

Practical manual on tuberculosis laboratory strengthening

2022 update



**World Health
Organization**

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Abbreviations

AFB	acid fast bacilli
AIDS	acquired immunodeficiency syndrome
aNAAT	automated nucleic acid amplification test
ASLM	African Society for Laboratory Medicine
BSC	biosafety cabinet
CLSI	Clinical and Laboratory Standards Institute
CRI	colorimetric redox indicator
DNA	deoxyribonucleic acid
DR-TB	drug-resistant tuberculosis
DST	drug-susceptibility testing
EQA	external quality assessment
ERPD	Expert Review Panel for Diagnostics (Global Fund)
FIND	Foundation for Innovative New Diagnostics
FL-LPA	first-line line-probe assay (for detecting resistance to rifampicin and isoniazid)
GLI	Global Laboratory Initiative
Global Fund	Global Fund to Fight AIDS, Tuberculosis and Malaria
HIV	human immunodeficiency virus
Hr-TB	isoniazid-resistant, rifampicin-susceptible tuberculosis
IATA	International Air Transport Association
IGRA	interferon-gamma release assay
ISO	International Organization for Standardization
LAMP	loop-mediated isothermal amplification
LF-LAM	lateral flow urine lipoarabinomannan
LIMS	laboratory information management system
LMIC	low- and middle-income countries
LPA	line-probe assay
MDR/RR-TB	multidrug- or rifampicin-resistant tuberculosis
MDR-TB	multidrug-resistant tuberculosis
MGITTM	Mycobacterial Growth Indicator Tube
MODS	microscopic observation of drug susceptibility
MoH	ministry of health
MTBC	<i>Mycobacterium tuberculosis</i> complex bacteria (e.g. <i>M. tuberculosis</i> or <i>M. bovis</i> bacteria)
mWRD	molecular WHO-recommended rapid diagnostic test
NAAT	nucleic acid amplification test
NGS	next-generation sequencing

Abbreviations

NRA	nitrate reductase assay
NRL	national tuberculosis reference laboratory
NSP	national strategic plan
NTM	nontuberculous mycobacteria
NTP	national tuberculosis programme
PLHIV	people living with HIV/AIDS
PPE	personal protective equipment
pre-XDR-TB	MDR/RR-TB that is also resistant to fluoroquinolones
QA	quality assurance
QC	quality control
QI	quality improvement
QMS	quality management system
QSE	quality system essential
RR-TB	rifampicin-resistant tuberculosis
SLIPTA	Stepwise Laboratory Quality Improvement Process Towards Accreditation
SL-LPA	second-line line-probe assay (for detecting to resistance to fluoroquinolones and amikacin)
SLMTA	Strengthening Laboratory Management Toward Accreditation
SOP	standard operating procedure
SRL	supranational reference laboratory
SRLN	Supranational Reference Laboratory Network
SWOT	strengths, weaknesses, opportunities and threats
TB	tuberculosis
TB SLMTA	Strengthening TB Laboratory Quality Management Towards Accreditation
TOR	terms of reference
US CDC	United States Centers for Disease Control and Prevention
WHO	World Health Organization
WRD	WHO-recommended rapid diagnostic test
XDR-TB	extensively drug-resistant TB (i.e. MDR/RR-TB that is also resistant to a fluoroquinolone and one other Group A drug: bedaquiline or linezolid)

About the GLI

The Global Laboratory Initiative (GLI) is a network of international partners dedicated to accelerating and expanding access to quality-assured tuberculosis (TB) laboratory services. GLI has been a working group of the Stop TB Partnership since 2007.

Coordinated by its core group with support from a secretariat at the World Health Organization (WHO) Global TB Programme, the GLI's mission is to serve as a collaborative platform for the development and uptake of practical guidance and tools for building and sustaining high-quality TB diagnostic networks, in the areas of:

- implementation of WHO policy guidance on TB diagnostics and laboratory strengthening;
- health system solutions and innovations for ensuring rapid, accurate testing and linkage to appropriate patient management;
- continuous quality improvement at all levels of the laboratory network;
- integration of laboratory diagnostic networks;
- human resource capacity development; and
- advocacy and resource mobilization.

The GLI core group has representation from key constituencies including national and supranational reference laboratories, country programmes from countries with a high TB and multidrug-resistant TB (MDR-TB) burden, technical partners, donors and civil society. More information about GLI can be found on its website¹ or by contacting the secretariat (gli_secretariat@who.int).

¹ See <https://stoptb.org/wg/gli>.

Purpose of the handbook

This handbook offers practical guidance for providing technical assistance on the implementation of WHO recommendations and international best practices for TB laboratory services. It is not intended to be a comprehensive manual or to repeat information provided by other guidance documents; hence, it contains references and links to original resources. In particular, the reader is referred to the WHO consolidated guidelines on TB for the most recent policies and to the WHO operational handbooks on TB (especially Module 3) for guidance on implementing the policies.¹

An important function of the GLI is harmonization of technical assistance provided to TB laboratories by its many partners. Thus, the intended audience for this handbook includes implementing partners and providers of technical assistance, who may also use it as a reference for available resources and tools; the final chapter includes guidance specific for consultants before, during and after a mission. Others who may find the handbook useful are TB laboratory managers and technicians, programme managers and other officials from ministries of health, and their partners.

This *GLI handbook for TB laboratory strengthening* is available online² and it contains hyperlinks to cited resources. Because many of these resources may be revised from time to time, the reader is advised to refer to the GLI or other websites where the latest versions of these resources will be available.

¹ The most up-to-date WHO policy guidance on TB diagnostics and laboratory strengthening can be found on the WHO Global TB Programme website (<https://www.who.int/teams/global-tuberculosis-programme>).

² See <https://stoptb.org/wg/gli/gat.asp>.

Summary of changes in this update

Since publication of the second edition of this handbook in 2017, WHO has approved or updated guidance on several diagnostic tests for TB, revised definitions of extensively drug-resistant TB (XDR-TB) and pre-XDR-TB, and developed guidance on new treatment regimens. This third edition of the handbook has been updated to incorporate the following:

- recent or updated WHO recommendations for tests to diagnose TB and detect drug resistance, including:
 - automated nucleic acid amplification tests (NAATs) (Xpert® MTB/RIF, Xpert Ultra, Xpert MTB/XDR, Truenat® MTB, MTB Plus and MTB-RIF Dx);
 - a low complexity automated NAAT for the detection of resistance to isoniazid and second-line drugs;
 - moderate complexity automated NAATs for the detection of TB and resistance to rifampicin and isoniazid (Abbott RealTime MTB and MTB RIF/INH, BD MAX MDR-TB, Hain FluoroType® MTB and MTBDR, Roche cobas® MTB and MTB RIF/INH);
 - a high complexity reverse hybridization NAAT (Genoscholar™ PZA-TB II);
- updated guidance for the use of lateral flow urine lipoarabinomannan (LF-LAM) and interferon-gamma release assays (IGRAs);
- updated diagnostic algorithms aligned with most recent WHO recommendations;
- updated critical concentrations for phenotypic drug-susceptibility testing (DST);
- updated phenotypic and molecular DST needed to support new treatment regimens and new definitions of pre-XDR-TB and XDR-TB;
- updated information on building quality-assured TB testing and management capacity using the *Stepwise Laboratory Quality Improvement Process Towards Accreditation* (SLIPTA) approach (*Score-TB package*¹);
- advances in assessing, analysing and optimizing TB diagnostic networks; and
- advances in the use of next-generation sequencing (NGS) to detect mutations associated with drug resistance for surveillance purposes.

¹ See <https://stoptb.org/wg/gli/accreditation.asp>.

Finally, the focus of the handbook has changed slightly, to emphasize the provision of technical assistance and to reduce the overlap between the previous version of the handbook and the recently published WHO consolidated guidelines and operational handbooks on TB (particularly Module 3). Hence, portions of the *GLI practical guide to TB laboratory strengthening*¹ (e.g. detailed discussions of molecular testing methods and of diagnostic algorithms) were updated and incorporated into Module 3 of the operational handbook.² Therefore, material discussed in depth in the WHO consolidated guidelines and operational handbooks is summarized briefly in this handbook, and the reader is referred to the relevant WHO publications for details. Also, where appropriate, material, figures and tables have been reproduced from the relevant WHO publications with permission.

The WHO website has recently been updated and links to many publications have changed. The validity of the links cited in this handbook were confirmed before the document was published.

¹ GLI practical guide to TB laboratory strengthening. Geneva: Global Laboratory Initiative; 2017 (<https://stoptb.org/wg/gli/default.asp>).

² Operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342369>).

1. Background

This section discusses laboratory tests for tuberculosis (TB) recommended by the World Health Organization (WHO) (Section 1.1), TB laboratory networks (Section 1.2), diagnostic algorithms (Section 1.3) and the targets and indicators used for TB laboratory strengthening (Section 1.4).

1.1 WHO-recommended laboratory tests for TB

1.1.1 WHO-recommended testing for diagnosing TB and detecting drug resistance

For decades, resource-constrained countries have relied on sputum smear microscopy as the primary method for detecting TB. Although microscopy is inexpensive and requires minimal biosafety precautions, it is not a sensitive test – particularly for people living with HIV/AIDS (PLHIV) and children – and it provides no information on the drug-resistance profile of the bacilli. Furthermore, microscopy cannot distinguish between *Mycobacterium tuberculosis* complex bacteria (MTBC) and nontuberculous mycobacteria (NTM).

Bacteriological culture is considered the reference standard for detecting MTBC but has disadvantages: results take weeks to obtain, and testing requires a well-equipped laboratory, highly trained staff, and an efficient transport system to ensure specimens with viable bacteria. Phenotypic drug-susceptibility testing (DST) on cultured specimens is the conventional method used to detect resistance to first-line and second-line TB drugs, and fast commercial liquid culture systems are now available. However, building adequate culture capacity in many countries with a high burden of TB has been slow, given the cost and infrastructure requirements.

In recent years, a growing number of molecular WHO-recommended rapid diagnostic tests (mWRDs) to aid in the diagnosis of TB and drug-resistant TB (DR-TB) have become available, to replace or complement existing conventional tests. A point-of-care rapid test using a lateral flow urine lipoarabinomannan (LF-LAM) assay is also available to assist with the diagnosis of TB among PLHIV. Despite the advantages of these newer tests, conventional microscopy and culture are still necessary for monitoring the response of a patient to treatment. Conventional culture and phenotypic DST are also needed to address gaps in the approved rapid test repertoire, including molecular DST, for many important TB drugs (e.g. bedaquiline and delamanid), as well as tests approved for use with the full range of respiratory and non-respiratory specimens.

WHO's global strategy for TB prevention, care and control for 2015–2035 (known as the End TB Strategy) calls for the early diagnosis of TB and universal DST (1). The

End TB Strategy highlights the critical role of laboratories in the post-2015 era; it also emphasizes that, to meet the End TB targets, WHO-recommended rapid diagnostic tests (WRDs) for TB should be available to anyone with signs and symptoms of TB, and that all bacteriologically confirmed TB cases should receive DST at least for rifampicin and then for fluoroquinolones among cases where rifampicin resistance is found.¹ To meet these targets, all national TB programmes (NTPs) should prioritize the development of a network of TB laboratories that have adequate biosafety, use modern methods of diagnosis, use standard operating procedures (SOPs) and appropriate quality assurance (QA) processes, and have qualified and sufficient human resources. These priorities should be comprehensively addressed in national strategic plans (NSPs) and should be adequately funded.

WHO, in collaboration with the Global Laboratory Initiative (GLI), has developed indicators and targets to assess a country's progress towards reaching the laboratory strengthening goals of the End TB Strategy (increase access to rapid and accurate detection of TB, reach universal access to DST and strengthen quality of laboratory services) (2). To meet these goals, WHO recommends using modern methods, particularly more rapid and sensitive diagnostic methods that provide information on drug resistance in addition to detecting MTBC (e.g. the Xpert® MTB/RIF assay). Making the change to using such techniques requires a large-scale effort coordinated by ministries of health (MoHs) and supported by local and international partner organizations.

Over the past decade, WHO has issued many new or updated recommendations on a wide range of laboratory tests, as well as on clinical and programmatic aspects; for example, revised definitions of extensively drug-resistant TB (XDR-TB) and pre-XDR-TB, new treatment regimens, detection and treatment of TB infection and screening for TB disease. To facilitate dissemination and uptake of the many new policies and guidelines, WHO has recently published consolidated guidelines on TB in which the policies and guidelines are described in four modules.

- *Module 1: Prevention – tuberculosis preventive treatment (3)*
- *Module 2: Screening – systematic screening for tuberculosis disease (4)*
- *Module 3: Diagnosis – rapid diagnostics for tuberculosis detection (5) and test for TB infection (6)*
- *Module 4: Treatment – drug-resistant tuberculosis treatment (7)*
- *Module 5: Management of tuberculosis in children and adolescents*

¹ The original End TB Strategy called for the testing of all rifampicin-resistant TB (RR-TB) patients for susceptibility to second-line injectable agents (amikacin, capreomycin and kanamycin). However, WHO currently recommends that injectable medicines be phased out as a priority in all treatment regimens and replaced by bedaquiline, which makes rapid DST for amikacin unnecessary.

Policies related to laboratory testing:

- Module 1: Use of interferon-gamma release assays (IGRAs) for detecting TB infection
- Module 2: Use of mWRDs in screening
- Module 3: Use of rapid diagnostic tests and tests for TB infection
- Module 4: DST needed for the new regimens
- Module 5: Diagnostics for children including the use of Xpert Ultra on stool

Each module has a companion operational handbook to guide the implementation of the policies and guidelines (9–11). It is anticipated that these modules will be regularly updated and that the most up-to-date WHO policy guidance on TB will be available on the WHO Global TB Programme website.¹

Table 1.1 presents a summary of WHO-recommended methods for diagnosing TB and drug resistance. Annex 1 and the references cited provide more details about the tests and manufacturers.

Table 1.1 Summary of WHO-recommended methods^a

Procedure and use	Test
Conventional tests used as the initial diagnostic test in people being evaluated for pulmonary and extrapulmonary TB	
AFB smear microscopy to detect MTBC for diagnosis of TB or for monitoring therapy ^b	Conventional light microscopy with Ziehl–Neelsen staining
	Conventional fluorescence microscopy
	LED fluorescence microscopy
Culture to detect MTBC for diagnosis of TB or for monitoring therapy or for isolating MTBC for DST ^c	Solid media: Löwenstein–Jensen; Middlebrook 7H10 or 7H11
	Liquid media: e.g. BACTEC™ MGIT™ 960 TB System
Rapid tests used as the initial diagnostic test in people being evaluated for pulmonary TB to detect MTBC without drug-resistance detection	
NAAT to detect MTBC ^d	Loopamp MTBC detection kit
	Fluorotype MTB assay
Rapid antigen detection test for TB ^{d,e} in PLHIV	LF-LAM assay – e.g. Alere Determine™ Urine TB LAM Ag
Rapid molecular tests used as the initial diagnostic test in people being evaluated for pulmonary TB to detect MTBC and resistance to rifampicin	
aNAAT to detect MTBC and resistance to RIF ^d	Xpert® MTB/RIF assay
	Xpert MTB/RIF Ultra assay
	Truenat® MTB, MTB Plus and MTB-RIF Dx

¹ See <https://www.who.int/teams/global-tuberculosis-programme>.

Table 1.1 Continued

Rapid molecular tests used as the initial diagnostic test in people being evaluated for pulmonary TB to detect MTBC and resistance to rifampicin and isoniazid	
MC-aNAAT to detect MTBC and resistance to RIF and INH ^d	RealTime MTB and MTB RIF/INH assays
	BD MAX MDR-TB assay
	FluoroType [®] MTB and MTBDR assay
	cobas [®] MTB and MTB-RIF/INH assays
Conventional diagnostic tests used to detect resistance to anti-TB drugs	
Phenotypic DST (indirect method) ^f	Solid media: Löwenstein–Jensen; Middlebrook 7H10 or 7H11
	Liquid media: e.g. BACTEC [™] MGIT [™] 960 TB System
Rapid molecular tests used to detect resistance to anti-TB drugs in people with bacteriologically confirmed pulmonary TB	
FL-LPA: reverse hybridization assay to detect resistance to INH and RIF ^d	GenoType [®] MTBDRplus
	NTM+MDRTB detection kit
SL-LPA: reverse hybridization assay to detect resistance to FQs and AMK ^d	GenoType MTBDRsl test
LC-aNAAT to detect resistance to INH and second-line anti-TB drugs (FQ, ETO and AMK) ^d	Xpert MTB/XDR test
HC-rNAAT to detect PZA resistance ^d	Genoscholar [™] PZA-TB II test

AFB: acid fast bacilli; AMK: amikacin; aNAAT: automated NAAT; DST: drug-susceptibility testing; ETO: ethionamide; FL-LPA: first-line line-probe assay; FQ: fluoroquinolones; HC-rNAAT: high complexity reverse hybridization NAAT; INH: isoniazid; LED: light-emitting diode; LC-aNAAT: low complexity automated NAAT; LF-LAM: lateral flow urine lipoarabinomannan; MC-aNAAT: moderate complexity automated NAAT; MTBC: *Mycobacterium tuberculosis* complex bacteria; NAAT: nucleic acid amplification test; PLHIV: people living with HIV/AIDS; PZA: pyrazinamide; RIF: rifampicin; SL-LPA: second-line line-probe assay; TB: tuberculosis; WHO: World Health Organization.

^a The tests included in this table are up to date as of the publication of this handbook.

^b See *Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook* (<https://www.stoptb.org/wg/gli/gat.asp>) (12).

^c See *GLI mycobacteriology laboratory manual* (<https://www.stoptb.org/wg/gli/gat.asp>) (13).

^d See *WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update* (<https://apps.who.int/iris/handle/10665/342369>) (10).

^e See *Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV* (<https://www.stoptb.org/wg/gli/gat.asp>) (14).

^f See *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis* (<https://apps.who.int/iris/handle/10665/275469>) (15).

Each of the mWRDs listed in Table 1.1 used as the initial diagnostic test for TB are approved for use with sputum specimens and bronchioalveolar lavage fluids in adults and children (5). The Xpert MTB/RIF and Ultra tests are also recommended for use with gastric aspirate, nasopharyngeal aspirate and stool specimens as the initial diagnostic test to detect pulmonary TB in children. For extrapulmonary TB, the Xpert MTB/RIF test is recommended for use with cerebrospinal fluid, lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine specimens as the initial diagnostic test for the corresponding form of extrapulmonary TB in adults and children. The Xpert Ultra test is recommended for use with

cerebrospinal fluid, lymph node aspirate, lymph node biopsy specimens as the initial diagnostic test for the corresponding form of extrapulmonary TB. Insufficient data were available to make recommendations for the other mWRDs with the additional specimen types.

The growing number of approved mWRDs for TB can present a challenge to countries for deciding which test or tests to implement. Recently, GII and WHO published a manual that describes a stepwise process and decision pathway to assist countries to identify which mWRDs may be suitable for addressing the diagnostic needs in their specific setting (16). The stepwise approach is suitable for use in any country and can be customized to tailor selection efforts to the local context. The process and decision pathway include considerations of national policies and goals, epidemiology of TB and DR-TB, diagnostic network structure and capacity, facility and infrastructure requirements, and implementation considerations. These factors may lead to the adoption of one or more mWRDs for use in a country, to ensure that testing needs for all clients are met.

In addition to the methods shown in this table, WHO has conditionally recommended selected noncommercial liquid culture systems for detecting MTBC and for detecting resistance to rifampicin as an interim solution pending the development of genotypic or automated liquid culture and DST capacity (17). These methods include microscopic observation of drug susceptibility (MODS), nitrate reductase assay (NRA) and colorimetric redox indicator (CRI). They are suitable for use at central level or reference laboratories and require highly trained personnel. However, their use is not intended to replace conventional culture and DST. Their implementation should be phased and include validation against standard methods. Scaling up the use of MODS, NRA and CRI is not recommended, nor is decentralizing their use to lower level laboratories.

1.1.2 WHO-recommended tests for use in DR-TB surveys

Table 1.2 Laboratory tests for use in DR-TB surveys

Type of test	Specific tests
Phenotypic methods	Any of the phenotypic DST methods listed in Table 1.1
Molecular tests	Any of the mWRDs listed in Table 1.1
	Next-generation sequencing ^a

DR-TB: drug-resistant TB; DST: drug-susceptibility testing; mWRD: molecular WHO-recommended rapid diagnostic test; TB: tuberculosis; WHO: World Health Organization.

^a See *Guidance for the surveillance of drug resistance in tuberculosis, 6th edition* (<https://apps.who.int/iris/handle/10665/339760>) (18).

In addition to the methods recommended for testing to support diagnosis and patient care, WHO has recommended the use of next-generation sequencing (NGS) to detect mutations associated with resistance in surveys of DR-TB (18) (Table 1.2). Global policy for the routine use of NGS-based DST to guide patient care decisions has not yet been developed.

NGS refers to techniques that rely on the sequencing of multiple DNA fragments in parallel, followed by bioinformatic analyses to assemble the sequences. The

technologies can be used to determine the nucleotide sequence of an entire genome (i.e. whole genome sequencing) or part of a genome (i.e. targeted NGS) in a single sequencing run (19). Because of the complexity of instrumentation and testing, NGS is only suitable for use in a central reference laboratory.

For DR-TB surveillance, the primary use of NGS is to detect mutations known to confer resistance to rifampicin and other first-line and second-line drugs, and thereby categorize a strain as drug resistant or drug susceptible. To be an effective approach for DR-TB surveillance, NGS-based DST requires a comprehensive knowledge base of the genetic determinants of phenotypic resistance before sequence data can be accurately interpreted and used to produce a drug-resistance profile. WHO has developed a comprehensive catalogue of confidence-graded mutations associated with drug resistance to harmonize interpretation of NGS data (20).

NGS can provide much more information than just the prevalence of DR-TB – it can contribute strongly to the surveillance of TB and DR-TB and to globally important research and development. For example, combining NGS data with phenotypic DST data can provide important information for assessing the performance (sensitivity and specificity) of the available molecular DST products in given settings and can help to identify novel mutations and resistance-conferring loci that could lead to the development of new diagnostic tests or improvement of existing tests.

Table 1.3 Immunological tests to detect TB infection

Type of test	Specific tests
Tests for TB infection ^a	Tuberculin skin test (TST)
	Interferon-gamma release assay (IGRA)

TB: tuberculosis.

^a See *WHO consolidated guidelines on tuberculosis Module 1: Prevention – tuberculosis preventive treatment* (<https://apps.who.int/iris/handle/10665/331170>) (3).

Recently, WHO has recommended testing to detect TB infection in individuals at high risk of developing active TB and for whom TB preventative therapy would be beneficial (3). The currently recommended tests for TB infection are the tuberculin skin test (TST, also known as the Mantoux test) and interferon-gamma release assays (IGRAs) (Table 1.3). Both tests measure the immune sensitization to mycobacterial protein antigens that occurs following infection with *M. tuberculosis* bacteria. There is no strong evidence that one test should be preferred over the other in terms of predicting progression from TB infection to TB disease. Neither test is recommended for use in diagnosing active TB disease.

1.1.3 Tests that are not recommended for use

The following tests were evaluated but not recommended by WHO owing to insufficient evidence to support the proposed uses:

- sputum concentration and decontamination methods for acid fast bacilli (AFB) smear examinations;
- phage plaque method for rapid detection of rifampicin resistance; and
- thin layer agar methods for rapid culture and DST.

The following tests were evaluated and recommended NOT to be used in low- and middle-income settings:

- commercial serodiagnostic tests for TB; and
- IGRAs for detecting active TB in all settings.

1.1.4 WHO review process

In accordance with current WHO standards for evidence assessment in the formulation of policy recommendations on TB diagnostics, WHO engages in a systematic, transparent process using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach. GRADE provides a structured framework for evaluating diagnostic test accuracy and the impact of new diagnostic tests on patient and public health. For more information, see *Implementing tuberculosis diagnostics: policy framework (21)*.

Most of the policies and recommendations issued by WHO related to diagnostic tests were based on a review of an individual test, and typically they included considerable detail about suitable uses and specimens. Recently, WHO has begun issuing policies and recommendations using a class-based approach, in which classes are defined by the type of technology, the complexity of the test for implementation and the target conditions. In the review process, the performances of the individual members of the class are combined and reviewed. The recommendations then apply to each member of the class.

As new members of a class arise, WHO will review them for suitability for inclusion in the class. The product will then be reviewed by the Expert Review Panel for Diagnostics (ERPD) of the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund) – hosted by the WHO Regulation and Prequalification Department – to assess the potential risks and benefits associated with the use of the product. ERPD approval of TB diagnostic tests is intended as an interim approval mechanism on the pathway to WHO prequalification; it allows countries to use funding from the Global Fund to procure products for a time-limited period with the possibility of renewal. ERPD approval of products is categorized as Risk Category 1 or 2. Although products in both categories meet established ERPD standards around manufacturing site quality and risk management systems, and have adequate evidence of analytical performance, products in Risk Category 2 have limited clinical performance data in the settings of intended use or limited stability data to assign shelf-life.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Provide training on WHO policies and guidelines related to laboratory testing
- Provide training on WHO-recommended methods
- Assist with selection of which tests to implement
- Assist with implementation of WHO-recommended tests

References for Section 1.1

(Key resources and suggested reading highlighted in bold font)

- 1 The End TB Strategy – global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2016 (<https://apps.who.int/iris/handle/10665/331326>).
- 2 Framework of indicators and targets for laboratory strengthening under the End TB Strategy. Geneva: World Health Organization; 2016 (<https://apps.who.int/iris/handle/10665/250307>).
- 3 WHO consolidated guidelines on tuberculosis Module 1: Prevention – tuberculosis preventive treatment. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/331170>).
- 4 WHO consolidated guidelines on tuberculosis Module 2: Screening – systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/340255>).
- 5 WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342331>).
- 6 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - tests for TB infection. Geneva: World Health Organization. 2022. <https://apps.who.int/iris/>
- 7 WHO consolidated guidelines on tuberculosis Module 4: Treatment – drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/332397>).
- 8 WHO operational handbook on tuberculosis Module 1: Prevention – tuberculosis preventive treatment. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/331525>).
- 9 WHO operational handbook on tuberculosis Module 4: Treatment – drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/332398>).
- 10 WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342369>).
- 11 WHO operational handbook on tuberculosis Module 2: Screening – systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/340256>).
- 12 Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).
- 13 GLI mycobacteriology laboratory manual. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).
- 14 Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV. Geneva: Global Laboratory Initiative; 2019 (<https://stoptb.org/wg/gli/gat.asp>).

