Viscosity

**Size**
The smaller the aerosol, the longer it is capable of staying airborne.

- Smaller aerosols may penetrate deeper into the lung increasing the risk of infection
- Aerosols are infective to human beings only when airborne

**Settling of water droplets in saturated air**

<table>
<thead>
<tr>
<th>Droplet diameter (um)</th>
<th>Time to fall 2 meters (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10000</td>
</tr>
<tr>
<td>10</td>
<td>1000</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1000</td>
<td>10</td>
</tr>
<tr>
<td>1000</td>
<td>1</td>
</tr>
</tbody>
</table>

**Bacillary load**
The bacillary load will vary according to the concentration of organisms in the material being manipulated.

- For smear positive specimens, the bacillary load of a scanty specimen is $10^3-10^4$ organisms per ml but may rise to $10^6$ per ml for a 3+ smear positive specimen
- Positive cultures may have a much higher bacillary load ($10^8-10^{10}$ organisms per ml), hence aerosols from cultures pose a greater risk of infection

**Viscosity**
The viscosity of a material will affect the ability to form aerosols. The viscosity of sputum specimens reduces the likelihood of generating aerosols. In contrast, the aerosolisation risk is much higher when manipulating a positive liquid culture.
Creating aerosols

Putting energy into a liquid creates aerosols.

More energy = more, and smaller aerosols = more droplet nuclei = increased risk

High-risk procedures and practices that may increase the potential of creating aerosols (which then become droplet nuclei) include:

- Mechanical (vortexing, centrifugation, shaking)
- Pouring/tipping
- Pipetting

Minimising aerosol production

Working safely to minimise production of aerosols is one of the most important actions when working in a TB laboratory.

Containers

Any container that will be vortexed, centrifuged, or shaken, must have a leak proof lid and be strong enough to resist the mechanical forces exerted upon it.

Post-vortexing or shaking

Specimens

- Do not open the lid of a vortexed or shaken specimen for at least 10 minutes

Cultures

- Do not open the lid of a vortexed or shaken culture or organism suspension for at least 15 minutes

ALWAYS OPEN A VORTEXED OR SHAKEN SPECIMEN, CULTURE, OR ORGANISM SUSPENSION IN A BSC
**Centrifugation**

Provided the sample has been centrifuged in a biosafety bucket with lid, the biosafety bucket may be taken into a BSC and then opened immediately.

---

**CENTRIFUGES WITHOUT A BIOSAFETY BUCKET AND LID MUST NOT BE USED FOR TB**

---

Only open biosafety buckets in a BSC
Pouring/tipping
Any action that moves liquid from one container into another.

Never pour a solution directly onto another; it will create aerosols.

IF YOU ARE CREATING BUBBLES YOU ARE CREATING AEROSOLS

Examples include
• Putting a decontaminant solution into a specimen container or centrifuge tube
• Post-centrifugation pouring of the supernatant into a disinfectant
• Putting sterile water or phosphate buffered saline into a decontaminated sample
• Diluting an inoculum of MTB during preparation for DST

Always pour the liquid from one container to another by running the liquid down the inner wall of the container
The same applies when using a pipette to transfer liquid from one container to another

Never pour a liquid from one container directly into another container
Correct, expel liquid above the meniscus and down the side of the tube

Wrong, pipette tip below meniscus

Wrong, expelling liquid directly onto another liquid

Wrong, pipette is outside of the tube

When pouring liquid into a discard container, use a funnel to increase the inner wall surface area to minimise pouring liquid directly into the discard bucket within the BSC
**Pipetting**

Using pipettes (e.g. Pasteur) or micropipettes creates a high risk for aerosol production.

Under air pressure from a bulb or plunger of a micropipette, moving fluid from the larger storage chamber or barrel through the tip may accelerate the fluid to high speed creating aerosols.

To minimise aerosols creation
- Slowly expel liquid from the pipette
- Direct it down the inside wall of the container
- Ensure the pipette tip is above the meniscus

These principles apply to all pipette types.
Around 20% of laboratory acquired infections have an obvious cause; the remaining 80% are due primarily to aerosol production created by unsafe work practices. Minimising aerosols is a vital skill for technicians working in the TB culture or DST laboratory.

Minimising aerosol production is vital for the well-being of the laboratory staff undertaking the work, and co-workers. It protects also the patient from false-positive laboratory results that occur when aerosols contaminate other specimens, cultures, or reagents and consumables.

**Summary**

Understanding how aerosols are created is the first step to minimising their production. Most aerosols are invisible and laboratory staff are frequently unaware that they are being produced.
CONTAMINATION CAUSES AND PREVENTION

This chapter describes how contamination occurs, and the steps required to prevent it from happening in your laboratory.
Laboratory contamination incidents may be dangerous for staff, for laboratory credibility, and potentially for the patient.

Good working practices reduce the risk of contamination occurring. Quality programs that include data analysis may enable unsuspected contamination to be identified. Moreover, regular observation of work practices by laboratory supervisors will enable unsafe practices to be corrected.

Contamination may be caused by unsafe work practices that allow
- Environmental microorganisms (bacteria, fungi, non-tuberculous mycobacteria) to enter consumables or reagents, or to soil equipment surfaces, or personal protective equipment
- Aerosolised material (specimens/cultures/inocula) to contaminate adjoining specimens, cultures or reagents

Handling containers

Some areas of a container must never be touched such as the inside of a container or its lid. Other places may be less obvious such as the thread area of a container.

During specimen collection, it is possible for the outside of the thread area (and the external tube surface) to become contaminated with sputum; closing the lid will spread the specimen all over the thread, as will undoing the lid. The problem is more dangerous when working with positive cultures, especially liquid cultures.

Sputum leakage outside the container
Use of correctly sized gloves is vital. Keep gloved fingers away from the thread area. When handling any container, including centrifuge and culture tubes, hold the container in the middle, well away from the thread area or mouth of the container. When pouring from one container to another, first check that the labels match, and then turn the labelling away from the field of view.

When removing a lid from a container or tube, never place the lid downwards. The thread area may be contaminated and will transfer a portion of specimen or culture onto the work area.
Using pipettes and micropipettes

Contamination by pipette or micropipette may occur in three ways.

1. **From the pipette to the specimen**
   Using a contaminated pipette or tip may result in contamination of a specimen, culture, or inoculum. Prevent this by
   - Using sterile pipettes/tips
   - Holding the pipette correctly
   - Considering each pipette or tip as single-use only

2. **From the specimen to the pipette**
   The specimen, inoculum, or aerosols may enter the internal workings of the micropipette or inside the bulb of a pipette. Prevent this by using pipettes or tips with filters to prevent liquids/aerosols from leaving the end of the pipette/tip.

3. **From specimen to specimen (carryover contamination)**
   This type of contamination occurs when dispensing the specimen or inoculum. Carryover occurs when part of the specimen/inoculum remains attached to the inside of the pipette/tip in droplet form. The same pipette/tip is then used to handle another specimen/inoculum. Prevent carryover contamination by replacing the pipette/tip after inserting it into any liquid that is potentially non-sterile.

**Pipettes**
Single use, plastic disposable pipettes are recommended. Single-piece, moulded bulb pipettes are best. Bulbs should be part of the pipette ensuring that a separate bulb is not required. Plastic disposable pipettes may be individually packed or be in bags. Once opened, reseal the bag when not in use.

Glass Pasteur pipettes are not recommended as they are easily broken creating sharp edges and require a separate bulb which may become contaminated, causing cross-contamination.
Holding a pipette correctly is vital to ensure that the liquid is delivered safely to a container.

Hold the pipette with thumb and forefinger, using the middle finger to guide placement.

Poor pipette control

NEVER TOUCH THE TIP OF A PIPETTE OR MICROPIPETTE
For inoculating both solid and liquid media, micropipettes must not be used as the very small tip opening is likely to become clogged, creating a risk of the tip being blown from the pipette creating an infectious spill within the BSC. Use a sterile, plastic graduated pipette to inoculate decontaminated specimens onto media as the tip opening is much larger and will allow the contents to pass through.

Use pipettes with a larger tip opening for inoculating media

After transferring the liquid from the pipette, put it directly into a discard container containing disinfectant.

Do not use glass pipettes in the TB laboratory.

If there is no alternative, the ends must be plugged with cotton wool.

Cotton wool plug fully inserted
**Micropipettes**

Micropipettes are a precision instrument to collect and dispense liquids accurately using disposable sterile plastic tips. They must be used only on non-viscous solutions.

The shape and size of the disposable tip will depend on the volume being collected, and the shape and size of the container holding the liquid.

