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NTEP Guidance Document for NABL Accreditation Assessment of Tuberculosis Laboratories in Private Sector

April 2023

National TB Elimination Programme
Central TB Division,
Ministry of Health and Family Welfare,
Govt. of India, New Delhi

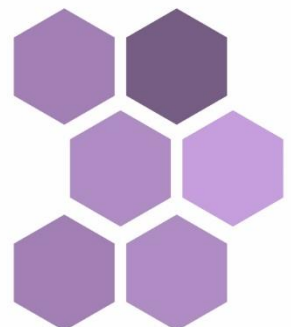


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List of Abbreviations

ACH	Air Change Per Hour
AFB	Acid Fast Bacilli
AST	Antimicrobial Susceptibility Testing
BSC	Biosafety Cabinet
BD	Becton Dickinson
BMW	Bio-Medical Waste
C&DST	Culture and Drug Susceptibility Testing
COVID	Coronavirus Disease
CLSI	Clinical and Laboratory Standards Institute
DNA	Deoxyribonucleic Acid
DMC	Designated Microscopy Centre
DR-TB	Drug-resistant Tuberculosis
DST	Drug Susceptibility Testing
EQA	External Quality Assurance
FL	First Line
ICT	Immunochromatographic Test
IEC	International Electrotechnical Commission
IRL	Intermediate Reference Laboratory
ISO	International Organization for Standardization
IQC	Internal Quality Control
GoI	Government of India
LC	Liquid Culture
LJ	Lowenstein Jensen
LPA	Line Probe Assay
LQMS	Laboratory Quality Management System
MGIT	Mycobacteria Growth Indicator Tube
MoHFW	Ministry of Health and Family Welfare
MTB	<i>Mycobacterium tuberculosis</i>
NAAT	Nucleic Acid Amplification Test
NABL	National Accreditation Board for Testing and Calibration Laboratories
NRL	National Reference Laboratory
NSP	National Strategic Plan
NTEP	National Tuberculosis Elimination Program

PHFID	Provider's Health Facility ID
PPE	Personal Protective Equipment
PT	Panel Testing
QC	Quality Control
QMS	Quality Management System
QSE	Quality System Essentials
RBRC	Random Blinded Rechecking
SOP	Standard Operating Procedure
SL	Second Line
TAT	Turnaround Time
TB	Tuberculosis
TDC	Tuberculosis Detection Centre
WHO	World Health Organization

1. Introduction

Tuberculosis (TB) is one of the leading causes of infectious disease deaths worldwide, with an estimated 1.6 million deaths occurring in 2021 (which was the highest number of deaths due to any infectious organism, after COVID-19). India is among the high-burden TB countries, accounting for one-fourth of the global burden of TB and multi-drug resistant TB. In response to the Global END-TB Call, the government of India has committed to End-TB by 2025.

To accelerate the efforts of TB programme in meeting the goals of End-TB, the Government of India (GoI) implemented the National strategic plan (NSP) for 2017-2025 to introduce interventions to expand and improve diagnosis, treatment and care for patients with TB. One of the key strategies is to engage the private sector (including laboratories) as the majority of TB patients (60-70%) seek care in the private sector. It is, therefore, necessary for the National TB Elimination Program (NTEP) to ensure that patients in private sectors receive quality diagnostic services, drugs, appropriate treatment, protection from high out-of-pocket expenditure, and other public health services such as co-morbidity testing, contact investigation, counselling, adherence monitoring, nutritional support, and outcome reporting as public-sector patients.

Strengthening of TB laboratories is a key component of the NSP for TB elimination goal. NTEP has expanded the diagnostic network covering over 30,000 facilities in the public health system (Fig 1). The laboratory network of NTEP is equipped with World Health Organization (WHO)-recommended rapid TB diagnostic tests and complies with WHO-recommended quality assurance protocols and biosafety requirements for all TB test procedures. In addition to this, NTEP laboratory network is progressing towards implementing the quality management system (QMS) in accordance to International Organization for Standardization (ISO)-15189. More than 35 TB laboratories under NTEP have obtained accreditation from National Accreditation Board for Testing and Calibration Laboratories (NABL) and the number continues to increase.

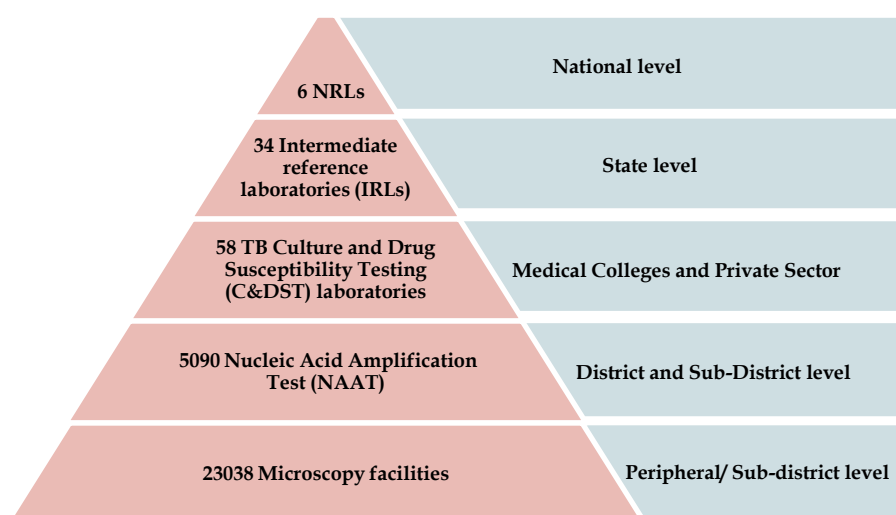


Figure 1. Network of TB laboratories – National TB Elimination Programme (NTEP)

Private laboratory networks are contributing significantly in notifying cases of TB alongside the public health laboratory system. However, it is crucial to ensure that the diagnostic services provided by these laboratories are of high quality. One way to ensure quality is through accreditation by the NABL, which has accredited over 2300 medical testing laboratories (both public and private) in India as of

December 31st, 2022. NABL assessors verify the Quality Systems documentation and its implementation by the laboratories for its compliance in accordance with ISO 15189 standard.