1. Background

- 15 Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva: World Health Organization; 2018 (<https://apps.who.int/iris/handle/10665/275469>).
- 16 Manual for selection of molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis. Geneva: World Health Organization; 2022. (<https://apps.who.int/iris/handle/10665/353596>)
- 17 Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis: policy statement. Geneva: World Health Organization; 2011 (<https://apps.who.int/iris/handle/10665/44601>).
- 18 Guidance for the surveillance of drug resistance in tuberculosis, 6th edition. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/339760>).
- 19 The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex: technical guide (WHO/CDS/TB/2018.19). Geneva: World Health Organization; 2018 (<https://apps.who.int/iris/handle/10665/274443>).
- 20 Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/341981>).
- 21 Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (<https://apps.who.int/iris/handle/10665/162712>).

Additional resource for Section 1.1

The GLI website includes links to guidance and tools, implementation manuals, training packages, SOPs and resources of partners (<https://www.stoptb.org/wg/gli/default.asp>).

1.2 TB laboratory networks

1.2.1 Organization of laboratory services in a country

TB laboratory services are typically managed through a national TB reference laboratory (NRL), which may or may not be under the NTP. When an NRL is managed separately from the NTP, coordination between both entities is essential to ensure that the priorities and strategies of the NTP are reflected in the NRL activities and vice versa.

Countries vary widely in how they set up and manage laboratory services under their MoH. In some countries, laboratories do not fall under a specific unit of the MoH, in which case the management, coordination and supervisory roles and responsibilities may not be clearly defined and may be spread across different sections and levels of the MoH. When laboratories are part of a single unit of the MoH, they can fall within a section of the MoH such as “public health”, “disease control” or “public health laboratory”. There may be a separate “clinical services” unit of the MoH through which certain clinical laboratory services are organized. Management of private sector laboratories can fall under the MoH or another ministry of the government; alternatively, such laboratories may not be specifically regulated or managed by any governmental unit.

Countries support a network of laboratories that provide services for TB diagnosis and treatment monitoring for patient care. The number and distribution of laboratories within the network will vary dramatically, depending on factors such as geography, disease burden, economic setting and political implications. The network comprises laboratories with varied testing capacity, depending on location, infrastructure and the particular roles and responsibilities assigned to a specific laboratory. Early access to diagnostic testing and treatment monitoring is often at the community or district level, whereas more sophisticated extensive testing is based at regional or central level facilities. The primary role of the network is to provide quality services within the population that will support the NTP.

Country capacity for diagnostic testing was previously monitored according to global targets that described numbers of microscopy centres per 100 000 population, and culture or DST laboratories per 5 million (1). These global targets are no longer used because of advances in diagnostic technologies and the need for country-specific targets that take into account epidemiology and patient access (e.g. urban and rural populations, and specimen referral systems). A recommended method for calculating country-specific targets for numbers of tests and facilities for each of the main diagnostic technologies – microscopy, WRDs (including Xpert MTB/RIF), culture and DST – is provided in Annex 1 of the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy*¹ (2); also, a Microsoft Excel-based tool to assist in calculations is available online.²

¹ See <https://apps.who.int/iris/handle/10665/250307>.

² See <https://www.who.int/tb/publications/labindicators>.

1.2.2 TB laboratory network structure

A network of TB laboratories within the public health system is typically organized into a tiered or pyramid structure; Fig. 1.1 illustrates the general tiers associated with a conventional TB laboratory network. The structure has several peripheral laboratories (Level 1 laboratories) accessible to most individuals being evaluated for TB, a moderate number of intermediate laboratories (Level 2 laboratories) that are usually located in mid-sized population centres and health facilities, and a central Level 3 laboratory at the provincial, state or national level. Large countries may have several Level 3 laboratories. Each level or tier has specific requirements for infrastructure and biosafety that are defined by the various activities and diagnostic methods being performed in the laboratories (see the WHO *Tuberculosis laboratory biosafety manual*¹ (3)). In addition, as the level of the laboratory increases from Level 1 to Level 3, the technologies become more advanced; hence, the necessary skills, proficiency and training requirements for technicians increase. The organization and operations found at different levels of the laboratory network for TB services are described in publications listed at the end of this section.

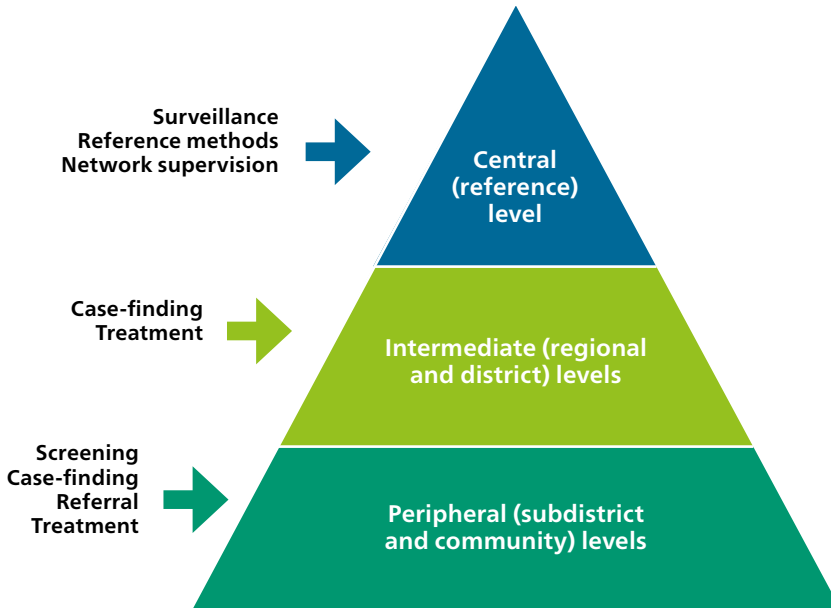
WHO recommends that NTPs prioritize the development of a network of TB laboratories that use modern diagnostics, have efficient referral systems, use SOPs and appropriate QA processes, and have adequate biosafety and sufficient human resources (2). These priorities should be comprehensively addressed in NSPs and should be adequately funded.

Many countries have governance over laboratory services at regional, state or provincial level. These entities may not coordinate with central or national level laboratories, but develop services and practices essential for their province, state or region. This situation makes it challenging to coordinate and provide services according to national guidelines.

The private sector also plays a significant and increasing role in TB control and laboratory services in many low- and middle-income countries (LMIC), in parallel to the public health laboratory system. Private sector facilities may directly inform the NTP of new TB cases; however, in many countries private laboratories are not linked with the NTP, and thus may not follow national guidelines or quality standards, or report TB case data. This scenario causes substantial challenges with the “quality” of diagnostic services and the accuracy of testing because the private laboratories are not under national QA programmes. In addition, these facilities are often not reporting cases or resistance data, which limits the NTP’s ability to accurately assess the burden of TB disease and to define the levels of drug resistance within the population. Initiatives to develop links between public and private sector laboratories are important to facilitate higher quality services and to provide necessary reporting and data sharing to optimize TB control.

¹ See <https://apps.who.int/iris/handle/10665/77949>.

Fig. 1.1 The three tiers of the network of TB laboratories (4)



- All tests performed at lower levels; culture using liquid media, phenotypic DST, MC-aNAAT, HC-rNAAT, FL-LPA, SL-LPA and sequencing
- All tests performed at the lower level and possibly culture on solid media, FL-LPA, SL-LPA, MC-aNAAT, HC-rNAAT and specimen referral
- Xpert MTB/RIF or Ultra, Truenat MTB, TB-LAMP, AFB smear microscopy, LF-LAM, LC-aNAAT and specimen referral

AFB: acid fast bacilli; aNAAT: automated NAAT; DST: drug-susceptibility testing; FL-LPA: first-line line-probe assay; HC-rNAAT: high complexity reverse hybridization NAAT; LAMP: loop-mediated isothermal amplification; LC-aNAAT: low complexity automated NAAT; MC-aNAAT: moderate complexity automated NAAT; NAAT: nucleic acid amplification test; SL-LPA: second-line line-probe assay; TB: tuberculosis.

1.2.3 Diagnostic services within the tiered TB laboratory network structure

Table 1.4 summarizes the functions and responsibilities that are generally attributed to each level. At the lower levels of laboratory networks, services tend to be integrated rather than having TB-specific laboratories. Commonly, peripheral laboratories will offer a range of basic diagnostic tests, including one or more of the following: AFB smear microscopy; Xpert MTB/RIF and Ultra; TB-LAMP; Truenat® MTB, MTB Plus and MTB-RIF Dx; low complexity automated nucleic acid amplification tests; and LF-LAM. Patients may self-refer to these facilities or may be referred from rural health posts for initial testing. Further testing may be accomplished through referral of specimens to a higher level testing facility.

At the intermediate level, testing requiring greater infrastructure, technical skills or biosafety precautions is offered; such testing includes culture on liquid or solid media,

first-line line-probe assay (FL-LPA) or second-line LPA (SL-LPA) using sputum specimens or moderate complexity automated nucleic acid amplification test. The tests offered at the peripheral level are also often available at intermediate level laboratories. Samples requiring additional testing may be referred to a higher level laboratory.

At the central level, testing requiring advanced skills, infrastructure and biosafety precautions is offered, such as culture using solid and liquid media, phenotypic DST using solid or liquid media, FL-LPA or SL-LPA, moderate complexity automated nucleic acid amplification tests using respiratory specimens and high complexity reverse hybridization NAAT using isolates and NGS. The tests offered at the lower level laboratories are also often available.

Table 1.4 Functions and responsibilities

Level 1. Peripheral (or community) laboratory
• Receives specimens
• Prepares, stains, and examines smears with Ziehl–Neelsen or LED fluorescence microscopy
• May use the Xpert MTB/RIF or Ultra assay, or the Truenat MTB/Truenat MTB-RIF Dx assays, as the initial diagnostic test to detect TB and resistance to rifampicin
• May use the TB-LAMP test as a replacement for smear microscopy for the diagnosis of pulmonary TB in adults and children with signs and symptoms of TB according to national guidelines
• May use LF-LAM assays to assist in the diagnosis of TB in patients who are HIV-positive
• May use LC-aNAATs as a follow-on test to detect resistance to fluoroquinolones, isoniazid, ethionamide and amikacin
• Records and report results according to national guidelines
• Maintains laboratory registers
• Cleans and maintains equipment
• Manages reagents and laboratory supplies
• Uses appropriate QC and QA procedures
• Participates in EQA programmes (e.g. blinded rechecking, panel testing and supervisory visits)
• Has appropriate biosafety measures in place
• Packages specimens appropriately for referral to other laboratories for testing
Level 2. Intermediate (or regional) laboratory
• Performs all of the functions of a Level 1 laboratory ^a
• May use MC-aNAATs as the initial diagnostic test to detect MTBC and resistance to rifampicin and isoniazid
• May use FL-LPA for direct detection of MTBC and mutations for isoniazid and rifampicin resistance from processed sputum samples that produce AFB-positive smears
• May use SL-LPA as the initial test to detect resistance to fluoroquinolones and amikacin in sputum specimens from people with MDR/RR-TB
• Performs digestion and decontamination of specimens, and inoculates cultures
• Uses culture to isolate and identify MTBC
• Refers positive cultures to an appropriate reference laboratory for DST
• Trains microscopists and supervises peripheral level staff in microscopy and the use of WRDs
• Prepares and distributes reagents for microscopy to peripheral laboratories
• Engages in proficiency testing and quality improvement activities for peripheral laboratories

Level 3. Central (or national) laboratory

- Performs all the functions of Level 1 and Level 2 laboratories
- Collaborates closely with the central level of the NTP
- Provides strategic oversight to ensure the effective management of laboratories in the network, the quality of the testing, and the efficient use of the network's services and TB diagnostics
- Performs DST of *M. tuberculosis* isolates to determine resistance to first-line and second-line anti-TB agents
- Performs molecular testing for rifampicin resistance on positive cultures (alone or in combination with testing for resistance to isoniazid)
- May use FL-LPA for direct detection of MTBC and mutations for isoniazid and rifampicin resistance from positive cultures
- May use SL-LPA as the initial test to detect resistance to fluoroquinolones and amikacin in positive cultures from people with MDR/RR-TB
- May use HC-rNAATs as a follow-on test to detect resistance to pyrazinamide from positive cultures
- May perform NGS to detect mutations associated with drug resistance in support of DR-TB surveillance, in accordance with WHO policies and recommendations
- Identifies NTM
- Arranges for a specialist to periodically check, calibrate and repair laboratory equipment
- Updates and disseminates laboratory manuals, including guidelines on diagnostic methods, equipment maintenance, training and supervision, and QA
- May distribute reagents and consumables when asked to do so by intermediate or peripheral level TB laboratories
- Supervises intermediate level laboratories' implementation and use of bacteriological methods, and the laboratories' performance monitoring of peripheral laboratories
- Undertakes QA of all procedures performed at intermediate level laboratories including microscopy, WRDs, culture and DST
- Ensures that an appropriate human resources development programme is in place, including training, retraining and competency assessment
- Conducts drug-resistance surveillance
- Undertakes operational and applied research relating to the laboratory network, and coordinates this with the requirements and needs of the NTP
- Establishes a formal collaboration agreement with a TB SRL for panel testing, support in implementing and validating new diagnostics, assistance with laboratory development and expansion strategies, and referral for challenging cases who require specialized testing

AFB: acid fast bacilli; aNAAT: automated NAAT; DR-TB: drug-resistant TB; DST: drug-susceptibility testing; EQA: external quality assessment; FL-LPA: first-line line-probe assay; HC-rNAAT: high complexity reverse hybridization NAAT; HIV: human immunodeficiency virus; LAMP: loop-mediated isothermal amplification; LC-aNAAT: low complexity automated NAAT; LED: light-emitting diode; LF-LAM: lateral flow urine lipoarabinomannan assay; LPA: line-probe assay; *M. Mycobacterium*; MC-aNAAT: moderate complexity automated NAAT; MDR/RR-TB: multidrug- or rifampicin-resistant TB; MTBC: *Mycobacterium tuberculosis* complex; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; NTM: nontuberculous mycobacteria; NTP: national TB programme; QA: quality assurance; QC: quality control; SL-LPA: second-line line-probe assay; SRL: supranational reference laboratory; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

^a Tests used in lower level laboratories may be placed at higher level laboratories for diagnostic purposes and for testing the proficiency of reference level staff responsible for supervision.

The levels and functions described above are useful conceptually, but there may be considerable variability both among and within countries in the structure and functions of the different levels. In general, the structure of the laboratory network and testing packages available at each level should be tailored to meet the needs of the community and the local epidemiology of TB. Services and testing targets should be based on demand rather than population, and the structure of the laboratory network must emphasize access to quality care. Such access can be accomplished by placing testing sites near the people who need to be tested (e.g. in Level 1 laboratories) or by implementing an efficient specimen referral system to transport specimens from collection sites to centralized testing centres (e.g. Level 2 laboratories).

Specimen referral systems play a critical role in ensuring access to laboratory services by allowing patients to receive care and treatment at a single location, while their specimens are transferred to various levels of a tiered laboratory system for testing. Referral systems can make it easier to access diagnostic tests in areas where testing is not available, prevent the need for patients to travel (and the associated costs of travel) and lead to equity in access to health care. Furthermore, for certain tests, centralized or regionalized testing and a robust specimen referral system may be more cost-effective than placing staff and procuring and maintaining equipment to conduct testing at lower levels. The *GLI Guide to TB specimen referral systems*¹ (5) and the *GLI specimen referral toolkit*² (6) are good sources of information for designing, implementing and monitoring systems for specimen referral and reporting of results.

1.2.4 TB diagnostic network

The TB laboratory services described above are a key component of a well-functioning diagnostic network. However, a laboratory test is just one part of the diagnostic process. A diagnostic network encompasses all points where community members seek care (in both the public and private sectors and among formal and informal providers), engages all health care facilities and workers in the diagnostic process, and has efficient linkages between steps in the diagnostic process. Strengthening the entire diagnostic network and patient pathway can reduce the time between a patient seeking care to a clinician making a patient care decision, reduce loss to follow-up and increase access to quality-assured laboratory services for all patients.

A diagnostic network analysis and optimization exercise can be performed to understand how diagnostic services are organized in a country, identify gaps in access to diagnostic testing and identify opportunities to optimize the delivery of diagnostic services. The *Framework of indicators and targets for laboratory strengthening under the End TB Strategy*³ can serve as a guide for implementing and monitoring improvements to TB testing and TB laboratory networks (2).

¹ See <https://stoptb.org/wg/gli/gat.asp>.

² See <https://stoptb.org/wg/gli/srt.asp>.

³ See <https://apps.who.int/iris/handle/10665/250307>.

TB diagnostic network analysis and diagnostic network optimization

Diagnostic network analysis and optimization is a three-step process. Step 1 (geographical mapping plus baseline model creation) and Step 2 (creation of alternative scenarios) cover analysis, and Step 3 (comparison of the scenarios to identify the optimal network design) covers network optimization → ✕ 📄 ✕ 📄.

- Step 1 aims to understand how diagnostic services are organized in a country, identify gaps in access to diagnostic testing and identify opportunities to optimize the delivery of diagnostic services. It includes mapping (spatial analysis) of the population needing testing; number and locations of health facilities where people seek care; number, locations, capabilities and capacities of testing sites; and referral linkages.
- Step 2 involves developing alternative scenarios to the baseline model, in consultation with key stakeholders. These scenarios should reflect decision points; for example, the question of where should testing be placed to maximize detection rates for TB or DR-TB, or to address the goals and priorities of the NSP for improving TB testing in underserved or priority populations.
- Step 3 relies on specialized software (e.g. supply chain management software or OptiDx) and modelling approaches to evaluate alternative network configurations, the aim being to increase access and optimize the delivery of diagnostic services. This optimization process can help to identify which diagnostic tests should be placed in which testing facility; design efficient specimen transport systems; develop strategies for expansion of diagnostic testing to address testing priorities and close gaps in coverage; and identify opportunities for integration of diagnostic testing and specimen referral across disease programmes.

A full analysis and network optimization exercise often requires considerable human resources and time (3–6 months) and expert technical assistance.

Assessing the functioning of a TB diagnostic network

A set of standards have been developed that define a comprehensive, well-functioning diagnostic network. The standards are based on the national TB diagnostic network standards that were developed and piloted by the GLI and partners (9), and which were based on an earlier GLI assessment tool focusing on TB microscopy laboratory networks (10). For each standard, core capacities and components are used to define essential features and functions of a national diagnostic network that is designed to detect, assess, notify and respond to TB (Table 1.5).

A comprehensive evaluation of a diagnostic network must use an assessment tool that addresses the entire diagnostic network, not just one component (e.g. laboratory testing). A network assessment tool differs from the tools used to assess the quality of individual laboratories, such as the WHO *Laboratory assessment tool*¹ (11). One such TB diagnostic network assessment tool, the United States Agency for International Development (USAID) TB-Net tool (12), was developed to assess the functionality of a national TB diagnostic network from the perspective of its ability to meet the needs of the country's NSP for TB. The TB-Net tool builds on:

¹ See <https://apps.who.int/iris/handle/10665/70874>.

Table 1.5 TB diagnostic network standards, core capacities and components

Core capacity 1: Political, legal, regulatory and financial framework
Standard 1. The country has a fully endorsed political, legal and regulatory framework in place that supports the achievement of the NSP, and that organizes and controls all public and private diagnostic services to support the NSP, with sufficient dedicated funding available. Policies are in place that enable continuous, countrywide availability of free, quality-assured diagnosis according to the national guidelines.
Components: Legislation and policies, national policies and plans, governance, financing and budgeting
Core capacity 2: Structure and organization of the diagnostic network
Standard 2. A sustainable, rational and efficient TB diagnostic network provides integrated, essential, quality diagnostic services for patient care and public health. The TB diagnostic network is coordinated by a national reference or public health laboratory, and includes the public and private sector as well as community level diagnostic services. All facilities have clearly defined terms of reference and are adequately supervised.
Components: Diagnostic network, coordination and management, programmatic and operational research
Core capacity 3: Coverage
Standard 3. The national TB diagnostic network provides complete coverage and universal access to TB diagnostic services to the entire population of the country. Referral mechanisms exist to rapidly and safely refer specimens to the appropriate level for testing, and to provide timely results to enable initiation of appropriate treatment. An efficient diagnostic–clinical interface allows for appropriate diagnostic tests to be ordered and performed, and ensures the timely linkage of diagnosed patients to appropriate care and treatment.
Components: Diagnostic network coverage, sample referral system, linkages and emergency preparedness
Core capacity 4: Diagnostic algorithm
Standard 4. A national TB diagnostic algorithm(s) that is responsive to the epidemic, is patient-centred, includes appropriate use of diagnostic technologies and is based on the current structure of the health system is enforced at all levels of the TB diagnostic network. A minimum package of tests and quality standards is defined for each level of the network. Laboratory staff, health care workers and TB programme staff are trained in the application of the algorithm.
Components: Algorithms, detection of TB and detection of DR-TB
Core capacity 5: Biosafety
Standard 5. Testing is performed in a manner and in facilities that ensure safety for the staff, customers, community and environment. Sufficient materials, means and skills are available throughout the system to ensure safe and secure procurement, handling, storage, transportation and disposal of samples and materials, both in routine and emergency circumstances.
Components: Facilities, biosafety and biosecurity manual, biosafety systems and waste management
Core capacity 6: Equipment and supplies
Standard 6. Testing is performed with state-of-the-art and well-maintained equipment and an uninterrupted supply of quality reagents and consumables, using standardized testing methods throughout the country.
Components: Supply chain management and equipment management
Core capacity 7: Workforce
Standard 7. Adequate numbers of competent, well-trained and motivated technical and managerial staff are available at all levels of the diagnostic network.
Components: Education and training, staffing, human resources development strategies and plans, and competency-based job descriptions

Core capacity 8: Diagnostics data management

Standard 8. Interoperable and interconnected electronic recording and reporting systems are in place that generate reliable data that are monitored and analysed in real time. These systems comply with international standards to allow the rapid exchange of information in standardized formats at national and subnational level. An LIMS provides up-to-date information about the status of the laboratories and is linked to the country's HMIS.

Components: Data collection forms, reporting, diagnostics connectivity and remote monitoring, data analysis and sharing, surveillance and epidemiology, security and confidentiality of information

Core capacity 9: Quality of the diagnostic network

Standard 9. High-quality diagnostic services producing accurate and reliable results are available throughout the network. Continuous quality improvement targets all facilities within the network and includes quality indicator monitoring, external quality assessment and regular on-site supervision. A system of national certification is in place for all public and private laboratories within the network, and reference and referral level laboratories are accredited according to national or international standards.

Components: Documents and document control, quality assurance, QMS, certification and accreditation

Core capacity 10: TB/HIV

Standard 10. A comprehensive approach is needed to combat the twin epidemics of HIV/AIDS and TB. All persons being evaluated for TB should receive free HIV testing and, if found to be positive, referred to appropriate counselling and care. All HIV-positive persons should be screened for TB and linked to appropriate diagnostic testing. Coordination and communication between the national AIDS control programme and NTP are essential. The TB diagnostic network should collaborate with the HIV diagnostic network regarding laboratory and diagnostic services (e.g. specimen transport, shared diagnostic platforms and referrals for testing).

Components: Legislation and policies, structure and organization of the network, coverage, diagnostic algorithm, workforce and diagnostic data management

AIDS: acquired immunodeficiency syndrome; DR-TB: drug-resistant TB; HIV: human immunodeficiency virus; HMIS: health management information system; LIMS: laboratory information management system; NSP: national strategic plan; NTP: national TB programme; QMS: quality management system; TB: tuberculosis.

- the National Laboratory Network Assessment (LABNET) (13) scorecard, which was designed to look broadly at national laboratory networks in Africa with respect to achieving global health security goals; and
- the national TB diagnostic network standards and assessment tools developed and piloted by the GLI and partners (9) and the GLI assessment tool for TB microscopy networks (10).

The TB-Net tool uses semiquantitative scoring procedures to identify the capability stage of various aspects of the diagnostic network, to help in measuring current capabilities and identifying areas for improvement.

The initial assessment of the TB diagnostic network in a country often involves external technical assistance and assessors, to identify a baseline of performance and areas for improvement. Typically, the objectives of a TB diagnostic network assessment are to:

- holistically review the diagnostic network, current practices and algorithms;
- identify challenges that prevent the diagnostic network from performing efficiently and effectively; and

- propose evidence-based interventions to improve the overall ability of the diagnostic network to meet the goals and targets of the NSP.