Filter tips provide effective protection from contamination of micropipettes. They prevent aerosols or liquid containing micro-organisms from entering the inner workings of the barrel.

Always ensure that the tip is placed inside a container and above the meniscus, before slowly releasing the contents. Never insert the barrel into the container.

**PLACE ONLY THE DISPOSABLE TIP INTO A CONTAINER, NEVER THE BARREL**

- [ ] Insert the tip only
- [ ] Never insert the barrel into the tube
CONTAMINATION CAUSES AND PREVENTION

The inner diameter of a micropipette tip is narrow (<1mm diameter) and processed specimens are frequently not homogeneous in consistency and do contain portions that are >1mm. Forcing a processed specimen through a micropipette tip will block the tip and create sufficient back pressure to blow the tip from the barrel, creating infectious aerosols and a spill.

DO NOT USE MICROPIPETTES TO INOCULATE PROCESSED SPECIMENS INTO MEDIA

When to use a micropipette
For culture, a micropipette should be used only for
• Adding 800μl of PANTA supplement into a MGIT tube

For DST, a micropipette should be used only for
• Preparing the inoculum dilution
• Adding drug solution to a MGIT tube
• Adding inoculum into a MGIT tube or onto solid media

Bacteriological loops
Used in preparation of sputum smears and for handling isolates.

Two types are available: single use, plastic loops and reusable wire loops.

Reusable wire loops that are sterilised in an electric incinerator between each use
• Wire can be heated but not the handle

Single use, plastic loops – strongly recommended.
Inserting the handle of a disposable loop is acceptable as it will be discarded immediately after use
Never insert the handle
Match the tube size to the wire length

Insert only the wire into a tube
Failing to sterilize the wire correctly, or placing the handle of the bacteriological loop into the container may result in contamination.

If disposable loops are temporarily unavailable, an alternative is to use a moistened, sterile cotton swab to prepare inocula.
Using data to detect contamination

Reviewing laboratory data provides a valuable opportunity to identify an otherwise undetected contamination event.

These organisms may come directly from a specimen, mishandling a positive culture, or from contaminated reagents, consumables such as pipettes, or from equipment.

Culture cross-contamination
Caused by MTB
Example: A smear positive specimen becomes culture positive within one week. Multiple subsequent specimens are smear negative but become culture positive, usually requiring a longer incubation time before signaling positive (Table 6.1).

- LRN# 243: A 3+ AFB positive specimen signals positive for MTB after one week (1W) of incubation – True positive
- LRN# 244-248 are all smear negative and become culture positive after four-five weeks
  - Probable contamination
  - All are smear negative yet culture positive at four-five weeks
  - All follow directly after a 3+ smear positive specimen

Table 6.1 Culture cross-contamination with MTB

<table>
<thead>
<tr>
<th>LRN</th>
<th>Date</th>
<th>Name</th>
<th>Age/Sex</th>
<th>Dx/FU</th>
<th>Smear</th>
<th>Culture</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>236</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>237</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>238</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>239</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>241</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>242</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>243</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3+</td>
<td>MTB1W</td>
<td>True positive result</td>
</tr>
<tr>
<td>244</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>MTB4W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>245</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>MTB4W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>246</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>MTB5W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>247</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>MTB5W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>248</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>MTB5W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>249</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
</tbody>
</table>

Notes
- **N6W** – No growth (N) after 6-weeks (6W) incubation
- **MTB1W** – Growth of MTB after one week (1W) of incubation
## Table 6.2 Culture cross-contamination with non-mycobacterial microorganisms

<table>
<thead>
<tr>
<th>LRN</th>
<th>Date</th>
<th>Name</th>
<th>Age/Sex</th>
<th>Dx/FU</th>
<th>Smear</th>
<th>Culture</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>238</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>239</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>240</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>241</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>N6W</td>
<td></td>
</tr>
<tr>
<td>242</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS1W</td>
<td></td>
</tr>
<tr>
<td>243</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3+</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>244</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>245</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>246</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>247</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>248</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>249</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>250</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>251</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Neg</td>
<td>CDIS3W</td>
<td>Probable contamination</td>
</tr>
</tbody>
</table>

### Notes
- **N6W** – No growth (N) after 6-weeks (6W) incubation
- **CDIS3W** – Cultures discarded (CDIS) after three (3) weeks incubation
Because contaminants grow faster than MTB, note that the 3+ smear positive specimen was also contaminated. Such a result delays diagnosis and slows down obtaining a DST result.

Specimens from other patients were also contaminated and had to be discarded resulting in delayed diagnosis, repeat testing, or looking for a differential diagnosis.

**Quality indicators for culture**

These quality indicators (Table 6.3) are recommended for culture and should be collected and analysed on a monthly basis. Indicators should be collected by type of culture medium if more than one type is used, and also by specimen type if the laboratory processes a range of specimens.

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Possible solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosols released into BSC</td>
<td>Do not open any vortexed or shaken specimen or centrifuged sediment for at least 10 minutes</td>
</tr>
<tr>
<td>• Opening a centrifuge tube immediately after vortexing or shaking to mix the sample decontaminant with the specimen or to resuspend the sediment post-centrifugation</td>
<td></td>
</tr>
<tr>
<td>A bottle of reagent opened at the start of processing becomes contaminated with specimen LRN# 242 and it subsequently contaminates all remaining specimens being processed</td>
<td>Use only small reagent volumes; a limit of 5-10 volumes is recommended</td>
</tr>
<tr>
<td>• Shows the importance of using small reagent volumes</td>
<td></td>
</tr>
<tr>
<td>A new bottle of (contaminated) reagent is opened and used from LRN# 242 onwards</td>
<td>Always check unused reagents for obvious turbidity or fungal growth</td>
</tr>
</tbody>
</table>
## Table 6.3 Quality indicators for culture

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Description</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number and proportion of diagnostic specimens (new and relapse) that were MTBC positive</strong></td>
<td>Number of diagnostic specimens culture positive for MTBC/Number of diagnostic specimens processed for culture</td>
<td>10-15%</td>
</tr>
<tr>
<td><strong>Number and proportion of diagnostic AFB smear positive specimens (new and relapse) that were culture positive for MTBC</strong></td>
<td>Number of AFB smear positive specimens culture positive for MTBC/Number of smear positive diagnostic specimens processed for culture</td>
<td>95-98% (liquid) 85-90% (solid)</td>
</tr>
<tr>
<td><strong>Number and proportion of diagnostic AFB smear negative specimens that were culture positive for MTBC</strong></td>
<td>Number of AFB smear negative specimens culture positive for MTBC/Number of all specimens, regardless of smear result, that were culture positive for MTBC</td>
<td>20-30% (liquid) 10-20% (solid)</td>
</tr>
<tr>
<td><strong>Number and proportion of contaminated cultures leading to uninterpretable results</strong></td>
<td>Number of inoculated culture tubes or plates discarded due to contamination/Total number of inoculated tubes or plates inoculated for culture</td>
<td>3-5% (solid) 8-10% (liquid)</td>
</tr>
</tbody>
</table>
DST cross-contamination

During DST

Cross-contamination can occur when a drug-susceptible MTB is inoculated into another culture or culture dilution. This type of cross-contamination is almost impossible to detect because it is not obvious that it has occurred. Drug-susceptible MTB cross-contamination of another drug-susceptible MTB will be ‘invisible’ as it will be inactivated by the specific anti-TB drugs.

