Biosafety Manual for Tuberculosis Laboratories under National TB Elimination Programme

April 2023

National TB Elimination Programme
Central TB Division, Ministry of Health & Family Welfare
Government of India, New Delhi

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Ministry of Health & Family Welfare
Government of India

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Deputy Director General
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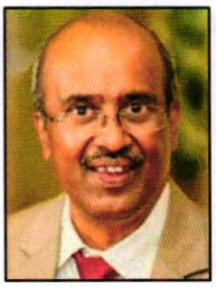


Message from DDG (TB), Central TB Division

The Government of India vision towards ending TB by 2025 has gained global attention. Achieving the goal of ending TB requires a holistic approach to ensure enhanced screening, and increased access to quality-assured diagnosis and availability of global standards of care for diagnosed patients. As India intensifies its efforts to improve diagnosis and treatment and close the incidence–notification gaps, the proportion of notified cases, that are bacteriologically confirmed needs to be monitored to ensure that people are correctly diagnosed and started on the most effective treatment regimen as early as possible.

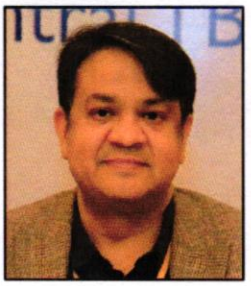
India is one of the high TB and drug-resistant TB burden countries; there is a programmatic demand for early screening and diagnosis. This demand is being met through the world's most extensive TB diagnostic networks, established across 30,000 healthcare facilities in the public as well as the private sector. In this network, there are Designated Microscopy Centres, NAAT sites, LPA, and TB Culture & Drug Susceptibility Testing Laboratories.

As TB spreads through the air, it is crucial to prioritize preventing its transmission. TB laboratory personnel are at increased risk of contracting the disease. Thus, handling TB specimens and cultures necessitates being extra vigilant, strictly adhering to safety protocols, and ensuring safe working conditions.

This Biosafety Manual has been prepared to promote the implementation of Biosafety practices in NTEP Laboratory tiers. This manual describes the minimum Biosafety requirements for each type of laboratory under the NTEP framework as well as strategies by which Biosafety and Biosecurity in TB Laboratories can be further strengthened with the aim of minimizing the risks of laboratory-acquired TB infection and ensuring that all laboratory workers are educated with the necessary knowledge and skills to handle TB specimens safely. By implementing the biosafety measures described in this Manual, laboratory personnel can help to ensure the safety of themselves, their colleagues, and the wider community, while contributing to the country's effort for ending TB.

Dr. Nishant Kumar

Joint Director (TB)
Central TB Division



Message from JD (TB), Central TB Division

The National TB Elimination Programme (NTEP) recognizes the crucial role of laboratories in providing universal access to TB diagnosis, Liquid Culture and Drug Susceptibility Testing (LC-DST), and also for monitoring response to the DR-TB treatment. To implement the national TB diagnostic algorithm, the NTEP has set up a vast network of state-of-the-art TB laboratories that follow a tiered system and deployed WHO recommended rapid diagnostic technologies. This algorithm involves conducting a cascade of rapid NAAT, first- and second-line LPA, and LC-DST.

TB poses an occupational hazard for healthcare workers, including laboratory personnel. The risk of laboratory-acquired TB infection can be high in programmatic settings where large numbers of TB specimens are handled. Therefore, it is crucial to understand the risks and hazards present at different levels within the NTEP laboratory network. Appropriate implementation of biosafety measures can minimize this risk, prevent the spread of TB, and ensure the accuracy and reliability of TB laboratory test results.

We are delighted to introduce this Biosafety Manual which is intended to serve as a comprehensive source for all levels of TB laboratories under NTEP. This manual provides guidance and Programmatic recommendations for implementing appropriate biosafety measures specific to different laboratory tiers and TB test procedures (such as smear microscopy, NAAT, LPA and culture DST).

I trust that this manual will promote a safe working environment for health staff working in TB Laboratories while advancing towards our efforts to enhance access to high-quality TB diagnostics under NTEP.

Dr. Ranjani Ramachandran

National Professional Officer
World Health Organization

Message from Dr. Ranjani Ramachandran

The implementation of the updated TB diagnosis algorithm, which includes the integration of NAAT and other extended drug-susceptibility tests, requires a greater emphasis on ensuring that these tests are conducted accurately and safely at every level of the laboratory network. The volume of samples being tested for TB is also increasing across all tiers of the diagnostic network, creating a greater demand for safe working conditions.

It is worth noting that personnel working in TB laboratories or healthcare workers who come into contact with TB patients are at a higher risk (3-9 times) of contracting Mycobacterium tuberculosis. Therefore, it is essential to prioritize the implementation of appropriate safety measures to prevent unintentional exposure to pathogens or their accidental release into the environment.

In response to this challenge, World Health Organization (WHO) and other international organizations have developed laboratory biosafety manuals to provide guidance on how to safely operate TB diagnostic laboratories. However, it is important to note that the global TB laboratory biosafety documents may not always be directly applicable to the unique needs and circumstances of each country. This realization has prompted the development of the present biosafety manual, which is in alignment with international guidelines while being tailored appropriately to the local context.

I hope this biosafety manual can help to promote a culture of safety within TB diagnostic laboratories, where laboratory personnel are aware of the risks associated with TB and are committed to following appropriate safety measures. Ultimately, this can lead to improved laboratory performance, greater confidence in laboratory results, and better patient care.

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Acknowledgment

The Biosafety manual for tuberculosis laboratories in NTEP is the result of series of meetings, collaborative efforts and inputs from many national institutions/laboratories and national expert individuals, without whose help, guidance, and support this would not have been possible.

Under the leadership of **Dr. Nishant Kumar**, Joint Director, the Central Tuberculosis Division (CTD) took the lead in the process of developing this comprehensive TB Laboratory Biosafety manual and its monitoring mechanism, with the technical support from USAID's Infectious Disease Detection and Surveillance (IDDS) project and all six National Reference Laboratories (NRLs). The manual has been developed following a series of meetings and deliberations with experts from the NRLs, external experts and key technical and development partners (USAID, IDDS, WHO). These meetings were conducted by the CTD, Ministry of Health and Family Welfare (MoHFW), GoI.

An extensive review of literature was undertaken, aligning with the current national laboratory network under NTEP standard guidelines and resource materials from the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC), the Global Laboratory Initiative (GLI)-Stop TB Partnership and others were adopted.

We would like to appreciate the efforts of the core team from the CTD and USAID'S IDDS project who took the lead in this process. The writing group included:

- Dr. S Anand, National Consultant Labs, WHO NTEP
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- Dr. Jyoti Kayesth, Tuberculosis Manager, IDDS India
- Dr. Pravin Kumar Singh, Consultant, IDDS India and
- Dr. Kavitha A.K, Consultant, IDDS India

We express our gratitude to Directors of all NRLs (in alphabetical order) for providing leadership, encouragement, and support. Their commitment will play a major role towards strengthening country's TB laboratory biosafety and to improve the quality and safety management in the NTEP laboratory network.

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- Dr. C. Padmapriyadarsini, Director, National Institute for Research in TB (NIRT) Chennai
- Dr. N. Somashekar, Director, National Tuberculosis Institute (NTI) Bangalore

- Dr. Prabha Desikan, Director, Bhopal Memorial Hospital and Research Center (BMHRC), Bhopal
- Prof.(Dr.) Ravindra Kumar Dewan, Director, National Institute of Tuberculosis and Respiratory Diseases (NITRD), Delhi and
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- Dr. Ranjani Ramachandran-NPO WHO India
- Dr. S. Siva Kumar, Scientist D & Head, Bacteriology Division, NIRT Chennai

Overall coordination for developing this manual was done by **Dr. Sanjeev Saini**, IDDS India Team lead.

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Abbreviations

ACH	Air Changes Per Hour
AFB	Acid Fast Bacilli
AHU	Air Handling Unit
AIC	Airborne Infection Control
ANSI	American National Standards Institute
ART	Aerosol Resistant Micropipette Tips
BCG	Bacille Calmette-Guerin
BMW	Bio Medical Waste
BSC	Biosafety Cabinet
BSL	Biosafety Level
CBNAAT	Cartridge Based Nucleic Acid Amplification Test
CCTV	Closed-Circuit Television
CDC	Center for Disease Control and Prevention
C-DST / C&DST	Culture and Drug Susceptibility Testing
CTD	Central Tuberculosis / TB Division
DMC	Designated Microscopy Center
DNA	Deoxyribonucleic Acid
DR	Drug Resistant
DST	Drug Susceptibility Testing
DTC	District Tuberculosis Center
DTO	District TB Officer
EMB	Ethambutol
EP	Extra Pulmonary
EPTB	Extra Pulmonary Tuberculosis
FFP	Filtering Facepiece
FM	Fluorescence Microscope
FPM	Feet Per Minute
GLI	Global Laboratory Initiative
GMLP	Good Microbiological Laboratory Practice
GUV	Germicidal Ultraviolet
HCW	Health Care Workers
HEPA	High-Efficiency Particulate Air
HIV	Human Immunodeficiency Virus
HVAC	Heating, Ventilation, and Air Conditioning
HWC	Health and Wellness Centers
IATA	International Air Transport Association
ICAO	International Civil Aviation Organization
IGRA	Interferon Gamma Release Assay
INH	Isoniazid
IPC	Infection Prevention and Control
IRL	Intermediate Reference Laboratory
LC	Liquid Culture
LIMS	Laboratory Information Management System
LJ	Lowenstein–Jensen Medium
LPA	Line Probe Assay
LPG	Liquid Petroleum Gas

MDR	Multi-Drug Resistant
MGIT	Mycobacterium Growth Indicator Tube
MODS	Microscopic Observation Drug Susceptibility
MoHFW	Ministry of Health and Family Welfare
MSDS	Material Safety Data Sheet
MTBC	Mycobacterium Tuberculosis Complex
NAAT	Nucleic Acid Amplification Test
NABL	National Accreditation Board for Testing and Calibration Laboratories
NCBI	National Center for Biotechnology Information
NDRF	National Disaster Response Force
NDRS	National Anti-Tuberculosis Drug Resistance Survey
NIH	National Institutes of Health
NRA	Nitrate Reductase Assay
NRL	National Level Reference Laboratories
NSF	National Sanitation Foundation
NTEP	National TB Elimination Programme
OSHA	Occupational Safety and Health Administration
PCR	Polymerase Chain Reaction
PHC	Primary Health Care
PMDT	Programmatic Management of Drug Resistant Tuberculosis
PPE	Personal Protective Equipment
PRT	Platinum Resistance Thermometer
PZA	Pyrazinamide
QMS	Quality Management System
RIF /RMP	Rifampicin
RR	Rifampicin Resistance
RRV	Relative Risk Value
SDG	United Nations Sustainable Development Goals
SDS	State Drug Stores
SOP	Standard Operating Procedure
SRL	Supra National Reference Laboratory
TB	Tuberculosis
TBI	TB Infection
TDC	Tuberculosis Detection Center
TNF	Tumor Necrosis Factor Alpha
TST	Tuberculin Skin Tests
U-DST	Universal Drug Susceptibility Testing
UPS	Uninterrupted Power Supply
UPU	Universal Postal Union
USAID	United States Agency for International Development
UV	Ultraviolet
VBM	Valuable Biological Materials
WHO	World Health Organization
WRD	WHO Endorsed Rapid Diagnostic
XDR	Extensive Drug Resistance
ZN	Ziehl–Neelsen Staining

Executive summary

The Joint National TB Diagnostic Network Assessment conducted in 2017 has recognized TB diagnostic laboratory networks in NTEP as most extensive networks globally, spanning all health system tiers. Although TB laboratory biosafety has been integrated into NTEP's laboratory quality assurance program, the team recognized the need of a standardized and comprehensive TB biosafety guidance manual or national policies and its enforcement at all levels of the laboratory network. NTEP has taken up this recommendation and developed this manual to develop national codes of practice for the safe handling of TB pathogens in all tiers of laboratories. To recommend the most feasible and effective safety practices, safety equipment, and infrastructure, this manual took into account standard international guidelines and literature [from the World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), Global of Laboratory Imitative (GLI), and others], as well as laboratory services and resources at various tiers within the NTEP setting. It is accepted internationally that the risk of exposure with infectious aerosol depends on the types of tests procedures being performed and it can be assessed by considering the bacterial load of materials (such as specimens and cultures) being handled, the viability of the bacilli, whether the material handled is prone to generate aerosols during the activity, the laboratory's workload, the epidemiology of the disease, and the health of laboratory workers. Although laboratory specific risk assessment is crucial, the NTEP laboratory network can be broadly classified into low-risk TB laboratories (district/sub-district and peripheral level TB Detection Centres), moderate-risk TB laboratories (C&DST laboratories where drug susceptibility testing is performed directly using clinical specimens and not the culture isolate), and high risk TB laboratories (Intermediate Reference Laboratories and National Reference Laboratories where all diagnostic modalities are available, including culture manipulation for drug susceptibility testing).

This manual describes the minimum biosafety requirements for each type of laboratory under the NTEP framework as well as suggested the strategies by which biosafety and biosecurity in TB laboratories can be further strengthened. The intended audience for this manual is laboratory supervisors, program manager as well as the laboratory technicians who test for TB in different tiers of laboratory network. The recommendations are specific to laboratories that follow well defined procedures to test samples potentially containing Mycobacterium tuberculosis.

This manual has twelve chapters and the key areas starts with basics of TB transmission, pathogenesis and principle for preventing TB infection in healthcare settings, classification of risk and differentiation of TB laboratories in NTEP based on risk of activities performed, discussion on basic and essential laboratory facilities and equipment's and good microbiology practices to be followed in different risk areas of laboratory activities inclusive of biosafety measures, safe practices, medical surveillance of health care workers, laboratory emergency preparedness, strategic response to chemical, fire, electrical and radiation emergencies and effective monitoring and evaluation biosafety programme

This manual was approved by the Central TB Division, MoHF&W, and the Government of India, and it presents the NTEP-level requirements and standards for biosafety in laboratories offering TB tests. The recommendations do not supersede any local or national rules or regulations.

Background

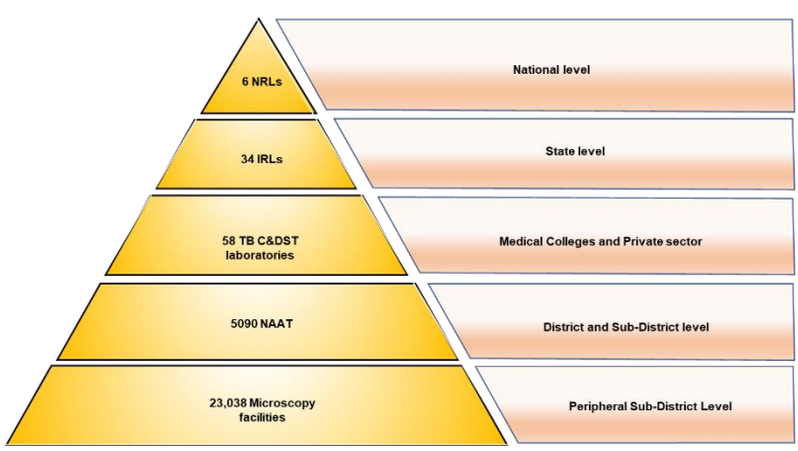
Tuberculosis (TB) has affected humans for centuries and continues to remain a major public health problem around the world. Every year about 10 million people globally fall ill due to TB. India has the largest burden of TB with an estimated incidence of 29.5 lakh cases (28% of global incidence) in 2021.[1] The National TB Prevalence Survey in India (2019-2021) revealed higher than expected prevalence of 312 cases per lakh population.[2]

Ending the TB epidemic by 2030 is one of the health targets of the United Nations Sustainable Development Goals (SDGs) and India is committed to ending TB by 2025, five years ahead of the global timeline. However, emergence of drug resistant forms of TB has posed a significant challenge to global TB elimination efforts. In 2021, India accounted for about 26% of total multi-drug resistant (MDR) or rifampicin resistant TB (RR-TB) cases detected globally.[1] According to the National Anti-Tuberculosis Drug Resistance Survey (NDRS; 2018) in India, 28% of TB patients are resistant to any one drug (22% among new and 36.8% among previously treated) and 6.2% have multi-drug resistant-TB (2.8% among new and 11.6% among previously treated).[3]

The National TB Elimination Programme (NTEP) in India has implemented universal drug susceptibility testing (U-DST) to at least rifampicin for all diagnosed TB patients by providing free rapid nucleic acid amplification tests (NAAT). Molecular and phenotypic DSTs are also being used extensively in the selection of appropriate regimens for drug resistant TB patients. Considering the high burden of TB and drug resistant-TB, there is a huge demand for TB detection and drug susceptibility testing in the country. To meet this demand, NTEP has impressively established one of the world's most extensive TB diagnostic networks, spanning all health system tiers.

TB laboratories network under National TB Elimination Program (NTEP) in India

TB laboratories network is organized in a tier system: National level Reference Laboratories (NRLs), State level Intermediate Reference Laboratories (IRLs), Culture and Drug Susceptibility Testing (C&DST) laboratories in public and private sector and peripheral level TB Diagnostic Centres (TDCs; that include NAAT laboratories and Designated Microscopy Centers (DMCs).[4]



Note: The numbers of these facilities are not constant and subject to change. Please refer to latest TB reports for updated numbers. TDCs include both NAAT sites and microscopy facilities.

Figure 1: TB laboratory network in NTEP

Source: From CTD, MoHFW

NRLs, IRLs and C&DST laboratories are more complex, with specialized and expensive equipment. DST is performed in these labs using a variety of technologies, including liquid culture, Line Probe Assay (LPA), and NAAT (GeneXpert and Truenat). Furthermore, these labs offer culture services for monitoring the treatment response. On the other hand, district level and peripheral laboratories are simple and usually have smear microscopy with or without rapid NAAT facilities. Although TB laboratories are increasingly available, WHO endorsed rapid diagnostic (WRD) technologies such as line probe assay, liquid culture for second line and newer extended drugs, in addition to conventional solid culture have further increased the country's need for TB containment laboratories.[5]

Biosafety in TB laboratories

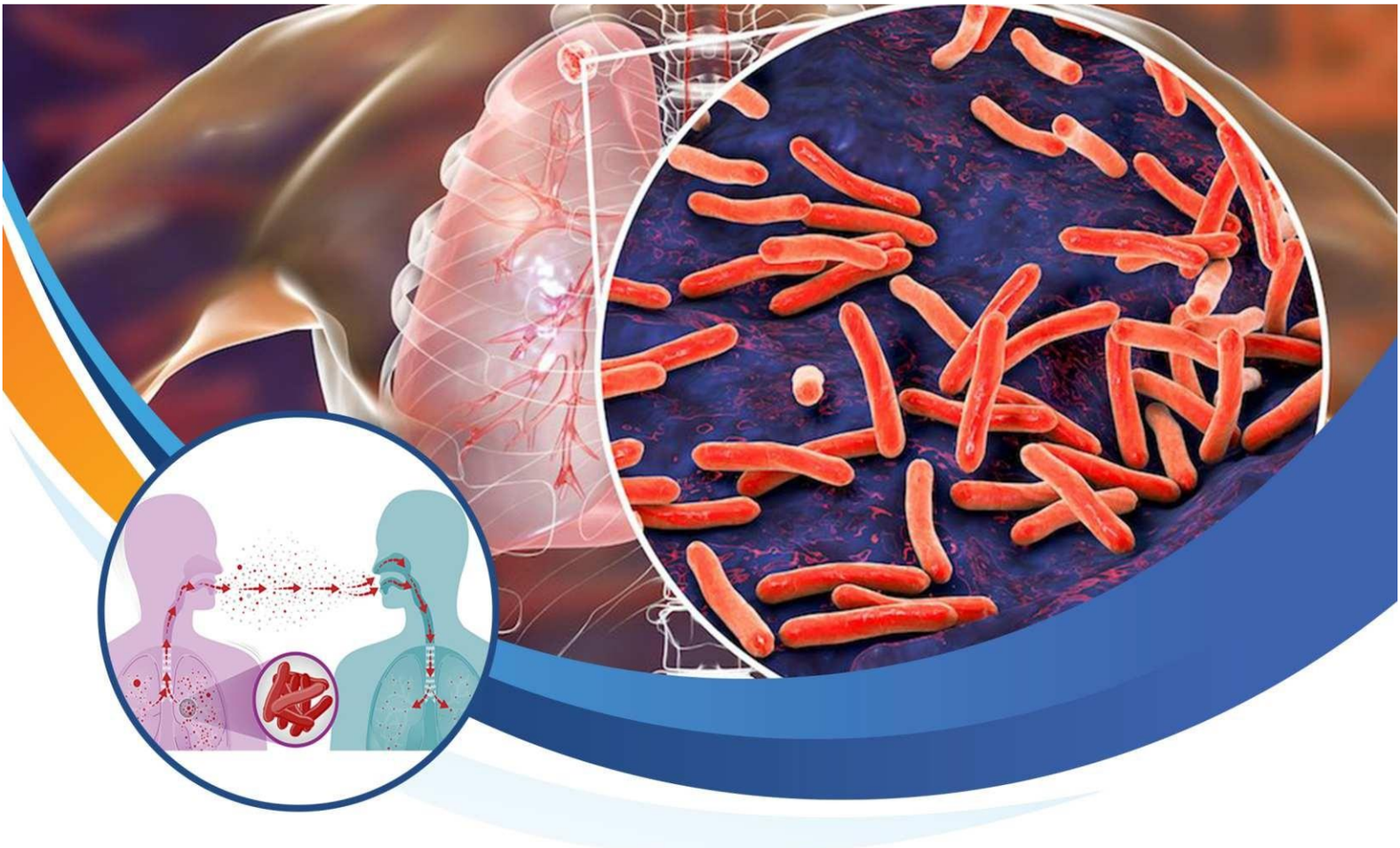
Healthcare workers who come into contact with TB patients, as well as those who work in the TB laboratory, are at an increased risk of contracting *Mycobacterium tuberculosis* (MTB). The risk of infection with MTB is 3-9 times higher for TB lab personnel than for other lab personnel.[6] Infection can also occur as a result of unrecognized production of infectious aerosols as well as needle sticks, broken skin, etc. Because samples from drug resistant (DR)-TB patients now account for an increasing proportion of the workload in TB laboratories, ensuring safe working conditions in all levels of TB laboratories in the country has become more important than ever before.

Laboratory biosafety is the process of implementing administrative controls, containment principles, practices and procedures, safety equipment and facilities to work safely with potentially infectious microorganisms. It also aims to prevent unintentional exposure to pathogens or their accidental release into the environment.

Biosafety manual for TB laboratories

The Joint National TB Diagnostic Network assessment conducted in 2017 has identified gaps related to biosafety requirements in the NTEP-diagnostic network. The team recognized the need for national TB biosafety policies and manuals, as well as the execution of the policies at all levels of the laboratory network, despite the existence of international manuals on biosafety and biosecurity. This biosafety manual for TB laboratories is developed with the aim

- To develop national codes of practice for the safe handling of TB pathogens in all tiers of laboratories.
- To encourage and guide the laboratory manager and program manager to implement the recommended biosafety policy, procedures, and practices at their respective laboratories.



Chapter 1
BASICS OF TB INFECTION AND PREVENTION

ABOUT THE CHAPTER

Tuberculosis (TB) laboratories pose a risk to both employee and the environment. Working safely in a TB laboratory requires an understanding of some basic concepts. This chapter discusses how TB is spread from person to person (transmission), how TB disease develops in the body (pathogenesis), the different types of TB, risk of TB infection among health care workers and the basic principles for preventing TB infection in the healthcare settings.

OBJECTIVE

To gain insight on the fundamental concepts of TB infection and prevention that laboratory personnel and managers can use to develop a safe working environment.

1. Basics of TB infection and prevention

TB, an airborne disease, remains a global public health threat and one of the leading infectious causes of death world-wide. TB is caused by any one of a group of bacteria known as the ***Mycobacterium tuberculosis complex*** (MTBC) but most commonly by ***Mycobacterium tuberculosis*** (MTB). MTB, often called tubercle bacilli, are aerobic, rod-shaped, 2-4 micron in length and 0.2-0.5 micron in width, and slow-growing (divide once every 15 to 20 hrs.). TB primarily affects the lungs (pulmonary tuberculosis), but it can affect virtually any organ outside the lungs (extrapulmonary tuberculosis). TB symptoms differ based on the affected area. Pulmonary TB is characterized by cough (especially if lasting 2 weeks or more), hemoptysis (coughing up blood), chest pain, shortness of breath, mild fever, night sweating, unexplained weight loss and weakness. Symptoms and clinical presentation of extrapulmonary TB are variable, site-specific and may need a high index of suspicion for diagnosis.

1.1 Transmission

TB spreads from person to person primarily through the air. Tubercle bacilli are carried in airborne particles of less than 5 microns in diameter called droplet nuclei. These are dried tiny particles that can accommodate one or a few tubercle bacilli only. When pulmonary TB patients cough, sneeze, shout, or sing, droplets of various sizes are generated and expelled into the air. While large droplets settle out of the air quickly, tiny droplet nuclei settle very slowly and often remain suspended in the air for several hours depending on the environment.[1,2] Transmission takes place when a person inhales infectious droplet nuclei and these droplet nuclei pass through the mouth/ nasal route, upper respiratory tract, and bronchi to reach the lungs alveoli (Figure 1.1).

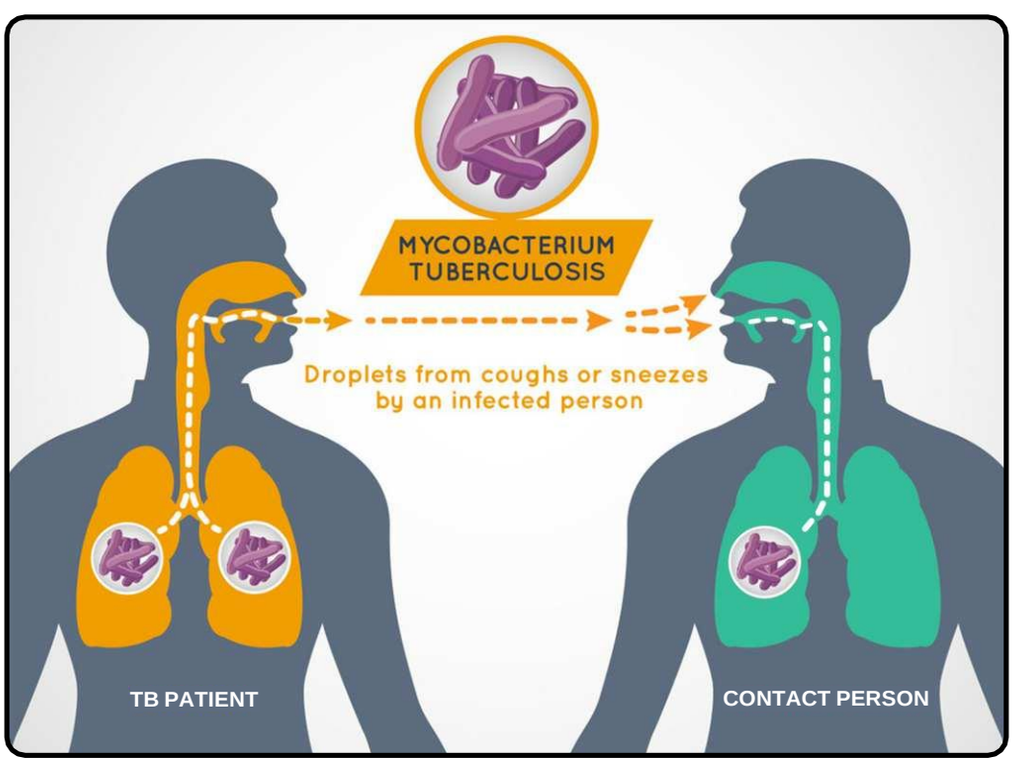


Figure 1.1. Transmission of TB: *M. tuberculosis* containing droplet nuclei generated by a TB patient (by coughing or sneezing) and transmitted to a close contact person.

1.1.1 Infective dose

The infective dose is the number of tubercle bacilli-laden particles that cause infection. The risk of acquiring tuberculosis is determined by the likelihood of inhaling an infectious dose and progressing to an established infection (active disease).

MTB has a very low infectious dose in humans, with 1-10 bacilli capable of causing infection.[3,4] The precise infectious dose, however, varies with microbial strain virulence (i.e the degree to which it can cause disease) and host immunity (including general immunocompetence, BCG immunization and prior exposure to MTB, or other mycobacteria).[5]

1.1.2 Factors determining the likelihood of transmission

The highest risk of transmission exists among family members and close contacts of a TB patient. Transmission can also occur between coworkers who work closely.[6] The key determinants of TB transmission are the infectiousness of the TB patient, the environment in which the exposure occurred, the frequency and duration of the exposure, and the immune status of the exposed person (Table 1.1).[7]

Table 1.1: Factors determining the likelihood of TB transmission

Factors	Description
Infectiousness	• Infectiousness of TB patients is directly proportional to the number of tubercle bacilli expelled into the air. • Pulmonary TB patients with positive smear/culture are considered more infectious than smear/culture negative patients. • Patients with non-respiratory TB disease are usually non-infectious.
Environment	• Since the concentration of infectious droplet nuclei is diluted by ventilation, transmission usually occurs indoors (with rare exception of outdoor). • Small space, poor ventilation, or re-circulation of air, as well as inadequate sunlight (ultraviolet rays) contribute to an increased risk of transmission. Therefore, people living in slum areas and congregate settings (like shelters, old age homes, refugee camps and correctional facilities) are at higher risk of TB transmission. • Forceful expiration (e.g., coughing, sneezing or singing),certain medical procedures (e.g., bronchoscopy and sputum induction) and laboratory procedures (e.g.,shaking, pouring and centrifugation) can result in high concentration of TB bacteria being released into the surrounding airspace.
Exposure	• This risk of transmission is higher with longer duration of exposure, more frequent exposure, and closer proximity. • Contact persons that may include family members, roommates, or coworkers are generally more exposed. • Contacts in crowded or poorly ventilated areas and those without any respiratory protection are at higher risk of transmission.

Factors	Description
Immune status (Susceptibility)	• Immuno-compromised individuals (e.g., HIV, diabetes) are more likely to become infected and re-infected than immuno-competent individuals. • Vaccination (*Bacille Calmette-Guerin*; BCG) and exposure to environmental mycobacteria may provide some protection against MTB infection.

1.2 Pathogenesis

The pathogenesis of TB can be divided into four distinct events (Figure 1.2).

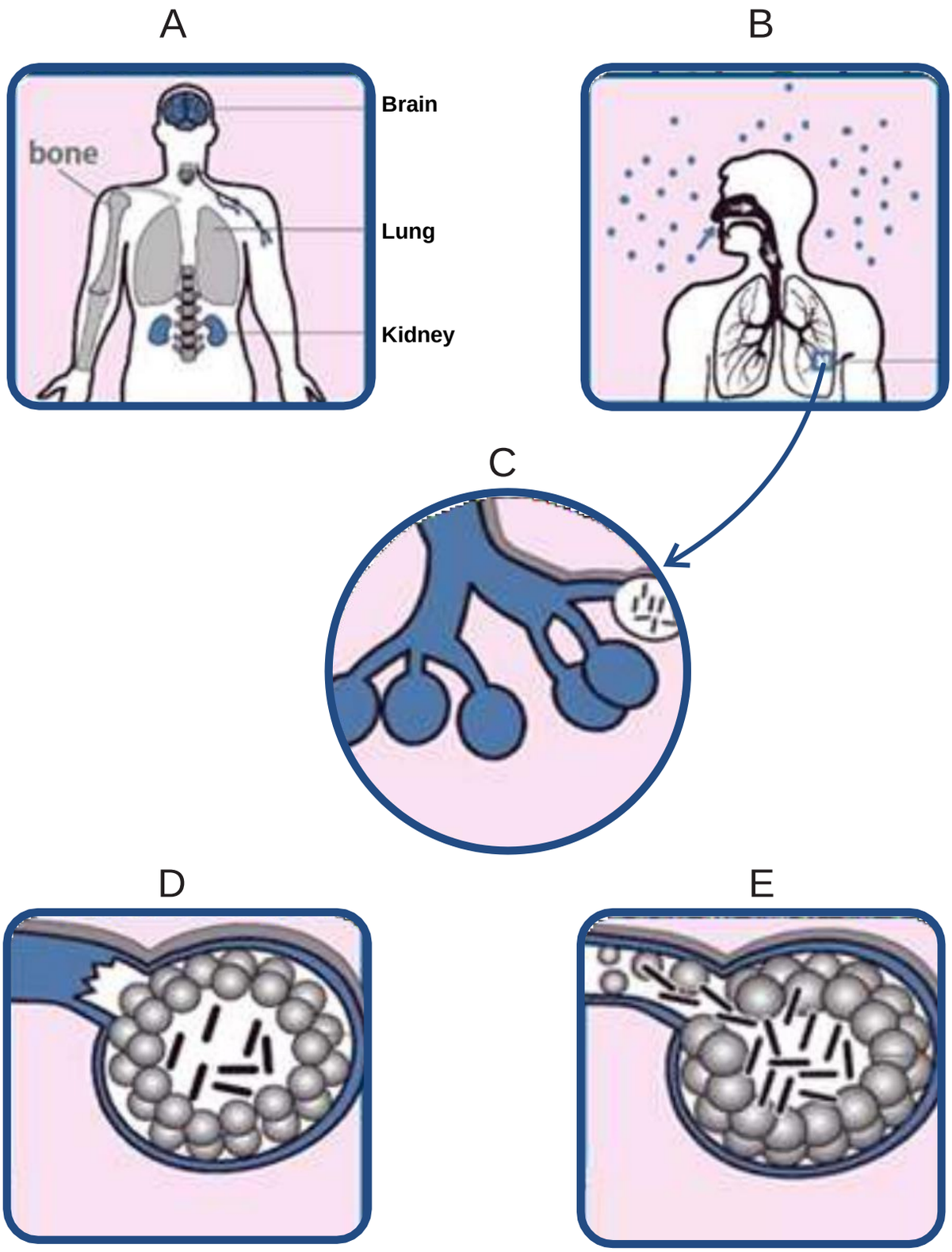
1) **Ingestion of tubercle bacilli by alveolar macrophages:** Once inhaled, tubercle bacilli reach the alveoli of the lung and interact with alveolar macrophages through cellular receptors. Macrophages engulf the tubercle bacilli and activate its bactericidal mechanism by releasing reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI). Although, majority of tubercle bacilli in the alveoli, are ingested by alveolar macrophages, a small number may multiply within macrophages and be released when the macrophages die. The efficient killing of tubercle bacilli depends on virulence of inhaled bacilli, bactericidal capacity of macrophages as well as inflammatory micro-environment at the site of infection.

2) **Recruitment of inflammatory cells:** Surviving tubercle bacilli proliferate/ multiply within macrophages and can spread to distant tissues and organs via lymphatic channels or the bloodstream (like regional lymph nodes, apex of the lung, kidneys, brain, bone etc.). Proliferating bacilli induce the production of proinflammatory cytokines that facilitate recruiting several types of immune cells including monocytes, neutrophils, and dendritic cells to the site of infection, in order to halt the multiplication. An increased level of TNF-α contributes to control mycobacterial growth.

3) **Control of tubercle bacilli proliferation (TB infection):** The recruitment of immune cells (including T cells) to the site of infection, ingest and surround the tubercle bacilli to form a characteristic barrier shell known as granuloma that contain tubercle bacilli and prevent further spread. Tubercle bacilli contained within granuloma eventually leads to a stable TB infection (latent TB) state.

4) **Progression towards TB disease:** If host immune system fails to control the tubercle bacilli, the latent TB reactivates, causing damage to nearby bronchi and spreading to other areas of lungs; thus progressing to active TB disease.

TB infection (TBI) can develop into TB disease at any time, but it is most common within the first two years of infection or in presence of certain medical conditions. The medical conditions that increase the risk of developing TB disease are listed in Table 1.2. [8]



A. Organs where TB disease is more likely to develop; **B.** Droplet nuclei containing tubercle bacilli being inhaled, entering the lungs, and travelling to the alveoli; **C**. Tubercle bacilli multiplying in the alveoli; **D.** Macrophage surrounding the tubercle bacilli; **E.** Tubercle bacilli rapidly multiplying and progressing from TB infection to TB disease

Figure 1.2: Illustration of TB pathogenesis

Source: Adapted from CDC (2019)[7]

Table 1.2: Risk factors for the development of active TB

High Risk	Moderate Risk
• HIV/AIDS • Transplantation (related to immune-suppressant therapy) • Silicosis • Chronic renal failure requiring hemodialysis • Carcinoma of head and neck • Recent TB infection (≤2 years) • Abnormal chest X-ray (fibronodular disease)	• Tumor necrosis factor alpha (TNF-) inhibitor therapy • Diabetes • Glucocorticoid therapy • Infection at younger age (0-4 years)
Slightly Increased Risk	**Low Risk**
• Heavy alcohol consumption • Underweight (<90% ideal body weight; body mass index ≤20) • Smoking • Abnormal chest X-ray	• A positive TST (without any risk factor abnormality in chest X-ray)

People with TB infection (TBI) do not feel sick and are not contagious. The infection cannot spread from these people to others. The key similarities and differences between TBI and TB disease are described in Table 1.3.[7]

Table 1.3: TB infection and TB Disease

TB Infection (TBI)	TB Disease (Pulmonary)
Few TB bacteria in the body that are alive, **but under control**	**Large number** of TB bacteria in the body that are **actively dividing**
TB bacteria **cannot spread** to other people	TB bacteria may **spread** to other people
No symptoms	Cough, fever, chest pain, weakness and weight loss are key **symptoms**
Usually **normal** chest X-ray	Usually **abnormal** chest X-ray
Sputum smears/ cultures **negative**	Sputum smears/cultures may be **positive**
Usually, **positive** test results in Tuberculin skin test or interferon-gamma release assay	Usually, **positive** test results in Tuberculin skin test or interferon-gamma release assay
Person **does not** require respiratory isolation	May **require** respiratory isolation
Consider treatment for TB infection(preventing the progress to TB disease)	Consider treatment for TB disease
Not a case of TB disease	Not a case of TB infection

1.3 Drug resistant tuberculosis (DR-TB)

Drug-resistant tuberculosis occurs when a strain of MTB is resistant to one or more drugs commonly used to treat the disease. This means those drugs will no longer be able to kill the bacteria.

The first line of drugs includes rifampicin (RIF), isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZA). DR-TB is classified into following types based on MTB resistance pattern
.

- **Mono-resistant TB:** resistance to one first-line anti-TB drug only
- **Poly-resistant TB:** resistance to more than one first-line anti-TB drug, other than both INH and RIF.
- **Rifampicin resistant (RR) TB:** resistance to RIF detected using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs
- **Multidrug resistant (MDR) TB:** resistance to at least both INH and RIF
- **Pre- extensive drug resistant (Pre-XDR) TB:** MDR/RR-TB that is also resistant to any fluoroquinolone (levofloxacin or moxifloxacin)
- **Extensive drug resistant (XDR) TB:** MDR/RR-TB that is also resistant to any fluoroquinolone (levofloxacin or moxifloxacin) and at least one additional Group A drug [presently to either bedaquiline or linezolid (or both)

Drug-resistant TB disease can develop in two ways: primary and secondary drug resistance.

- **Primary resistance** develops through the transmission of TB bacteria that are already resistant to a particular drug/(s).
- **Secondary resistance or acquired resistance** develops during TB treatment. Individuals in this situation have drug-susceptible TB bacteria that eventually become drug resistant during treatment. Acquired resistance is considered to be man-made and develops usually due to
    - Nonstandard treatment regimen (patient was not treated with appropriate or standard treatment regimen)
    - Non-adherence to prescribed treatment regimen (either taking the drugs incorrectly or irregularly)
    - Malabsorption of drugs
    - Drug to drug interactions (causing low serum levels)

Box 1.1: Mechanism involved in the development of drug resistance in MTB[9]

Drug resistance in MTB develops largely due to mutations in chromosomal genes in growing bacteria. It may also be developed due to epigenetic changes in gene expression and protein modification that cause drug tolerance in non-growing bacteria.]

In non-treated populations of TB bacteria, random mutations that confer resistance to key anti-TB drugs occur spontaneously at predictable frequencies

Isoniazid (INH): 1.84 $X10^{-8}$
Rimapicin (RIF): 2.20 $X\ 10^{-10}$

A 2-cm diameter TB cavity in lungs harboring 10^8 bacteria may contain a few (about 100) bacteria resistant to INH, a few (about 10) resistant to RIF, and a few resistant to other drugs. Hence, the likelihood of a bacterium spontaneously developing resistance to two unrelated drugs will be the product of probabilities.

For example, the probability of developing spontaneous mutation both for INH and RMP resistance will be 10^{18} [(INH: 1 in 10^8) × (1 in 10^{10}) = (1 in 10^{18})].

Because the total number of bacteria in the body rarely approaches this number (10^{18}), spontaneous MDR-TB development is extremely rare. Therefore, treatment with two or more drugs prevents the emergence of drug-resistant TB.

1.4 Incidence and prevalence of TB among healthcare workers

Although TB has long been recognized as a serious occupational hazard for healthcare workers (HCWs), it has received less attention than is required. An estimated 81% of TB cases among HCWs are due to occupational exposure.[10] Previous reports suggest that HCWs are at an increased risk of acquiring TB compared to the general population, regardless of local TB incidence and economic setting.[10-12] However, the risk is higher in 'low-resource and high TB-burden settings', where HCWs have frequent and prolonged contact with TB patients.[13-18] According to the global TB report, 9,299 TB cases were reported among HCWs in 60 countries in 2017; and the notification rate of health care-associated TB infections was twice that of the general adult population.[19]

Surveillance data are limited for occupationally acquired TB in HCWs in low-resource high-burden settings. This is mainly due to[20-21]

- Limited or absence of HCW medical surveillance systems
- Inadequate implementation for HCW safety measures
- Fear of stigma and discrimination prevents HCWs from seeking TB care on time

Overall, compared to the general population, HCWs may have a threefold increased risk of acquiring TB.[22] The risk of acquiring TB varies by occupation, but frontline workers caring for undiagnosed TB patients or those diagnosed with TB (who do not respond to treatment) are at highest risk.[12,15,21,23-28] TB laboratory personnel are also a high-risk occupational category, particularly when occupational biosafety measures, such as safe practices, safety equipment, and facility design, are compromised.[24,25]

This risk may be variable (relative risk value 2.0 to 21.5) depending on the type of activities performed by the laboratory staff. Manipulating positive cultures for DST poses the highest risk. Medical and nursing students are often overlooked but they are also at severe risk. A study conducted in India reported a high prevalence of TB infection in nursing trainees, with an annual rate of TB infection of 7.8% compared to the national average of 1.5%.[17]

Additionally, HCWs have a higher risk of developing DR-TB than the general population. For instance, hospitalization for MDR-TB is up to six times more common in HCWs than in the general population.[30] HCWs with XDR-TB had high mortality rates, delayed diagnosis and poor treatment outcomes.[31]

1.5 Principles of TB infection and prevention in healthcare settings

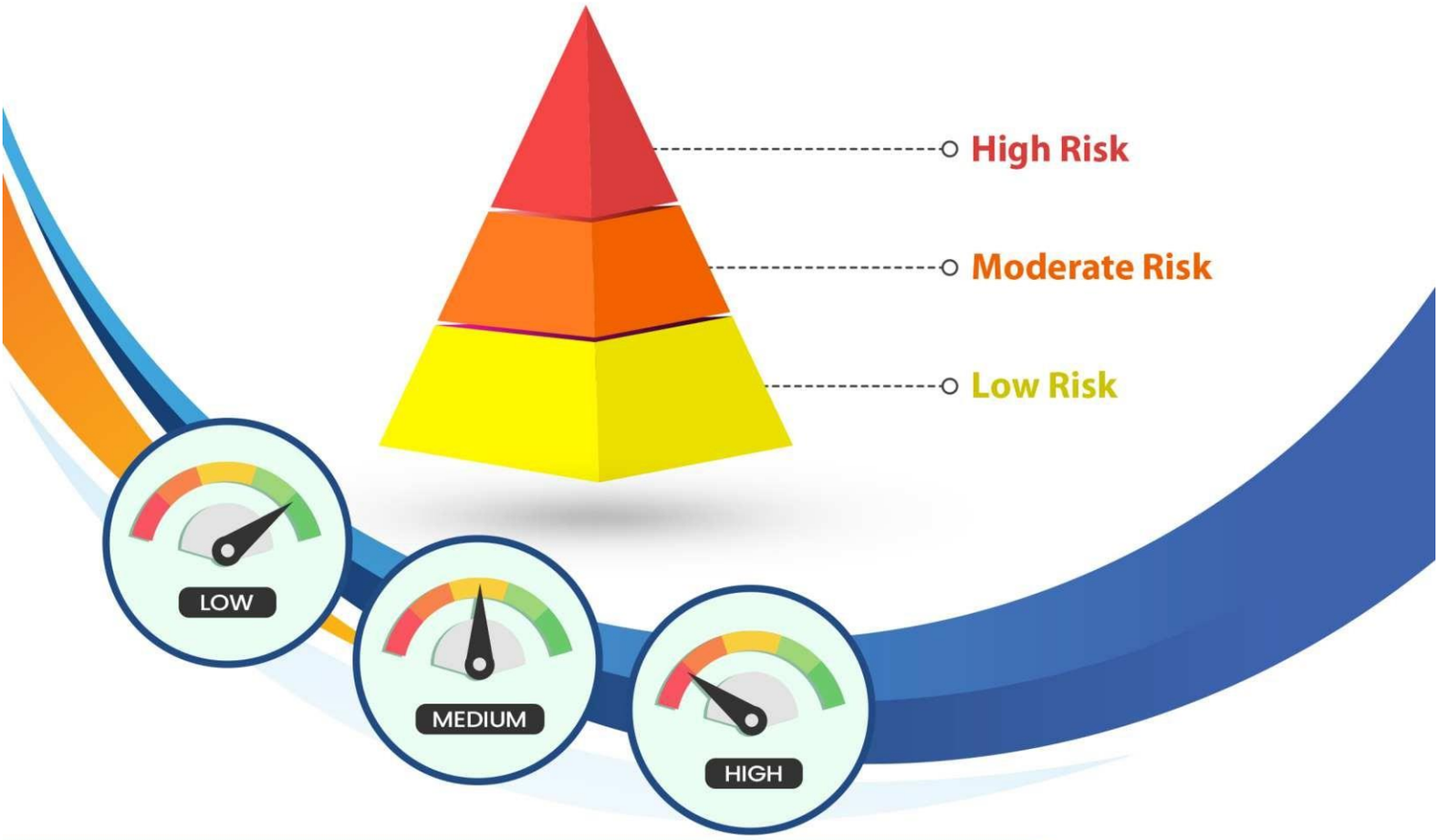
TB transmission in healthcare facilities poses risk not only to HCWs but also to patients and visitors. Breaking the transmission cycle is crucial and this requires a set of interventions with the following aims

- To rapidly identify source cases
- To minimize the concentration of infectious airborne particles and the exposure time that vulnerable people are exposed; and
- To initiate the appropriate TB treatment as soon as possible.

The above interventions are the basic principles for effective infection prevention and control (IPC) in all health care settings. The TB infection control programme in healthcare settings is based on a three-level hierarchy of control measures and includes administrative controls, environmental controls, and respiratory protection. In 2019, the World Health Organization released an updated guideline on tuberculosis infection and prevention in health care settings. [32] Key recommendations in the guidelines are summarized in Table 1.4.

Table 1.4 TB infection control in health care facilities

Administrative control	Environmental control	Respiratory protection
• Triage of patients with TB disease or symptoms suggestive of TB • Isolation or respiratory separation of patients with TB, whether suspected or confirmed. • Initiation of effective TB treatment for people with TB disease at earliest • Respiratory hygiene (including cough etiquette) in patients with suspected or confirmed TB	Strategic use of • Ultraviolet germicidal irradiation (UVGI) • Ventilation systems (including natural, mechanical or mixed-mode) • High efficiency particulate air (HEPA)	• Implementing respiratory protection program and use of particulate respirators.



Chapter 2

CLASSIFICATION OF TB LABORATORIES ACCORDING TO RISK OF INFECTION

ABOUT THE CHAPTER

All TB tests have procedural biosafety risk which can be low, moderate or high. In NTEP, there is a network of TB laboratories that vary in the types of activities performed and thus inthe level of associated risk. This chapter describes how TB laboratories can be classified broadly on the basis of risk associated with activities being performed.

OBJECTIVE

To enable TB laboratories (NTEP) to understandi their class and plan appropriate measures based on the procedural risks associated with various activities/ TB tests.

2. Classification of TB laboratories according to risk of infection

The infectious microorganisms are classified into four risk groups depending on virulence, transmissibility, and availability of treatments in humans (Table 2.1).[33] Tubercle bacilli are considered as 'Risk group 3' microorganism and the major risk of TB infection in the laboratory is inhalation of infectious aerosols generated by TB testing activities. Therefore, minimizing the aerosol production is one of the most effective ways to stay safe. Aerosols do not re-aerosolized, once they have settled on a surface. They may, however, contaminate specimens or cultures, consumables, reagents, equipment or PPE, posing a risk of cross-contamination.

Table 2.1: Classification of infective microorganisms by risk group

Risk group	Description	Example
Risk group 1 (no or low individual or community risk)	Microorganisms that are unlikely to cause human or animal disease	*Aspergillus niger, Escherichia coli* -k12*, Lactobacillus acidophilus,*
Risk group 2 (moderate individual risk, low community risk)	Microorganisms that can cause human or animal disease but are rarely serious or for which effective treatment and preventive measures are often available and the risk of spread of infection is limited.	Pathogenic *Escherichia coli Streptococcus pneumonia, Salmonella choleraesuis*
Risk group 3 (high individual risk, low community risk)	Microorganisms that usually cause serious human or animal disease but effective treatment or preventive measures are available . They do not ordinarily spread from one infected individual to another.	*Mycobacterium tuberculosis Brucella* species *Yersinia pestis, Francisella tularensis*
Risk group 4 (high individual and community risk)	Microorganisms that usually cause serious human or animal disease and effective treatment and preventive measures are not usually available. They can be readily transmitted from one individual to another, directly or indirectly.	Ebola virus Hendra virus Marburg virus Lassa virus

2.1 Procedural risk of aerosolization in TB laboratories

There are various TB test procedures, and each poses a variable level of aerosolization risk in TB laboratories. A better understanding on procedure risk associated with TB tests enables the laboratory managers and staff to meet the minimum biosafety requirement and carry out procedures safely.