Key components of the TB diagnostic network are assessed through review of data, documents, strategic plans, annual reports, etc.; site visits to a representative sample of facilities carried out using standardized checklists; focused discussions with laboratory and programme staff at the state and district levels; and consultations with government, technical and other key stakeholders.

Repeating the assessment after 2–4 years, perhaps as part of regular programme reviews or as preparation for Global Fund applications, should measure progress in improving the network and identify areas for further improvement. The *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (2)* can serve as a guide for implementing and monitoring improvements to TB testing and TB diagnostic networks.

1.2.5 Network development: capacity-building and strengthening

Generally, resource limitations restrict the ability to rapidly establish complete networks of TB laboratories that will meet all of a country's needs during the early stages of development. Thus, it is best to implement a network or build capacity in stages, over a time frame agreed by consensus among the programme's managers and knowledgeable laboratory personnel.

As a rule, rather than establishing full national capacity at the beginning of implementation, countries and territories with small populations of TB patients may find it more practical to outsource specific services to neighbouring countries or territories, while building their own capacity, expertise and proficiency.

Several considerations will guide the placement and the expansion of services when implementing new technologies within the current laboratory network structure. Factors to consider when positioning technologies include:

- available resources for implementation;
- infrastructure requirements;
- biosafety requirements;
- current and planned testing algorithms;
- specimen types and collection procedures;
- projected testing volumes;
- minimum number of tests needed to maintain expertise and optimal use of instruments;
- trained human resource capacity;
- links to other laboratories for further testing;
- specimen referral and result reporting mechanisms; and
- possibility of integration with testing, specimen referral and reporting systems for other diseases.

Generally, microscopy is found at the lower levels of the laboratory network or in smaller testing facilities, because of the minimal biosafety and infrastructure requirements for performing the test and the need for community level access to ensure rapid screening. The Xpert MTB/RIF test, Truenat MTB tests and TB loop-mediated isothermal amplification (TB-LAMP) test may be implemented at this level in facilities that can meet infrastructure requirements for the test. These mWRDs are also suitable for implementation at the intermediate and central levels, provided suitable sample referral mechanisms with short turnaround times are in place from lower level laboratories or community health services. The other technologies placed at intermediate and higher level laboratories cannot be extended down to the lower levels of the network because of infrastructure requirements, biosafety concerns, test complexity and the need for trained staff.

The priority for the use of culture is usually to perform DST and to monitor the response to treatment of patients with multidrug-resistant TB (MDR-TB) and XDR-TB. Cultures are required monthly during the intensive phase of treatment and less frequently (according to country guidelines) during the continuation phase. At a minimum, quality-assured culture must be established at the central TB laboratory with the appropriate equipment, biosafety measures, infrastructure and referral mechanisms in place. If there is no central level laboratory with culture capacity, then mechanisms for transporting specimens to a WHO TB supranational reference laboratory (SRL) or to a neighbouring country's NRL for culture-based testing and drug-resistance evaluations should be in place.

A strategic process with measurable sequential objectives to carefully implement a network of TB laboratories or build capacity for such a network is less likely to result in wasted resources. Past experience can guide an effective and efficient approach for gradual expansion of a network. The process of designing an overall strategic plan for laboratory strengthening, capacity-building and expansion is discussed in Section 2.10.

1.2.6 TB networks and human resources

As networks are developed and capacity strengthened, it is essential to develop human resources on-site. Each laboratory will have specific requirements for trained and competent staff needed to perform the various tests that the laboratory runs. Higher levels of skill and training are needed to perform advanced testing for DST and surveillance at central and intermediate level laboratories. WHO has recommended limiting the number of tests performed by technicians, to reduce errors and ensure quality performance. It is important to ensure that each level has enough trained staff to efficiently perform daily routine workloads. Further support staff are also required to assist with non-testing activities, such as preparation of media and reagents, housekeeping and maintenance, waste management, data management, quality management, QA activities and various administrative tasks. It is essential that laboratories at all levels are well staffed to support the necessary demands for testing, to have a successful system for patient management and care.

Table 1.6 provides information that may be helpful in determining the number of personnel needed to perform various tests in a TB laboratory. During some phases of

the testing processes, personnel may be able to perform additional tasks. In addition, it may take almost the same amount of time to test one or two specimens as to test several specimens, depending on the experience of the technician. The numbers provided in Table 1.6 are estimates based on the assumption that staff are proficient and well trained. Testing is often batched in smaller units throughout the workday. Daily workload and testing will depend on the availability of equipment and biosafety

Table 1.6 Estimated number of tests that can be performed during an 8-hour workday

Procedure	No. of tests per day ^a	
AFB light microscopy	20–25 ^b	Per technician
AFB fluorescence microscopy	40–50 ^b	Per technician
Culture (liquid/solid media, including specimen processing)	20–40 ^c	Per technician
DST (using liquid media)	10–20	Per technician
DST (using solid media)	10–20	Per technician
FL-LPA (manual method)	12–24	Per instrument
SL-LPA (manual method)	12–24	Per instrument
Loopamp MTBC detection (TB-LAMP) test	12–18 ^d	Per instrument
Xpert MTB/RIF, Ultra or MTB/XDR assay (using four-module instrument)	12–16 ^e	Per instrument
Truenat MTB, MTB Plus and MTB-RIF Dx (using Quatro instrument)	Up to 36	Per instrument
RealTime MTB and MTB RIF/INH	Up to 94	Per instrument
FluoroType MTB and MTBDR	Up to 288	Per instrument
BD MAX MDR-TB	Up to 48	Per instrument
cobas MTB and MTB RIF/INH	384–1056	Per instrument
Genoscholar PZA-TB (using Multi-Blot NS-4800)	Up to 48	Per instrument

AFB: acid fast bacilli; DST: drug-susceptibility testing; FL-LPA: first-line line-probe assay; LAMP: loop-mediated isothermal amplification; MTBC: *Mycobacterium tuberculosis* complex; QA: quality assurance; SL-LPA: second-line line-probe assay; TB: tuberculosis.

- ^a The number of tests that can be performed in a day is given as an indication; it will vary according to local conditions. The ranges provided are estimates based on the assumption that a technician would work on all parts of the given procedure.
- ^b The recommendations provided for the maximum number of AFB smear examinations that can be performed by a single, competent laboratory worker are based on staining a maximum of 12 smears per batch, and examining smears stained with Ziehl–Neelsen (light microscopy) for 5 minutes each and smears stained with auramine O (fluorescence microscopy) for 2 minutes each; these specifications have been taken from *Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook* (<https://stoptb.org/wg/gli/gat.asp>) (14). Additional time will be required to engage in QA activities and to prepare reagents and reports. As a rule, the maximum number of Ziehl–Neelsen smears that can be examined by a microscopist in a single day should not exceed 25 because beyond that eye fatigue may lead to a deterioration in reading quality. However, proficiency in reading Ziehl–Neelsen smears should be maintained through regular examination of at least 10–15 smears per week.
- ^c To maintain overall laboratory proficiency in culture, laboratories should process at least 20 specimens per week with a minimum of five cultures per person. The same minimum requirements hold for maintaining proficiency in culture-based DST.
- ^d Six samples per cycle, with each cycle taking 1.5 hours. The proposed numbers are for 2–3 cycles per day.
- ^e One technician could perform more than 12 Xpert MTB/RIF tests per day (up to 24) assuming that more than one instrument was available in the laboratory. Where one instrument is available, a single technician may have time to perform other duties, such as reading smears.

cabinets. Often, laboratories have routine daily and weekly schedules for cabinet use, which allows for the efficient management of routine activities.

Section 2.7 provides more detail on the practical considerations TB laboratories face in relation to human resources.

References for Section 1.2

(Key resources and suggested reading highlighted in bold font)

- 1 The Global Plan to Stop TB 2011–2015: transforming the fight towards elimination of tuberculosis. Geneva: World Health Organization; 2010 (<https://apps.who.int/iris/handle/10665/44437>).
- 2 Framework of indicators and targets for laboratory strengthening under the End TB Strategy. Geneva: World Health Organization; 2016 (<https://apps.who.int/iris/handle/10665/250307>).
- 3 Tuberculosis laboratory biosafety manual (WHO/HTM/TB/2012.11). Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/handle/10665/77949>).
- 4 Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (<https://apps.who.int/iris/handle/10665/162712>).
- 5 Guide to TB specimen referral systems and integrated networks. Geneva: Global Laboratory Initiative; 2017 (<https://stoptb.org/wg/gli/srt.asp>).
- 6 GLI specimen referral toolkit. Geneva: Global Laboratory Initiative; 2022 (<https://stoptb.org/wg/gli/gat.asp>).
- 7 Albert H, Purcell R, Wang YY, Kao K, Mareka M, Katz Z et al. Designing an optimized diagnostic network to improve access to TB diagnosis and treatment in Lesotho. *PLoS One*. 2020;15(6):e0233620 (<https://pubmed.ncbi.nlm.nih.gov/32492022/>).
- 8 Nichols K, Girdwood SJ, Inglis A, Ondoa P, Sy KTL, Benade M et al. Bringing data analytics to the design of optimized diagnostic networks in low- and middle-income countries: process, terms and definitions. *Diagnostics*. 2021;11(1)(<https://www.mdpi.com/2075-4418/11/1/22>).
- 9 Albert H. Essential standards for a TB diagnostic network. 2016.
- 10 TB microscopy network accreditation: an assessment tool. Geneva: Global Laboratory Initiative; 2013 (<https://stoptb.org/wg/gli/gat.asp>).
- 11 Laboratory assessment tool. Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/handle/10665/70874>).
- 12 Piatek A. Assessment of TB diagnostic networks: a new tool. Washington DC: United States Agency for International Development; 2018 (https://www.teachepi.org/wp-content/uploads/Courses/ADV-2018/APiatek_adv_TB_diag2018June20.pdf).
- 13 Ondoa P, Datema T, Isadore J, Oskam L, Keita-Sow M-S, Ndiokubwayo J-B et al. A new matrix for scoring the functionality of national laboratory networks in Africa: introducing the LABNET scorecard. *Afr J Lab Med*. 2016;5(3):1–9 (<https://pubmed.ncbi.nlm.nih.gov/28879141/>).
- 14 Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).

Additional resources for Section 1.2

Narvaiz de Kantor I, Kim SJ, Frieden TR, Laszlo A, Luelmo F, Norval P-Y et al. Laboratory services in tuberculosis control. Geneva: World Health Organization; 1998 (<https://apps.who.int/iris/handle/10665/65942>).

Operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342369>).

Laboratory Mapping Program (LabMaP) [website]. African Society for Laboratory Medicine (ASLM) (<https://aslm.org/what-we-do/labmap>).

1.3 Diagnostic algorithms

Effective and efficient TB diagnostic algorithms are key components of a diagnostic cascade that ensures patients with TB are accurately and rapidly diagnosed and are promptly placed on appropriate therapy. In turn, such diagnosis and therapy should reduce morbidity and mortality, improve patient outcomes, reduce transmission and avoid development of drug resistance.

As a region's laboratory capacity improves or new diagnostic tests are implemented, algorithms will need to be modified. Modifications to algorithms must be put in place only after a formal evaluation, review and approval by officials within the MoH and the NTP. Often, nationally appointed thematic working groups are used to evaluate new technologies and develop implementation plans (which typically include revising current algorithms). These groups comprise local ministry officials and professionals (laboratory and medical) who will decide the optimal use and placement of the new technology within the current network structure. A technical consultant may be a part of this working group (either formally or informally) as an expert adviser to assist with evaluation, training, implementation or expansion activities.

The following points should be considered when designing or reviewing algorithms for testing at different levels of the laboratory network:

- characteristics (risk groups) of the population being served – these should be derived from population-based studies (if available), including the proportions that have DR-TB, are HIV-positive, have extrapulmonary TB or are children;
- the specific diagnostic tests in use or being considered for use;
- whether the tests are recommended by WHO and, if so, for what uses;
- the current and planned capacity of the country's laboratories, the laboratory infrastructure and the availability of competent personnel to conduct the tests;
- the adequacy of systems for specimen collection and transport, and average turnaround time between sites;
- the capacity of clinical services to offer diagnosis and treatment; and
- the drugs used for the treatment of TB.

Algorithms should be designed to use existing laboratory services, so that specimens can be referred to the appropriate level for tests that are not available at the peripheral level laboratories. Such referrals are particularly important when people are being evaluated for DR-TB or HIV-associated TB, when children are being evaluated for TB, or when people are being evaluated for extrapulmonary disease.

Model algorithms that incorporate the goals of the End TB Strategy and the most recent WHO recommendations for the diagnosis and treatment of TB and DR-TB have been available since 2015 (1) and have been updated several times (2, 3). In this section, the most recent recommended algorithms that emphasize the use of WRDs for TB are briefly described. The *WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update*¹ (3) provides

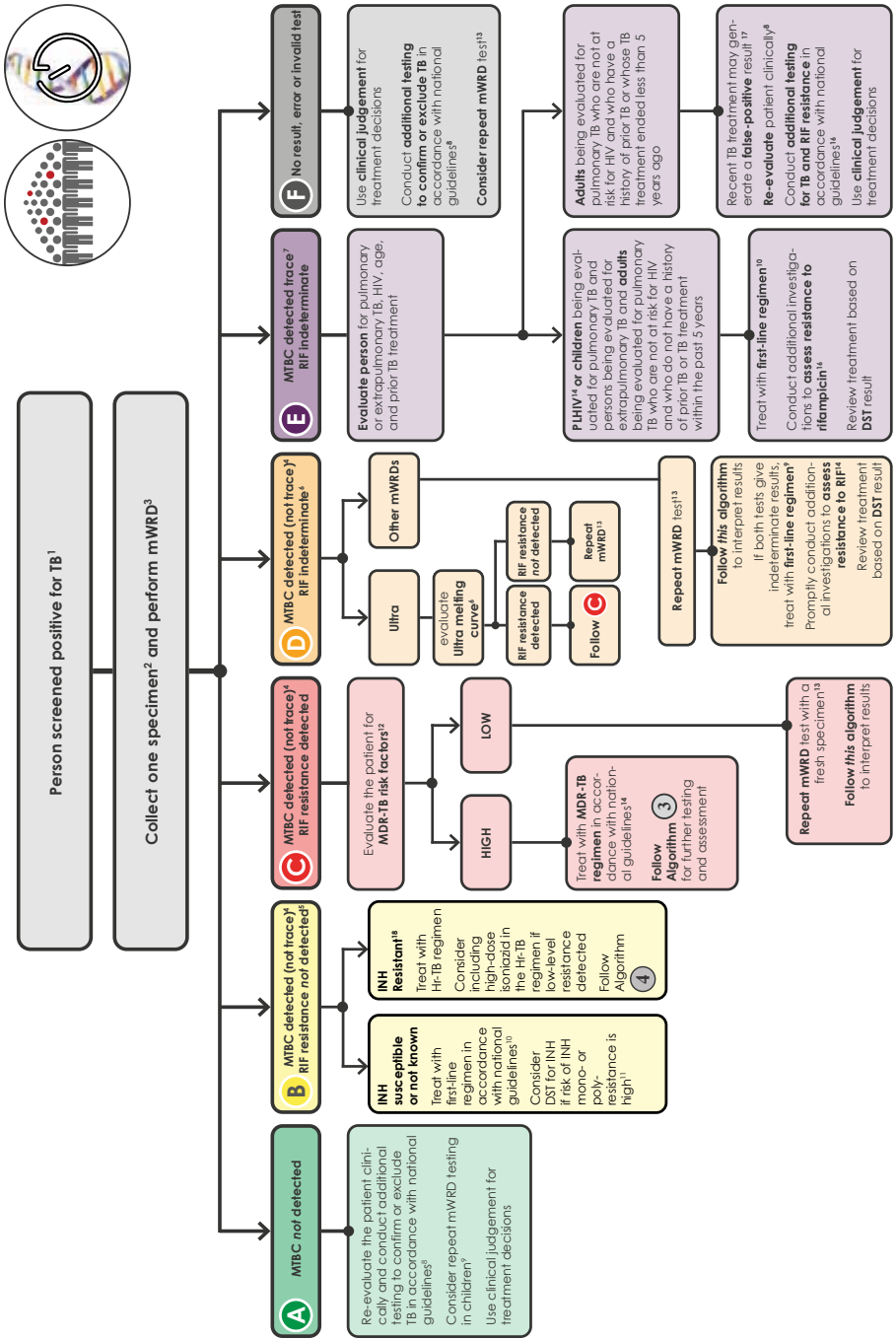
¹ See <https://apps.who.int/iris/handle/10665/342369>.

a thorough discussion of the algorithms, including explanatory notes and a decision pathway that details the various decisions to be taken. The algorithms are illustrative and must be adapted by countries to the local situation. The remainder of this section discusses each of the four algorithms.

1.3.1 Algorithm 1 – mWRD as the initial diagnostic test for TB

Algorithm 1 is the preferred algorithm for testing to support the diagnosis of TB in individuals being evaluated for pulmonary and extrapulmonary TB, and to achieve universal DST. In this algorithm, mWRDs are used as the initial diagnostic test to detect TB, resistance to rifampicin (except if TB-LAMP is used) and resistance to isoniazid if MC-aNAATs are used (i.e. this algorithm meets the goals of the End TB Strategy for the use of mWRDs and universal DST). The algorithm is designed to be used with any of the mWRDs for detection of MTBC (Xpert MTB/RIF, Xpert Ultra, Truenat MTB, Truenat MTB Plus and TB-LAMP, and MC-aNAAT), although it may need to be modified, depending on which mWRD is used and the population being tested. For example, in a setting with a high burden of MDR-TB, it would be preferable to use an mWRD that detects MTBC and rifampicin resistance simultaneously (e.g. Xpert MTB/RIF or sequentially Truenat MTB then Truenat MTB-RIF Dx) rather than one that detects only MTBC (e.g. TB-LAMP). In a setting with a well-functioning referral system and a high risk of isoniazid-resistant, rifampicin-susceptible TB (Hr-TB), an MC-aNAAT may be preferred as the initial test because of the ability to test for resistance to isoniazid and rifampicin simultaneously. This algorithm is feasible when the mWRD testing can be conducted on-site or can be accessed through a reliable referral system with short turnaround times. Algorithm 1 is shown in Fig. 1.2.

Fig. 1.2 Algorithm 1: mWRD as the initial diagnostic test for TB



AIDS: acquired immunodeficiency syndrome; CSF: cerebrospinal fluid; DNA: deoxyribonucleic acid; DST: drug-susceptibility testing; EMB: ethambutol; HIV: human immunodeficiency virus; HREZ: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide; Hr-TB: isoniazid-resistant, rifampicin-susceptible tuberculosis; INH: isoniazid; LAMP: loop-mediated isothermal amplification; LPA: line-probe assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant tuberculosis; MTB: *Mycobacterium tuberculosis*; MTBC: *Mycobacterium tuberculosis* complex bacteria; mWRD: molecular WHO-recommended rapid diagnostic test; PLHIV: people living with HIV/AIDS; PZA: pyrazinamide; RIF: rifampicin; RR-TB: rifampicin-resistant tuberculosis; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

- ¹ People screened positive for TB include adults and children with signs or symptoms suggestive of TB, with a chest X-ray showing abnormalities suggestive of TB, a positive mWRD used as a screening tool or positive C-reactive protein test (>5 mg/L) in PLHIV (4). A person with a positive mWRD used as a screening tool and a low pretest probability should be clinically assessed and, if deemed a presumptive TB patient, should have a repeat mWRD performed and follow Algorithm 1. If the pretest probability is high and the clinical picture is consistent with TB disease, then this test could be considered diagnostic and the patient should be managed based on the result of the test and, if relevant, should continue on to Algorithm 3 or 4. This algorithm may also be followed for the diagnosis of extrapulmonary TB using CSF, lymph node and other tissue specimens. However, mWRDs that are recommended for use in the diagnosis of extrapulmonary TB investigations are currently limited to Xpert MTB/RIF and Xpert Ultra.
- ² Programmes may consider collecting two specimens upfront. The first specimen should be promptly tested using the mWRD. The second specimen may be used for the additional testing described in this algorithm. For individuals being evaluated for pulmonary TB, sputum is the preferred specimen. Tissue biopsy samples are difficult or impossible to obtain repeatedly; therefore, they should be tested with as many methods as possible (e.g. mWRD, culture, DST or histology).
- ³ mWRDs or classes appropriate for this algorithm include Xpert MTB/RIF, Xpert Ultra, Truenat MTB, Truenat MTB Plus, MC-aNAAT and TB-LAMP.
- ⁴ "MTBC detected (not trace)" includes MTBC detected as high, medium, low or very low. These categories apply to the Xpert MTB/RIF and Xpert Ultra tests. Results of the Truenat MTB and MTB Plus tests, MC-aNAAT and the TB-LAMP test also fall into the category of "MTBC detected (not trace)". The MC-aNAAT provides additional resistance detection for isoniazid and leads to additional considerations in Box B.
- ⁵ Determination of RIF resistance occurs simultaneously in the Xpert MTB/RIF, Xpert Ultra and some MC-aNAAT tests. A second test is needed to determine RIF resistance in the Truenat MTB or MTB Plus test, using the same DNA isolated for the Truenat MTB tests (Truenat MTB-RIF Dx test) and in the TB-LAMP test, which requires a fresh specimen to be collected and a molecular or phenotypic DST to be conducted. In the case of MC-aNAAT, INH resistance detection would also occur simultaneously with RIF detection.
- ⁶ The interpretation and follow-up testing for "MTBC detected rifampicin indeterminate" results for the Xpert Ultra test differs from the interpretation of results for other mWRDs. MTBC detected that RIF indeterminate results obtained with the Xpert Ultra test (especially those with high and medium semiquantitative results) may be due to large deletions or multiple mutations that confer RIF resistance. Analysis of the Ultra melt curves can detect such resistance-conferring mutations. In some cases, culture and DST, sequencing or alternative mWRD will be needed to confirm or exclude RIF resistance. Indeterminate results for the other mWRDs are usually related to very low numbers of bacilli in the sample.
- ⁷ "MTBC detected trace" applies only to the Xpert Ultra test.
- ⁸ Further investigations for TB may include chest X-ray, additional clinical assessments, repeat mWRD testing, culture or clinical response following treatment with broad-spectrum antimicrobial agents.
- ⁹ In children with signs and symptoms of pulmonary TB in settings with a pretest probability of 5% or more, and an Xpert MTB/RIF or Xpert Ultra negative result on the initial test, repeat testing with Xpert MTB/RIF or Ultra (for a total of two tests) in sputum or nasopharyngeal aspirate. Furthermore, repeated testing with Xpert MTB/RIF may only be used only in gastric fluid, and stool specimens. No data were available to assess the performance of Xpert Ultra in gastric fluid and stool specimens. Programmes are encouraged to use Xpert Ultra in gastric fluid and stool specimens under operational research conditions. The mWRD should be repeated at the same testing site with a fresh specimen, with the result of the repeat test interpreted as shown in this algorithm. The result of the second test is the result that should be used for clinical decisions.
- ¹⁰ Patients should be initiated on a first-line regimen according to national guidelines, unless the patient is at very high risk of having MDR-TB. Such patients should be further investigated and initiated on an MDR-TB regimen. In situations where INH results are available (e.g. MC-aNAAT) and INH resistance has not been detected, the probability of having MDR-TB would be lower.
- ¹¹ A sample may be sent for molecular or phenotypic DST for INH if there is a high prevalence of INH resistance not associated with RIF resistance (i.e. INH mono- or poly-resistance) in this setting. Where a result for INH resistance is "not detected" (e.g. MC-aNAAT), and the pretest probability for Hr-TB is high, phenotypic DST

1. Background

for INH should be performed because 6–14% of resistance can be missed by molecular assays.

- ¹² Patients at high risk for MDR-TB include previously treated patients, including those who had been lost to follow-up, relapsed or failed a treatment regimen; non-converters (smear-positive at end of intensive phase); MDR-TB contacts; and any other groups at risk for MDR-TB identified in the country.
- ¹³ The mWRD should be repeated at the same testing site with a fresh specimen, and the result of the repeat test should be interpreted as shown in this algorithm. The result of the second test is the result that should be used for clinical decisions.
- ¹⁴ PLHIV include those who are HIV-positive or whose HIV status is unknown, but who present with strong clinical evidence of HIV infection, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all those with unknown HIV status, HIV testing should be performed according to national guidelines.
- ¹⁵ Patients should be promptly initiated on an MDR-TB regimen in accordance with national guidelines. Algorithm 3 should be followed for additional testing for any patient with RR-TB.
- ¹⁶ Phenotypic (culture and DST) and molecular (e.g. mWRDs, LPAs and DNA sequencing) methods are available for evaluating drug resistance. Rapid molecular methods are preferred.
- ¹⁷ In patients with a prior history of TB within the past 5 years or whose TB treatment was completed less than 5 years ago, Xpert Ultra trace results (and occasionally Xpert MTB/RIF “MTBC detected low or very low”) may be positive, not because of active TB but because of the presence of non-viable bacilli. Clinical decisions must be made based on all available information and clinical judgement.
- ¹⁸ Patients diagnosed using an MC-aNAAT and whose result is RIF resistance not detected and INH resistance detected should be treated for Hr-TB with RIF/EMB/PZA (REZ) and levofloxacin. For practical purposes, HREZ fixed-dose combination tablets may be used instead of REZ. Consider including high-dose INH in the Hr-TB regimen if low-level resistance is detected (inhA mutation only). Follow Algorithm 4.