In contrast contamination caused by drug resistant TB organism is more readily detected. (Table 6.4)

<table>
<thead>
<tr>
<th>LRN</th>
<th>Date</th>
<th>Name</th>
<th>Age/Sex</th>
<th>STR</th>
<th>INH</th>
<th>RIF</th>
<th>EMB</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>270</td>
<td>12/12/2018</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>275</td>
<td>12/12/2018</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>279</td>
<td>12/12/2018</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>285</td>
<td>12/12/2018</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>290</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>True result</td>
</tr>
<tr>
<td>296</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>303</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>311</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>315</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>326</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>333</td>
<td>16/12/2018</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>Probable contamination</td>
</tr>
<tr>
<td>252</td>
<td>18/12/2018</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>True result</td>
</tr>
<tr>
<td>253</td>
<td>18/12/2018</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>True result</td>
</tr>
</tbody>
</table>

Notes
- The DST cross-contamination occurred on a single day (16/12/2018)
- Uncommon DST profile (resistance to S/H/R) supports cross-contamination
- If genotyping is available, isolates 290-333 should be tested to determine whether they have the same profile
### Contamination Causes and Prevention

<table>
<thead>
<tr>
<th>Possible cause</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerosols released into BSC environment</td>
<td>Do not open any vortexed or shaken culture for at least 15 minutes</td>
</tr>
<tr>
<td>• Opening a tube immediately after vortexing</td>
<td></td>
</tr>
<tr>
<td>• Shaking of a positive culture to prepare a DST inoculum</td>
<td></td>
</tr>
<tr>
<td>A reagent was contaminated with aerosols from LRN# 290 which was used for the rest of the day</td>
<td>Use small volume reagents</td>
</tr>
<tr>
<td>A reagent was contaminated via a pipette tip or another consumable entering a positive culture</td>
<td>Always use good pipetting technique and appropriately sized containers relative to tip length</td>
</tr>
<tr>
<td>If DST cross-contamination occurs across multiple days, then check if reagents are being used across multiple days</td>
<td>Discard all incompletely used reagents after each DST run</td>
</tr>
</tbody>
</table>

In the example above, if cross-contamination is not identified, patients 296-333 may be falsely defined as having MDR-TB and treated with a more toxic, and less effective, drug regimen of longer duration. The result may be catastrophic financially for patients and their families.
Assessing a drug-resistant result
Care must be taken whenever a drug-resistant result is observed. In MGIT-DST, contaminated DST tubes usually, but not always, yield a result within four days and the MGIT machine will declare the result to be invalid. Some non-mycobacteria microorganisms will take longer than four days and thus yield a DST result.

Whenever a drug resistant result occurs, and especially when all anti-TB drugs tested yield a resistant result, do the following
- Ensure that the MGIT broth is clear
  - Small white granules on the bottom of the tube is common
  - If turbid, prepare two smears, one for ZN staining, the other for Gram’s staining
- Discuss with the laboratory supervisor
  - Set up a blood agar or nutrient agar plate to check for growth of non-mycobacteria microorganisms
- If resistant to rifampicin (RIF), prepare a 1:100 dilution of the RIF tube broth
  - Perform a GeneXpert or LPA test to confirm RIF-resistance
  - If rpoB gene sequencing is available, undertake an urgent sequence analysis
- Advise clinician of the results and indicate that DST will be repeated or the isolate sent to a higher-level laboratory for second-line DST

Quality indicators for phenotypic DST
These indicators (Table 6.5) should be collected and analysed on a monthly basis. Other secondary indicators may be collected on a less frequent basis (e.g. quarterly), such as the number and proportion of unusual drug resistance patterns. However, always be aware of the DST results and be especially suspicious whenever groupings of unusual DST profiles are recorded.

Assessment of work practices
Supervising laboratory staff are responsible for the training of junior staff to a level of competency that includes correct standard operating procedures, performing quality assurance, and use of safe working practices. Once training is completed, supervisors should regularly check work practices, especially for staff that have just completed training.

Record keeping
Collecting and analysing laboratory data including work practices and quality indicators must be part of any laboratory Quality Management System.
Table 6.5 Quality indicators for phenotypic drug susceptibility testing

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Description</th>
<th>Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number and proportion of mono-resistance and multidrug resistance to all combinations of drugs tested (e.g. INH mono-resistance, RIF mono-resistance, MDR)</td>
<td>Number of isolates resistant to single or multiple drug combination/Total number of isolates tested</td>
<td>Dependent on population tested and country drug resistance prevalence and patterns</td>
</tr>
<tr>
<td>Number and proportion of isolates inoculated for DST that were discarded due to contamination</td>
<td>Number of isolates discarded due to contamination/Total number of isolates inoculated for DST</td>
<td>&lt;3%</td>
</tr>
<tr>
<td>Number and proportion of isolates inoculated for DST that were uninterpretable due to lack of growth of control (drug-free) tubes/plates</td>
<td>Number of isolates discarded due to lack of growth on drug-free media/Total number of isolates inoculated for DST</td>
<td>&lt;3%</td>
</tr>
<tr>
<td>Laboratory turnaround time (TAT)</td>
<td>Time between inoculation of DST and result reporting (mean, range and 90th centile). For total DST TAT, add this value to culture TAT</td>
<td>Solid media: 8–16 weeks Liquid media: 4–6 weeks</td>
</tr>
</tbody>
</table>

**Summary**

Specimens or cultures contaminated with environmental microorganisms may result in diagnostic delays. Of much greater concern are specimens or cultures contaminated with MTB. Patients may be given false-positive results resulting in unnecessary, or extended on-going treatment, or may be falsely diagnosed as having MDR/XDR-TB; a potentially catastrophic event for the patient and their family.
CONTAINERS AND REAGENTS

How you use containers, and how you manage reagents reduces the risk of cross-contamination.

The term container includes items such as tubes, bottles, flasks, and pipettes of all types.
Containers for liquids

Containers must be ‘fit for purpose’.

Key features include
- Lids and caps that reduce the risk of aerosolization
- Pouring lips for accurate pouring
- A suitable size
- Reusable, or single-use
- Glass or plastic

Lids and caps
Use screw-cap, leak-proof lids for all liquid reagents.

Flip-top closures are not recommended as they may spray aerosols when opened or snapped shut.

<table>
<thead>
<tr>
<th>Screw-cap</th>
<th>Flip top</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Screw-cap" /></td>
<td><img src="image2.png" alt="Flip top" /></td>
</tr>
</tbody>
</table>
Do not use cotton wool, waxed cotton wool or rubber closures for cultures or reagents.

The pouring lip
Containers with a ‘sharp’ edge not a rounded edge reduce the risk of uncontrolled pouring.
Size
Consider the size, shape and construction of containers as well as the method of transferring reagents to, or from, a container.
- Clear glass or plastic enables you to see the reagent, and helps you place a pipette tip or bacteriological loop
- The mouth of the container is wide enough for pouring, or receiving of liquid reagents, or pipettes
- Containers should be large enough to hold sufficient reagent, and to enable micropipette tips to collect reagents
  - Never insert the barrel of a micropipette into a container
Reusable or single use
Single use containers should be used wherever possible. Once used, the containers must be discarded to eliminate the risk of cross-contamination.

Reusable containers such as McCartney bottles (for solid culture), must be strong enough to be autoclaved, disinfected, washed, and repackaged multiple times.

Glass or plastic containers
A glass container may be reused, a plastic container cannot. Your choice of either a plastic or glass container will be determined by its use.

**Plastic**
Commonly used for
- Specimen container
- Centrifugation
- Smaller reagent volumes (<100ml)

**Glass**
Commonly used for
- Tubes with screw-caps (e.g. McCartney bottles) for solid media
- Larger reagent volumes (>100ml)
- May be reusable
Other types of containers

Single-use items such as micropipette tips present a major contamination risk if not used correctly.

Always put the lid on a tip box container when tips not being removed.

- Open the box to select a tip, then close the lid
- Risk of contamination – never touch unused tips with fingers or equipment
Reagents

The number of samples processed each day will determine your reagent volumes.

To minimise risk of contamination during use, consider the following
- Labelling
- Reagent volume requirements
- Workload
- Managing unused reagents

Labelling
Clear labelling is essential to provide the user with confidence that reagents are ‘fit-for-purpose’.

The label must include
- Name of the reagent
- When it was made
- Who made it
- Expiry date
- Batch number
- Specific storage conditions

Never erase or modify an existing label
Use new labels
Reagent volumes
Cross-contamination may occur when a volume of reagent is accessed to process multiple specimens. The more times a reagent is accessed, the greater the likelihood of cross-contamination occurring.

Reducing the number of times that the reagent is accessed limits how many specimens may be affected if cross-contamination occurs. A limit of 5-10 volumes is recommended. A maximum volume of 250ml is recommended for any working reagent volume. Larger volumes become unmanageable and difficult to pour or handle accurately.

<table>
<thead>
<tr>
<th>Reagent/specimen</th>
<th>Usual standard volume requirement (ml)</th>
<th>Recommended maximum volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decontaminant</td>
<td>&lt;5</td>
<td>50</td>
</tr>
<tr>
<td>PBS or sterile distilled water for neutralization step/centrifuge tube</td>
<td>≤45</td>
<td>250</td>
</tr>
<tr>
<td>PBS for resuspending centrifuged deposit</td>
<td>≤2</td>
<td>20</td>
</tr>
</tbody>
</table>

Some laboratories will have 50ml of sterile water (or PBS) in a Falcon tube to be used for one decontaminated specimen only.
- Most effective strategy but also the most expensive

**Workload**
Based on the average number of specimens processed each day, calculate the volume of each reagent used and use that as the maximum reagent volume (plus 10% reserve). Do not exceed the 250ml volume. Very high volume laboratories should use these principles as a guide to work practice.