The bacillary load of the materials being manipulated and the likelihood of producing infectious aerosols from the materials are two crucial factors in assessing the risk of aerosolization. The most common specimen tested for TB is sputum, and the bacillary load for these specimen ranges from 0 (applicable for up to 90% of diagnostic samples) to 10^3-10^4/ml in sputum specimens with scanty smear grading, to 10^6/ml in sputum specimens with 3+ smear grading. On the other hand, the bacillary load in a culture recovered from sputum specimen is considered to be quite high and may even exceed 10^8/ml. Furthermore, compared to liquid cultures, manipulation with sputum specimens has a significantly lower risk of generating infectious aerosols due to their high viscosity. Taking the above facts into account, the risk of TB procedures involving sputum manipulation are much lower than those involving culture manipulation. Table 2.2 summarizes the quantitative and/or qualitative risks posed to laboratory personnel performing various tests/(s) versus administration personnel who have no contact with TB testing activities.

Table 2.2: Procedural risks to laboratory personnel and key consideration while conducting a specific test procedure or determining the procedure specific precautions

Procedures	Relative risk value (RRV) [@]	Remarks
Administration	1.0*	Relative risk value (RRV) of administrative staff is similar to the general community
Microscopy only	1.4* (95% CI 0.2–10.0) **Low risk**	• A low risk of generating infectious aerosols when safe microbiological practices are followed • Tubercle bacilli load is variable in materials manipulated • Viability of bacilli is uncertain but it is assumed to be high • May be performed on an open bench.
Gene Xpert and Truenat	≤ 1.4** **Low risk**	• The risk of generating infectious aerosols is equivalent to that of sputum smear microscopy • Tubercle bacilli load is variable in materials manipulated • Viability of bacilli - Lysis buffer (Gene Xpert) reduces AFB viability by 10^6 within 15 min; DNA extraction step inactivates AFB • May be performed on an open bench if environmental conditions are as per manufacturer's recommendations
Culture only	2.0* (95% CI 0.2–13.3) **Moderate risk**	• Marginally increased risk of generating infectious aerosols as compared to sputum smear microscopy • Tubercle bacilli load is variable in materials manipulated • Viability of bacilli - Processing can kill 70-90% of bacilli • Should be performed preferably in BSCs and with good microbiological practices

Procedures	Relative risk value (RRV) [@]	Remarks
LPA (direct)	≤ 2.0** **Moderate risk**	• Marginally increased risk of generating infectious aerosols as compared to sputum smear microscopy • Tubercle bacilli load is uniformly high (>10^3/ml sputum)[#] in materials manipulated • Viability of bacilli - Processing can kill 70-90% of bacilli • Specimen processing should be performed preferably in BSCs and with good micro-biological practices
Combined culture and DST	7.8* (95% CI 1.7–34.9) **High risk**	• A high risk of generating infectious aerosols even when good microbiological technique is used • Tubercle bacilli load is uniformly high (>10^8/ml sputum) in materials manipulated • Viability of bacilli is high • Should always be done in BSC within a TB containment laboratory with good microbio-logical practices
DST only	21.5* (95% CI 4.5–102.5) **High risk**	
LPA (in-direct)	≤ 21.5** **High risk**	• Since it requires manipulation of a positive culture a risk may be as high as for DST • Tubercle bacilli load is uniformly high (>10^8/ml sputum) in materials manipulated • Viability of bacilli is high (although the subsequent step of DNA extraction inactivates AFB). • Should always be done in BSC preferably within a TB containment laboratory.

[@] relative risk value is a measure of the risk of a certain event happening in one group compared to the risk of the same event happening in another group;
* estimated in a retrospective study (Kim et al., 2007);[29]
** suggestive estimate as per GLI (2019)[34] and WHO (2012);[35]
# considering only smear positive samples are subjected to LPA

2.2 Classification of TB laboratories

According to WHO (2012),[35] TB laboratory facilities can be classified into low-risk, moderate-risk and high-risk (TB containment) laboratories based on the activities being done and the risks involved. A careful decision about the most appropriate biosafety measures for a specific TB laboratory is essential, which can be undertaken using the procedural risk assessment approach.

Risk-assessment for a TB laboratory should consider the followings:

- The bacillary load in materials (eg. sputum and cultures), as well as their viability
- The mode of transmission
- The likelihood that the handling or manipulation of the material required for the procedure would produce infectious aerosols

- The number of manoeuvre for each technique that may potentially generate aerosols
- Testing workload of the laboratory as well as on individual laboratory technicians
- The location of the laboratory
- The epidemiology of TB and the patient population that the lab serves
- The level of expertise and competence of the laboratory technicians
- The health of the laboratory staff (especially those who are HIV-positive and other immune suppression conditions)
- The availability of containment equipment and their operational integrity
- The facility's safeguards
- The availability and adherence to standard operating procedures

Note: While underestimating risks can result in biosafety hazards, stringent safeguard measures may burden laboratories with unnecessary cost and human resource demands.

The TB laboratory network in NTEP is organized into a multi-tier system that varies in the availability of testing modalities. For complex tests like LPA, culture and DST, samples are transported from district or sub-district level labs to higher level or referral laboratories. In accordance with WHO's classification of TB laboratories,[35] the laboratories under the NTEP network can also be grouped into the three main levels of procedural risks (low, medium, and high) and are described in Table 2.3.

Table 2.3: Risk precaution levels, TB laboratories in NTEP and available procedural activities

Risk level of the laboratory	TB Laboratory in NTEP		Laboratory activities
	Hierarchy level	Type of laboratory	
Low risk	Peripheral or sub-district or district level	TB Detection Centres (TDCs)	• Sputum-smear microscopy and/or • Rapid NAAT (Truenat or CBNAAT)
Moderate risk	Sub-state or state level	• Culture and DST (C&DST) laboratories • Intermediate Reference Laboratory (IRL) *(without liquid culture and DST facility)*	• All activities of low-risk TB laboratory; and • Processing and concentration of specimens for inoculation on primary culture media. and/or • Solid culture manipulation for identification. and/or • Direct LPA (i.e LPA on sputum sample)
High risk (TB containment laboratory	Sub-state level	• Culture and DST (C&DST) laboratories • Intermediate Reference Laboratory (IRL) • National reference laboratories (IRL) *(with liquid culture and DST facility)*	• All activities of moderate-risk TB laboratory; and • Liquid culture manipulation for identification. and/or • Liquid culture DST and indirect LPA (*i.e.* LPA on culture isolate)

Sample collection centers: Many health centers both in the public and private sector don't have TB testing facilities but provide the diagnosis services using the 'Hub and Spoke' model. Samples from these centers (spoke) are collected, packed, and transported to linked TB testing laboratories (Hub). These facilities have very low biosafety risk, provided that samples are handled carefully and packed, transported in accordance with national/ international guidelines (see chapter 7: Sample collection and transportation).

Health and Wellness Centers

Under the Ayushman Bharat (AB) initiative in India, the primary care facilities including Primary Health Centers (PHCs) and Sub Health Centers (SHCs) are being strengthened as Health and Wellness Centers (HWCs) to provide comprehensive primary health care services, including for Tuberculosis care close to the community. HWCs without TB testing facility serve as sample collection sites and will ensure that a good quality specimen is collected, packaged, and safely transported to linked TB testing laboratory.

Chapter 3
COMPONENTS OF BIOSAFETY IN TB LABORATORIES

ABOUT THE CHAPTER

Laboratory biosafety is containment of biological agents to prevent exposure to laboratory workers, surrounding community and the environment. Good microbiological techniques, safety equipment, and facility design are critical in achieving containment. This chapter discusses all essential components of an effective biosafety program for TB laboratories.

OBJECTIVE

To provide a clear understanding of the essential biosafety components that TB laboratories must implement.

3. Components of biosafety in TB laboratories

Every clinical laboratory, including TB laboratories have risk of exposure to hazardous biological agents. An effective biosafety program in the laboratory helps in reducing or eliminating exposure to laboratory workers, other people, and the environment. Containment and risk assessment are the core principles of biosafety.[36]

- **Containment:** It refers to safely managing infectious materials in the laboratory environment where they are being handled or maintained. Containment is achieved through a combination of primary and secondary containment measures. Primary containment is provided by good microbiological technique and by the use of safety equipment, including personal protective equipment, whereas secondary containment is achieved by operational procedures and facility design (Figure 3.1).

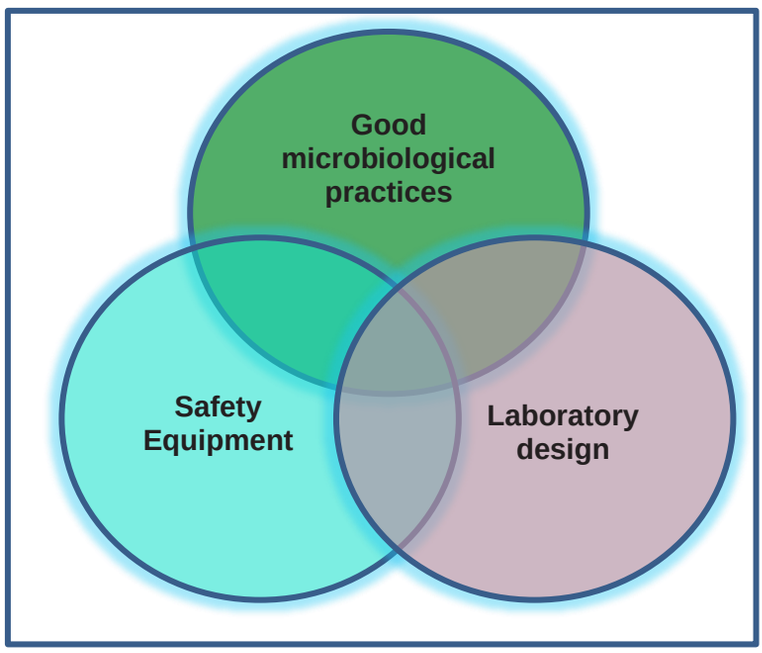


Figure 3.1: Components of biosafety

- **Risk assessment**: It refers to a process that allows for the appropriate selection of microbiological practices, safety equipment, and facility safeguards in order to prevent laboratory-associated infections. The level of biosafety risk varies by laboratory, depending on the procedures/activities being carried out. A comprehensive risk assessment identifies all potential risks which must be addressed through combination of containment measures.

Essential components of biosafety in TB laboratories include risk assessment, code of practices (good microbiological practices), laboratory design, and administrative policies as well as the use of safety equipment and disinfection/sterilization of infectious agents or biomedical waste. Staff training, occupational health program, biosecurity, emergency preparedness and safety to chemicals, fire, electrical and radiation are also included. All the above components are discussed in following sections and chapters.

3.1 Good microbiological laboratory practices (GMLP)

Good microbiological laboratory practices (GMLP) is a code of practice that contains the most essential biosafety risk control measures in all types of work with biological agents. This includes general behavior, technical procedures, best work practices etc., that must always be followed in the laboratory. When working with biological agents, the use of GMLP can help in protecting the laboratory personnel and the community from infections as well as prevent contamination of the environment and the product. The laboratory in-charge must ensure that all laboratory personnel receive GMLP training and understand the rules.

The following concepts of GMLP should be considered at all levels of TB laboratories.

3.1.1 Best behavioral practices

- Only authorized personnel should be permitted access to the laboratory.
- The working area/ refrigerator of the laboratory should not be used for storing food or drink. Eating, drinking, smoking, applying cosmetics, and handling contact lenses are all prohibited inside the laboratory.
- While in the lab, never put any objects like pens or pencils in the mouth.

- Wounds and cuts, if any, should be covered before entering the lab.
- Any jewelry that could tear gloves or easily become contaminated should be covered or removed.
- Hands should be thoroughly washed with soap after handling biological materials, before donning and doffing personal protective equipment (PPE), before leaving the laboratory, or whenever hands are visibly or believably soiled or contaminated (**Annexure 1**).
- Use personal protective equipment (e.g. gloves, gowns etc.) depending on the anticipated exposure in the laboratory (see Chapter 3.2.1).
- The open flames or heat sources should never be near the flammable supplies.
- The work area should be clean and free of unnecessary materials/ equipment.
- Avoid using portable electronic devices (such as a mobile phone, tablet, laptop, etc.) unless it is necessary for the laboratory procedures being performed. If necessary, keep them in areas where they can't easily become contaminated and act as fomite to spread infection. Before leaving the laboratory, ensure that devices have been decontaminated or protected by a physical barrier.
- Ensure that the work is done carefully and without haste.
- After any spill of potentially infectious material and at the end of each work session, work surfaces must be decontaminated using suitable disinfectants (see Chapter 9).

3.1.2 Technical procedures

A) Preventing inhalation of biological agents (minimizing aerosol generation)

- When using a pipette to mix liquid, always do so gently and never vigorously.
- When adding a reagent to a potentially infectious liquid, place the pipette against the inner wall of the tube and gently expel the liquid.
- Avoid carelessly tapping or flipping open tubes. A brief centrifugation of the tubes prior to opening can help in moving any liquid away from the cap.
- Reusable loops should be avoided because putting them on a flame (heat sterilization) might cause infectious material to spatter. Always, prefer disposable transfer loops/ wooden stick for preparing smear.
- Adhere to additional safety precautions/procedures that are relevant to the specific risk level of TB laboratories (see Chapters 4, 5, and 6).
- TB testing procedures involves aerosol generating activities and therefore special safety equipment (e.g. biological safety cabinet, biosafe centrifuge etc.), appropriate personnel protective equipment (e.g. respirator, gloves etc.) and special facility design (e.g. containment lab) are required to prevent the exposure to infectious aerosol (see Chapters 3.2 and 3.3).

B) Preventing ingestion of biological agents and contact with skin and eyes

- Mouth pipetting must be prohibited.
- When handling specimens or biological agents, always wear disposable gloves. Disposable gloves should never be reused.
- Avoid making contact with the face with gloved hands.
- After using gloves, remove them aseptically and thoroughly wash your hands.
- Face-shield/ safety goggles must be used during any operation where splashes may occur, such as during the preparation of disinfectant solutions.

C) Preventing injection of biological agents

- Avoid using syringes as a substitute for pipetting devices
- Avoid recapping or removing needles from disposable syringes.
- Replace any glassware with plastic-ware, wherever possible. If glassware is to be used, check it on a regular basis for integrity and discard it if it is cracked/damaged.
- Sharps (such as needles, blades, broken glass etc.) should always be discarded in puncture-proof container fitted with sealed covers. The container must have a suitable disinfectant and must not be filled more than three-quarters full.

3.1.3 Safe handling of specimens

Improper specimen collection, transport, and handling not only increase the risk of infection for laboratory personnel but can also lead to inconclusive or inaccurate test results. Sputum should never be collected inside the laboratory; instead, it should be collected in an open well-ventilated area away from people.

A) Specimen collection:

- Specimens should be collected in sterile, screw-capped, plastic containers properly sealed to prevent spillage or leakage.
- To avoid spills, always hold specimen containers by the outside of the container rather than the cap.
- Specimen containers should be correctly capped and labelled. It should not be kept on test requisition form.
- Always handle the specimens with gloved hands. If the outside of the container is visibly soiled with specimen, it should be cleaned with disinfectant (5% phenol).

B) Specimen transport:

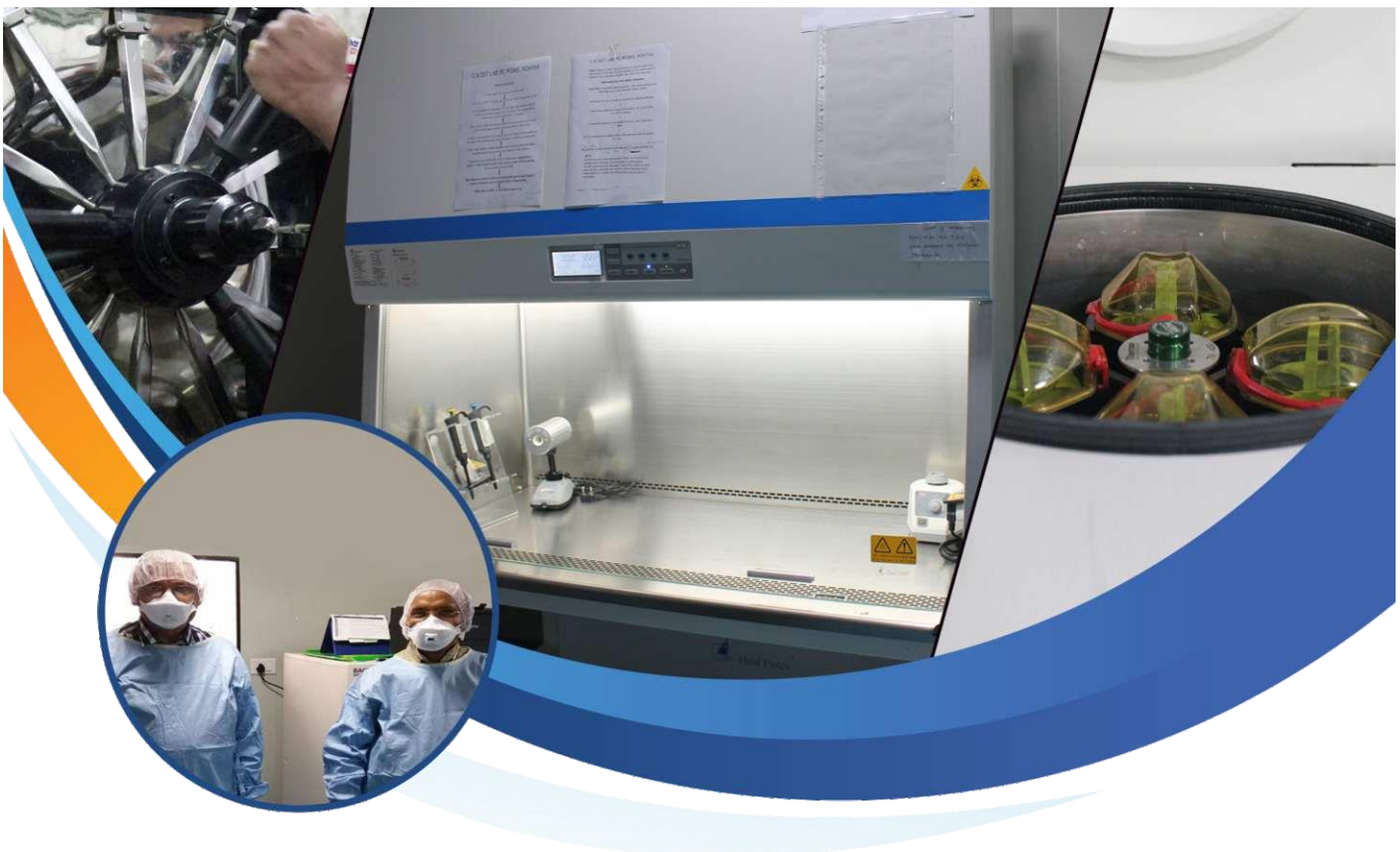
- Transport and storage of samples should be under conditions that maintain the integrity of the sample (see Chapter 7).

- If specimens are transported within the facility, secondary leak proof containers should be used so that accidental spillage and leakage can be avoided.
- Secondary containers should be un-breakable, autoclavable or resistant to chemical disinfectants, and have a tightly sealed lid. They should be decontaminated on a regular basis.
- If specimens are transported between the facilities, follow standard triple layer packaging and NTEP guideline (see Chapter 7).

C) Specimen receipt:

- There should be a designated area for laboratories that receive a large number of specimens.
- In case of anticipated delay, specimen should be stored in appropriate condition (2-8^{0}C) till further processing. This will reduce the growth of unwanted microbial population present in the specimen.
- Leaking specimen containers, specimen-smeared requisition forms, and incorrectly labelled containers should be avoided.
- Personnel who handle the specimens should be trained to take standard precautions and be aware of the potential health hazards involved.

3.2 Safety equipment



In TB laboratories, different safety equipment are used to eliminate or minimise the exposure risk to biohazards. However, to ensure the safety of users and the environment, the user's competency, adherence to standard operating procedures, and frequent equipment maintenance are essential. There are various types of safety equipment, and the need for specific equipment is determined by the procedure/activities to be performed and the level of risk involved. The major safety equipment used in TB laboratories includes biological safety cabinets (BSC), autoclaves/sterilizers, centrifuges with safety buckets, relevant personal protective equipment (PPE) etc.

3.2.1 Personal protective equipment

PPE is 'specialized clothing' worn by laboratory personnel to protect against hazardous or infectious materials. PPE acts as a physical barrier, reducing the exposure risk to aerosols, splashes and accidental inoculation. However, PPE does not protect the users from the risk associated with unsafe work practices. The choice of PPE depends upon the specific procedure conducted in the laboratory.

PPE, that is inappropriate, poorly fitted or worn incorrectly, reduces its effectiveness and may give the user a false sense of safety. A suitable PPE must be worn at all times in the laboratory and never outside of the laboratory. Users must remove their PPE and wash their hands before leaving the laboratory. PPE should never be brought home for washing or disposal. Donning and doffing of PPE should be done carefully to avoid contamination of personal clothes/ belongings (**Annexure 2**).

3.2.1.1 Laboratory coats and gowns

Laboratory coats and gowns protect personal clothing from contamination. Laboratory coat fasten/buttoned in the front, provide comparatively less protection than gown, and therefore it should be worn in laboratory settings where there is a low or moderate risk. On the other hand, laboratory gowns fasten at the back, provide protection across the front-side and should be worn in a high-risk TB laboratory (especially when conducting culture and drug susceptibility testing). On the other hand,

A) Laboratory coat:

Both disposable and non-disposable lab coats, including those with or without elasticised wrist-bands, are commercially available. In TB Laboratories, long sleeves and fully buttoned (preferably elasticised) lab coats should be used but only for low to moderate risk procedures (Figure 3.2).

Note: It should never be worn while manipulating the culture or conducting drug susceptibility testing (DST).

Figure 3.2: Laboratory coat

B) Laboratory gowns:

Laboratory gowns used in TB laboratories should have following characteristics (Figure 3.3):

- Ties at the neck and waist
- Full cover to the front
- Long elasticised cuffs
- Made of non-absorbent material (impermeable to liquid).
- Long enough to cover the lap completely with the gown (when staff is seated) or extends below the height of the workbench (when staff is standing).

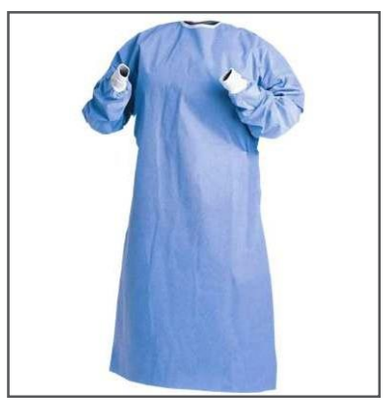


Figure 3.3: Laboratory gown

In TB laboratories, disposable gowns are preferred. However, in the case of re-usable gowns the following points must be considered

- Before being removed for cleaning, the re-usable gowns need to be autoclaved (at 121°C for 15 minutes)
- Each staff member must have at least three gowns (in-use, being cleaned and ready for use)
- Should be changed at least once every week or earlier if a spill occurs
- Should be available in different sizes (small, medium, and large)

Ideally, laboratory should have a separate changing area; and clean gowns and used gowns should be kept separately. Laboratory clothing and personal clothing must not be stored in the same cupboards. Extra gowns should always be available in case of accidental contamination/ spill.

3.2.1.1 Respirators

Respirators, if used correctly may protect users from aerosol inhalation. Although, respirators are not usually required for work in a low-risk TB laboratory, they must be worn when manipulating the cultures within a TB-containment laboratory. When performing high-risk procedures, wearing a respirator will never be a substitute for a well-maintained biological safety cabinet (BSC).

A) Features:

More than 95% of infectious particles larger than 0.2 mm are filtered by respirators. The N95 and FFP2 respirators are lightweight, disposable and cover the nose and mouth.

If respirators are used in a laboratory, all personnel should be informed and trained on how to use and fit them properly, as well as their limitations. Ideally, the user must undergo a "respirator fit test" to ensure the right selection of respirator and there is no leakage. Fit testing requires specialized equipment, skilled personnel and a range of respirators (**Annexure 3**). Alternatively, after wearing the respirator, the user can perform a simple fit test by blowing air. If the user can feel the air escape, then it does not fit him/her correctly. The respirator should be snugly fitted to the user.

B) Wearing a respirator:

Always check a respirator before using it to make sure there are no holes, the strap connection is intact, the surface of respirator is clean, and the straps have not been overstretched. If respirator is in good condition following steps should be followed (Figure 3.4).

1. Hold the respirator in the palm of your hand and straps underneath.
2. Place the respirator under the chin and then over the face gently.
3. Pull the upper headband over the head and place it high at the back of the head.
4. Pull the lower headband over the head and place it under the ears.
5. Adjust and mold the metal strap to nose with the help of the fingertips of both hands. Using only one hand may result in incorrect fit.
6. Breath in and out to confirm pressure and detect any leaks.

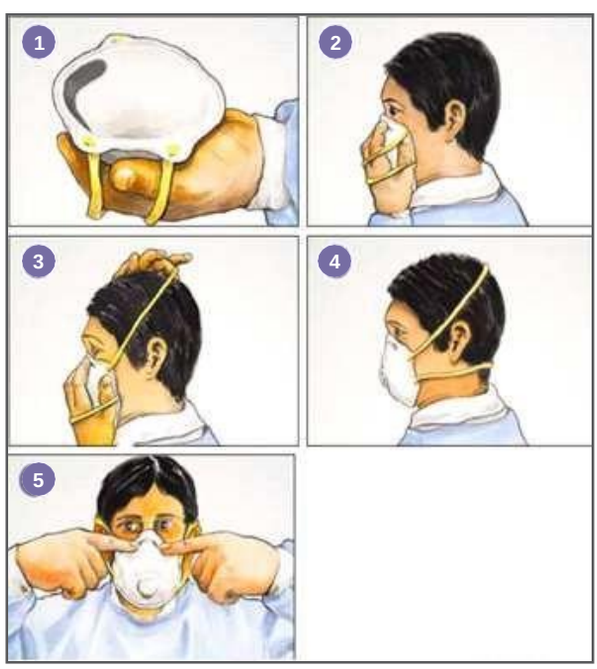


Figure 3.4: Donning of respirator
Source: From GLI (2019)[34]

Cautions: Respirators should not be used by people with thick beards. After putting on a respirator, the wearer should never touch the front of it. Avoid placing respirator under the chin or on the head to talk or attend the phone.

C) Removing a respirator

Before removing a respirator, remove the gloves first and then thoroughly wash the hands. Follow the steps given below (Figure 3.5)

1. Locate and pull over the lower headband strap; release the strap tension and hold it in one hand.
2. Remove the respirator from the face and hold both straps in one hand.
3. Store or dispose of the respirator and wash the hands.

Figure 3.5: Doffing of respirator
Source: From GLI (2019)[34]

D) Storing a respirator

A respirator can be reused if stored correctly. Recently, a study demonstrated that protective efficacy of N95 respirator is not affected until three days of use (8 hrs./day).[37]

After usage, if the respirator is still in good condition, store it in a well-ventilated place in a paper bag with air holes to let moisture evaporate. Respirator shouldn't be hung by the strap, placed in a plastic bag, or kept in a pocket.

Note:

- It is recommended not to store/ reuse the respirators, if they are used for liquid culture and DST.
- Surgical masks are not recommended for laboratory personnel.

3.2.1.2 Gloves

Gloves must be worn at all times when working with specimens, cultures, or other potentially infectious items. Gloves reduce the chances of hands contamination but cannot protect against injuries (piercing) from needles and other sharp instruments. There should be small, medium, and large-sized gloves available, and the user should wear gloves that fit properly and cover their wrists. Gloves that are too small tear easily and restrict finger movement, whereas gloves that are too large get easily contaminated and make fine work difficult.

There are various types gloves available, including those that give protection against biological agents, thermal protection, and protection from sharps or chemicals. Gloves made of vinyl, nitrile, and latex materials are frequently used to protect against biological agents. Latex gloves may cause allergic reactions (such as skin rash or hypersensitive reaction). If it happens to someone, alternative glove materials should be used. Staff should consider following do's and don'ts of wearing gloves (Table 3.1).

Table 3.1: Do's and don'ts of wearing gloves

DO'S	DON' TS
• Wear disposable gloves only and before use check that they are intact • Gloves should always cover the gown cuff • Remove torn or visibly contaminated gloves immediately and discard them into biohazard bin. ▪ After use, remove the gloves aseptically and wash hands immediately	• Do not bring gloves outside of the lab. ▪ Do not re-wear used gloves. ▪ Never handle the door knobs/ handles, telephones, pens and/ or paper work with gloved hands (because contaminated gloves may spread the infection to others)

Aseptically removing gloves (after use) is important and laboratory staff should be trained by following steps (Figure 3.6)

1. Remove one glove by grasping it under the cuff and rolling the glove off the hand. In this way, the majority of the contamination surface of the glove will be on the inside.
2. Hold the removed glove with your opposite gloved hand.
3. Carefully tuck exposed fingers inside the cuff of the gloved hand to avoid touching the infected glove's surface.
4. Peel the glove off from the inside out and roll it over the other used glove to form a bag of used gloves with contamination inside.
5 Dispose of gloves into a biohazard waste bin and wash the hands immediately.

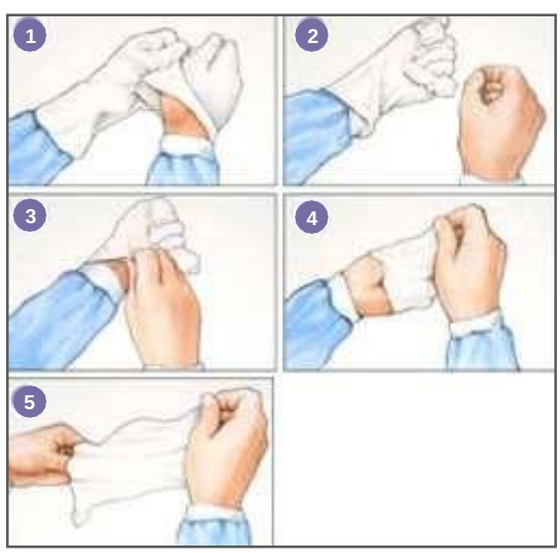


Figure 3.6: Removing gloves safely
Source: From GLI (2019)[34]

3.2.1.4 Eyes and face protector

To protect the eyes and face from splashes, safety glasses/goggles, face shields (visors) or other protective gear must be worn (Figure 3.7). The choice of eyes and face protector is based on type of laboratory activities to be performed (Table 3.2).

Figure 3.7: Safety goggles and face protector

Table 3.2: Type of eye and face protector

Type of Protector	Laboratory Activities	Remarks
Safety glasses/ goggles (It is made of shatterproof plastic with curved sides.)	• Dilution of strong acids (such as preparation of decolourisation reagents-smear staining). • Preparation of strong alkaline solutions (such as 4% NaOH for decontamination of TB specimens) • Preparation of disinfectants (such as 5% phenol or 1% hypochlorite solution) • Response to spill incidence (spill kit)	• Always add strong acids to water gently. Acid and water react vigorously exothermically, releasing heat and occasionally boiling the liquid. • NaOH pellets should be slowly added and mixed to avoid excess heat generation.
Face shield (visor) (It is composed of shatterproof plastic and covers the front and sides of the face, with a head strap to keep it in place)	• Unloading of an autoclave	• Post-autoclaving, large liquid volumes may boil over if moved before cooling.

Note:

- After each use, eye/face protector must be cleaned. If it has been splashed, it must be disinfected using an appropriate disinfectant.
- Do not use prescription glasses and contact lenses as eye protection.
- Face shield to protect the eyes from UV-light exposure is also available that must be used while using the UV illuminator equipment.

3.2.1.5 Footwear

Laboratory personnel must always wear foot protection in laboratory especially in the areas where chemical, biological and physical hazards are present. Laboratory shoes must cover the toes, the upper part of the feet, and have a fastening at the back of the heel (to prevent easy removal of footwear). Sandals, flip-flops, and perforated shoes are not appropriate since they provide no protection against hazardous materials or broken glass. Leather shoes can absorb chemicals and become spoiled or may have to discarded if they are contaminated with a hazardous material.

Figure 3.8: Shoe cover

Note:

- Street footwear must not be used in moderate and high-risk TB laboratories because they are a potential vector for infection transmission or contamination. To minimize cross contamination, separate shoes and/or disposable elasticized shoe covers may be required for different sections of the TB laboratory (Figure 3.8).

- To prevent potential exposure to caustic chemicals, huge amounts of solvents, or water that could penetrate regular footwear, chemical-resistant overshoes or boots may be used.

- Chemical-resistant overshoes or boots may be worn to prevent exposure to potentially harmful chemicals, large amounts of solvents, or water that could seep through ordinary footwear (e.g., during spill cleanup).

3.2.2 Biological safety cabinet

Biological safety cabinet (BSC) is one of most effective and widely used primary containment devices in high aerosol risk laboratories. Aerosol-generating procedures such as shaking, stirring, pouring, pipetting, opening of pressurized containers, etc should be carried-out safely inside the BSC.

BSC is an enclosed, ventilated safe working space to handle or manipulate infectious materials. It offers protection to the operator, the laboratory environment and/or the work materials (product). BSCs are highly effective in reducing the cross-contaminations of cultures or specimens and laboratory-acquired infections. However, failing to adhere to safe working practices in BSC may expose the user to potential infection.

3.2.2.1 Safety features

Containment through BSC is achieved by segregation of the work from the main area of the laboratory, use of engineered high efficiency particulate air (HEPA) filters, air curtains and controlled unidirectional airflow mechanisms. HEPA filter, an essential component of BSC consists of a metal or wood frame holding a long, folded strip of cellulose or borosilicate fibre. It removes 99.97% of all particles at a 0.3 um size from air passing through them. Thus, HEPA filters in BSC can effectively trap all known infectious agents, ensuring that only microbe-free exhaust air is released. BSC provides three types of protections

- **Personnel protection:** With an inward air-flow at the front opening, the user is protected from infectious aerosol (that could have come from the BSC work area).

- **Product/sample protection:** BSCs protect the samples/product from unsterile laboratory air by allowing only HEPA-filtered downward air flow onto the work area inside the BSC. It also helps to prevent cross contamination within the BSC work area by utilizing unidirectional downflow or re-circulation of filtered air onto the BSC work area. A particular class of BSC (Class I), however doesn't have product protection feature.

- **Environmental protection:** A HEPA-fitted exhaust system at the top of the BSC ensures that only microbe-free exhaust air is released into the lab environment.

3.2.2.2 Classification of BSC

Based on design, use and containment capabilities BSCs are classified into Class I, Class II, or Class III. Different regions of the world have their own standards for BSCs. NSF/ ANSI 49 – 2008 and EN 12469:2000 are the globally accepted standards.

Class I BSCs: These are open-fronted cabinets that draw unfiltered room air in through the front opening (at a standard velocity; minimum 0.38m/sec), pass it over the work surface, and then pass it through a HEPA filter before discharging out-side (Figure 3.10). They protect personnel and the environment, but do not protect objects put in the work area (since unfiltered room air enters the BSC and pass over the work surface/area). The front opening of cabinet enables the user's arm to reach the work surface while user observes the surface through glass window (sash). This window can also be fully raised for cleaning or other purposes. Class I BSC is not recommended for conducting TB culture and DST as an increased contamination rate may be observed.

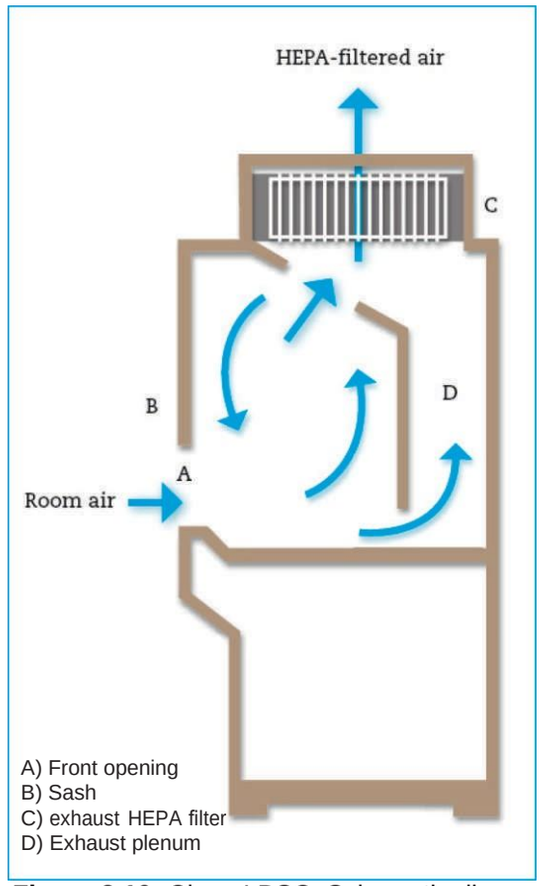


Figure 3.10: Class-I BSC: Schematic diagram of a Class I biological safety cabinet.

Source: From WHO (2012) [35]

Class II BSCs: These are open-fronted cabinets that differ from Class I BSCs by allowing only HEPA-filtered (sterile) air supply to flow over the work surface; thus, providing product protection. NSF standards specify four types of Class II cabinets (A1, A2, B1, and B2) based on airflow patterns and velocities, HEPA air filter position, ventilation rates, and exhaust system. Similarity and differences among 4 types of Class II BSCs (as per NSF/ANSI 49 2011 standard) are described in Table 3.3.

As per WHO (2012),[35] most commonly used and suitable BSC for all TB work is the 'Class II Type A2' with movable sash.

A schematic diagram of Class II Type A2 BSC is shown in Figure 3.11. This class of BSC, pulls unsterile room air (supply air) into the cabinet through the front opening and then into the front intake grill (under the work surface) and goes up the backwall plenum with the help of motor blower. This supply air is then pushed into the central plenum, where about 30% of air is exhausted out through a HEPA filter at the top of cabinet. The remaining about 70% air is re-circulated and it flows downward to the work zone through a HEPA filter.

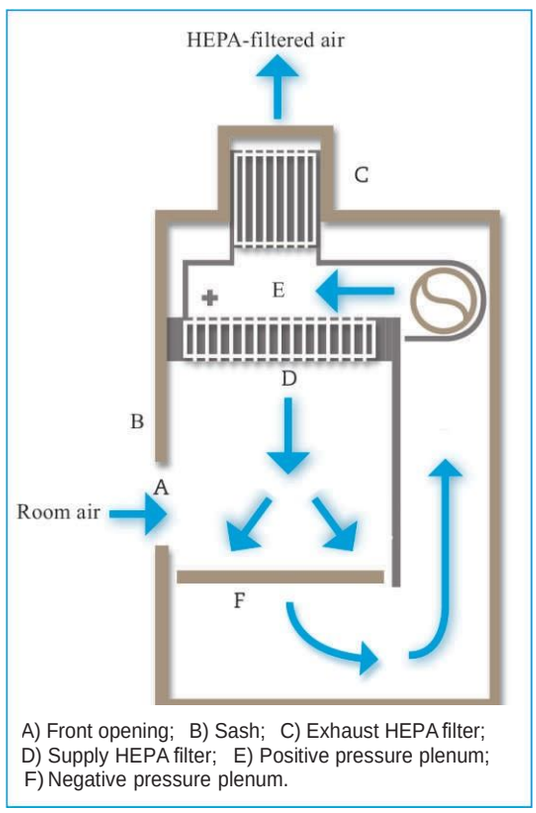


Figure 3.11: Schematic diagram of a Class II type A2 BSC.

Source: From WHO (2012) [35]

The sterile air splits as it flows downward at a height of 6-18 cm above the work surface, with roughly half of the air volume passing through the front exhaust grill and the other half via the rear exhaust grill. Any aerosols generated while working are captured in the downward airflow and expelled from the working zone through front or rear exhaust grills, ensuring the highest level of product protection..

Table 3.3: Features of different Class II BSCs

Features			Type A1	Type A2	Type B1	Type B2
Contain-ment and Protection	Protection from aerosols	Personal	Yes			
		Product	Yes			
		Environment	Yes			
	Protection from Chemical vapors & Gases	Personal	Not suitable, if chemicals are used	Yes, if exhausted outside the building	Yes	
		Product	Not suitable, if chemicals are used	No	Minimized exposure	Yes
		Environment	Not suitable, if chemicals are used	Yes, if exhausted outside the building	Minimized exposure	Yes, if exhausted outside the building
Air-flow Charact-eristics	Cabinet face velocity		minimum 75 FPM	minimum 100 FPM		
	Normal percentage	Recirculated	~70%		~30%	0%
		Exhausted	~30%		~70%	100%
Plenum	Plenum Pressure (Biologically contaminated)		Negative to room or surrounded by negative pressure			
	Cabinet exhaust source		Common plenum		Exhaust plenum	
Exhaust Charact-eristics	Exhaust destination	To room	Yes		No	
		Vented outside	Optional		Yes	
		Connection type	Thimble/ canopy		Hard ducted	

The HEPA filtered air (~30%) may be exhausted into the laboratory or outside the building (through a thimble connected to a dedicated duct; it must NOT be discharged through the building's exhaust system). A separate, dedicated ventilation system, however, is required in a containment laboratory where air from the Class II BSC is recirculated to the room in order to ensure unidirectional air flow with 6–12 air changes per hour (ACH). In a TB containment lab, a thimble connection is recommended for exhausting the air from the cabinet to the outside of the building.

Box 3.1: Thimble ducting- Exhaust system of BSC Class II Type A2

The thimble is designed to fit over the cabinet's exhaust casing. The extractor fan installed at the end of thimble duct draws the cabinet's air and discharge outside. A gap of about 5 cm is kept between the thimble and cabinet's exhaust casing. This gap enables the room air to enter thimble exhaust system and reducing the air pressure in the room. This improves the pressure cascade in the laboratory room; however, it must be ensured that the capacity of thimble exhaust (extractor fan) is adequate to draw both room air and cabinet exhaust.

Thimble connected BSCs have advantages in that the performance of the BSC and the pressure in the laboratory/containment room are not greatly affected by the changes in the building's airflow and switching on or off the BSC, respectively. Another advantage of employing a thimble connection is that at the time of power outage, the air flowing back into the low-pressure room only pass through the thimble air intake, rather than washing microorganisms off the HEPA filter. Thus, a valve is installed in the duct to prevent the air backflow.

The thimble ducts should be removable or carefully designed to facilitate proper certification, service, repair and maintenance of the BSC.

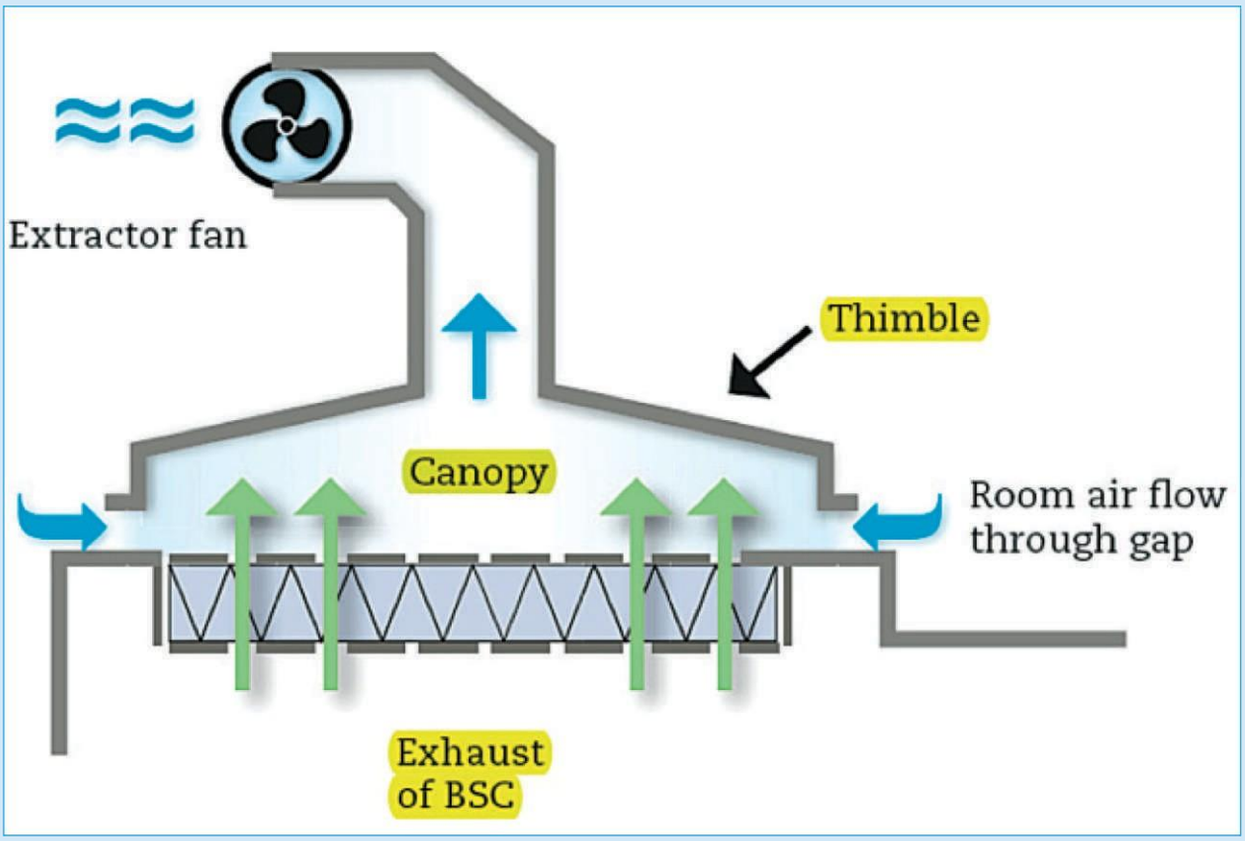


Figure 3.12: Schematic diagram of thimble ducting
Source: From WHO (2012) [35]

BSC Class III: It is a closed-fronted design (also known as glove boxes) that is generally used in high containment laboratories. This class of BSC is not recommended for TB laboratories.

In Class III BSC, there is total separation between the biohazardous material being handled and the operator or laboratory environment. Work-surface is accessed with the help of strong rubber gloves attached to cabinet ports. These cabinets are airtight, have HEPA filtered supply and exhaust air and high air change rate within the cabinet. A dedicated exhaust system outside the cabinet maintains airflow and creates negative pressure inside the cabinet (compared to surrounding space).

3.2.2.3 Installation of a BSC

A) Placement of BSC in the laboratory: The inward airflow curtain of BSC protects the users but it is fragile and affected by factors such as cabinet positioning, room ventilation and pressure differences. While installing the BSC following precautions should be taken to ensure its effectiveness.

- The cabinet should be located away from traffic areas as people walking disrupt air flow. Keep a distance of 1.5 meters between a BSC and laboratory traffic areas. When using the BSC in a small laboratory, restrict people's movement within the laboratory.
- Opening and closing the doors and windows can cause disruptive inward airflow of BSC, they should be kept closed when the BSC is in use. A biosafety cabinet should not be installed directly opposite of door. Windows must be locked.
- External air currents reduce the effectiveness of BSC. Therefore, cabinets should be placed where air supply inlets or other equipment (that creates air movement) will not interfere with performance. BSCs should not be installed in a face-to-face position.
- To allow easy access for maintenance, a clearance of 30 cm should be provided behind and on either side of the cabinet.
- A space of 30-35 cm above the cabinet may be needed to change exhaust filter or to accurately measures the exhaust air velocity.

B) Ergonomics: Good ergonomics is important as it enables the operator to focus on the work and to work safely for several hours a day. Users should take the following considerations (Figure 3.13) into account in order to achieve good ergonomics

1) There should be enough space under the bench for a person to sit comfortably and move their legs.
2) Foot rest should be used if user is unable to put their feet flat on the floor.
3) Chair height should be adjusted so that operator's forearms rest horizontally on the front of the BSC.

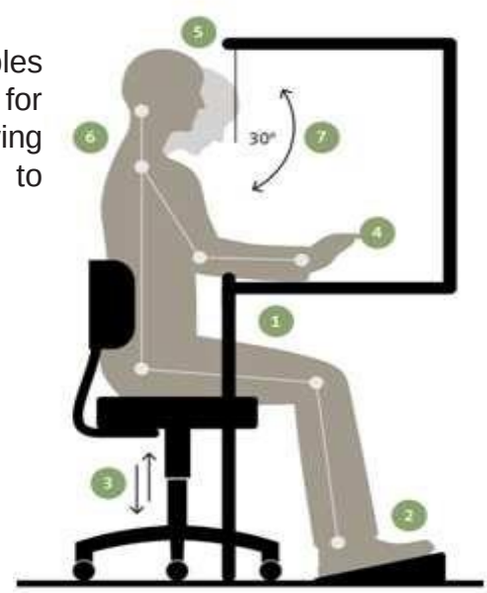


Figure 3.13: Ergonomics when working at the BSC
Source: From GLI (2019) [34]

4) Electrical outlets and other services should be easily accessible so that users don't have to twist or bend to reach them.

5) BSC light should be shielded to protect the user from light and heat.

6) Operators should sit with their back straight and their neck, shoulders, and arms relaxed.

7) Twisting of head and body should be minimized and it should be less than 30° for routine activities.

C) Others:

- Ideally, BSC should be connected to a dedicated UPS that provides enough power backup to keep the BSC operational for at least 20 minutes.

- While the manufacturer certifies the BSC's structural integrity prior to shipment, stress during shipment can compromise the BSC's integrity and efficiency. Thus, BSCs should be tested and certified prior to initial use.

- The procedures and requirements for testing BSCs differ depending on the cabinet's . design and intended application. The key tests include, HEPA filter leak test, air tightness test, airflow measurement in context to velocity, directional flow and balance of intake and exhaust air.

3.2.2.4 Working in a BSC

When BSCs are utilized improperly, their protective effects may be significantly reduced, and in certain cases, the risk to the laboratory worker may even increase.

- **Personal protective equipment:** Before using the BSC, the proper personal protection equipment (PPE) must be worn. Only one person should operate the BSC at a time. When two or more people operate the BSC at the same time, the protective front air curtain may be damaged, and infectious aerosols may be released outside the cabinet.