Source: Reproduced from Fig. 4.2 of *WHO operational handbook on tuberculosis Module 3 (3)*.¹

¹ See <https://apps.who.int/iris/handle/10665/342369>.

1.3.2 Algorithm 2 – LF-LAM testing to aid in the diagnosis of TB among PLHIV

Algorithms 2a (inpatient settings) and 2b (clinic and outpatient settings) are the preferred algorithms for testing to support the diagnosis of TB in PLHIV. The algorithms are appropriate for use in settings with a high burden of HIV and for use with individual patients living with HIV who meet the testing criteria, regardless of the overall HIV burden. These algorithms emphasize the use of the LF-LAM assay to quickly identify patients needing TB treatment; they also emphasize that all individuals with signs and symptoms of TB should receive an mWRD (Algorithm 1). LF-LAM assay results (test time <15 minutes) are likely to be available before mWRD results, and treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests. The ease of use of the LF-LAM test makes it suitable to be implemented outside of the laboratory (e.g. in outpatient clinics).

The currently available urinary LF-LAM assays have sufficient sensitivity and specificity to aid in the diagnosis of TB among individuals coinfecting with HIV (5). The urinary LF-LAM assays are recommended as the initial diagnostic tests:

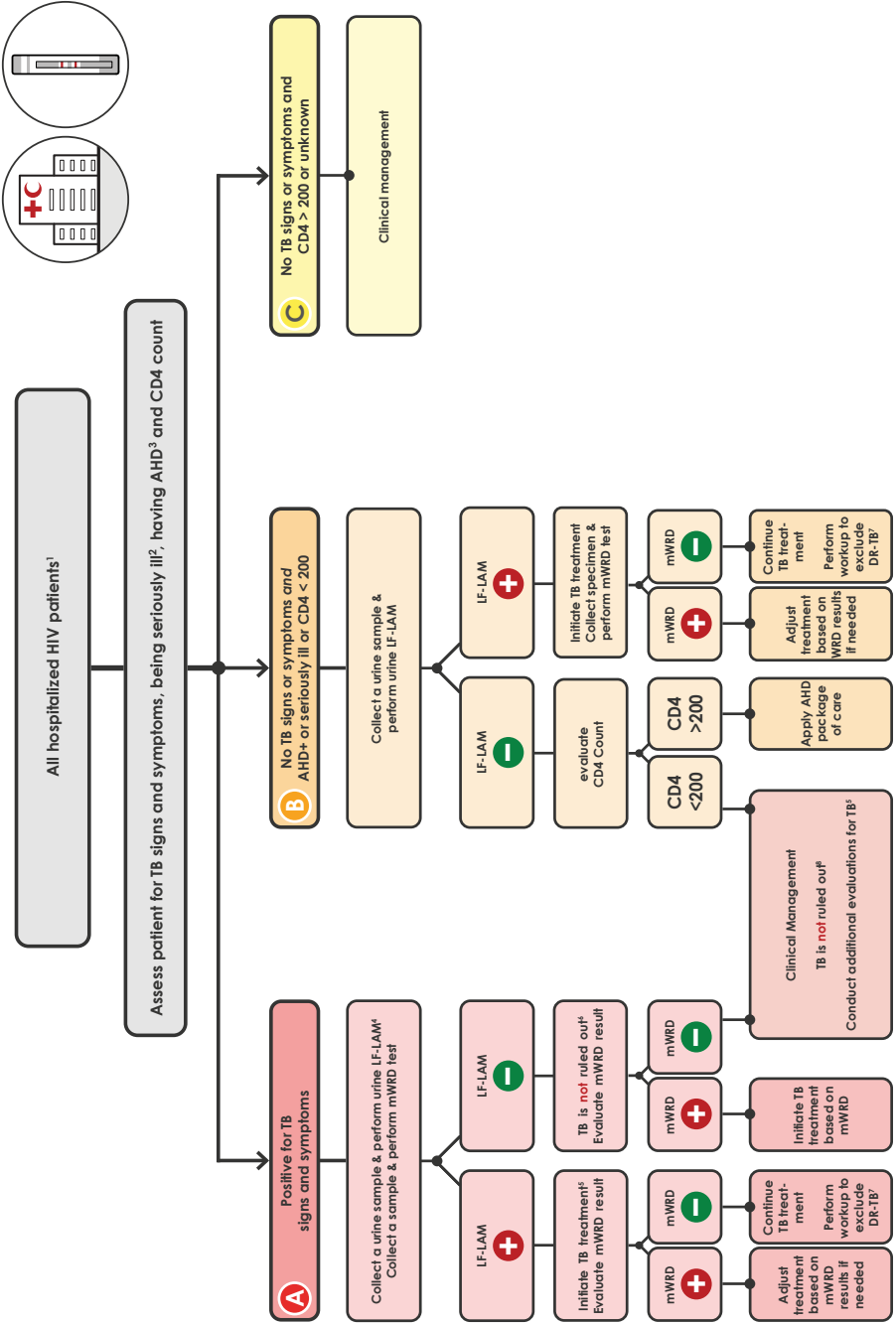
- for all PLHIV with signs and symptoms of TB;
- in inpatient settings for HIV-positive adults, adolescents and children with advanced HIV disease or who are seriously ill, or PLHIV with a CD4 cell count of less than 200 cells/mm³, irrespective of signs and symptoms of TB; and
- in outpatient settings, for HIV-positive adults, adolescents and children who are seriously ill, or PLHIV with a CD4 cell count of less than 100 cells/mm³, irrespective of signs and symptoms of TB.

WHO recommends against using LF-LAM:

- to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children without TB symptoms, and an unknown CD4 cell count or a CD4 cell count greater than 100 cells/mm³ in outpatient settings; and
- for the diagnosis of TB in HIV-negative persons, because of suboptimal sensitivity and specificity in HIV-negative persons.

Algorithms 2a and 2b are shown in Fig. 1.3 and Fig. 1.4, respectively.

Fig. 1.3 Algorithm 2a: LF-LAM to aid in the diagnosis of TB among PLHIV in inpatient settings



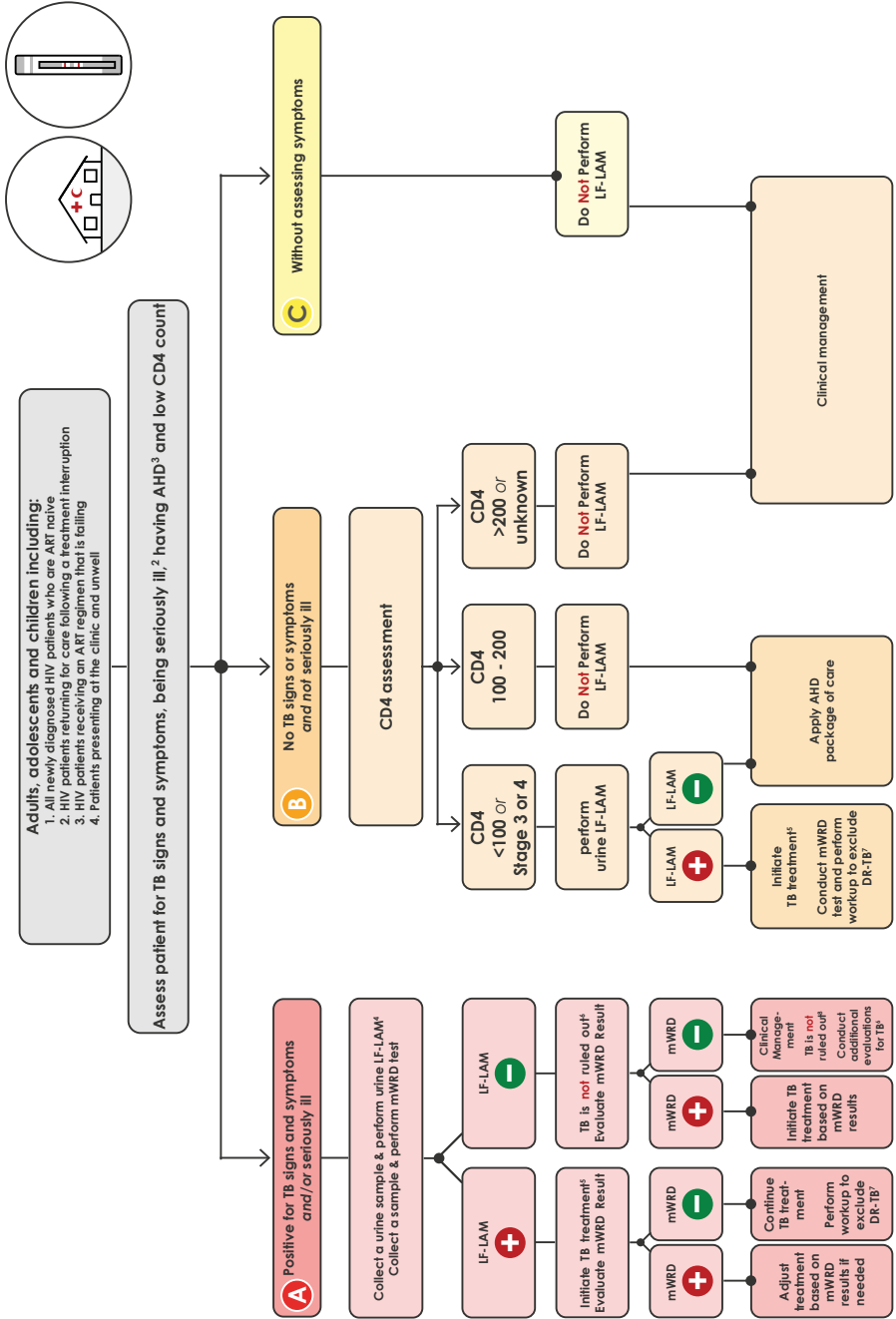
AHD: advanced HIV disease; AIDS: acquired immunodeficiency syndrome; DNA: deoxyribonucleic acid; DR-TB: drug-resistant tuberculosis; DST: drug-susceptibility testing; FQ: fluoroquinolone; HIV: human immunodeficiency virus; LF-LAM: lateral flow urine lipoarabinomannan assay; LPA: line-probe assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic test; PLHIV: people living with HIV/AIDS; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

- ¹ PLHIV include persons who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all people with unknown HIV status, HIV testing should be performed in accordance with national guidelines. PLHIV with TB may also present with signs and symptoms of extrapulmonary TB, including lymphadenopathy, meningitis or other atypical presentations that warrant evaluation.
- ² "Seriously ill" is defined based on four danger signs: respiratory rate >30 per minute, temperature >39 °C, heart rate >120 beats per minute and unable to walk unaided.
- ³ For adults, adolescents and children aged >5 years, AHD is defined as CD4 cell count <200 cells/ml³, or WHO stage 3 or 4 event at presentation for care. All children aged <5 years are considered as having AHD.
- ⁴ The LF-LAM test and mWRD should be done in parallel. The LF-LAM results (test time <15 minutes) are likely to be available before the mWRD results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.
- ⁵ Patients should be initiated on a first-line regimen according to national guidelines, unless they are at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen.
- ⁶ Negative LF-LAM results do not rule out TB in symptomatic persons. The mWRD result should be evaluated when it becomes available for treatment decisions. See Algorithm 1 for interpretation of mWRD results.
- ⁷ Phenotypic (culture and DST) and molecular (e.g. mWRDs, LPAs and DNA sequencing) methods are available to evaluate drug resistance. Rapid molecular methods (e.g. Xpert MTB or Truenat MTB-RIF Dx or MC-aNAAT tests) are preferred.
- ⁸ Negative mWRD and LF-LAM results do not rule out TB in symptomatic persons. Conduct additional clinical evaluations for TB. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, and additional mWRD testing or culture. Consider initiating treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (FQs should not be used) and for *Pneumocystis pneumonia*. The clinical response should be evaluated after 3–5 days of treatment.

Source: Reproduced from Fig. 4.3 of *WHO operational handbook on tuberculosis Module 3 (3)*.¹

¹ See <https://apps.who.int/iris/handle/10665/342369>.

Fig. 1.4 Algorithm 2b: LF-LAM to aid in the diagnosis of TB among PLHIV in clinic and outpatient settings



AHD: advanced HIV disease; ART: antiretroviral therapy; DNA: deoxyribonucleic acid; DR-TB: drug-resistant tuberculosis; DST: drug-susceptibility testing; FQ: fluoroquinolone; HIV: human immunodeficiency virus; LF-LAM: lateral flow urine lipoarabinomannan assay; LPA: line-probe assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic test; PLHIV: people living with HIV/AIDS; RIF: rifampicin; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

- ¹ PLHIV include persons who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all people with unknown HIV status, HIV testing should be performed in accordance with national guidelines. PLHIV with TB may also present with signs and symptoms of extrapulmonary TB, including lymphadenopathy, meningitis or other atypical presentations warranting evaluation.
- ² "Seriously ill" is defined based on four danger signs: respiratory rate >30 per minute, temperature >39 °C, heart rate >120 beats per minute and unable to walk unaided.
- ³ For adults, adolescents and children aged >5 years, AHD is defined as CD4 cell count <200 cells/mL3 or WHO stage 3 or 4 event at presentation for care. All children aged <5 years are considered as having AHD.
- ⁴ The LF-LAM test and mWRD should be done in parallel. The LF-LAM results (test time <15 minutes) are likely to be available before mWRD results, and treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.
- ⁵ Patients should be initiated on a first-line regimen according to national guidelines, unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. Treatment regimens should be modified as needed based on the results of the mWRD testing.
- ⁶ Negative LF-LAM results do not rule out TB in symptomatic persons. The result of the mWRD should be evaluated when it becomes available for treatment decisions. See Algorithm 1 for interpretation of mWRD results.
- ⁷ Phenotypic (culture and DST) and molecular (see Algorithms 1 and 3) methods are available to evaluate drug resistance. Rapid molecular methods (e.g. mWRDs) are preferred.
- ⁸ The mWRD negative and LF-LAM negative results do not rule out TB in symptomatic persons. Conduct additional clinical evaluations for TB. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, and additional mWRD testing or culture. Consider initiating treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (not FQs) and those for *Pneumocystis pneumonia*. The clinical response should be evaluated after 3–5 days of treatment.

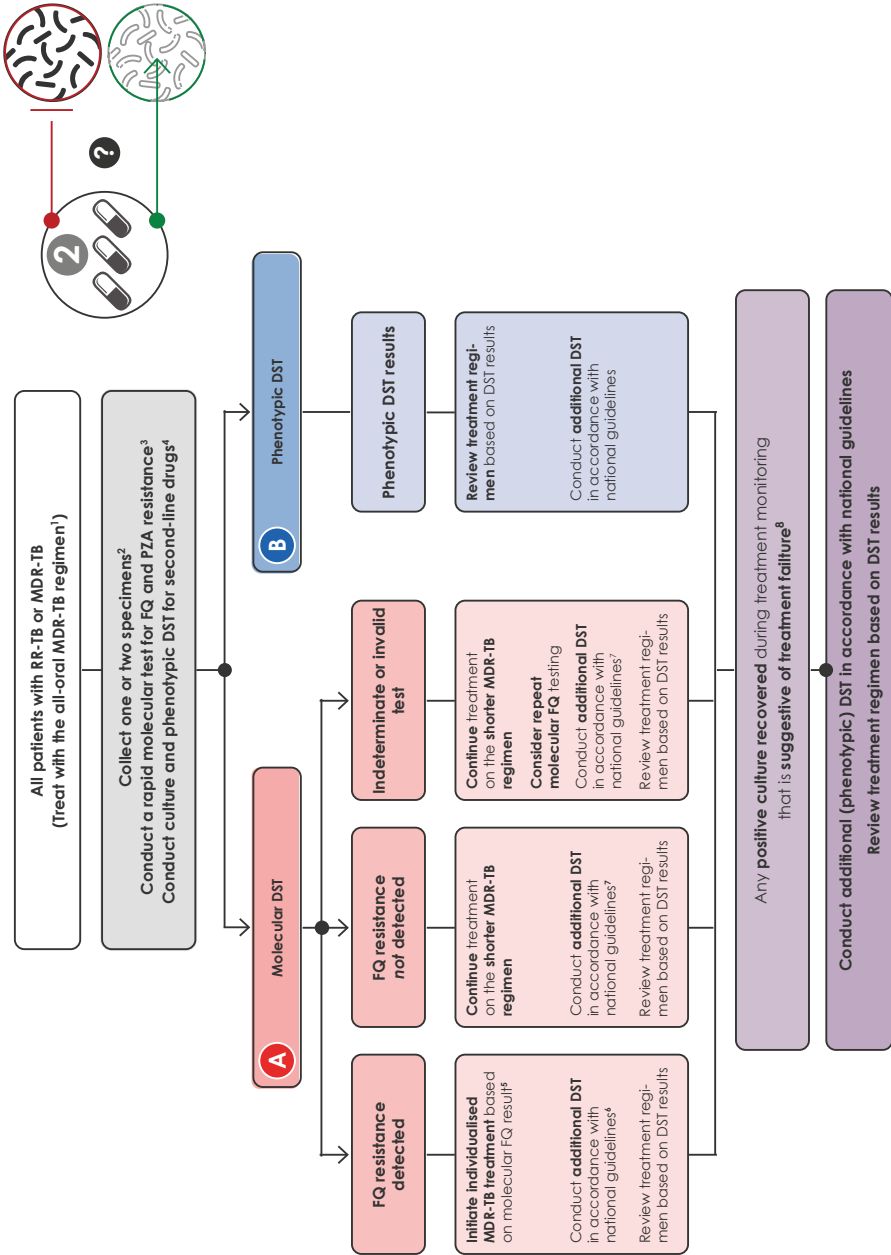
Source: Reproduced from Fig. 4.4 of *WHO operational handbook on tuberculosis Module 3 (3)*.¹

¹ See <https://apps.who.int/iris/handle/10665/342369>.

1.3.3 Algorithm 3 – DST for second-line drugs for persons with MDR/RR-TB

Algorithm 3 is for further evaluation of patients with multidrug- or rifampicin-resistant TB (MDR/RR-TB). In its most recent recommendations (6), WHO stresses the importance of DST before starting a patient on the preferred shorter, all-oral, bedaquiline-containing MDR-TB regimen, especially for the medicines for which mWRDs are available (currently, fluoroquinolones, isoniazid and rifampicin). In addition, WHO stresses the need to scale up laboratory capacity for DST for medicines for which there are accurate and reproducible phenotypic methods (7), including bedaquiline, clofazimine, delamanid and linezolid. As in any potentially life-saving situation, treatment for DR-TB should not be withheld from a patient due to a lack of complete DST results. Algorithm 3 is shown in Fig. 1.5.

Fig. 1.5 Algorithm 3: DST for second-line drugs for MDR/RR-TB patients



1. Background

BDQ: bedaquiline; CFZ: clofazimine; DLM: delamanid; DST: drug-susceptibility testing; FQ: fluoroquinolone; INH: isoniazid; LC-aNAAT: low complexity automated NAAT; LZD: linezolid; MDR-TB: multidrug-resistant tuberculosis; MDR/RR-TB: multidrug- or rifampicin-resistant tuberculosis; NAAT: nucleic acid amplification test; PZA: pyrazinamide; RR-TB: rifampicin-resistant tuberculosis; SL-LPA: line-probe assay for second-line drugs; TB: tuberculosis; WHO: World Health Organization.

- ¹ Patients should be promptly initiated on an MDR-TB regimen in accordance with national guidelines and WHO recommendations. A shorter all-oral BDQ-containing treatment regimen of 9–12 months in duration is the preferred option for eligible MDR/RR-TB patients [8].
- ² If molecular and phenotypic testing are performed in the same laboratory, one specimen may be sufficient. If testing is performed in two laboratories, two specimens should be collected, and the molecular and phenotypic testing conducted in parallel.
- ³ WHO recommends getting the rapid DST results for FQs before the start of treatment, although this testing should not delay the start of treatment. Currently, LC-aNAAT and SL-LPA are the WHO-approved rapid molecular tests for detecting FQ resistance.
- ⁴ Phenotypic DST should be conducted for each of the drugs included in the treatment regimen for which there are accurate and reproducible methods. Reliable phenotypic DST methods when performed in a quality-assured laboratory are available for BDQ, FQ, CFZ, INH, PZA, DLM and LZD. A new molecular class of tests, the reverse hybridization high complexity NAAT, is available for PZA resistance detection on culture isolates. The initiation of treatment should not be delayed while awaiting the results of the phenotypic DST.
- ⁵ For more details regarding individualized regimens, see the WHO consolidated guidelines on drug-resistant tuberculosis treatment [8].
- ⁶ For FQ-resistant MDR/RR-TB, a specimen should be collected and submitted for phenotypic DST to the WHO Group A (BDQ and LZD), B and C drugs, if not already being done as described in note 4.
- ⁷ In settings with a high underlying prevalence of resistance to FQs or for patients considered at high risk of FQ resistance, a specimen should be referred for culture and phenotypic DST for FQs.
- ⁸ If resistance to an individual drug (e.g. BDQ) is suspected and DST for these drugs is not available in the country, laboratories will need to have mechanisms to store the isolate and ship it to a WHO supranational laboratory for DST.

Source: Reproduced from Fig. 4.5 of *WHO operational handbook on tuberculosis Module 3* (3).¹

¹ See <https://apps.who.int/iris/handle/10665/342369>.

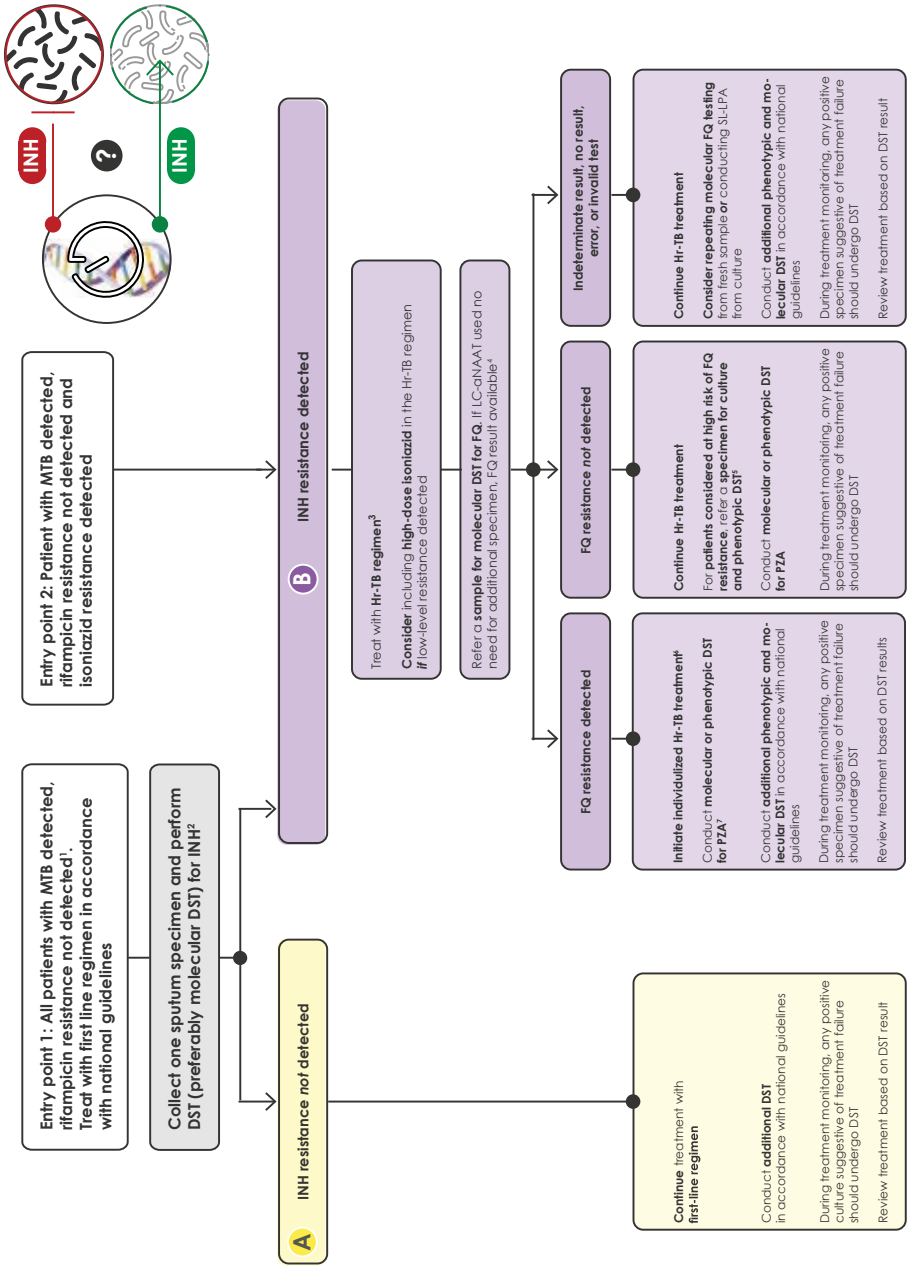
1.3.4 Algorithm 4 – Follow-on test to detect Hr-TB

Algorithm 4 is for either detection of Hr-TB or follow-on testing for individuals shown to have Hr-TB. This algorithm was developed in response to the 2018 publication *WHO treatment guidelines for isoniazid-resistant tuberculosis*¹ and Module 4 of the WHO consolidated guidelines (8, 9). The publication focuses on the treatment of Hr-TB; it emphasizes that the successful treatment of Hr-TB, the prevention of the spread of Hr-TB and the acquisition of resistance to additional drugs (e.g. rifampicin) relies on rapidly detecting individuals with Hr-TB and placing them on effective treatment regimens. Compared to people with drug-susceptible TB, individuals with Hr-TB who are treated with the recommended regimen for drug-susceptible TB have a much higher risk of treatment failure (11% versus 2%), relapse (10% versus 5%) and acquiring additional drug resistance (8% versus 1%). This testing algorithm incorporates the testing requirements to ensure that the recommended Hr-TB treatment regimen (rifampicin, ethambutol, pyrazinamide and levofloxacin for 6 months) will be effective.