**Managing partly used reagents**
After the processing run is completed, discard all partly used reagents.

**Summary**
Purchase containers based on function not price alone.
USING EQUIPMENT SAFELY

This chapter provides a practical approach to using and maintaining laboratory equipment. Laboratory equipment is expensive and once purchased, has to operate reliably and for a long period of time. Equipment must be ‘fit for purpose’ and used correctly to prevent damage.

<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrifuges</td>
<td>112</td>
</tr>
<tr>
<td>Incubators</td>
<td>121</td>
</tr>
<tr>
<td>Vortex</td>
<td>125</td>
</tr>
<tr>
<td>Racks</td>
<td>127</td>
</tr>
<tr>
<td>Micropipettes</td>
<td>129</td>
</tr>
<tr>
<td>Sharps</td>
<td>130</td>
</tr>
<tr>
<td>Summary</td>
<td>130</td>
</tr>
</tbody>
</table>
Failure to maintain and safely use equipment presents a risk to laboratory staff through production of aerosols or physical injury, and to patients by generating false results.

It is good practice to have equipment registered in the vendor’s system and/or to be registered as a client. The vendor distributes information on equipment updates or critical information on quality issues such as advice on a manufacturing defect.

For all equipment, keep a copy of the manufacturer’s instructions in the laboratory.

**ALWAYS READ AND FOLLOW THE MANUFACTURER’S INSTRUCTIONS**

**Centrifuges**

Because centrifuges produce aerosols, it is mandatory that samples be contained within a biosafety bucket with sealed lid.

The centrifuge must be able to reach and maintain 3040 RCF (relative centrifugal force) for 15-20 minutes to sediment the majority of acid-fast bacilli.

**RCF vs RPM**

Revolutions per minute (RPM) and RCF are not the same. RCF is used to specify centrifuge settings.

RCF is determined by RPM and centrifuge radius.

For example, a centrifuge with radius 17cm at
- 2000 RPM produces 760 RCF: <50% of AFB sedimented
- 4000 RPM produces 3040 RCF: >95% of AFB sedimented

RCF may be calculated using the formula:

\[1.118 \times 10^5 \times \text{radius} \ (\text{cm}) \times \text{RPM}^2\]
For a given RPM, RCF increases non-linearly with radius.

<table>
<thead>
<tr>
<th>Radius (in cm)</th>
<th>Relative centrifugal force (in g)</th>
<th>Revolutions/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>3</td>
<td>20,000</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>15,000</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>10,000</td>
</tr>
<tr>
<td>50</td>
<td>50</td>
<td>6,000</td>
</tr>
<tr>
<td>100</td>
<td>120</td>
<td>5,000</td>
</tr>
<tr>
<td>200</td>
<td>240</td>
<td>4,000</td>
</tr>
<tr>
<td>300</td>
<td>360</td>
<td>3,000</td>
</tr>
<tr>
<td>500</td>
<td>540</td>
<td>2,000</td>
</tr>
<tr>
<td>1,000</td>
<td>1,080</td>
<td>1,000</td>
</tr>
<tr>
<td>2,000</td>
<td>2,160</td>
<td>500</td>
</tr>
<tr>
<td>3,000</td>
<td>3,240</td>
<td>200</td>
</tr>
<tr>
<td>5,000</td>
<td>5,400</td>
<td>10</td>
</tr>
<tr>
<td>10,000</td>
<td>10,800</td>
<td>3</td>
</tr>
</tbody>
</table>

RPM 4000

RCF >3040<
Refrigerated centrifuges should be considered in hot environments, or when multiple centrifugation runs are conducted each day.
- TB bacilli may be killed if exposed to temperatures above 38°C for even a short time
- Set a refrigerated centrifuge to 10-15°C

If using a refrigerated centrifuge
- Turn on at least 30 minutes before use
- Keep buckets inside the centrifuge during the cooling down period

**Biosafety buckets**
These are mandatory when the centrifuge is used for TB culture.

Each manufacturer produces biosafety bucket parts specifically for their centrifuges
1. Lid
2. Bucket
3. Insert
Inserts
Bucket inserts hold tubes in place during centrifugation. It is crucial that the shape of the insert matches the base shape of the centrifuge tube.

For example, a flat-bottomed insert will damage V shaped centrifuge tubes, potentially cracking the plastic and spilling the tube contents.
Before each use, check O-rings to ensure that they are not cracked or broken. O-rings may be located in the lid or the bucket.

Do not use damaged or broken lids. Replace cracked, broken, or missing O-rings.
Centrifuge placement
A centrifuge must be located

- Within the ‘dirty’ part of the laboratory
- Close to the BSC
- On a solid, stable bench able to take the weight and vibrations generated during use
- In an ergonomically correct work position
- Away from water, sinks, or chemicals to avoid splashes or spills
- In a dust-free area

Set on a solid bench

Do not place a centrifuge on the floor

- Risk of dust and insects entering equipment
- Trip hazard
- Poor ergonomics
Using a centrifuge
A balanced, symmetrical load is essential for all centrifuges.

An unbalanced load creates vibration which will damage the centrifuge. Each load must be balanced equally about the center axis.

Most centrifuges have four positions for swing-out buckets; all positions must be filled with the same type and specification of bucket, insert, and lid. Do not use components from another manufacturer unless specifically approved.

**NEVER OPERATE A CENTRIFUGE WITH THE LID OPEN**

**STOP THE CENTRIFUGE IMMEDIATELY IF ANY UNUSUAL NOISE IS HEARD**

---

**Unequal tube numbers**
- Use water-filled centrifuge tubes to balance the load
- Clearly label blank tubes so that they are not confused with samples
- Some laboratories use pre-prepared tubes of varying volumes

---

- All positions loaded identically
- Each bucket must contain the same number of tubes and be loaded identically
- All bucket positions must be loaded
Cleaning and care
Daily – after use
- Turn off and leave lid open
- Allow centrifuge bowl to reach room temperature
- Wipe out any moisture
- Close the lid
- Separate the buckets, inserts and lids and place on a paper or cloth towel to dry

Weekly
If there have been no spills, clean the centrifuge bowl, rotor, buckets, inserts and lids weekly
- Check centrifuge basin for condensation
- Check rubber pad at base of centrifuge bowl is not cracked, worn, or damaged
- Check rotor for wear and corrosion
- Replace as required

Check the swing out rotors and trunnions for cracks
- Remove old grease and any debris
- Lubricate the rotor trunnions and bucket lugs
- Use a small amount of manufacturer’s lubricant or neutral pH grease

DO NOT ADD TOO MUCH LUBRICANT – IT WILL BE SPRAYED ONTO THE WALL OF THE CENTRIFUGE

Trunnions are located on the centrifuge rotor

Lugs are located on each side of a centrifuge bucket
Check the buckets for any signs of corrosion
- Pitting
- Plaque formation
- Change in color
- Cracks

![Centrifuge bucket showing early signs of corrosion and poor cleaning](image1)

![Severe corrosion Replace immediately](image2)

Check the lids
- O-rings intact and correctly positioned
- Carefully remove any debris from the O-rings
- No cracks or breaks
- Replace cracked or worn O-rings and lids immediately
- Lightly rub talcum powder over O-rings
- Clips are not bent or damaged

REPORT ALL DAMAGE TO THE LABORATORY SUPERVISOR

DO NOT USE THE CENTRIFUGE IF THE BUCKETS AND LIDS DO NOT FORM A TIGHT SEAL
Disinfection

- Check manufacturer’s instructions
- Autoclave buckets and inserts at 121°C for 15 minutes maximum
- Disinfect lids with a phenolic or chlorine-based disinfectant for 15 minutes
  - If a chlorine-based disinfectant is used, wash in water or 70% v/v alcohol and dry

For spills clean up see Chapter 10.

Other considerations

When ordering a new centrifuge, include at least two spare sets of lids and O-rings in the tender as they are the most fragile items of equipment.

Only purchase a centrifuge with a locking lid that cannot be opened whilst in use.

The centrifuge must be serviced annually by a qualified service technician who must ensure that the unit operates safely and properly. The service should include
- Cleaning condenser coils, fans, screens and filters
- Checking the centrifuge brushes, bearings, timer, temperature and speed, and checking for electrical integrity

The service technician must issue an inspection certificate indicating compliance with safety and proper operation.

Incubators

Consider the following features when purchasing an incubator.