- **User's competency and BSC readiness:** User must be competent and receive training on how to handle the BSC, procedure to be performed as well as all bio-safety precautions prior to BSC use. Confirm that the BSC annual certification is not pending and there is no visual/audible alarms flagged by BSC

- **Preparing the BSC :**

  - Before starting the work, cabinets should be turned on at least for 5 minutes. This allows the contaminated air to be evacuated from the cabinet.

  - Following this, clean the BSC using appropriate disinfectant (5% phenol followed by 70% alcohol or sterile waster). Before starting work in the BSC, ensure that the drain valve under the work surface is closed.

  - Plan in advance to have all required equipment inside the BSC and avoid overloading. If the BSC is small or it is not possible to keep all materials inside the BSC, place clean items on a trolley so they are easily accessible without interfering with work.

- Nothing should be placed over the intake or rear exhaust grille.

- **BSC operation:**

  - Operator should maintain the integrity of airflow and minimize the movement of arms/ hands into and out of BSC. When required, operator should move their arms slowly and keep them perpendicular to the front opening (never in a sweeping motion).

  - Operator should wait for two minutes after placing their hands inside the BSC before starting to manipulate biohazardous materials; this will allow the airflow to be adjusted and swept over the hand surface

  - Front grill of BSC must not be obstructed while working, and the operation must always be carried out at least 4 inches inside the front grill.

  - To reduce the possibility of cross-contamination, clean material should be kept a distance (at least one foot) away from the work surface area in the BSC where aerosol-generating activities are being done.

  - Workflow inside the BSC must be "clean to dirty." While working, restrict the movement of dirty items over clean items. Paperwork inside the BSC must be avoided.

  - Power generator backup coupled with UPS (minimum 20 min power back-up) is critical for ensuring uninterrupted BSC operation. When only UPS backup is available (in case non-functional generator), operations in the BSC must be halted immediately in the event of a power outage and BSC must be allowed to purge for 15 minutes.

  - When a spill occurs inside a BSC, it should be handled appropriately (see Chapter 9) without turning the BSC off. It is important to properly disinfect/ dispose of any materials that come into contact with spilled biohazardous material.

  - Do not use an open flame inside a BSC; if required, an electric incinerator or disposable loop should be used. An open flame (e.g. Bunsen burner) can disrupt the unidirectional airflow, damage the filter or catch fire.

  - Prefer the BSCs equipped with visual and audible alarms for certain risks like sash alarm (which indicate improper position) and airflow alarm (which indicate disruption of the cabinet's normal airflow). If an alarm sounds while working, it must be responded immediately. In case of sash alarm, the sash must be returned to its proper position; in case of airflow alarm, work must stop immediately and the laboratory manager must be notified for corrective action as per manufacturer's instruction manual. Never, press the mute button of the alarm and continue the work.

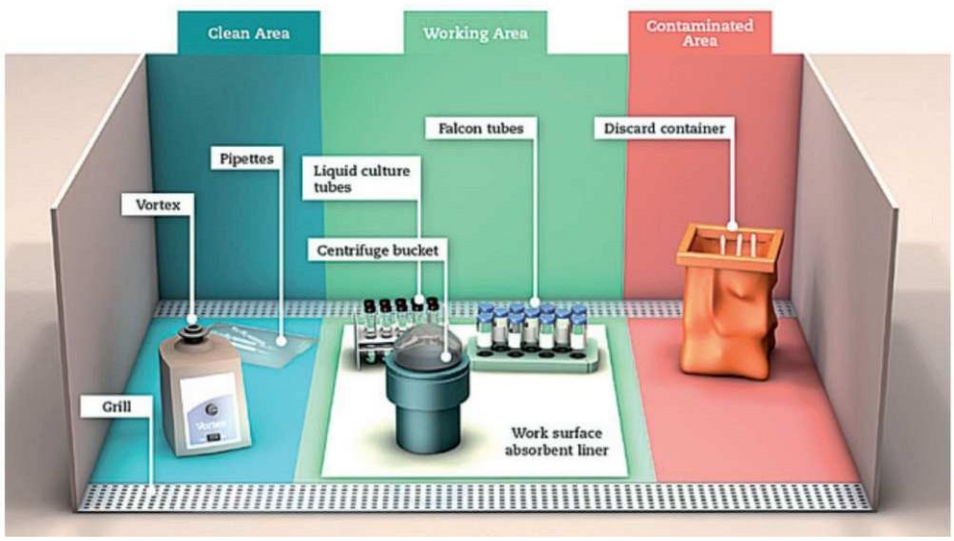
3.2.2.5 Material placement inside the BSC

- TB testing procedures (like specimen processing for culture or conducting drug susceptibility testing) are divided into different steps. Because each step of the workflow requires specific items, we should place only those items that are required.

- Work should be performed on disinfectant-soaked absorbent towels (to catch splatters/ splashes).

- Items that are required for the operation (including aerosol generating device such as vortex) should be placed as far back in the BSC as possible without obstructing the rear grill. Discard container should be placed to one side – inside the BSC.

- In terms of materials arrangement, BSC can be functionally divided into three areas: clean (for small equipment and consumables), working (for manipulation activity), and contaminated (for discard bin) (Figure 3.14).



Clean materials are placed to the left of the cabinet; samples are inoculated in the centre of the cabinet and contaminated pipettes and other materials are placed into waste containers on the right side of the cabinet. This arrangement can be reversed for left-handed people.

Figure 3.14: Typical layout for working from clean areas to dirty areas within a Class II BSC.
Source: From WHO (2012) [35]

3.2.2.6 Cleaning the BSC

- When work is completed, leave the BSC running for 15 min (to clear the aerosol).

- Before removing the potentially contaminated materials out of the cabinet, they must be surface decontaminated. Liquid or solid biohazard waste (treated with appropriate disinfectant) should be closed before bringing them out and transferred for autoclaving.

- The work surface and internal walls of the BSC should be wiped down uni-directionally (using 5% phenol followed by 70% alcohol) before and after each use.

- The final surface decontamination at the end of the workday should include wiping down the work surface as well as the sides, back, and interior of the glass. Use a long-handled tongue or forceps to reach the difficult areas.

3.2.2.7 Decontamination, maintenance and certification

- **Decontamination:** Sometimes, BSC require special decontamination (gaseous/vapors based) prior to performing maintenance work, repair, testing, moving, changing filters, changing work programs, and after gross spills. The most common decontamination method uses formaldehyde gas. However, it is carcinogenic and the BSC downtime for this procedure is higher (18-24 hrs).

Therefore, alternate options (like Chlorine di-oxide and hydrogen peroxide) may be used as per NSF/ANSI 49 recommendations. Decontamination procedures are described briefly in **Annexure 4** and it should be performed by a qualified professional.

- **Maintenance and certification:** Regular monitoring and maintenance of BSC is critical for proper functioning of BSC and consequently operator safety (Table 3.4). While majority of maintenance activities can be performed by laboratory technician, annual certification and if necessary, changing the HEPA filters (and subsequent re-certification) would required to be done only by authorized engineers based on the corresponding standards (EN 12469, NSF 49 or others).

Table 3.4: Maintenance plan for BSC

S.No.	Maintenance activity	Frequency	Responsibility
1	Disinfecting the interior surface of BSC with 5% phenol followed by70% alcohol(or sterile water)	Daily (before and after usage)	Lab staff
2	Checking the airflow parameters on the display (vaneometer may be used for checking the inward air velocity)	Daily (before usage)	Lab staff
3	After using the BSC, turn on the built in UV lamp (if present).	Daily (after usage for 30 min)	Lab staff
4	Air sampling on agar plates (product protection testing)-(Annexure-5)	Every 6 months	Lab staff
5	Smoke test for visual examination of airflow (inflow and downflow)	Monthly	Lab staff
6	Surface disinfection and cleaning of external parts	Monthly	Lab staff
7	Disinfection and cleaning of internal surface area (below the working surface)	Quarterly	Lab staff
8	Installation testing	At the time of installation and relocation of BSC	Qualified engineer/ technician
9	Calibration and certification of BSC as per standard (EN 12469, NSF/ANSI 49or other); Calibration should include at least following parameters • Thorough visual examination to ensure that there are no surface defects or other damage. • Smoke test for down flow and inflow visualization (as per the corresponding Standard). • Checking the downflow and inflow air velocity in accordance with the manufacturer's requirements and its adjustment if necessary. • Performing an aerosol leakage test (for HEPA filters) using a particle counter or photometer. Each filter should be tested independently. • Checking the alarm indicators according to the manufacturer's specifications. The alarm device should be calibrated, if necessary. • Careful visual examination of extraction duct system to ensure that it is free from defects, cracks and other damage.	Annually	Qualified engineer/ technician
10	Replacement of HEPA filters (with recalibration and recertification of BSC)	On recommenda-tion of service engineer	Qualified engineer/ technician

Source: Adapted from WHO (2017) [38]

3.2.3 Autoclave

Autoclaves use 'saturated steam under pressure (moist heat)' to destroy or inactivating biological agents including TB organism. It is most effective and reliable equipment available to sterilize the laboratory materials (like glassware, media and reagents) prior to use or decontaminate the biohazardous waste before disposal. The requirement of an autoclave in different risk-level TB laboratories is summarized in Box 3.2.

Box 3.2: Use of autoclave in different risk-level TB laboratories

Low-risk TB Laboratories: The availability of an autoclave in a low-risk TB testing laboratory is not mandatory. However, all waste should be properly disinfected using chemical methods before its final disposal (burial pits or hand over to a biomedical waste management agency).

Moderate-risk TB laboratories: Autoclave is an essential equipment for moderate risk laboratories and all infectious waste must be autoclaved before being removed from the facility.

High-risk TB laboratories: An autoclave must be available in every facility where TB cultures are performed or manipulated, and it should ideally be located within (or adjacent) the TB containment laboratory.

All laboratories require a secure space to temporarily store laboratory waste before it is subjected to autoclaving. High-workload laboratories should have enough space for the storage of heavy loads before and after autoclaving, as well as for a trolley (for waste transportation).

3.2.3.1 Key considerations while using an autoclave

- Ideally, a laboratory should have separate autoclaves for 'clean' loads (such as media preparation and glassware sterilization) and 'dirty' loads (infectious/ contaminated waste). If one autoclave is non-functional, plan autoclaving on separate days for dirty and clean loads.
- Laboratory technician authorized for operation and maintenance of autoclave must be trained on how to safely use the autoclave, sterilization program as well as maintaining the loading plan (information on the contents, number, volume/mass of the sterilized product) for each cycle.
- Proper run conditions and packing are critical for effective sterilization. In general, clean items are sterilized for at least 15 minutes at 121°C and 15 psi. Infectious biomedical waste on the other hand is autoclaved for at least 60 minutes (for gravity flow autoclave) or 45 minutes (for vacuum autoclave) at 121°C and 15 psi. [39]
- A reliable steam source such as distilled or RO water should be used. Chemical contamination or high salt in water may compromise the efficiency or damage the pipe of chamber of autoclave.
- Materials or waste placed in the autoclave must be in containers that allow for easy air evacuation and good heat penetration.
- Autoclave should never be overloaded.
- Hazardous chemicals should never be autoclaved.

- At the end of holding time switch off the power supply and allow the autoclave to cool slowly until the pressure decreases to zero reading.
- When opening the autoclave, operators must wear heat resistant gloves and eye protection even when temperature is dropped to level appropriate for opening the chamber.
- Autoclave pressure valves and drains should be checked regularly to ensure that they are not clogged with paper, plastic, or other materials.

3.2.3.2 Quality control

To ensure that the autoclave is functioning properly, the time, temperature, and pressure should be recorded each time it is used. Chemical and biological indicators should be used regularly to validate the efficacy of the autoclave to achieve sterilization.

- **Chemical indicators:** These are tapes, card or papers that confirm that the temperature conditions were met by simply visualizing the change in colour printed on the indicator tape, but they do not confirm that microbial killing has occurred. Visual indicators should be used in every run and for every item in every sterilized load.

- **Biological (bacterial spore) indicators:** This is most reliable method of assuring autoclave performance. This test uses most thermally resistant microorganisms (spores of *Geobacillus stereothermophilus* or Bacillus species), and it determines whether or not the spores were killed during sterilization. These tests are standardized tests and should be done as per manufacturer's instruction. In general, biological indicators are placed deep within the load to challenge. Once the autoclave run is completed, spores are allowed to grow in a suitable growth medium for an extended period of time (8 to 72 hours). The absence of growth in the media indicates that the sterilization process was successful. Biological indicators should be used at least weekly.[39]

If any of the above quality control tests fail, the load should be considered infectious, and autoclaving (with the same or a different autoclave) and indicator confirmation should be performed again.

3.2.3.3 Maintenance and calibration

Following minimum maintenance activity should be performed by designated laboratory staff or qualified technician/engineer (external agency) within the suggested time period (Table 3.5).

Table 3.5: Maintenance plan for autoclave

S.No.	Maintenance activity	Frequency	Responsibility
1	Sterilization report (if digital autoclave is being used) and quality control test (chemical indicator) for each sterilization cycle	Daily	Lab staff
2	Cleaning the exterior of the autoclave with a damp cloth	Daily	Lab staff
3	Cleaning the interior of autoclave (sterilization chamber and drainage filter) with non -chlorine/noncorrosive disinfectants	Weekly	Lab staff
4	Cleaning the exterior with a mild detergent	Weekly	Lab staff
5	Lubricating the gasket rubber (O -ring)	Weekly	Lab staff
6	Draining the vapour generator	Weekly	Lab staff

S.No.	Maintenance activity	Frequency	Responsibility
7	Verify the sterilization efficiency using biological indicator	Weekly	Lab staff
8	Checking the manometer function	Every 6 months	Qualified engineer / technician
9	Checking the safety valve and lubrication of gasket	Every 6 months	Qualified engineer / technician
10	Calibration of control unit (pressure gauge and temperature by thermal mapping)	Annually	Qualified engineer / technician

Source: Adapted from WHO (2017) [38]

3.2.4 Biosafe centrifuge

Biosafe centrifuge is an essential equipment in moderate and high-risk TB laboratories. It is mainly used for processing (especially concentration) of specimen for TB tests like culture, line probe assays etc. Since, centrifuge generates aerosol, it is mandatory that samples be contained within containment rotors or a safety-buckets with sealed lid.

The recommended centrifuge for use in moderate to high-risk TB laboratories (especially for specimen processing) is one with

- A fixed angle rotor with the minimum speed of 3000 xg or higher (to achieve desired pellet/ sediment).
- Sealed biosafety buckets that can hold 50 ml screw-cap conical centrifuge tubes (to protect users from infectious aerosols and to avoid transferring specimens to another container compatible with centrifuge buckets).
- Cooling feature during centrifugation (to maintain optimal temperature and minimize the killing of bacteria).

For DNA extraction (like line probe assay), bench top micro-centrifuge is used that can hold 1.5 - 2.5ml micro-centrifuge tubes. The rotor should be fixed angle with a sealed lid.

3.2.4.1 Placement of centrifuge

Infectious materials can be centrifuged in the open laboratory; however, it must be ensured that sealed centrifuge safety cups are used and buckets are loaded or unloaded within a BSC. In TB laboratory, the safety centrifuge must be located close to the BSC, away from the water, sinks or chemicals and in a dust-free area. Centrifuge must be placed in an ergonomically correct work position and on a sturdy bench/ platform that withstand the weight and vibrations during use. Never place a bench top centrifuge on floor.

3.2.4.2 Using the centrifuge

- Centrifuge can be dangerous equipment if not operated appropriately. Therefore, operator must have received the training on centrifuge operation, balancing of tubes, associated hazards and response strategy for emergency situations. Always wear the relevant PPE while using the centrifuge.
- Before loading buckets of centrifuge, ensure that lids are not cracked and that O-rings (silicon sealing ring) are present and intact.

- During centrifugation heat is generated that can affect the quality of temperature-sensitive samples. Therefore, rotor and rotor chamber should be appropriately pre-cooled before centrifuging the temperature sensitive specimen. Centrifuge should be connected to UPS.
- In order to ensure that a rotor runs smoothly and safely at its operating speed, the load that a rotor is carrying needs to be symmetrical and balanced (Box 3.3).

Box 3.3: Balancing the rotor before centrifugation

Rotor must have buckets at opposing position (running with any missing bucket, adaptor or lid must be avoided). All opposing load must balance within a certain weight range as specified by manufacturers. Therefore, it is important to add equal volume of materials in tubes placed in opposite side. If opposing buckets are run with a partial load of tubes these tubes must be arranged symmetrical. Alternatively, one or more tubes of the same size with water or a denser liquid may be used to balance the load symmetrically.

- Before operating the centrifuge, the caps for each bucket and tubes must be properly closed. Always use a centrifuge with the lid closed.
- Use only genuine components from the manufacturer of the centrifuge, unless otherwise authorised.
- Stop the centrifuge immediately if any unusual noise is heard.
- To prevent aerosol exposure, centrifuge buckets should be loaded and unloaded only inside a BSC. Never open the lid of a safety bucket immediately so as to allow the aerosol to settle. down.

3.2.4.3 Response to spill inside the safety buckets

- The breakage of the centrifuge tube inside the safety buckets is the primary cause of the centrifuge spill. It can be avoided by using high-quality tubes (which resist the applied centrifugal force) and buckets/tube adaptors with conical bottom designs (which better support the conical bottom centrifuge-tubes and exert the high force during centrifugation).
- If tube breakage occurs, use squeeze bottle to apply disinfectant to all contaminated surfaces of buckets and centrifuge chamber, taking care to minimize splashing. Broken tubes must be discarded in a puncture-proof container. Decontaminate centrifuge buckets by soaking them in a suitable disinfectant (such as phenol). Bleach should not be used to disinfect metal parts as it may corrode them. Buckets can also be decontaminated by autoclaving.

3.2.4.4 Maintenance

Following minimum maintenance activity should be performed by designated laboratory staff or qualified technician/engineer (external agency) within the suggested time period (Table 3.6).

Table 3.6: Maintenance plan for biosafe centrifuge

S.No.	Maintenance activity	Frequency	Responsibility
1	Balancing of buckets before centrifugation	Every run	Lab staff
2	After use, drying of condensed water in the rotor chamber; separation of safety buckets, lid and adaptor and putting it on absorbent sheet to dry	Daily	Lab staff
3	Disinfecting the rotor buckets and centrifuge chamber	Weekly	Lab staff
4	Lubricating rotor trunnions	Weekly	Lab staff
5	Cleaning the exterior of centrifuge with a general -purpose cleaner	Weekly	Lab staff
6	Careful disinfection after spillage	As needed	Lab staff
7	Maintenance (including cleaning of condenser coils, fans, screens and filters; and checking the centrifuge brushes, bearings, timer, temperature and speed, and checking for electrical integrity) and calibr ation (for the parameters of rotation speed, temperature and time)	Annually	Qualified engineer/ technician

Source: Adapted from WHO (2017) [38]

3.2.5 Pipettes

Pipettes are essential and most extensively used lab equipment. They are used to measure out or transfer small amount of liquid. A wide range of pipettes are available, from simple serological and Pasteur pipettes (disposable or re-usable) to more complex design, with a plunger and piston i.e called micropipettes. It may be fixed volume or variable volume (based on ability to dispense only a fixed volume or variable volume by adjusting the pipette within a range), single channel or multi- channel (based on ability to aspirate and dispense the liquid between two or more containers at a time) with mechanical or electronic control.

Key advantages of using pipettes (especially micropipette) include

- **Accuracy and precision:** Pipettes can accurately measure and dispense the liquid. Micro-pipettes are designed to measure the liquid even in micro-litre. Pasture pipettes, however, are used when precision is not critical.
- **Protecting the sample integrity:** Pipettes offer a safe method to aspirate, transfer and dispense liquid samples. Micropipettes are generally used with disposable tips, thus helping to minimize the risk of contamination.
- **Protecting the personnel:** Pipettes eliminate direct contact of laboratory personnel with hazardous liquid samples and protecting them from splashing when hazardous/infectious material is poured between the containers.

3.2.5.1 Using the pipettes

Pipetting is associated with a high risk of aerosol generation; therefore, laboratory personnel must be appropriately trained in the correct pipetting technique to minimize the aerosol generation and improve both safety and quality.

A) Pasture pipette: In general, single use, plastic, graduated, disposable pipettes are recommended that can be used for certain activities like sample preparation in Xpert and Truenat testing; reverse hybridization steps in line probe assay, as well as inoculation of liquid or solid culture media.

- It is NOT recommended to use glass pasteur pipettes because they are prone to breaking, leaving sharp edges, and because they need a separate bulb that could become contaminated and cause cross-contamination.
- Plastic disposable pipettes are available either individually packed or in bags. In case of bag, the pipettes should be taken out aseptically and the bag should be resealed when not in use.
- Pasteur pipettes must be used in an upright position. The bulb goes on top, the tip of the pipette is pointed down. Never hold a filled pipette upside down or horizontally.
- Syringes with needles should not be used as substitute for pipetting devices.

B) Micropipette:

- Always use compatible microtips with the micropipette.
- Majority of pipetting activities in TB lab are performed by micro-pipettes. Users must be trained in correct pipetting technique (**Annexure 7**).
- When dispensing the liquid, touch the pipette's tip to the inner wall of the receiving tube at 30-45 degree angle and 8–10 mm above the surface of liquid (if any) in the receiving tube. Dispense the liquid from pipette gently (never forcibly).
- Disposable pipette tips must not be touched with hand and it must be carefully removed to avoid formation of any aerosols.
- Placing a disinfectant-soaked towel at the bottom of the dispensing container will minimize the risk of aerosol generation caused by possible biological agent droppage from a pipette tip or tip bouncing when ejecting from the pipette. The wet towel must be disposed of as infectious waste after use.
- When mixing with pipette is essential for a procedure, it must be done slowly and gently (not vigorously). Before opening the microtube (containing liquid), briefly spin it so that any liquid in the cap can settle to the bottom of the microtube.

3.2.5.2 Preventing cross-contamination

- To avoid contamination of sample from contaminated pipette or tips
  - Use sterilized filter tips as they prevent aerosols and biological materials from entering into the barrel of a pipette.
  - Never insert the barrel into the container.
  - After pipetting each sample, change the tip to avoid carry-over contamination.
- To avoid contamination of pipette from sample
  - When pipetting, keep the pipette vertical to avoid liquid from entering the pipette body.
  - Slowly release the piston.

3.2.5.3 Cleaning, decontamination and maintenance

A) Cleaning: Check the pipette for cleanliness on a daily basis. If dirty, it must be cleaned with a suitable solvent, mild detergent solution, or any other solution recommended by the manufacturer.

B) Decontamination: Decontamination of pipette is required at the time of detailed service or repair as well as if there is visible contamination of pipette or to resolve the cross-contamination event, if occurred.

- Place re-usable pipettes horizontally in a pan and submerge them in disinfectant solution recommended by the manufacturer.
- Some pipettes are autoclavable and they can be sterilized by standard autoclaving procedure.
- If a pipette has come into contact with hazardous materials, user must ensure that it is properly decontaminated before being used in other procedures.

C) Maintenance: Following minimum maintenance activities should be performed by designated laboratory staff or qualified technician/engineer (external agency) with in the suggested time period (Table 3.7).

Table 3.7: Maintenance plan for pipette

S. No.	Maintenance activity	Frequency	Responsibility
1	Examining the integrity and adjusting the mechanism	Daily or per procedure	Lab staff
2	Leakage control testing (by filling the tip with distilled water)	Daily or per procedure	Lab staff
3	Pipette exterior cleaning and decontamination with a mild detergent	Weekly	Lab staff
4	Sterilizing or complete decontamination of the pipette according to the manufacturer's instructions (when contamination of pipette is noticed; when pipette is handed over to an external agency for calibration)	As needed	Lab staff
5	Disassembling and cleaning all pipette parts. Checking the O rings and replaced, if needed. Lubrication of plunger and piston followed by assembling of parts.	Bi-annually	Qualified engineer/ technician
6	Calibrating pipette using a standardized procedure	Bi-annually	Qualified engineer/ technician

Source: Adapted from WHO (2017) [38]

3.2.6 Inoculation loop and micro-incinerator

Bacteriological or inoculation loop is a simple handheld device that is used primarily to take and transfer a small quantity of culture (inoculum) streaked onto, or stabbed into, solid agar-based media, or simply introduced and dispersed into liquid media. An inoculation loop features a handle with a terminal loop that can be used to pick up and transfer a small amount of inoculum (usually 1-10 microliter; depending on loop diameter). Calibrated loops may be used for transferring the semi-quantitative amounts of inoculums. Inoculation loops are broadly available in two types i.e disposable plastic loops, and reusable wire loops.

- In TB laboratories, inoculation loops are also used especially for preparation of smear from sputum/culture isolate as well as handling of culture isolates (on solid media). Disposable / plastic loops are strongly recommended to be used in TB laboratories.
- Reusable loop requires repeated sterilization using open flame and this can cause spatter and release of aerosols and droplets. Disposable loop on the other hand can be discarded immediately after the use.
- Use of wire loop (reusable) and its sterilization with open flame inside the BSC should be avoided. Open flames produce turbulence and disrupt the directed HEPA-filtered air being provided to the work surface, and they might be dangerous (fire accident) when volatile, flammable substances are also present.
- Although a micro-incinerator is an alternative to an open flame, installing it inside the BSC requires extra space and care.

3.2.6.1 Working with inoculation loop

A) **Disposable plastic loop:** These are generally made of biologically inert polymer (like polystyrene/polypropylene) and have several advantages over reusable wire loop. Although there are no special precautions with inoculation, they must be handled carefully to avoid spillage on the work surface and discarded immediately after use in an effective disinfectant (phenol).

B) **Reusable wire loop:** This is a thin metal device with a handle at one end and a twisted metal (such as nichrome, tungsten or platinum) wire with loop at the other end. Following aspects should be considered while handling the reusable wire loop

- Never touch or place the handle of reusable loop into the container as it may result in contamination of loop.
- Ensure the sterilization of loop after every use.
- A flame should not be used to sterilize reusable wire loops. To sterilize metal transfer loops, an enclosed electric micro-incinerator that can also be placed inside the BSC without interfering with directional airflow, should be used.

3.2.6.2. Micro-incinerator

Micro-incinerators are used to sterilize metal inoculation loops and needles. In general, infrared heating is utilized inside the ceramic device without an open flame that protects the user from dangerous gases, flames, and splatters. Moreover, it is quite safe to use inside the BSC as compared to flame-based sterilization. Metal inoculation loop reach the optimum sterilization within 10 sec due to high temperature inside the micro-incinerator (around 900^0C).

Following precautions should be taken into account while using the micro-incineration.

- Insert the loop or needles into the micro-incinerator (insertion port) gently and without scraping the sides of the heater element.
- To avoid spattering, the loop must be inserted toward the rear of the heating element. The loop must be kept inside the heating element for a minimum of 3-7 seconds (or as per recommendation by manufacturer). Intense heat exposure to loops over an extended period of time should be avoided as it can damage the loops and needles.

- Inoculation loop with insulated loop holders should be used.
- Avoid any contact with the heat shield of micro-incinerator during operation.
- Always follow the manufacturer's instructions while using the equipment

Maintenance

Visual examination of the heater element should be done on a regular basis to detect if the heater element core is worn. This examination should be done in both cool and heated conditions. If any defects are discovered, the heating element should be timely replaced.

3.2.7 Containers for infectious materials

When biological or infectious materials are transported, there is a risk of biohazard release and its exposure to the people and the environment through which the material passes. There are international and national level guidelines and/or regulations that must be followed for correct packaging and transportation within a facility or between facilities.

To ensure safety and containment throughout the transport process, use of correct vessels and container is critical as it prevents the release of microbes (primary containment). The type of vessel/container used is mostly determined by the type of material being transported, the level of risk involved during transit, and environmental factors affecting the material's integrity. The key features of vessels/container used in TB laboratories for transportation and disposal of biomedical waste or receiving TB specimen within or between the facilities are as follows

A) Containers for transporting infectious material for decontamination

- Considering significant risk of aerosol, spill and leaks, the containers must be of leak-proof and shatterproof construction with secure lid or cover.
- Different color-coded disposable biohazard bags are also used in the TB laboratories which is intended to segregate the waste before disposal. However, when transporting these biohazard bags to onsite/off-site sterilization units these must be tide and placed in leak-proof secondary container with a secure lid.
- Disposal containers must be cleaned and decontaminated before reusing them.
- A dedicated cart or vehicle should be used for safe transporting the waste especially when amount of waste or the transportation distance is significant.

Note: Decontamination with a suitable disinfectant or autoclaving must be ensured before removing any biological/ infectious waste from the facility.

B) Containers for sharp disposal

- Dispose of any sharp materials (for example, broken slides, used slides, needles, metal casing on drug vials and blades) in puncture-proof containers with sealed covers.
- While discarding the sharp it must be in contact with appropriate disinfectant at least for minimum recommended time. Before reusing, discard containers must be decontaminated and cleaned.
- Discard containers must be puncture-proof, not filled more than the capacity (three-quarters full) and must be disposed of as per standard guideline.[39]

C) Containers for specimen collection

TB specimens are collected in a screw-cap, leak-proof plastic container. The specification recommended in NTEP is as follow

50 ml polypropylene (PP) tubes: Centrifuge tubes-50 ml, made of polypropylene, conical shape bottom with tight screw cap, walls thick enough to allow centrifugation (maximum RCF) up to 5000xg, solvent resistant, with white labeling area and black graduations, flat PE caps which can close with quick 3-4 turns, allowing one handed operation. Tubes should be radiation sterilized to ensure non- cytotoxicity.

Note: Refer Chapter 7 for sputum packaging and transportation

Figure 3.15: Polypropylene tubes (50ml)

3.3 Facility design and administrative policies

3.3. 1 Facility design

The design and construction of laboratory contributes to the safety of all laboratory personnel while at the same time providing a barrier that protects the community and environment from the infectious aerosols that might be generated there.

Laboratory facilities are usually designated as Biosafety Level 1 (Basic facility), Biosafety Level 2 (Basic facility), Biosafety Level 3 (containment facility), and Biosafety Level 4 (Maximum containment facility). This classification is based on a combination of design elements, construction, containment facilities, equipment, practices and operational procedures needed to work with agents from the any of four risk groups (as per WHO risk group category). The key facility design requirements for the different biosafety levels are provided in Table 3.8.

Microorganisms are also classified into four risk groups (based on virulence, transmissibility, and the availability of treatments), but organism risk groups do not correspond to laboratory biosafety levels. Globally, it is accepted that the minimum biosafety requirement including facility design for TB laboratories is based on 'Procedural risk assessment'. Different TB test procedures have different level of biohazard risk such as smear microscopy has low risk than culture or drug-susceptibility testing. Based on procedural risk, TB laboratories are classified into low-risk, moderate-risk, and high-risk laboratories rather than biosafety levels 1, 2, and 3.

While detailed features of low-risk, moderate-risk and high-risk TB laboratories are described in subsequent chapters (see Chapter 4, 5 and 6), the basic recommended features of a TB laboratory are as follow

Table 3.8: Key biosafety level requirements (facility design and safety equipment)

Key design features and safety equipment	Biosafety level			
	1	2	3	4
Functional isolation of laboratory	No	No	Yes	Yes
Room sealable for decontamination	No	No	Yes	Yes
Ventilation				
• Inward airflow	No	Desirable	Yes	Yes
• Controlled ventilation system	No	Desirable	Yes	Yes
• HEPA filtered air exhaust	No	No	Yes	Yes
Double door entry	No	No	Yes	Yes
Anteroom	No	No	Yes	-
Autoclave				
• On-site	No	Desirable	Yes	Yes
• In laboratory room	No	No	Desirable	Yes
• Double ended	No	No	Desirable	Yes
Biological safety cabinet	No	Desirable*	Yes	Yes
Personnel safety monitoring capability	No	No	Desirable	Yes

* Required when infectious aerosol may be generated (such as TB culture activity).

3.3.1.1 Structural design of basic TB laboratory

- Ample space should be available for laboratory work as well as cleaning and maintenance.
- Designated hand-washing basin should be available preferably near the exit door.
- Laboratory walls, floors, and furniture need to be smooth and easy to clean.
- Laboratory bench tops have to be impervious to liquid and resistant to laboratory chemicals, disinfectants and moderate heat. The edges of bench tops should be rounded to reduce injury.
- Access to the laboratory must be restricted. Laboratories doors should preferably have view panels (to monitor and take action in case of any accident), be appropriate fire-rated, and be self-closing.
- The lighting in the laboratory should be reliable and adequate. Wherever possible, daylight should be utilized effectively. Undesired reflections and glare should be avoided. The availability of emergency lighting is important for safely stopping work and exiting the laboratory.
- TB laboratory should be adequately ventilated (either natural and/or mechanical) with unidirectional airflow. Airflow direction and speed (minimum 6-12 room air exchange per hour) must be considered to ensure safe working conditions. Turbulent airflow should always be avoided.
- Laboratory furniture that does not allow the laboratory personnel to work safely and comfortably are undesirable and they must be removed. It is important to have enough space between and under work benches, cabinets and equipment for cleaning.
- Bench tops and aisles should be clutter-free and there should be adequate storage space to hold supplies for immediate use. Space for long-term storage should also be available in a convenient location.
- In order to decontaminate the biomedical waste, the laboratory should have facilities (such as effective disinfectants and autoclaves) in close proximity to the laboratory. A proper waste management system (including temporary storage and transportation for removal) should be considered in the design.

- Storage facility for food, beverages and personal items/clothing must be provided outside the laboratory. Similarly, a place to eat and drink should be outside the laboratory.
- Suitably equipped first-aid facilities/box should be easily accessible.
- The design must take emergency situations into account, as indicated by the local risk assessment, and should take the geographical and meteorological context into account (see Chapter 9).
- The safety system of laboratory must cover chemical, fire, electrical, radiation emergencies as well as incidence response facilities based on risk assessment (see chapter 10).

3.3.1.2 Dedicated 'Clean' and 'Dirty' areas

The term "clean" refers here to a laboratory area or item that is less likely to contain or be contaminated by infectious agents. While "dirty" refers to a location or item in the laboratory that is more likely to contain infectious agents or be contaminated by them

- In general, the entry area of a laboratory should be reserved for low-risk activities (clean), while high risk activities (dirty) should be performed at the end of laboratory.
- The laboratory should be designed so that air can flow from clean to dirty areas. This rule should also be considered for multi-room laboratories that have separate rooms for specific activities or tests with varying risk levels (Figure 3.16). As you move deeper into the room, the level of risk increases from the entry point

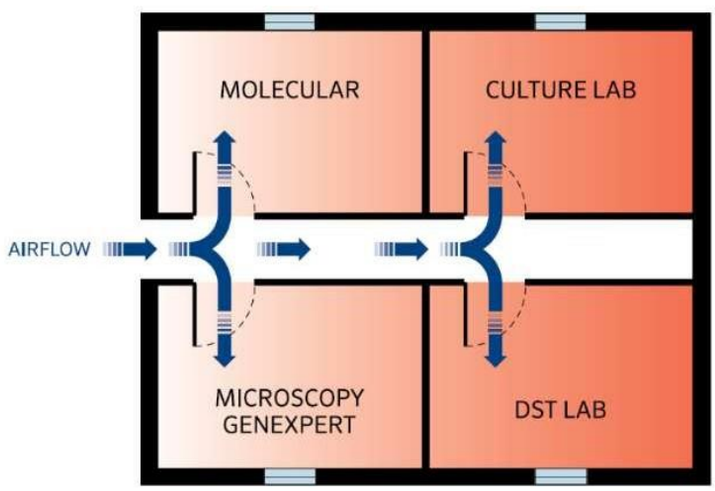


Figure 3.16: Airflow from 'clean to dirty' area in a multi-room laboratory
Source: From GLI (2019) [34]

- Separate benches should be used for clean and dirty activities. For example, the bench/areas used for receiving specimens and paperwork should be separated from the bench/areas used for manipulating the specimen or conducting the tests.

To prevent carry-over contamination of reagents/samples some of test procedures (especially molecular test) require physical separation of laboratory areas and strict adherence to good practices. For example, line probe assay (LPA) for TB requires ideally 4-room facility or minimum 3-room facility (Figure 3.17). Workflow between these rooms must be unidirectional i.e from clean to dirty/contaminated area and never in opposite direction. Staff must change the laboratory coats and gloves and wash their hands between areas.

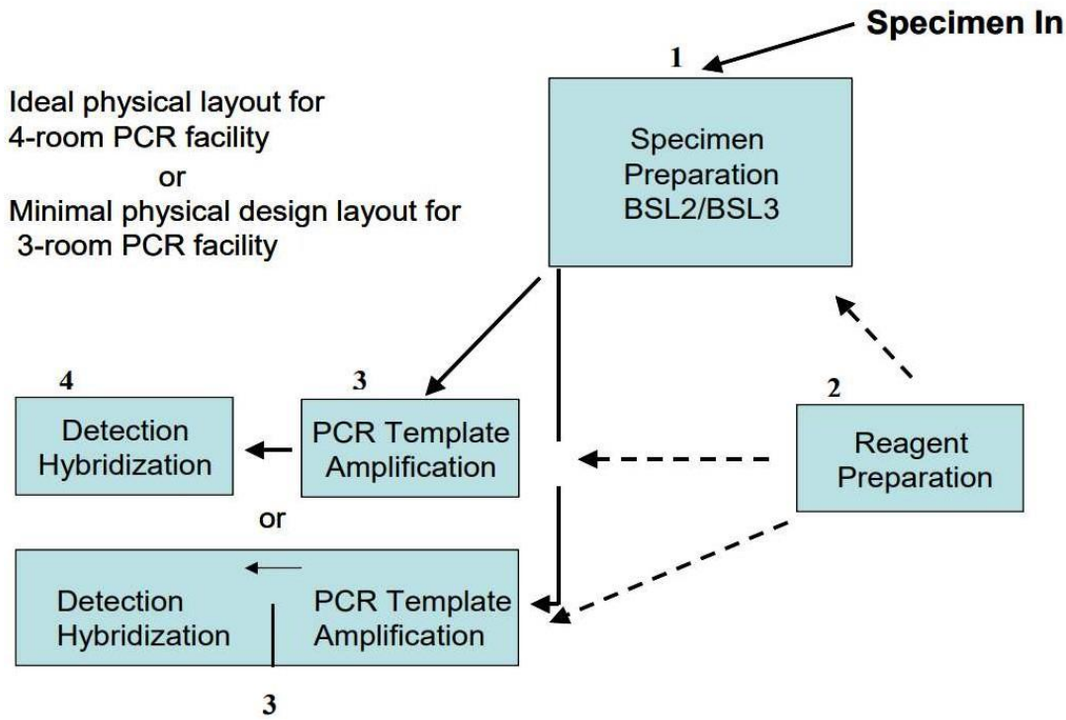


Figure 3.17: Illustration of the work flow for both the ideal physical design layout (4-room PCR facility) and a basic 3-room PCR facility.
Source: From Barnard et al (2012) [40]

3.3.1.3 TB containment facility

TB-containment laboratory refers to a facility that has the minimum of design features essential to manipulate TB cultures. A TB containment facility may or may not meet all of the requirements of a biosafety level 3 laboratory as described in the WHO Biosafety Manual (2004).[3] The basic features of a TB containment laboratory include.

A) Location: It should be physically separated from building areas or corridors with unrestricted traffic flow

B) Access: Only trained and authorized staff should have access.

C) Anteroom/Doors: Entry should only be via an anteroom *i.e* a set of two doors. Doors should be airtight and equipped with view panel.

D) Floor/walls/windows:

- Floors, walls, and structural joints must be smooth, easy to clean, non-porous to liquids, and resistant to laboratory chemicals and disinfectants.
- Windows should be sealed so they cannot be opened and are airtight.

E) Air-flow:

- The supply and exhaust components of the ventilation system are designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory.

- A visual monitoring device showing proper directional airflow is maintained at all times.

- Air from the containment lab should not be re-circulated to other areas in the building. Exhaust air should be filtered (through high-efficiency particulate air; HEPA) before being discharged outside.

F) Safety equipment:

- An on-site disinfection facility (i.e autoclave) should be available in the close proximity to the TB containment laboratory.
- A biological safety cabinet, ducted outside, should be available.
- A sink for handwashing (with hands-free operation) should be available.
- Eye wash station, emergency shower and communication device should be available.

Note: Refer to the following link to access the detailed specifications of the TB containment laboratory laid down by the Central TB Division, New Delhi. Govt. of India.[41]

3.3.1.4 Appropriate signage

Wherever biohazardous materials are handled and stored, doors must be appropriately marked with the international biohazard warning symbols (**Annexure 6**). The 'red-orange biohazard symbol' label must be placed on refrigerators, freezers or other equipment used with infectious agent, specimen/culture shipping container, biomedical waste bins or any other surface that are likely to encounter surface contamination from infectious materials.

3.3.2 Administrative policies:

Administrative policies or controls are an important component of a laboratory biosafety management plan. It includes written policies and procedures for safe laboratory work practices (including use of safety equipment, emergency preparedness and occupational safety), establishes the standard for behavior within the laboratory and authorizes the individuals to access the laboratory or containment facility and perform the assigned task. Once developed, administrative control must be implemented and adhered to by all personnel working in the laboratory.

Laboratory manager/supervisor must ensure that laboratory personnel working under their supervision are informed and adhere to laboratory specific safety policies and procedures – such as the requirements covered in this TB laboratory biosafety manual. He should promote the safe laboratory environment and ensure that everyone understands that the biosafety is a shared priority. This will help the laboratory personnel in recognizing the significance of reporting their concerns, sharing information openly, and taking action when needed. Each TB laboratory, particularly those with moderate and high-risk, should appoint a qualified biosafety officer (either on full time basis or part-time). He or she must be knowledgeable about the risks related to the laboratory activities being conducted, as well as biosafety standards in TB laboratories and pertinent national/state and local regulations. The duty of the biosafety officer should be to oversee biosafety and biosecurity practices, including the overall management of the biosafety program, which may include developing policies, implementing the program, ensuring compliance, conducting risk assessments and safety audits, overseeing and documenting biosafety-related trainings as well as continual improvement of the program.

3.4 Sterilization and disinfection

Sterilization is a process of destroying or eliminating all forms of microbial life using physical or chemical methods. On the other hand, disinfection is the process that eliminates most or all pathogenic microorganisms from inanimate objects with exception of bacterial spores[1]. Sterilization and disinfection are critical for preventing the spread of infectious pathogens to laboratory personnel, patients and visitors. The following factors influence the effectiveness of both sterilization and disinfection:

- physical nature of the object (e.g., crevices and hinges)
- presence of organic and inorganic load
- type and level of microbial contamination
- concentration of and exposure/contact time to the germicide used
- existence of biofilms
- temperature and pH during the process

3.4.1 Sterilization methods

Sterilization destroys all microorganisms on the surface of an article or in a fluid. The various methods of sterilization are as shown in Figure 3.18

3.4.1.1 Physical methods:

A) Heat sterilization: This method involves the elimination of enzymes and other vital cell components. It is the most widely used and dependable physical sterilization method (thermostable materials).

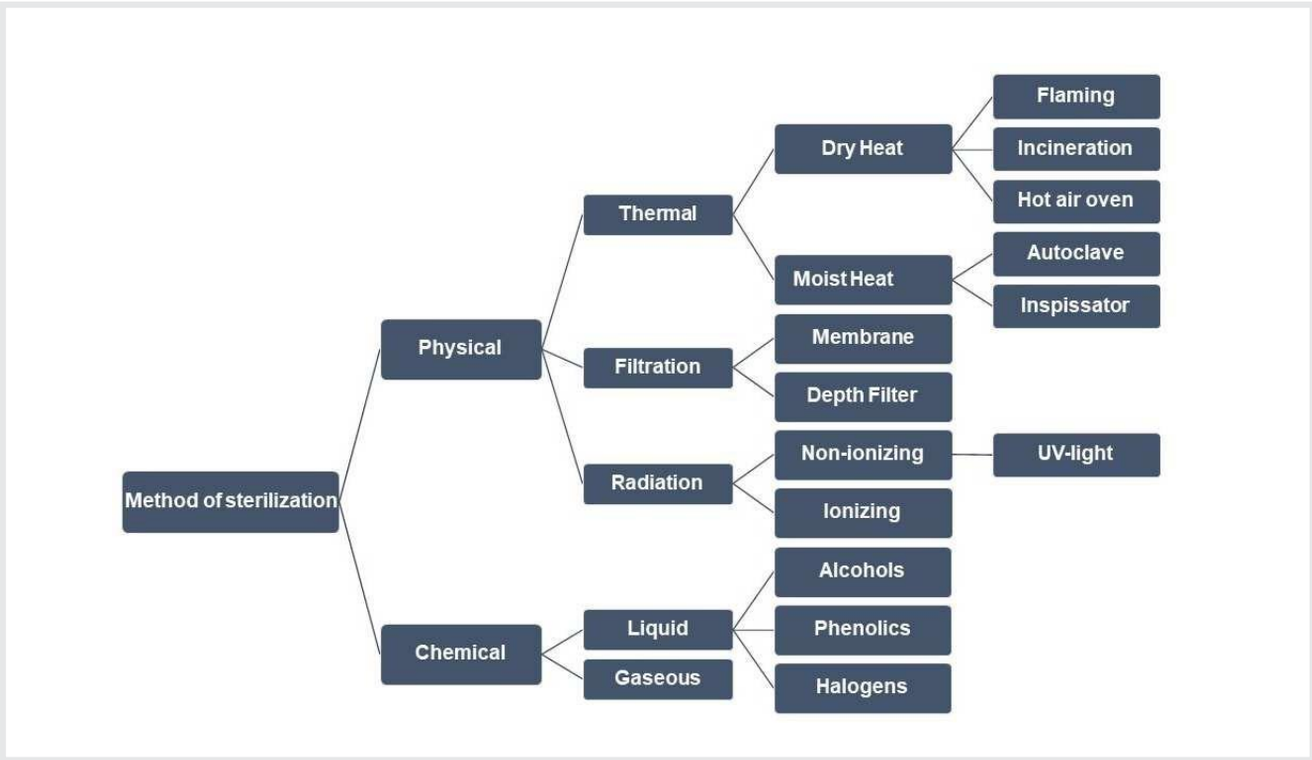


Figure 3.18: Methods of sterilization

Heat sterilization can be performed either through dry heat or moist heat.

i. **Dry heat**: Dry heat sterilization kills the microorganism through oxidation effect. The different procedures are as follow

- Flaming: Inoculation loops or wire, as well as the tips of forceps and spatulas, are held in open flame (like Bunsen burner) until red hot.
- Microincinerator: An equipment designed to sterilize metal inoculating loops and needles without the use of an open flame.
- Hot air oven: An equipment used to sterilize forceps, scissors, scalpels, non-graduatedglassware, petri-dishes, test tubes. A holding period of 160^{o}C for 1 hour is desirable.

ii. **Moist heat:** Microorganisms are destroyed by moist heat by coagulating their proteins. The common methods used are:

- Inspissation: It sterilizes by heating at 85^{0}C for 50 minutes holding time. This method is used to sterilize Lowenstein-Jensen media.
- Autoclaving: It works on the principle of steam under pressure. Sterilization is carried out under 15 lbs pressure at 121^{o}C for 15-20 minutes.

B) Filtration methods: Filtration helps to remove microorganisms from heat labile liquids such as antibiotic solutions and reagents. The primary filtration mechanisms include sieving, adsorption, and trapping inside the matrix of the filter material.

- Membrane filters: These filters are made of cellulose acetate, cellulose nitrate, polycarbonate, and polyvinylidene fluoride, or other synthetic materials with pore sizes between 0.2- 0.45 µm.

- High efficiency particulate air (HEPA) filters: HEPA filters are often used in biological safety cabinets where it traps infectious airborne agents and only allow clean air to pass through. HEPA filters out 99.97% of particles with diameters greater than 0.3 micrometers. In general, contaminated air is first passed through prefilters to remove larger particles and then passed through HEPA filters.

iii) Radiation method: Radiation sterilization is also called cold sterilization and it targets microbial DNA. Non-ionizing radiation such as UV light are low energy with poor penetrative power, at wavelength between 330nm and 400nm causes sterilizing effect by inducing excitation of electrons. Used in rapid mass sterilization of prepacked syringes or other materials. It is also used for surface sterilization of laminar flow hoods as well as clean rooms.

3.4.1.2 Chemical sterilization:

Chemical sterilizers work by causing protein coagulation, cell membrane breakdown, sulfhydryl group removal, or substrate competition. The key chemical sterilizers include:

A) Ethanol/Isopropyl alcohol: It kills bacteria and fungi by denaturing proteins and disrupting cell membranes. The optimal concentration is 70% and it is used to mechanically wipe microbes off skin and for surface sterilization.

B) Phenolics: It causes cell lysis by altering the permeability of cell membranes. Synthetic phenolics are mostly used in discard containers as an effective alternative to alcohol- and chlorine-based disinfectant and for decontaminating the environmental surfaces.[34]

C) Halogens: The oxidizing properties of halogens like chlorine and iodine are known to exert bactericidal and sporicidal effects. Sodium hypochlorite solution, commonly called as household bleach, is the most commonly used halogen. In TB laboratory, a solution containing 0.5% or 1% (5000 ppm or 10,000ppm) of available chlorine, depending on its intended use, is recommended. It is a general-purpose disinfectant used for contaminated non-metallic materials.

D) Formaldehyde: It is a gas that kills all microorganisms as well as spores. Effective killing, however requires temperature above 20°C and relative humidity about 70%. Formaldehyde based sterilization is commonly used for decontamination of enclosed volumes such as safety cabinets and rooms.

Whatever sterilization procedures are used, it is critical to ensure that the sterilization process is effective. To validate this, different indicator viz., mechanical, chemical, and biological indicators are used. The recommended frequency of usage of quality control indicators in autoclaving procedure are:[33].

- Mechanical: Temperature, time, pressure in each sterilization cycle
- Chemical: Each cycle and each package in the load
- Biological: At least weekly (or as per the autoclave load)

3.4.2 Disinfectants

In the health-care settings, many disinfectants are used such as alcohols, chlorine and chlorine compounds, formaldehyde, glutaraldehyde, ortho-phthalaldehyde, hydrogen peroxide, iodophors, peracetic acid, phenolics, and quaternary ammonium compounds. These are used alone or in combination (for example, hydrogen peroxide and peracetic acid).