In Algorithm 1, individuals tested with mWRDs that detect MTBC and rifampicin resistance simultaneously (e.g. Xpert MTB/RIF) or sequentially (e.g. Truenat MTB then Truenat MTB-RIF) with a result of 'RIF resistance not detected' will enter Algorithm 4, to assess isoniazid resistance. Individuals tested with MC-aNAATs in Algorithm 1 with already identified Hr-TB (i.e. resistance to isoniazid and susceptibility to rifampicin) will enter Algorithm 4 for follow-on testing. Algorithm 4 is shown in Fig. 1.6.

¹ See <https://apps.who.int/iris/handle/10665/260494>.

Fig. 1.6 Algorithm 4: mWRD as the initial test to detect Hr-TB in patients with rifampicin-susceptible TB



DST: drug-susceptibility testing; EMB: ethambutol; FL-LPA: line-probe assay for first-line drugs; FQ: fluoroquinolone; HC-rNAAT: high complexity reverse hybridization NAAT; HREZ: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z); Hr-TB: isoniazid-resistant, rifampicin-susceptible tuberculosis; INH: isoniazid; LC-aNAAT: low complexity automated NAAT; LFX: levofloxacin; MC-aNAAT: moderate complexity automated NAAT; MTB: *Mycobacterium tuberculosis*; MTBC: *Mycobacterium tuberculosis* complex bacteria; NAAT: nucleic acid amplification test; PZA: pyrazinamide; REZ: rifampicin (R), ethambutol (E) and pyrazinamide (Z); RIF: rifampicin; SL-LPA: line-probe assay for second-line drugs; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

- 1 All patients with MTBC detected, RIF resistance not detected and INH resistance unknown should be initiated on a first-line regimen according to national guidelines.
- 2 Patients at high risk for Hr-TB should be given priority for molecular testing for INH resistance. Patients at high risk of Hr-TB include previously treated patients such as those who had been lost to follow-up, relapsed and failed a treatment regimen; Hr-TB contacts; and any other groups at risk for Hr-TB identified in the country (e.g. from populations with a high prevalence of Hr-TB). Molecular DST is preferred and includes MC-aNAAT, LC-aNAAT or FL-LPA.
- 3 Patients should be initiated on an Hr-TB regimen in accordance with national guidelines. The preferred regimen is 6 months of RIF-EMB-PZA-LFX (6 REZ-LFX) after confirmation of INH resistance, so long as RIF resistance has been reliably excluded. INH may be included in the regimen to enable the use of an HREZ fixed-dose combination tablet. The use of high doses of INH (up to 15 mg/kg) may be useful for patients whose isolate displays low-level resistance to INH (e.g. isolate with mutations in the *inhA* promoter region only).
- 4 For each patient with Hr-TB, a specimen should be referred for molecular DST for FQs. The LC-aNAAT can simultaneously detect INH and FQ resistance. The alternative for FQ resistance detection is the SL-LPA. PZA resistance detection should also be performed where phenotypic or molecular (e.g. HC-rNAAT) DST for PZA is available, reliable and quality assured. Sequencing of the *pncA* if available is another option.
- 5 Despite good sensitivity of LC-aNAAT (93%) and SL-LPA (86%) for detecting FQ resistance, culture and phenotypic DST may be needed for patients with a high pretest probability for FQ resistance (e.g. setting with a high underlying prevalence of resistance to FQs or patient risk factors) when the resistance is not detected by the molecular test.
- 6 Patients with FQ-resistant Hr-TB may be treated with a 6-month regimen of (H)REZ or an individualized Hr-TB regimen.
- 7 For all Hr-TB patients with concurrent resistance to FQ, phenotypic or molecular DST (e.g. HC-rNAAT) for PZA is desirable if a reliable DST for PZA has been established in the country. When resistance to PZA is confirmed, appropriate treatment regimens may have to be designed individually, especially if resistance to both FQ and PZA are detected.

Source: Reproduced from Fig. 4.6 of *WHO operational handbook on tuberculosis Module 3 (3)*.¹

¹ See <https://apps.who.int/iris/handle/10665/342369>.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT:

- Provide training on WHO-recommended algorithms
- Assist with selection of tests to implement
- Assist with implementation of WHO-recommended tests

References for Section 1.3

(Key resources and suggested reading highlighted in bold font)

- 1 Implementing tuberculosis diagnostics: policy framework. Geneva: World Health Organization; 2015 (<https://apps.who.int/iris/handle/10665/162712>).
- 2 GLI model TB diagnostic algorithms. Geneva: Global Laboratory Initiative; 2018 (https://stoptb.org/wg/gli/assets/documents/gli_algorithms.pdf).
- 3 WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342369>).
- 4 WHO consolidated guidelines on tuberculosis Module 2: Screening – systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/340255>).
- 5 WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342331>).
- 6 Rapid communication: key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB). Geneva: World Health Organization; 2018 (<https://apps.who.int/iris/handle/10665/275383>).
- 7 Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva: World Health Organization; 2018 (<https://apps.who.int/iris/handle/10665/275469>).
- 8 WHO consolidated guidelines on tuberculosis Module 4: Treatment – drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/332397>).
- 9 WHO treatment guidelines for isoniazid-resistant tuberculosis: supplement to the WHO treatment guidelines for drug-resistant tuberculosis. Geneva: World Health Organization; 2018 (<https://apps.who.int/iris/handle/10665/260494>).

1.4 Targets and indicators for TB laboratory strengthening

The WHO End TB Strategy calls for the early diagnosis of TB including universal DST, which is currently defined as DST for at least rifampicin among all patients with bacteriologically confirmed TB, and further DST for at least fluoroquinolones among all TB patients with rifampicin resistance. A prerequisite for any NTP to reach this goal is a quality-assured laboratory network equipped with rapid diagnostics. The WHO *Framework of indicators and targets for laboratory strengthening under the End TB Strategy*¹ serves as a guide for all countries to use when developing plans for laboratory strengthening in 2016–2025 (1). The indicators measure the programme's capacity to detect patients accurately and rapidly using new diagnostic tests (i.e. WRDs), provide universal DST and ensure quality of testing. Table 1.7 shows the 12 core indicators grouped under three objectives (increase access to rapid and accurate detection of TB, reach universal access to DST and strengthen quality of laboratory services). These indicators will be monitored at the global level by WHO to assess a country's progress towards reaching targets; additional stratified indicators are also included for monitoring at country level when recording and reporting systems allow.

Laboratory strengthening efforts in country must be aware of these indicators and prioritize activities that address the accomplishment of the goals of the End TB Strategy.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Provide training on indicators and targets
- Assist with collection of data and calculations for indicators

References for Section 1.4

(Key resource and suggested reading highlighted in bold font)

- 1 **Framework of indicators and targets for laboratory strengthening under the End TB Strategy.** Geneva: World Health Organization; 2016 (<https://apps.who.int/iris/handle/10665/250307>).
- 2 **ISO 15189. Quality management system implementation: look before you leap.** Geneva: International Organization for Standardization; 2015 (https://challengetb.org/publications/tools/lab/ISO15189_QMS_Implementation.pdf).

¹ See <https://apps.who.int/iris/handle/10665/250307>.

Table 1.7 Indicators for laboratory strengthening under the End TB Strategy^a

Objective 1: Increase access to rapid and accurate detection of TB	
Indicator 1.	Does the national diagnostic algorithm indicate a WRD as the initial diagnostic test for all people with signs and symptoms of TB?
Indicator 2.	Percentage of notified new and relapse TB cases tested with a WRD as the initial diagnostic test
Indicator 3.	Percentage of notified new and relapse TB cases with bacteriological confirmation ^b
Indicator 4.	Percentage of testing sites using a WRD at which a data connectivity system has been established that transmits results electronically to clinicians and to an information management system
Indicator 5.	Does national policy indicate that TB diagnostic and follow-up tests provided through the NTP are free of charge, or that fees can be fully reimbursed through health insurance, for all people with signs and symptoms of TB?
Objective 2: Reach universal access to DST ^c	
Indicator 6.	Does national policy and the diagnostic algorithm indicate universal access to DST?
Indicator 7.	Percentage of notified bacteriologically confirmed TB cases with DST results for rifampicin
Indicator 8.	Percentage of notified RR-TB cases with DST results for fluoroquinolones and second-line injectable agents
Objective 3: Strengthen quality of laboratory services	
Indicator 9.	Percentage of diagnostic testing sites that monitor performance indicators and are enrolled in an EQA system for all diagnostic methods performed
Indicator 10.	Percentage of DST sites that have demonstrated proficiency by EQA panel testing for all DST methods performed
Indicator 11.	Percentage of laboratories conducting either culture, LPA or phenotypic DST (or all of these), in which a formal QMS towards achieving accreditation according to international standards is being implemented
Indicator 12.	Is the NRL currently accredited according to the ISO 15189 ^d standard?

DST: drug-susceptibility testing; EQA: external quality assessment; FQ: fluoroquinolones; LPA: line-probe assay; NRL: national reference laboratory; NTP: national TB programme; QMS: quality management system; RR-TB: rifampicin-resistant TB; TB: tuberculosis; WRD: WHO-recommended rapid diagnostic test; WHO: World Health Organization.

^a Indicators are not listed in order of priority.

^b A bacteriologically confirmed TB case is one from whom a biological specimen is positive by smear microscopy, culture or WRD.

^c Universal access to DST is currently defined as DST for at least rifampicin among all patients with bacteriologically confirmed TB and further DST for at least FQs among all TB patients with rifampicin resistance. DST methods include genotypic (molecular) and phenotypic methods.

^d See *ISO 15189. Quality management system implementation* (https://challengtb.org/publications/tools/lab/ISO15189_QMS_Implementation.pdf) (2).

2. Key technical areas for guidance

2.1 Procurement and supply chain management

Effective care and treatment of TB requires support from fully functioning laboratory services that provide accurate, reliable and timely results. To be fully functional, laboratory services require a continuous uninterrupted supply of commodities. These commodities include equipment and supplies (e.g. reagents, diagnostic kits and various consumables).

Effective supply chain management is a complex process that includes:

- product specification;
- product selection;
- forecasting of needs (based on past and projected consumption);
- procurement;
- customs clearance, if applicable;
- distribution; and
- storage and use.

In most LMIC, provision of uninterrupted supplies at laboratories continues to be a significant challenge. Reasons for this situation include heavy reliance on direct donor procurement; lack of coordination and standard procedures for procurement and distribution of supplies by government, donors and other partners; lack of accurate consumption data on which to estimate actual supply needs; lack of up-to-date guidance with regard to the necessary technical specifications, International Organization for Standardization (ISO) regulations, or essential quality parameters; and long and bureaucratic procedures for procurement within the government, involving several government ministries and levels of approval. This situation has led to:

- frequent stock-outs, leading to interruptions in service delivery and delays in treatment or patient management decisions;
- waste, because of expiry of reagents;
- poor quality of materials or reagents, which in turn can lead to inaccuracy of testing or test failure; and
- equipment that is inappropriate or non-functional.

An inadequately managed supply chain may result in either understocking or overstocking of supplies, both of which have serious detrimental effects. If access to supplies is interrupted, a laboratory may have to suspend services or divert patients to other testing sites. This may result in delayed diagnosis of patients, added cost and inconvenience to patients who are referred to other sites, and confusion and lack of confidence in the laboratory among clinicians. Overstocking also has negative consequences, including waste of resources when stock reaches its expiry date before being consumed. Furthermore, poor selection of equipment and supplies leads to inadequate or poor-quality goods being used.

Effective management of laboratory equipment and supplies is essential at every level of the network. Such management requires planning, understanding of the routine consumption rates of supplies, and anticipation of changes in the workload (e.g. due to seasonal or annual trends). Donors and partners who are procuring laboratory supplies directly must also be coordinated. Thus, the NTP, NRL and others involved in commodities decision-making, forecasting and procurement must work together to ensure a continuous flow of supplies to support testing. Some countries have instituted a central pooled procurement process, to better manage procurement of certain supplies (e.g. Xpert MTB/RIF cartridges). Such a system ensures that the needs of all laboratories are met while reducing waste due to reagents expiring before they are used. Other countries have implemented a logistics strategy to ensure that sufficient numbers of cartridges that are within the expiry date are available; when a laboratory has accumulated excess stock, it is reallocated to other laboratories within the network.

The NRL, NTP or other central institution – working with national medical supply services or procurement agencies – usually sets standards for commodities management, and provides QA and reporting mechanisms. They should also evaluate the quality, accuracy and performance of equipment and supplies. More specifically, the NRL, NTP and supportive commodities management organizations should be responsible for:

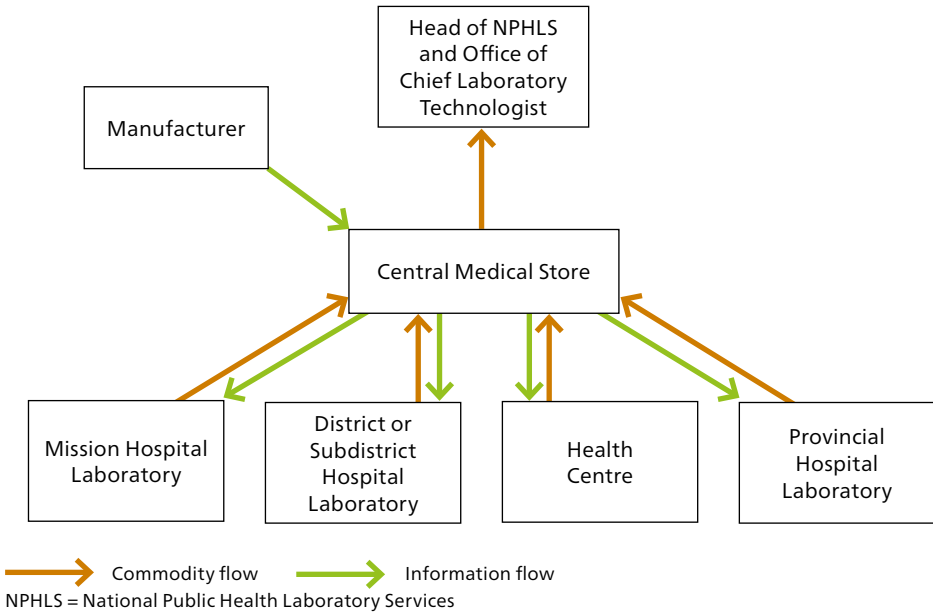
- selecting equipment and supplies, and setting specifications and quantities;
- participating in budgeting and planning – including verifying tenders, bids and contracts;
- working with local and national procurement organizations; and
- arranging and training laboratory managers and staff in commodity management activities.

Managing a laboratory's commodities involves careful planning and coordination, and should follow the well-recognized cycles of selection, procurement, distribution and use. General guidance can be obtained from publications such as *Guidelines for managing the laboratory supply chain*¹ (1) and *Logistics supply management tool*² (2). The flow of information and supplies in a laboratory network is illustrated in Fig. 2.1.

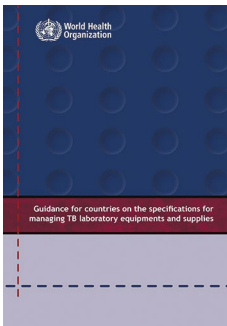
¹ See https://publications.jsi.com/JSIInternet/Inc/Common/_download_pub.cfm?id=11109&lid=3.

² See <https://www.challengeb.org/library/lab>.

Fig. 2.1 Flow of information and supplies in a laboratory network



Reproduced with permission from *Guidelines for managing the laboratory supply chain (V2) (1)*.



WHO has developed guidelines and specifications¹ that provide standard guidance on procuring TB laboratory equipment, consumables and supplies for TB microscopy, culture and DST equipment and supplies (3).

In well-functioning TB programmes, forecasting, procurement and distribution are regulated through a national system and are well documented using electronic data systems that monitor both distribution and consumption rates for all laboratories. In addition, materials and supplies are stored in well-organized national warehouses with proper climate control conditions. Distribution is then provided on a schedule that correlates to usage rates. Shipments to laboratories are organized on a central calendar to ensure timely delivery. Often, regional hubs are established to facilitate local transit or pickups. Such systems are rare in most resource-limited settings, but their presence is increasing owing to the increased use of molecular testing technologies that have specific storage specifications for reagents and materials necessary for quality testing. Countries are encouraged to develop commodities management guidelines and national distribution systems to limit issues of expiry, stock-outs or wastage due to inappropriate storage, poor forecasting and inefficient shipment. Factors influencing procedures for storage and distribution include expiry dates and storage requirements.

¹ See http://whqlibdoc.who.int/publications/2011/9789241503068_eng.pdf.

2. Key technical areas for guidance

Each laboratory (at all levels) should have a comprehensive list of equipment, reagents and consumables for the tests being performed. This list should include detailed specifications (including catalogue and lot numbers) for each item in stock; such specifications are required where laboratory goods need to be procured by tender. To maintain a consistent record, these data should be managed by a single person in the laboratory; although the data can be kept in a paper register, it is better to have this information in a database or Microsoft Excel register if possible (supply chain management tools include (1) and (2)). Any materials or equipment provided directly to a laboratory by a donor or partner organization should be placed in this inventory and reported to the NTP or national procurement systems services. All commodities should be registered with the national authorities, to maintain equity within the system and avoid overstocking or expiry of materials.

Product lead time (i.e. the time from the placing of an order to the delivery of the goods) may be long and involve complex procedures, contributing to stock-outs and service interruption, which may be further extended by customs clearance procedures for imported products. The procedures often require special knowledge and skills; if this is missing, there may be unanticipated costs and materials may be stored incorrectly. Delays in clearing customs have resulted in supplies being unfit for use. In some countries, customs clearance is handled by specialized public or private entities; in others, TB laboratory or MoH staff may dedicate considerable time to ensuring that supplies are cleared from customs in a timely fashion.

Each country will have its own process and distribution system. It is essential to understand the existing system before implementing new technologies or adapting current testing capacity. Existing processes should be strengthened and parallel systems should be avoided.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Advise on developing specifications for TB laboratory supplies and equipment and product selection criteria
- Review existing supplies management practices and advise on improvements
- Assist with measuring consumption and establishing inventories
- Develop technical specification for equipment, consumables and reagents
- Assist with developing forecasting strategies
- Support development of internal commodities registers
- Implement laboratory information management systems (LIMS) to assist with commodities management
- Provide training on various aspects of supply chain management
- Support establishment and management of the laboratory storage and distribution system
- Help to develop national guidelines for commodities management

References for Section 2.1

- 1 Guidelines for managing the laboratory supply chain (V2). Washington, DC: United States Agency for International Development; 2008 (https://publications.jsi.com/JSIInternet/Inc/Common/_download_pub.cfm?id=11109&lid=3).
- 2 Logistics supply management tool. Challenge TB; 2011 (<https://www.challengetb.org/library/lab>).
- 3 Guidance for countries on the specifications for managing TB laboratory equipments and supplies. Geneva: World Health Organization; 2011 (https://apps.who.int/iris/bitstream/handle/10665/44798/9789241503068_eng.pdf?sequence=1).

Additional resource for Section 2.1

Planning and budgeting tool for TB and drug resistant TB testing: calculation tool. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/349949>).

2.2 Specimen collection, transportation and reception

Well-designed specimen referral systems¹ (1, 2) underpin a strong diagnostics network and can help to:

- optimize access to services;
- improve promptness of testing, use of instruments, biosafety and biosecurity, maintenance of proficiency and QA;
- facilitate linkages to care;
- provide solutions adapted to the local geography and epidemiology; and
- make it possible to integrate sample transportation with testing for other diseases, thus providing broader testing services in underserved settings.

Lower level laboratories must be linked to higher level laboratories if follow-up testing is to provide efficient patient management, ensure optimal use of different technologies at different levels of the tiered network and maintain staff competence in these techniques. Optimization of the specimen referral network requires careful consideration to balance access, costs and turnaround time.

Efforts should be made to integrate TB specimen transport and referral systems with systems used for other specimens and testing purposes.

The initial steps in the process are to collect, label, transport and register the clinical specimens. These steps must be done in a timely manner to expedite treatment initiation or regimen changes.

Regardless of the location of the laboratory, proper steps for sputum collection are important to obtain specimens of good quality, to ensure accurate and reliable test results. Use of good-quality specimen containers is critical; specifications should be clearly defined to ensure that good-quality specimen containers are available at all sites. Specimen containers must always be labelled before the specimen is collected from the patient.

¹ See <https://stoptb.org/wg/gli/gat.asp>.

Having a well-functioning sample reception unit that checks the quality of each sample and rejects those that are of inadequate quality is an essential step in ensuring that the testing process runs correctly and produces a quality result. The reception unit must check each sample for compliance with quality criteria and completed documentation. Once accepted, each specimen must be recorded in the laboratory register with all the necessary information. If the sample does not completely comply with the criteria, it must be rejected and a request should be made for a new sample. If documentation is incomplete, efforts must be taken to contact the referring physician or clinic to acquire all the necessary information to complete the register.

When testing cannot be conducted at the site of collection, collected specimens must be properly labelled, and efficiently and safely transported to the nearest laboratory for testing. Transporting of sputum samples must be done according to recommended protocols, taking into account the distance and transit time, to ensure integrity.

The NTP and NRL should determine the information to be included on the specimen container and requisition form, drawing on WHO publications *Guidance on regulations for the transport of infectious substances*¹ (3) and Tuberculosis laboratory biosafety manual² (4). It is critical that the NTP and NRL train and monitor provincial health units and other referring facilities to ensure that proper and safe collection practices are in place, proper transit protocols are used and documentation is complete. Laboratories should develop a laboratory handbook that includes information relating to collection, labelling and transporting of specimens (with target turnaround times and specimen rejection criteria), and the handbook should be distributed to all referring facilities.

Important considerations for collection, labelling, transporting and registering specimens can be found in GLI-approved training programmes³ for Xpert MTB/RIF, culture and DST. Target turnaround times should be set locally and monitored for adherence. Section 2.3.2 has more information on quality indicators.

2.2.1 Collection

Good-quality specimens are necessary for proper laboratory diagnosis of TB. Non-salivary sputum specimens of about 3–5 mL are optimal. However, collecting sputum represents a significant hazard because coughing produces potentially infectious aerosols. Therefore, specific measures must be taken to minimize the health care worker's exposure to these aerosols. Wherever possible, sputum specimens should be collected outdoors where infectious droplets will be rapidly diluted and ultraviolet light can rapidly inactivate TB bacilli. Specimens should never be collected in laboratories, toilets or washrooms, waiting areas, reception rooms or any other enclosed space where people congregate. When ventilated sputum collection rooms (or booths) are correctly used and maintained, they provide a safe alternative to outdoor collection. Maintenance of these rooms or booths requires proper modes of ventilation during expectoration, and appropriate decontamination and disinfection procedures.

¹ See <https://apps.who.int/iris/handle/10665/339825>.

² See <https://apps.who.int/iris/handle/10665/77949>.

³ See <https://stoptb.org/wg/gli/trainingpackages.asp>.

Staff must be trained to provide patients with appropriate instructions about how to collect a quality specimen. Although instructions on posters and leaflets in designated sputum collection areas are helpful, it is necessary to supervise the first specimen collection process to help the patient understand the protocol. While supervising, the health care worker must stand behind the patient, away from any possible exposure to aerosolized droplets. Detailed guidance on safe collection of good-quality sputum specimens is provided in *Laboratory diagnosis of tuberculosis by sputum microscopy: the GLI handbook*¹ (5).

Recently, the use of stool specimens for the diagnosis of TB in children has received considerable attention. The collection and processing of stool specimens for use with the Xpert MTB/RIF and Xpert Ultra tests are described in the *GLI Practical manual of processing stool samples for diagnosis of childhood TB*² (6).

The LF-LAM assay is a WHO-approved test for detecting TB that is unique in that it uses urine as the patient sample. Guidance on collecting and storing of urine samples for the LF-LAM can be found in the Alere Determine™ TB LAM Ag information website.³

2.2.2 Transport and packaging

Triple packaging is required for the safe transport of infectious material; that is, the container should be wrapped in absorbent material (cotton or paper towels), protected by secondary packaging (e.g. a ziplock bag), and then placed in shock-resistant outer packaging. Special requirements for local and international transit are discussed below.



Local transit

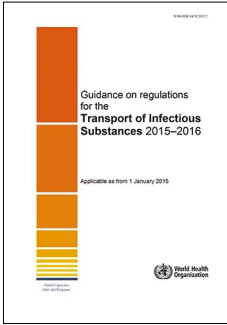
Local transit may be done by, for example, courier, health facility vehicles, other means of transport (e.g. motorcycles) or “hand delivery” by district TB officers or other cadres. All individuals transporting specimens should be provided with training on biosafety and should have spill kits accessible in case of accidents. All transporters should follow local regulations where applicable. Use of specimen transport logs is recommended, to provide adequate budgeting for a sustainable system and to identify high service areas that may need an additional laboratory or referral hub.

¹ See <https://stoptb.org/wg/gli/gat.asp>.

² See <https://stoptb.org/wg/gli/gat.asp>.

³ See <https://www.globalpointofcare.abbott/en/product-details/determine-tb-lam.html>.