- Electronic controls on the outside
- Double outer doors for larger incubators
- Glass inner doors allows pre-selection of cultures
- Trolley wheels (with foot-lock) for ease of movement
- Check noise level produced when in use
- How many shelves are included? Order more if required

The appropriate incubator size (volume) depends on
- Workload
- Size of the media tube (e.g. McCartney)
- Number of tubes per rack

Incubator location

Incubators are a biohazard risk because they may contain many positive cultures.

Consider the following
- Place within the ‘dirty’ part of the laboratory
- Close to specimen processing or DST
- Away from water, sinks, or chemicals to avoid splashes or spills
- In a dust-free area
- Away from direct sunlight
Shelves
Consider ordering additional shelves from the manufacturer when you place your order. Custom built shelves are often unsuitable
• Made from sheet metal with sharp edges or wood
• Solid shelves or those with small holes may interfere with airflows

Loading an incubator
Heated air must circulate freely within the incubator so that ‘hot or cold spots’ do not occur
• Do not put racks or cultures on the incubator floor as they may overheat
• Use racks of similar size and arrange them vertically
• Always use suitably-sized racks to hold culture tubes securely
• Do not overload racks

Correct loading ensures even air movement and heat distribution
Uneven loading can create uneven air movement and heat distribution
Open wire racks and trays allow effective airflow around all tubes

Solid shelves or trays block airflow adversely affecting incubation and organism growth
Routine cleaning and care
Read the manufacturer’s instructions.

Monthly, wipe down interior and exterior surfaces, including shelves and racks, with 70% v/v alcohol.

Spills
For spills clean up see Chapter 10.
Vortex

The vortex is perhaps the greatest aerosol generator; always use with extreme care.

- Only vortex tubes/containers that have a leak-proof cap
  - Most specimen containers do not have a leak-proof cap
- Always use a vortex inside a BSC
- Do not open vortexed specimens for at least 10 minutes
- Do not open vortexed cultures of MTB for at least 15 minutes

Always use a vortex inside a BSC
Never use a vortex outside a BSC

Use a timer to ensure that minimum times are adhered to
It is easier to generate a vortex with longer tubes (e.g. 50 ml centrifuge tube)

It is difficult to generate a vortex in a short and wide container such as a specimen container or when the sample is viscous

Cleaning and care
Check manufacturer’s instruction manual
- Before use check the rubber pad for damage
- After use wipe down with 70% v/v alcohol

If a spill occurs, clean the affected areas using a phenolic or chlorine-based disinfectant for at least 15 minutes, then wipe down with 70% v/v alcohol.

For spills clean up see Chapter 10.
Racks

The design of the humble rack is often ignored however, it has a major impact on working safely. A well designed and constructed rack will provide years of service and a safer working environment.

Poorly designed or manufactured racks create a high risk of aerosol generation and cross-contamination.

Suitable racks should
- Be made of metal or autoclave/chemical resistant plastic
- Support containers from the base
- Have holes slightly larger than the tube diameter
- Allow tubes to be physically separated without touching
- Allow room for fingers to pick up the container without touching either
  - The thread area
  - Adjacent tubes
- Allow labels to be read easily

Unsuitable racks have one or more of the following problems
- Made from wood
  - They absorb spills, allow fungus to grow, and can’t be decontaminated
- Racks without a base
  - Hold the container by the thread
  - Containers fall out when racks are lifted
- Mismatched hole size, large holes fail to hold smaller tubes upright
- Containers in physical contact
- Difficult to pick up a single container without touching another

Tubes are held upright, are separated from each other, have a base, with room for fingers to pick-up a tube
Tubes held by thread, no base and mismatched hole size

Tubes must be held upright

Unsupported culture bottles are a spills risk
Micropipettes

Micropipettes are precision tools designed to collect and deliver specific reagent volumes.

Do not use micropipettes for inoculating processed specimens onto media because it may be viscous, not homogenous and block the tip.

When releasing a micropipette tip, always aim it downwards into the discard bucket.

Tip selection

There are many types of tips and manufacturers. You must check that the tips you order are suitable for the micropipettes you are using. If in doubt request a sample to test.

Using an unsuitable tip may result in inaccurate volumes being delivered or leaks that create a biohazard risk.

To prevent barrel damage or contamination, always use filtered tips.

Record keeping

All preventive maintenance procedures must be documented on a maintenance log, signed and dated.
Sharps

A high degree of care must always be taken with contaminated sharp items including, slides, pipettes, and scalpels.

Dispose of sharps directly into designated containers.

Infection risk
The risk of needle stick injury and infection is very high if you attempt to manipulate a needle.

DO NOT USE NEEDLES/SYRINGES IN A TB LABORATORY

Broken glassware
Use a brush and dustpan or forceps to collect broken glassware.

NEVER PICK UP BROKEN GLASS WITH YOUR FINGERS

Summary
Correct placement, use, and maintenance of laboratory equipment will make a substantial difference to your safety and working environment. Understanding how to correctly use equipment will help protect you from harm.
MANAGING LABORATORY WASTE

Waste is anything that is to be discarded from the laboratory. To minimise health risks to staff and the community, laboratory waste must be disposed of properly. Procedures must comply with relevant local and national regulations.

<table>
<thead>
<tr>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types of waste</td>
</tr>
<tr>
<td>Within the laboratory</td>
</tr>
<tr>
<td>Outside the laboratory</td>
</tr>
<tr>
<td>Autoclave</td>
</tr>
<tr>
<td>Summary</td>
</tr>
</tbody>
</table>
Waste must not be allowed to accumulate within a laboratory. Daily work activities include waste management and staff time must be allocated to complete the work.

It is the responsibility of staff to manage waste correctly.

Before waste is removed from a laboratory, the Laboratory Manager must be satisfied that
- The waste has been effectively disinfected using the correct procedure, or
- It has been packed in a sealed container or bag for immediate on-site incineration or autoclaving
- There are no additional risks of any kind to anyone who must handle, or may come into contact with, the disinfected material

**Types of waste**

**Low-level waste**
Waste that has not been in direct contact with infectious materials such as inoculated cultures or used test kits.

For example
- Packaging for consumables, reagents, or test kits
- Materials used to send specimens to the laboratory (plastic bag or absorbent material provided no specimen leakage has occurred)
- Any item removed from a BSC that has been disinfected prior to removal
- Waste that has already been disinfected or autoclaved
- Double-bagged waste where the outer bag surface has been disinfected

**High-level waste**
- Anything that has been in direct contact with infectious materials
- An item removed from a BSC that has not been disinfected

**AFTER AUTOCLAVING, HIGH-LEVEL WASTE BECOMES LOW-LEVEL LABORATORY WASTE**

**Within the laboratory**
Laboratory staff are responsible for managing low- and high-level waste.

- A procedure for correct packaging of high- and low-level waste must be available within the laboratory
- Staff must be competent and comply with the waste management procedures
- Appropriate materials (bags, boxes, containers with lockable lids) must be available within the laboratory. The procedure must be reviewed regularly by the Supervisor to ensure that it remains up to date
• Staff must be assessed regularly to confirm that the procedure is being followed
• Cleaning staff must not handle high-level waste
  - They have little or no understanding of infectious risk
  - Do not have technical knowledge on managing an infectious spill correctly

Infectious waste which has not been autoclaved or decontaminated must be double bagged and sealed then placed within a sealable and lockable container for removal from the laboratory. An autoclave bag marked with a biohazard logo is suitable
**Outside the laboratory**

Waste bins should be located outside but near to the laboratory exit, and within the facility. They should be emptied regularly. Do not allow laboratory waste to be stored outside of the bin. Bins must be secure so that only authorized staff can access them.

**Autoclave**

Use of high-pressure, saturated steam is a very efficient way of killing TB organisms. In an autoclave, all of the air in the chamber is replaced by steam under pressure (usually 115kPa or 15psi) and at 121°C.

Ideally, a laboratory should have separate autoclaves for ‘clean’ (media preparation, glassware sterilization) and ‘dirty’ (infectious waste from the laboratory) loads. If only one autoclave is available, designate separate days for dirty and clean autoclave runs.

**Moderate-risk TB laboratories**

All high-level waste must be autoclaved before being released from the facility. The autoclave should be within the TB laboratory. If packaged correctly, high-level waste may be moved to an autoclave within the facility but outside of the TB laboratory. Low level waste may be removed from the laboratory for incineration or burial if local regulations allow.