The commonly used disinfectants in TB laboratories, its preparation, correct uses and safety precautions are described in Table 3.4.1.

Table 3.4.1: A brief description of commonly used disinfectants in TB laboratories

Parameters	Disinfectant commonly used in TB laboratory		
	Phenol	**Sodium Hypo -chlorite (NaOCl)**	**Alcohol**
Availability in market	In crystal form (100% conc.); in liquid form (~40% conc.)	4-6% (40-60g/L; 40,000 - 60,000 ppm) liquid form (household bleach)	Absolute alcohol (ethanol/ethyl alcohol)
Hazard	Highly corrosive; toxic if swallowed; skin exposure can cause severe skin burns and eye damage	Highly alkali and corrosive; exposure can cause severe skin burns and eye damage	Highly inflammable liquid and vapor; exposure can cause serious eye irritation
Where to use?	Surface decontamination , decontamination of biomedical waste and spill management.	Surface decontamination in molecular testing area; should NOT be used on metal surfaces as it may be corroded.	Routine surface cleaning (comparatively clean area of laboratory)
	may leave residue on surface thus need to be cleaned with distilled water/alcohol	may leave residue on surface thus need to be cleaned with distilled water/alcohol	do not leave any residue on surface
What is effective concentration?	5% in water (preferably distilled water)	1% in water (preferably distilled water)	70% in water (preferably distilled water)
What is minimum exposure time?	15 min (overnight for decontamination of sputum cup)	15 min	-
How to prepare disinfectant solution?	19 parts of water for each part of melted phenol crystal; or [%phenol in liquid solution/ % phenol desired] − 1 = Total parts of water for each part phenol solution Example: [40% liquid phenol solution/ 5% phenol desired] -1= 7 parts of water for each part liquid phenol	[% chlorine in liquid sodium hypochlorite / % chlorine desired] − 1 = Total parts of water for each part sodium hypochlorite Example: [4% in liquid sod. Hypochlorite/1% chlorine desired] -1= 4 parts of water for each part of sodium hypochlorite.	7 parts of water for each three parts of absolute alcohol
	Melt phenol crystal (100%) in water bath to get it in liquid form	Check the conc. of hypochlorite before calculation	Ensure that the alcohol is almost 100%

continued...

Parameters	Disinfectant commonly used in TB laboratory		
	Phenol	Sodium Hypo -chlorite (NaOCl)	Alcohol
How frequently to prepare the working solution?	Preferably on weekly basis	On daily basis	Preferably on weekly basis
How to store the disinfectant?	Tightly closed; ventilated place away from acids	Tightly closed; well - ventilated and dry place.	Ventilated place and away from heat and ignition source

3.4.3 Precautions during handling and storage

- Incompatible chemicals must not be mixed with chemical disinfectants. For example, bleach should never be mixed with ammonia or with acidic products, including drain, toilet bowl, and metal cleaners, since toxic fumes (potentially fatal) will be released.

- Working concentrations shouldn't exceed the recommended concentration because doing so poses a risk to operators.

- When transferring concentrated disinfectant for dilution, the proper dispensing device/ equipment should be used to avoid spills. Disinfectants should be transferred in ventilated area.

- Chemical disinfectants should be stored away from incompatible chemicals in a cool and ventilated place protected from direct sunlight.

Chapter 4
BIOSAFETY MEASURES FOR LOW-RISK TB LABORATORIES

ABOUT THE CHAPTER

This chapter describes the minimum biosafety requirements for laboratories performing specific procedures with a low risk of TB exposure. These requirements are applicable to TB laboratories at district and sub-district level as well as peripheral level (TB Detection Centers). Additional measures, however, may be required based on site specific risk assessment.

OBJECTIVE

To recommend minimum biosafety requirements in TB laboratories, when performing low risk tests procedures.

4. Biosafety measures for low-risk TB laboratories

This chapter describes the biosafety requirements for low-risk TB laboratories where test procedures involve the handling and manipulation of clinical specimens, especially sputum. Sputum manipulation, due to its viscous nature, is less likely to produce aerosols if good microbiological practices are used.

4.1 TB testing procedures

Low-risk TB laboratories can manipulate.

- Sputum specimen for direct sputum-smear microscopy
- Sputum specimens are for the Xpert MTB/RIF® assay and Truenat MTB/RIF® assay
- Designated microscopy center (DMCs)/ TB Detection Centers (TDCs)

Low-risk TB laboratories under NTEP

- Designated microscopy center (DMCs)/ TB Detection Centers (TDCs)

A single unprotected cough poses a much higher risk of aerosol generation and transmission than the test procedure performed in low-risk TB laboratories. Therefore, specimens should never be collected within the laboratory.

Low risk laboratories may face following key challenges, all of which increases the risk

- Inadequate working and storage space
- Inadequate ventilation/ cross ventilation
- Relevant personal protective equipment either not available or incorrectly/infrequently used.
- When collecting, handling, or transporting the specimen, safety precautions are often overlooked
- Biomedical waste segregation, disinfection, and disposal are not properly practiced, or personnel are not trained.

4.2 Biosafety measures to be established.

To address the biosafety risks in a low-risk TB laboratory, the following requirements should be implemented.

4.2.1 Facility design

Good laboratory design and construction not only contributes to the safety of laboratory staff butalso provides a barrier that protects the surrounding area or community from TB aerosols that may be generated inside the lab. Laboratory design and facilities are secondary containment measures; and recommended features for a laboratory is based on the procedures performed and the risk of transmission associated with them. In a low-risk TB laboratory, the secondary barriers must consider following features (Figure 4.1)

- Laboratory has door for controlling the access.
- A sink for hand washing.
- Ample bench space for safe conducting of work as well as cleaning and maintenance. No cloth covering of furniture and table.
- Ergonomic chairs with adjustable height.
- An area for the safe preparation, handling and storage of acids, stains and other supplies.
- Adequate supply of water and electricity (to avoid interruption of services).
- Proper illumination in the work area (no cloth curtains).
- Color coded biohazard bags/ foot operated bins for waste segregation, disinfection and further disposal.

1) Restricted access 2) Hand washing sink 3) Standard safety precautions /procedures 4) personal protective equipment 5) Adequate work space 6) Adequate storage space 7) Bins for biomedical waste.

Figure 4.1: Design and facilities at low-risk level laboratory

Source: Adapted from CDC (2021)[42]

A suggestive layout design for a TB laboratory with smear microscopy and/or Truenat testing, as well as a GeneXpert testing facility, is given in **Annexure 7**.

4.2.2 Procedures and Biosafety Practices

In general, laboratories must conduct the test procedures in such a way that aerosols generation can be avoided or minimized (see section 4.3.5). In addition, following good practices should be followed

- Mouth pipetting and putting any objects in the mouth should be prohibited.
- In laboratory, only self-adhesive labels should be used. It is preferable to write directly on the container with a permanent marker.
- Use of needles and syringes should be limited. Avoid receiving the specimens in syringes.

- Syringes should never be used as a substitute for pipetting.
- Lab registers or any documents in work area should be protected from contamination.
- Before disposal or cleaning, the contaminated materials should be appropriately decontaminated.
- Standard operating procedures should be readily available (or displayed on wall), and staff must follow them while carrying out the procedures.
- Standard guidelines should be followed for sputum packing and transportation (see chapter 7).

Figure 4.2: Laboratory good practices

4.2.3 Use of Bench Spaces

- The bench where specimens are processed for direct sputum-smear microscopy or the NAAT (GeneXpert/ Truenat) assay should be located apart from places where specimens are received or administrative areas where papers and computer/telephone are used.
- Bench for smear preparation or staining area should be separated from microscopic examination area and Truenat testing area.
- The bench top should be impermeable to, and resistant to, chemicals, disinfectants, and moderate heat.
- Laboratory bench should be sturdy and cluttered free. No cloth covering should be used for benches or furniture.
- Open spaces should be available between and under benches, cabinets, and equipment for easy cleaning.

Figure 4.3: Laboratory bench

4.2.4 Ventilation

The primary goal of ventilation is to dilute and remove possibly contaminated air from the laboratory. The laboratory should be functionally divided into two regions: 'functionally clean and 'potentially contaminated (dirty)'. As a general rule, the entry area of laboratory room should be reserved for 'clean' activities (such as administrative and preparatory works) while 'dirty' activities (such as specimen manipulation) should be performed furthest away from the entry.

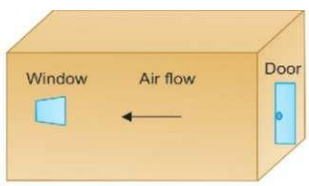
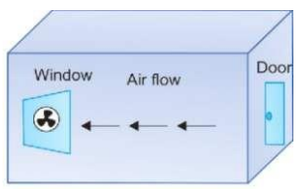
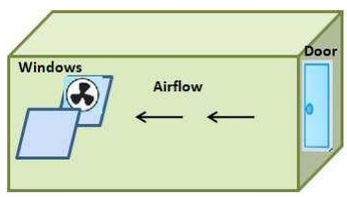
Adequate ventilation for TB laboratories is described as **directional airflow with 6–12 air exchanges per hour.**

- Directional air-flow: refers to the flowing of air in the direction from clean areas to those areaswhere aerosols might be generated, and then outside the room.

- Air exchange per hour (ACH): refers to the number of times that the total air volume in a room or space is completely removed and replaced with clean air.

Ventilation in TB laboratories may be achieved by natural, mechanical or hybrid (mixed) model depending on the design of the laboratory, climate, the procedure and the way it is performed by laboratory staff. Following considerations (Box 4.1) must be taken into account while determining the type of ventilation.

Box 4.1 Methods of ventilation

Natural Ventilation	Mechanical Ventilation	Hybrid ventilation
		
- Outdoor air is drawn into the laboratory naturally through open doors and windows. - More economical and provide high rate of ventilation	- Laboratory air is expelled from the laboratory by installing mechanical fans (exhaust fan) in windows or on walls, or in ducts or ventilated work station. - Reliable method in delivering desired rate of airflow.	- Laboratory relies on natural ventilation; but when natural air-flow is low or not suitable, it uses mechanical airflow. -Reliable and economic method of ventilation.
● Directional control of contaminants in air may be achieved if air moves at least 0.5 m/s. ● Method to determine the air -exchange rate is described in **Annexure-8**		

Ventilation requirements for different procedures:

- **Direct sputum smear Microscopy:** Natural ventilation is sufficient if air flow directionally (from clean to dirty area), climate condition allows opening of windows (cross ventilation) and procedures are performed using good microbiological practices.

- **GeneXpert:** This equipment is sensitive to environmental condition (temperature, humidity and dust) and therefore air conditioner is required which should be placed so that air flows away from the technician. The steps of specimen preparation can be carried out in naturally or mechanically ventilated area (or where sputum smear preparation is performed).

- **Truenat:** Truenat is less sensitive to environmental conditions than GeneXpert. Ventilation requirement for Truenat is similar to that of smear microscopy.

4.2.5 Minimizing the generation of aerosols

Some of practical steps for reducing the aerosols generation in low-risk TB laboratories are

- Specimen containers should not be opened immediately because the specimen may have been shaken during transportation (risk of aerosol exposure).
- When preparing a sputum smear with a wooden stick or disposable loop, move it slowly and smoothly to avoid aerosol generation.
- Smear preparation should be done within 6 inches radius of flame-spirit lamp
- Wooden sticks or disposable loops should be used rather than reusable loops that need open flame sterilization.

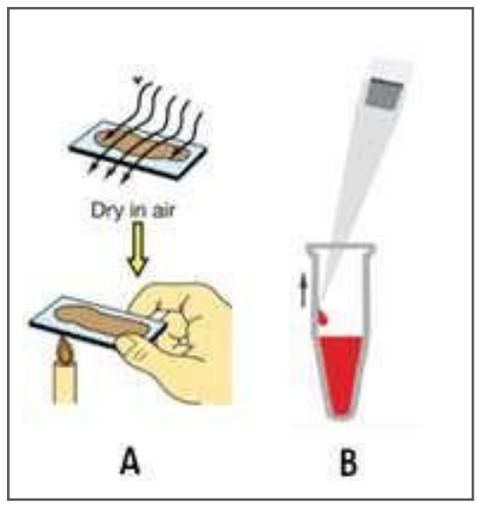


Figure 4.4: Safe practices to minimize the aerosol generation

- Smears should not be dried over an open flame because infectious materials may spatter. It is recommended to let the smears air-dry first and then use a flame to fix the smears. (Figure 4.4. A)
- Pipette careful while conducting NAAT. Do not forcibly expel or aspirate infectious liquid from a pipette. When adding a reagent to a potentially infectious liquid, place the pipette against the inner wall of the container and gently dispense the liquid. (Figure 4.4. B)
- Never shake or vortex an open tube; before shaking or vortexing, always make sure that the tubes are securely capped.

4.2.6 Handling specimen containers

- Appropriate patient counselling (see Chapter 7.1) is required to obtain a quality specimen with no leakage/ visible contamination on the exterior of container.
- When specimen containers are delivered to the laboratory, their integrity must be carefully checked. Leaked containers should be discarded (in biohazard bin containing disinfectant,5% phenol), and a fresh specimen should be requested. If possible all efforts should be made to test the extra pulmonary.
- Even if there is no visible contamination or leakage, the exterior of the specimen container should be cleaned with disinfectant (5% phenol) soaked cotton/ absorbent tissue paper.

Figure 4.5 Handle the container with gloved hand; tighten the screw cap

- Improperly fitted cap of the specimen container is the major reason of leakage; therefore, ensure that specimen containers are properly capped and transported to the laboratory, preferably in an upright position. In the laboratory, specimen containers should always be handled with gloved hands. (Figure 4.5).

4.2.6 Personal protective equipment

- In the laboratory, protective lab coats must be worn at all times.
- If re-usable lab coats are used, it must be maintained and washed regularly on biweekly basis or on a weekly basis (if used heavily). Important consideration related to re-use of lab coat is described in Box 4. 2.
- Ideally three lab coats should be available for each technician considering the first one is in-use, second one is in laundry and third one is for emergency use when the coat that is worn gets contaminated.
- Gloves must be worn at all times during procedure that involve direct contact, or may involve unintended contact with specimens or other potentially infectious material.
- Gloves should not be reused and should be always changed when it gets ripped, torn, contaminated or believed to be contaminated.
- Follow proper hand hygiene (**Annexure 1**).

Box 4.2 Important consideration while re-using the lab coat

Lab coats should not to be taken home for laundry. Whenever chemical or biological spill occur lab coat should be washed. If large amounts of concentrated acids or corrosives damaged the lab coat, it is better to discard the lab coat.

Lab coat must be washed in hot water with bleach and laundry detergent with in lab/hospital premises.

4.2.7 Decontamination

- Always disinfect the contaminated materials/waste before final disposal.
- Unbreakable discard containers, that are unbreakable should be placed at each workstation.
- Waste materials must remain in contact (submerged) with effective concentration of disinfectants. The commonly used disinfectants in TB laboratories, its preparation, correct uses and safety precautions are described in Chapter 3.4.
- The discard container should be decontaminated, washed and reused
- Liquid waste (like sputum, body fluid etc.) should also be treated with effective disinfectant (5% phenol) before disposal.

4.2.8 Waste handling and disposal

- Any waste generated inside the laboratory should be considered as infectious and they must be disinfected, segregated and disposed as per the BMW guidelines from Govt. of India (2016).[39]
- All contaminated materials (such as plastic sputum containers, cartridges used for molecular analysis, wooden applicator sticks) materials except sharps should be placed in appropriate color coded disposable non-chlorinated plastic bags before being transported for final disposal (Box 4.3).

- Non-hazardous liquids can be disposed of in the sink and rinsed thoroughly with running water. The containers are washed and rinsed thoroughly before re-use.

- Expired chemicals/ reagents must be disposed of in the biohazard waste bag, unless stated otherwise in the Safety Data Sheet (SDS).

- Even after decontamination, waste generated in TB laboratories should not be disposed in a landfill. In rural and remote areas where there is no access to a common biomedical waste treatment facility, disposal by burial can be done with prior approval from competent authority[39] and as per guidelines issued from time to time by the Central Pollution Control Board (https://cpcb.nic.in/).

- **Liquid waste**: The disposal of liquid infectious waste (ex- leftover sputum) should be done in a safe and responsible manner to prevent the spread of infections and protect public health. Following general steps to follow
    - Ensure that the waste is collected in leak-proof and puncture-resistant containers that are labelled appropriately as infectious waste. Use containers that are specifically designed for infectious waste disposal.
    - Decontaminate with 5% phenol (for overnight) before disposal. Double bucket system (having outer leakproof bucket and inner perforated bucket) that allows the infectious waste remain in contact with disinfectant and easily segregation of solid waste (ex- sputum cup), if any.
    - After pre-treatment discharge the liquid waste in separate sanitary sewer followed by flushing enough water (open the water tap for 10 min). Disposal of liquid chemical waste (eg. expired sulfuric acid) requires neutralization/dilution before disposal.
    - If the waste is to be transported to another facility for disposal, ensure that twin-buckets (outer and inner both should be leakproof) are used.
    - Proper personal protective equipment (PPE) should be worn during the handling and disposal of the waste, including gloves, masks, and eye protection.

Highlights

Testing Procedures: Sputum smear microscopy , GeneXpert and Truenat.

Special Practices: Access to laboratory area to authorized persons only; Standard microbiological practices and adherence to standard operating procedures; safe technique to avoid/ minimize aerosol generation; no specimen collection inside the laboratory; use of quality specimen container; transportation of specimen in triple layer packaging; use of disposable loop/wooden stick; disinfection, segregation (colour coded and leak proof biohazard bags/bin) before removal from the laboratory.

Primary barrier and PPE: No special safety equipment (like BSC or Safety centrifuge) required; minimum PPE includes gloves and lab coats.

Secondary barrier (facility design): Laboratory doors with biohazard signage; sink for hand washing; sturdy and impervious laboratory bench; adequate water and electrical supply; ventilation (open windows or exhaust), directional air flow from clean to potentially contaminated area; lighting adequate for all activities and suitable environmental condition for GeneXpert machine.

Box 4.3: Segregation of biomedical waste - Low risk TB laboratory

Yellow Bin	Red Bin	Blue Bin	Black Bin
			
Infected waste (for incineration)	**Infected plasticwaste** (after disinfection)	**Glass waste**	**General waste**
• Truenat chips • Disposable coat • Mask • Wooden applicator stick • Chemical	• Specimen collection tubes/ sputum cup; • Specimen packaging material (plastic) • Pipette tips • Pasteur pipettes • Truenat cartridges • Elute collection tube • PCR tubes • Used gloves • CBNAAT cartridges	• Glass slide in Truenat machine, • Used slides (ZN/FM) • Broken glass **Note:** Metallic sharps (e.g. needles, scalpels, blades, etc.) are discarded into white puncture proof containers.	• Cartridge pouches • Chip pouches • Transfer pipette wrappers • Desiccant pouches • Sleeves

Chapter 5

BIOSAFETY MEASURES FOR MODERATE-RISK TB LABORATORIES

ABOUT THE CHAPTER

This chapter describes the minimum biosafety requirements for laboratories performing specific procedures with a moderate risk of TB exposure. These requirements are applicable to sub-state level TB laboratories (Culture DST laboratories performing solid culture or line probe assays but not the liquid culture DST) in NTEP. Additional measures, however, may be required based on site specific risk

OBJECTIVE

To recommend minimum biosafety requirements in TB laboratories, when performing moderate risk tests procedure.

5. Biosafety measures for moderate risk TB laboratories

TB laboratories performing procedures with a moderate risk of aerosol exposure should adhere to the requirements described in this chapter.

5.1 TB testing procedures

Moderate risk TB laboratories can:

- Process specimens for inoculation on primary culture media
- Perform DST directly on processed sputum such as line probe assay (LPA)

In addition to the risks or challenges that low-risk laboratories face, moderate-risk laboratories may face following additional challenges:

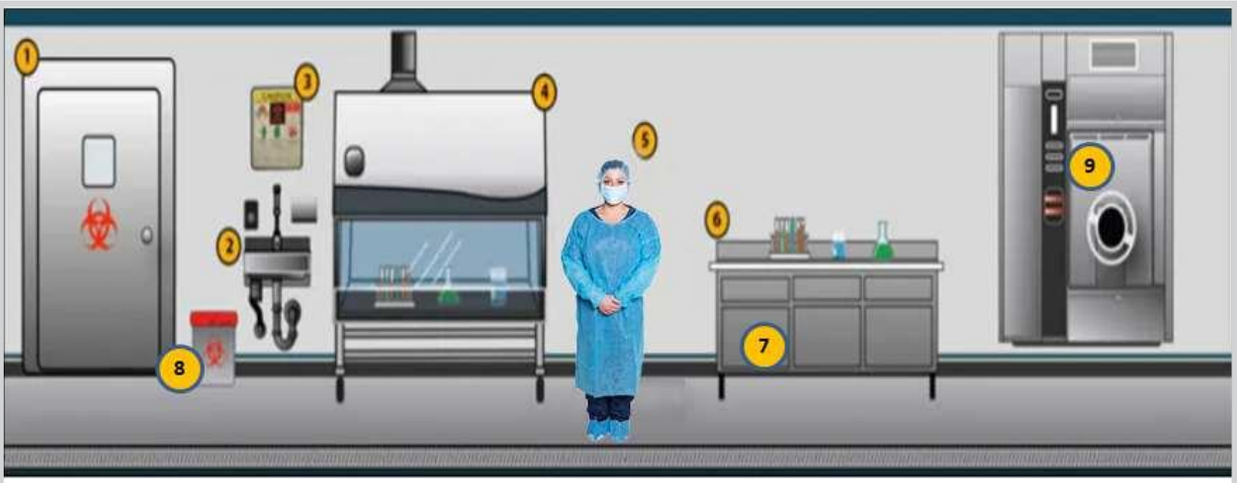
- Available safety equipment (like BSC, bio-safe centrifuge, pipettes, etc.) may be poorly maintained or not certified/calibrated.
- BSCs may not be properly placed and ducted.
- The laboratory environment may be dusty, and BSC high-efficiency particulate air (HEPA) filters may become clogged.
- Non-compliance of safety technique for specimen manipulation inside the BSCs.
- Vortex and Centrifuge safety precautions may not be followed adequately (for example, vortexing the specimen outside the BSC or without securing the cap of container; opening the biosafe centrifuge buckets outside the BSC).
- Breakage of specimen containers during centrifugation.
- Biohazard signage, safety instructions and emergency contact number may not be available or displayed prominently.

5.2 Biosafety measures to be implemented

To address the biosafety risks in a Moderate-risk TB laboratory, the following requirements should be implemented

5.2.1 Facility design

In a laboratory with moderate risk of aerosolization, there are two levels of containment: the biological safety cabinets- BSCs (primary containment) and laboratory/ facility design itself (secondary containment). On-site autoclaving facility is important for moderate risk laboratories and biomedical waste should be autoclaved before handing it over to authorize biomedical agency for final disposal. Doors should be self-closable and windows in the room should be sealed or fitted with screen. Other considerations for laboratory design and facilities are similar to those for low-risk TB laboratories (see chapter 3; Figure 5.1)



1) Self-closing door and restricted access; 2) Hand washing sink; 3) Standard safety precautions /procedures; 4) Biological safety cabinet; 5) personal protective equipment; 6) Adequate work space; 7) Adequate storage space; 8) Bins for biomedical waste; 9) Autoclave

Figure 5.1: Design and facilities at moderate-risk level laboratory
Source: Adapted from CDC (2021)[42]

In **Annexure 9**, a suggestive layout design for a Line Probe Assay facility as well as specimen processing area is provided.

5.2.2 Biological safety cabinets

Sputum processing (digestion, concentration or manipulation of liquefied sputum) must take place in a well maintained BSC. Although BSC provides primary containment when specimens are processed for culture inoculation or direct DST (LPA), inappropriate use of the cabinet may result in infectious aerosols being released into the laboratory. Hence, good microbiological practices and appropriate use of the cabinet are critical for conducting the work safely (see Chapter 3-Section 3.2.2).

The key considerations while installing a BSC are as follow

- Class II Type A2 cabinets are most suitable for all procedures as they offer protection to the operator/technician, the media being inoculated (product protection) and the environment.
- Appropriate placement of BSC is critical to ensure the effectiveness of BSC. A suitable place should be identified considering the factors (such as air movement, people movement, doors and windows, location of other equipment, surrounding clearance, etc) that compromise the effectiveness of BSC (see section 3.2.2.).
- BSC exhaust should be ducted outside the building, and air should be passed through a HEPA filter before being discharged.
- The BSC must be connected to a reliable power supply, preferably through a dedicated UPS with sufficient capacity to keep the BSC running for at least 15 minutes.
- When there is a power outage, work should be stopped immediately and the 15 minutes UPS power backup should be used to clear the aerosol in the cabinet. A backup of the power generator for the cabinet as well as other critical equipment is useful.

5.2.3 Ventilation

Appropriate ventilation in moderate-risk TB laboratory is achieved by the use of BSC as well as the directional airflow into the laboratory with minimum 6-12 ACH. Following considerations may be taken into account to ensure the required ventilation

- A simple way of ensuring unidirectional airflow is to place a vent that allows air to flow in the clean area and let the thimble-fitted BSC run constantly so that it draws air from the dirty area, passes through HEPA filters in the BSC and discharges it outside the building. Thimble ducting has an advantage as the extractor motor in the duct pulls air from both the cabinet and the room especially when BSC is kept on. When the BSC is turned off, the expelled air is extracted exclusively from the room. This feature allows to maintain the directional airflow in the laboratory without any adjustment in the BSC or installation of additional equipment.

- Laboratory air when vented outside must be dispersed away from occupied building and air intakes. Windows in the laboratory must be kept closed at all times.

- Monitoring the directional airflow in the biosafety cabinet is important. Daily log sheet may also be maintained for the same.

5.2.4 Minimizing the generation of aerosols

Specimen processing for culture or LPA is one of the major activities in moderate-risk TB laboratories. Because samples are liquefied during this procedure, it is more likely that aerosols will be produced; therefore, steps to reduce aerosol generation are critical. Following good practices should be considered to minimize the aerosols generation

- When dispensing infectious liquids from a pipette, place the pipette against the inner wall of the container and expel the liquid gently (never forcibly).

- The lid must be completely sealed when operating the centrifuge. Before centrifugation, individual centrifuge buckets must be inspected to ensure that there is no damage of bucket, lid and O-ring (gasket) and that they are tightly closed.

- Centrifuge buckets must be loaded and unloaded in a BSC. Refrigerated centrifuges with swinging buckets are recommended for processing of TB specimen.

- Never shake or swirl an open tube; always ensure that screw caps are securely fastened to tubes. Use of cotton plugs or rubber stoppers should be avoided when shaking the tubes or other containers.

- The tubes containing infectious materials should not be immediately opened after centrifugation, vortexing or shaking. Let them stand for 10 minutes (to settle down the aerosol) and open in a BSC. This will help in preventing the generation of and exposure to infectious aerosols.

- Vigorous pipette mixing (repeatedly filling and fully emptying a pipette) should be avoided for infectious materials.

- Only the disposable tip of a micropipette should be put in a tube or container (never put the barrel of a micropipette).

- When decanting infectious liquid, the tube should be held at an angle so that the liquid runs down the side of the discard container to minimise any splashes.

- Staff must be trained in safe technique when using inoculation loops, pipetting, opening specimen containers, handling leaking containers, centrifuging and vortexing to prevent infectious aerosol exposure.

- Instead of using a metal reusable loop, a sterile disposable transfer loop should be preferred. The risk of spattering the infectious material when metal loops are sterilized in open flame can also be prevented by using an enclosed electric micro-incinerator.

5.2.5 Handling leaked specimen containers

In addition to the safety precautions described for low-risk laboratories, specimen leakage during centrifugation is a critical incident that can occur in both moderate and high-risk laboratories.

- It is crucial to use good quality specimen containers for centrifugation to avoid such incidents. Specimen containers must resist the given centrifugal force (at least 5000 X g)

- The standard practice to load and unload centrifuge buckets in a BSC protects users from infectious aerosol generated due to accidental breakage or leakage of tube during centrifugation. If a tube breaks, it must be discarded in a puncture-resistant container and disposed of immediately. Decontaminate the centrifuge bucket by immersing it in a disinfectant solution (preferably 5% phenol). Alternatively, buckets may be decontaminated by autoclaving (if recommended by the manufacturer).

5.2.6 Personal protective equipment (PPEs)

When performing a moderate-risk procedure, technicians must consider followings points

- In laboratories where there is a moderate risk of infection, a respirator (eg. N95 mask), protective lab gown, and gloves must be worn at all times.

- Gloves should never be reused and staff must wash their hands after removing gloves and before leaving the laboratory.

- All the procedures must be carried out inside BSC with appropriate PPE. Wearing a respirator, however, does not eliminate the necessity for a BSC to perform aerosol-generating operations.

- Before performing any splash-prone activities, eye and face protection (e.g., safety glasses, goggles or face shield) should be used.

- The use of safety shoes and shoe covers contributes to the safety of staff as well as cleanliness of the laboratory. Dust may increase the rate of contamination of culture medium and may also clog the HEPA filters of BSCs.

5.2.7 Decontamination

- Types of disinfectants to be used are same as described for low risk level laboratories (i.e phenol, hypochlorite and alcohol).

- Moderate-risk laboratories should have an autoclave facility and all infectious waste should be autoclaved first before removal from the laboratory.

- After spills, splashes, or other possible contamination, laboratory equipment must be decontaminated using an appropriate disinfectant (5% phenol). Decontamination of equipment also needed before repair, maintenance or removal from the laboratory.

5.2.8 Waste handling and disposal

- Any waste generated inside the laboratory should be considered as infectious and they must be disinfected, segregated and disposed as per the BMW guidelines from Govt. of India (2016).[39]

- All the staff should receive training on biomedical waste management. An authorized biomedical agency should be contracted for removal and final disposal of biomedical waste generated in to the laboratory.

- Biomedical waste should not be disposed of in burial pits and should be autoclaved first before removal from the laboratory. The precautions in handling of biomedical waste are similar to those described for low-risk laboratories and colour coding bags/buckets should be used for waste segregation.

- Biomedical waste should be removed from the laboratory within 48 hrs. The area where the biomedical waste is temporarily stored must be secured.

- A daily log sheet of biomedical wastes removed or handed over to BMW management agency should be maintained.

- **Liquid waste:** Moderate-risk TB laboratories handle more specimens than the low-risk TB laboratories. The processing of sputum samples in moderate-risk TB laboratories produces a significant amount of liquid waste. These wastes are highly infectious and require proper disinfection before disposal. To do so, the liquid waste must be collected in a robust and watertight container with sufficient volume of phenol, with an overall concentration of 5%. The container should be left for overnight contact time before discharge into the sanitary sewer system, preferably through an effluent treatment plant, in accordance with the Biomedical Medical Waste Management Rule 2018. For low-volume infectious liquid waste, a certified autoclave can be used for disinfection. For general steps and precautions related to liquid waste management, please refer to Chapter 4.

Moderate risk procedures generate following waste and these must be segregated as shown below

Yellow Bin	Red Bin	Blue Bin	Black Bin
			
Infected waste (for incineration)	**Infected plastic waste** (after disinfection)	**Glass waste**	**General waste**
● Disposable gown ● N-95 Mask ● Hair cover ● LJ media (after autoclave) ● Chemical	● Specimen collection tubes ● Specimen packaging material (plastic) ● Pipette tips ● Pasteur pipettes ● PCR tubes ● Used gloves ● Disposable loop	● Used slides ● Broken glass *Note:* Metallic sharps (e.g. needles, scalpels, blades, etc.) are discarded into w hite puncture proof containers .	● Transfer pipette wrappers ● Packaging box of consumable s, kits, reagents. ● Test requisition slip (after retention period)

Highlights

Testing Procedures: Specimen processing and inoculation for primary culture media; drug susceptibility testing directly on specimens (LPA).

Special Practices*: Limited access to testing area; all procedures that may generate an aerosol or splash conducted in a BSC; decontamination of laboratory equipment; autoclaving of infectious waste before removal from laboratory.

Primary barrier and PPE: BSCs (preferably Class II Type A2 with thimble ducting) used for manipulations of specimen that may cause splashes or aerosols; Centrifuge with safety lid/ buckets are used during specimen processing; use of laboratory gown, gloves (other PPEs including N-95mask).

Secondary barrier (facility design): Self-closing doors with biohazard signage; sink located near exit; windows sealed or fitted with screens; unidirectional airflow in the laboratory, and ensuring there are a minimum of 6–12 ACHs (that may be maintained by BSC itself) and availability of autoclave.

* Including the recommendations of the low risk-laboratory in the same context.

Chapter 6
BIOSAFETY MEASURES FOR HIGH RISK TB LABORATORIES

ABOUT THE CHAPTER

This chapter describes the minimum biosafety requirements for laboratories performing specific procedures with a high risk of TB exposure. These requirements are applicable for sub-state, state and national level TB laboratories (performing liquid culture DST and other TB tests). Additional measures, however, may be required based on site specific risk assessment.

OBJECTIVE

To recommend minimum biosafety requirements in TB laboratories, when performing high risk tests procedures.

6. Biosafety measures for high risk TB laboratories

High-risk TB laboratories are also known as TB-containment laboratories- which refer to a laboratory that has the minimum design features required to manipulate TB cultures safely. A high-risk TB laboratory may or may not meet all of the requirements of a standard "Biosafety Level 3 laboratory," as described by the World Health Organization.[33]

6.1 TB testing procedures

TB containment laboratories are designed to safely work with high concentrations of MTB and to perform the procedures that are associated with high-risk infectious aerosol exposure. High-risk TB laboratories can

- Manipulate cultures to identify MTB
- Manipulate cultures or suspensions of tubercle bacilli for drug susceptibility testing (DST) including indirect molecular DST.

High -risk TB laboratories under NTEP

- Culture and DST (C-& DST) laboratories (with liquid culture DST facility)
- Intermediate reference laboratories (IRLs)
- National reference laboratories (NRLs)

In addition to the hazards described for low and moderate risk TB laboratories, high-risk (or TB containment) laboratory has following additional challenges:

- Opening of culture positive vials/tubes
- Preparation of smear from positive cultures
- Extraction of DNA from positive culture
- Manipulation of cultures for identification and conducting DST
- Accidental spillage of culture when handling the same
- Spill clean-up and disposal of broken culture tubes

6.2 Biosafety measures to be implemented.

To address the biosafety risks and challenges in a high-risk TB laboratory (or TB containment laboratory), the following requirements should be implemented.

6.2.1. Facility Design

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

In high-risk TB laboratories, all procedures of handling viable liquid suspensions of TB bacilli for identification and indirect DST must be performed within a BSC in a TB containment facility.

In addition to the facility design features (including BSC) required for a moderate-risk laboratory, TB containment laboratories require the following additional enhancements.

Location:

- The TB containment laboratory should be physically separated from building areas or corridors with unrestricted traffic flow. This will minimize the risk of exposure to the staff and others who are not directly involved in the laboratory activities.

Access:

- Access to TB containment facility should be restricted and the area should be under continuous surveillance; only staff who have been trained to work inside the laboratory and respond to emergency situations should be permitted/authorized.

- Access may be controlled by key cards, biometric passes and entry-exit register.

- Biohazard signage should be displayed at the entrance of TB containment laboratory. Emergency information and important caution may also be displayed at strategic location in the laboratory.

Entrance/anteroom and containment facility:

- Entry to the containment laboratory should be through a set of two doors (creating an anteroom), which creates a physical barrier between the containment laboratory and the outer areas. The anteroom is generally considered a part of the containment area since it shares the same ventilation system as the containment laboratory. However, it is actually a transition zone between clean and potentially contaminated areas. When there is no space constrain, anteroom may be divided into clean and unclean zone use the facility accordingly (like donning/doffing of PPE).

- Doors should preferably be self-closing, airtight, and equipped with a vision panel and an interlocking system (allowing only one door open at a time, thus preventing leakage of potentially contaminated air from the lab to the corridor). Windows should be sealed.

- Floors, walls, doors and working surfaces of laboratory should be impervious, resistant to chemicals and disinfectants and easy-to-clean. If there are any gaps, and cracks, it should be completely sealed.

- An emergency door should also be provided in containment room for emergency exit.

- The laboratory should be built in a way that allows it to be fumigated and prevents insect intrusion. Internal laboratory surfaces should be resistant to liquids and chemicals used for cleaning and decontaminating the area.

- Eye wash station should be readily available and a hands-free sink (along with soap dispenser and paper towel) should be provided near the exit door. In addition, a shower station may be provided for use in case of emergency.

- Apart from the above requirements, any paper-based communication between the TB containment laboratory and the area outside the laboratory should be avoided. Instead, a computer based laboratory management information system should be preferred. It is desirable to have an intercom or hands-free phone in the containment laboratory that can be utilized in any emergency situations.

A layout plan for TB containment laboratory is provided in **Annexure 10.**

6.2.2 Ventilation

In addition to the requirements described for moderate risk TB laboratory, followings must also be considered

- The mechanical ventilation of the laboratory is designed to maintain the laboratory at negative pressure to surrounding areas. The ventilation system must ensure differential pressure, directional airflow, as appropriate, between adjacent areas within the laboratory. Minimum pressure difference between anteroom and TB containment lab should be 15 Pa (e.g. -15 Pa in the anteroom and -30 Pa in the laboratory)

- The supply and exhaust components of the ventilation system are designed to maintain the laboratory at negative pressure to surrounding areas and provide differential pressure or directional airflow, as appropriate, between adjacent areas within the laboratory. Minimum pressure difference between anteroom and TB containment lab should be 15 Pa (e.g. -15 Pa in the anteroom and -30 Pa in the laboratory)

- Visual monitoring device showing that proper directional airflow is maintained at all times. Monitoring device should be displayed outside the laboratory, preferable next to the entrance. It preferable to have an audio-visual alert if the negative pressure goes out of range.

- An independent air duct with HEPA filter should be available to draw air from anteroom and containment lab and discharge them outside. Although a HEPA filter is not required for the air intake duct but it should have a one-way valve to stop any backflow in the event that the negative pressure is not maintained.

- Air from the containment lab should not be re-circulated to other areas in the building. There should be at least 6-12 air exchange per hour.

- A heating, ventilation, and air-conditioning (HVAC) control system may be installed to provide thermal comfort to laboratory personnel along with the required safe ventilation. This system should be equipped with audio-visual alarms to notify personnel of HVAC system failure.

6.2.3 Minimizing the generation of aerosols

In addition to the procedures/ safety precautions described for moderate risk TB laboratory, the followings must also be considered

- All the additions and handling of cultures should be done only inside a biological safety cabinet (BSC). Positive MGIT tubes must be sampled cautiously to minimize aerosol generation since broth culture contains high concentration of tubercle bacilli.

- Smear from specimen and culture growth may require to be prepared. When needed, slides should be prepared and heat-fixed inside the BSC before removing them from BSC. Slides can be heat fixed on a slide warmer set to 65-75°C for at least two hours. Heat-fixed smear may contain viable bacilli but they wouldn't be aerosolized easily, if fixed properly.

- A bubble or film in an open culture tube should never be disrupted. To remove the bubble, secure the tube's cap first and then gently tap the top of the tube. Let the tube rest for 10 min to allow any generated aerosols to settle down before opening.

- Use of aerosol resistant micropipette tips (ART) must be preferred and eject micropipette tips down inside the discard bucket.

- Avoid poor practices that can cause spillage, such as hand-carrying tubes, vials, and bottles, or improperly stacking racks or baskets. Infectious items should be transported within the laboratory in protected racks, baskets, or trolleys.

6.2.4 Handling leaked specimen containers

Same as described for low and moderate risk TB laboratory.

6.2.5 Personal protective equipment

- Similarly, in a moderate-risk TB laboratory, technicians must use laboratory gowns, gloves, and respirators while working in a TB containment laboratory.
- Gowns should have solid front, long sleeves with an elasticized cuff and a back closure. Gown should be impermeable to liquid.
- Gloves must be worn (preferably double pair when performing high risk activities).
- Respirators (N-95 mask) must be worn as they offer additional protection from aerosols with high concentrations of infectious particles during manipulating liquid cultures for identification and DST.
- The use of hair coverings, google/face shield, shoe covers or dedicated shoes may also be considered when needed. Any PPE used in the TB-containment laboratory must not be worn in any other section of laboratory.
- After removing PPE and before existing laboratory, staff must wash their hands.

6.2.6 Decontamination

- Equipment should be routinely decontaminated (5% phenol). Decontamination of equipment should also be considered after spills, splashes, or other potential contamination and before repair, maintenance, or removal from the laboratory.
- An autoclave must be available on site or in the vicinity of the TB-containment laboratory to minimize the risk of exposure or release during waste transportation. All tubes and vials with cultures of TB bacilli should be sterilized prior to being removed for disposal.

6.2.7 Waste handling and disposal

After disinfection, all waste must be transported in sealed non-chlorinated plastic bags or containers for final disposal to authorized biomedical agency (as per Biomedical Waste Management Rule 2016). Standard practices as described for moderate-risk TB laboratory should be followed.

Liquid waste: Apart from the liquid waste produced in low and moderate risk TB laboratories, high-risk TB laboratories also generate extremely infectious microbiological waste, such as liquid culture tubes. These wastes must be autoclaved in a certified autoclave before final disposal as per Biomedical Medical Waste Management Rule 2018. For other liquid wastes refer the steps and precautions as described for low and moderate-risk TB laboratories (in the Chapter 4 and Chapter 5). Furthermore, it is important to note that the handling and disposal of infectious waste should be carried out by trained professionals who are familiar with the procedures and guidelines for safe handling and disposal of infectious waste.

High risk TB laboratories generate following waste and these must be segregated as shown below

Yellow Bin	Red Bin	Blue Bin	Black Bin
			
Infected waste (for incineration)	**Infected plastic waste** (after disinfection)	**Glass waste**	**General waste**
● Truenat chips ● Gown ● N-95 Mask ● Hair cover ● Chemical ● Expired drugs	● MGIT tubes ● Rapid Immunochro-matographic test card ● Specimen collection tubes ● Specimen packaging material (plastic) ● CBNAAT cartridges ● Pipette tips ● Pasteur pipettes ● PCR tubes ● Used gloves ● Shoe cover (plastic) ● Disposable loop	● Used glass slides ● Broken glassware ● Glass petri-plates *Note:* Metallic sharps (e.g. needles, scalpels, blades, metal casing on drug vials etc.) are discarded into white puncture proof containers.	● Pouches and wrappers of kits, consumables. ● Test requisition slip (after retention period)

Highlights

Testing Procedures: Manipulation of cultures to identify *M. tuberculosis* or for all indirect DST methods and molecular assays.

Special Practices*: Access of TB containment facility to trained staff only; all procedures for handling cultures are conducted within a BSC in a TB-containment laboratory; sterilization of cultures prior to being removed for disposal.

Primary barrier and PPE: Use of BSCs and safety centrifuge; use of laboratory solid front gown, gloves (preferably double pair), N-95 respirators (and other PPEs as per site risk assessment).

Secondary barrier (facility design): Physical separation from access corridors; access through two consecutive self-closing airtight doors; hands-free sink near exit; windows are sealed; dedicated air ventilation system with negative airflow into laboratory with no re-circulation; autoclave available on site in the vicinity of the TB-containment laboratory (preferably in the lab).

* Including the recommendations of the preceding level (low or moderate risk-laboratory) in the same context.

Chapter 7
SPECIMEN COLLECTION AND TRANSPORTATION

ABOUT THE CHAPTER

Good quality TB specimens serves as basis for quality test result. Since, TB specimens are infectious and are transported to various levels of a tiered laboratory system for testing, a safe, secure and reliable method of sample collection, packaging and transportation is crucial; and are discussed in this chapter.

OBJECTIVE

To ensure the collection of good quality TB samples as well as their safe and secure handling packing, and transportation.

7. Specimen collection and transportation

TB specimens constitute a major hazard in all levels of TB laboratories, therefore it is important that specimens are collected, handled, stored or transported with all safety precautions and as per applicable national and international guidelines. All TB specimens are potentially infectious, even when they are collected from asymptomatic patients and the same standards of practice should be used routinely to prevent exposure to these specimens. Although smear negative specimens are less infectious, we must remember that even 1-10 bacilli can cause infection,[43] which may go undetected by standard TB tests. Moreover, the quality of sputum and the way it is handled and transported impacts on the yield and quality of test results.

7.1 Patient counseling for good quality specimen

7.1.1 Sputum collection

More than 85% of TB disease in high-TB burden countries (including India) is pulmonary and therefore most specimens received by the TB laboratory are sputum samples. Under NTEP, two sputum specimens, one early morning (sample B) & one spot specimen (sample A) are recommended for diagnosis. Alternatively, two spot specimens are collected with a gap of at least one hour if the patient is coming from a long distance or patient is unlikely to return to give the second specimen. It is vital to explain clearly to the patient how to collect the sputum specimen, using simple and easily understood words, and written instructions may also be provided. While adhering to airborne infection control (AIC) measures, laboratory technicians should counsel the patient about the followings.

- The importance of sputum examination and the number of specimens required for diagnosis of TB or follow-up of TB treatment.
  - Two samples (spot and morning) for diagnosis and one sample for follow-up.
- The requirement of actual sputum, not saliva.
  - Sputum in 2-5 ml quantity, preferably mucopurulent and not heavily blood stained or visually contaminated.
- Where to collect the sputum?
  - Sputum collection is aerosol generating procedure, thus it should be collected always in dedicated properly open ventilated/open areas under direct sunlight and never in laboratories, washrooms, waiting areas or any other enclosed space.
- How to open and close the containers?
- How to produce good quality sputum?
  - Repeated deep inhalation and exhalation of breath followed by cough from as deep inside the chest as possible).
- How to avoid contamination of the exterior of the container?
  - Carefully spitting and closing the container.

Note: It is important to designate a place for spot sputum collection (either outdoor or indoor). If indoor ventilated sputum collection rooms (or booths) are used, they must be disinfected regularly and well maintained.

7.1.2 Extra-pulmonary specimens

In case of presumptive extra-pulmonary TB (EP-TB), the laboratories may receive a variety of specimens. These specimens may be classified into two categories:

- **Sterile:** specimen that are collected aseptically; usually free of other microorganisms (eg. pleural, spinal, synovial, pericardial, ascitic, pus, bone marrow, tissues/biopsies, fine needle aspirates etc.)

- **Non- sterile:** specimens that were not collected aseptically; likely contaminated by normal flora (eg. gastric lavage, bronchial washings, urine, pus etc.).

Most EP-TB specimen collections involve invasive procedures, and it must be done by a physician using appropriate aspiration techniques or surgical procedures. Some of key considerations while collecting the EP-TB specimens are as follows.

- Specimens should be collected in sterile container and transported to the laboratory as quickly as possible.

- Tissue/biopsy specimen should be sent in normal saline and not in formalin (as formalin kill the bacilli).

- Blood samples should be discouraged.

- Large volume specimens (like pleural fluid, urine) and tissues may require special processing (like centrifugation or tissue homogenization). Since, processing of such specimens generates aerosol, it must be done in laboratories where safety equipment (like safety centrifuge and BSC) are available.

- No preservative should be used for any extra-pulmonary specimen. Gastric lavage/ aspirate is acidic in nature, thus it should be collected in a container with a neutralization buffer or it must be neutralized before processing the specimen.

For more details, refer to 'Standard Operative Procedure for collection, transport and processing and inoculation of Extra-pulmonary specimens" by Central TB Division, Ministry of Health and Family Welfare, New Delhi.[45]]

7.2 Specimen container

Specimen leakage or breakage of specimen containers (during centrifugation) is the potential biohazard exposure risk encountered commonly in TB laboratories. These risks however can be avoided using quality specimen containers and safe practices while handling the specimen. Specimen container should be

- rigid enough to prevent breaking during transportation or centrifugation (must withstand centrifugal force at least 5000 xg).

- leakproof and have screw-caps, to prevent leakage and contamination.

- wide-mouthed, so that a patient can expectorate easily into a container without contaminating the outside.

- have a capacity of 50 ml and be made of transparent material so that specimen volume and quality can be checked without the need to open the container.
- made of single-use, combustible material to facilitate disposal.
- have easily-labeled walls to allow permanent identification.

(Refer to the specification of specimen containers as recommended in the NTEP) [41]

Note: Sterile specimen containers (generally sterilized using gamma irradiation or ethylene chloride) should be preferred, though it may be more expensive than clean containers (likely to be free from microbes but sterility is not guaranteed). Specimens for conducting TB culture and DST should always be collected into sterile containers.

7.3 Specimen handling, labelling and storage

- Always handle the specimen with a gloved hand. Disinfect the surface of all specimen containers received, whether visibly contaminated with specimen or not, with 5% phenol-soaked cotton wool or paper towels.
- When labelling the specimen container, ensure that it is: (i) labelled on the side of the container (never on the lid only); (ii) written clearly with permanent marker; and (iii) contains at least one unique identifier (like Ni-kshay ID) as well as other identifiers such as patient name, gender, and age. The patient information on the specimen request form should match the information on the specimen container.
- Ideally specimens should be processed on the same day of sample collection. However, If a delay is unavoidable the specimens should be kept in the refrigerator (to inhibit the growth of other microorganisms) till further processing.

7.4 Specimen packaging (triple layer packaging)

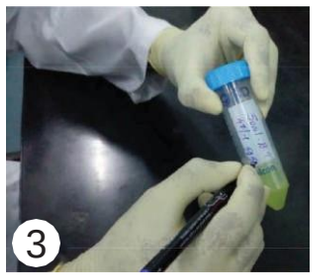
Material required for packing the specimen for transportation. Specimen collection tubes containing the sputum samples; 5% phenol; test tube rack; tongs; parafilm; absorbent cotton; tissue paper; self-sealing covers; thermocol boxes; gel packs; permanent marker pens; scissors; rubber bands; scotch tapes; biohazard stickers etc. It is to be ensured that the coolant gel packs have been frozen prior to packaging samples for transportation. It is to be ensured that the coolant gel packs have been frozen prior to packaging samples for transportation.

Standard operating procedure for specimen packaging under NTEP [44] is as follows:

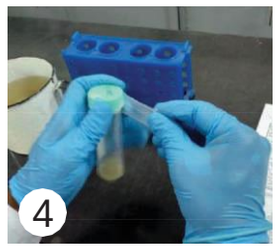
Step 1. Make sure that the specimen collection tube is tightly closed after the sample has been collected from the patient.