International transit

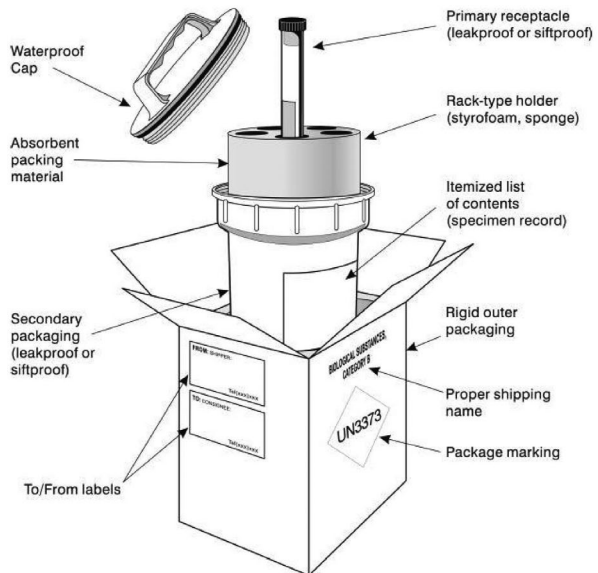


International transportation requires proper packaging according to carrier specifications for shipping infectious materials and must comply with international regulations. Fig. 2.2 illustrates the elements required for packaging and shipping through international postal carriers. The package should be labelled according to regulations for the transport of infectious materials and logged into a transportation register by the carrier, with a copy given to the referring centre for tracking. International organizations such as the Universal Postal Union (UPU), the International Civil Aviation Organization (ICAO) and the International Air Transport Association (IATA) follow specific guidelines to facilitate the safe shipment of infectious materials. Shipping *M. tuberculosis* cultures internationally (e.g. for diagnostic DST, retesting or proficiency testing) is subject to international regulations and to specific national import and export regulations. International protocols and guidelines for safe transit are well established and are described in the WHO *Guidance on regulations for the transport of infectious substances 2021–2022*¹ (3).

If delays in transport are anticipated, specimens should be transported to the laboratory in a cool box. This is especially relevant for specimens for TB culture. Sample contamination due to inappropriate storage and long transport times is less of a concern with smear or rapid molecular tests than with conventional culture-based approaches.

Shipment of infectious materials is an expensive process, and it is critical to ensure that shipments are not delayed by bureaucratic or packaging errors. Shipments may be rejected or suffer excessive delays that render the samples useless for subsequent laboratory investigations.

Fig. 2.2 Example of triple packaging for IATA Category B infectious substances



IATA: International Air Transport Association.

Source: *Guidance on regulations for the transport of infectious substances 2021–2022* (3).

¹ See <https://apps.who.int/iris/handle/10665/339825>.

2.2.3 Specimen logs, registers and examination request forms

Proper documentation of samples being transported and received is critical to tracking and managing referral activities, while also providing a structure for collecting essential patient information. Primary forms of documentation include referral logs or registries, specimen registries and test request forms. All forms and registers need to be complete and well maintained. Although some laboratories may use electronic registries, most settings still rely on paper-based systems.

The following documentation is generally needed for shipments outside the country (e.g. to an SRL) and should be checked before initiating a shipment:

- customs declarations;
- evidence that staff have a current IATA certification (if air shipment is required);
- current import permits; and
- contact details of the person to whom shipment is being sent.

All copies of paperwork should be sent to the consignee in advance (e.g. airway bill, customs, quarantine and dangerous goods declaration).

Where transport registers or logs are lacking, it is important to encourage programmes to develop these within their systems. Transport registers help to provide a tracking system and should record the name of the referring clinic; the date of transport; the number of specimens being transported; if the transport system is integrated, the type of specimens transported (e.g. containing blood, urine or extrapulmonary tissue or fluids in addition to sputa for TB testing); distance (km) transported (to assist with budgeting for fuel and manage efficient travel routes); and incidents or accidents during transport that cause delays or promote contamination. A sample form is provided at Fig. 2.3.

Specimen registers are required for all laboratories. The register contains 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 the 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 or identification numbers and therefore the patient's internal records. Registers will vary depending on the level of the laboratory and the tests performed at the facility. Fig. 2.4 shows a sample register for a peripheral laboratory, and illustrates the use of the Xpert MTB/RIF test. Laboratory registers will need to be modified to accommodate the use of other mWRDs.

Test specimen examination request forms contain information about the patient and the tests requested by the physician. These forms identify the patient as a new case for diagnosis or a patient requiring follow-up testing to manage treatment. These forms are critical and must be complete to capture data for routine surveillance activities and proper patient record management. Fig. 2.5 illustrates a request form used by a peripheral level laboratory to request conventional TB tests, Xpert MTB/RIF and LPAs. Specimen examination forms will need to be modified to accommodate all tests available in the receiving laboratory (in particular, other mWRDs).

Fig. 2.5 Sample examination request form for a peripheral laboratory

Request for examination of biological specimen for TB

Treatment unit: _____ Date of request: _____

Patient name: _____

Age (years): _____ Date of birth: _____ Sex: Male Female

Patient address: _____

Telephone: _____

Reason for examination:

Diagnosis. If diagnosis, presumptive RR-TB/MDR-TB?: Yes No

OR Follow-up. If follow-up, month of treatment: _____

HIV infection? Yes No Unknown

Previously treated for TB? Yes No Unknown

Specimen type: Sputum Other (specify): _____

Test(s) requested: Microscopy Xpert MTB/RIF
 Culture Drug susceptibility Line probe assay

Requested by (Name and signature): _____

Microscopy results (to be completed in the laboratory)

Date sample collected <i>(filled by requestor)</i>	Specimen type	Laboratory serial number(s)	Visual appearance (blood-stained, mucopurulent or saliva)	Result <i>(tick one)</i>				
				Negative <i>(0 AFB/100 HPF)</i>	1–9/100 HPF <i>(scanty; report no. of AFB)</i>	+	++	+++

Examined by (name and signature): _____

Date of result: _____

2. Key technical areas for guidance

Xpert MTB/RIF test result (to be completed in the laboratory)

Date sample collected: _____

M. tuberculosis: Detected Not detected Invalid / No result / Error

Rifampicin resistance: Detected Not detected Indeterminate result

Examined by (name and signature): _____

Date of result: _____

Culture results (to be completed in the laboratory)

Date sample collected (filled by requestor)	Media used (liquid or solid)	Laboratory serial number(s)	Result (tick one)						
			Negative (0 colonies)	1–9 (<10 colonies)	+ (10–100 colonies)	++ (>100 colonies)	+++ (Innumerable/ confluent growth)	NTM ¹	Contaminated

Examined by (name and signature): _____

Date of result: _____

Drug susceptibility test (DST) and line probe assay (LPA) results (to be completed in the laboratory)

Date sample collected (filled by requestor)	Method ^a	Laboratory serial number(s)	Results ^b (mark for each drug)														
			H	R	E	S	Amk	Km	Cm	FQ:	Other:	Other:	Other:	Other:			

^a Specify: solid media DST; liquid media DST; direct LPA; indirect LPA

^b Results codes: R = Resistant S = Susceptible C = Contaminated — = Not done

Examined by (name and signature): _____

Date of result: _____

AFB: acid fast bacilli; AMK: amikacin; C: capreomycin; DST: drug-susceptibility testing; E: ethambutol; FQ: fluoroquinolones; H: isoniazid; HPF: high power fields; LPA: line-probe assay; MDR-TB: multidrug-resistant TB; NTM: nontuberculous mycobacteria; RR-TB: rifampicin-resistant TB; S: streptomycin; TB: tuberculosis.



*The Definitions and reporting framework for tuberculosis*¹ (7) should be used as a template for devising request and report forms for referring specimens and reporting results from AFB smear microscopy, culture, WRDs or DST. Countries are encouraged to modify these forms to include additional tests such as TB-LAMP, moderate complexity automated nucleic acid amplification tests or SL-LPA. For example, the form shown in Fig. 2.5 could be modified to add TB-LAMP to the Xpert MTB/RIF column and to add a set of TB-LAMP reporting values to the footnotes.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Offer training on proper collection for quality specimens
- Develop aids for patients on specimen collection and safe practices
- Offer training on specimen packaging and transportation
- Develop specimen referral systems with proper tracking systems
- Analyse recent shipments to determine cost, efficiency and service received
- Establish a data system to record referral activities to assess the process; allow adequate budgeting for materials, personnel and fuel; identify limitations to access; and stratify referral to define access for risk groups
- Develop and update templates of logs, registers and specimen examination request forms

References for Section 2.2

(Key resources and suggested reading highlighted in bold font)

- 1 GLI specimen referral toolkit. Geneva: Global Laboratory Initiative; 2020 (<https://stoptb.org/wg/gli/gat.asp>).
- 2 Guide to TB specimen referral systems and integrated networks. Geneva: Global Laboratory Initiative; 2017 (<https://stoptb.org/wg/gli/srt.asp>).
- 3 Guidance on regulations for the transport of infectious substances 2021–2022. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/339825>).
- 4 Tuberculosis laboratory biosafety manual (WHO/HTM/TB/2012.11). Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/handle/10665/77949>).
- 5 Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).
- 6 Practical manual of processing stool samples for diagnosis of childhood TB. Geneva: World Health Organization; 2022 (<https://apps.who.int/iris/handle/10665/353599>).
- 7 Definitions and reporting framework for tuberculosis – 2013 revision: updated December 2014 and January 2020. Geneva: World Health Organization; 2013 (<https://apps.who.int/iris/handle/10665/79199>).

¹ See <https://apps.who.int/iris/handle/10665/79199>.

2.3 Quality assurance

2.3.1 Introduction to QA

A comprehensive and systematic QA programme should be implemented, to enable laboratories to achieve and maintain high levels of accuracy and proficiency in testing, which will ensure the reliability and reproducibility of results, and thus inspire confidence in clinicians and patients who use the laboratory’s services.

QA has been defined as follows:

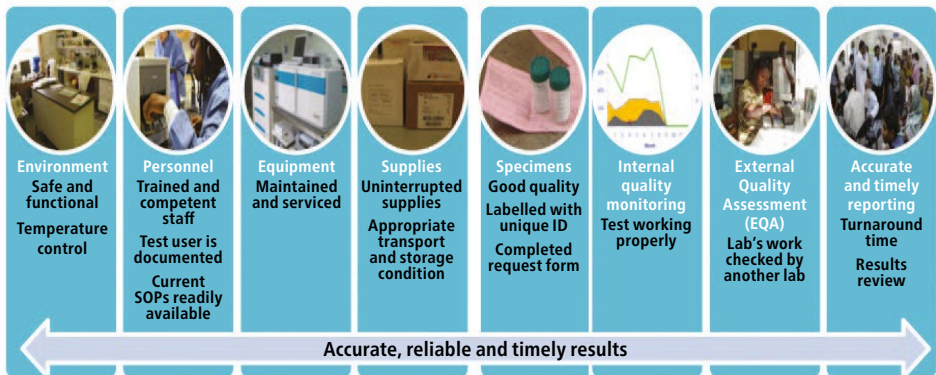
Planned and systematic activities to provide confidence that an organization fulfils requirements for quality.¹ (1)

Encompasses a range of activities that enable laboratories to achieve and maintain high levels of accuracy and proficiency despite changes in test methods and the volume of specimens tested.²

In many resource-limited settings, comprehensive QA for TB diagnostic tests is often limited or absent, performed sporadically and poorly documented. Monitoring of laboratory indicators may not be done routinely, and quality control (QC) and external quality assessment (EQA) may only be performed in a limited way or only on certain tests. Even when such procedures are in place, the results of QA activities are frequently not reported back to the laboratories in a timely fashion, or support for corrective actions is not available, leading to missed opportunities for quality improvement. The content and quality of training provided in a country may vary widely, and training participants are often not assessed for competency. Implementation of a holistic QA programme can significantly improve TB laboratory services.

Fig. 2.6 illustrates the essential elements of a QA programme applied to any technology. Some requirements are general to all technologies whereas others have test-specific requirements or definitions.

Fig. 2.6 Essential elements of a comprehensive QA programme



ID: identifier; QA: quality assurance; SOP: standard operating procedure.

¹ See https://clsi.org/media/1523/qms01a4_sample.pdf.

² See <https://www.cdc.gov/labstandards/index.html>.

QA activities should be seen as an integral part of the routine workload rather than as a separate activity. All QA activities must be documented. Providing feedback to testing sites and implementing corrective and preventive measures are the most critical aspects of any QA programme; they are also the aspects that are often poorly implemented. However, QA is just one part of a laboratory quality management system (QMS), which is required to ensure the quality of all the processes undertaken in a laboratory.

2.3.2 Key QA activities

Specific QA activities can be defined beyond the general elements already mentioned. The following are considered essential QA activities for any TB laboratory. They are also ISO 15189 (2) requirements.

Key QA activities are as follows (each activity is discussed below):

- training and competence assessment;
- instrument verification;
- equipment maintenance;
- method validation;
- QC;
- lot testing (also known as incoming QC or new batch testing);
- EQA;
- quality indicator monitoring; and
- continuous quality improvement (QI).

Training and competence assessment

Training materials have been developed and are freely available for most WHO-approved TB diagnostics, including smear microscopy (light and fluorescence), solid and liquid culture, DST, LPA and Xpert MTB/RIF. They may be downloaded from the training packages of the GLI website.¹ QA procedures associated with each technology are included in each package and should be part of any trainings. Some specific training materials have also been developed which deal exclusively with QA in more detail; for example, *External quality assessment for AFB smear microscopy*² (3) and *Practical guide to implementing a quality assurance system for Xpert MTB/RIF Testing*³ (4).

The training materials may require customization based on the country situation, resources and existing policies and guidelines. Where possible, such customization should be done in close collaboration with the NRL and NTP (often through a

¹ See <https://stoptb.org/wg/gli/trainingpackages.asp>.

² See <https://stoptb.org/wg/gli/assets/documents/External%20Quality%20Assessment%20for%20AFB%20Smear%20Microscopy.pdf>.

³ See <https://stoptb.org/wg/gli/gat.asp>.

laboratory technical working group), to ensure local ownership and country relevance. A standardized training package and tools should be implemented in all laboratories in the country. Where the country has a process for national approval of the training materials, that process should be followed, and all organizations providing training in the country should follow the approved training materials, ensuring consistent quality and content.

At the country level, a common approach is “training the trainers”, in which selected participants (usually from the NRL or regional referral laboratories) are provided with intensive content training and are coached in how to deliver the training to other personnel. This may be done regionally or by country. Once the trainers are deemed competent, they can provide training to staff in peripheral laboratories within the country.

In planning to conduct training it is important to liaise with the NRL, to ensure that the appropriate personnel are invited to the training. For example, for laboratory training in DST, personnel who will conduct the testing on a routine basis should be trained, rather than managers or nonlaboratory personnel.

All trainings should include a competency assessment of the participants. Competency is defined as a “demonstrated ability to apply knowledge and skills”, and clear criteria for competency should be set in advance. Staff competency should be monitored on a regular basis, with refresher training provided where necessary.

Instrument verification

Instruments should be evaluated as being “fit for purpose” through verification with known positive and negative material, both before they start to be used for testing of clinical specimens and after calibration or repair of instruments. Verification testing should be repeated if there is any deviation from expected results, and suppliers should be contacted for troubleshooting if there are repeated errors.

Equipment maintenance

A schedule of preventive maintenance and calibration should be designed for each piece of equipment. If calibration and maintenance are easy to perform, then a staff member or a designated equipment officer may perform the tasks, with or without additional technical training. If the equipment is sensitive and maintenance or calibration is complex, it is better to hire an external, specialized company to perform these tasks. In some cases, manufacturers offer maintenance and calibration services.

Method verification

All tests used in the laboratory must be verified as being fit for their intended use. For commercial tests, in which the test is used according to the manufacturer’s intended use, additional large-scale laboratory evaluations are not necessary. Rather, small-scale method verifications, in line with requirements for national or international accreditation schemes, may be warranted. However, some laboratories do conduct such large-scale evaluation studies to confirm performance if they believe that country-specific factors (e.g. the prevalence of different mutations) may cause

performance to deviate substantially from the manufacturer's results or the results from other evaluation studies.

Where laboratories perform non-standard or modified methods, use tests outside their intended scope (e.g. specimens for which the test has not been validated) or use methods developed in-house, then more extensive method validation is required before starting to test clinical specimens. Such validation usually comprises testing either a well-characterized panel of known positive and negative samples (in a blinded fashion), or prospective testing of the current gold standard and new test in parallel on clinical specimens.

Quality control

QC monitors activities related to the examination (i.e. analytical) phase of testing. The goal of QC is to detect, evaluate and correct errors due to test system failure, environmental conditions or operator performance, before patient results are reported. QC involves examination of control materials or known substances at the same time as patient specimens and in the same manner, to monitor the accuracy and precision of the complete analytical process. If QC results are not acceptable, patient results must not be reported.

QC materials are most commonly the following: well-characterized strains of MTBC or NTM, water or decontamination solutions (i.e. negative QC samples), known positive or negative clinical samples, or aliquots of DNA extracts from known strains. Controls may also be built into the test device (sometimes referred to as an "internal control") and are performed automatically with each test (e.g. Xpert MTB/RIF assay). However, internal controls may only monitor a portion of the procedure, and additional, traditional QC may be needed from time to time.

QC is one element of process control (i.e. control of the activities employed in the handling and examination of samples). QC ensures accurate and reliable testing, and it is a requirement for all testing for accreditation. Other aspects of process control apply to the other stages of testing (e.g. pre-analytical and post-analytical testing).

In TB laboratories in resource-limited settings, there may be limited use of QC, or QC may be used only with certain tests. A commonly cited reason for the absence of QC is a lack of funding; however, local solutions can be found to fulfil QC requirements, and reliance on expensive commercial solutions is not usually necessary. Other barriers include the cost of additional reagents and supplies needed to perform the QC testing. Technical support may be needed to develop local solutions using available resources; for example, strains obtained from well-characterized panels received from SRLs as part of an EQA programme.

QC in a TB laboratory can include monitoring of activities such as preparation of stains and media, staining and examining of AFB microscopy slides, decontamination and inoculation of culture, DNA extraction and LPA procedure, sample processing control and probe check control in the Xpert MTB/RIF assay. Table 2.1 provides more examples of general quality indicators.

Lot testing

Lot testing is also known as incoming QC or new batch testing. Such QC testing should be performed on new kits or lots of reagents before their use for testing patient samples, to ensure that they perform as expected. Incoming QC testing is a requirement of ISO 15189 (2). If kits are centrally procured and then distributed to peripheral sites (e.g. Xpert MTB/RIF cartridges), the incoming QC testing requirement may be fulfilled by centralized testing before distribution to outlying sites. However, caution is required because transportation of reagents and kits to an end-user site may damage or inactivate the products; QC testing is strongly advised at the end-user site before a test is used on clinical samples.

In addition to QC testing of new lots, continuous monitoring at site level of the performance indicators of tests, including error rates, is important. Continuous monitoring allows for the early detection of any problems with different lots due to factors such as local storage conditions.

External quality assessment

EQA (assurance) is defined as follows:

Inter-laboratory comparisons and other performance evaluations that may extend throughout all phases of the testing cycle, including interpretation of results; determination of individual and collective laboratory performance characteristics of examination procedures by means of inter-laboratory comparison; NOTE: the primary objectives of EQA are educational and may be supported by additional elements.¹ (5)

EQA for TB laboratories may include on-site supervision, proficiency testing and blinded rechecking. These factors are discussed below.

All laboratories should ensure that all tests are part of an EQA programme; however, monitoring performance using laboratory quality indicators (also known as performance indicators) is the most effective way to assure the quality of the laboratory results and identify areas for improvement. Quality indicator monitoring should always be implemented in conjunction with an EQA programme. Section 2.4 has more information on quality indicators.

On-site supervision

Site visits should be planned at regular intervals to assess the laboratory and testing site practices and adherence to protocols. Usually conducted by the NRL, NTP or partners, these visits may be conducted by national, regional or district level staff, and should be integrated with other on-site supervision where possible (e.g. quarterly NTP site visits). A standardized checklist must be used for consistency and completeness of information. On-site supervision should form part of the EQA processes for all TB diagnostic technologies. The visits provide motivation and support to staff, especially in peripheral settings. Establishing strong relationships with staff encourages rapid reporting of any problems, which in turn allows rapid troubleshooting, retraining and

¹ See <https://webstore.ansi.org/standards/cls/clsigp27a2>.

corrective actions. When planning on-site visits, sufficient time should be allocated for each visit, including travel time.

The extent of the evaluation during each visit will depend on the frequency of the visits, the capacity of the staff and the performance of the laboratory, with more extensive evaluation needed in poorly performing sites. During the visit, all components of testing and laboratory workflow should be evaluated, including pre-analytical and post-analytical stages (i.e. specimen collection, recording and reporting results, and confirmatory testing); also, a review and analysis of trends in quality indicators should always be conducted. Supervision visits are an opportunity to discuss concerns and solve problems, and to mentor staff on troubleshooting.

A schedule for site visits should be drawn up in advance, preferably integrated with other supervision activities. Where there is sufficient capacity, responsibilities for on-site supervision may be decentralized to regional or district staff. All staff conducting supervision visits need appropriate training and should use standardized checklists. Reports should be shared with the testing site and the NRL or NTP, according to local practices.

Failed proficiency testing or out-of-range quality indicators can help to identify testing sites that are performing poorly; those sites should be prioritized for on-site visits. However, proficiency testing and monitoring of quality indicators do not negate the need for on-site supervision. On-site visits are especially critical during the early stages of implementation of a new technology.

Proficiency testing

Proficiency testing is defined as follows:

A programme in which multiple specimens are periodically sent to members of a group of laboratories for analysis and/or identification, in which each laboratory's results are compared with those of other laboratories in the group and/or with an assigned value, and reported to the participating laboratory and others.¹ (5)

Ideally, a proficiency testing programme checks key processes (pre-analytical, analytical and post-analytical) occurring at the testing site. A number of samples are sent to the laboratory or testing site several times per year. Testing is performed as it would be with patient specimens, and results are compared with expected results and across several testing sites. Results are monitored for trends over time. Although proficiency testing does not measure routine laboratory performance, it may identify laboratories with major deficiencies. Proficiency testing is recommended at least once per year, and is an ISO 15189 (2) requirement. Feedback regarding proficiency testing results should be provided in a timely manner to the testing sites and to supervisory staff. Rapid feedback is needed to enable prompt initiation of corrective actions. Although on-site supervision and routine monitoring of quality indicators are the most critical components of QA, proficiency testing helps to identify major nonconformities, allowing supervisors to target the most poorly performing laboratories for on-site supervision.

¹ See <https://webstore.ansi.org/standards/clsi/clsigp27a2>.

Proficiency testing may be used, in conjunction with quality indicator monitoring, where human or financial resources are not sufficient for implementing a regular on-site supervision programme. Proficiency testing panels may also be used to evaluate the performance of technicians after training.

Blinded rechecking

Blinded rechecking is usually applied to AFB smear microscopy; it involves the re-examination of a sample of routine smears at a higher level laboratory. Slides are usually sampled on a quarterly or monthly basis. The technician rechecking the slides does so in a blinded fashion (i.e. not aware of the original diagnostic results) and the percentage of agreement is calculated. Extensive information on establishing a blinded rechecking programme as well as other EQA elements is given in *External quality assessment for AFB smear microscopy*¹ (3).

Blinded rechecking assesses the routine performance of microscopy, and is thus an important component of an EQA programme. However, this rechecking is resource intensive, because it requires sampling slides and re-reading, and many countries face challenges with widescale implementation. Also, collecting the necessary data and providing timely feedback to sites for corrective actions remains challenging in many settings.

NTPs should have data on the following performance indicators, which provide an insight into the participation of laboratories in the network, both regionally and nationally:

- proportion of laboratories participating in the blinded smear rechecking activity;
- proportion of participating laboratories participating in all quarters for a given year; and
- proportion of laboratories with an error rate of less than 5% and no high false errors.

Quality indicator monitoring

Routine monitoring of quality indicators (also known as performance indicators) is a critical element of QA for any diagnostic test; it is also an ISO requirement. All laboratories should collect and analyse testing data at least monthly, using a standardized format. Targets should be set for all indicators monitored, and any unexplained change in quality indicators (e.g. an increase in error rates, a change in the rate of MTB positivity or rifampicin resistance, or a significant change in volume of tests conducted) should be documented and investigated. A standard set of quality indicators should be used for all sites conducting a particular test, to allow for comparison. Quality performance indicators should be reviewed by the laboratory manager and must always be linked to corrective actions if any unexpected results or trends are observed. It is crucial to document the corrective actions and subsequent improvement and normalization of laboratory indicators following the corrective actions taken.

¹ See https://www.aphl.org/AboutAPHL/publications/Documents/External_Quality_Assessment_for_AFB_Smear_Microscopy.pdf.

A system should be in place for centralized reporting of monthly quality indicators to the NRL or NTP. Some of the newer diagnostic tests produce electronic data; such tests include GeneXpert®, BACTEC™ MGIT™, MC-aNAATs, LPAs with automated readers and other mWRDs that use diagnostics connectivity solutions (see Section 2.6). Electronic data allow for real-time remote monitoring of sites within a network, and for easy and accurate stratification of data, as needed for analysis of performance.

The indicators discussed in this section focus on laboratory testing. However, laboratories need to work with clinicians and programme managers to develop and monitor quality indicators that reflect the whole diagnostic process, such as the proportion of patients started on treatment or the turnaround time from the collection of a specimen to treatment initiation. This was discussed in Section 1.4 and is also covered in Section 2.8.