Where the autoclave is within the facility but outside the laboratory, there must be adequate protection from unauthorized access. Ideally, high-level waste should be handed directly to the staff for immediate autoclaving.
MANAGING LABORATORY WASTE

FOR MODERATE-RISK TB LABORATORIES, AN AUTOCLAVE MUST BE AVAILABLE WITHIN THE FACILITY – IDEALLY WITHIN THE TB LABORATORY

High-risk TB laboratories
For high-risk TB laboratories, an autoclave must be located within the laboratory, and all waste, including low-level waste, must be autoclaved before removal from the laboratory.

FOR HIGH-RISK TB LABORATORIES, AN AUTOCLAVE MUST BE LOCATED WITHIN THE TB LABORATORY

Autoclave operation
The laboratory requires an area for

- Temporary storage of laboratory waste before it passes through the autoclave
- Post-autoclaving where it can cool before being removed

Laboratories with a pass-through autoclave may store sterilized waste outside of the laboratory but must ensure that it is stored securely.

Infectious waste
Sterilized waste
For high-workload laboratories, enough space must be provided for the storage of large loads pre and post autoclaving and for a trolley.

**Run conditions for TB cultures**
Run conditions and correct packing of an autoclave determine effective sterilization. Run conditions vary according to autoclave size, and load.

As a guide the following apply:
- Low-level waste: minimum 121°C at 115kPa for at least 15 minutes
- High-level waste: minimum 121°C at 115kPa for at least 45 minutes

READ THE MANUFACTURER’S INSTRUCTIONS TO ESTABLISH THE CORRECT SETTINGS FOR THE LOAD BEING STERILIZED

**Loading the autoclave**
To ensure effective sterilization do not overload the autoclave
- Use only autoclave buckets provided by the manufacturer
- Immediately before starting the autoclave run
  - Carefully open bags or closed containers
  - Carefully add 50-100mls of water to assist the sterilization
  - Close the bags or containers

**Correct loading**
An open wire mesh basket allows steam to contact all of the waste

**Incorrect loading**
A solid bucket limits steam contact with all of the waste
Clear plastic shield to cover the face and an adjustable strap for the head.

A heavy-duty apron to be worn when unpacking the autoclave
- Made from waterproof and heat-resistant material
- Tied at the neck and waist
- Full covering of the chest, abdomen, and legs

Ensure that the run has been completed before opening the autoclave.
Partially open the lid and allow the load to cool down.
Monitoring performance

Visual and biological systems are used to assess the performance of an autoclave.

- Visual systems such as tapes, cards, or papers confirm that the temperature conditions were met but do not confirm that microbial killing has occurred
- Biological indicators show that microbial killing has occurred

Visual systems

All loads should include a visual check that shows the required temperature was reached. Some visual systems also provide information about steam conditions.
**Biological indicators**
The bacterial spore technique is the most widely accepted method of checking autoclave performance. It tests whether spores of *Geobacillus stereothermophilus* or *Bacillus* species have been killed.

After incubation, growth (failure) or no growth (success).

- **Unused biological indicator**
- **Pass** No growth after autoclaving
- **Fail** Growth after autoclaving

Bacterial enzyme indicators work in a similar way. They are tested post-autoclaving and once a substrate is added, a colour change indicates if the run conditions were met.

All biological indicators should be placed deep within the load to challenge the run conditions.

If visual or biological tests fail, the load remains potentially infectious and must be:
- Autoclaved again with indicator confirmation of a successful run
- Referred to another autoclave within the facility
- Held securely until the autoclave is repaired

BIOLOGICAL INDICATORS SHOULD BE USED WEEKLY
Thermocouples
Validation of autoclave run conditions using thermocouples provides a more detailed measure of performance and should be conducted at least annually.

Thermocouples measure temperature providing a continuous readout of data; they are placed at multiple sites within the load to determine the effectiveness of sterilization.

Maintenance
Read, understand, and follow the manufacturer’s instructions on maintenance procedures for an autoclave.

Some activities may be performed by laboratory staff
Daily
• Check the control panel is operational with no warning lights
• Door seals/gaskets/O-rings are in place and undamaged
• Water levels are satisfactory
• Waste container is not full

Weekly
• Clean exterior with a mild detergent and dry
• Check printer paper and ink levels where present

Maintenance checks should be performed every 6 months by a qualified engineer who follows manufacturer instructions.

Record keeping
All maintenance and performance checks must be documented, signed, and dated.

Summary
Effective waste management is an integral part of daily laboratory activities that helps keep both laboratory staff and the community safe.
10

MANAGING INFECTIONOUS SPILLS

Infectious spills of positive cultures and/or high-titre inocula present a high risk to staff.
10 MANAGING INFECTIOUS SPILLS

A SPILL OUTSIDE OF A BSC IS A MAJOR INCIDENT AND PLACES STAFF AT GREATEST RISK

SPILLS USUALLY INVOLVE LIQUIDS AND AEROSOLS OF INFECTIOUS DROPLET NUCLEI ARE GENERATED

Spill management

The Laboratory Manager is responsible for ensuring

- Regular health surveillance of staff
- All laboratory staff are aware of the symptoms of TB
- Staff are trained to safely manage spills
  - Refresher training is conducted at least annually
- Sufficient resources are provided for clean-up
  - Equipment
  - PPE
  - Consumables and reagents
- A standard operating procedure for managing infectious spills is available which should be reviewed annually
- Spills incident recording forms are available
- A post-incident review is conducted to identify underlying cause(s) and corrective actions to prevent a repeat incident
- There is clinical management of staff involved in the spill

Senior staff are responsible for cleaning up spills and preparing a report to the Laboratory Manager.
Spill kit – what you need

At least two spill response kits must always be maintained.

- One inside the laboratory
- Another in the airlock or weather vestibule

![Spill response kit in a lockable container](image)

A contents list detailing each item, the quantity, and expiry dates for stock solutions should be placed on the container lid and checked quarterly by staff.

SUPERVISORS ARE RESPONSIBLE FOR ENSURING THAT SPILL RESPONSE KITS ARE CHECKED QUARTERLY
Spill response kit contents

**Standard operating procedure**
- At least one printed copy, reviewed annually

**Sign**
- Biohazard symbol and in large letters *do not enter*

**Disinfectant**
- Concentrated synthetic phenolic or hypochlorite solution
- Minimum of 500ml
- Expiry date must be clearly written on the side of the container
- A working solution will be made at the time of clean-up

**Respirator**
- A selection of different types of N95/FFP2 respirators stored in a ziplock bag
- N95/FFP2 respirators must be suitable for faces of different size and shape
- Pack near the top of the container to avoid crushing

**Eye protection**
- At least 2 pairs safety glasses with full eye cover

**Gloves**
- 3 bags of small, medium and large gloves (10 per bag), write expiry dates on each bag

**Gowns**
- At least 2 gowns each; small, medium and large sizes
- Disposable, long sleeved, elasticized cuff, and back opening
- Made from liquid-resistant material

**Hair and shoe coverings**
- 4-6 of each
- Disposable, elasticized

**Sharps container (disposable)**
- At least 500ml, clip-lock closure, puncture-proof and autoclavable

**Biohazard bags**
- Minimum of 6 large and 6 small strong autoclavable plastic bags

**Absorbent materials**
- Roll of cotton wool
- Roll of paper towel
- Absorbent towel

**Miscellaneous**
- One pair of scissors (disposable)
- Two pairs of tweezers or forceps (disposable)
Spills clean-up

When spilt a high-titre MTB liquid separates into three parts.

- The largest part into pools of liquid
- A smaller part separates into splashes
- Some is aerosolized
  - Large aerosols (>5 μm in diameter) settle quickly and are not re-aerosolised
  - Small aerosols (<5 μm in diameter) dry and may remain airborne for some time
  - Aerosols may circulate within the BSC or laboratory

Spills inside a BSC

Spills inside a BSC have a lower risk as they are contained and infectious aerosols generated are removed by the BSC.

1. Keep the BSC running – do not switch off
2. Do not disturb the front air curtain

- Wait 15 minutes for aerosols in the BSC to clear before starting the clean-up procedure
- Wear a long-sleeved gown and gloves that overlap the cuffs
- Soak absorbent material with disinfectant, then cover the spill
- If the walls of the BSC are contaminated, wipe down with disinfectant

Cover the spill with disinfectant soaked absorbent material
MANAGING INFECTIOUS SPILLS

LEAVE DISINFECTANT FOR AT LEAST 30 MINUTES

1. Use forceps to pick-up sharp objects and place into a sharps container
2. Place unused disposable items within the BSC into a biohazard bag – do not reuse
3. Wipe down equipment and reusable items with disinfectant (e.g. vortex, micropipettes, centrifuge buckets, inserts, lid etc.)