Step 2. Wipe the outer surface of the 50 ml conical tube with 5% phenol followed by absorbent tissues and allow it to air dry.

Step 3. Write the patient details on the opaque area (white area) of the specimen collection tube using a permanent marker pen, preferably in capital letters.



Step 4. Cut the parafilm strip, wrap one of the strips at the joint between the cap and the neck of the specimen collection tube such that a secure seal is formed. **(Primary receptacle/ package)**



Step 5. Open the absorbent cotton roll and spread out on the work bench; separate the cotton into two equal layers. Roll the specimen collection tube containing the sample tightly in the absorbent cotton such that the tube is covered completely.

step 6. Put this roll containing the specimen collection tube into the ziplock pouch. Roll the whole into a tight bundle, ensuring that there is no air in the pouch. This bundle should be secured with the rubber bands. **(Secondary receptacle/ package)**

Step 7. Repeat steps 5–7 for the second sample of the patient.

Step 8. Insert the test request form in to a separate ziplock pouch after ensuring that the details on the form and the sample tubes match, with the writing facing outside (details should be visible through the package). Seal the ziplock pouch.

Step 9. Place the pre-frozen gel packs in the thermocol box, followed by the sample tubes packed in ziplock pouches (on the gel packs) and the pouch containing the test request form (on the top). Close the lid of box and wrap tightly with brown duct tape. ***(Tertiary receptacle / package).***

Step 10. Stick the BIOHAZARD sign over the lid and "To and From" stickers on the exterior of thermocol box. Complete the 'From' and 'To' addresses on the stickers, using a permanent marker pen.

Box 7.1: Material checklist for specimen collection and packaging

- Gloves, 5% phenol, sprit/alcohol swab.
- Specimen container (screw capped 50 ml polypropylene tube)
- Permanent Marker
- Parafilm tape
- Sealable poly bags (zipped pouch, white)
- Tissue paper/cotton
- Biohazard stickers
- Brown tape for box packaging
- Scissors
- Rubber bands
- Properly filled test request form

Box 7.2 Technical specification of transportation box

- Thickness of box-2.5 cm
- Outer dimensions:
  Length-18.5cm, breadth-13cm, height-12 cm (without lid), height-14 cm (with lid)
- Inner dimensions:
  Length-14.5cm, breadth-8cm, height-12cm (without lid), height-13cm (with inner part of lid)
- Number of gel pack required: 2
- Weight of fully packed consignment box: 400 grams

In tightly packed thermocol boxes, gel packs maintain a temperature between 12-20^{0}C for up to 48 hours (provided that average outside temperature 35^{0}C; gel packs are pre-freezed at -20^{o} C for 48 hours before use.

Note: This is a single use box, thus thermocol boxes and gel packs should not be reused.

7.1 Transport of specimens

The use of triple packaging is required to safely transport the specimens – that is, the container should be wrapped in absorbent material (cotton or paper towels), protected by secondary packaging (e.g., ziplock pouch) and then placed in shock resistant outer packaging (thermocol box). For rapid NAAT, the majority of samples are tested at TDCs (sub-district level), but some extra-pulmonary samples, such as tissues or biopsies, may need to be transported to C&DST laboratories or IRL because processing the sample (grinding with a mortar and pestle) poses a moderate to high risk of aerosol exposure and thus requires special safety equipment (BSC).

In NTEP, TB specimens are transported across the country by courier, post or human carrier. Before engaging transporter/personnel, the program manager should ensure that All persons transporting specimens are sensitized on i) symptoms of TB disease and its transmission; ii) hand hygiene requirement; iii) precautions to deliver the specimen safely; and iv) response to emergency situation (e.g. spillage).

Box 7.3. Steps to be taken by the transporter in case of accidental damage/ spillage[44,46]

- Inform sender/receiver whoever is nearer.
- Do not leave the damaged/broken container unattended. Cover it with cloth/paper and immediately inform the nearest NTEP laboratory for assistance and spill management as per standard SOP.
- In the event of exposure to any infectious substance, wash hands and affected part with soap and water. Seek medical advice from Medical officer/ District TB Officer.

International transport: Infectious materials are not only transported within country but also across international border. MTB cultures may be needed to ship internationally (e.g. for diagnostic DST or external quality assurance purpose) and therefore adherence to international regulations as well as to specific national import and export regulations is necessary to preserve the integrity of materials, and facilitate their timely arrival at destination. Refer the WHO "Guidance on regulations for the transport of infectious substances 2017–2018".[47]

7.2 Specimen logs, registers and test request forms

Proper documentation of samples being transported and received is critical for tracking and managing referral activities as well as providing a framework for collecting necessary patient information. The common types of documentation include referral logs or registers, specimen registers and test request forms. All forms and registers need to be completed and well maintained. While some laboratories may use electronic registries, the majority of settings still use paper-based systems.

Transport registers: Transport registers help provide a tracking system and should record the name of the referring clinic, the date of transport, the number of specimens being transported, type of specimens transported if the transport system is integrated (e.g., containing blood, urine, or extra-pulmonary tissue or fluids in addition to sputum specimens for TB testing), distance travelled (to assist with budgeting for fuel and managing efficient travel routes), and incidents or accidents during transport that cause delays or promote contamination.

Specimen registers: This register is required for all laboratories and it records the information from the test request form for each patient and queues the specimen into the laboratory testing schedule. Each specimen is assigned a number which is then used as the identifier throughout all testing processes. The specimen identification number ensures patient confidentiality and eliminates preferential queuing for certain clients. The identification number is linked to patient TB registration (Ni-kshay ID) and consequently with the patient's internal records.

Test request forms: It usually contains information about the patient and the tests requested by the doctor. In NTEP, the test request form collects information related to the patient, registration ID, risk factors, reason for testing (diagnosis or follow-up of TB or DR-TB) and requested test. This is the most important form, and it must be completed in order to capture data for routine surveillance activities and proper patient record management.

Chapter 8
HUMAN RESOURCE AND OCCUPATIONAL HEALTH

ABOUT THE CHAPTER

Health workers, including laboratory personnel globally are at elevated occupational risk of tuberculosis infection and disease. This chapter provides guidance on skills and the training required to be competent to work in TB laboratories and the requirements for medical surveillance of the health care workers.

OBJECTIVE

To help the laboratory manager to remain vigilant about occupational hazard and implementing policies that ensure occupational safety.

8. Human resource and occupational health

The risk of developing TB as an occupational disease is well established among health care workers (HCWs). Personnel competence and biosafety training is one of the most important considerations for TB laboratories because poor technical skills can compromise even the best safeguards put in place to protect laboratory workers. Considering risk of occupational exposure, laboratory should have occupational health program in-place, and workers should be provided with occupational medical services such as medical evaluation, surveillance, and treatment, as appropriate.

8.1 Personnel competence

Human error and poor technique can result in aerosol exposure. Thus, competent and safety-conscious laboratory personnel, who understand how to identify and respond to or control laboratory risks, are critical for the prevention of laboratory associated infections and/or other incidents. The competence of laboratory staff includes not only skills that can be taught and developed, but also judgement and the ability to recognize the limitations of the work environment as well as one's own and others' skills in the laboratory.[48] The framework for competencies includes four skill domains (Table 8.1).

Table 8.1: Competencies of laboratory personnel

Domain	Skills
Domain I: Potential hazards	• Biological material • Chemical materials • Radiologic al materials • Physical environment
Domain II: Hazard controls	• Personal protective equipment • Engineering controls – equipment (primary barriers) • Engineering controls – facility (secondary barriers) • Decontamination and waste management
Domain III: Administrative control	• Hazard communication and signage s • Guidelines/SOP and regulatory compliance • Safety program • Occupational health – medical surveillance • Risk management
Domain IV: Emergency preparedness and response	• Emergencies and incident response • Exposure prevention and hazard mitigation • Emergency response – exercises and drills

8.1.1 Potential hazards

This domain focuses on the skills required to understand the hazards in a laboratory setting. Recognizing hazards is the first step toward preventing occupational and environmental exposures. (see also in Chapter 11)

8.1.2 Hazard control

This includes use of primary and secondary barriers (to prevent exposure to hazardous materials) as well as competencies related to decontamination and management of hazardous waste.

Primary barriers include personal protective equipment and specialized laboratory equipment (like BSC, biosafe centrifuge etc.) to protect against accidental exposures. Secondary barriers include actual design and construction features of the laboratory facility to prevent exposure both within and outside laboratory areas. Competencies for waste management include guidelines related to biological, chemical, and radiological hazardous, including decontamination, sterilization, and safe removal of potentially contaminated laboratory equipment (see also in Chapters 3 and 10).

8.1.3 Administrative control

This domain is with a variety of administrative controls that can be used to reduce the duration, frequency, and severity of exposure to hazardous materials or situations. These controls include hazard communication, removal of hazard or use of its safer alternative, safety signages, institutional guidelines and work process training, regulatory requirements, the overall safety program, occupational health and medical surveillance, and the management of risk (see also in section 8.3 and chapter 11).

8.1.4 Emergency preparedness and responses

This domain consists of competencies to manage emergency preparedness and response. Institutions are responsible for referring applicable standards of practice for their operations and for the development of response plans for incidents that affect them. (see also in Chapter 9)

8.2 Biosafety training

It is crucial to ensure that the biosafety practices and operational working procedures are being followed by appropriate laboratory personnel. To ensure this, laboratory management should provide financial and administrative support for training of all laboratory personnel. Following the training, personnel must demonstrate competency before working independently. All laboratory personnel must receive laboratory biosafety training, though the components of the training module may differ depending on the procedural risks (or risk level of TB laboratories) and the nature of the job assigned to them (such as administrative, data entry, handling/ manipulation of biological materials, etc.).

Basic biosafety training should be included into the induction program for new hires in the laboratory. This should be followed by job specific and detailed biosafety and biosecurity training (Table 8.2).[49]

Training should include a combination of didactic classroom training, supervised practical training and exercises, mentorship training, and refresher training.[50] Supervising laboratory staff are responsible for the training of junior staff to a level of competency, thus it may require a mentorship period. Competency of the staff in any procedure must be verified before allowing them to work independently. Once training is completed, supervisors should regularly check work practices, especially for staff that have just completed training. Refresher training for all staff should be undertaken at least once in two years. Training records should be maintained for all the lab personnel.

Table 8.2: Training for Laboratory Personnel

Training	Areas to be covered
General familiarization and awareness training *(Mandatory for all personnel - new hires)*	An introduction to: • Laboratory layout, features and equipment • Laboratory code(s) of practice • Applicable local guidelines and legislative obligations (including biomedical waste management) • Institutional policies • Potential risk/hazard and safety signages • Safety or user manual(s) • Emergency/incident response procedures • Medical surveillance and occupational health
Job specific training	• Training to be determined based on job description or assigned job (including relevant personal protective equipment, safety equipment and requirement of special environment or facility design) • All personnel involved in the handling of biological agents must be trained on good microbiological practices and special technique to avoid or minimize the aerosol generation. • Information on new procedures, equipment, technologies and knowledge must be communicated to concerned personnel as and when available
Safety and security training *(Mandatory for all personnel)*	• Awareness of hazards present in the laboratory and their associated risks • Safe working procedures • Security measures • Emergency preparedness and response s

8.3 Healthcare worker surveillance

Under the employee occupational health program, a baseline medical checkup and provision for regular follow-up should be considered for all staff prior to commencing work in the TB laboratory. It is the responsibility of laboratory management that

- Medical services are readily available to allow timely and appropriate evaluation and treatment.
- Highly susceptible individuals (e.g. pregnant women or immune-compromised persons are excluded from high risk laboratory work.
- Relevant personal protective equipment and facility design is in place.

The medical personnel providing occupational health services should be knowledgeable about the nature of potential health risks in TB laboratories and have access to experts for consultation. Pre-placement medical examination may include symptoms screening, tuberculin skin test (TST) or Interferon gamma release assay (IGRA), Chest X-ray and routine general physical examination. Medical clearance of staff for using N-95 mask may be needed for high-risk laboratories. If a newly inducted laboratory personnel had been radiographed less than 6 months previously, the chest X-ray may not need to be done. Instead, the previous chest X-ray report may be provided to complete the process.

Laboratory staff those are symptomatic or have abnormal chest X-ray should be offered upfront NAAT to rule in or rule out in the first instance, and also during periodic screening. Screening frequency may vary depending on the level of risk exposure (low, moderate or high). Active screening should be recorded with details of who conducted the screening, when and where screening was done and who were screened, the result of screening and who were diagnosed and treated for TB. Active screening of HCWs should be undertaken as per the risk, based on the guidelines indicated in the table below: [51]

Table 8.3: Active surveillance among Health Care workers with variable risk of exposure

Risk of TB Disease	+	++	+++	++++
HCW type	HCW with minimal contact with patients, e.g., storekeeper	DOT provider, pharmacist	LTs at TDCs, laboratory supervisors, ART centre staff, TB/Chest OPD/ IPD staff/doctors/sweepers	LTs at CDST laboratories, MDR-TB ward/staff/nurses/doctors /sweepers
Screening type and frequency	Screening at the time of joining, then annual symptom screening	Screening at the time of joining, then biannual symptom screening	Screening at the time of joining, then annual symptom screening + CXR	Screening at the time of joining, then biannual symptom screening + CXR
Diagnosis Algorithm	NTEP diagnosis algorithm (CXR+NAAT)	NTEP diagnosis algorithm (CXR+NAAT)	NAAT and C-DST (first and second line)	NAAT and C-DST (first and second line)
Note: -- Each TB episode to be notified (entered in Nikshay) -- Ensure to complete the appropriate treatment regime				

Abbreviations: DMC- designated microscopy centre: ART - antiretroviral treatment; CDST – culture

In addition to active TB surveillance, laboratory personnel should be encouraged to present themselves for examination, if they are symptomatic.

Chapter 9
EMERGENCY PREPAREDNESS AND RESPONSE

ABOUT THE CHAPTER

Emergencies can occur at any time and can present a variety of hazards in the impacted area. Therefore, expecting an unexpected situation and preparing to respond efficiently is vital for all laboratories. This chapter focuses on key emergency situations in TB laboratories (like spillage of infectious material) and how to mitigate them.

OBJECTIVE

To enable the TB laboratory to efficiently respond to the emergency situation.

9. Emergency preparedness and response

Safety procedures must include emergency preparedness and response. Staff must be trained and practice responding appropriately to accidents or incidents such as fires or power outages, accidental spill exposures, and the need for emergency medical treatment and evacuation. Emergency preparedness plans should be devised following a risk assessment that evaluates;

- Which laboratory areas are considered to be high risk?
- Which personnel are at risk and which personnel should be involved in responding to incidents?
- What medical treatment and emergency transport is available? and
- Which equipment and supplies are needed for each specific response?

Safety procedures and emergency preparedness plans should be written, readily available, and even displayed at locations visible and easily accessible to all staff. At a minimum, annual trainings on emergency procedures should be implemented, including practical spill exercises. All staff, including house-keeping staff, staff who handle or transport biomedical waste, clerks, and other support staff need biosafety training. A written contingency plan for dealing with emergency and accidents is a necessity in high-risk TB laboratories.

9.1 Emergency preparedness plan

The emergency plan should provide operational procedures for:

- Precautions against natural disasters, e.g. fire, flood, earthquake and explosion
- Biohazard risk assessment
- Incident-exposure management and decontamination
- Emergency evacuation from the premises
- Emergency medical treatment of exposed and injured persons
- Medical surveillance of exposed persons
- Clinical management of exposed persons
- Epidemiological investigation
- Post-incident continuation of laboratory operations

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

- Identification of hazard (e.g. MTB)
- Location of high-risk areas, (e.g. containment laboratory; storage areas of culture isolate)
- Identification of at-risk personnel
- Identification of responsible personnel and their duties, e.g. biosafety officer, laboratory supervisor/microbiologist and lab director.

In addition to this, contact number of emergency services like fire services, hospital/ ambulance, police, water, gas and electricity services should be prominently displayed in the laboratory facility.

- Provision of emergency equipment, e.g. protective clothing, disinfectants, biological spill kits, fire extinguisher and first-aid kits. Basic first-aid procedures are described briefly in **Annexure 11.**

9.2 Emergency response procedures

It is the responsibility of the Laboratory Director/Supervisor to ensure that all laboratory personnel have read and understood the emergency plan, and that all necessary training/drills have taken place. Annual refresher training should also be considered.

All laboratory incidents must be reported to the biosafety officer and laboratory supervisor, in a timely manner. A written record of incidents must be maintained and investigated in a timely manner with information of root cause analysis, corrective action, and preventive action (**Annexure 12**).

Root cause analysis should be focused to identify not only what and how an event occurred, but also why it happened. Understanding why an event occurred is the key to specify workable corrective measure as well as preventive measure that prevent future events of the type observed. Results from incident investigations should be used to update laboratory procedures and emergency response. Response procedure to different emergency situations is as follow.

9.2.1 Puncture wounds, cuts and abrasions

The affected person should remove protective clothing, wash their hands and any affected areas, apply a suitable skin disinfectant and if necessary, seek medical attention. The cause of the wound and the organism/ hazard involved should be reported after adequate laboratory investigations, treatment initiated, and the medical records kept.

9.2.2 Ingestion of potentially infectious material

Medical attention should be sought after removing the protective clothing. The identification of the ingested material as well as the circumstances of the incident should be reported, and appropriate and complete medical records should be kept.

9.2.3 Fire and natural disasters

Fire and other services should be involved in the development of emergency preparedness plans. They should be told in advance which rooms contain potentially infectious materials. It is beneficial to arrange for these services to visit the laboratory to become acquainted with its layout and contents.

When an external emergency response team (such as police or fire service) needs to enter the laboratory building following a natural disaster, they should be warned of the potential hazards in the laboratory. They should only enter the laboratory if accompanied by a trained laboratory worker. When needed, infectious materials should be collected in leakproof boxes or strong disposable bags for transportation to another secure storage area.

9.2.4 Biological spill response

When a spill occurs, the laboratory staff must quickly assess the situation, severity of spill (major/ minor) and decide how to respond. The actions required are dependent on the following variables:

- Where the spill occurred (inside or outside the BSC)?
- The severity of the spill as determined by the type of hazard (e.g. spillage of specimen produces less aerosol than culture; spillage of solid culture produces less aerosol than liquid culture) and the number/amount of live organisms (e.g. solid culture contain less number of live bacilli than liquid culture).

9.2.4.1 Spill kits

The biological spill kits should be readily accessible to laboratory personnel and it should contain the following items (Figure 9.1):

- Instructions (SOP) for spill clean-up
- 5% phenol
- N-95 mask
- Gown or jumpsuit
- Shoe covers
- Gloves (different sizes)
- Autoclave bags
- Absorbent towels/cotton
- Forceps/ tongs
- Biohazard signs for laboratory doors to keep people from entering the room where the spill occurred

Figure 9.1: Spill kit

In addition to keeping a spill kit outside the laboratory, it is important to have a spill kit near the BSC so all supplies are readily available in case a spill occur in the BSC.

9.2.4.2 Spill inside the BSC: Minor spill

Spills that occur inside the BSC are usually less dangerous because they are already contained. If the BSC is working properly and the technologist is using the BSC correctly, they pose little risk to laboratory personnel. In the event of a minor spill inside the BSC, follow the steps described below.

- Let the BSC 'ON' and clean up spill with absorbent paper towel saturated 5% phenol
- Place contaminated absorbent paper towel into a disposable bag while in the BSC, tie the bag and place it in a container to be autoclaved
- Change gloves if they gloves have been contaminated
- Immediately wipe the interior surface and walls of the BSC and any items or equipment within the cabinet with a paper towel soaked with 5% phenol

9.2.4.3 Spill inside the BSC: Major spill

If a large spill occurs in the BSC, then more extensive decontamination needed. First of all, ensure that the drain valve is closed and BSC is kept on. After that, following steps should be taken to clean up the spill

- Change the gloves (as it may be contaminated) and remove all items from BSC after decontaminating their surface.
- Gently pour disinfectant (5% phenol) over the area concentrically (i.e., starting from the outside edges of the spill and then toward the center). Aerosol generation is reduced by using a gentle flooding action.
- Wipe down and remove front and rear grates and work surface.
- Pour disinfectant into the bottom pan.
- Allow at least 30 min for decontamination.
- Wipe down all walls and inside of glass sash.
- Empty the drain pan into a collection vessel containing disinfectants.
- Follow up by wiping all surfaces with 70% alcohol to remove residues.
- Reassemble the grates and work surface in the cabinet.

9.2.4.4 Spill outside biological safety cabinet

Any infectious material spilled outside of a BSC is considered a major event. Infectious aerosols are produced when infectious liquids spill. The risk of spilling a liquid culture, such as a TB positive MGIT vial, is far greater than that of dropping a LJ slant containing TB colonies. When the liquid is splashed, it more easily aerosolizes and can cause dangerous aerosol to become suspended in the air.

If there is spillage outside the BSC, hold the breath and leave the affected area immediately. The laboratory supervisor and biosafety officer should be notified immediately. No one should enter the room for an appropriate period of time (about 4 hours) to allow aerosols and heavier particles to settle. Make sure that the BSC is on. If the spillage area lacks BSC or central air exhaust system, entry should be delayed (for about 24 hour). Signs indicating that entry is prohibited should be placed. After the appropriate time, decontamination should proceed with spill kit and full PPE, and it should be under supervision of biosafety officer.

Broken containers contaminated with infectious substances and spilled infectious substances should be covered with paper towels. After that, a disinfectant (5% Phenol) should be poured concentrically over these and left for at least 30-40 minutes. The cloth or paper towels and the broken material can then be cleared away; glass fragments should be handled with forceps. The contaminated area should then be swabbed with disinfectant. If dustpans are used to clear away the broken material, they should be autoclaved. Cloths, paper towels and swabs used for cleaning up should be placed in a contaminated-waste container. During the entire process, full PPE (respirator, gown, gloves, shoe cover, goggles and head cover) must be worn. If laboratory forms or other printed or written matter become contaminated, the information should be copied onto another form and the original should be discarded into the contaminated-waste container. All potentially infected materials should be autoclaved before removal for final disposal. Anyone who was exposed to the spill should be referred for medical advice and a record should be kept of the incident.

9.2.4.5 Spill cleanup: If tube breakage during centrifugation

Take the following steps to safely clean the spill.

- Tube should be contained within the safety cup
- Place unopened safety cup in BSC
- Let sit undisturbed for 30 minutes
- Disinfect all inside surfaces of centrifuge
- Carefully open the safety cup in which break occurred, place lid next to cup, top down
- Remove uncompromised tubes and disinfect outer surface of tubes by wiping with disinfectant -soaked cotton/gauze
- Pour disinfectant into the cup and lid taking care not to splash
- Let stand for 30 minutes, pour off disinfectant and broken tube into discard container to be autoclaved
- Disinfect the entire surface of BSC
- Wash cup, lid and insert in hot soapy water, remove O-ring to clean underneath, air dry and put the silicone O-ring.
- All infectious waste must be autoclaved before removal from laboratory for final disposal.

9.3 Eye wash and showers in TB containment lab

Emergency shower and eye wash station should be available at a strategic location. Shower and eye wash stations should be hands-free, and they should be checked on a regular basis to ensure adequate water supply.

9.3.1 Splash to face response (Eye wash station)

Flush affected area in eye wash for 15 minutes. If an eyewash is not immediately available, use another source of clean water and follow up with a full 15-minute eyewash as soon as feasible. Each laboratory shall contain a facility for hand washing and an eye wash facility which is readily available within the work area.

9.3.2 Shower

Although an emergency shower is primarily to deal with exposure to chemical hazards, it may be used to help disinfect personnel who may have been exposed to a large volume of biological agent.

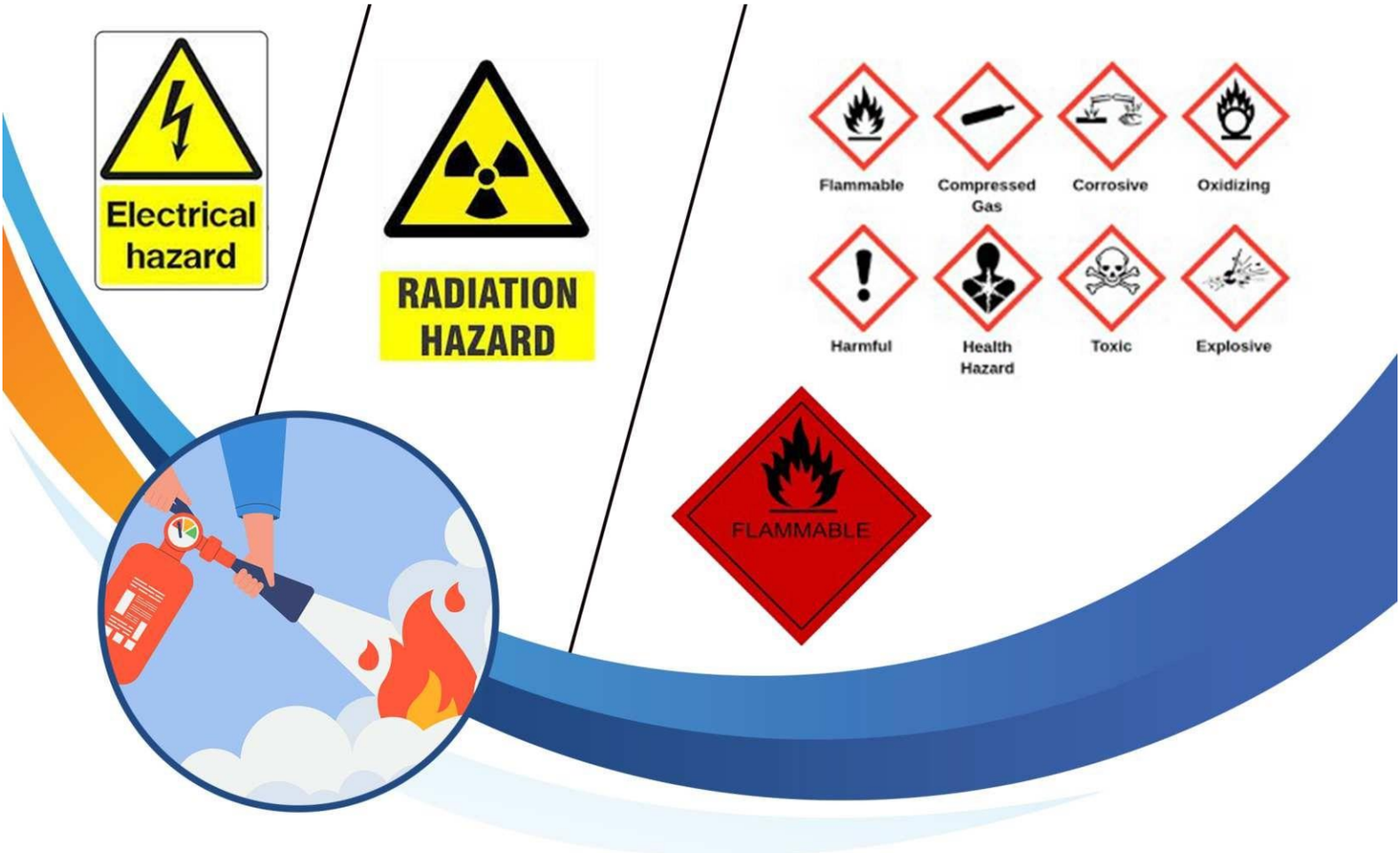
9.4 Electric backups (un-interrupted power supply)

A central UPS console should be provided to cater to the extreme essential power requirement of the laboratory. All critical components like lights, door Interlocks, exhaust blowers of BSCs and critical equipment should be provided with UPS that support the air-handling unit (AHU) of TB containment facility for 30 minutes.

Power required for the TB containment laboratory should be tapped from the existing feeder lines (through its expansion and laying of required power cablings) or panels. Three phase power supply and with proper earthing is required for AHU Unit of TB containment lab. Adequate provision for power back up in the form of diesel generator set of about 120-150 KVA capacity (to be re-calculated based on requirement at time of procurement/assessment) is important to keep the lab functional all time.

9.5 Substitution in case of equipment failure

Backup of critical equipment (BSC, biosafe centrifuge, thermocycler, micro-centrifuge, incubators, GT-Blot, MGIT etc.) need to be placed and maintained to tackle emergencies due to equipment failure/damage.



Chapter 10
CHEMICAL, FIRE, ELECTRICAL AND RADIATION SAFETY

ABOUT THE CHAPTER

Chemical, fire, electrical, and radiation risks (in addition to biosafety risks) are inherent in TB laboratories. Hence, a careful planning with an emphasis on safety can help the laboratory personnel in dealing with emergency situations, saving lives and minimizing losses. This chapter discusses the safety and response strategy to the aforementioned emergencies.

OBJECTIVE

To ensure that laboratory personnel are aware of safety and how to respond to emergencies such as chemical, fire, electrical and radiation incidents / accidents.

10 Chemical, fire, electrical and radiation safety

It is imperative for the lab personnel (lab technicians, microbiologists etc.,) to have full understanding of the hazards and risks.[49] The hazards frequently encountered are given in Figure 10.1

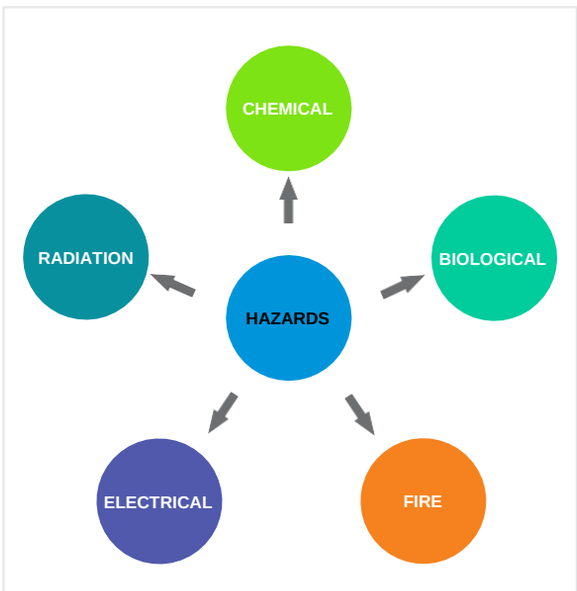


Figure 10.1: Classes of hazards

10.1 Chemical hazard

Handling reagents is a routine activity, but hazardous chemicals can cause physical and/or health threats to the lab personnel. Hazardous chemicals can often be identified from their labels, which could state “Danger,” “Warning,” “Caution” or words to that effect, or the label could have a symbol which indicates a hazard.[52] The chemicals can enter the body through respiratory system, skin absorption or ingestion.[53] Chemicals will have different hazard signs, depicted with pictograms as shown in Figure 10.2.

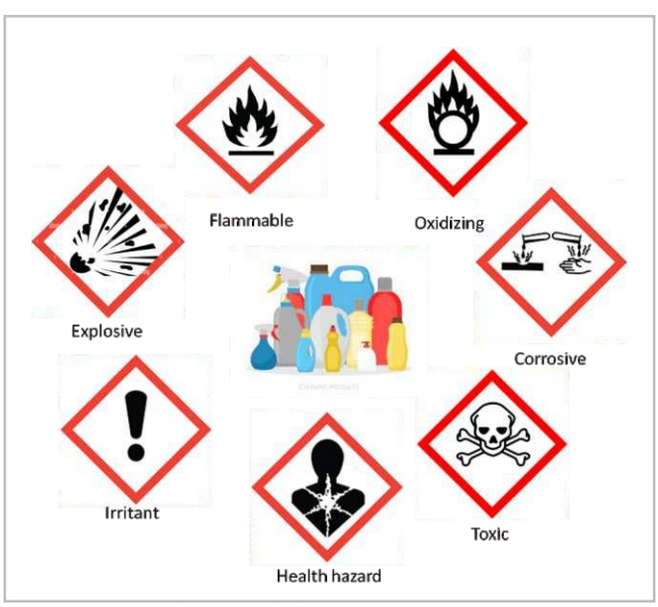


Figure 10.2: Hazard signs

10.1.1 Safety practices

- Use appropriate container and check its integrity regularly. Minimize clutter in working areas to avoid inadvertent exposure.
- All the containers with chemicals should be labeled clearly. Labels on chemical containers must not be removed or defaced.
- Chemical containers should be kept closed when not in use, and with restricted access to relevant laboratory personnel.
- After finishing the work, the work bench must be cleaned, and leftover chemicals are to be removed or disposed properly.
- Pipetting by mouth must be strictly forbidden. Maintain hand hygiene.
- Storing foods and drinks anywhere in the laboratory working areas must be prohibited.
- All laboratories must have access to a spill kit and first aid box.
- Emergency equipment, such as eye wash station, safety shower, fire extinguisher must be easily accessible, and periodically checked to ensure that they are functional.

10.1.2 Chemical inventory, procurement and storage

- Identify all the chemicals that will be encountered in the lab, and inventory list should be maintained. Chemicals that are commonly used in TB laboratories are provided in **Annexure 13** along with potential risks and precautions.[54]
- Safety data sheets (SDS) for chemicals received by the laboratory must be supplied by the manufacturer, distributor or importer and must be maintained and readily accessible to lab personnel. The information that can be obtained in the SDS are given in Figure 10.3.

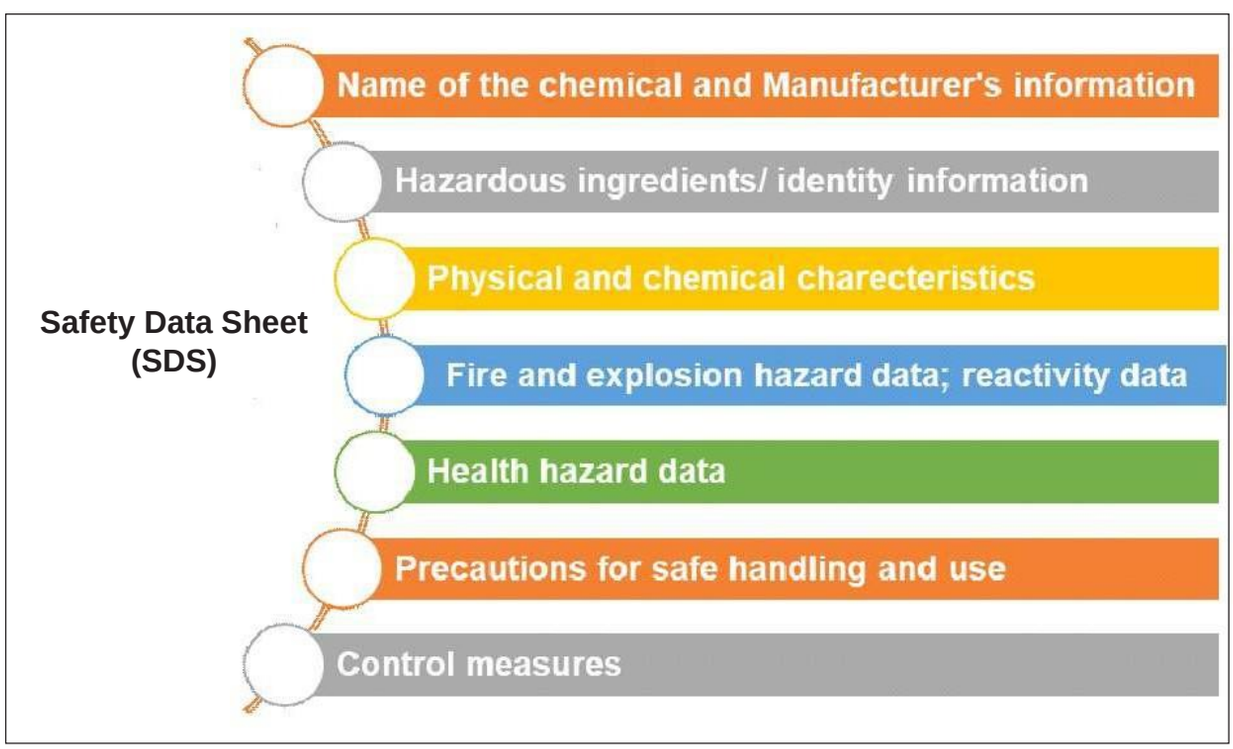


Figure 10.3: Components of safety data sheet

- In cold rooms where air re-circulate, keep all toxic and flammable substances tightly closed.[55]
- Chemical necessary only for daily use should be stored in the laboratory. Bulk stocks should be kept in specially designated rooms or buildings. Chemicals should not be stored in alphabetical order. [33]
- A clearly labelled folder with hard copies of SDS of all the chemicals should be kept accessible to the lab personnel.
- Chemical should be arranged properly on the shelves.
- Do not store chemicals in direct sunlight or next to heat sources. No chemicals should be stored above eye level.
- Avoid storing incompatible chemicals together as that can result in a fire or explosion or release of toxic gases. See Table 10.1 for chemical storage recommendation.

Table 10.1: Chemical storage recommendation

Type of chemical	Storage
Flammable	Store in approved safety cans or cabinets. Do not store incompatible materials in the same cabinet. Keep away from any source of ignition: heat, sparks, or open flames. Flammable solids must be segregated from flammable liquids
Acids	Do not store with flammable solvents or combustibles. Ideally, store in a cabinet designed for acids; do not store acids on metal shelving.
Bases	Store in corrosives cabinet or on protected shelving away from acids.
Light Sensitive Chemicals	Store in amber color bottles in a cool, dry, dark place.

- Inspect all incoming shipments to ensure proper labels are attached, accurate and that the containers are intact and in good condition.
- Never store food and drink in refrigerators and freezers used to store chemicals. Refrigerators used to store chemicals should be labeled as “Chemicals Only – No Food”.

10.1.3 Hazard control

The control measures are as follow:

Engineering controls: Use fume hoods if the chemical's are highly volatile and boiling point is below 120^{0}C or highly volatile. Keep the chemicals away from water source .

Administrative controls: Make sure appropriate signages are in place and SOPs are available and followed.

PPE: Make sure that appropriate PPE (such as lab coats, gloves etc.) are used when handling the hazardous chemical.

10.1.4 Spill response

Chemical spills must be cleaned up as soon as possible by properly protected and trained personnel. Clean up spills using contents of the laboratory spill kit. Spill kits with appropriate instructions, adsorbents, and protective equipment must be made available in the laboratory so that laboratory employees may safely clean up a minor chemical spill found in that lab.

Equipment in the spill kit could include:

- Wisk broom and dust pan (available at home improvement stores)
- Sponge
- pH paper
- Bags - for collection of spill cleanup material
- Small and large Ziploc bags – for collection of spill cleanup material or to enclose leaking bottles/containers.
- Safety goggles
- Thick and thin Nitrile gloves
- Hazardous waste labels

Minor chemical spills (10-50ml of spill)

- Alert people in the immediate area of the spill. Avoid breathing vapors from spill. Turn off ignition and heat sources if spilled material is flammable. Put on appropriate PPE , such as safety goggles, suitable gloves, and long-sleeved lab coat. Confine spill to small area. Slowly add absorbent material (Table 10.2) on and around the spill and allow the chemical to absorb. Use appropriate kit to neutralize and absorb acids and bases spill before its final disposal.

Table 10.2: Neutralizing/absorbent materials for chemical spill

Type of chemical	Absorbent material for neutralizing
Acid and corrosive chemicals	Sodium carbonate – Na_2CO_3 Sodium bicarbonate – $NaHCO_3$
Alkali	Sand

Major Chemical Spill (>50ml-5 liters)

When a spill occurs that you are not capable of handling:

- Alert people in the immediate area of the spill and evacuate the room
- If an explosion hazard is present, do not unplug, or turn electrical equipment on or off -doing so can result in a spark and ignition source
- Confine the hazard by closing doors as you leave the room
- Use eyewash station or safety showers as needed to rinse spilled chemicals off people or yourself
- Evacuate any nearby rooms that may be affected
- If the hazard will likely affect the entire building, then evacuate the entire building by activating the fire alarm

Mercury spills: Do not dispose of mercury or mercury contaminated spill debris in the regular trash or down the drain. There is no absorbent material available. Physical removal processes are best for removing and collecting mercury. Cover small droplets in inaccessible areas with powdered sulfur. Place the residue in a labeled container and dispose it separately. Use appropriate kit or spill pads for other chemicals. Collect residue, place in appropriate container, and dispose as chemical waste. Clean spill area with water. All efforts need to be taken to minimize the use of mercury containing devices in the lab.

Hazardous material splashed in the eye

Immediately rinse eyeball and inner surface of eyelid with running water for 15 minutes. Forcibly hold eye open to effectively wash behind eyelids. Use eyewash station or tap/sink for washing. All work-related injuries and illnesses (including chemical spills onto the body), regardless of the severity, must be reported to the supervisor.

10.1.5 Medical consultations

When a chemical exposure occurs, medical consultations and medical examinations should be made available to laboratory workers who work with hazardous chemicals as required. All work-related medical examinations and consultations should be performed by or under the direct supervision of a licensed physician of the institute or nearby health care facility.

An accurate record of exposure monitoring activities and exposure measurements as well as medical consultations and examinations, including medical tests and written opinions should be maintained for each individual in the lab.

10.1.6 Do's and Don'ts of chemical safety

Laboratory personnel should consider following do's and don't when handling or storing the chemicals.

Table 10.3: Do's and don'ts of chemical safety

Do's	Don'ts
Know the chemical hazards and the first aid method using the Safety Data Sheet before handling.	Don't store incompatible chemicals together.
Post the chemical compatibility chart near the chemical storage area.	Don't leave chemicalcontainers with the lid open unattended.
Chemicals reactive to water should not be stored outside of the shed or where there is a risk of exposure to water.	Don't mix different wastes or contaminated chemicals in the same container to avoid violent reactions.
Label all chemicals used and seal the container tightly.	Don't try to siphon the chemical through your mouth.
Train all the people who are responsible for handling chemicals.	Acid canisters should never be moved without protection.
Follow standard procedure for handling, storing, and disposing of chemicals.	Don't store leaked or damaged containers.
Maintain good housekeeping in and around the chemical storage area.	Don't store chemicals near heat or flames.

10.2 Fire hazard

Heat, fuel and oxygen must combine in a precise way for a fire to start and continue to burn.[56] The key to preventing fires is to keep heat and ignition sources away from materials, equipment and structures that could act as fuel to complete the fire triangle (Figure 10.4).

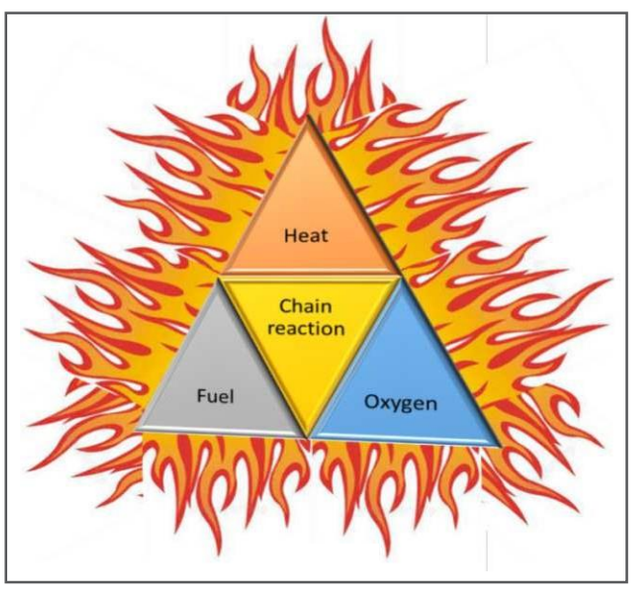


Figure 10.4: Fire triangle

10.2.1 Sources of fire

Small bench-top fires in lab spaces are typical and not uncommon. Large lab fires are rare. Flammable liquids and hazard levels in labs are variable. Labs using solvents in any quantity, have a very high potential for flash fires, explosion, rapid spread of fire, and high toxicity of products of combustion (heat, smoke, and flame).[53]

Classification of fire and the example for each class is given in Table 10.4

Table 10.4: Classes of fire

Class	Category	Examples
A	**Ordinary combustibles**	paper, trash, some plastics, wood and cloth etc.,
B	**Flammable liquids or gases**	acetone, methanol, ethanol, LPG etc.,
C	**Electrical equipment**	autoclaves, incubators, refrigerators, or freezers, UPS, incinerators etc.,
D	**Flammable metals**	Sodium, potassium, calcium, magnesium etc.,

10.2.2 Safety practices

Following SOP makes work organized and prevents lab fires due to procedural errors.

- Wear proper clothing and personal protective equipment.
- No-smoking signs posted in all regulated areas
- Aisles, doors, and access to emergency equipment should be always kept unobstructed.
- Work areas should be uncluttered and cleaned. Minimum quantity of materials as required for the procedure should be kept in the work area.
- Store solvents properly in approved flammable liquid storage cabinets.
- Combustibles should be kept away from open flames.
- All the lab personnel should be trained to implement the emergency plan and learn to use the emergency equipment provided. Training is required upon employment and at least annually thereafter.
- Fire-prevention training sessions should be documented. If an outside organization conducts the training, obtain training certificates for the attendees.
- Fire drills to be conducted at least annually and all staff should participate. Every fire alarm that sounds should be treated as a true emergency.

10.2.3 Hazard response

Remember the “RACE” rule in case of a fire:

- R= Rescue/remove all occupants
- A= Activate the alarm system
- C= Confine the fire by closing doors
- E= Evacuate/Extinguish

Small fires:

Small fires are defined as those fires confined to a specific, small area or piece of equipment where flames cannot easily reach other combustibles. These types of fires can be extinguished without evacuation. However, an immediate readiness to evacuate is essential in the event the fire cannot be controlled. If the conditions, as shown in figure 10.5, are not fulfilled, then laboratory workers should evacuate the premises and call fire department immediately.

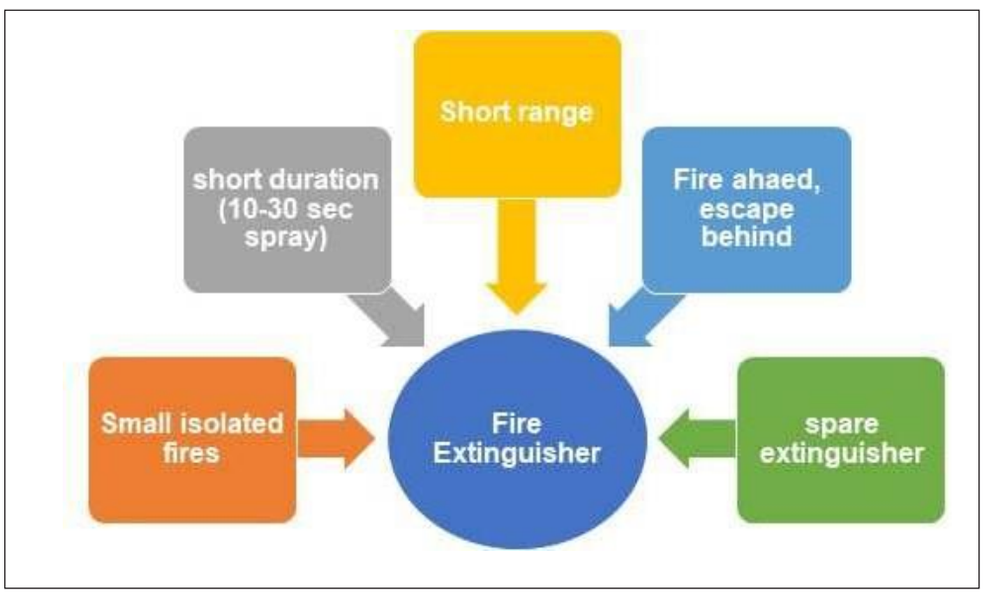


Figure 10.5: Considerations for use of fire extinguisher

Large fires

- Fire-fighting equipment should be placed near room doors and at strategic points in corridors and hallways. This equipment may include hoses, buckets (of water or sand) and a fire extinguisher.
- Emergency exit plan for safe evacuation should be displayed along with marked Fire exit, evacuation route, fire alarms, location of fire extinguishers.
- Elevators should not be used in any circumstances. Walk down the stairs in a single file do not panic, do not push or block others leaving.
- Call fire service emergency number and inform the location and extent of the fire.

10.2.4 Fire extinguisher and proper usage

The lab personnel should know the components and operation of fire extinguisher. Before using an extinguisher, all lab personnel should be trained and familiar with the PASS (pull, aim, squeeze, sweep) method of firefighting (Figure 10.6).

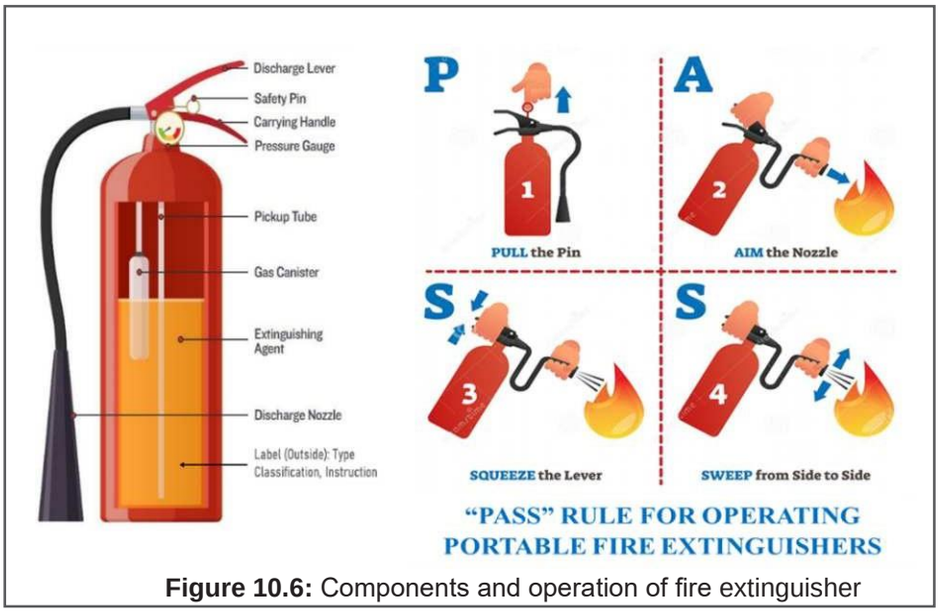


Figure 10.6: Components and operation of fire extinguisher

There are different types of fire extinguishers designed to put out the different classes of fire (Figure 10.7). The wrong extinguisher may make a fire emergency worse. The two most common types of extinguishers in the laboratory are pressurized dry chemical (Type BC or ABC) and carbon dioxide.[53] Co_2 extinguishers are preferred for electronics such as computers and scientific instrument as they don't leave any harmful residue.