NTEP recognises the diagnostic network and efficiency in the private sector and encourages public-private partnerships. NTEP is leveraging private sector services, including laboratory testing, and has issued guidelines for engaging private sector in the "Guidance Document on Partnerships."¹ In addition to engaging private laboratory networks, NTEP is striving towards improving the quality of TB services in the private sector by training to private providers on TB diagnosis and treatment guidelines, establishing referral networks from public to private sectors and providing quality-assured free diagnostic services and medicines.

Moving forward, NTEP is promoting the certification of private laboratories providing Line Probe Assay (LPA) and liquid culture - drug susceptibility testing (LC-DST) services to TB/Drug-resistant TB (DR-TB) patients. For certification, NTEP has defined quality standards for public/private sector laboratories and certification is awarded for both first line (FL) and second line (SL) LPA, as well as FL and SL LC-DST.

NTEP certification ensures that patients receive the best quality diagnostic services for TB and that appropriate treatment is initiated. The process of NTEP laboratory certification includes several steps. First, the application is reviewed to determine whether the laboratory has met the prerequisites. Second, an on-site visit is conducted by the NTEP National Reference Laboratory (NRL) to validate the information provided in the application. Third, a proficiency testing (consisting of panel testing and re-testing) exercise is conducted with the NRL. Fourth, the laboratory is certified if desired concordance is achieved in proficiency testing and all recommendations by NRL is addressed. Fifth, the laboratory's testing data is monitored post-certification, and annual on-site evaluation visits and panel testing exercise are conducted to maintain certification and renew it further (Annexure 1).

2. Purpose and scope of document

Handling of TB specimen/culture and TB testing procedures involve a significant occupational risk (exposure to infectious aerosol) and it requires a set of biosafety measures as recommended by WHO. Furthermore, some of TB tests are complex, require special skill sets to perform or interpret the results in accordance with national and international guidelines that are periodically updated. While these aspects are carefully considered and monitored within the NTEP network of laboratories, it is important that laboratories in the private sector also comply with TB testing standards/guidelines and are supervised.

This guidance document is intended for "**NABL Assessors**" to consider it as supplement document when conducting assessments of any private sector medical laboratory seeking ISO-15189 accreditation with a TB test included in their scope.

TB tests under the scope of this document include

1. Smear Microscopy (*Ziehl-Neelsen (ZN)/Fluorescence*)
2. GeneXpert, Cepheid
3. Truenat, Molbio Diagnostic Pvt. Ltd.
4. Line Probe Assay (FL/SL), Hain Lifesciences
5. Solid Culture (Lowenstein Jensen (LJ) medium)
6. Mycobacteria growth indicator tube (MGIT)-LC DST, Becton Dickinson (BD) (*First line and second line drugs*)



Figure 2. TB tests under the scope of document

3. Guidance for assessment of private sector TB laboratories

The Laboratory Quality Management System (LQMS) is a set of procedures and practices that contribute to the overall quality of laboratory test results. The Clinical and Laboratory Standards Institute (CLSI) has developed the framework of the LQMS which is divided into 12 quality system essentials (QSEs). These QSEs are foundational elements that are critical for implementing an effective LQMS.

The ISO 15189 standard guides the development of the laboratory's quality management system, technical solutions, and administrative aspects, all of which are critical to the laboratory's operation. Although any medical laboratory, regardless of test, can use the ISO 15189 standard, Table 1 highlights additional considerations under 12 QSEs that can be taken into account by NABL assessors when assessing private sector TB laboratories.

Table 1. TB test specific consideration under 12 quality system essentials (QSEs)

Quality System Essentials (QSEs)		Test in scope					
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture
1 Organization	Ni-kshay HFID	<ul style="list-style-type: none"> Any laboratory (public or private) offering TB testing services shall have a “Ni-kshay- Health Facility ID” (laboratory can contact District TB Centre for Ni-kshay-HFID as well as relevant training) 					
2 Personnel	Training and competency	<ul style="list-style-type: none"> In addition to testing procedures, staff must be trained and competent in reducing aerosol generation, using safety equipment, responding to specimen/culture spillage, and avoiding aerosol exposure. Laboratory training program should also cover ‘Ni-kshay-Training’ (especially for those who are authorized to access Ni-kshay web-portal). 					
3 Laboratory equipment	Key equipment and their maintenance/certification/calibration	Microscope	GeneXpert	Trueprep Device and Truelab Analyzer	Biosafety Cabinet (BSC), Thermocycle, GT-blot/ Twincubator, Hot air oven and Microlitre Centrifuge	Inspissator, BSC, Incubator and Refrigerated Centrifuge	BSC, BD-MGIT machine, Incubator, Refrigerated Centrifuge
		<ul style="list-style-type: none"> Schedule of preventive maintenance and calibration should be implemented for all equipment. as per manufacturers’ recommendation/NABL 112. Maintenance of proprietary equipment should be done by an authorized agency MGIT (BD): Replacing the MGIT calibrator tubes before the expiry GeneXpert- Calibration yearly/after every 2000 tests/module (whichever happens first) Critical equipment should have un-interrupted power backup for sufficient time 					
4 Purchase and inventory	Kits, consumables and quality control (QC) strains	<ul style="list-style-type: none"> Standard kits and consumables as recommended by manufacturer (Cepheid/ Molbio / Hain Lifescience/ BD) TB specimen container should be screw capped, leak proof and made of plastic (preferably 50 ml conical bottom centrifuge tube that resist at least 5000g centrifugal force) Availability of QC culture strains (<i>M. tuberculosis</i> H37Rv, <i>M. fortuitum</i> and <i>M. kansasii</i>) known <i>M. tuberculosis</i> culture strains /DNA extract (of variable resistance) for batch verification of newly procured diagnostic kits, media, reagents and pure anti-TB drugs (for LC-DST). 					