LEAVE DISINFECTANT FOR AT LEAST 30 MINUTES CONTACT TIME

4. Remove equipment from the BSC
5. Wipe down the walls, floor, and inside of the glass viewing panel with the disinfectant and leave for minimum of 30 minutes contact time
6. Remove the grille cover and wipe with disinfectant. Check the sump for contamination; if contaminated, add sufficient disinfectant solution to cover the sump floor
7. Leave for 30 minutes before cleaning up
8. Place all clean-up materials into a biohazard bag
9. Remove gloves inside the BSC and place into the biohazard bag
10. Place the biohazard bag into a second bag and autoclave
11. Electrical power outlets
   Check circuit breaker and earth-fault interrupters
   Report faults to the Laboratory Manager
12. Do not use the BSC until the Laboratory Manager and senior staff have approved its use
   A BSC fumigation may be necessary
Spills outside a BSC

1. **Immediately** evacuate everyone from the laboratory
   - If wearing a respirator leave it on
   - Remove all other PPE and place on the laboratory floor
   - Once outside remove and discard the respirator if worn
   - Wash your hands

2. Immediately advise the Laboratory Manager that a spill outside of the BSC has occurred

3. Prevent re-entry
   Place one staff member at the laboratory door to stop anyone entering

4. Open the Spill Kit and place the **do not enter** sign on the outer door of the laboratory

5. Note the time – wait for one-hour before re-entering the laboratory to enable the ventilation system to remove aerosols

6. During the one-hour exclusion period
   - Senior staff determine who will be involved in the clean-up and assign roles
   - Discuss the nature of the spill
   - The location of the spill
   - Estimate of the volume and the concentration of AFB/ml
   - Review the SOP for clean-up
   - Review the contents of the Spill Kit
   - Prepare absorbent material and a working solution of disinfectant
   - Check to see if additional items are required

7. Three people are required
   - One to monitor the door and to observe the clean-up from outside of the laboratory
   - Two to do the clean-up
     - One person is the primary cleaner
     - The second person provides the items required for clean-up as directed by the primary cleaner
After the one-hour exclusion period, and before entering the laboratory, two members of the clean-up team, put on PPE from the spill kit.

You must be dressed like this:
1. Hair cover
2. Eye protection
3. N95/FFP2 respirator
4. Long-sleeved gown
5. Disposable gloves
6. Shoe covers
9. Enter the laboratory and assess the situation
   - Confirm the location and size of the spill
   - Provide a brief verbal summary to the person at the door of the laboratory and confirm that plans for the clean-up are appropriate
   - Collect all PPE left behind during the evacuation and place into a biohazard bag

10. Cover the spill area with disinfectant soaked absorbent material
11. Carefully pour additional disinfectant over the spill area, beginning at the outer edge of the spill moving to the centre. Avoid splashing the disinfectant.

12. Wait at least 30 minutes for the disinfectant to work.

13. Using tweezers collect any fragments of broken glass, other sharps, and small items and place into a sharps container.

14. Carefully pick-up the absorbent towels and place into a biohazard bag.

15. Clean up remaining liquid by wiping from the edges and towards the centre. Put used absorbent material into a biohazard bag.

16. Repeat steps 10 to 15.

17. Wipe down area with 70% v/v alcohol. Place used towel into a biohazard bag. Allow to dry.

18. Collect all biohazard bags and sharps containers and place into a second biohazard bag then autoclave.

19. Remove all PPE and place into a biohazard bag for autoclaving.

20. Wash hands, and leave the laboratory.
After the clean-up

- Conduct a debrief with senior management and laboratory staff involved in the spill
  - Confirm the laboratory is safe
  - Describe the cause of the incident
  - Detail the clean-up procedure used
- Arrange clinical assessment and follow-up for all staff in the laboratory during the spill
- Restock the spill kit
- Write an incident report for management
- Identify corrective actions required
- Conduct a formal meeting with staff to describe the incident, and to advise the corrective actions being implemented
- File the incident report

Spill management procedures and training are a fundamental requirement for working safely in a TB laboratory. Management must ensure procedures are in place, reviewed regularly and that staff are trained in the use of the spills kit. Conducting an effective debrief and implementation of corrective actions will reduce the likelihood of future spills.

Summary

Even in the best laboratories, infectious spills do occur. Spill management procedures and training are a fundamental requirement for working safely in a TB laboratory. Management must ensure procedures are in place, reviewed regularly and that staff are trained in the use of the spills kit. Conducting an effective debrief and implementation of corrective actions will reduce the likelihood of future spills.
### APPENDICES

<table>
<thead>
<tr>
<th></th>
<th>Specimen containers</th>
<th></th>
<th>154</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Sputum collection</td>
<td></td>
<td>156</td>
</tr>
<tr>
<td>3</td>
<td>Specimen tracking</td>
<td></td>
<td>157</td>
</tr>
<tr>
<td>4</td>
<td>Disinfectants and their use</td>
<td></td>
<td>162</td>
</tr>
<tr>
<td>5</td>
<td>Washing hands</td>
<td></td>
<td>166</td>
</tr>
<tr>
<td>6</td>
<td>References</td>
<td></td>
<td>167</td>
</tr>
</tbody>
</table>
Ideal specimen container

The quality of specimen containers makes a significant contribution to safety in the laboratory.

Container specifications

- Break resistant polyethylene/polypropylene plastic
  - Polystyrene containers crack/shatter easily – do not use
- Wide mouth $>35$mm width
- Volume $\geq 50$mm
- Waterproof with a leakproof lid
- Multiple threads
- Frosted area or label for writing with a permanent marker

SPECIMEN CONTAINERS ARE SINGLE-USE
DO NOT REUSE
Clean versus sterile container

Some laboratory tests rely for their accuracy on the use of a sterile specimen container. For other tests, a clean container may be sufficient.

Clean specimen container
• Likely to be free from microbes but sterility is not guaranteed
• Suitable for smear microscopy and for GeneXpert testing
• Much cheaper than sterile containers

Sterile specimen container
• Sterilized using gamma irradiation or ethylene chloride
• Required for TB culture and/or DST
• Much more expensive than clean containers

Storage

Key issues for storage include.

• Restricting access to the laboratory and store room to authorized staff
• Controlled humidity to minimise fungus growth
• Clean, tidy and vermin-proof

Procurement

It is essential that procurement departments do not substitute poor quality containers simply because they are cheaper.
1. Take a deep breath in
2. Then breathe out hard
3. Do the same again
4. On the third time, cough deeply from your chest
5. Place the open container close to your mouth to collect the sputum

You may be asked to try again for a better specimen

From the lungs

Not the nose or mouth

Screw the lid on tightly

GOOD SPECIMENS – ACCURATE DIAGNOSIS
Specimen tracking is the process that ensures the link between the patient, the specimen request form and the specimen remains intact through every step in the laboratory. Specimen tracking must be used at all times regardless of the laboratory test or methodology. It must be part of any laboratory quality management system.

**Labelling**

Labelling is the primary way of connecting the specimen with the request form and any slide, tube, or cartridge back to the specimen request form, and ultimately, back to the patient.

Before labelling anything in the laboratory, confirm that the patient details on the specimen request form are the same as on the specimen container.

1. Check patient details on the container match the Laboratory Request Form
2. Transfer patient details from the Laboratory Request Form to the Laboratory Register
3. Write the Laboratory Register Number (LRN) on the side of the specimen container
4. Write the LRN on the Laboratory Request Form
Labelling must be

- Written clearly
- Pencil for frosted-end slide, **not** a permanent marker
  - Permanent marker writing will wash off with solvent
- Permanent marker for specimen-and media-containers
- Using at least one unique identifier for every patient
  - LRN
  - An additional option is to include the first four letters of the patient’s surname also

Always write on the side of the container, never on the lid only. Writing on both the side and lid is acceptable.
For numbers that can be read the same way (e.g. 66 or 99; 106 or 901), place a line underneath the number to indicate the correct way to read the number.

**Working with specimens**

There are a number of things that may be done to minimise the potential for a transcription errors to occur during the diagnostic process.