Figure 10.7: Types of extinguishers and their use

Each fire extinguisher should be inspected monthly to make sure it is in its designated location and has not been tampered with or actuated. Each fire extinguisher should be clearly visible with nothing obstructing or obscuring it from view.[56]

All fire extinguishers should be examined at least yearly and/or recharged or repaired to ensure operability and safety. A tag as shown in Figure 10.8 should be attached to show the maintenance or recharge date and the signature or initials of the person performing the service.[53]

Figure 10.8 Example of tag and guage of fire extinguisher

- Observe that pressure is at the recommended level on extinguishers equipped with a gauge that means the needle should be in the green zone - not too high and not too low.
- The pin, nozzle, tamper seal and other parts are intact.
- Recharge all extinguishers immediately after use regardless of how much they were used.

10.2.5 Personal injury

- If the floor is not on fire, STOP, DROP and ROLL to extinguish the flames or use a fire blanket or a safety shower if not contraindicated (i.e., there are no chemicals or electricity involved).
- If a coworker's clothing catches fire and he/she runs down the hallway in panic, tackle him/her and smother the flames as quickly as possible, using appropriate means that are available (e.g., fire blanket, fire extinguisher).
- Obtain immediate medical attention.
- Report the incident to the Safety officer.

10.2.6 Do's and Don'ts of fire safety

Laboratory staff should consider following (Table 10.5) do's and don't related to fire safety .

Table 10.5: Do's and don'ts of fire safety

Do's	Don'ts
Evacuation drills should be conducted at regular intervals.	Don't mix contaminated chemical inhibitor sand with other wastes to avoid fire hazard.
Always keep a bucket of water and sand ready	Don't leave open flames unattended
Know the locations of fire extinguishers, fire alarm pull stations, and exits.	Don't engage unqualified contractors for the servicing of fire fighting system.
Know your building's evacuation plan.	Don't use elevators during an evacuation.
Keep means of escape cle ar of obstructions.	Don't ignore any building alarm.

10.3 Electrical hazard

A TB lab contains wide variety of electrically powered equipment including microscopes, stirrers, shakers, centrifuge, biosafety cabinets, autoclaves and even computers and air-conditioners. While electricity is in constant use within and outside the laboratory, mishandling or damage can cause significant physical harm or death.

All electrical points in the laboratory needs to be checked periodically. Faulty electrical equipment/instruments wiring, damaged receptacles and connectors, overloaded circuits or unsafe work practices present a serious safety risk and can cause incidents and injuries if left ignored.[53] The electrical hazards produced are shown in figure 10.9.

It is important that miniature circuit-breakers (MCB) and earth leakage circuit breaker (ELCB) are installed. MCB does not protect people;they are intended to protect wiring from being overloaded with electrical current and hence to prevent fires. ELCB is intended to protect people from electric shock.

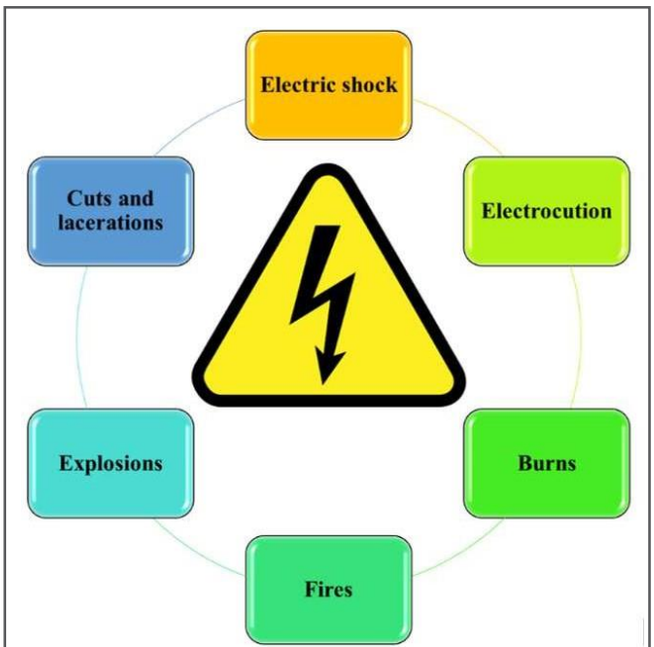


Figure 10.9: Electrical hazard

10.3.1 Sources of hazard

The list of different sources that can lead to electrical hazard are as shown in Figure10.10

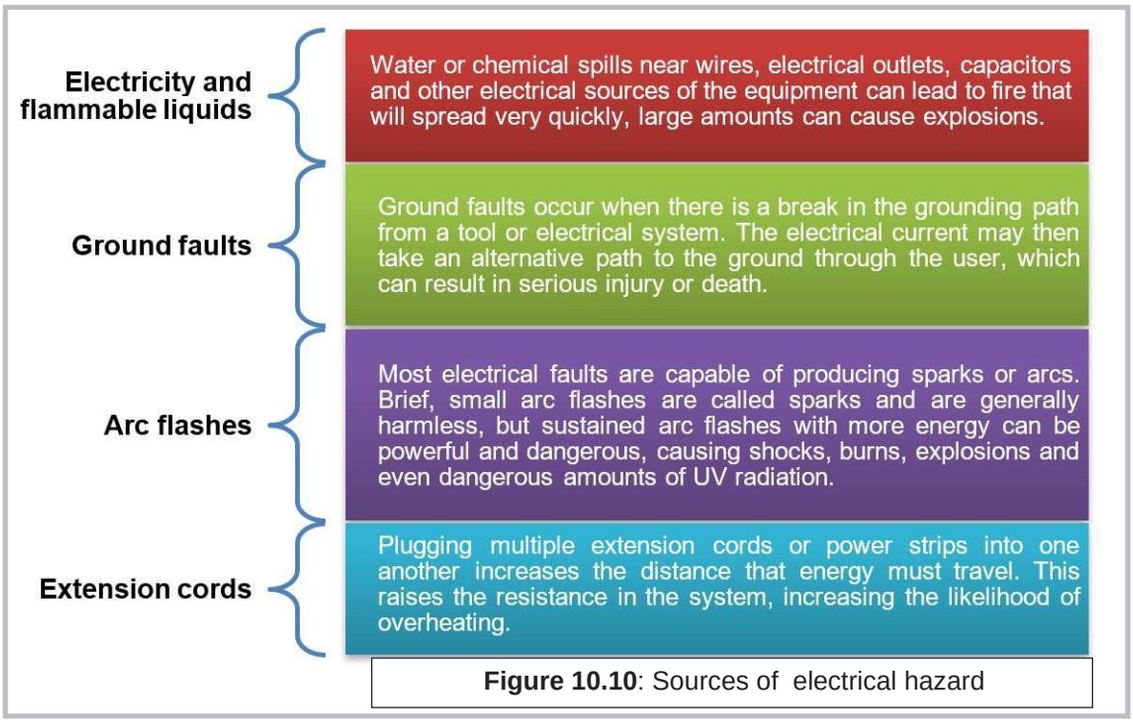


Figure 10.10: Sources of electrical hazard

10.3.2 Safety practices

- Always follow manufacturer's recommendations for using electrical equipment. Inspect equipment wiring prior to use.
- Replace worn or damaged cords immediately
- Only equipment with three prongs (ground) should be used in the laboratory.
- Avoid extension cords and multipoint sockets. There should be a dedicated socket for each equipment.
- Minimize the chances of water or chemical spills near electrical equipment
- Ensure that Ground-Fault Circuit Interrupter (GFCI) outlets are installed and used when water is present within 6 feet
- Conduct a periodic inspection of laboratory electrical equipment to be sure that they are in good condition. Replace frayed cords promptly and properly coil up loose cords

10.3.3 Hazard response

- Know the location and how to operate shut-off switches and/or circuit breaker panels. Use these devices to shut off equipment in the event of a fire or electrocution.
- If you see a person being electrocuted, DO NOT TOUCH him /her! The electricity can go through you, too. If possible, turn off the power (pull the plug or trip the circuit breaker), or use an item made of non-conductive material (e.g., wooden broom handle) to pry him or her away from the contact.
- Obtain immediate medical attention. Report the incident to the Safety officer.

10.3.4 Do's and Don'ts of electrical safety

Laboratory staff should consider following (Table 10.6) do's and don't related to electrical safety .

Table 10.6: Do's and don'ts of electrical safety

Do's	Don'ts
All electrical equipment and electrical equipment must comply with the classification of electrical hazard.	Don't plug too many electric appliances in one socket.
Before servicing o r maintenance , completely drain or bleed and flush lines of equipment.	Don't hide any information concerning hazards in the premises or laboratory make them known to all.
Correct rated wire and electrical appliance etc. should be used.	Don't use temporary wiring.
Faulty electrical appliances should be repaired or replaced immediately.	Don't touch anything electric al appliances or equipment's with wet hands.
Read and follow manufacturer instructions for electrical equipment	Don't let cords get twisted or tangled.
Report any electrical tool, equipment, or wire problems immediately	Don't try to poke, probe, or fix electrical equipment with objects like pencils or rulers because the metal in them can serve as a form of conductor.
Clearly label all circuit breakers and fuse boxes.	Don't use nails to tape cords to the wall or floor.

10.4 Radiation hazard

Non-ionising radiation is found in a wide range of occupational settings and can pose a considerable health risk to potentially exposed workers if not properly controlled.[53] The hazard is dependent on the frequency. The hazards can be either thermal or photochemical as shown in figure 10.11.

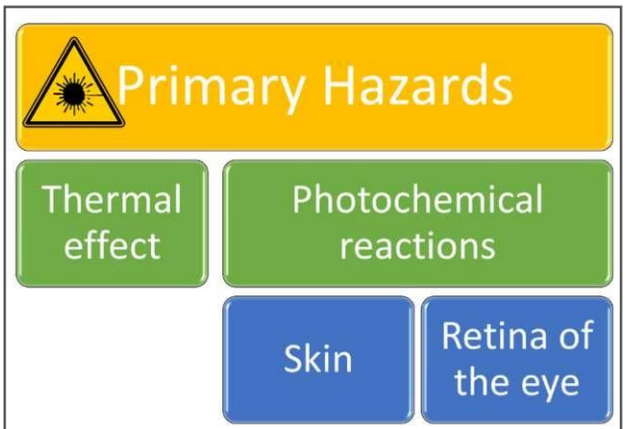


Figure 10.11: Hazards of non-ionizing radiation

10.4.1 Sources of hazard

There are many sources of non-ionizing radiation such as microwaves ovens, UV lamps, fluorescent objects. UV radiation sources in the TB laboratory include BSC and transilluminators (Figure 10.12).

Figure 10.12: Sources of UV radiation

10.4.2 Safety practices

Precaution to be taken near BSC include:

- Avoid working in or around the safety cabinet when the UV light is on or avoid using the room when UV light is on.
- Always close the sash completely when the UV light is on. Even small opening of the sash can cause skin damage and other biological effects.
- Ensure the UV light is off prior working at the cabinet. Control access to the room/UV light area while the lamps are operating to prevent exposure.
- PPE should be always worn when there is potential for UV exposure.

Laboratories using UV equipment should consider the following [57]

1. Engineering controls: UV can easily be shielded by materials such as polycarbonate, metal, cardboard, and wood. Ordinary glass blocks most UV light of wavelengths less than 330 nm but may also transmit most of the UV for longer wavelengths. It should not be relied for UV protection unless UV shielding is verified.

2. Administrative controls: UV exposure may also be minimized by limiting exposure time and increasing the distance between personnel and the UV source. Access to the UV radiation area/UV room/lab should be limited to only authorized personnel who is properly trained on the procedures. If experiments using the UV radiation is conducted in shared spaces, all occupants must receive prior notification and warning signs must be clearly posted.

3. PPE: The suitable PPE include

- Long-sleeved clothing to protect arms, hands and neck
- Nitrile Gloves
- A polycarbonate face shield and/or eyeglasses (wrap around lens) with Z87 marking (ANSIZ87.1 UV certification) must be worn to protect the eyes and face.

4. Signs and labels:

Any equipment that emits UV radiation must be conspicuously labeled with a caution label.

A warning sign must also be posted on entrances to the lab/ room during UV irradiation and/or on the BSC.

Figure 10.13: UV hazard signage

Safety precautions- microwave oven

Most microwaves have intact doors and door seals that do not produce excessive microwave leakage. The following control measures or care must be taken while using microwaves.[58]

- Do not use a metal stirrer or plastic-coated magnetic stirrer bars, aluminum foil wires, cables in a microwave oven.
- Do not attempt to heat flammable liquids or solids, hazardous substances or radioactive material in a microwave oven.
- Do not heat-seal containers; pressure can build up can cause an explosion either in the oven or shortly after removal.
- Do not use plastic containers in a microwave oven.
- Do not overheat liquids in a microwave oven. It is possible to raise water to a temperature greater than the normal boiling point; when this occurs, any disturbance to the liquid can trigger violent boiling that could result in severe burns.
- No unauthorized repairs on a microwave oven should be allowed or permitted. If a unit is suspected to be malfunctioning, disconnect it from the power supply, remove it from service and label it with an appropriate tag while awaiting repair or disposal. If the door hinges, latch or seals are damaged, it is safer and cheaper to replace the entire unit.

10.4.3 Hazard response

The skin or eye injury will heal relatively quickly, but a doctor should assess the severity of the injury and can prescribe medications to reduce the pain of the injury. Report the incident to the Laboratory Safety officer

10.4.4 Do's and don'ts of radiation safety

Laboratory staff should consider following (Table 10.7) do’s and don’t related to radiation safety .

Table 10.7: Do’s and don’ts of radiation safety

Do’s	Don’ts
Make sure you are aware of the differer potential sources of radiation in you workplace	Don’t override any interlocks preventin access
Ensure appropriate shielding andPPE is used to reduce exposure	Don’t expose any object beyond the threshold limit

Chapter 11
MONITORING AND EVALUATION

ABOUT THE CHAPTER

An effective monitoring and evaluation program assesses the effectiveness of a laboratory's biosafety and biosecurity measures. This chapter focuses on the monitoring and evaluation program component in TB laboratories, as well as its effective implementation and review to ensure safe and secure laboratory operations.

OBJECTIVE

To guide TB laboratory managers in identifying risks/hazards, implementing appropriate preventive measures, and monitoring them on a regular basis in order to create a safer workplace.

11. Monitoring and evaluation

11.1 Baseline facility risk assessment

Risk assessment is a systematic process of gathering information and evaluating the likelihood of mishappening again and consequences of exposure to or release of workplace hazard(s) and determining the appropriate risk control measures to reduce the risk to an acceptable risk (Figure 11.1).

Figure 11.1. The risk assessment framework
Source: Adapted from WHO (2020)[49f]

The various steps of the risk assessment process are as follows

11.1.1. Gather information (Hazard identification)

Key information to be gathered should include about:

- The biological agents and other potential hazards (e.g. transmission, infectious dose, treatment/preventive measures, pathogenicity).
- The laboratory procedures/activity that might cause exposure to biohazard to be used (e.g. culturing, centrifugation, waste handling, frequency of performing the laboratory activity).
- The types of personal protective equipment (PPE) to be used (e.g. gloves, masks, gown)
- The types of equipment to be used (e.g. centrifuges, autoclaves, biological safety cabinets).
- The type and condition of the facility where work is conducted (e.g. TB containment lab).
- Relevant human factors (e.g. competency, training, experience and attitude of personnel).
- Any other factors that may affect laboratory operations

All the above-mentioned information collectively informs a much broader, multi-factorial evaluation of risk that may exist in the laboratory. A suggestive template with filled information for different procedures and different risk-levels of TB laboratories under NTEP are provided in **Annexure-11** (11.A, 11.B and 11.C).

11.1.2 Evaluate the risks

After gathering all available information on the circumstances of the work to be performed, it is necessary to use that information to identify and evaluate any risks that exist.

The goal of the risk evaluation step is to:

- Determine the likelihood of an exposure to and/or release of a biological agent occurring and the severity of the consequences of such an event
- Establish how the likelihood and consequence contribute to the initial risk of the work to be performed
- Decide, based on the gathered information of the risk assessment, whether these risks are acceptable or not; this decision must be justified and documented comprehensively.

If the evaluated risks are not acceptable, those performing the risk assessment should develop an appropriate risk control strategy.

11.1.3 Develop a risk control strategy

There are several different strategies that may be used to reduce and control risks. Often, more than one risk control strategy may need to be applied to reduce the risks effectively. Table 11.1 shows the most common strategies employed for risk control and examples of the risk control measures

Table 11.1: Strategies for risk control

Strategy	Example
Isolation	Isolate the hazard: • Elimination and reduction might not be possible, particularly in clinical setting, therefore isolate the biological agent(s) (e.g. in a primary containment device)
Protection	Protect personnel/the environment • Use engineering controls (e.g. BSC) • Use PPE • Vaccinated personnel
Compliance	Have administrative controls and effective biosafety programme management in place such as: • Good microbiological practices observed by personnel • Good communication of hazards, risk and risk control measures • Appropriate training • Clear SOPs • An established safety culture
Abbreviations: BSC- Biological safety cabinet; PPE- Personal protective equipment; SOPs- Standard operating procedures.	

Source: From WHO (2020)[49]

11.1.4 Implement risk control measures

Once a risk control strategy has been developed, risk control measures must be selected and then implemented to fulfill the risk control strategy. While selecting control measures, following should also be taken into consideration

- Measures that are required by national legislation or regulations (if any).
- Measures that are recommended by national/international guidelines, policies and strategies (if any).

It is important to note that risk can never be completely eliminated unless the work is not performed at all, so it should be reduced to acceptable levels (Table 11.2). Most of the clinical and diagnostic laboratory work will require the core requirements that are safety practices to effectively control the risks.

Table 11.2 Examples of laboratory activities, their initial risk, and risk control measures

Procedure	Initial risk (Likelihood/ consequence)	Risk control measures(s)
Smear microscopy or rapid NAAT of sputum specimen	Low (unlikely/ moderate)	Low risk control measure - LRCM
Processing of sputum specimen for culture	Medium (possible/ moderate)	Moderate risk control measure -MRCM (e.g. LRCM plus BSC and respirators)
Culture in liquid media for strain characterization a nd DST	High (likely/ moderate)	High risk control measures - HRCM (e.g. MRCM plus containment lab)
Note: After implementing risk control measures, the residual risk should be evaluated; if the residual risk is acceptable, work can proceed.		

Source: Adapted from WHO (2020)[49]

Evaluate the residual risk that remains after risk control measures have been selected to determine if the risk is now acceptable and whether work should proceed. If the residual risk is still unacceptable, further action is necessary such as additional risk control measures, based on the initial risk evaluated in STEP 2, redefining the scope of work such that it is acceptable with existing risk control measures in place or identifying an alternative laboratory with appropriate risk control strategies already in place that is capable of conducting the work as planned.

11.1.5 Review risks and risk control measures

Biosafety precautions already in place should be reviewed at least annually; they should be revised when necessary, following a risk assessment, and always after the introduction of a new procedure or technique. Typically, an annual review is adequate; however, some situations may prompt an ad hoc review, such as a biosafety incident, or feedback from the laboratory personnel on the effectiveness and ease of use of the risk control measures that have been implemented. Recording the results of the reassessment is also important to document the decisions made, which will facilitate future reviews and performance evaluations.

Table 11.3 covers an example of low-risk laboratory work involving smear preparation and microscopy of sputum specimens.[49]

Table 11.3: Example of the risk assessment process flow

Step 1 Gather information	**Step 2** Evaluate risk	**Step 3** Develop a risk control strategy	**Step 4** Select and implement risk control measures	**Step 5** Review risks and risk control measures
Routine smear preparation and microscopy of sputum specimens Biological agent with a low infectious dose transmitted through aerosols Conducted by competent personal in a diagnostic laboratory	**Low** Specimen volume and concentration are small Aerosol production is unlikely Slide containing the smear has been heat-fixed resulting in partial inactivation	**Core requirements** Core requirements should be adequate to bring this low risk to an acceptable risk	Prepare SOPs on good microbiological practices and core requirements Ensure proper operation and maintenance of microscope, including written SOPs Train personnel on SOPs	Observe laboratory work to ensure good microbiological practices and core requirements are followed Conduct a review in the event of an incident, or changes to the characteristics of the biological agent or the procedures

Source: From WHO (2020)[49]

Refer to annexure 16 for detailed TB Laboratory Biosafety supervisory and monitoring Checklist under National TB Elimination Programme.

Chapter 12
LABORATORY BIOSECURITY

ABOUT THE CHAPTER

Laboratory biosecurity refers institutional and personnel security measures to prevent unauthorized access, loss, theft, misuse, diversion or intentional release of biological agents and/or the equipment, skills and data being handled in the laboratory. This chapter focuses on biosecurity measures relevant to TB laboratories under National TB Elimination program setting in India.

OBJECTIVE

To help the laboratory manager and program manager to conduct risk assessment, identify the potential biosecurity risk, develop and implement effective control strategy for individual TB laboratories.

12. Laboratory biosecurity

Biosafety and biosecurity are concepts that are related but not identical. While biosafety aims to protect personnel and the environment from potentially hazardous biological agents, biosecurity intends to prevent unauthorized access, loss, theft, misuse, or intentional release of potentially hazardous biological agents. As per WHO (2020),[49] laboratory biosecurity may be defined as "Principles, technologies and practices that are implemented for the protection, control and accountability of biological materials and/or the equipment, skills and data related to their handling." Dealing with laboratory biosecurity risk is similar to and complementary to dealing with biosafety risk in many ways. Effective biosafety practices serve as foundation for laboratory biosecurity, and both are implemented in an integrated manner.

MTB, an airborne pathogen that causes tuberculosis, is classified as risk-group-3 pathogen (see also Chapter 3). Thus, the laboratories dealing with this pathogen (or clinical specimen) have inherent biosafety and biosecurity risks which may vary depending on the procedures/ tests being performed. With respect to laboratory biosecurity program, it is important to first determine all 'valuable biological materials' (VBM) in the laboratory followed by comprehensive risk assessment and the development, implementation and review of effective control measures.

12.1 Valuable biological materials (VBMs):

Any material comprised of, containing, or that may contain biological agents and/or their harmful products, such as toxins and allergens is called biological materials. These materials may further be classified as valuable biological materials (VBM), if they require specific protective and monitoring measures in laboratories to protect their economic and historical (archival) value, and/or the population from their potential to cause harm.[59]

In addition to biological materials, sensitive documents/information and equipment are also under the scope of VBM and must be considered for biosecurity. The laboratory should collect information on the type of biological agents available, their physical location, the personnel required to access either to handle the agents or for other reasons such as service and maintenance, and those responsible for the VBM security management. The key VBM available in TB laboratories (of various hierarchies) under NTEP is listed in Table 12.1.

Table 12.1: Viable biological materials (VBMs) available in TB laboratories (NTEP)

Valuable biological material (VBM; under biosecurity scope)	Availability (Yes, Likely, No)		
	TDCs	C-DST Labs or IRLs (without culture testing services)	C-DST Labs, IRLs or NRLs (with culture and DST services)
Clinical and reference MTB strain	No	No	Yes
Clinical and reference non - tubercle mycobacteria strain	No	No	Yes
Clinical specimen	Yes	Yes	Yes
Genetic material (DNA sample)	Yes	Yes	Yes
Test result, patient information, LIMS, computer/ laptop/tablets	Yes	Yes	Yes
Laboratory quality management system related document	Likely	Yes	Yes
Equipment	Yes	Yes	Yes
Biologicals/reagents (like phenol, acids, alcohol, test kits etc)	Yes	Yes	Yes

The amount of VBM and the risk of unauthorized access may differ between laboratories in different hierarchies as well as within the same hierarchy. Therefore, each individual lab should gather all VBM related information and relates to the likelihood of unauthorized access to the identified biological agents and the consequences of a deliberate release of those agents (Table 12.2). This exercise will help to determine the severity of risk and to select and implement appropriate biosecurity measures.

Table 12.2: Physical biosecurity measure

Biosecurity risk assessment steps		Description	Remark/ Example
VBM identification	Inventory	VBM inventory include information on type of VBM, associated risk, quantity, storage location, accountability to handle etc.	Mycobacterial isolates, clinical specimen, DNA specimen, sensitive documents/data (physical or electronic), test results, patient information, equipment.
Risk identification and assessment	Risk scenario	Assume what could happen as a result of an incident occurring with the VBM	Deliberate or accidental loss, theft, misuse, unauthorized access and release etc.
	Likelihood	Possibility of the risk to occur if a risk control measure is not in place. *Consider including:* • potential adversary (both internal and external individuals), • their motivation and capability to act, • predicted or known frequency of the scenario.	Very low, low, moderate, high or very high
	Consequences	Consequences of risk and the extent of the effects.	Harm, disease, death, financial losses, reputational harm to the organization, scientific losses etc.
Risk control	Risk control measures	Description of the applicable risk control strategies to be implemented. Consideration should be given to prevention and response actions	Physical control, inventory control, information control, personnel control, transport control, emergency or incident control
	Vulnerability assessment	Assessment of the effectiveness of the risk control strategy in relation to the impact of the likelihood and consequences	Very low, low, moderate, high or very high
Risk acceptance and review	Strategy review	Based on the risk assessment and risk control measure, determine the overall risk associated with the VBM. Review whether the risk is acceptable or not. If not, additional risk control measures are necessary. The biosecurity risk assessment should be reviewed periodically.	Acceptable or not acceptable

12.2 Securing valuable biological materials (VBMs):

Securing of VBM may involve different types of biosecurity measures like physical, inventory, personnel, information, transport, and emergency control measures.

12.2.1. Physical Security Measure

Physical security is used to prevent unauthorized access of outsiders (who do not have a legitimate presence in the facility and may have malicious intent) and also to minimize the threat from insiders (who have a legitimate presence in the facility such as employees and approved visitors) who do not require access to a particular VBM. An effective physical security system includes a variety of elements like boundaries, access controls, intrusion detection, alarm assessment and response. These elements should be graded as general security area (negligible or low biosecurity risk), restricted area (moderate biosecurity risk) and high security area (high biosecurity risk).

Figure 12.1: Physical biosecurity measures

Table 12.3: Physical biosecurity measure

Security areas	Control measures	Remarks
General area	• Should be enclosed by physical barrier/boundary • Should be equipped by access control in the form of manual key, electronic key -card, showing staff -ID to security guards etc. • May or may not be equipped by 24 hrs. intrusion detection system (like CCTV camera)	Applicable to TDCs, C&DST lab, IRLs and NRLs
Restricted area (Storage of VBM presenting moderate biosecurity risk)	Additional layer of security and access • Should be enclosed all side (separated from public area) • Should be equipped with access control device/ measure allowing access of staff only • Should be equipped by 24 hrs. intrusion detection system (like CCTV camera) – at least for those laboratories where mycobacterial cultures are handled or stored	Applicable to TDCs, C&DST lab, IRLs and NRLs
High security area (Storage of VBM presenting high biosecurity risk	Additional layer of security and access • Should be within the restricted area • Should be equipped with access control device/ measure allowing access of authorized staff only. • Should be equipped by 24 hrs. intrusion detection system (like CCTV camera) • Equipment and facility log should be maintained (including cleaning and maintenance personnel)	Applicable to C&DST lab, IRLs and NRLs (with TB containment lab facility)

12.2.2 Inventory security measure:

- **Detailed inventory**: A detailed inventory of VBM should be maintained in high-risk TB laboratories that include description of the biological agent(s), its quantities, associated biosecurity risk, location for storage and use, the person responsible (safety officer/quality manager/designee), documentation of internal and external transfers, and an inactivation and/or disposal of the materials. A simpler form of inventory (stock register) may be adopted by low or moderate risk laboratories.

- **Periodic review**: A periodic review of the VBM inventory is essential, and any discrepancies that are discovered should be investigated and resolved by Laboratory supervisor or designee. The inventory should be up-to-date, complete and accurate to ensure that there is appropriate control and accountability.

Implementation of inventory control measure is applicable for all TB laboratories and both employees and employer should take responsibility for biosecurity.

12.2.3 Information security measure

Figure 12.2: Information security measure

It is important to protect the confidentiality and integrity of sensitive information held in the laboratory that could be used with malicious intent. Laboratory should identify, label, and protect sensitive information against unauthorized access. Sensitive information includes diagnostic results, laboratory information management system (LIMS), research information, laboratory personnel information (like medical records), security plans, quality management system documents, quality access codes, passwords, storage locations and biological agent inventories etc. Sharing sensitive information with unauthorized individuals must be strictly prohibited. The following practices can be implemented and are applicable to all levels of TB laboratories.

- Sensitive paper or physical information should be kept under lock and key, but authorized users should have access (document the access) to it whenever they need it (routine work should not be hampered due to biosecurity related restrictions).

- Sensitive electronic information (such as LIMS, NIKSHAY app/web-portal, computers, and official email accounts) should always be password protected and it should be with those only who are authorized to access. Computer/laptop/ tablet should have genuine operating software and anti-malware/virus software and it should be updated. External electronic storage devices can serve as back-up and should be under lock and key in a separate building/area. Server security should also be considered to protect electronic data from adversary attack.

12.2.4 Personnel security measure

Personnel control management must include following measures

- Procedures should be in place to define the roles, responsibilities, and authorities of laboratory personnel who need to handle, use, store, transfer, and/or transport VBM.

- Document the training, experience, competency and suitability requirements for individuals who have access to VBM, ensuring that members of the workforce have appropriate personal and technical qualifications and skills.

- Clear documented procedures for personnel recruitment, including background checks, conflicts of interest, and due diligence, should be followed. A mechanism should be developed to ensure that the integrity of the facility is not compromised by the absence of key personnel.
- Establishing a system of security control (eg. entry & exits logs) for visitors that allows them to enter the facility and classify whether they should be escorted or unescorted. In general visitors should not be permitted in high security areas. Procedures and training for visitors, contractors, suppliers, cleaning and maintenance staff should be available and followed.

12.2.5 Transport security measure

Procedures and practices for correctly categorizing, packaging, documenting, and transporting VBM from one location to another should be available. VBM presenting moderate to high biosecurity risk (like transporting the clinical sample or culture isolates for TB diagnosis, drug susceptibility testing or proficiency testing purpose) require fulfillment of national and/or international regulations (see Chapter 7). Following measures should be taken in account while transporting the VBM

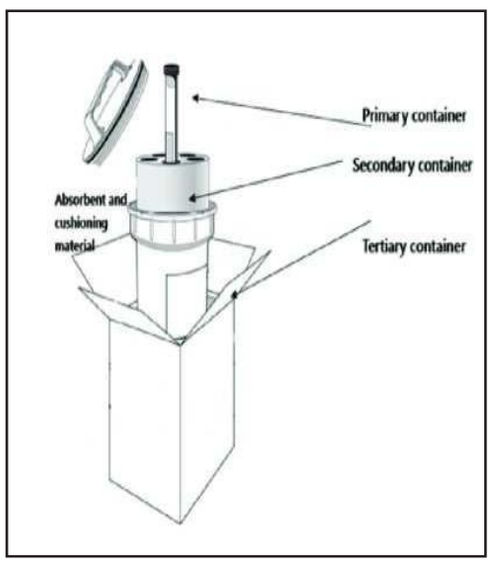


Figure 12.3: Triple layer packaging

- VBM that poses high risk should neither be left unattended nor temporarily stored outside the high security area.
- All infectious biological materials should be transported in triple layer packaging, in accordance with applicable national and/or international regulations.
- Ensure that VBM is ordered from legitimate providers and that it arrives at its destination via competent and approved couriers.
- Procedures should be available and followed to ensure that shipper, carrier, and receiver responsibilities for controlling biosecurity risks are met.
- Transfer of VBM should be pre-approved and relevant custodian documents/ records should be kept.
- Inventories must be updated to reflect incoming and outgoing specimens, including internal and external transfers. A security check (through gate passes) for entry and exit key VBM from the facility can be implemented.
- If disposal of VBM is needed, standard biomedical waste management rule must be followed (see Chapter 4, 5 and 6).

12.2.6 Emergency/incident response:

Despite existing prevention or risk control measures, laboratory may experience unintentional or intentional incidents or emergencies. Therefore, emergency response plans to deal with unexpected situations should be available (see Chapter 9 and 10). The response plan should be developed considering following aspects.

- Assess unusual situations and establish criteria for determining when outside involvement is warranted (like fire incident, threats, theft of commodity). Following this, establish a mechanism to allow emergency responders access while ensuring continuous and uninterrupted laboratory biosecurity, control, accountability, and traceability of VBM.

- The response plan should be available for potential incidents such as discrepancies found in inventories, missing biological agents, unauthorized persons in the laboratory etc.

- Response plan should contain

  i) name and contact number of supervisory staff along with their roles and responsibilities, including during non-office hours

  ii) a floor plan of the facility/building/laboratory along with emergency routes for safe evacuation

  iii) methods for the safe removal of dangerous substances and proper decontamination procedures

  iv) information about emergency response personnel (external) and contact information.

- An incident response should be documented and communicated properly (such as maintain the incidence register) to facilitate investigation, root-cause analysis, corrective action and process improvement.

- Drills and exercises can also be used in the planning and preparation stages to test the responses to simulated incidents or emergencies. Moderate and high-risk TB laboratory should conduct drill at least once in a year. They can help identify gaps and other improvement opportunities.

- Plans should be reviewed and updated at least once a year, and insight from drills, incident reports, and investigations should be used to make necessary adjustments and improvements.

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Annexure 1

Hand hygiene

The role of hands in the transmission of infections has been well established and can be minimized with appropriate hand hygiene. Hands must be washed appropriately.

- after handling biological material or removing the gowns/coats and gloves,
- before eating/drinking and leaving the laboratory, or
- when hands are visibly soiled or believed to be contaminated

In most situations, thorough washing of hands with ordinary soap and water is sufficient to decontaminate them. Hands should be thoroughly lathered with soap, using friction, for at least 40 to 60 seconds, rinsed in clean water and dried using a clean paper or cloth towel (Figure A1).

Other considerations:

- Alcoholic hand rubs are not a substitute for hand washing, except for rapid hand decontamination between patient contacts.
- Ensure the availability of washbasins with proper water supply (and preferably with hands free controls and anti-splash devices).
- Ideally, liquid soap dispensers should be provided in the laboratories. If not feasible, soap bars (eg. carbolic soap) after washing should be left in a dry tray to prevent contamination with microorganisms which grow in moist conditions.
- Suitable materials should be used for hand drying (disposable towels, reusable sterile single use towels or well-maintained roller towels) and if not available air dry.

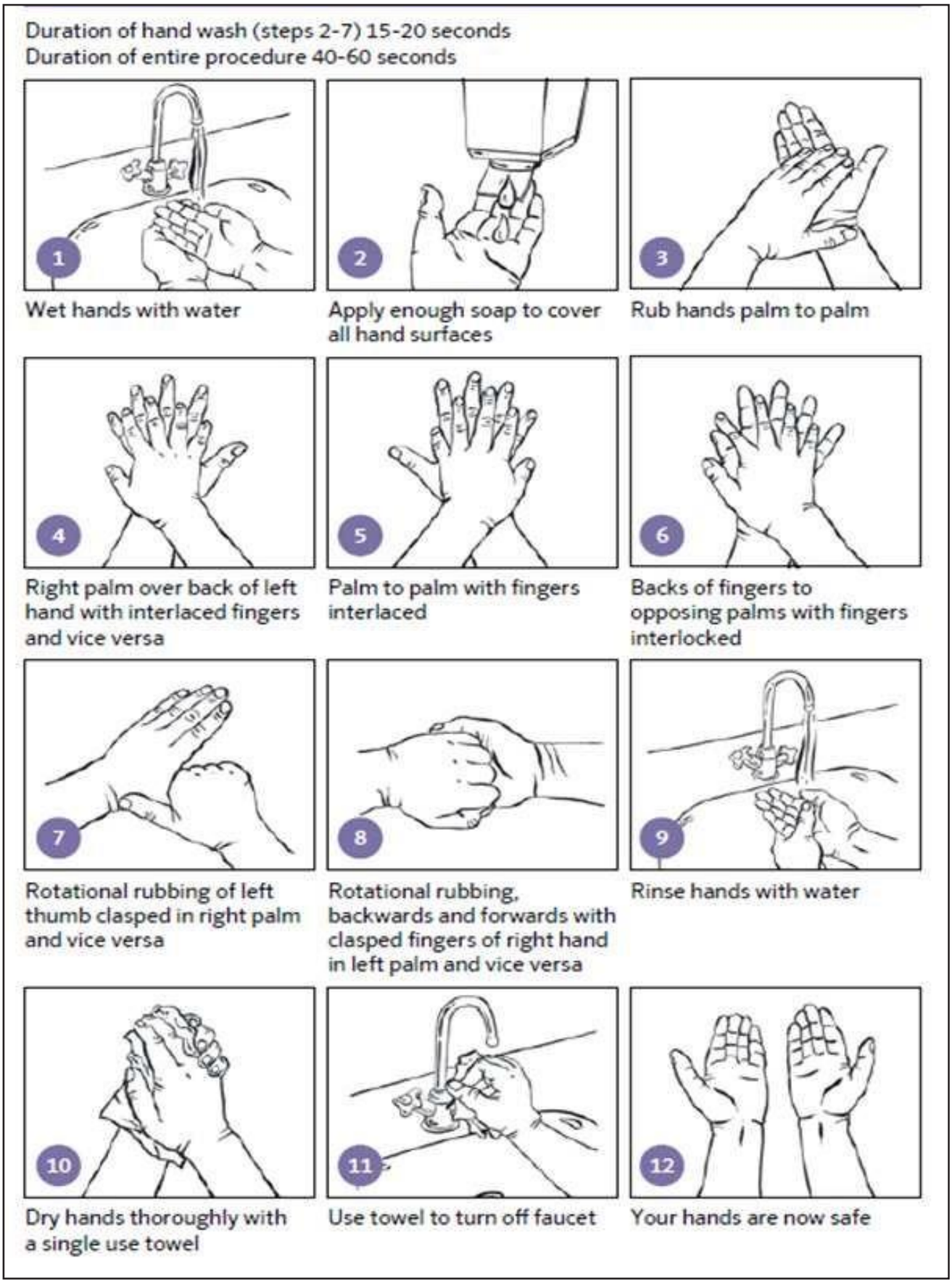


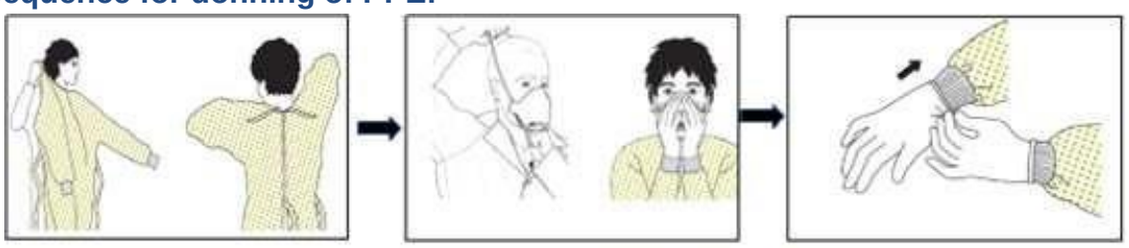
Figure A1: Steps for proper hand washing
Source:https://www.stoptb.org/file/9717/download

Annexure 2

Sequence for donning and doffing of PPE

The selection of personal protective equipment (PPE) depends on the type of procedures performed or level of risk involved. Following sequence should be considered for donning and doffing of PPE.

A) Sequence for donning of PPE:



Gown

- Completely cover the torso (from neck to knee, arms to end of wrist) and wrap around the back
- Fasten in back of neck and waist.

Mask/ Respirator

- Secure elastic bands at the middle of head and neck
- Fit flexible band to nose bridge
- Fit mask to face and below chin. Seal-check the mask/ respirator.

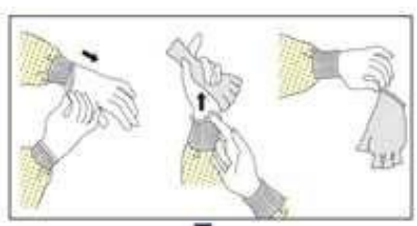
Gloves

- Always use new gloves and extend it to cover the wrist of gown.
- Some procedure may require to use double pair of gloves.

B)Sequence for doffing of PPE:

Gloves

- If the outside of the gloves is contaminated, grasp the outside of the glove with the opposite gloved hand and peel it off.
- Place the removed glove in the gloved hand. Slide the fingers of the un-gloved hand under the remaining glove at the wrist.
- Remove gloves over first glove and discard in waste bin

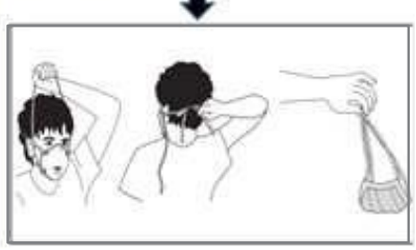


Gown

- Unfasten ties if the gown front and sleeves are contaminated.
- Pull away from the neck and shoulders, only touching the inside of the gown.
- Flip the gown inside out. Fold or roll into a bundle and discard.

Mask/Respirator

- If front of mask/ respirator is contaminated – Don't Touch
- Grasp the bottom, then top elastic band and pull it off.
- Discard the respirator in waste bin.



Source: https://www.cdc.gov/hai/pdfs/ppe/ppe-sequence.pdf

Note: Full PPE should be worn when conducting high risk procedures (like TB culture and DST). Following sequence of PPE donning and doffing should be considered

- **Donning:** First pair of gloves → Clean Gown → Hair cover →N-95 mask → Second pair of gloves
- **Doffing:** Outer gloves → Contaminated Gown → N-95 mask → Hair cover → Inner gloves

Annexure 3

Respirator fit testing

A fit test is a procedure used to ensure that a respirator is both comfortable and offers the intended level of protection for the user. Occupational Safety and Health Administration (OSHA) recommends respirators fit test for each user at initial time point (and may further be conducted on annual basis) using quantitative or qualitative methods.

A) General requirement for respirator fit testing:

- A sufficient number of respirator models and sizes are required so that the respirator is acceptable to the user and fits correctly.
- The user should be guided/demonstrated how to put on a respirator, position it on the face, set strap tension, and determine an acceptable fit.
    - The most comfortable mask is donned can be assessed by i) position of the mask on the nose, face and chin; ii) room for eye protection; and iii) room to talk.
    - The criteria used to determine the adequacy of the respirator fit are i) chin properly placed; ii) adequate strap tension, not overly tightened; iii) fit across nose bridge; iv) respirator of proper size to span distance from nose to chin; v) self-observation in mirror to evaluate fit and respirator position

B) Quantitative respirator fit test: A quantitative fit test uses a fit testing instrument(s) to provide quantitative, or numerical, measurements of the amount of face seal leakage present when a user wears a respirator. Quantitative fit testing requires a hole punched in the respirator to perform the test. Access to quantitative fit testing may not be readily available in India.

C) Qualitative respirator fit test: A qualitative fit test is popular procedure. It uses a test agent that can be qualitatively detected by the user's sense of taste and smell, if there is a gap in the seal of respirator to user's face. OSHA accepted protocol may be accessed from https://www.osha.gov/lawsregs/regulations/standardnumber/1910/1910.134AppA.a

Figure A3: Respirator fit test

There are some commercial kits (ex. 3MTM qualitative fit test) available that works by spraying a sweet or bitter solution into the hood environment. If the wearer does not detect the test agent's taste, an acceptable fit has been demonstrated (Figure A3).

Apart from the respirator fit test, it is critical to perform the 'respirator user seal check' procedure every time a respirator is worn to ensure that the respirator is properly donned. This is a quick procedure and user can be checked by either a positive pressure or negative pressure procedure.

- **Positive pressure user seal check:** The user wearing the respirator gently exhales while blocking the paths for air to exit the respirator (to ensure that there are not any leaks). When a slight pressure builds up in the respirator without any leakage, the test is considered successful.

- **Negative pressure user seal check:** The user wearing the respirator inhales quickly while blocking the airways into the facepiece. A successful check is considered when the facepiece slightly collapses under negative pressure.

Annexure 4

BSC decontamination

Decontamination (gas/vapor based) methods for BSC

Decontamination of the BSC using a gas/vapor-based method (fumigation) may be necessary prior to performing maintenance work, repair, moving, changing filters, or after gross spills. The three most commonly used chemicals in BSC fumigation are formaldehyde (CH2O), chlorine dioxide (ClO2), and hydrogen peroxide (H2O2). Each chemical has advantages and disadvantage in this application (Table 1).

Table 1: Advantages and disadvantage of different chemicals used for BSC fumigation

	Formaldehyde	Chlorine dioxide	Hydrogen peroxide
Material (BSC) compatibility	+	-	+/-
Carcinogenic	Yes	No	No
Permissible exposure leve[$]l	0.75 ppm	0.1 ppm	1 ppm
Humidity requirement	>60%	60-80%	70-90%
Sealing of cabinet	Airtight seal of BSC	Airtight seal of BSC	Small gaps are acceptable when method operates BSC at negative pressure
Generation of gas/ vapor	Heated pan/vaporizer is used to vaporize a formalin solution	Chemical reaction take place in a beaker using water and sodium chlorite or by passing chlorine gas through sodium chlorite salt	Generator needed to flash vaporize a hydrogen peroxide solution
Kill time	6-12 hr.	60-85 min[#]	30-110 min[#]
Deactivation step*	Neutralization with ammonia is required	Gas is catalyzed with Charcoal	Hydrogen peroxide vapor will break down naturally over time to oxygen and water but can be accelerated via catalyzer
Clean-up step	Wiping formaldehyde residue may be necessary	No	No
Duration of BSC down time	18-24 hr.	3 hr.	2-3 hr.

$ or as per local authority; * important step when BSC is not ducted; # depending on the types of method used

The procedures for BSC decontamination/ fumigation using different chemicals are as follows-

A) Formaldehyde based BSC fumigation:

Formaldehyde is a colourless gas with a pungent odour. Since it is carcinogenic in nature, it is crucial to take precautions to avoid exposure.

Formaldehyde penetrates well and is effective in decontaminating spores and microorganisms. However, the entire formaldehyde fumigation process is very time-consuming (typically 2 days).

Fumigation process includes following steps

i) **Preparation of the BSC for fumigation:** BSC (Class II Type A2) should be sealed as leak proof as possible with tape and plastic. If the BSC is ducted, the damper must be closed and air tight. This will eliminate the loss of gas into the exhaust system. Once the cabinet is properly sealed, warning placards placed, and generator connected, the decontamination process can be started.

ii) **Fumigation process:** To ensure proper decontamination with formaldehyde gas, the cabinet must first be conditioned with relative humidity (RH) as >60%. Humidity can be raised using a small steam generator, ultrasonic humidifiers, or a hot plate and some water. Once the RH is at the proper level, the gas can be introduced. This is typically done by heating up (by using hot plate or commercially available automatic generators) the proper amount of paraformaldehyde to release formaldehyde gas. The amount of paraformaldehyde to be used is proportional to volume within the BSC (~0.30 g/ft3 or 11 g/m3). BSC must be exposed to the concentrations for at least 6 hr, preferably 12 hr. The BSC blower should be activated for 10-15 seconds during the heating process when 25%, 50%, 75%, and 100% of the paraformaldehyde has depolymerized.

iii) **Release of BSC:** Once the exposure time has elapsed, the gas needs to be vented or neutralized. If the cabinet is ducted, the damper can be opened to exhaust the gas. In case there is no ducting, formaldehyde should be neutralized by heating up the ammonium bicarbonate that release the ammonium vapor. The amount of ammonium bicarbonate used should be 110% of the original weight of paraformaldehyde. During the process, the blower must be turned on to ensure that the ammonium vapour reaches all areas. The neutralization process produces a residue of repolymerized formaldehyde that must be hand-cleaned. The entire process takes 18-24 hr to complete, thus access to the room is restricted while the decontamination cycle is in process.

B) Chlorine dioxide based BSC fumigation:

Chlorine dioxide (ClO2) is a synthetic, green-yellowish gas with irritating chlorine-like odour. It is extremely effective in decontaminating microorganisms and spores. This fumigation procedure is faster (than formaldehyde) and can be completed within three hours. Chlorine dioxide, on the other hand, is highly corrosive and has been known to react with steel and plastics. Thus, multiple fumigations are likely to deteriorate key components of BSC. The following steps are involved in fumigation of BSC

i) Preparation of the BSC for fumigation: The same as with formalin-based fumigation.

ii) Fumigation process: To achieve proper decontamination, the relative humidity (RH= 60%-85%) within the BSC must be optimised using methods described for formaldehyde-based fumigation. During humidification, the BSC blower or a recirculation system must be running. Once the humidity has been stabilized at the proper levels, the chlorine dioxide gas can be introduced using any of the methods described below.

- **Method 1:** Similar to formaldehyde-based fumigation, in this method the volume of the cabinet determines how much Chlorine dioxide gas must be generated. The gas can be generated by either creating a concentrated solution of chlorine dioxide and allowing it to off-gas through bubbling or effervescence, or it can be generated by passing dilute chlorine gas over a cartridge of sodium chlorite which converts the chlorine to pure chlorine dioxide. The gas generated should reach 0.13 g/ft3 or 4.7 g/m3. Following that, every 15 minutes of contact time, the cabinet blower should be activated for 1 minute. It is recommended that a total of 85 minutes of gas exposure or contact time be considered.

- **Method 2:** This method requires the capability of monitoring the gas concentration and adding more gas if concentration drops. A particular concentration of gas (3 mg/L [1086 ppm] or 5 mg/L [1810 ppm]) is produced by passing diluted chlorine gas over sodium chlorite cartridges, and it is maintained for the duration of the exposure period (60 minutes for 3 mg/L and 45 minutes for 5 mg/L). This technique needs equipment that can produce the gas on demand, monitor the gas, and keep the concentration of the gas constant.

i) **Release of BSC:** After decontamination time has elapsed, the gas is removed from the cabinet by opening the duct damper. The ducted cabinets can be aerated in 5-10 minutes. Before removing the cabinet sealing and further cleaning, a safety sensor is used to ensure that the cabinet is below the exposure level of 0.1 ppm.