Quality System Essentials (QSEs)		Test in scope					
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture
5 Process Control	Pre-examination	<ul style="list-style-type: none"> • Staff must be trained to provide patients with appropriate instructions about how to collect a quality specimen. • Quality and quantity of specimen should be monitored. Laboratory should define sample acceptance/ rejection criteria in line with recommendations from manufacturers and national/ international guidelines. Smear microscopy, direct LPA on extrapulmonary or smear negative specimens, and GeneXpert or Truenat on blood-stained specimens are discouraged in NTEP. • Patients should be guided to collect 2-5 ml of quality sputum (<i>i.e</i> mucopurulent and free of blood, food, beetle and tobacco particles). Two sputum samples (one spot and another morning sample) should be collected/tested for Acid Fast Bacilli (AFB) microscopy. In case of extra-pulmonary TB patient, specimen from the site of infection should be collected. • Spot sputum should be collected in well-ventilated/ open place (away from public traffic, never in washroom) • Standard triple layer packaging should be followed while transporting specimen (from specimen collection centres to laboratory). • Proper documentation of samples being transported and received is critical. Specimen should be transported in cool chain if there is delay of >4 hr. 					
	Examination	Standard Operating Procedures (SOPs)					
		It should be in accordance with manufacturers' instructions as well as reference documents/updated guidelines as given below					
		Ref. Doc. 1, 2, 3	Ref. Doc. 4, 5	Ref. Doc. 6, 7, 8	Ref. Doc. 9, 10, 11	Ref. Doc. 12	Ref. Doc. 13, 14
Internal quality control (IQC)							
		<ul style="list-style-type: none"> • Availability of QC slides (+ve and -ve) and its usage with each batch of testing. • QC of stains/ reagents with each new batch. 	<ul style="list-style-type: none"> • Machine/ cartridges has in-built IQC features. • No need of any IQC with each batch of testing. 	<ul style="list-style-type: none"> • Periodic testing of +ve and -ve controls (preferably after every 50 tests) 	<ul style="list-style-type: none"> • Negative controls (DNA extraction and master-mix preparation) and positive control should be run in each batch of testing 	<ul style="list-style-type: none"> • QC of media (LJ or MGIT) batch-sterility and performance testing. • A negative control to be inoculated preferably with each batch of culture inoculation 	<ul style="list-style-type: none"> • QC strain/s (susceptible and resistant <i>Mycobacterium tuberculosis</i> (MTB) strain) should also be tested preferably with each batch of LC-DST.

Quality System Essentials (QSEs)		Test in scope						
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture	MGIT Liquid Culture DST
		Other critical considerations during testing						
		<ul style="list-style-type: none"> • Appropriate thickness & size of smear. • Avoiding sunlight exposure to auramine stain and stained slides. 	<ul style="list-style-type: none"> • Ratio of specimen and reagent buffer and further incubation as per manufacturers' recommendations 	<ul style="list-style-type: none"> • The rifampicin resistance detection step should be done only if DNA is confirmed as positive in the Truenat MTB test. • Pipettes replacement / calibration every six months 	<ul style="list-style-type: none"> • LPA should be conducted on smear positive sputum or MTB culture isolates. • Interpretation of blot result as per recent WHO guidance (Ref. Doc 11) 	<ul style="list-style-type: none"> • Periodic reading of inoculated slant after inoculation for detecting culture growth/contamination • LJ growth should be confirmed for MTB using smear & rapid immunochromatographic test (ICT)/biochemical tests). 	<ul style="list-style-type: none"> • 4% NALC-NaOH method (centrifugation at 3000Xg for 20 min at 4°C) is recommended for sputum processing. • The amount of PANTA and supplements as per standard protocol. • Positive tube flagged by MGIT should be confirmed for MTB by smear & ICT. 	<ul style="list-style-type: none"> • DST Inoculation of MTB strain must be pure (checked by BHI-agar plate), and it should be 1-5 days old. • No dilution if DST is conducted within 1-2 days; dilution in ratio of 1:5 if DST is conducted during 3-5 days.
	Post Examination	<ul style="list-style-type: none"> • The laboratory needs to update the test results against the Ni-kshay IDs which was available along with the receipt of specimens. In case the IDs are not available laboratory may create a new ID, result entry in Ni-kshay or communicate the results to the respective district TB centre and the referring physician via email. Please note that once the tests results are entered in Ni-kshay, the updated information can easily be accessed by NTEP and the referring physician/healthcare facility. • Bio-Medical waste (BMW) generated must be autoclaved first before handing over to a biomedical agency for final disposal. In place of autoclaving, chemical disinfection (with 5% Phenol) may be used for waste generated during AFB microscopy, GeneXpert or Truenat testing. 						
6 Information Management	Standard	In accordance to ISO 15189						