General quality indicators

The set of quality indicators shown in Table 2.1 apply to all technologies; they should be collected, analysed on a monthly basis and disaggregated according to the test. These indicators are provided as a guide – laboratories should review and set locally appropriate targets.

Table 2.1 General quality indicators

Indicator	Target
Number of tests performed, by type of test	–
Service interruptions	No interruptions
Stock-outs	No stock-outs leading to service interruption
Equipment down time	No equipment downtime leading to service interruption
Turnaround time	90% of results meet test-specific turnaround time
Test statistics (quality indicator) report	100% of reports are completed by the defined due date
EQA results	>90% of EQA panels are passed
QC results	>90% of QC results meet expected criteria
Specimen rejection	<1% of specimens are rejected ^a
Customer satisfaction	>80% of surveyed customers are satisfied
Technician productivity	Report average number of tests performed per month per technician

EQA: external quality assessment; QC: quality control.

^a Where resources allow, some laboratories may collect additional secondary indicators, such as volume and quality of sputum specimens. This may be important for certain tests (e.g. >1 mL sputum is required for the Xpert MTB/RIF test). Some laboratories apply specimen rejection criteria related to quality of specimen or to incompletely labelled or leaking specimens.

Test-specific quality indicators

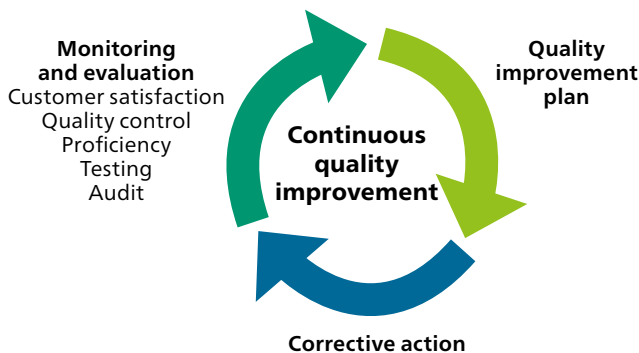
Annex 2 provides recommended quality indicators for each WHO-approved method; the indicators listed in Annex 2 are additional to the general quality indicators listed in Table 2.1. Data for the quality indicators should be collected and analysed monthly. Targets provided in Annex 2 are intended as a guide, and laboratories should determine their own targets. These targets – especially isolation rates – will vary based on factors such as the local situation and the patient population tested. Laboratories should monitor indicators and establish baseline performance and acceptable ranges. The indicators should be reviewed by the laboratory manager; any deviations from baseline or results outside the acceptable range should be investigated and corrective actions taken. Documentation of corrective actions and subsequent improvement and normalization of laboratory indicators following the corrective actions are critical components of quality assurance.

Continuous quality improvement

Quality improvement is a critical but often neglected part of the quality assurance process. Key components of the quality improvement process are the identification of nonconformities through data collection, subsequent data analysis and creative problem-solving. In addition to continual monitoring, continuous quality improvement involves identifying and analysing actual and potential defects. Nonconformities may be identified in many ways, including proficiency testing, reviewing quality indicators, reporting of issues identified by staff members and audits.

The quality improvement cycle is shown in Fig. 2.7. Nonconformities identified during routine testing and QA activities (summarized in Annex 3 for TB tests) should be analysed, then corrective actions should be implemented and the results monitored over time. These four steps should be repeated regularly to ensure continuous improvements in laboratory processes. For many laboratories, this process is difficult to implement in a routine and systematic way, but it is an essential part of implementing quality services. This is a key area in which technical assistance may be required.

Fig. 2.7 The continuous quality improvement cycle



Adapted from WHO LQMS handbook.¹

¹ See <https://www.who.int/publications/i/item/9789241548274>.

It is important to clearly define the procedures for identifying nonconformities, determining responsibility, recalling the results associated with the nonconformities and resuming routine testing following corrective actions. Follow-up actions to prevent the same nonconformity from occurring in the future must also be put in place.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Provide guidance on implementing international best practices for TB laboratory QA
- Provide training and mentoring in establishing QA practices
- Assess QA procedures and practices in individual laboratories, and provide recommendations for improvement
- Review criteria for on-site supervisory visits and assist with planning an on-site supervisory programme to be established in conjunction with other QA activities
- Support an EQA programme for smear microscopy and mWRDs, including establishing processes for provision of timely feedback to sites on performance and corrective actions
- Provide support to establish systems for quality indicator monitoring, to identify nonconformities and implement corrective and preventive actions

References for Section 2.3

(Key resource and suggested reading highlighted in bold font)

- 1 Quality management system: a model for laboratory services (Approved guideline, 4th edition). Wayne, PA: Clinical and Laboratory Standards Institute; 2011 (https://clsi.org/media/1523/qms01a4_sample.pdf).
- 2 ISO 15189. Quality management system implementation: look before you leap. Geneva: International Organization for Standardization; 2015 (https://challenge.tb.org/publications/tools/lab/ISO15189_QMS_Implementation.pdf).
- 3 Ridderhof J, Humes R, Boulahbal F. External quality assessment for AFB smear microscopy (https://www.aphl.org/AboutAPHL/publications/Documents/External_Quality_Assessment_for_AFB_Smear_Microscopy.pdf).
- 4 Practical guide to implementing a quality assurance system for Xpert MTB/RIF testing. Geneva: Global Laboratory Initiative; 2019 (<https://stoptb.org/wg/gli/gat.asp>).
- 5 Using proficiency testing to improve the clinical laboratory: approved guideline, 2nd edition (CLSI document GP27-A2). Pennsylvania, USA: Clinical and Laboratory Standards Institute; 2007 (<https://webstore.ansi.org/standards/clsi/clsigp27a2>).

Additional resource for Section 2.3

Laboratory quality management system: handbook. Geneva: World Health Organization; 2011 (<https://apps.who.int/iris/handle/10665/44665>).

2.4 Implementing a quality management system

This section covers all aspects of a quality management system (QMS), from key activities in a QMS to monitoring and assessment.

2.4.1 Introduction to QMS

A QMS is defined as “coordinated activities to direct and control an organization with regard to quality”. This definition is used by the ISO and the Clinical and Laboratory Standards Institute (CLSI), both of which are internationally recognized laboratory standards organizations. In a QMS, all aspects of laboratory operation, from the organizational structure to the processes and procedures, need to be addressed to ensure quality (Fig.2.8). The whole workflow must be considered, from the patient through to the reporting of results (1). WHO has produced a laboratory QMS training toolkit that can be customized to fit local needs (2).¹ Quality system essentials (QSEs) are a set of coordinated activities that form the building blocks of a QMS (Fig. 2.9). In order to have a functioning QMS, all QSEs must be in place.

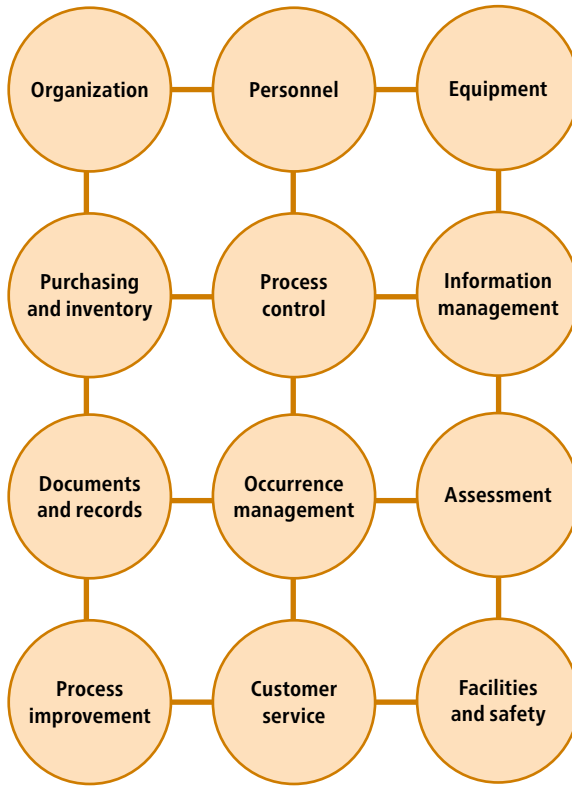
Fig. 2.8 A QMS incorporates pre-examination, examination and post-examination phases of testing



QMS: quality management system.

¹ See <https://extranet.who.int/hslp/content/LQMS-training-toolkit>.

Fig. 2.9 The 12 QSEs



QSE: quality system essential.

2.4.2 Accreditation

Accreditation is defined as a procedure by which an independent, authorized body gives formal recognition that a laboratory is competent to perform specific tasks. Laboratory accreditation recognizes a laboratory's technical capability and is usually specific about the systems, products, components or materials for which the laboratory claims proficiency. Accreditation allows a laboratory to determine whether it is performing its work correctly and according to appropriate standards. This does not guarantee that a given analytical result is correct, but it does establish standards that must be met and a framework within which nonconformities are identified and addressed.

The accreditation of clinical or medical laboratories is achieved by measuring performance against ISO 15189, which addresses the 12 QSEs (3)⁶⁶. Those 12 QSEs are described in *Laboratory quality management system: handbook*¹ (1).

¹ See <https://apps.who.int/iris/handle/10665/44665>.

The accreditation of clinical or medical laboratories is provided by an independent organization that has achieved the standards of ISO 17021 (4) and that is affiliated with or a member of the International Laboratory Accreditation Cooperation.¹ Some of the organizations that provide accreditation to medical laboratories include:

- the College of American Pathologists;²
- the Kenya Accreditation Service;³ and
- the South African National Accreditation System.⁴

Accredited laboratories are recognized as meeting certain quality standards, and having the necessary technical processes and administrative systems in place to ensure high-quality results. A strong laboratory QMS is critical to ensuring the quality of testing. Weak laboratory systems have a direct impact on patient care; for example, laboratory errors may lead to overdiagnosis or underdiagnosis of TB; poor stock management or lack of equipment maintenance systems may lead to interruptions in service; and failure to meet biosafety standards puts laboratory workers, patients and the community at risk of infection. Appropriate turnaround times for results are critical for optimal patient management, while a strong reporting system and referral network ensures that results reach clinicians in time to deliver appropriate care and treatment. Such requirements can only be consistently met by concerted efforts to develop and maintain a QMS within the TB laboratory.

Every NRL should be engaged in implementing a QMS towards national or international accreditation. National laboratory strategic plans should articulate the goals for accreditation of NRLs and regional referral laboratories, where applicable. Working to achieve international accreditation standards is a complex and time-consuming task for any laboratory, especially when starting from a low baseline where resources are limited and staff have limited capacity. If plans to work towards accreditation are to be successful, they need to be realistic and budgeted for appropriately. The first step towards strengthening a country's TB laboratory network is to improve the quality management of the NRL so that it has the capacity to support the other laboratories in the network. Regional referral laboratories should then be targeted for quality improvement initiatives, given that they provide culture and DST services, in addition to supervising peripheral laboratories in their region.

In most resource-limited settings, it is not realistic for peripheral laboratories to meet the quality standards required for international accreditation. However, meeting minimum standards to ensure accurate and reliable testing is still important, and quality improvement plans should be developed, documented and monitored over time to ensure that minimum quality standards can be consistently achieved. GLI has developed an AFB microscopy network accreditation tool⁵ (5) that can be used to assess and improve the quality of the whole laboratory network. Although it applies only to microscopy laboratory networks, the tool can be adapted for use in laboratory

¹ See <https://ilac.org/>.

² See <https://www.cap.org/>.

³ See <https://www.kenas.go.ke/>.

⁴ See <https://www.sanas.co.za/Pages/index.aspx>.

⁵ See <https://stoptb.org/wg/gli/gat.asp>.

networks performing tests other than microscopy, such as Xpert MTB/RIF. The tool has been developed for self-assessment, and it is not currently linked to a formal accreditation programme.

The *Global Plan to Stop TB 2011–2015*¹ (6) noted that less than 5% of national TB reference laboratories worldwide were accredited to international standards, and it identified a target of 50% of NRLs meeting international accreditation standards by 2015; that target was not met. The *Framework of indicators and targets for laboratory strengthening under the End TB Strategy*² (7) included an indicator for accreditation of NRLs, with a target of 100% of NRLs in high TB burden countries being accredited by 2020. Progress is already being made in some resource-limited countries, but in many countries there is limited awareness or implementation of QMS.

Implementing a QMS is a complex process that requires committed laboratory and facility management; appropriate infrastructure, personnel, equipment and supplies; and the establishment of standardized procedures and documentation of all processes. All stages of the diagnostic process must be monitored, from specimen handling to testing and reporting. Good management practices are essential to ensuring that the quality of a laboratory's services remains high and that improvements are made as deficiencies are identified. At the national level, regulations and accreditation programmes that outline standards and guarantee accountability are necessary factors for ensuring that high-quality services are maintained.

2.4.3 Approaches and tools for implementing a QMS

Factors to consider when selecting an approach or tool include what is already being done on QMS in the country, in both TB and non-TB laboratories; which national organizations and people are responsible for the accreditation; and the country's current capacity to support TB laboratories in training and mentoring. Local ownership of the programme will be a critical factor for success, because working towards accreditation is a long process that may outlive any individual person or organization providing support. A laboratory's goal may be formal national or international accreditation, or it may simply be implementing or strengthening a QMS to improve the quality of results without intending to become formally accredited.

There are several frameworks that can be used to help laboratories that are preparing for accreditation, or simply wish to implement or improve their QMS without the ultimate goal of accreditation. This section briefly describes these tools. It may be prudent to decide to follow one overall framework, to avoid confusion. However, because each framework has different strengths and may offer different activities and tools, consultants should be aware of what each package or approach has to offer and should use components from each accordingly. This decision-making process should be led by the country authorities, with technical input from partners and consultants. The guidance document, *ISO 15189 Quality management system implementation: look before you leap: best practice guidance document*³ (8), describes the deployment of QMS in three NRLs in Africa and recommends best practices.

¹ See <https://apps.who.int/iris/handle/10665/44437>.

² See <https://apps.who.int/iris/handle/10665/250307>.

³ See https://challengetb.org/publications/tools/lab/ISO15189_QMS_Implementation.pdf.

2. Key technical areas for guidance

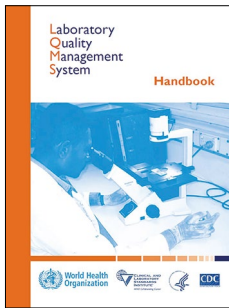
Key resources that can be used to assist TB laboratories in developing and maintaining a QMS include the following publications, discussed below:

- *ISO 15189:2012. Medical laboratories (3)* – which details the requirements for quality and competence;
- WHO's *Laboratory quality management system: handbook (1)* and training toolkit;¹
- the *GLI stepwise process towards TB laboratory accreditation*;²
- WHO's *Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA) (9)*;³
- *Strengthening Laboratory Management Toward Accreditation (SLMTA)*;⁴
- *Strengthening TB Laboratory Quality Management Towards Accreditation (TB SLMTA) (10)*;⁵ and
- the *Score-TB package*⁶ (11).

ISO 15189:2012. Medical laboratories – particular requirements for quality and competence

ISO 15189 (3) is used by medical laboratories in developing their QMSs and assessing their competence. It can also be used by laboratory customers, regulating authorities and accreditation bodies for confirming or recognizing the competence of medical laboratories. ISO 15189 is not intended to be used as the basis for certification of laboratories. ISO standards are copyrighted and should not be reproduced without permission, and they may be difficult for individual laboratories to purchase. The latest version of the standard was issued in 2012 (3).

WHO's *Laboratory quality management system handbook and toolkit*



The handbook (1) and training toolkit⁷ were developed by the WHO Lyon Office for National Epidemic Preparedness and Response, the United States Centers for Disease Control and Prevention (US CDC) – Division of Laboratory Systems and CLSI. They provide an introduction to QMS and are applicable to all medical laboratories.

The toolkit includes a manual and training modules. It is based on US CDC and WHO field experience, and CLSI guidelines for implementation of ISO 15189. Trainers can customize the materials to meet local training needs.

¹ See <https://extranet.who.int/hslp/content/LQMS-training-toolkit>.

² See <https://stoptb.org/gli-guidance-and-tools/gli-stepwise-process-towards-tb-laboratory-accreditation>.

³ See <https://apps.who.int/iris/handle/10665/204423>.

⁴ See <https://slmta.org/>.

⁵ See <https://www.finddx.org/wp-content/uploads/2016/11/TB-SLMTA-flyer-28NOV16.pdf>.

⁶ See <https://stoptb.org/wg/gli/gat.asp>.

⁷ See <https://extranet.who.int/hslp/content/LQMS-training-toolkit>.

GLI stepwise process towards TB laboratory accreditation



The *GLI stepwise process towards TB laboratory accreditation*¹ was originally developed by the Royal Tropical Institute, DATOS and GLI partners as a tool to help NRLs gradually implement all the requirements of a properly functioning QMS in compliance with the ISO 15189:2012 standard. Subsequently, DATOS further developed this into the *GLI stepwise process towards tuberculosis laboratory accreditation* and in 2020 merged the GLI tool with the WHO *Laboratory quality stepwise implementation tool*² (the LQSI tool) to create a tool that offers TB laboratory specific information and document templates, in addition to information and document templates for medical laboratories in general.

The LQSI tool covers technical, managerial and TB-specific requirements. It provides implementation guidance and user-friendly guidelines, roadmaps, checklists and links to support materials to address each requirement of the ISO 15189 standard. Within the tool, the ISO 15189 requirements are translated into specific activities in a TB laboratory context. These activities are grouped along the 12 QSEs as defined by CLSI.

Activities are divided into four phases, with each phase having a specific focus. Laboratories are encouraged to complete one phase before proceeding to the next. However, the tool is constructed so that even if a laboratory does not fully implement a QMS, it will still improve the quality of its services. The four phases of implementation are as follows:

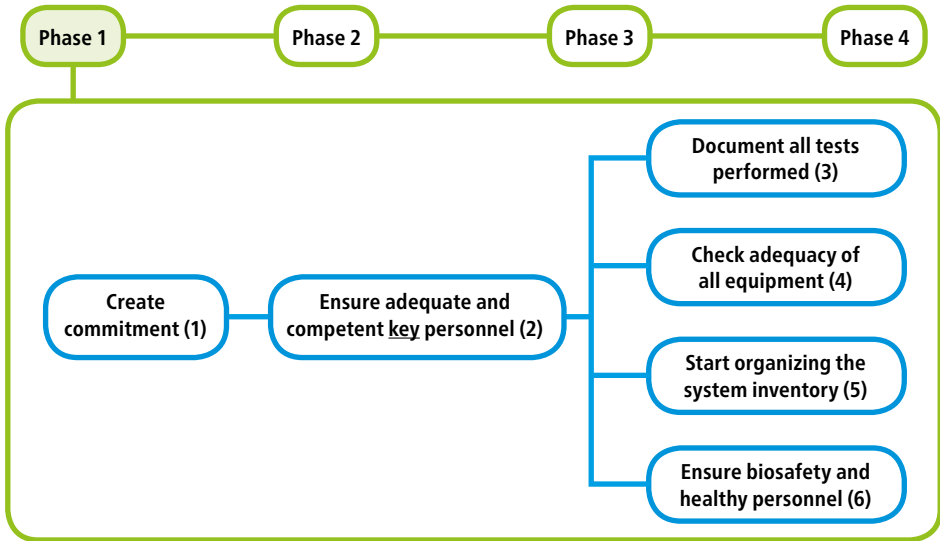
- Phase 1: ensuring that the primary processes of the laboratory are operating correctly and safely. During this phase, the basic elements necessary to enable safe and adequate laboratory practices are established. These are procedures that all laboratories should have in place, regardless of their size or location.
- Phase 2: quality control and assurance, and creating traceability. The fundamentals of the QMS are established (i.e. QC and QA).
- Phase 3: ensuring that the laboratory is properly managed, is well organized and has strong leadership. Effective organizational systems, management practices and leadership are implemented.
- Phase 4: creating continual improvements over time and preparing for accreditation. Systems are implemented that enable passive and active identification of needs for improvement; these are used to optimize the quality of services.

Phase 1 of the process is shown in Fig. 2.10.

¹ See <https://stoptb.org/gli-guidance-and-tools/gli-stepwise-process-towards-tb-laboratory-accreditation>.

² See <https://extranet.who.int/lqsi/>.

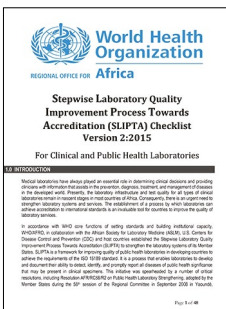
Fig. 2.10 Sample roadmap for the implementation of elements of a QMS – Phase 1: Ensuring that the primary process operates correctly and safely



QMS: quality management system.

A training course (*Introduction to TB laboratory quality management*¹) is available through DATOS to help countries to use this tool. The course may be used in conjunction with other approaches (e.g. SLMTA) or as a technical resource for mentoring by experienced laboratory mentors supporting individual laboratories.

Stepwise Laboratory Quality Improvement Process Towards Accreditation (SLIPTA)



SLIPTA is a monitoring and auditing framework that was originally developed by WHO's Regional Office for Africa (9).² In Africa, a SLIPTA certification programme is administered by the African Society for Laboratory Medicine (ASLM). ASLM is not an accreditation body, and this is a stepwise certification process. SLIPTA is based on ISO 15189:2007 and the CLSI *Quality management system: approved guideline* (12). The checklist was developed to monitor the progress and improvement of a laboratory quality system. It is directly applicable to all laboratory settings and disciplines. SLIPTA is based on the 12 QSEs identified by CLSI, and the assessment

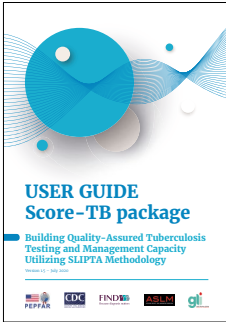
is scored and rated on a scale of 1 to 5 stars. It is considered an indicator of readiness for international accreditation. SLIPTA has been implemented in 160 laboratories in 18 African countries, and many other countries are using SLIPTA as the basis of quality improvement initiatives (with or without the SLMTA programme, see below). ASLM

¹ See <https://datos-advise.nl/what-2/TB%20laboratory%20quality%20management%20training>.

² See <https://apps.who.int/iris/handle/10665/204423>.

has a training programme for certified SLIPTA auditors, and a number of African countries have local capacity for certified SLIPTA audits.

Score-TB package



The Foundation for Innovative New Diagnostics (FIND) has developed a TB harmonized checklist based on the SLIPTA checklists, which incorporates specific elements related to TB laboratory testing (13). Recently, the checklist was updated to include new TB testing methods and incorporated into the *Score-TB package* (11).¹ The Score-TB package combines the TB harmonized checklist, TB test-specific scorecards and the SLIPTA checklist (version 2:2014) in traditional and automated e-tool formats, to facilitate user-friendly quality assessment of modern TB laboratories. The e-tool is user-friendly and reduces the risk of errors by automating the calculation of assessment scores and presenting these in a reporting worksheet, to visualize strengths and weaknesses of a laboratory's QMS (the SLIPTA score) and TB testing methods.

Strengthening laboratory management toward accreditation (SLMTA)



Developed by the US CDC, in collaboration with the American Society for Clinical Pathology (ASCP), the Clinton Health Access Initiative (CHAI) and the WHO Regional Office for Africa, SLMTA is a task-based training and mentoring toolkit that is provided to laboratory personnel in a multi-workshop implementation model.²

The foundation of this programme is a framework that defines the tasks a laboratory must perform to deliver quality laboratory services that support optimal patient care. Training activities are designed to enable laboratory managers to accomplish those tasks using tools and job aids to enhance their management routines. The framework empowers laboratory managers to initiate immediate laboratory improvement measures, even without additional resources.

The framework has two stages: the first stage is a training-of-trainers workshop (10 days), and the second is countrywide implementation. The training can be delivered as three interactive workshops that last 1 week each or as a facility-based approach in which the modules are taught in blocks at each facility. Between each of the workshops or blocks, the trainer or consultant conducts site visits, and the laboratory's staff complete improvement projects. Auditors conduct a baseline and final assessment of the laboratory, and improvement projects are developed based on the findings of the baseline assessment. Baseline and exit assessments are conducted using the SLIPTA checklist to document improvement and the impact of SLMTA.

¹ See <https://stoptb.org/wg/gli/gat.asp>.

² See <https://slmta.org/>.

Strengthening TB Laboratory Quality Management Towards Accreditation (TB SLMTA)



FIND has developed a TB-specific programme, TB SLMTA¹ (10), that incorporates the GLI tool into the SLMTA programme. It includes a TB laboratory QMS towards accreditation harmonized checklist, and specific training modules and tools that meet the differing requirements of TB laboratories – for example, with regard to QA and biosafety. The harmonized checklist incorporates GLI checklist clauses within the SLIPTA checklist, and results in the same SLIPTA score as with the official SLIPTA checklist.

All of the above-mentioned tools help laboratories to meet the requirements of ISO 15189. They can be used individually or in combination.

GLI recommends that the NRL undertake a baseline assessment, using the SLIPTA checklist or the TB harmonized checklist, and develop action plans based on any nonconformities identified. There are several ways to implement quality improvements, depending on the resources and support available. TB laboratories may work through activities provided in the GLI online tool, often with external consultant support. Alternatively, where the SLMTA programme is being implemented in a whole country, TB laboratories may be integrated into that country's general SLMTA programme, and may complement this programme with TB-specific elements from the GLI tool. Several countries are following the specific TB SLMTA programme with or without additional on-site mentoring.