C) Hydrogen peroxide based BSC fumigation:

Hydrogen Peroxide (commercially available in 30-50%) is a colourless, clear liquid that is a strong oxidizer and corrosive. It is commonly used as a laboratory reagent and also as a steriliser in its vapour form. It is a safer and more efficient decontamination method for BSC and containment room than formaldehyde. Following steps should be considered when fumigating the BSC

I) **Preparation of the BSC for fumigation:** BSC should be sealed properly using sealing tape and plastics. Damper to the cabinet should also be closed and sealed. A sash adapter and plenum adapter are installed on the cabinet to allow for the connection of the hydrogen peroxide vapor generator to the BSC. The decontamination process can begin once the cabinet has been properly sealed, warning placards have been placed, and the generator has been connected.

ii) **Fumigation process:** To avoid condensation and ensure effective decontamination, it is critical to stabilise the temperature and humidity before beginning the fumigation process. Following that, a 35% hydrogen peroxide solution is vaporized and released into the BSC using a generator. Vaporized hydrogen peroxide is very sporicidal at low concentrations (typically 0.1-3 mg/L at 25°C). The hydrogen peroxide concentration is kept at its maximum during the fumigation process. The proportion of hydrogen peroxide "consumed" by absorption or decomposition is replenished in the BSC. The contamination process may take 30-110 minutes depending on the type of generator.

Release of BSC: Before removing placards and unsealing the cabinet, a hydrogen peroxide sensor or tube is used to determine that the hydrogen peroxide vapor concentration is ≤1 ppm as measured at the cabinet sash. Once this limit is reached, the cabinet can be completely unsealed and further cleaned.

Annexure 5

Correct pipetting technique

In order to minimize the aerosol generation and to obtain precise and reliable results users must be aware of the correct pipetting technique. There are two types of pipetting: forward pipetting and reverse pipetting.

A) Forward mode of pipetting:
The forward mode is the most common method of pipetting and involves the following steps. (Figure A5.1)

1. Preparation:
   - Before using a pipette, make sure it is calibrated and appropriate for the volume of liquid that needs to be transferred. If a variable volume pipette is used, the volume to be dispensed must first be selected.
   - Place a new sterile tip on the pipette tip holder in accordance with the pipette specifications and ensure that it is properly fitted. Always use aerosol barrier tip when transferring infectious liquid.
   - Hold the pipette in a nearly vertical position and then press the plunger gently until it reaches the first limit. Until this point, the pipette tip should not come into contact with the liquid.

2. Aspiration:
   - Immerse the pipette tip in the liquid and allow the plunger to move up smoothly to the rest position. Wait one second before removing the pipette's tip from the liquid (so that all the liquid has time to move up into the tip).
   - If the tip is immersed either too deep or not deep enough, it will impact on test result. In general, the immersion depth for volumes '0.1-1l,' '1-100 l,' '101-1000 l,' and '1001 l -10 ml'

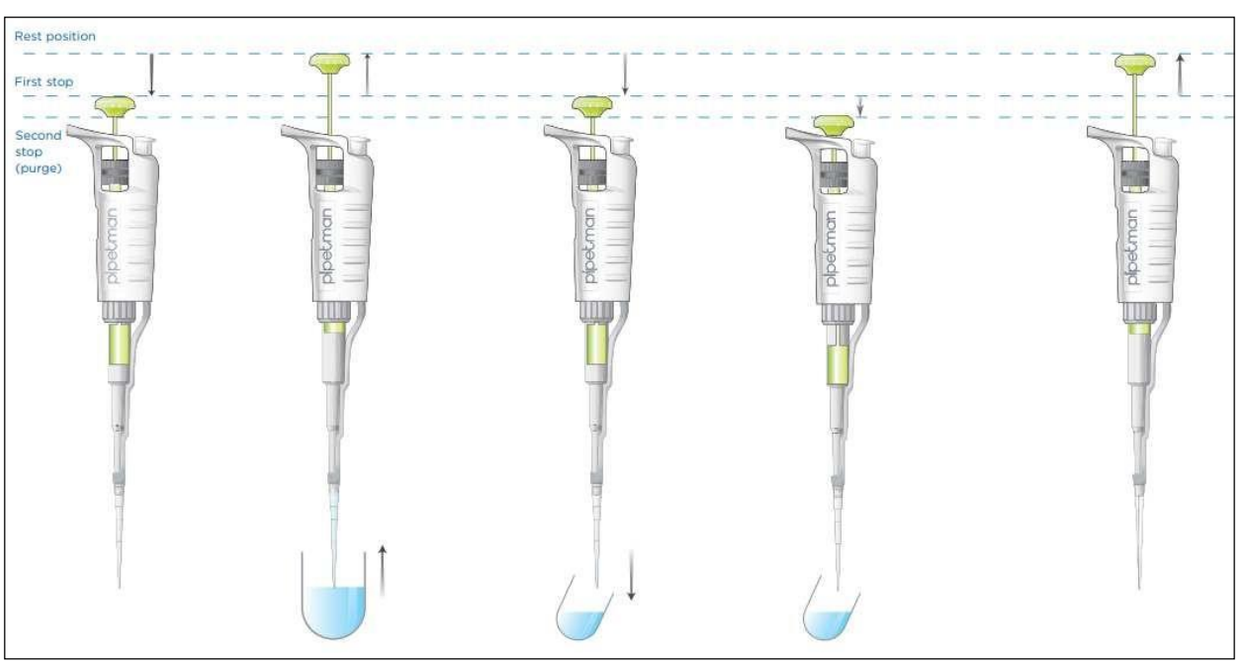


Figure A5.1: Forward mode of pipetting

Source: https://www.gilson.com/pub/media/docs/GuideToPipettingE.pdf

3. Dispense:
   - Place the pipette's tip against the inside wall of the receiving vessel/ tube. Verify that the angle formed between the pipette's tip and its wall is between 30 and 45°. If the receiving tube already contains liquid, avoid the pipette's tip from being submerged.
   - Press the plunger smoothly until reaching the first limit. Make sure that the pipette's tip is constantly in touch with the wall of receiving tube.

4. Purge:
   - Wait one second, then press the plunger to the second stop position. This purge stroke removes any remaining sample from the tip. Pipette tip end should be carefully removed from sidewall by moving it up (8 to 10 mm from tube edge) ensuring that there are no liquid drops remaining on it.
5. Rest:
   - Once the pipette is removed, gently release the plunger to the higher limit position. Discard the pipette's tip by press the expulsion mechanism's button with thumb.

B) Forward mode of pipetting:

The reverse mode is sometimes used to pipette slightly viscous liquids and it include following steps (Figure A5.2)

1. Preparation: Hold the pipette in a nearly vertical position and press the plunger gently to the second stop position.
2. Aspiration: Immerse the pipette tip into liquid and gently release the plunger to move up to the rest position. Wait for one second so that desired amount of the liquid reaches the tip.
3. Dispense: Place the pipette tip against the inner wall of the receiving vessel/tube at an angle 30-450. Gently press the plunger to reach the first stop position and wait for a second.
4. Re-aspiration If the pipette tip is to be reused for the same sample, maintain the plunger in the intermediate position for subsequent immersion for the next pipetting cycle.

or

5. Purge: Wait one second and purge. If the pipette tip is not to be re-used, release the plunger to rest position and then eject the tip in discard bin.

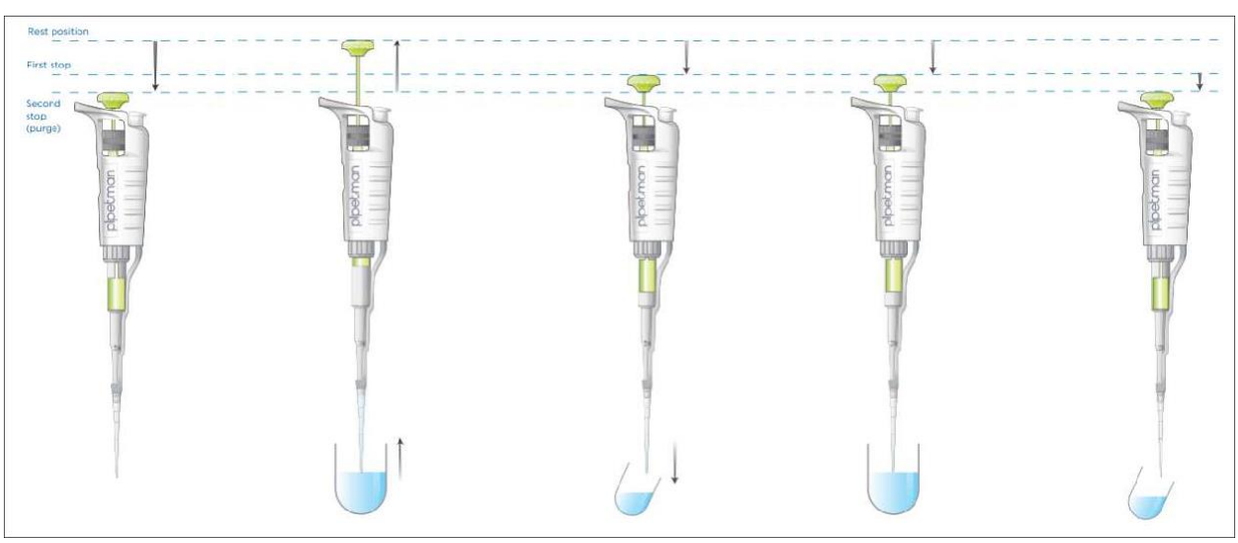


Figure A5.2: Reverse mode of pipetting

Source: https://www.gilson.com/pub/media/docs/GuideToPipettingE.pdf

Annexure 6

Biohazard signage

CAUTION
Authorized Personnel Only

This containment zone contains infectious materials

Containment level:	
Primary contact person (lab in charge):	
Phone number:	
Alternate emergency contact person:	
Phone number:	
Entry requirements:	

Annexure 7

Suggestive layout design of a TB laboratory

A) Designated Microscopy Centre

The basic requirements for designated microscopy centre include

1. Good ventilation and directional airflow (contaminated air flows away from staff and out of the laboratory)
2. A sturdy table/ bench to prepare smears
3. A sink to stain smear
4. A sturdy table/bench to examine the smears
5. A sturdy table/bench for paperwork
6. Basin for hand washing
7. Proper illumination (light)
8. Anti-skid floor
9. An area for receiving specimen
10. Storage cupboard
11. Waste bin

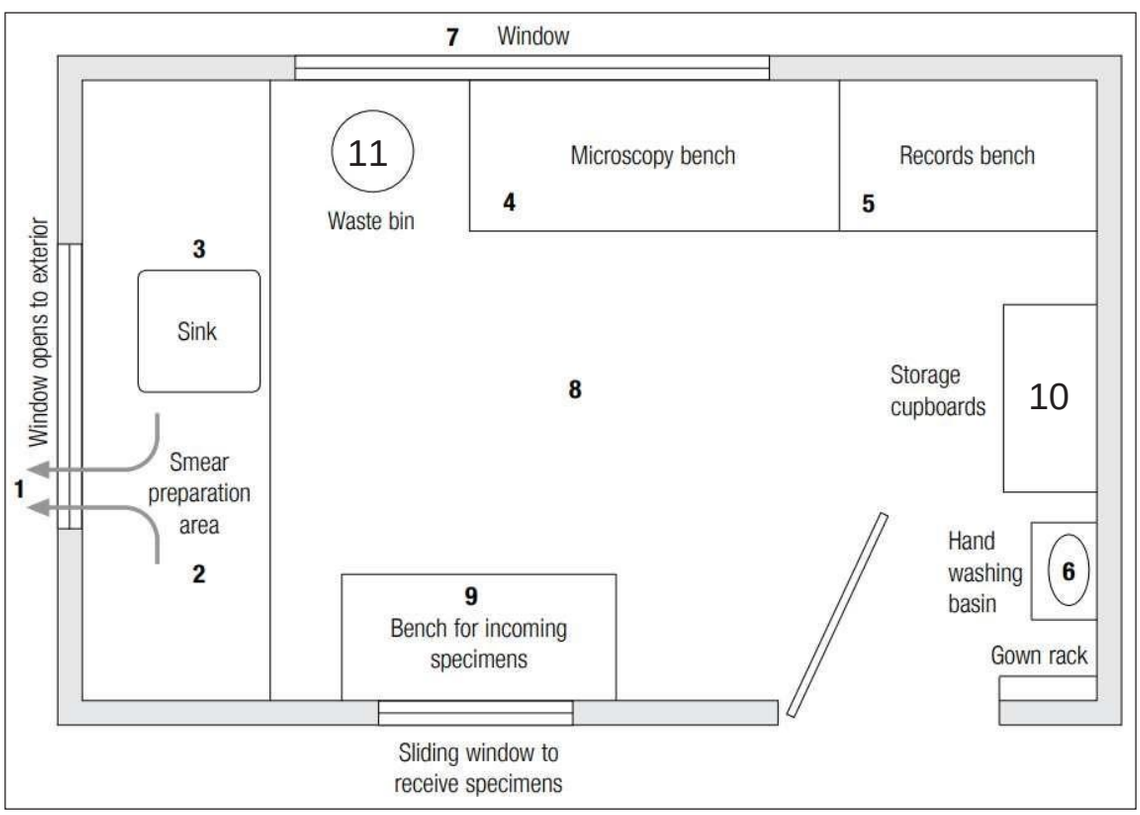


Figure A7.1: Sample layout of a Designated Microscopy Centre (DMC)

Source: https://stoptb.org/wg/gli/assets/documents/TB MICROSCOPY HANDBOOK_FINAL.pdf

Note: The size of the laboratory should be adequate to the function and provide a safe working environment. The size of laboratory depends on the testing load and and types of tests procedures being performed. However, a minimum area of 10 m^2 should be available for DMC laboratory.

B) Truenat Laboratory

Truenat testing has biosafety requirements similar to smear microscopy. Furthermore, with minor adjustments, Truenat testing can be performed in the same laboratory area as smear microscopy (like separate bench space).

1. Good ventilation and directional airflow (contaminated air flows away from staff and out of the laboratory)
2. A sturdy table/ bench to prepare smears
3. A sink to stain smear
4. Waste bin
5. A sturdy table/bench to examine the smears
6. A sturdy table/bench for paperwork
7. A sturdy table/bench for Truenat testing (more than 2 feet long)
8. Proper illumination (light)
9. Anti-skid floor
10. An area for receiving specimen
11. Storage cupboard
12. Basin for hand washing
13. Refrigerator

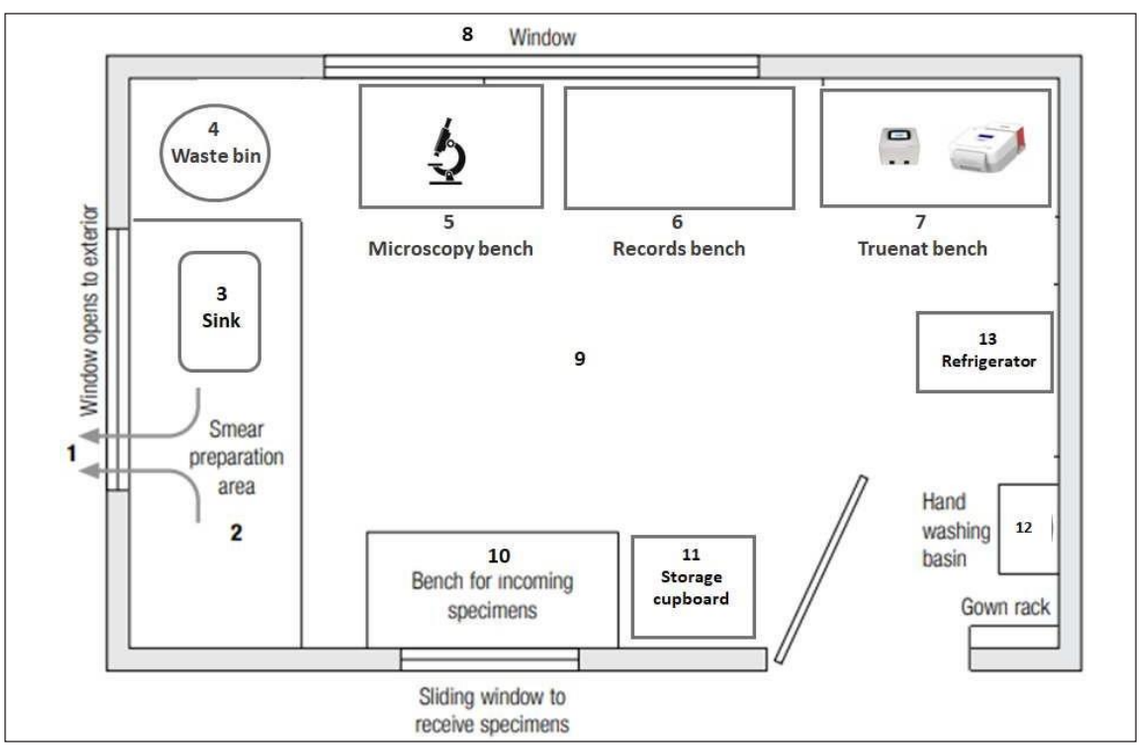


Figure A7.2: Sample layout of a laboratory with both smear microscopy and Truenat testing facility

Note: The size of the laboratory should be adequate to the function and provide a safe working environment. The size of laboratory depends on the testing load and types of tests procedures being performed. However, a minimum area of $10m^2$ should be available for both smear microscopy and Truenat testing.

C) GeneXpert Laboratory

The biosafety requirements for GeneXpert testing are similar to those for smear microscopy, but the machine operation requires dust free and temperature/humidity controlled (15°-30°C) environment; thus, require an air-conditioner. The machine can be installed in a separate air-conditioning room or cubicle, whereas specimen preparation may be performed in a ventilated area such as the smear preparation area.

1. Good ventilation and directional airflow (contaminated air flows away from staff and out of the laboratory)
2. A sturdy table/ bench to prepare smears (for microscopy) or specimen (for GeneXpert)
3. A sink to stain smear
4. Waste bin
5. A sturdy table/bench to examine the smears
6. A sturdy table/bench for paperwork
7. A sturdy table/bench for GeneXpert testing (5-10 cm of clearance surrounding the instrument from the walls or other instruments)
8. Refrigerator
9. Air-conditioner
10. Proper illumination (light)
11. Anti-skid floor
12. Basin for hand washing
13. Storage cupboard
14. An area for receiving specimen

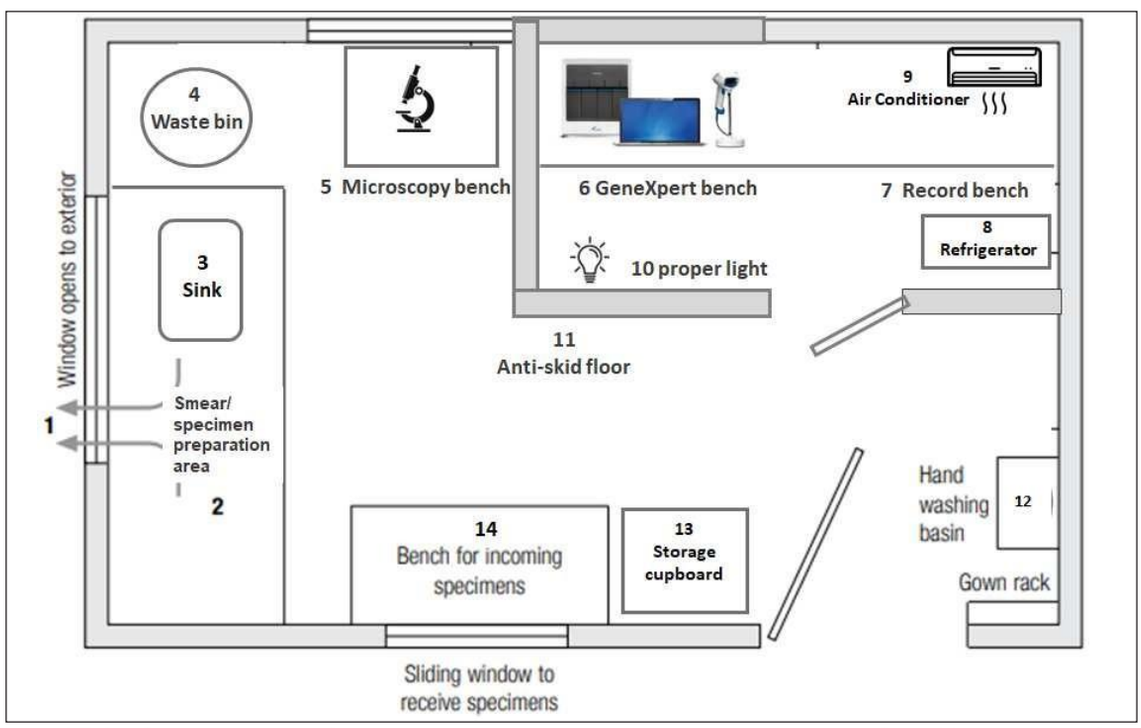


Figure A7.3 : Sample layout of a laboratory with both smear microscopy and GeneXpert testing facility

Note: The size of the laboratory should be adequate to the function and provide a safe working environment. The size of laboratory depends on the testing load and types of tests procedures being performed. However, a minimum area of 15m^2 (covering two rooms; or one big room with at least 4m^2 inside cubical) should be available for both smear microscopy and GeneXpert testing

Annexure 8

Method to determine the air exchange rate (ACH)

The number of room volumes of air expelled per hour and replaced with clean air is referred to as ACH. A simple method to measure ACH (when mechanical ventilation is used) is to -

ACH refers to the number of room volumes of air expelled per hour and replaced with clean air. When mechanical ventilation is used, one method of measuring ACH is to:

a. identify the air exhaust vent or vents.
b. cover the vent with a piece of cardboard that has an opening of 10 cm x 10 cm;
c. measure the out-flowing air velocity with a vaneometer or anemometer;
d. calculate the volumetric airflow rate for each air exhaust port

$$\mathbf{Q = V \times A \times 3600}$$

Q = Volumetric air-flow rate in m^3/h
V = Velocity of air in m/s
A = Area of opening in m^2 (for example, 10 cm [0.1 m] x 10 cm = 0.01 m^2)
3600conversion of seconds to hours;

e. sum up all the exhausts for the room;
f. measure the volume of the room

$$\mathbf{Vol = Length \times Width \times Height = m^3 \text{ (measure in meters);}}$$

g. calculate the ACH

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

Note: When natural ventilation is used, ACH measurements are too variable to provide a reliable measure of ventilation. It is preferable to use directional airflow to ensure safe working conditions. Air should flow past the worker, across the work area where potentially infectious materials are present, and away from occupied areas of the room to protect against aerosols generated in the work area.

Annexure 9

Suggestive layout design for LPA facilityand specimen processing area

Line Probe Assay Laboratory

The work flow in Line Probe Assay diagnostic lab is as follow:

- Sample preparation (DNA extraction)
- preparation of regents for PCR set-up (master mix or pre-mix preparation)
- PCR amplification, and hybridisation
- Post-PCR analysis of results.

Preventing amplicon contamination is most crucial need of the facility (for reducing the risk of cross-contamination and false results). This is achieved by physically separating the source of amplicons' (e.g., post-PCR) activities from the Pre-PCR activities.

The facility is located close to the C&DST facility. Separate rooms are the ideal arrangement for the molecular diagnostic facility. Separate rooms for each step of the work-flow may not be possible due to space constraints, and the existing facility may need to be renovated. It is suggested that two to three separate rooms be used. The rooms are quite small. Alternatively, a large room could be appropriately partitioned. All efforts are made to prevent dust by restricting access to the facility, sealing off exterior wall openings, and meticulously following cleaning procedures for rooms and work-benches. Dedicated supplies (such as micropipettes, sterile disposable plugged pipette tips, microcentrifuge tubes, reagents, distilled water, cleaning solutions and floor cleaning mops etc.,) need to be established separately in each room. An almirah underneath benches to keep dedicated pipette sets for all three rooms is advisable. A minimum area of 20-25 m^2 should be considered for LPA diagnostic lab (excluding culture and DST room).

Facility detail (fig 1):

- **Sample preparation (DNA extraction room):** This activity does not require a separate room. It is carried out within the biological safety cabinet, in the culture and DST room. Heating and/or sonication are used to extract DNA directly from processed sputum sediment. A dedicated workbench and the use of disposable gloves (changed frequently) would aid in avoiding sample-to-sample or DNA contamination. Before beginning the work, the work surfaces should be cleaned with 1% bleach, followed by 70% alcohol.

- **Pre-mix or master-mix room:** This work area is used to prepare master-mix for PCR setup (e.g., dNTPs, 10X buffer, distilled water, taq DNA polymerase). It is preferable to have two doors. In this work area, a clean-work bench is required. A small refrigerator and/or a -20^{0}C freezer (small but double sleeve) as well as a quick-spin should be available.

- **Amplification room:** This work area is meant for the PCR amplification of target DNA. Thermocycler with clean bench is needed.

- **Hybridisation room:** This work area is meant for handling the amplified DNA for hybridisation (using Twin-incubator and/or GT-Blot machines). Larger room may be considered for this lab (compared to Pre-mix and Amplification Room).

(Wherever extra space is not available, the amplification and hybridisation work is carried out on separate work -benches with in a single room (fig 2). One work bench/area is meant for PCR amplification. The second work bench/area is meant for those activities which involve the handling of amplified DNA- i.e., hybridisation with Line probe assay strips).

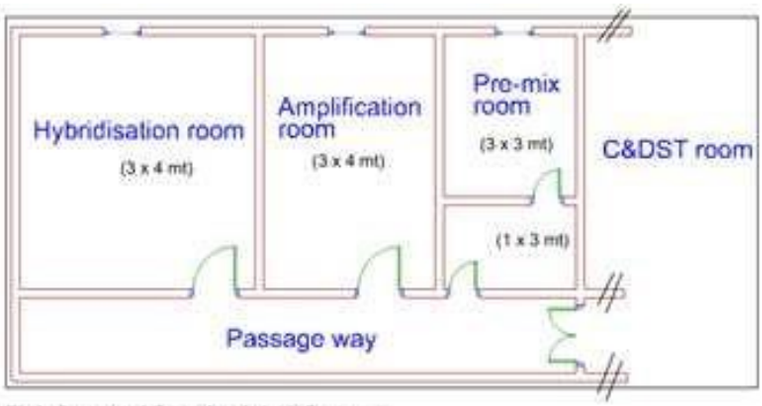


Fig1: Suggested floor sketch 1 (6x7 meters)

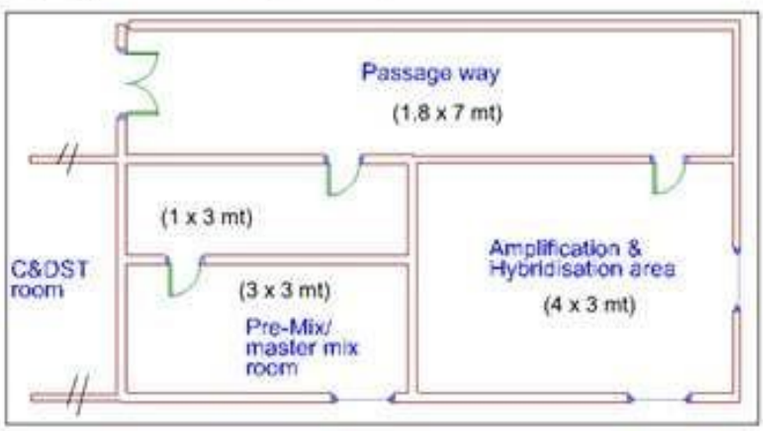


Fig 2: Suggested floor sketch 2 (6x7 meters)

Source: https://tbcindia.gov.in/WriteReadData/l892s/90464237417th%20(July%202009).pdf

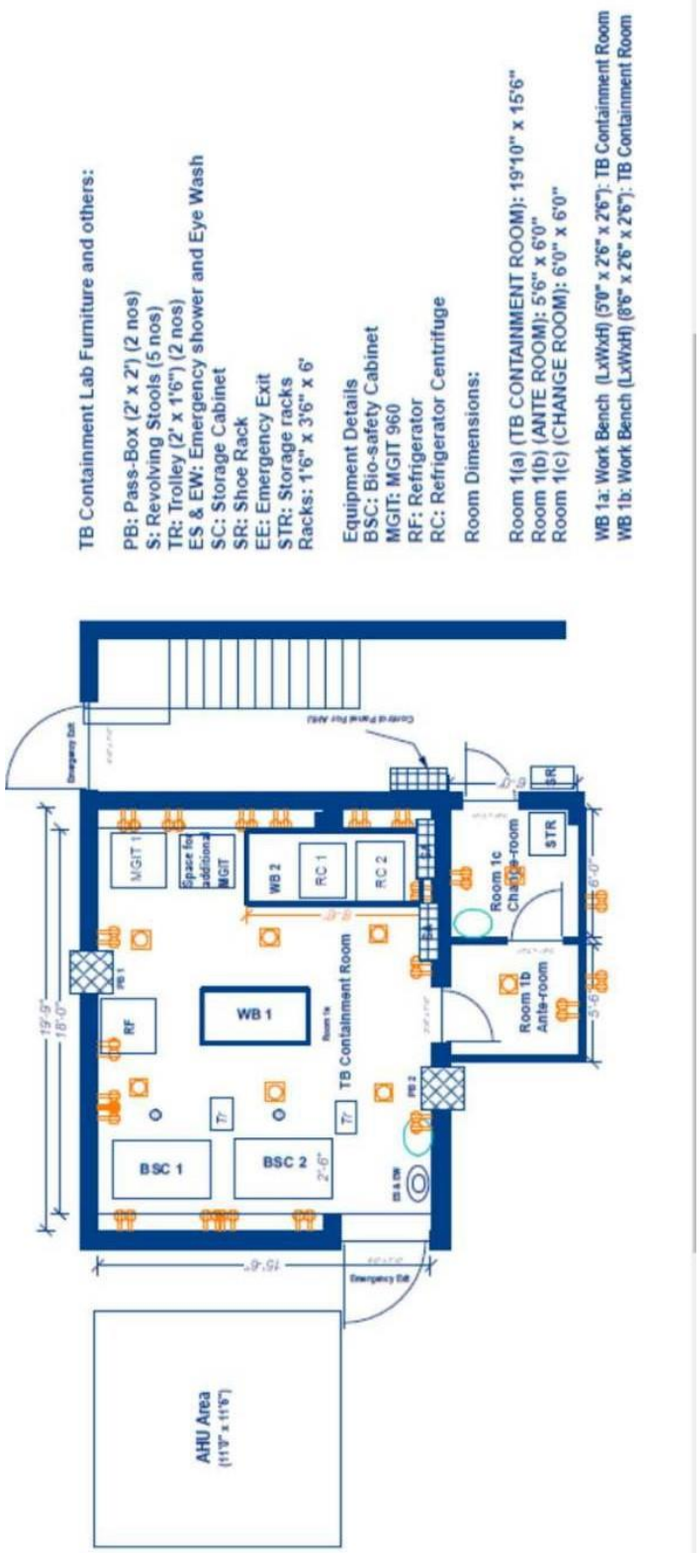
Annexure 10

A layout plan for TB containment laboratory

Based on testing load (number of equipment needed) the area size of TB containment varies. However, a minimum of 50 m^2 area should be considered for TB containment laboratory.

Detail specification of TB containment laboratory may be accessed using below link.

https://tbcindia.gov.in/index1.php?lang=1&level=3&sublinkid=5350&lid=3443



Annexure 11

Basic first-aid procedures

1. **Bleeding:** *(Sign- steady flow of blood from wound/cuts)*
   - Apply direct pressure to the wound/ cut using a sterile gauze pad or clean cloth.
   - Elevate the injured area above the level of heart, if there is no fracture.
   - Cover the wound/cut with pressure bandage. If bleeding does not stop apply additional dressings and seek medical attention.
2. **Burns- Thermal or Chemical:** (Sign- redness, pain, blisters, swelling, skin damage)
   - Hold burned area under cool running water or immerse for 10-15 minutes.
   - Cover the burn with sterile cloth or non-adhesive bandage.
   - Do not break blister and put spray or ointment for second/ third degree burn.
3. **Sprains:** (Sign: Pain, limited ability to move the affected joint)
   - Rest the ankle of injured area and apply ice or cold packs (wraps in cloth or put cloth under to protect the skin)
   - Compress by lightly wrapping an elastic bandage around the injured area.
   - Elevate the injured area above the heart level to reduce swelling.
4. **Fracture:** (Sign: Pain, inability to move the limb, swelling)
   - Support above and below fracture, apply well-padded splint and seek medical attention.
5. **Gas Poisoning:** (Sign: Giddiness, weariness, throbbing heart)
   - Move to fresh air, keep an open airway; administer CPR, if needed; loosen clothing and seek medical care.
6. **Heat Exhaustion:** (Sign: Fatigue, pale face, shallow breathing, cold sweat)
   - Move to cool areas; lay down victim; raise feet; loosen clothing; give sip of cool salt water; use wet cloth to cool victim.
7. **Electric Shock:** (Sign: Unconscious; difficulty in breathings; burn at contact point, muscle spasms)
   - Turn off electric current; break contact with electric source using a dry non-metallic object; don't touch victim until he is free from wire; begin CPR and seek medical attention immediately.
8. **Fainting:** (Sign: Temporary unconscious; blurred vision; nausea, paleness, sweating)
   - Lay on back, loosen clothing, if victim vomit tilt head to side; bath the face with cool water.
9. **Shock:** (Sign: partly or totally unconscious; dazed condition, pale face, nausea)
   - Keep victim laying down and elevate feet (Apply direct pressure to the wound/ cut using a sterile gauze pad or clean cloth.
   - Elevate the injured area above the level of heart, if there is no fracture.
10. **Eye injury:**
    - If eye is splashed by a chemical, flush eye immediately for 15 minutes. While flushing lift eyelid as well. After flushing close eyelid and cover eye with moist dressing and seek medical attention immediately.

Table: Suggestive contents of first aid kit

Contents	Minimum Quantity
1.Tube silver sulfadiazine ointment (15g)	1
2. Band aid strips	10
3. Roller bandage (5x5 cm)	1
4. Package absorbent sterilized cotton (15g)	1
5. Scissor 7cm (sharp/blunt edge)	1
6. Paracetamol Tablets	10
7. Plastic mouth-to-mouth resuscitator	1
8. Triangular bandage (90 cm)	1
9. Antiseptic lotion-100ml	1
10. Safety pins	10
11. Adhesive tape	1
12. Splints	3 to 4
13. ORS sachets	2
14. Medical exam gloves	2 pairs
15. Hand sanitizer	1
16. First Aid kit checklist	1

Annexure 12

Documentation of laboratory incidents/accidents

Laboratory Incident

Date of incident reporting:_____Date of incident/accident occurred:_____

Personnel reporting the incident/accident:_____Incident/accident No: _____

Category of incident/accident – Please "√" on relevant category									
Spill & Splashes	Needle Stick	Fall & slip	Fire	Electrical	Chemical	Infrastructure	Testing Related	Equipment related	Others

Details of incident/accident:
Root cause analysis:
Corrective action taken (with date):
Preventive action taken (if applicable)
Date Signature of Laboratory Supervisor/In-charge

Annexure 13

Chemical used in TB laboratory: Hazards and precautions

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
Agar agar	M, H	Transparent odorless solid; [Merck Index] Light yellowish orange, yellowish grey to pale yellow, or colorless solid in various forms; [JECFA] Beige powder; [Sigma-Aldrich MSDS]. Insoluble in cold water; soluble in boiling water	Not a hazardous substance	Flash point data for this chemical are not available. It is probably combustible.	If inhaled: fresh air. In case of skin contact: Rinse skin with water/ shower. In case of eye contact: rinse out with plenty of water. If swallowed: drink water (two glasses at most).
Amikacin disulfate	H	White crystalline powder. m.p: 203-204°C. b.p: 632.23	H317: May cause an allergic skin reaction. H318: Causes serious eye damage. H372: Causes damage to organs through prolonged or repeated exposure.	When heated to decomposition it emits toxic fumes of nitrogen oxides.	P260, P261, P264, P264+P265, P270, P272, P280, P302+P352, P305+P354+P338, P317, P319, P321, P333+P313, P362+P364, and P501
Auramine O	L,M,H	Yellow flakes or powder; m.p. 136 °C; insoluble in water	H302: Harmful if swallowed. H311: Toxic in contact with skin. H319: Causes serious eye irritation. H351: Suspected of causing cancer. H411: Toxic to aquatic life with long lasting effects	Flash point data for this chemical are not available. It is probably combustible.	P203, P264, P264+P265, P270, P273, P280, P301+P317, P302+P352, P305+P351+P338, P316, P318, P321, P330, P337+P317, P361+P364, P391, P405, and P501
Basic Fuchsin	L, M, H	Green to dark green powder or crystals. m.p.268-270 ℃. Soluble in water	H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H351: Suspected of causing cancer. H411: Toxic to aquatic life with long lasting effects.	May be combustible at high temperature.	P203, P264, P264+P265, P270, P273, P280, P301+P317, P302+P352, P305+P351+P338, P318, P321, P330, P332+P317, P337+P317, P362+P364, P391, P405, and P501
Brain heart infusion agar	M, H	Beige powder	Not a hazardous substance or mixture	Combustible. Development of hazardous combustion gases or vapours possible	After inhalation: fresh air. After eye or skin contact: rinse out with plenty of water. Remove contact lenses. After swallowing: make victim

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
				in the event of fire.	drink water (two glasses at most).
Capreomycin sulfate	H	White to slightly yellowish white, amorphous powder. Odorless. Freely soluble in water. Practically insoluble in most org solvents.	H302 + H312 + H332: Harmful if swallowed, in contact with skin or if inhaled. H360: May damage fertility or the unborn child.	Hazardous combustion products formed under fire conditions	P203, P261, P264, P270, P271, P280, P301+P317, P302+P352, P304+P340, P317, P318, P321, P330, P362+P364, P405, and P501
Dihydrostreptomycin sulfate	H	White to Off-white powder. Soluble in water	H302 + H312 + H332: Harmful if swallowed, in contact with skin or if inhaled H360: May damage fertility or the unborn child	During a fire, highly toxic gases may be generated by thermal decomposition or combustion	P203, P260, P261, P264, P270, P271, P280, P301+P317, P302+P352, P304+P340, P317, P318, P319, P321, P330, P362+P364, P405, and P501
Di-Sodium Hydrogen Phosphate	M, H	white or colorless hygroscopic crystals or powder. m.p: > 450 °C Freely soluble in water.	A mild skin, eye, and respiratory tract irritant	not flammable	If inhaled: fresh air. In case of skin contact: Rinse skin with water/ shower. In case of eye contact: rinse out with plenty of water. If swallowed: drink water (two glasses at most).
Ethambutol dihydrochloride	H	Solid. m.p: 201.8 – 202.6 °C. Soluble in water	H302: Harmful if swallowed. H360: May damage fertility or the unborn child.	Hazardous decomposition products formed under fire conditions.	P203, P280, P318, P405, and P501
Ethanol	M, H	clear colorless liquid with a characteristic vinous odor and pungent taste. m.p: -114 °C; b.p: 78 °C. completely miscible in water	H319: Causes serious eye irritation. Harmful if ingested. May affect central nervous system.	Flash point 13 °C H225: Highly flammable liquid and vapor	P210, P233, P240, P241, P242, P243, P280, P303+P361+P353, P370+P378, P403+P235, and P501
Glycerol	M, H	Clear, colorless, odorless, syrupy liquid or solid. b.p: 290 °C (decomposes); m.p: 18 °C miscible in water at 25 °C	Not a hazardous substance	Flash point 177 °C Combustible. Gives off irritating or toxic fumes (or gases) in a fire.	Eye: Irrigate immediately Skin: Water wash Breathing: Fresh air
Hydrochloric acid (10-37%)	L,M,H	Colourless fuming liquid with a pungent odour; b.p. –121 °C; miscible with water	H314: Causes severe skin burns and eye damage H331: Toxic if inhaled	Fire may produce irritating or poisonous gases. Containers may explode in heat of fire. At high temperatures, it decomposes into hydrogen and chlorine.	P260, P261, P264, P271, P280, P301+P330+P331, P302+P361+P354, P304+P340, P305+P354+P338, P316, P321, P363, P403+P233, P405, and P501
Isoniazid	H	odorless colorless or white crystals or white crystalline powder. Taste is slightly sweet at first and then	H302: Harmful if swallowed. H315:Causes skin irritation.	Flashpoint >250 °C Combustible. Development of hazardous combustion gases or vapors possible	P264, P270, P280, P301+P317, P302+P352, P321, P330, P332+P317, P362+P364, and P501

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
		bitter. m.p: 170-173 °C. Soluble in water.		in the event of fire	
Kanamycin sulfate	H	Solid crystals. Miscible with water at 25 °C	H360: May damage fertility or the unborn child	Not flammable or combustible	P203, P280, P318, P405, and P501
L-asparagine monohydrate	H	White solid; mp: 235 °C	Not a hazardous substance	Combustible. Fire may cause evolution of: nitrogen oxides Development of hazardous combustion gases or vapours possible in the event of fire.	After inhalation: fresh air. After eye or skin contact: rinse out with plenty of water. Remove contact lenses. After swallowing: make victim drink water (two glasses at most).
Levofloxacin	H	Light yellowish - white to yellow-white crystal or crystalline powder; m.p.: 218 - 220 °C. sparingly soluble in water	H302: Harmful if swallowed. H317: May cause an allergic skin reaction. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H361: Suspected of damaging fertility or the unborn child H362: May cause harm to breast-fed children	Combustible. Development of hazardous combustion gases or vapors possible in the event of fire	P203, P260, P261, P263, P264, P270, P272, P280, P284, P301+P317, P302+P352, P304+P340, P318, P321, P330, P333+P313, P342+P316, P362+P364, P405, and P501
Malachite green oxalate	L, M, H	Crystalline; m.p: 144 - 150 °C. Soluble in water	H301: Toxic if swallowed. H318: Causes serious eye damage. H361d: Suspected of damaging the unborn child. H410: Very toxic to aquatic life with long lasting effects.	Hazardous decomposition products formed under fire conditions. - Carbon oxides, Nitrogen oxides (NOx)	P264, P264+P265, P270, P280, P301+P317, P305+P354+P338, P317, P330, and P501
Methanol	M, H	Colorless liquid with a characteristic pungent odor. m.p. –98 °C b.p. 65 °C; miscible with water	H225: Highly Flammable liquid and vapor H301: Toxic if swallowed H311: Toxic in contact with skin H331: Toxic if inhaled H370 **: Causes damage to organs	H225: Highly Flammable liquid and vapor; flashpoint –16.3 °C flammable range 7–37%.	P210, P233, P240, P241, P242, P243, P260, P261, P264, P270, P271, P280, P301+P316, P302+P352, P303+P361+P353, P304+P340, P308+P316, P316, P321, P330, P361+P364, P370+P378, P403+P233, P403+P235, P405, and P501
Methylene blue	L, M, H	dark green powder. m.p 374 °F. Soluble in water.	H302: Harmful if swallowed. H315: Causes skin irritation H319: Causes serious eye irritation H335: May cause	Flash point data for this chemical are not available; however, it is probably combustible.	P260, P261, P264, P264+P265, P270, P271, P280, P301+P317, P302+P352, P304+P340, P305+P351+P338, P319, P321, P330, P332+P317, P337+P317, P362+P364, P403+P233, P405, and P501

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
			respiratory irritation H373: Causes damage to organs through prolonged or repeated exposure.		
Moxifloxacin hydrochloride	H	Colorless, odorless, crystal, m.p: 331 °C	H302: Harmful if swallowed. H319: Causes serious eye irritation. H412: Harmful to aquatic life with long lasting effects.	Combustible. Development of hazardous combustion gases or vapors possible in the event of fire.	P264, P264+P265, P270, P273, P280, P301+P317, P305+P351+P338, P330, P337+P317, and P501
N-Acetyl-L-cysteine	M, H	White crystalline power with slight acetic odour. m.p: 109.5 °C b.p:143.9–145.3°C. soluble in water	H315: Causes skin irritation. H319: Causes serious eye irritation H335: May cause respiratory irritation	Violent reactions possible with: Strong oxidizing agents Development of hazardous combustion gases or vapors possible in the event of fire.	P261, P264, P264+P265, P271, P280, P302+P352, P304+P340, P305+P351+P338, P319, P321, P332+P317, P337+P317, P362+P364, P403+P233, P405, and P501
Nutrient Broth	M, H	Beige powder	Not a hazardous substance or mixture	-	If breathed in, move person into fresh air. In case of eye or skin contact: Wash off with plenty of water. If swallowed: Never give anything by mouth to an unconscious person. Rinse mouth with water.
Ofloxacin	H	Off-white to pale yellow crystalline powder. m.p: 250-257°C. Soluble in aqueous solutions with pH between 2 and 5. Sparingly to slightly soluble in aqueous solutions with pH 7 (solubility falls to 4 mg/mL) and freely soluble in aqueous solutions with pH above 9.	H302: Harmful if swallowed H312: Harmful in contact with skin H317: May cause an allergic skin reaction H332: Harmful if inhaled H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled H341: Suspected of causing genetic defects H361: Suspected of damaging fertility or the unborn child	Combustible. Emits toxic fumes under fire conditions.	P203, P261, P264, P270, P271, P272, P280, P281, P284, P301+P317, P302+P352, P304+P340, P317, P318, P321, P330, P333+P313, P342+P316, P362+P364, P405, and P501
Oxalic acid dihydrate	M, H	Colorless hygroscopic crystals. m.p:101-102 °C. b.p: 149 - 160 °C	H302 + H312: Harmful if swallowed or in contact with skin. H315: Causes skin irritation H318: Causes serious eye damage. H373: Causes damage to organs through	Combustible. Gives off irritating or toxic fumes (or gases) in a fire.	P260, P264, P264+P265, P270, P280, P301+P317, P302+P352, P305+P354+P338, P317, P319, P321, P330, P332+P317, P362+P364, and P501

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
			prolonged or repeated exposure		
Phenol	M, H	Colorless to light-pink, crystalline solid with a sweet, acrid odor. m.p. 41 °C b.p. 182 °C; soluble in water	H301: Toxic if swallowed H311: Toxic in contact with skin H314: Causes severe skin burns and eye damage H331: Toxic if inhaled H341: Suspected of causing genetic defects H373 **: Causes damage to organs through prolonged or repeated exposure	Flashpoint 80 °C flammable range 1.7–6%. Combustible material: may burn but does not ignite readily. When heated, vapors may form explosive mixtures with air. Contact with metals may evolve flammable hydrogen gas. Containers may explode when heated.	P203, P260, P261, P264, P270, P271, P280, P281, P301+P316, P301+P330+P331, P302+P352, P302+P361+P354, P304+P340, P305+P354+P338, P316, P318, P319, P321, P330, P361+P364, P363, P403+P233, P405, and P501
Potassium di Hydrogen phosphate	M,H	Odorless, colorless crystals or white granular or crystalline powder. m.p: 253 °C; Freely soluble in water	A skin, eye, and respiratory tract irritant;	not flammable. Not combustible. Gives off irritating or toxic fumes (or gases) in a fire.	If inhaled: fresh air. In case of skin contact: Rinse skin with water/ shower. In case of eye contact: rinse out with plenty of water. If swallowed: drink water (two glasses at most).
Potassium dichromate	L,M,H	orange red crystals; No distinctive odor. m.p 398 °C. Decomposes at about 500 °C; soluble in water.	H301: Toxic if swallowed. H312: Harmful in contact with skin. H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction. H330: Fatal if inhaled. H334: May cause allergy or asthma symptoms or breathing difficulties if inhaled. H335: May cause respiratory irritation. H340: May cause genetic defects. H350: May cause cancer. H360FD: May damage fertility. May damage the unborn child. H372: Causes damage to organs (Cardio-vascular system) through prolonged or repeated exposure if inhaled.	H272: May intensify fire; oxidizer Not combustible but enhances combustion of other substances. Risk of fire and explosion on contact with combustible substances.	P203, P210, P220, P260, P261, P264, P270, P271, P272, P273, P280, P281, P284, P301+P316, P301+P330+P331, P302+P352, P302+P361+P354, P304+P340, P305+P354+P338, P316, P317, P318, P319, P320, P321, P330, P333+P313, P342+P316, P362+P364, P363, P370+P378, P391, P403+P233, P405, and P501

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
			H410: Very toxic to aquatic life with long lasting effects.		
Potassium permanganate	L,M,H	Purple crystals. m.p. 240 °C (decomposes); readily soluble in water	H302: Harmful if swallowed. H361d: Suspected of damaging the unborn child. H400: Very toxic to aquatic life H410: Very toxic to aquatic life with long lasting effects	H272: May intensify fire; oxidizer. Powerful oxidizing agent; may ignite combustible materials.	P203, P210, P220, P264, P270, P273, P280, P301+P317, P318, P330, P370+P378, P391, P405, and P501
Rifampicin	H	Orange-red-brown color Powder/crystal. m.p: 183 – 188 °C	H302: Harmful if swallowed. H315: Causes skin irritation. H319: Causes serious eye irritation. H335: May cause respiratory irritation	this material is combustible, but will not ignite readily	P261, P264, P264+P265, P270, P271, P280, P301+P317, P302+P352, P304+P340, P305+P351+P338, P319, P321, P330, P332+P317, P337+P317, P362+P364, P403+P233, P405, and P501
Sodium citrate	M, H	Crystalline white powder or colorless crystals. m.p: >300 °C; completely soluble in water	Not a hazardous substance	Combustible. Development of hazardous combustion gases or vapors possible in the event of fire.	If inhaled: fresh air. In case of skin contact: Rinse skin with water/ shower. In case of eye contact: rinse out with plenty of water. If swallowed: drink water (two glasses at most).
Sodium dodecyl sulfate	H	White or cream-colored crystals, flakes, or powder. m.p: 204 - 207 °C - lit. Insoluble in water.	H302 Harmful if swallowed. H302 + H332 Harmful if swallowed or if inhaled. H315 Causes skin irritation. H318 Causes serious eye damage. H319 Causes serious eye irritation. H332 Harmful if inhaled. H335 May cause respiratory irritation. H412 Harmful to aquatic life with long lasting effects.	Flash point 170 °C H228: Flammable solid. Combustible. Gives off irritating or toxic fumes (or gases) in a fire.	P210, P273, P280, P301+p312, P304+P312+P340, P305+P351+P338
Sodium hydroxide	M, H	Colorless to white, odorless solid (flakes, beads, granular form) m.p: 318 °C. b.p: 1390 °C. soluble in water	H314 Causes severe skin burns and eye damage. H315 Causes skin irritation. H318 Causes serious eye damage. H319 Causes serious eye irritation.	H290 May be corrosive to metals. Non-combustible, substance itself does not burn but may decompose upon heating to produce corrosive and/or toxic fumes. Contact with metals may evolve	P260, P264, P280, P301+P330+P331, P302+P361+P354, P304+P340, P305+P354+P338, P316, P321, P363, P405, and P501