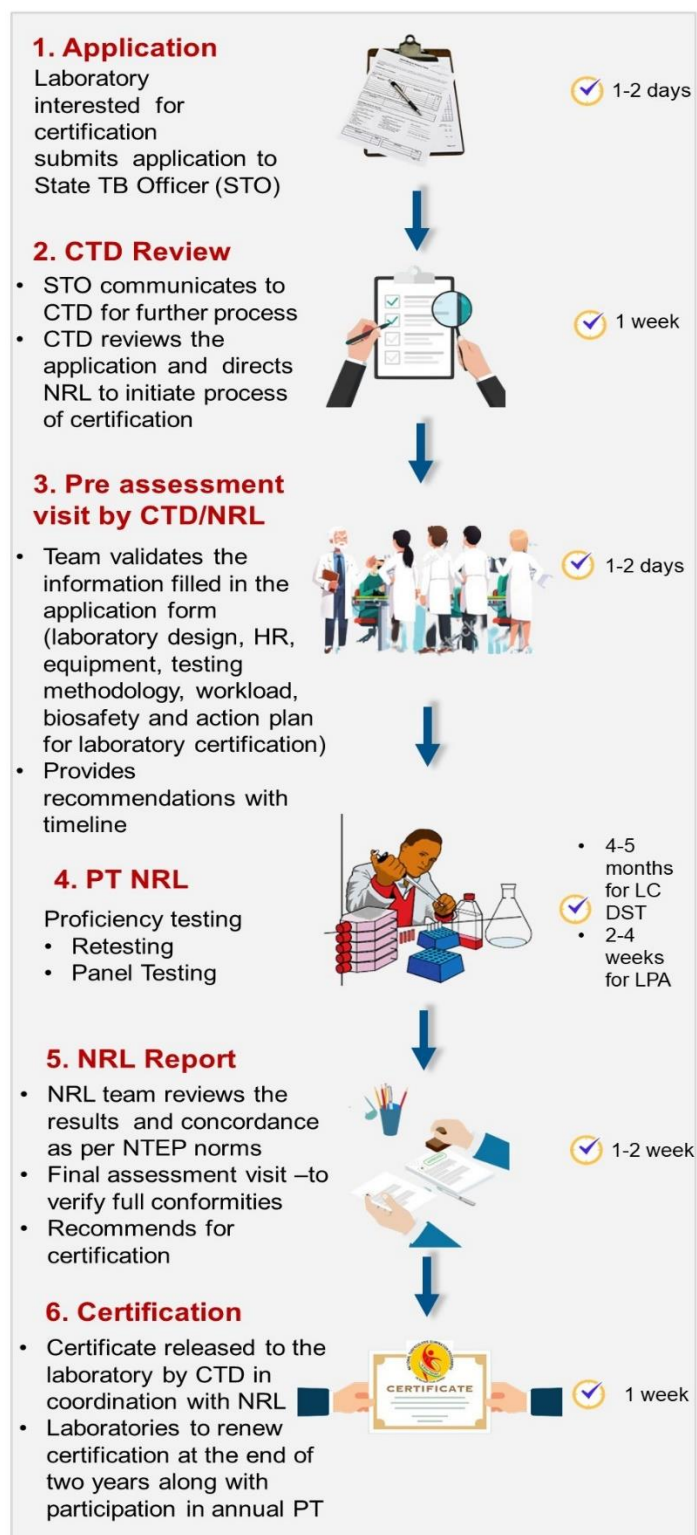
Quality System Essentials (QSEs)		Test in scope						
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture	MGIT Liquid Culture DST
7 Documents and records	Laboratory registers and others	Laboratory register	Laboratory register	Laboratory register	Laboratory register; GT-blot sheet having test and IQC strips (batch-wise)	Laboratory register; Media preparation and QC check (sterility & growth performance); smear, ICT or bio-chemical test record of solid culture growth	Laboratory register; Unloaded +ve/-ve MGIT report; BHI Agar, ICT and Smear report of MGIT +ve tube	Laboratory register; Unloaded Antimicrobial Susceptibility Testing (AST) report (MGIT)
8 Occurrence Management	Standard	In accordance to ISO 15189						
9 Assessment	Internationally accepted 'External Quality Assurance' (EQA program under NTEP)	Random blinded rechecking (RBRC) and Panel testing (PT) are offered by District TB Centres and IRLs to public/private sector TB laboratories	NTEP- NTI, Bangalore offers PT panel (5 dried tube specimen annual basis) to labs both in public and private sector	NTEP-NTI, Bangalore offers PT panel (5 dried tube specimen annual basis) to labs both in public and private sector	NTEP- Designated NRLs offer PT panel (annual basis) to labs both in public and private sector	PT exercise for solid culture alone is not available under NTEP-EQA program. <i>(Since, solid culture has lower sensitivity and higher turn-around time, NTEP prefer liquid culture).</i>	PT exercise for liquid culture alone is not available under NTEP-EQA program. However, EQA of this procedure is assessed as integral part in PT exercise for LC DST.	NTEP-NRLs offer PT panel (annual basis) to labs both in public and private sector
		Detailed EQA program offered by NTEP to laboratories both in public and private sectors are described in "Comprehensive guidance document for conducting EQA by laboratory supervisory staff in NTEP (2023)" by Central TB Division (CTD), Ministry of Health and Family Welfare (MoHFW), GoI [2]						

Quality System Essentials (QSEs)		Test in scope						
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture	MGIT Liquid Culture DST
10 Process Improvement	Key indicators	Turn-around time (TAT; 1-2 day), % positivity rate	TAT (1-2 day), % MTB positivity rate, % RIF resistance, % Test errors/invalid/no result (<5%), % RIF indeterminate result (<5%)	TAT (1-2 day), % MTB positivity rate, % unsuccessful DNA extraction, % RIF/INH, % invalid/error result (<5%), % Indeterminate result	TAT (2-3 days), % MTB positivity rate, % RIF/ INH resistance, % invalid result (<5%), % indeterminate result (<5%)	TAT (3-8 weeks), % positivity rate, % contamination rate (3-5%)	TAT (8-42 days), % positivity rate, % contamination rate 5-8%)	TAT (22-48 days; both culture from specimen and subsequent DST), % DST failed/ error (<3%), % resistance to particular drug
11. Customer Service	Standard	In accordance to ISO 15189						
12 Facilities and Safety	Minimum facility requirement (Refer Annexure 2-4)	<ul style="list-style-type: none"> • Lockable laboratory doors with biohazard signage • Directional airflow from clean to potentially contaminated area; minimum 6-12 air change per hour (natural or mechanical ventilation) • Adequate water supply and hand washing sink. 	<p>For sample preparation</p> <ul style="list-style-type: none"> • Lockable laboratory doors with biohazard signage • Directional airflow from clean to potentially contaminated area; minimum 6-12 air change per hour (natural or mechanical ventilation) 	<ul style="list-style-type: none"> • Lockable laboratory doors with biohazard signage • Directional airflow from clean to potentially contaminated area; minimum 6-12 air change per hour (natural or mechanical ventilation) • Adequate water supply 	<ul style="list-style-type: none"> • 3-rooms or 2-rooms LPA facility and separate sample processing room. • Uni-directional workflow; no DNA in master-mix preparation room. • Separate Personal Protective Equipment 	<ul style="list-style-type: none"> • Specimen processing room should have features - 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 	<ul style="list-style-type: none"> • Specimen processing room should have features- self-closing doors with biohazard signage, sink located near exit, windows sealed or fitted with screens, unidirectional airflow in the lab and ensuring there are a minimum of 6–12 ACHs (that may be maintained by 	<ul style="list-style-type: none"> • TB containment facility (Physical separation from access corridors; access through two consecutive self-closing airtight doors; biohazard signage; hands-free sink near exit; windows are sealed; dedicated air ventilation system with negative airflow into laboratory with no re-circulation;