Always

- Place specimen containers, centrifuge tubes, and media in a rack and in ascending LRN numerical order from lowest on the left to the highest on the right
- Ensure that the LRN is clearly visible; some racks may cover the LRN if the label is not located ideally on the tube
- Before transferring the specimen or any part thereof, compare the name and/or number on a specimen container with the number on the slide, culture tube or test kit

For reusable containers, ensure that old labels have been removed

Underline a number to ensure it is read correctly

66  99  901  106

Note tubes are in increasing LRN order (L–R)
• If tubes are reusable, ensure that the previous labelling has been removed during the cleaning process
• If using sticky labels, they must stay on the container, slide, or tube regardless of the chemicals/reagents used in the specimen processing
  - Be careful, some labels will peel off

Each tube for DST must be labelled with the LRN and the specific anti-TB drug.

1 Compare labelling on the specimen container and the slide
2 Compare labelling on the specimen container and the centrifuge tube
3 Compare labelling on the centrifuge tube and the MGIT/LJ media
4 Compare labelling on the positive culture and the DST tubes

ALL TUBES MUST BE LABELLED BEFORE USE
False-positives – Consequences
• Patients are treated unnecessarily
• Treatment may be continued longer than is necessary
• Medications will be wasted

False-negatives – Consequences
• Patients with TB may not be treated resulting in more sickness, disease progression or death
• An untreated smear positive patient may infect another 10-15 persons each year

**Summary**
Laboratory errors due to poor specimen tracking may cause false-positive or false-negative results which may have catastrophic impacts to an individual, their family, friends, and community.
Disinfectants are chemicals able to kill most of the micro-organisms on a surface or in a solution. For *Mycobacterium* species including MTB, their high fatty acid cell wall makes them less affected by some disinfectants. The choice of disinfectant depends upon the material to be disinfected, exposure time, and the relative advantages/disadvantages of a disinfectant.

**Alcohols**

Alcohols cause membrane damage and precipitate or coagulate protein. However, alcohols are ineffective against MTB in the presence of proteins in specimens such as sputum.

In sputum, protein is coagulated and this action may protect MTB from effective contact with the alcohol. Accordingly, alcohols are not suitable for disinfection of sputum spills.

They should not be used for inactivating suspensions of MTB or other *Mycobacterium* species as more effective agents such as chlorines and phenolics are available.

A 70% v/v solution of alcohol (denatured ethanol, methylated spirits) which is approximately equivalent to a 70% v/v isopropanol solution may be used for routine disinfection of laboratory benches and BSCs. For BSCs, it must not be sprayed or aerosolized due to the risk of fire.

There is no residual action after the alcohol has evaporated.

**Phenol**

Phenols change the permeability of cell membranes resulting in cell lysis.

Phenol (carbolic acid) is a well-established disinfectant for mycobacteriology laboratories. The ‘crude’ phenolics have a strong odour, are irritating to the skin, eyes and mucous membranes, and highly corrosive. In contrast, synthetic phenolics do not cause these irritations. Ingestion of phenolics of any type are toxic to human beings.

Organic materials such as proteins have a minimal effect on phenolic-based disinfectants and are affected to a lesser degree than occurs with chlorine-based disinfectants. The main use for synthetic phenolics is in discard containers within a BSC and as an alternative to alcohols- and chlorine- based disinfectants.

Prepare 5% phenol solutions every 2-3 days. Accuracy in diluting phenolics is important as small errors may result in substantial variations in activity.
Chlorine

Chlorines are a very active oxidizing agents responsible for inactivating enzymatic activities of proteins. They may also damage bacterial DNA and stop DNA synthesis.

Chlorine-based disinfectants are widely available, perhaps the most notable being domestic bleach with sodium hypochlorite being the primary agent. Sodium hypochlorite is available only as a liquid which is prepared by mixing chlorine with sodium chloride. The solution is highly alkaline and will corrode metal including stainless steel. Chlorine reacts rapidly with organic material and in such conditions, the concentration must be high enough to provide an effective residual concentration of chlorine to inactivate mycobacteria.

Concentrations of 0.5-1.0% chlorine must be prepared daily. For discard buckets, a higher concentration of disinfectant is added such that when full, the chlorine concentration is at the correct dilution. If used for discard containers or for cleaning up infectious spills, the material must not be autoclaved as the chlorine gas produced will quickly damage the equipment.

If chlorine is used to disinfect metal surfaces such as a BSC, a wipe-down rinse with sterile water or 70% v/v alcohol is required.

**Recommended applications for disinfectants**

<table>
<thead>
<tr>
<th>Laboratory infrastructure/equipment</th>
<th>Primary disinfectant</th>
<th>Alternative selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine cleaning of benches</td>
<td>70% v/v alcohol</td>
<td>5% synthetic phenolic</td>
</tr>
<tr>
<td>Routine cleaning of BSC</td>
<td>70% v/v alcohol</td>
<td>5% synthetic phenolic or 0.5-1% chlorine plus water wipe afterwards</td>
</tr>
<tr>
<td>Discard containers within BSC</td>
<td>5% synthetic phenolic</td>
<td>0.5-1% chlorine</td>
</tr>
<tr>
<td>Spill within centrifuge bio-safety bucket</td>
<td>Disinfectant not recommended</td>
<td>If a disinfectant must be used, wait for 15 minutes, then open within a BSC and use 5% synthetic phenolic</td>
</tr>
<tr>
<td></td>
<td>Autoclave at 121°C for 15 minutes</td>
<td></td>
</tr>
<tr>
<td>Spills inside a BSC*</td>
<td>5% synthetic phenolic</td>
<td>0.5-1% chlorine and water wipe afterwards</td>
</tr>
<tr>
<td>Spills outside a BSC*</td>
<td>5% synthetic phenolic</td>
<td>0.5-1% chlorine and water wipe afterwards</td>
</tr>
<tr>
<td>Equipment</td>
<td>Refer to manufacturer’s instructions</td>
<td>70% v/v alcohol if no ignition risk identified</td>
</tr>
</tbody>
</table>

* See Chapter 10 for clean-up procedure
## Using a disinfectant and contact times

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Concentration and contact time</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohols</strong></td>
<td>70% v/v alcohol (denatured alcohol or methylated spirits) 70% v/v isopropanol Wipe over surface and allow to dry</td>
<td>Concentration lessens as it evaporates No residual effect or residue Will damage rubber and plastics Do not use as a spray within BSCs and where sparks may occur</td>
</tr>
<tr>
<td><strong>Synthetic phenols</strong></td>
<td>5% concentration 15 minutes for routine disinfection 30 minutes for high-titre spills</td>
<td>Use only synthetic phenolics Made up every 2-3 days Dilute concentrated solutions carefully to ensure disinfectant activity</td>
</tr>
<tr>
<td><strong>Chlorine</strong></td>
<td>0.5-1% available chlorine 15 minutes for routine disinfection 30 minutes for high-titre spills</td>
<td>Concentration decreases with time; check expiry date Working solution made up fresh each day Solutions must not be autoclaved Highly corrosive to metals including stainless steel; if used, wipe down afterwards with sterile water or 70% v/v alcohol Dilute concentrated solutions carefully to ensure disinfectant activity Optimal pH range of 6-8</td>
</tr>
</tbody>
</table>
### Disinfectants and health risks

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Health risks</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alcohols</strong></td>
<td>Irritant to skin resulting in cracking and roughness. Flammable, especially in spray form.</td>
<td>Wear gloves and long-sleeved gown. Do not use as a spray. Do not use where spark risk is present.</td>
</tr>
<tr>
<td><strong>Synthetic phenols</strong></td>
<td>Potential carcinogen. Toxic if ingested.</td>
<td>Use in well ventilated areas only. Do not ingest.</td>
</tr>
<tr>
<td><strong>Chlorine</strong></td>
<td>Lung irritation, coughing due to gaseous chlorine. Irritant to skin and eyes.</td>
<td>Eye protection against splashes, especially when handling concentrated solutions. Use in well ventilated areas only. Wear gloves and long-sleeved gown.</td>
</tr>
</tbody>
</table>
Wash hands when visibly soiled, and before leaving the laboratory

Duration of hand wash (steps 2-7) 15-20 seconds
Duration of entire procedure 40-60 seconds

1. Wet hands with water
2. Apply enough soap to cover all hand surfaces
3. Rub hands palm to palm
4. Right palm over back of left hand with interlaced fingers and vice versa
5. Palm to palm with fingers interlaced
6. Backs of fingers to opposing palms with fingers interlocked
7. Rotational rubbing of left thumb clasped in right palm and vice versa
8. Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa
9. Rinse hands with water
10. Dry hands thoroughly with a single use towel
11. Use towel to turn off faucet
12. Your hands are now safe
These sources were used in preparing the handbook

https://www.aphl.org/programs/infectious_disease/tuberculosis/Documents


http://www.stoptb.org/wg/gli/gat.asp