Table 10.1: Chemicals used in TB lab: hazards and precautions

Chemicals	Type of facility	Physical property	Health hazard	Fire hazard	Precaution*
				flammable hydrogen gas. Containers may explode when heated.	
Sodium hypochlorite	L,M,H	Greenish-yellow liquid with a disagreeable, sweetish odor. m.p: -20 °C. b.p: 111 °C. soluble in water	H314: Causes severe skin burns and eye damage. H318: Causes serious eye damage. H335: May cause respiratory irritation. H411: Toxic to aquatic life with long lasting effects.	EUH031 Contact with acids liberates toxic gas. Store in a cool, dark place, away from combustible materials. Emits chlorine gas when burned.	P260, P264, P264+P265, P273, P280, P301+P330+P331, P302+P361+P354, P304+P340, P305+P354+P338, P316, P317, P321, P363, P391, P405, and P501
Sulphuric acid	L,M,H	Colorless, odorless viscous liquid; m.p. 10°C b.p. (decomposes) 340 °C.	H314: Causes severe skin burns and eye damage Corrosive to all body tissues. Inhalation of vapor may cause serious lung damage. Contact with eyes may result in total loss of vision. Skin contact may produce severe necrosis.	It is highly reactive and capable of igniting finely divided combustible materials on contact. When heated, it emits highly toxic fumes. Avoid heat, water, and organic materials.	P260, P264, P280, P301+P330+P331, P302+P361+P354, P304+P340, P305+P354+P338, P316, P321, P363, P405, and P501

L- Low Risk; M-Moderate risk; H-High risk ; m.p-melting point; b.p-boiling point
*For hazard and precaution codes refe:https://www.chemsafetypro.com/Topics/GHS/GHS_precautionary_statement_p_code.html
Note: For any other chemicals the details can be found at NCBI > Chemicals & Bioassays > Pub Chem Compound[54]

Annexure 14

Risk identification and in-place control measures

Annexure 14 A: Risk identification and in-place control measures: Low-risk TB laboratories

Steps- Risk assessment	Information or in place risk control measures (NTEP: TDCs)	Applicable procedures (Y/NA/P)		
		Micro-scopy	Gene-Xpert	True-nat
1) **Description of potential biological agent/ biohazard**	*M. tuberculosis* may be present in clinical specimens (eg. sputum)	Y	Y	Y
	Spread by airborne and percutaneous routes, ingestion, contact/ fomites	Y	Y	Y
	ID50 (infectious dose) is estimated to be < 10 bacilli	Y	Y	Y
	Transmissible	Y	Y	Y
	Effective immunization is not routinely available	Y	Y	Y
	Antibiotics are available for post-exposure prophylaxis	Y	Y	Y
	Drug resistant strains exist but very less in this setting	Y	Y	Y
	Susceptible to 5% phenol or 1% freshly prepared hypochlorite for 15 min	Y	Y	Y
2) **Laboratory procedures/ activities (that might cause exposure to biohazard)**	Specimen receipt, storage and recording; leaky specimen containers	Y	Y	Y
	Transportation of specimens/materials inside and outside the laboratory	Y	Y	Y
	Contact with contaminated surface /fomite	Y	Y	Y
	Accidental spilling/dropping/splashing infectious material	Y	Y	Y
	Cleaning up spill	Y	Y	Y
	Preparing smear, heat fixing or staining of slides	Y	NA	NA
	Pouring, splitting or decanting infectious liquids	NA	Y	Y
	Mixing or shaking or vortexing	NA	Y	Y
	Manipulation of infectious material with wooden applicator sticks, inoculation loops or pipettes.	Y	Y	Y
	Handling and disinfection (chemical or heat/autoclaving) of biomedical waste	Y	Y	Y
	Biomedical waste transportation and disposal (in burial pits or by external agency)	Y	Y	Y
3) **Types of PPE to be used**	Laboratory coat	Y	Y	Y
	Gloves	Y	Y	Y
	Mask-95 (during spill management)	Y	Y	Y
4) **Types of safety equipment to be used**	Disposable loop or wooden applicator sticks	Y	NA	NA
	Pipette (disposable/re-usable)	NA	Y	Y
	Refrigerator	P	Y	Y
	Handwashing sink	Y	Y	Y

Steps- Risk assessment	Information or in place risk control measures (NTEP: TDCs)	Applicable procedures (Y/NA/P)		
		Micro-scopy	Gene-Xpert	True-nat
5) Design and condition of laboratory/ facility where work is conducted	The building and laboratory are physically secured (eg. boundary wall, doors and windows are closable and are locked when un-occupied, restricted entry to laboratory)	Y	Y	Y
	Specimen collection area is designated that can easily be located (appropriate signage) by patients	Y	Y	Y
	Space is adequate to perform the activities in the laboratory	Y	Y	Y
	Floors are smooth (not slippery and cracked; preferably tiles fitted) and cleaned on daily basis; walls are also smooth and clean	Y	Y	Y
	Bench tops sturdy and impervious to disinfectants (eg. made of steel, granite without cracks etc.); surface is decontaminated before starting and completing the work.	Y	Y	Y
	Furniture are good in numbers and condition, ergonomically appropriate and without any fabric coverings.	Y	Y	Y
	Good natural cross ventilation (open doors and windows) in laboratory area; laboratory is organized in a way that air flows from clean area to dirty area and finally to outside.	Y	Y	Y
	Mechanical ventilation (exhaust fan) is installed in area with inadequate ventilation/ airflow direction.	Y	Y	Y
	Air-conditioner is installed (considering air flows away from technicians) in the room where controlled environmental condition is required.	NA	Y	NA
	Electrical and water supplies are adequate for laboratory work	Y	Y	Y
	Additional un-interrupted power supply (of sufficient back-up depending on the electricity outage pattern) is in-place for critical equipment	NA	Y	NA
	Dedicated sink for hand washing is available (preferably near the exit)	Y	Y	Y
	First aid box, spill kit and eye wash kit/equipment are readily available in the facility.	Y	Y	Y
6) Human resources related factor that need to be addressed	Staff have received relevant biosafety training and are adequately informed about the hazards in the laboratory	Y	Y	Y
	New hired or deputed staff are allowed to work or use the equipment independently only after successful completion of training and competency assessment.	Y	Y	Y
	None of the staff are with impaired immunity and are screened for TB symptoms periodically	Y	Y	Y
	Job aids (good microbiological practices and standard operating procedures) work instructions are displayed/ provided to perform the work safely and efficiently.	Y	Y	Y

Annexure 14B: Risk identification and in-place control measures: Moderate-risk TB laboratories

Risk identification Template (filled with appropriate in-place measures)- **Moderate-risk Laboratories**	
Institution/Laboratory Name:	
Laboratory Supervisor:	
Date:	

Steps- Risk Assessment	Information or in-place risk control measures (NTEP: Culture and DST laboratories)	Applicable procedures (Y/P/NA)	
		Direct LPA	Solid Culture
1) Description of potential biological agent/ biohazard	*M. tuberculosis* is likely to be present in all specimens (sputum) subjected for LPA (all specimens are POSITIVE as per NTEP diagnosis algorithm).	Y	NA
	M. tuberculosis may be present in clinical specimens (eg. sputum, other body fluids or infected tissues) subjected to solid culture; majority of specimens may be smear NEGATIVE if they are tested for treatment follow-up.	NA	Y
	M. tuberculosis culture may be isolated at lower rate from the specimens and may be lower in concentration (as compared to liquid culture)	NA	Y
	Spread by airborne and percutaneous routes, ingestion, contact/ fomites	Y	Y
	ID50 (infectious dose) is estimated to be < 10 bacilli	Y	Y
	Transmissible	Y	Y
	Effective immunization is not routinely available	Y	Y
	Antibiotics are available for post-exposure prophylaxis	Y	Y
	Drug resistant strains exist	Y	Y
	Susceptible to 5% phenol or 1% freshly prepared hypochlorite for 15 min	Y	Y
2) Laboratory procedures/ activities (that might cause exposure to biohazard)	Specimen receipt, storage and recording; leaky specimen containers	Y	Y
	Transportation of specimens/materials inside and outside the laboratory	Y	Y
	Handling of culture and transportation inside or outside the laboratory	Y	Y
	Centrifuging the specimen/infectious materials	Y	Y
	Media inoculation (sample testing load/rate of positivity)	Y	Y
	Contact with contaminated surface /fomite	Y	Y
	Accidental spilling/dropping/splashing infectious material (specimen, processed specimen or culture bottles)	Y	Y
	Cleaning up spill	Y	Y
	Preparing smear, heat fixing or staining of slides	Y	NA
	Pouring, splitting or decanting infectious liquids	NA	Y
	Mixing or shaking or vortexing	NA	Y
	Grinding (tissue specimen)	Y	Y
	Manipulation of infectious material with wooden applicator sticks, inoculation loops or pipettes.	Y	Y

Steps- Risk Assessment	Information or in-place risk control measures (NTEP: Culture and DST laboratories)	Applicable procedures (Y/P/NA)	
		Direct LPA	Solid Culture
	Handling and disinfection (chemical or heat/autoclaving) of biomedical waste	Y	Y
	Transportation of biomedical waste for final disposal	Y	Y
3) Types of PPE to be used	Laboratory coat (re-usable)	Y	Y
	Disposable laboratory gowns/coat	Y	Y
	Gloves	Y	Y
	Mask-95	Y	Y
	Protective eye wear	P	P
	Protective headwear and footwear	P	P
4) Types of safety equipment to be used	Biological safety cabinet (preferably Class II Type A2; well maintained and certified initially, annually and each time a unit is moved)	Y	Y
	Autoclave (validated periodically using chemical and biological indicators)	Y	Y
	Micro-pipette	Y	Y
	Disposable loop	Y	Y
	Micro-incinerator (if reusable loops are used)	Y	Y
	Refrigerator	Y	Y
	Freezer	Y	Y
	Incubator	Y	Y
	Biosafe centrifuges (with sealed rotors or safety cups)	Y	Y
	Leak-proof and puncture proof vessels (for transportation of infectious materials and collecting sharp waste)	Y	Y
	Handwashing sink (with handsfree operation)	Y	Y
5) Design and condition of laboratory/ facility where work is conducted	The building and laboratory are physically secured (eg. boundary wall, doors and windows are closable and are locked when un-occupied) and laboratory access is controlled (by lock and key, biometric system or showing staff ID card to security guard).	Y	Y
	Access to high risk or high security areas by authorized staff only (eg. entry-exit log or CCTV in areas where culture isolate or other valuable biological materials are handled or stored).	Y	Y
	Specimen collection area is physically separated and it is located at place convenient to the patients (eg. next to the patient waiting area or appropriate signage at strategic locations).	Y	Y
	Space for working and storage are adequate	Y	Y
	Floors are smooth (not slippery and cracked; preferably tiled) and cleaned with disinfectant on daily basis; the walls are also smooth and clean.	Y	Y
	Bench tops are sturdy, resistant to moderate heat and impervious to disinfectants (eg. made of steel, granite without cracks etc.); Work surfaces are decontaminated before starting and completing the work.	Y	Y
	Furniture is good in numbers and condition, ergonomically appropriate and without any clothes coverings.	Y	Y

Steps- Risk Assessment	Information or in-place risk control measures (NTEP: Culture and DST laboratories)	Applicable procedures (Y/P/NA)	
		Direct LPA	Solid Culture
	Laboratory has sufficient number of BSCs (preferably Class II Type A2 - with thimble/canopy connection for exhaust to outsides) and are installed at appropriate place.	Y	Y
	Mechanical ventilation (air-handling unit/ exhaust fan) system is working properly and does not impacting on efficiency of BSC or working with biological material.	Y	Y
	Unidirectional airflow (from clean to dirty area) into the laboratory, and a minimum of 6–12 ACHs is ensured (in area where high risk activity is performed).	Y	Y
	Ample lighting in laboratory with reliable electrical supply (or availability of power generator unit).	Y	Y
	Additional un-interrupted power supply (with sufficient power backup) to critical equipment (eg. BSC, centrifuge)	Y	Y
	Adequate water supply and handwashing sinks are available (preferably near to exit door).	Y	Y
	Dedicated clean rooms are available for LPA once the DNA is extracted in sample processing room. Each room are physically separated from each other to allow unidirectional workflow (Master mix preparation, amplification and hybridization room) and are with separate ventilation systems.	Y	NA
	First aid box, spill kit and eye wash kit/equipment are readily available in the facility.	Y	Y
6) Human resources related factor that need to be addressed	Staff have received relevant biosafety training and are adequately informed about the hazards in the laboratory	Y	Y
	Newly hired or deputed staff are allowed to work or use the equipment independently only after successfully completion of training and competency assessment.	Y	Y
	None of the staff are with impaired immunity and are screened for TB symptoms periodically.	Y	Y
	Job aids (good microbiological practices and standard operating procedures) work instructions are displayed/ provided to perform the work safely and efficiently.	Y	Y

Y-Yes; NA-Not applicable; P-Preferable

Annexure 14C: Risk identification and in-place control measures: High-risk TB laboratories

Risk identification Template (filled with appropriate in-place measures)- **High-risk Laboratories**			
Institution/Laboratory Name:			
Laboratory Supervisor:			
Date:			
Steps- Risk assessment	**Information or in-place risk control measures (NTEP: C&DST Laboratory/IRLs/NRLs)**	Applicable procedures (Y/P/NA)	
		In-direct LPA	Liquid culture DST
1) **Description of potential biological agent/ biohazard**	Concentration of *M. tuberculosis* in liquid culture is high and therefore handling or manipulation with it (for identification, DNA extraction or liquid culture DST) involve high risk.	Y	Y
	Positivity rate of specimen subjected to liquid culture is higher (than the solid culture).	Y	Y
	Spread by airborne and percutaneous routes, ingestion, contact/ fomites	Y	Y
	ID50 (infectious dose) is estimated to be <10 bacilli	Y	Y
	Highly transmissible	Y	Y
	Effective immunization is not routinely available	Y	Y
	Antibiotics are available for post-exposure prophylaxis	Y	Y
	Drug resistant strains exist	Y	Y
	Susceptible to 5% phenol or 1% freshly prepared hypochlorite for 15 min	Y	Y
2) **Laboratory procedures/ activities (that might cause exposure to biohazard)**	Specimen receipt, storage and recording; leaky specimen containers	Y	Y
	Centrifuging the specimen/infectious materials	Y	Y
	Grinding (tissue specimen)	NA	Y
	Media inoculation (sample testing load/rate of positivity)	Y	Y
	Handling of specimen or culture and their transportation inside or outside the laboratory	Y	Y
	Opening of culture tubes	NA	Y
	Preparation of smears from positive cultures.	NA	Y
	DNA extraction from positive culture	Y	NA
	Manipulation of cultures for identification and DST	NA	Y
	Contact with contaminated surface /fomite	Y	Y
	Accidental spilling/dropping/splashing infectious material (specimen, processed specimen or culture tubes)	Y	Y
	Cleaning up spill	Y	Y
	Preparing smear, heat fixing or staining of slides	NA	Y
	Pouring, splitting or decanting infectious liquids	Y	Y
	Mixing or shaking or vortexing	Y	Y
	Manipulation of infectious material with wooden applicator sticks, inoculation loops or pipettes.	Y	Y
	Handling and disinfection (chemical or heat/autoclaving) of biomedical waste	Y	Y
	Transportation of biomedical waste for final disposal	Y	Y
	Disposable laboratory gowns/coat	Y	Y

Steps- Risk assessment	Information or in-place risk control measures (NTEP: C&DST Laboratory/IRLs/NRLs)	Applicable procedures (Y/P/NA)	
		In-direct LPA	Liquid culture DST
3) Types of PPE to be used	Gloves	Y	Y
	Mask-95	Y	Y
	Protective eye wear	P	P
	Protective headwear and footwear	P	P
4) Type of safety equipment to be used	Biological safety cabinet (preferably Class II Type A2; well maintained and certified initially, annually and each time a unit is moved)	Y	Y
	Autoclave (validated using chemical and biological indicators)	Y	Y
	Micro-pipette	Y	Y
	Disposable loop	Y	Y
	Micro-incinerator (if reusable loops are used)	NA	Y
	Refrigerator	Y	Y
	Freezer	Y	Y
	Incubator/MGIT	NA	Y
	Centrifuges (with sealed rotors or safety cups)	Y	Y
	Leak-proof and puncture proof vessels (for transportation of infectious materials and collecting sharp waste)	Y	Y
	Handwashing sink	Y	Y
5) Design and condition of laboratory/ facility	Specimen collection area is physically separated and it is located at place easily accessed by the patients (eg. next to the patient waiting area or appropriate signage at strategic locations)	Y	Y
	Containment laboratory with adequate space is located within the restricted area of the laboratory (preferably where laboratory personnel traffic is minimal) and away from regions that could impact directional air-flow or differential negative pressure.	Y	Y
	Access to the containment laboratory is restricted to authorized personnel only (via biometric system or entry-exit log) and is monitored for 24 hr a day (using CCTV camera).	Y	Y
	Entry into the containment lab through an anteroom with two self-closing doors; windows are sealed.	Y	Y
	Ventilation by air-handling unit (directional air flow; air-conditioning; gradient negative air pressure) in both anteroom and containment room; supply and exhaust of room air through HEPA filter.	Y	Y
	Anteroom to containment lab features include i) security system for access ii) windows allowing a view of main containment room; iii) negative pressure to the outside area of anteroom but at positive pressure to the main containment room iv) airtight and interlocking doors; v) pressure gauge; vi) adequate space.	Y	Y
	Containment laboratory has i) essential equipment (including BSC, centrifuge, MGIT machine, Oven, etc) required to perform the work; ii) airlocked pass boxes for	Y	Y

Steps- Risk assessment	Information or in-place risk control measures (NTEP: C&DST Laboratory/IRLs/NRLs)	Applicable procedures (Y/P/NA)	
		In-direct LPA	Liquid culture DST
	transfer in/out of materials; iii) safety shower/eye wash station; iv) hand washing; v) communication system; vi) Alarm system to indicate that negative pressure within the laboratory is out of range; vii) easy access of autoclave unit.		
	Work surfaces, floors, walls, and ceilings are designed, constructed, and finished to facilitate easy cleaning and decontamination.	Y	Y
	The room is capable of being sealed for gaseous decontamination.	Y	Y
	Containment lab has sufficient number of BSCs (preferably Class II Type A2 - with thimble/canopy connection for exhaust to outsides) and are installed at appropriate place.	Y	Y
	Ample illuminous in the laboratory as well as reliable electrical supply (or availability of power generator unit).	Y	Y
	Additional un-interrupted power supply (with sufficient power backup) to critical equipment (eg. BSC, centrifuge, AHU)	Y	Y
	Adequate water supply; handwashing sinks are available both in containment as well as near exit door.	Y	Y
	Adequate number of incubators and freezers are available for storage of infectious materials (eg. culture isolates)	Y	Y
	Bench tops are sturdy, resistant to moderate heat and impervious to disinfectants (eg. made of steel, granite without cracks etc.); Work surfaces is decontaminated before starting and completing the work.	Y	Y
	Furniture is good in numbers and condition, ergonomically appropriate and without any clothes coverings.	Y	Y
	Spill kit are readily available in the laboratory	Y	Y
	Dedicated areas with three separated rooms are available for LPA once the DNA is extracted in a TB containment facility. Each room are physically separated from each other to allow unidirectional workflow (Master mix preparation, amplification and hybridization room) and are with separate ventilation systems.	Y	NA
6) Human resources related factor that need to be addressed	Staff have received relevant biosafety training and are adequately informed about the hazards in the laboratory	Y	Y
	New hired or deputed staff are allowed to work or use the equipment independently only after successfully completion of training and competency assessments.	Y	Y
	None of staff are with impaired immunity and are screened for TB symptoms periodically	Y	Y
	Job aids (good microbiological practices and standard operating procedures) work instructions are displayed/provided to perform the work safely and efficiently.	Y	Y

Y-Yes; NA-Not applicable; P-Preferable

S. No	Suggested biosecurity measures (Tools and its feature/ purpose)		TDCs	C&DST Labs*	C&DST labs/ IRLs/NRLs
1.0	**Physical security measure**				
1.1	*Biosecurity layers:* Three graded layers that divided into general area, restricted area, and high biosecurity areas		Two layers (general and restrict area)	All three layers	All three layers
1.2	*Tools in different biosecurity layer*				
	General area (Physical boundary; security guard; manual or electronic keys; staff ID; signage; CCTV; entry-exit logbook, etc)	Physical boundary/barrier that separates general area from public area.	Required	Required	Required
		Controlled access that allows entry of staff, patients, suppliers, equipment service providers and visitors (not those who have no valid reasons to enter).	Required	Required	Required
		24 hrs. intrusion detection mechanism (like CCTV and/or security guard maintaining entry- exist logbook).	Preferable	Preferable	Required
	Restricted Area *(Similar tools as described for general are)*	Physical boundary/ barrier within general area.	Required	Required	Required
		Controlled access that allows entry of staff and authorized persons only.	Required	Required	Required
		24 hrs. intrusion detection mechanism (like CCTV and/or security guard maintaining entry- exist logbook).	Preferable	Required	Required
	High security area *(Similar tools as described for general are)*	Physical boundary/ barrier within restricted area.	Not Applicable	Not Applicable	Required
		Controlled access that allows entry of only authorized staff (not all the staff).			Required
		24 hrs intrusion detection mechanism (like CCTV and entry- exist logbook).			Required
2.0	**Inventory security measure**				
2.1	VBM inventory and log sheets	Inventory of VBM must include information on - Type of VBM and associated bio-security risk - Quantity stored (and internal external transfer log) - Location of storage and use. - Person authorized to handle or to take custody.	Preferable	Required	Required

S. No	Suggested biosecurity measures (Tools and its feature/ purpose)		TDCs	C&DST Labs*	C&DST labs/ IRLs/NRLs
		- Disposal mechanism			
2.2	Periodic review	At least monthly review of VBM inventory; internal/external transfer log; VBM disposal log	Preferable	Required	Required
3.0.	**Information security measure**				
3.1	Protecting physical information (lockable cupboard/ almirah)	Physical documents to be secured - Diagnosis result/patient information - Laboratory registers - Documents-Quality management system - Security plan, VBM inventory etc.	Required	Required	Required
3.2	Protecting electronic information (password protection; use of anti-virus software and genuine software)	Electronic documents to be secured - NIKSHAY app/web portal - LIMS software (validation) - Email accounts (official) - Reliable cloud storage - Computer system; Laptop or tablet - External electronic storage device (under lock and key)	Required	Required	Required
4.0.	**Personnel security measure**				
4.1	Accountability, training and competency assessment	- Authorize, train and assess the competency of staff who will use, handle, store, transfer, transport and dispose the VBM. - Training of housekeeping and maintenance staff – biosecurity risk area/ equipment.	Preferable	Preferable	Required
4.2	Background verification, due diligence and conflict of interest.	Important to address before delegating accountability related to VBM management	Preferable	Preferable	Required
4.3	Policy/procedure for visitors and others	- Authorization by laboratory manager - Assessment for escorted/on-escorted visit - Entry-exit log - Relevant training and PPE	Preferable	Preferable	Required
5.0.	**Transport security measure**				

S. No	Suggested biosecurity measures (Tools and its feature/ purpose)		TDCs	C&DST Labs*	C&DST labs/ IRLs/NRLs
		- Disposal mechanism			
2.2	Periodic review	At least monthly review of VBM inventory; internal/external transfer log; VBM disposal log	Preferable	Required	Required
3.0.	**Information security measure**				
3.1	Protecting physical information (lockable cupboard/ almirah)	Physical documents to be secured - Diagnosis result/patient information - Laboratory registers - Documents-Quality management system - Security plan, VBM inventory etc.	Required	Required	Required
3.2	Protecting electronic information (password protection; use of anti-virus software and genuine software)	Electronic documents to be secured - NIKSHAY app/web portal - LIMS software (validation) - Email accounts (official) - Reliable cloud storage - Computer system; Laptop or tablet - External electronic storage device (under lock and key)	Required	Required	Required
4.0.	**Personnel security measure**				
4.1	Accountability, training and competency assessment	- Authorize, train and assess the competency of staff who will use, handle, store, transfer, transport and dispose the VBM. - Training of housekeeping and maintenance staff – biosecurity risk area/ equipment.	Preferable	Preferable	Required
4.2	Background verification, due diligence and conflict of interest.	Important to address before delegating accountability related to VBM management	Preferable	Preferable	Required
4.3	Policy/procedure for visitors and others	- Authorization by laboratory manager - Assessment for escorted/on-escorted visit - Entry-exit log - Relevant training and PPE	Preferable	Preferable	Required
5.0.	**Transport security measure**				

S. No	Suggested biosecurity measures (Tools and its feature/ purpose)		TDCs	C&DST Labs*	C&DST labs/ IRLs/NRLs
5.1	Appropriate packaging (triple layer)	Standard triple layer packaging to avoid release of VBM like specimen, culture strain etc. (that can affect personnel/ environment)	Required	Required	Required
5.2	Entry and exist log and passes	To ensure that VBM are transferred out/in by authorized person	Preferable	Required	Required
5.3	Transportation by authorized agency/ individual	To ensure that the agency is certified/authorized to transport VBM (infectious material) as per national and international standard/ rules.	Required	Required	Required
5.4	Inventory and custody documentation for VBM	To ensure proper record-keeping and initiate corrective action, if any incident occurred.	Preferable	Required	Required
6.0.	**Emergency/ incident control**				
6.1	Contingency or response plan	That include response plan to all potential incident (like fire, theft, threat, flood etc.) Must include contact number of internal and external emergency responders.	Preferable	Required	Required
6.2	Incidence register	To document and communicate the incidence for root cause analysis and taking corrective and preventive measures.	Preferable	Required	Required
6.3	Training and drills	At least for key incidents; annual refresher training	Preferable	Required	Required
6.4	Periodic review	To identify the gaps and improve the response plan; Conducted at least annually and at the time of every major incidence.	Preferable	Required	Required

* C&DST laboratory performing only LPA (not Liquid culture and DST)

Annexure 16

TB Laboratory Biosafety supervisory and monitoring Checklist
National TB Elimination Programme
(Strengthening of NTEP TB laboratory network towards ensuring laboratory safety)

Introduction:

The TB laboratories present a risk of direct and indirect exposure to infectious aerosols, in addition to safety risks associated with any clinical laboratories such as chemical, fire, electrical and the risks associated with radiation.

Tuberculosis is a well-known occupational hazard for health care workers and therefore, applying the standard good laboratory operating procedures, safety equipment, contingency plans, and other laboratory biosafety measures is important. Laboratory biosafety measures not only ensures the safe handling of potentially infectious microorganisms by laboratory workers but also strives to prevent the accidental exposure of potentially harmful pathogens to larger communities.

The level of risk in particular laboratory depends on what types of testing activities being carried out. Considering procedural risk involved, TB laboratories are classified into following categories:

- ***Low-risk laboratories***
  - Test procedures- Smear Microscopy; Truenat; CBNAAT
  - Type of NTEP laboratory - District and sub-district level TB laboratories
- ***Moderate-risk laboratories***
  - Test procedures- Processing and concentration of specimens for culture and direct DST such as Line Probe Assay
  - Type of NTEP laboratory – TB C&DST Laboratory (having facility of LPA with or without solid culture)
- ***High-risk laboratories***
  - Procedures- Culture manipulation for identification of TB or DST or indirect-LPA
  - Type of NTEP laboratory- C&DST laboratory, IRL and NRL (having LC-DST services with or without other testing services) available for safe laboratory operations.

The laboratory should aim to progress toward implementing all the listed measures in respective risk-level checklist. Individual laboratory may track their progress by conducting an on-site audit by laboratory supervisor or by higher laboratory using the provided checklists and scores. Each question carries a maximum score of “2” if the suggested measure is in place, “1” for partial fulfilment of measure and “0” if the measure is not in place. A few questions may be not applicable for particular type of laboratory, thus the maximum score will be adjusted accordingly. Based on percent cumulative score achieved, the laboratories are awarded number of stars in following manner

	*	**	***	****	*****
Cumulative score (%)	**<60%**	**60-70%**	**70-80%**	**80-90%**	**>90%**

Safety audit checklist- Low-risk TB Laboratory

Sr. No.	Safety measures (Low-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
	A) Laboratory Access				
1	Laboratory areas are restricted to authorized personnel only. *(Signages for restricted entry; lockable doors)*				
2	Sputum collection inside the laboratory building is not allowed *(Designated location in open and well-ventilated areas – outside the building)*				
	B) Infrastructure				
3	Laboratory space is adequate for safe operation, cleaning and maintenance				
4	Sperate area/window for sample receipt				
5	Hand-washing sink is available (preferably near the exit door). *(Soap, SOP for correct hand washing technique)*				
6	Laboratory workbench is sturdy, spacious, smooth, easy to clean, impermeable to liquids, and resistant to commonly used laboratory chemicals and disinfectants.				
7	Furniture is fit for purpose and without cloth covering. (*No damaged table/chair; chair for adjustable height specially for microscopy*)				
8	Laboratory has good cross ventilation (open windows and doors) with directional air-flow *(Mechanical ventilation such as exhaust fan should be considered when there is obstruction or inadequate natural ventilation).*				
9	Air-flow in laboratory areas is ensured to be unidirectional and it flows from clean to dirty area.				
10	Adequate storage space is available for immediate and long period storage of laboratory regents/materials.				
11	Adequate supply of water and electricity for safe operation in laboratory.				
	C) Safe laboratory practices				
12	Eating and drinking inside the laboratory is not practiced				
13	Food and drink are not stored in refrigerator/cupboard used for storage of laboratory materials				
14	Work bench is kept tidy, clutter-free and decontaminated (with appropriate disinfectant, before starting the work, immediately after completion the work, and if spillage occur).				
15	Staff are trained in hand hygiene practices and they follow the same. *(Washing of every time after handling biological material or removing the gloves; before eating/drinking and leaving the laboratory; or when hands are known or believed to be contaminated). Pictures depicting hand-washing steps are pasted / available at each wash station.*				
16	Staff are trained in correct pipetting technique to minimize aerosol generation *(Avoiding forcibly expelling of liquid; over-vigorous mixing, and carelessly flipping open tubes)*				
17*	Availability and use of disposable loop/ wooden stick for smear preparation *(Avoiding the use of disposable loop)*				
18	Electronic gadget/equipment (like telephone, tablet or computer) are protected to be contaminated; when not necessary kept away from work-place. *(Restricted handling of electronic gadgets with gloved hands)*				
	D) Personnel Protective Equipment (PPE)				
19	Availability of single use or reusable laboratory coat. *(If reusable coats are used, at least 3 lab coats should be available- one for in-use; one for emergency use; and one in laundry as per method described in TB laboratory biosafety manual)*				

Sr. No.	Safety measures (Low-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
20	Availability of disposable gloves (appropriate size)				
	E) Equipment				
21	Equipment in laboratory is certified for safe use *(Periodic calibration and/or maintenance of equipment is available).*				
22	Staff are trained to operate the equipment safely. *(SOP for safety precaution, use of equipment as well as in-house maintenance log for critical equipment such Truenat, CBNAAT and microscope)*				
	F) Sample handling, packaging and transportation				
23	Specimens are received in clear, leak-proof, unbreakable plastic containers with screw caps. *(Adhere to the NTEP specification for sample container)*				
24	When receiving or unpacking, the samples are inspected for their safe condition. *(Adherence to NTEP guideline for sample rejection criteria; availability of disinfectant soaked cotton for cleaning the exterior of container)*				
25	Triple layer packaging is used to transport the specimens between the facilities *(Availability of packaging materials and packaging as per NTEP guideline)*				
26	When required, samples are stored at required temperature (refrigerator) and away from clean material (like kits, consumables and reagents) *(Availability of refrigerator packaging materials and packaging as per NTEP guideline)*				
	G) Handling of biomedical waste and their disposal				
27	Wastes are segregated in color coded biohazard bags/bins. *(Availability of different color-coded bins preferably foot operated)*				
28	Wastes are disinfected (5% phenol) before disposal as per standard procedures				
29	Final disposal of wastes as per BMW rule (Govt. of India) *(Contract with BMW agency for collection and disposal of waste)*				
	H) Chemical, fire and electrical safety				
30	Chemicals are clearly labelled and stored i) not in excess amount; iii) not above eye level; iv) away from direct sunlight or heat; and v) separately if un-compatible.				
31	Storage of flammable liquids in ventilated place and away from fire source. (Safe storage of alcohol, sprit and other flammables).				
32	Fire extinguishers/buckets are available and staff are trained to respond the fire incidence.				
33	All flexible connecting electricity cables are as short as possible, in good condition, and not frayed, damaged, or spliced?				
	I) Biosecurity				
34	Doors and windows are break-proof and laboratory is securely locked when unoccupied				
35	Sensitive information either in physical or digital form are secured (using lock and key or passwords) *(Access to Nikshay- Tablet/laptop/computer; Lab register)*				
	J) Occupational Health				
36	Laboratory staff are screened for TB symptoms at least once a year (chest X-ray, NAAT or Tuberculin skin testing as per recommendation of medical officer)				
37	During pregnancy, alternative working arrangements (with minimal biosafety risk) are considered for women.				
38	First-aid facility/box is readily accessible. *(If laboratory located within the hospital premises, consider "Yes")*				

Safety audit checklist- Moderate-risk TB Laboratory

Sr. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
	A) Safety Training				
1	Laboratory safety is included into annual laboratory training/refresher training plan. All staff are trained and competent in laboratory safety. *(Training calendar and records, Training tools and agenda in line with TB Laboratory Biosafety Manual, CTD, Govt. of India)*				
	B) Laboratory Access				
2	Laboratory areas are restricted to authorized personnel only. *(Signages for restricted entry; lockable doors)*				
3	Sputum collection inside the laboratory building is not allowed *(Designated location in open and well-ventilated areas – outside the building)*				
4	Biohazard signage are displayed on the doors where higher-risk activities are performed *(Ex. Doors of specimen processing and culture area, biomedical disinfection/storage area)*				
	C) Infrastructure				
5	Laboratory space is adequate for safe operation, cleaning and maintenance.				
6	Sperate area/window for sputum receipt				
7	Dedicated space/room are available for incompatible laboratory activities. *[Example - dedicated rooms/cabins would require for i) LPA testing (Master mix preparation room; Amplification room; Hybridization room); ii) sample processing and culture activity; iii) smear microscopy; iv) media and reagent preparation, v) washing and sterilization- clean items, vi) disinfection/ sterilization- infectious materials; vii) administrative. recording and reporting]*				
8	Air-flow in laboratory areas is ensured to be unidirectional and it flows from clean to dirty area. *(Exhaust fan in area where ventilation/directional air-flow is inadequate)*				
9	Laboratory workbench is sturdy, spacious, smooth, easy to clean, impermeable to liquids, and resistant to commonly used laboratory chemicals and disinfectants.				
10	Hand-washing sink is available (preferably near the exit door). *(Soap, wall instructions/procedure for correct hand washing technique) Pictures depicting hand-washing steps are pasted / available at each wash station.*				
11	Floors and walls are cleaned. Daily housekeeping activities are in-place.				
12	Furniture is fit for purpose and without cloth covering. (*No damaged table/chair; chair for adjustable height specially for microscopy, and working inside a BSC*)				
13	Adequate storage space is available for immediate and long period storage of laboratory regents/materials. *(Separate storage of infectious material and clean/sterile materials)*				
14	Adequate supply of water and electricity for safe operation in laboratory.				
	D) Safety equipment (including personal protective equipment)				
15	Biological safety equipment (Class II type A2) is installed appropriately, and preventive maintenance and certification is done periodically. *(For placement of BSC, air movement, people movement, doors and windows, location of other equipment, surrounding clearance, etc should be considered; Annual validation and preventive maintenance report of BSC)*				
16	BSC exhaust is ducted (preferably thimble ducting) outside the building, and air passes through a HEPA filter before being discharged.				
17	The BSC is connected to a reliable power supply (preferably through a dedicated UPS with sufficient capacity to keep the BSC running for at				

Sr. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
	least 15 minutes).				
18	Safety centrifuge with safety buckets is available and fit for use. *(No damage of bucket, lid and O-ring; timely calibration and preventive maintenance reports)*				
19	Appropriate gowns are available and used during working in the lab				
20	Disposable gloves , in appropriate sizes, are available and used while handling infectious/contaminated materials.				
21	Respirators (N-95 mask) are available and worn appropriately while performing high-risk procedures. *(Appropriate storage of mask if re-used)*				
22	mittens/ heat resistant gloves (for handling heated materials) or heavy-duty gloves (for washing of glassware) are available and worn whenever required.				
23	Face-shield/ safety goggles are available and used during any operation where splashes may occur (e.g mixing of disinfectant solutions a).				
	E) Safe laboratory practices				
24	All activities involving manipulation of infectious materials are carried out inside the BSC				
25	Centrifuge buckets are loaded and unloaded in a BSC.				
26	The tubes containing infectious materials are not immediately opened after centrifugation, vortexing, or shaking.				
27	Staff are trained in correct pipetting technique to minimize aerosol generation. *(Avoiding for vigorous mixing, forcibly dispensing of liquid, contamination of the micropipette's barrel)*				
28	Staff are trained in hand hygiene practices, and they follow the same. *(Washing every time after handling biological material or removing the gloves; before eating/drinking and leaving the laboratory; or when hands are known or believed to be contaminated).*				
29	Availability and use of disposable loop. *(Use of micro-incinerator when reusable metal loop is used inside the BSC)*				
30	Correct donning-doffing of PPE and appropriate storage if re-used. PPE are removed before leaving the laboratory.				
31	Electronic gadget/equipment (like telephone, tablet or computer) as well as registers are protected to be contaminated; when not necessary kept away from work-place. *(Restricted handling of electronic gadgets with gloved hands)*				
32	Work bench is kept tidy, clutter-free and decontaminated (with appropriate disinfectant, before starting the work, immediately after completion the work, and if spillage occur). *(Use of 5% phenol as disinfectant for bacteriological work and 1% freshly prepared hypo chlorite for molecular work)*				
33	Eating and drinking inside the laboratory is not practiced. Foods and drinks are not stored in refrigerator/cupboard used for storage of laboratory materials. *(Dedicated place for refreshment out-side the laboratory)*				
	F) Sample handling, packaging, and transportation				
34	Specimens are received in clear, leak-proof, unbreakable plastic containers with screw caps. *(Adhere to the NTEP specification for sample container)*				
35	When receiving the specimen from courier agency, unpacking is done in a ventilated place and it is ensured that samples are in safe condition and were transported in triple layer packaging. *(Adherence to NTEP guideline for sample rejection criteria such as sample leakage)*				
36	Samples are stored before processing at required temperature				

Sr. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
	(refrigerator) and away from clean material (like kits, consumables and reagents) *(Separate refrigerator or infectious material and clean materials)*				
	G) Handling of biomedical waste and their disposal				
37	Wastes are segregated in color coded biohazard bags/bins. *(Availability of different color-coded bins preferably foot operated)*				
38	Infectious wastes are autoclaved before disposal. Effective autoclaving is ensured using chemical/ biological indicators.				
39	Biomedical waste is removed from the laboratory within 48 hrs and the area where the biomedical waste is temporarily stored is secured.				
40	Final disposal of wastes as per BMW rule (Govt. of India) *(Contract with BMW agency for collection and disposal of waste)*				
	H) Chemical safety				
41	Chemicals are clearly labelled and stored i) not in excess amount; iii) not above eye level; iv) away from direct sunlight or heat; and v) separately if un-compatible.				
	I) Fire safety				
42	Fire extinguishers are maintained (fully charged and in working order) and kept at strategic locations.				
43	Open flames are never used in BSC; and flammable items are always kept in a well-ventilated place away from open flame. *(Safe storage of alcohol, sprit and other flammables)*				
44	Corridors, and fire exit are always clear and unobstructed for movement of staff and fire-fighting equipment at the time of emergencies				
45	All laboratory staff are trained in using fire extinguisher and to respond fire emergencies (through periodic drill)				
	J) Electrical safety				
46	All flexible connecting electricity cables are as short as possible, in good condition, and not frayed, damaged, or spliced?				
47	Electrical pre-requisite is considered when equipment installed and operated.				
	K) Radiation safety				
48	Access to the UV radiation area/UV room/BSC is limited to only authorize personnel who knows harm from radiation and trained to avoid exposure.				
	L) Biosecurity				
49	Doors and windows are break-proof and laboratory are securely locked when unoccupied.				
50	Entry/exit log or gate pass system are in-place to ensure that valuable biological materials are transferred out/in by authorized persons only.				
51	Sensitive information either in physical or digital form are secured (using lock and key or passwords) *(Access to Nikshay- Tablet/laptop/computer; Lab register)*				
	M) Occupational Health				
52	Laboratory staff are screened for TB symptoms at least once a year (chest X-ray, NAAT or Tuberculin skin testing as per recommendation of medical officer)				
53	During pregnancy, alternative working arrangements (with minimal biosafety risk) are considered for women.				
54	First-aid facility/box is readily accessible. *(consider 'Yes' if laboratory is part of health facility/ hospital)*				

Safety audit checklist- Hight-risk TB Laboratory

S. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
Safety Training					
1	Laboratory safety is included into annual laboratory training/refresher training plan. All staff are trained and competent in laboratory safety. *(Training calendar and records, Training tools and agenda in line with TB Laboratory Biosafety Manual, CTD, Govt. of India)*				
Laboratory Access					
2	Laboratory areas are restricted to authorized personnel only. *(Signages for restricted entry; lockable doors)*				
3	Sputum collection inside the laboratory building is not allowed *(Designated location in open and well-ventilated areas – outside the building)*				
4	Biohazard signage are displayed on the doors where higher-risk activities are performed *(Ex. Doors of specimen processing and culture area, biomedical disinfection/storage area)*				
5	Access to the containment laboratory is restricted to authorized personnel only --via ● Biometric system ● Entry-exit log ● Monitored for 24 hr a day (using CCTV camera).				
6	Entry into containment lab through an anteroom with two self-closing doors; windows are sealed.				
A) Infrastructure					
7	Sperate area for sputum collection / Separate Area for specimen reception				
8	**Ventilation by air-handling unit** i) Directional air flow ii) Air-conditioning iii) Gradient negative air pressure in both anteroom and containment room iv) Supply and exhaust of room air through HEPA filter.				
9	**Anteroom to containment lab features include** i) security system for access ii) windows allowing a view of main containment room iii) negative pressure to the outside area of anteroom but at positive pressure to the main containment room iv) airtight and interlocking doors v) pressure gauge.				
10	**Containment laboratory has** i) Essential equipment (including BSC, centrifuge, MGIT machine, Oven, etc) required to perform the work **ii)** Airlocked pass boxes for transfer in/out of materials **iii)** Safety shower **iv)** Eye wash station **v)** Hand washing **vi)** Communication system **vii)** Alarm system to indicate that negative pressure within the laboratory is out of range **viii)** Easy access of autoclave unit				
11	Work surfaces, floors, walls, and ceilings are designed, constructed, and finished to facilitate easy cleaning and decontamination.				
12	Containment lab has sufficient number of BSCs (preferably Class II Type A2 - with thimble/canopy connection for exhaust to outsides) and				

S. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
	are installed at appropriate place.				
13	Additional un-interrupted power supply (with sufficient power backup) to critical equipment (e.g., BSC, centrifuge, AHU)				
14	Ample lighting in the laboratory as well as reliable electrical supply (or availability of power generator unit).				
15	*sterilization- infectious materials; vii) administrative. recording and reporting]*				
16	Air-flow in laboratory areas is ensured to be unidirectional and it flows from clean to dirty area. *(Exhaust fan in area where ventilation/directional air-flow is inadequate)*				
17	Laboratory workbench is sturdy, spacious, smooth, easy to clean, impermeable to liquids, and resistant to commonly used laboratory chemicals and disinfectants.				
18	Hand-washing sink is available (preferably near the exit door). *(Soap, wall instructions/procedure for correct hand washing technique)*				
19	Floors and walls are cleaned. Daily housekeeping activities are in-place.				
20	Furniture is fit for purpose and without cloth covering. (*No damaged table/chair; chair for adjustable hight specially for microscopy, and working inside a BSC*)				
21	Adequate storage space is available for immediate and long period storage of laboratory regents/materials. *(Separate storage of infectious material and clean/sterile materials)*				
22	Spill kit(s) are readily available in the laboratory				
Safety equipment (including personal protective equipment)					
23	Biological safety equipment (Class II type A2) is installed appropriately, and preventive maintenance and certification is done periodically. *(For placement of BSC, air movement, people movement, doors and windows, location of other equipment, surrounding clearance, etc should be considered; Annual validation and preventive maintenance report of BSC)*				
24	BSC exhaust is ducted (preferably thimble ducting) outside the building, and air passes through a HEPA filter before being discharged.				
25	The BSC is connected to a reliable power supply (preferably through a dedicated UPS with sufficient capacity to keep the BSC running for at least 15 minutes).				
26	Safety centrifuge with safety buckets is available and fit for use. *(No damage of bucket, lid and O-ring; timely calibration and preventive maintenance reports)*				
27	Gowns are available and used during working in the lab				
28	Disposable gloves in appropriate sizes, are available and used while handling infectious/contaminated materials.				
29	Respirators (N-95 mask) are available and worn appropriately while performing high-risk procedures. *(Appropriate storage of mask if re-used)*				
30	mittens/ heat resistant gloves (for handling heated materials) or heavy-duty gloves (for washing of glassware) are available and worn whenever required.				
31	Face-shield/ safety goggles are available and used during any operation where splashes may occur (e.g., mixing of disinfectant solutions a).				
Safe laboratory practices					
32	All activities involving manipulation of infectious materials are carried out inside the BSC				
33	Centrifuge buckets are loaded and unloaded in a BSC.				
34	The tubes containing infectious materials are not immediately opened after centrifugation, vortexing, or shaking.				
35	Staff are trained in correct pipetting technique to minimize aerosol generation. *(Avoiding for vigorous mixing, forcibly dispensing of liquid,*				

S. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
	contamination of the micropipette's barrel)				
36	Staff are trained in hand hygiene practices, and they follow the same. Pictures depicting hand-washing steps are pasted / available at each wash station. *(Washing of every time after handling biological material or removing the gloves; before eating/drinking and leaving the laboratory; or when hands are known or believed to be contaminated).*				
37	Availability and use of disposable loop. *(Use of micro-incinerator when reusable metal loop is used inside the BSC)*				
38	Correct donning-doffing of PPE and appropriate storage if re-used. PPE are removed before leaving the laboratory.				
39	Electronic gadget/equipment (like telephone, tablet or computer) as well as registers are protected to be contaminated, when not necessary kept away from work-place. *(Restricted handling of electronic gadgets with gloved hands)*				
40	Work bench is kept tidy, clutter-free and decontaminated (with appropriate disinfectant, before starting the work, immediately after completion the work, and if spillage occur). *(Use of 5% phenol as disinfectant for bacteriological work and 1% hypo chlorite for molecular work)*				
41	Eating and drinking inside the laboratory is not in practice. Foods and drinks are not stored in refrigerator/cupboard used for storage of laboratory materials. *(Dedicated place for refreshment out-side the laboratory)*				
Sample handling, packaging and transportation					
42	Specimens are received in clear, leak-proof, unbreakable plastic containers with screw caps. *(Adhere to the NTEP specification for sample container)*				
43	When receiving the specimen from courier agency, unpacking is done in a ventilated place and it is ensured that samples are in safe condition and were transported in triple layer packaging. *(Adherence to NTEP guideline for sample rejection criteria such as sample leakage)*				
44	Samples are stored at required temperature (refrigerator) and away from clean material (like kits, consumables and reagents) *(Separate refrigerator or infectious material and clean materials)*				
Handling of biomedical waste and their disposal					
45	Wastes are segregated in color coded biohazard bags/bins. *(Availability of different color-coded bins preferably foot operated)*				
46	Infectious wastes are autoclaved before disposal. Effective autoclaving is ensured using chemical/ biological indicators.				
47	Biomedical waste is removed from the laboratory within 48 hrs and the area where the biomedical waste is temporarily stored is secured.				
48	Final disposal of wastes as per BMW rule (Govt. of India) *(Contract with BMW agency for collection and disposal of waste)*				
Chemical safety					
49	Chemicals are clearly labelled and stored i) not in excess amount; iii) not above eye level; iv) away from direct sunlight or heat; and v) separately if un-compatible.				
Fire safety					
50	Fire extinguishers are maintained (fully charged and in working order) and kept at strategic locations.				
51	Open flames are never used in BSC; and flammable items are always kept in a well-ventilated place away from open flame. *(Safe storage of alcohol, sprit and other flammables)*				
52	Corridors, and fire exit are always clear and unobstructed for movement of staff and fire-fighting equipment at the time of emergencies				
53	All laboratory staff are trained in using fire extinguisher and to respond fire emergencies (through periodic drill)				

S. No.	Safety measures (Moderate-risk TB Laboratory)	Yes (2)	No (0)	Partial (1)	NA
Electrical safety					
54	All flexible connecting electricity cables are as short as possible, in good condition, and not frayed, damaged, or spliced?				
55	Electrical pre-requisite is considered when equipment installed and operated.				
Radiation safety					
56	Acce BSC is limited to only authorize personnel who knows harm from radiation and trained to avoid exposure.				
Biosecurity					
57	Doors and windows are break-proof and laboratory are securely locked when unoccupied.				
58	Entry/exit log or gate pass system are in-place to ensure that valuable biological materials are transferred out/in by authorized persons only.				
59	Sensitive information either in physical or digital form are secured (using lock and key or passwords) *(Access to Nikshay- Tablet/laptop/computer; Lab register)*				
Occupational Health					
60	Laboratory staff are screened for TB symptoms at least once a year (chest X-ray, NAAT or Tuberculin skin testing as per recommendation of medical officer)				
61	During pregnancy, alternative working arrangements (with minimal biosafety risk) are considered for women.				
62	First-aid facility/box is readily accessible. *(consider 'Yes' if laboratory is part of health facility/ hospital)*				