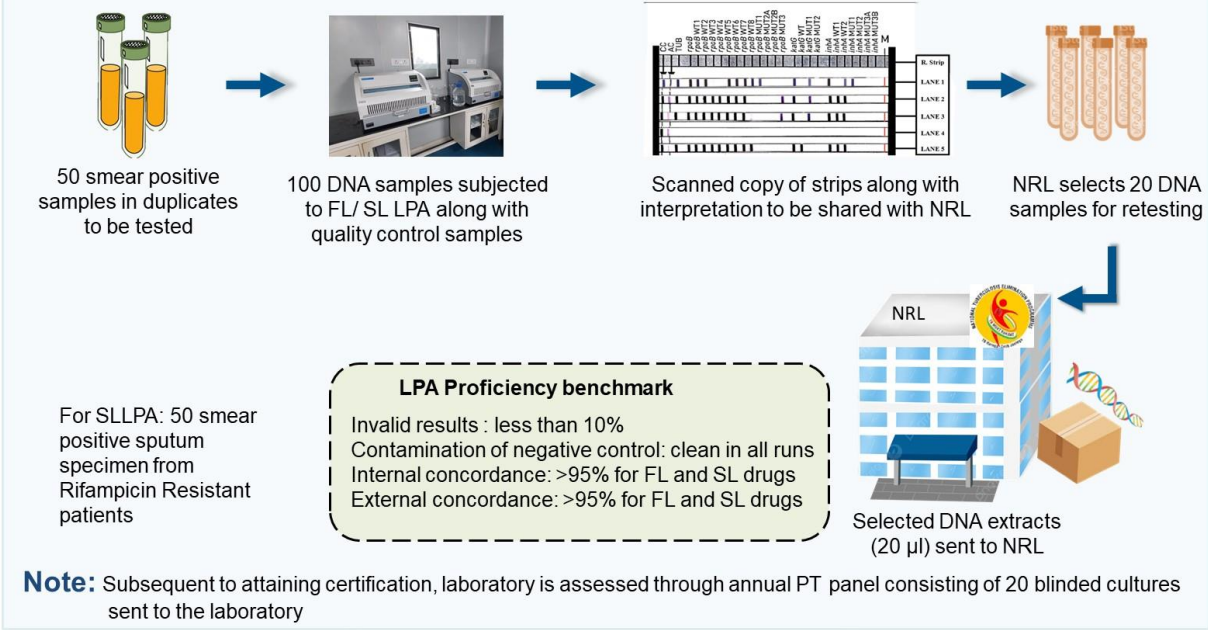
Quality System Essentials (QSEs)		Test in scope						
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture	MGIT Liquid Culture DST
		<ul style="list-style-type: none"> Sturdy and impervious bench; separate benches for smear preparation and microscopy. No cloth curtain and coverings on table/chair 	<ul style="list-style-type: none"> Sturdy and impervious bench Adequate water supply and hand washing sink. No cloth curtain and coverings on table/chair <p><u>For machine operation</u></p> <ul style="list-style-type: none"> Airconditioned room (~22-28°C) with sturdy and impervious bench 	<ul style="list-style-type: none"> and hand washing sink. Sturdy and impervious bench; separate benches for smear preparation and microscopy. No cloth curtain and coverings on table/chair 	<ul style="list-style-type: none"> (PPE) and pipettes for each room. <p><i>Note: Refer solid/liquid culture facility for sample processing room design</i></p>	<ul style="list-style-type: none"> minimum of 6–12 air change per hour (ACH; that may be maintained by BSC itself) and availability of autoclave. Specimen processing and solid culture manipulation or identification in BSC. 	<ul style="list-style-type: none"> BSC itself) and availability of autoclave. Specimen manipulation in BSC. (Culture manipulation require TB containment lab) 	<ul style="list-style-type: none"> autoclave available in the vicinity of the containment lab), intercom.
	Minimum safety equipment (including PPE)	No special safety equipment; prefer to use disposable loop/wooden stick for smear preparation.	No special safety equipment; PPE: Lab coat and gloves	No special safety equipment; PPE: Lab coat and gloves	No special safety equipment; PPE: Lab coat and gloves	BSC (preferably Class II Type A2 with thimble ducting); centrifuge with safety lid/ buckets; PPE: Laboratory gown, gloves and (other PPEs including N-95 mask as per risk assessment)	BSC (preferably Class II Type A2 with thimble ducting); PPE: Laboratory gown, gloves (preferably double pair), N-95	

Quality System Essentials (QSEs)		Test in scope					
		Smear Microscopy	GeneXpert	Truenat	Line Probe Assay	Solid Culture (LJ slant)	MGIT Liquid Culture
		PPE: Lab coat and gloves			<i>Note: Safety equipment in specimen processing room is similar to solid/ liquid culture lab</i>		respirators (and other PPEs as per risk assessment).
	Safe work practices	<ul style="list-style-type: none"> Adherence to universal laboratory safety precautions Safe laboratory techniques to minimize the creation and exposure to aerosols 					
	Spill management	<ul style="list-style-type: none"> Availability of standard spill kit and staff training to assess the severity of spill and clean up accordingly. Use of 5% phenol (min contact time 20 min) for spill management. Spill of DNA sample require cleaning with freshly prepared 1% Sodium hypochlorite 					
	BMW management	Infectious waste must be segregated and autoclaved (or at least disinfected with 5% phenol) before handing over to BMW agency. Final disposal of liquid/solid waste as per updated BMW Management Rules, Govt. of India.			Infectious waste must be segregated and autoclaved before handing over to BMW agency. Final disposal of solid and liquid waste as per updated BMW Management Rules, Govt. of India.		

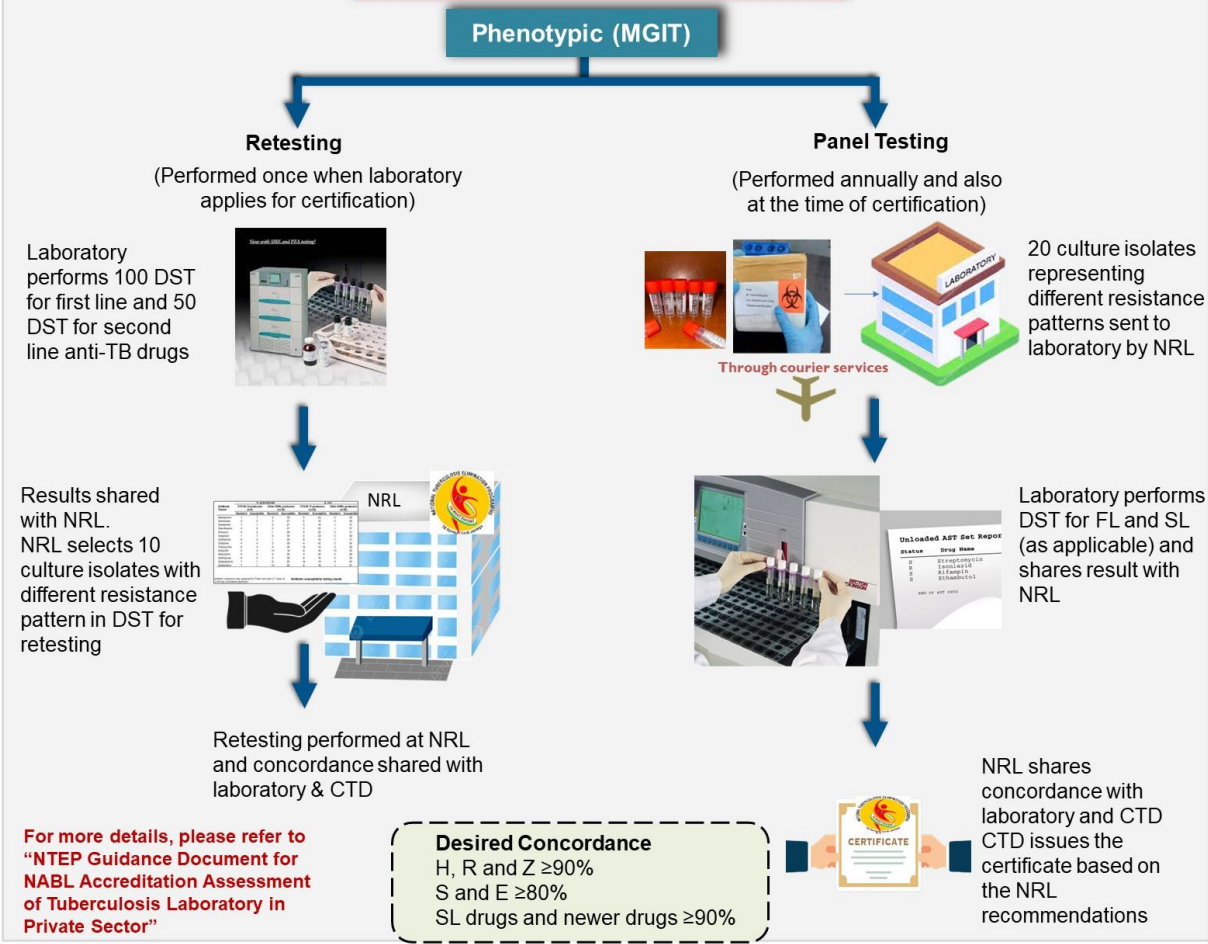
Annexure 1. Private laboratory Certification process



PROFICIENCY TESTING (LPA)

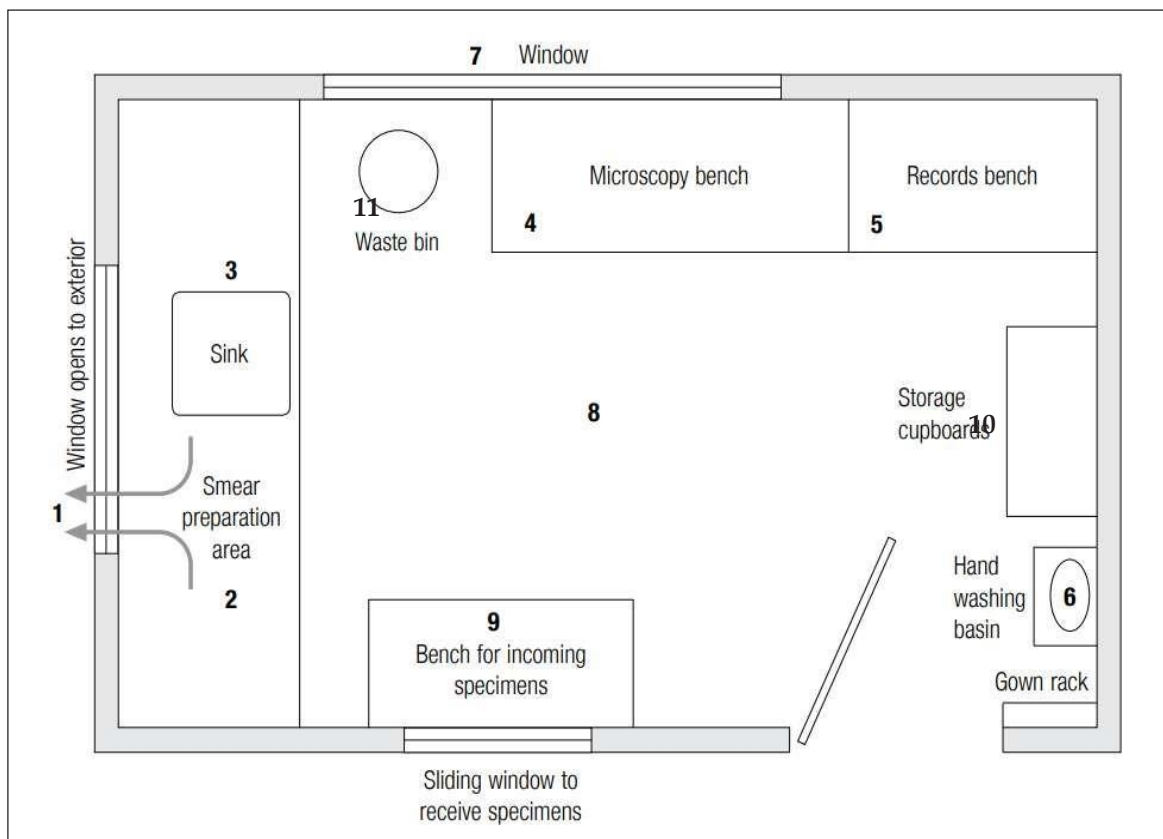


PROFICIENCY TESTING (LC-DST)



Annexure 2. Suggestive layout of Microscopy and NAAT facility

A. Microscopy facility

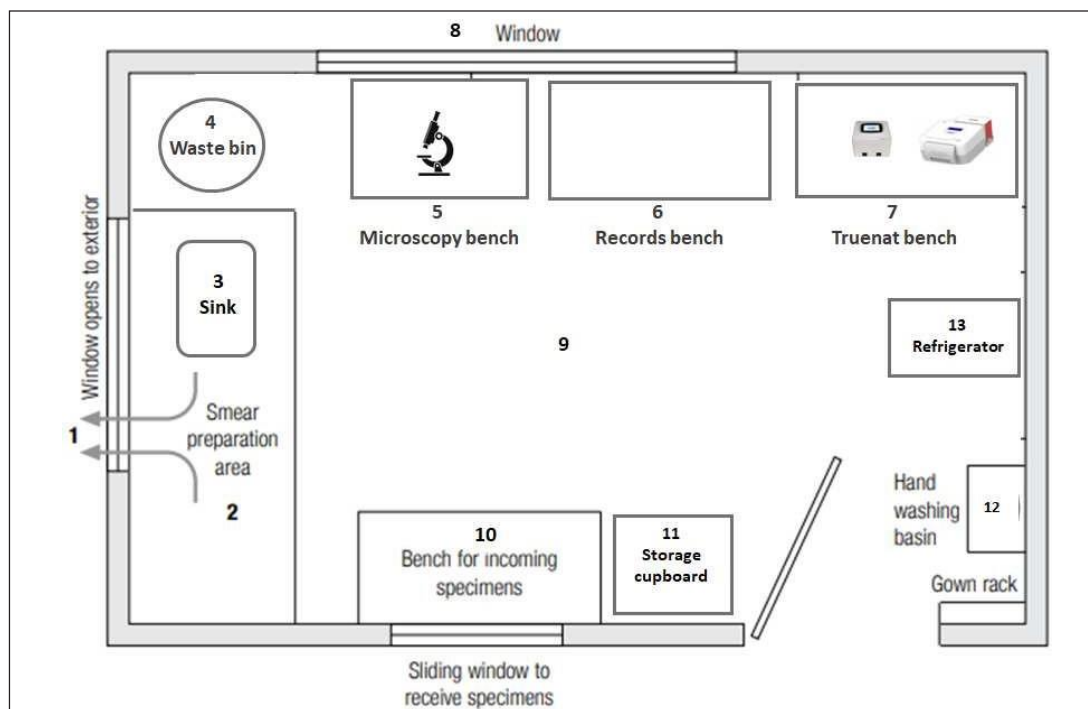


Sample layout of a Microscopy facility

Source: [https://stoptb.org/wg/gli/assets/documents/TB MICROSCOPY HANDBOOK_FINAL.pdf](https://stoptb.org/wg/gli/assets/documents/TB_MICROSCOPY_HANDBOOK_FINAL.pdf)

Note: The basic requirements for microscopy facility include: 1: Good ventilation and directional airflow (contaminated air flows away from staff and out of the laboratory), 2: sturdy table/ bench to prepare smears, 3: sink to stain smear, 4: sturdy table/bench to examine the smears, 5: 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. 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 10 m² should be available for DMC laboratory.

B. Truenat Laboratory

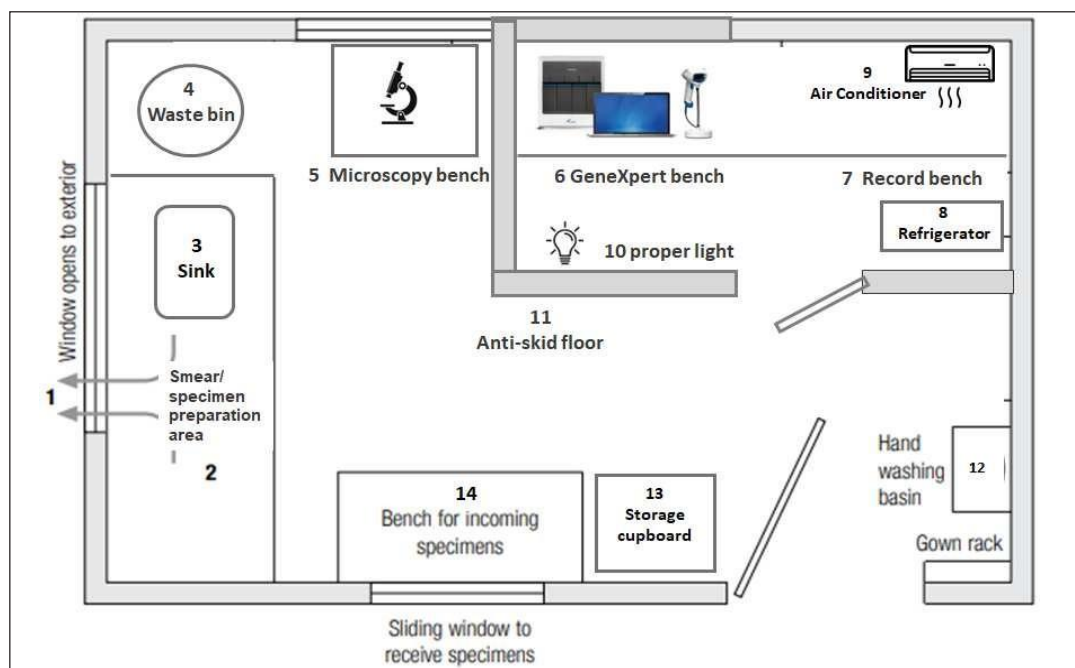


Sample layout of a laboratory with both smear microscopy and Truenat testing facility

Note: 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).

The area should have 1: Good ventilation and directional airflow (contaminated air flows away from staff and out of the laboratory), 2: sturdy table/ bench to prepare smears, 3: 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. 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 10m² should be available for both smear microscopy and Truenat testing.

C. GeneXpert Laboratory



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 15m² (covering two rooms; or one big room with at least 4m² inside cubical) should be available for both smear microscopy and GeneXpert testing.

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.

The area should have 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 smear, 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: Area for receiving specimen.

Annexure 3. Suggestive layout design for LPA facility and specimen processing area

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 workbenches. 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 m² should be considered for LPA diagnostic lab (excluding culture and DST room).

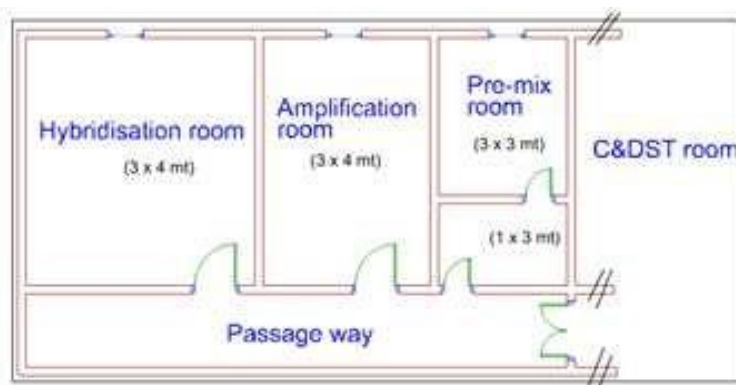


Fig 1: Suggested floor sketch 1 (6x7 meters)

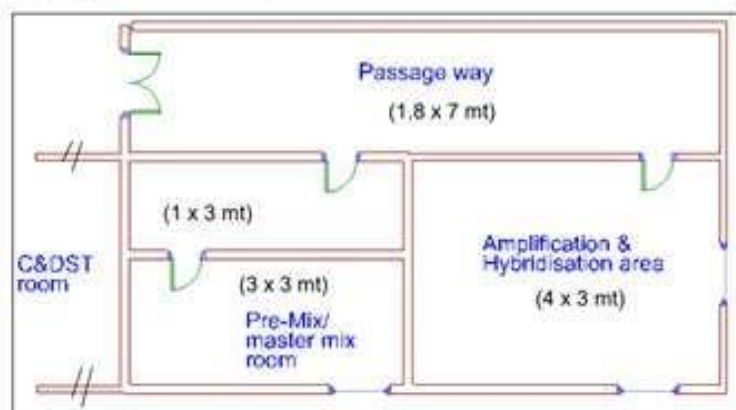


Fig 2: Suggested floor sketch 2 (6x7 meters)

Source: [https://tbcindia.gov.in/WriteReadData/l892s/90464237417th%20\(July%20202009\).pdf](https://tbcindia.gov.in/WriteReadData/l892s/90464237417th%20(July%20202009).pdf)

Note:

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.

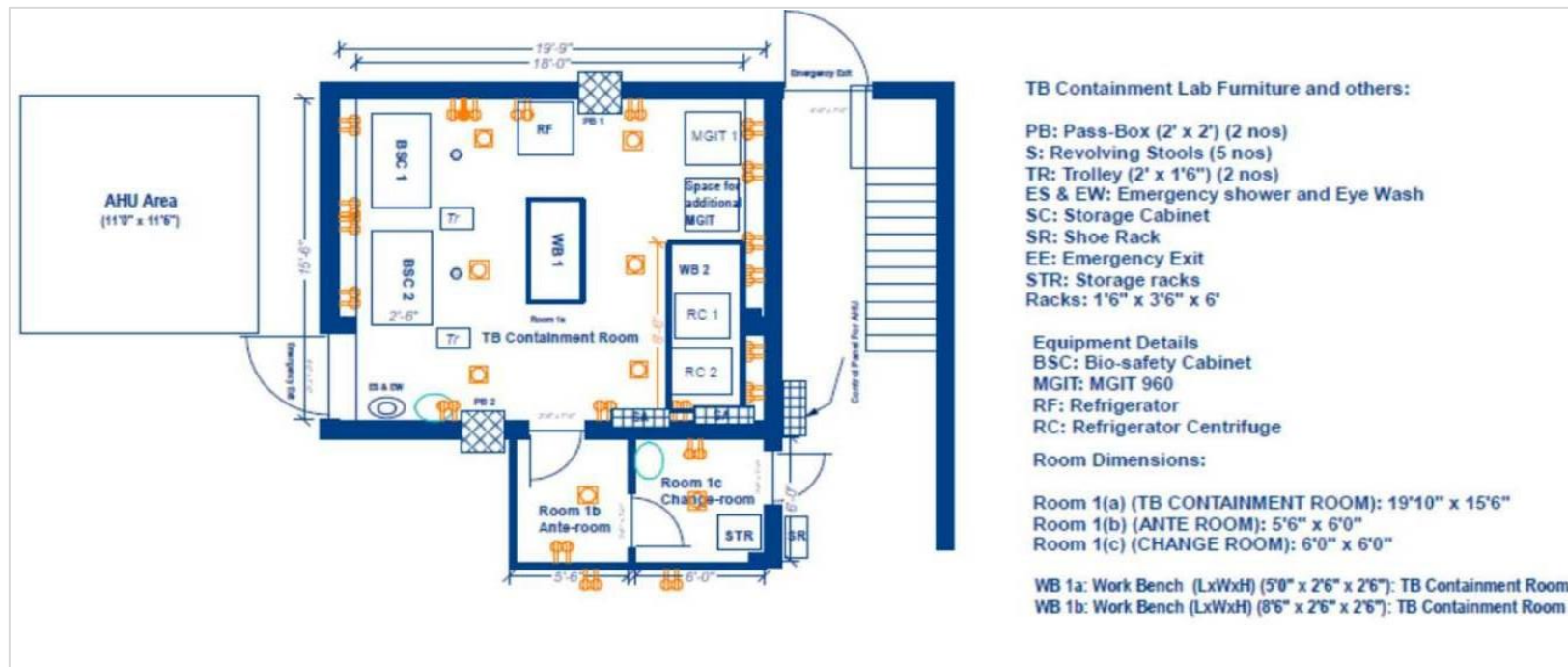
- **Pre-mix or master-mix room:** This work area is used to prepare master-mix for PCR setup (e.g., dNTPs, 10X buffer, distilled water, Taq DNA polymerase). It is preferable to have two-doors. In this work area, a clean-work bench is required. A small refrigerator and/or a -20°C freezer (small but double 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 workbenches within 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 Lineprobe assay strips).

Annexure 4. Suggestive 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 50m² 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>



Sample layout of TB containment laboratory

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