2.4.4 Mentoring

Structured mentoring can accelerate a laboratory's progress towards accreditation. Different models of mentoring have been used, depending on available resources, the availability of mentors and the number of laboratories being supported. The scope of mentoring should be clearly defined, and early engagement of facility and laboratory managers is critical to ensure that the mentor has the necessary authority to conduct the agreed-upon scope of work.

A clear mentoring schedule should be drawn up in advance and agreed to by all parties. Mentoring should always be conducted in a standardized way, with clear action plans and well-delineated responsibilities. Mentors should be experienced and should receive training not only in the technical aspects of assessment and the use of a structured mentoring approach, but also in "soft" skills, such as effective presentation, negotiation and conflict resolution. The mentor's role is to work alongside the laboratory staff and help them to implement the various activities and improvements. Although it may lead to more rapid results in the short term for the mentor to conduct activities on their own, this seldom leads to sustainable improvements and it does not help to foster a sense of ownership by the laboratory staff.

¹ See <https://www.finddx.org/wp-content/uploads/2016/11/TB-SLMTA-flyer-28NOV16.pdf>.

2.4.5 Assessment

Assessment is a process for examining laboratory performance and comparing it with standards, benchmarks or the performance of other laboratories. Assessments can be internal (i.e. performed by the laboratory's own staff) or external (i.e. conducted by a person or group outside the laboratory).

The above-mentioned checklists may be used for QMS assessments. Alternatively, shorter checklists may be used for more frequent assessments or to audit specific technical areas, such as biosafety. A proper audit of a laboratory takes at least 1–2 days; hence, it may be more efficient to use abbreviated checklists for more frequent internal audits. Audit reports, including nonconformities and recommendations for improvement, should be shared with the laboratory manager and staff, and support should be provided for planning how to act on them.

In addition to the checklists mentioned above, other checklists may be in use in the country, such as WHO's *Laboratory assessment tool*¹ (14) or the GLI's TB microscopy network accreditation assessment tool² (5). When providing technical support, consultants may be asked to review local checklists to assess whether they are comprehensive and conform to international standards. Alternatively, countries may wish to customize these standard checklists to make them more directly relevant to their setting.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Advocate within appropriate MoH structures for the need for NRL accreditation and QMS
- Prepare plans and budget for implementing QMS in TB laboratories, and assist in liaising with partners for funding and technical support
- Conduct laboratory QMS assessments, make recommendations for quality improvements and work with laboratory personnel to develop action plans
- Conduct basic QMS training (e.g. using the WHO Laboratory quality management system package)
- Conduct a training and mentoring programme for TB laboratories working towards accreditation
- Conduct training in laboratory assessment (auditing)
- In conjunction with regional bodies, such as ASLM, conduct formal external audits (e.g. WHO SLIPTA) – if appropriately qualified
- Coordinate with other partners providing support to TB laboratories in the country to ensure a harmonized approach
- Provide support for country customization of checklists used for laboratory assessments
- Review local checklists for conformance to international standards

¹ See <https://www.who.int/publications/i/item/WHO-HSE-GCR-LYO-2012.2>.

² See <https://stoptb.org/wg/gli/gat.asp>.

References for Section 2.4

(Key resources and suggested reading highlighted in bold font)

- 1 Laboratory quality management system: handbook. Geneva: World Health Organization; 2011 (<https://apps.who.int/iris/handle/10665/44665>).
- 2 Laboratory quality management system training toolkit [website]. Geneva: World Health Organization; 2021 (<https://extranet.who.int/hslp/content/LQMS-training-toolkit>).
- 3 ISO 15189:2012 Medical laboratories – requirements for quality and competence. Geneva: International Organization for Standardization; 2012 (<https://www.iso.org/standard/56115.html>).
- 4 ISO/IEC 17021-1:2015 Conformity assessment – requirements for bodies providing audit and certification of management systems — Part 1: Requirements. Geneva: International Organization for Standardization; 2015 (<https://www.iso.org/standard/61651.html>).
- 5 TB microscopy network accreditation: an assessment tool. Geneva: Global Laboratory Initiative; 2013 (<https://stoptb.org/wg/gli/gat.asp>).
- 6 The Global Plan to Stop TB 2011–2015: transforming the fight towards elimination of tuberculosis. Geneva: World Health Organization; 2010 (<https://apps.who.int/iris/handle/10665/44437>).
- 7 Framework of indicators and targets for laboratory strengthening under the End TB Strategy. Geneva: World Health Organization; 2016 (<https://apps.who.int/iris/handle/10665/250307>).
- 8 ISO 15189. Quality management system implementation: look before you leap. Geneva: International Organization for Standardization; 2015 (https://challengtb.org/publications/tools/lab/ISO15189_QMS_Implementation.pdf).
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- 10 TB laboratory accreditation. A training & mentoring programme. Geneva: Foundation for Innovative New Diagnostics; 2016 (<https://www.finddx.org/wp-content/uploads/2016/11/TB-SLMTA-flyer-28NOV16.pdf>).
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- 15 Maruta T, Motebang D, Mathabo L, Rotz PJ, Wanyoike J, Peter T. Impact of mentorship on WHO-AFRO strengthening laboratory quality improvement process towards accreditation (SLIPTA). *Afr J Lab Med.* 2012;1(1):6 (<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5644515/>).

2.5 TB laboratory biosafety

2.5.1 Introduction to biosafety

Many countries lack safe working environments in TB laboratories. Even where infrastructure has been upgraded, challenges remain in ensuring adequate servicing and maintenance of safety equipment such as biosafety cabinets (BSCs) and air handling systems, and uninterrupted supply of personal protective equipment (PPE) such as respirators and gloves.

Establishing and maintaining a safe working environment with best practices in a TB laboratory is essential. Administrative, environmental and personal protective controls must be in place to ensure the safety of workers and guarantee quality performance.

The WHO *Tuberculosis laboratory biosafety manual*¹ (1) should be consulted for the latest detailed recommendations for biosafety.

2.5.2 Assessing risk

To understand the level of risk involved in a laboratory, a formal assessment must be performed. A risk assessment is simply a careful examination to determine what in the laboratory's work could cause harm to people within the facility.

The identifiable risks differ according to the methods and activities being performed. In TB laboratories there are three established risk levels (low, moderate and high) for performing different standard procedures required for various testing, as shown in Table 2.2.

Table 2.2 Risk precaution levels associated with laboratory activities and risk assessment for TB laboratories

Risk level of TB laboratory ^a	Laboratory activities	Assessment of risk
Low risk	Direct sputum smear microscopy, processing of specimens for use in nucleic acid tests including Xpert MTB/RIF, Ultra or MTB/XDR; Truenat MTB, MTB Plus or RIF Dx; MC-aNAAT	Low risk of generating infectious aerosols from specimens; low concentration of infectious particles
Moderate risk	Processing and concentration of specimens for smear microscopy, inoculation of primary isolation cultures and use with LPAs	Moderate risk of generating infectious aerosols from specimens; low concentration of infectious particles
High risk	Manipulation of cultures for DST or use in LPAs	High risk of generating infectious aerosols from specimens; high concentration of infectious particles

DST: drug-susceptibility testing; LPA: line-probe assay; MTBC: *Mycobacterium tuberculosis* complex bacteria; TB: tuberculosis; MC-aNAAT: moderate complexity automated nucleic acid amplification test.

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

Source: *Tuberculosis laboratory biosafety manual* (<https://apps.who.int/iris/handle/10665/77949>) (1).

¹ See <https://apps.who.int/iris/handle/10665/77949>.

SL-LPA is similar to FL-LPA, in that direct testing of sputum specimens is considered moderate risk and testing of cultures is considered high risk. Xpert MTB/XDR is similar to Xpert MTB/RIF, in that testing of sputum specimens is considered low risk. Laboratories may consider using additional precautions against airborne transmission because the samples for SL-LPA and Xpert MTB/XDR are from patients with known or presumed MDR/RR-TB. The manipulation of urine for LF-LAM is considered to have minimal risk for the transmission of TB, and universal precautions are recommended for the handling of urine.

Low risk

Low-risk procedures should be performed in an adequately ventilated area or room (i.e. one with unidirectional airflow and 6–12 air changes per hour). If appropriate microbiological techniques are used, testing can be performed on an open laboratory bench or counter. If laboratory ventilation is inadequate, a ventilated work station¹ (2) or a BSC should be used.

Moderate risk

Procedures that have a moderate risk of generating aerosols include processing and concentrating sputum specimens for inoculation onto primary culture media or for performing direct DST (e.g. LPA testing on processed sputum). These procedures must be performed in a BSC because of their inherent risks. A separate laboratory area is required for moderate-risk procedures. The laboratory should have a sink for hand-washing, and adequate ventilation (i.e. unidirectional airflow into the laboratory with 6–12 air changes per hour). Infectious wastes should be sterilized before disposal. Centrifuges used for processing specimens must have sealed buckets that prevent leaks. Work with specimens must be carried out in a BSC of either Class I or Class IIA2.

High risk (TB-containment laboratories)

High-risk procedures must be performed in a TB-containment laboratory. These procedures include manipulating cultures or suspensions of MTBC for identification, indirect DST or molecular assays. Cultures contain large numbers of TB bacilli and they constitute a high level of risk for laboratory staff who manipulate them. Essential features of a high-risk facility include restricted access to essential personnel, a controlled ventilation system providing at least 6–12 air changes per hour and on-site autoclaving for waste management. Further details on requirements are provided in the WHO manual² (2).

2.5.3 Infrastructure

To reach minimum standards of safety for conducting culture and DST, countries often require infrastructure development or upgrades. With the support of donors and partners, many NRLs are undergoing such upgrades. Consultants may be asked to provide guidance on how to design laboratories to ensure safe and efficient

¹ See <https://stoptb.org/wg/gli/assets/documents/vwm4afbsm.pdf>.

² See <https://stoptb.org/wg/gli/assets/documents/vwm4afbsm.pdf>.

workflow. Upgrading infrastructure is a long process, and interim interventions may be conducted to improve the safety of the laboratory staff and the public. Sometimes, simple changes can contribute towards improved safety, before or in the absence of large capital investment, such as re-positioning equipment, dividing rooms and decluttering workstations. Where safety is seriously compromised, the consultant should clearly articulate the safety concerns and proposed solutions in written reports to management, donors and partners, and advocate for immediate action. In some circumstances, where the safety of staff or the public is put at risk, it may be that the only option available is to recommend that testing be interrupted until corrective actions can be implemented.

2.5.4 Personal protection

As with all laboratories, personal protection begins with an individual's understanding of laboratory policies and guidelines on safety, and the use of best practices while working in the laboratory. Additional protection can be obtained through proper use of recommended PPE. The particular types of equipment used in TB laboratories depend on the risks associated with the procedures being performed. For example, gloves and laboratory coats or gowns should be used for any work that involves handling specimens (sputum, blood and body fluids) and other potentially infectious materials (especially waste), manipulating cultures or preparing reagents using hazardous materials. Gowns that open at the back, are seamless in front and have long sleeves with elastic cuffs should be worn in moderate-risk and high-risk laboratories where cultures are being prepared or used for advanced testing. Shoe covers or shoe changes are recommended in entry and exit protocols designed for TB-containment laboratories. Protective eyewear should also be used during procedures where there is the risk of eyes being splashed by hazardous or infectious materials; for example, preparing acid or basic solutions, cleaning glassware that previously contained infectious materials or incinerating waste.

Respiratory equipment, either N95 (US standard) or FFP2 (European standard), may be used to provide additional protection during high-risk procedures that generate aerosols with high concentrations of infectious particles, such as manipulating cultures for identification and DST. Staff required to use respirators must undergo proper fit testing and understand proper procedures for donning and doffing the respirator.¹ Reuse of respirators is not recommended; however, resource limitations mean that some laboratories may implement a reuse policy. If reuse of respirators is necessary, laboratory administrators must ensure adherence to administrative and engineering controls (e.g. properly certified BSCs and adequate ventilation), to limit potential N95 respirator surface contamination. In addition, to reinforce the need to minimize unnecessary contact with the respirator surface, frequent training or the use of posters regarding strict adherence to hand hygiene practices, proper technique for donning and doffing PPE, physical inspection and performing a user seal check should be in place. Unfortunately, it is not possible to determine the maximum number of safe reuses. Safe N95 reuse is affected by variables such as exposure time and atmospheric bacterial load. Protective respiratory equipment is

¹ See <https://www.cdc.gov/niosh/nppt/default.html>.

not a substitute for a poorly functioning BSC or an uncertified BSC. In all cases, the use of good microbiological technique is essential to prevent aerosol production and minimize the risk of laboratory-acquired infections.

2.5.5 Emergency preparedness and response

Safety procedures must include emergency preparedness and response. Staff must be trained in and must 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 drivers transporting specimens, clerks and other support staff – need biosafety training.

2.5.6 Occupational health

The goal of occupational health programmes is to provide a safe workplace. For TB laboratories, such programmes include taking measures to minimize employees' risk of exposure to infectious aerosols and other materials, ensuring that employees know the signs and symptoms of TB, and ensuring that competent medical diagnosis and treatment are available if laboratory-acquired infections occur.

A medical evaluation should be undertaken before people are employed, to ascertain both the risk level and baseline of health for each staff member. Additional health surveillance strategies should be implemented to monitor staff on a regular basis. Strategies may include personal consultations with staff regarding their current health status or the use of medical surveys. If possible and applicable, regular follow-up with available diagnostic tests (e.g. X-ray and TST) can be implemented. A mechanism for occupational health surveillance is recommended, to provide a supportive working environment for staff, to ensure the health of staff, and to promote retention of well-trained staff.

2.5.7 Waste management

The appropriate management of laboratory waste is important to ensure safety for laboratory personnel, prevent contamination of the environment and eliminate the risk of exposing the community to harmful materials. Waste management procedures must comply with all pertinent local and national requirements and regulations; however, in some countries, such regulations may be non-existent or ill-defined.

In many countries there are limited options for waste disposal, especially for sharps (e.g. lancets, blades, syringes and hypodermic needles), broken glass (e.g. Pasteur pipettes and contaminated vials) or hazardous chemicals. Resources for proper

disinfection or sterilization procedures are often limited, especially in remote areas. Consequently, burial or open-pit burning practices are still widely used. These practices are problematic because they often result in incomplete disinfection or destruction of the waste; also, they produce emissions that contribute to local air pollution. In extremely poor settings, materials from these sites may be scavenged by locals and sold to buyers who wash, repack and recirculate items for reuse without proper sterilization. These dangerous practices lead to the transmission of infectious diseases and are extremely problematic for public health programmes. It is the responsibility of the consultant to educate and train national programme officials and laboratory personnel on how best to manage waste given the constraints of the local setting.

TB laboratories can produce various types of waste, from noninfectious general waste to hazardous chemical or biological infectious materials.

Infectious waste is all waste that has been in contact with infectious materials. This includes infected body tissues or fluids, used needles, PPE used in protocols handling infectious materials, and any instruments or consumables that have come in contact with infectious materials that cannot be sterilized and recycled. The overriding principle in minimizing risks from infectious waste is to decontaminate, sterilize by autoclave or incinerate all items.

Chemical waste in TB laboratories refers to reagents and solutions used for various protocols such as specimen processing, microscopy, media preparation and decontamination. Sometimes, chemical waste may need additional segregation, depending on the type or category. Chemical waste should be neutralized (in the case of an acid or base) or sent to a collection facility that has the appropriate knowledge and training on disposal (for organic solvents).

Noninfectious general waste comprises basic materials that can be disposed of in the general waste stream of the facility's trash; examples of such waste are paper, boxes and containers.

All waste must be segregated according to category into appropriate disposable bags or containers with proper markings and disposed of using appropriate protocols. Again, burial and open-pit burning should be discouraged, and the consultant should assist with training on proper methods of decontamination and sterilization. At lower level facilities in rural areas, access to equipment or disinfectants essential for proper disposal may be limited; thus, these methods may be the only options. In such cases, it is important to assist national programmes with designing waste segregation and pick-up strategies, encourage programmes to provide proper decontaminating agents, or have the programmes construct small incinerators to facilitate proper waste management practices.

In extreme situations, consultants may find that programmes are recycling materials. Some materials (e.g. glassware, instruments and laboratory clothing) can be reused or recycled after proper sterilization. However, sometimes there are attempts to recycle other items (e.g. microscope slides and sputum cups) by boiling and washing them. These items should never be reused; thus, it is the responsibility of the consultant to address these situations and educate both laboratory and programme officials regarding the problems associated with reusing these materials.

Often, cost and sustainability are the primary limitations to implementing proper waste management practices. Provision of expensive equipment such as sterilizers or the construction of incinerators for each facility may not be possible within the current programme budget. Under such circumstances, strategies for waste pick-up and transportation to larger waste management facilities may be an option. Having centralized facilities at regional or provincial levels with larger incinerators to handle the increased demand and volume should be considered. In this scenario, each laboratory would have waste accumulation and holding areas with restricted access and regular pickups would be scheduled. Depending on the local terrain and available infrastructure, this may or may not be more cost-effective for the programme. Consultants and laboratory managers should continue to encourage improved waste management.

As previously stated, it is important for programmes to establish policies, guidelines and protocols at the national level for laboratory waste disposal. In countries where such measures are not in place, it is recommended that the consultant assist and help direct these developments by working alongside national officials, providing the necessary resources on internationally recommended guidelines and devising education and training programmes. Implementing waste management programmes for TB laboratories is an essential step in providing the QMSs that are essential for accreditation. Thus, proper guidance on appropriate methods, writing guidelines and waste management documentation is often the responsibility of the consultant and is essential for laboratories and networks seeking to acquire official ISO 15189 accreditation.

Specific information on waste management in TB laboratories can also be found in the WHO *Tuberculosis laboratory biosafety manual*¹ (1). Local and international partners may provide biosafety training courses.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Review working practices within the laboratory and advise on improvement in safety
- Offer training on biosafety for laboratories at all levels
- Develop biosafety guidelines for TB laboratories
- Perform a risk assessment
- Develop guidelines for waste management practices and establish such practices
- Assist with SOP development
- Assist with development of an occupational health programme
- Assist with laboratory design and workflow for safe operations
- Assist with developing an emergency preparedness plan

¹ See <https://apps.who.int/iris/handle/10665/77949>.

References for Section 2.5

(Key resource and suggested reading highlighted in bold font)

- 1 Tuberculosis laboratory biosafety manual (WHO/HTM/TB/2012.11). Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/handle/10665/77949>).
- 2 Ventilated workstation manual for AFB smear microscopy. Geneva: Stop TB Partnership; 2009 (<https://stoptb.org/wg/gli/gat.asp>).

Additional resources for Section 2.5

GLI TB laboratory safety handbook. Geneva: World Health Organization; 2019 (<https://stoptb.org/wg/gli/gat.asp>).

Biosafety in microbiological and biomedical laboratories, 5th ed. Atlanta: United States Centers for Disease Control and Prevention; 2009 (https://www.cdc.gov/labs/pdf/CDC-Biosafety_microbiologicalBiomedicalLaboratories-2009-P.pdf).

2.6 Implementing systems to manage laboratory data

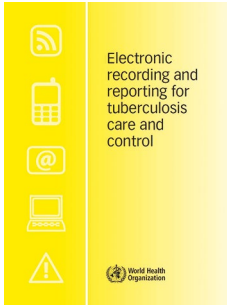
All laboratories need a system for managing their data, be it manual or electronic. In recent years, there has been increased interest and progress in implementing electronic data management systems, particularly in reference and referral laboratories. However, the norm in peripheral laboratories in many countries remains a manual recording and reporting system.

A laboratory information management system (LIMS) – also known as a laboratory information system (LIS) or laboratory management system (LMS) – whether paper-based or electronic, usually includes the following features:

- requisition, receipt and scheduling of tests;
- collection and management of samples, including chain of custody;
- reporting of test results to clinicians;
- other reporting, such as billing;
- workload statistics and laboratory performance;
- QC and EQA processes; and
- inventory management.

Additional functionalities may include audit management, a barcode reader, instrument calibration and maintenance, and time tracking to calculate laboratory turnaround times.

Electronic data management has several benefits over paper-based reporting, including improved data quality (e.g. by highlighting values that are outside the normal limits); decreased workload (by removing duplicate data entry); better access to data, data analysis and reporting; flexibility to modify reporting format; and ability to link multiple test results performed on a single patient. LIMS may be open-source applications or proprietary products, or they may be developed by individual developers for a particular laboratory. Open-source applications are more easily integrated with other electronic databases (e.g. electronic TB registers and electronic medical records), allowing laboratory data to be loaded directly into the application. Proprietary products may deliver the required set of features and may include



support for set-up and maintenance; however, changing or adding features once the system is installed requires additional fees that may be difficult for some laboratories once initial partner support for the installation expires. Self-developed or open-source applications require sufficient local information technology (IT) support to ensure upgradability and sustainability, and to make subsequent revisions to the system. Additional information can be found in the WHO publication *Electronic recording and reporting for tuberculosis care and control*¹ (1).

LIMS may be implemented within an individual facility; alternatively, a number of sites in a country may be networked. Several partners will be involved in improving the networking capabilities of laboratory networks.

2.6.1 Diagnostics connectivity

By taking advantage of opportunities to connect diagnostic test devices that produce results in a digital format – such as Xpert MTB/RIF, liquid culture (e.g. MGIT) and LPAs with automated readers – electronic data can be transmitted reliably to various users and can provide a highly cost-effective way to ensure that a diagnostic device network is functioning properly. The adoption of diagnostics connectivity solutions will be monitored as a core indicator for laboratory strengthening under the End TB Strategy.

Diagnostics connectivity solutions typically comprise three things: a connectable diagnostic device that produces electronic data; a software platform that receives and interprets data; and a means to transmit data from the device to the software platform and to a server. Systems have been developed by Cepheid, United States of America (C360), SystemOne (GxAlert™/Aspect™), Savics (DataToCare™) and FIND (Connected Diagnostics Platform). Importantly, the developers of these systems are collaborating to ensure their compatibility. The software can usually be configured so that subsets of data can be made available, securely, to those that need access to it. Security protocols also protect the privacy of the patient.

Key features of the systems are the ability to monitor performance remotely, conduct QA and manage inventory. With remote monitoring, designated individuals can use any internet-enabled computer to access the software platform, providing them with an overview of the facilities, devices and commodities in their network. For example, the head of an NRL or other authority can easily see how many tests are being performed and where; what the results are; and which sites are underperforming or experiencing abnormal results or errors (which may highlight a need for troubleshooting, device repairs, targeted on-site supervision or retraining of technicians). Software can track consumption and inventory to avoid stock-outs and expiring cartridges; potentially, it can also identify commodity lots or specific instruments with poor performance and abnormal error rates for QA purposes.

¹ See <https://apps.who.int/iris/handle/10665/44840>.

Test results can be sent to the NTPs as real-time data to assist with surveillance of trends on disease or resistance patterns, enhance the capacity of NTPs to generate performance indicators and provide the data needed for some of the top 10 indicators of the End TB Strategy. Another key feature of the system is to send results automatically to clinicians and to LIMS and electronic registers. Section 2.8 has more detail on this.

Whether laboratories are using paper-based or electronic data management systems, it is important for countries to have standardized recording and reporting formats, and to use a standard set of quality indicators to measure laboratory performance. Also, laboratory information needs to be integrated into data management systems used by the NTP.

The Association of Public Health Laboratories (APHL) has developed a series of documents to guide countries in the selection and implementation of an LIMS, including a guidebook¹ (2) for implementing an LIMS in resource-poor settings, a detailed toolkit for implementation and a software provider report.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- **Provide guidance to laboratories to strengthen and standardize paper-based and electronic LIMS**
- **Assist laboratories to implement paper-based and electronic LIMS**
- **Provide guidance on addition of new features or upgrades to an existing LIMS**
- **Provide guidance on diagnostic connectivity solutions**
- **Advise on integration of LIMS data into national data management systems, including electronic databases (e.g. electronic TB registers)**

References for Section 2.6

(Key resource and suggested reading highlighted in bold font)

- 1 **Electronic recording and reporting for tuberculosis care and control (WHO/HTM/TB/2011.22).** Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/handle/10665/44840>).
- 2 **Laboratory information systems project management: a guidebook for international implementations.** Silver Spring, Maryland: Association of Public Health Laboratories; 2019 (<https://www.aphl.org/aboutAPHL/publications/Documents/GH-2019May-LIS-Guidebook-web.pdf>).

Additional resource for Section 2.6

GLI quick guide to TB diagnostics connectivity solutions. Geneva: Global Laboratory Initiative; 2016 (<https://stoptb.org/wg/gli/gat.asp>).

¹ See <https://www.aphl.org/aboutAPHL/publications/Documents/GH-2019May-LIS-Guidebook-web.pdf>.

2.7 Human resources

A major challenge for TB programmes in resource-limited settings is developing and retaining well-trained laboratory staff. Qualified personnel who have extensive training, experience and advanced technical skills often take positions in the private sector or in other countries where they can earn higher salaries than in the public health sector. As a result, public sector laboratories have high staff turnover. In some places, personnel are rotated from laboratory to laboratory on a systematic basis, to cover staffing shortages. Although this may seem practical, rotation policies make it difficult to achieve consistent and reliable routine testing because newly cycled staff need training on the laboratory's methods and technologies. Under these circumstances, laboratories cannot establish the proper level of proficiency to provide continuous quality testing. Finally, as laboratories receive more MDR-TB and XDR-TB samples, awareness of personal risk is rising, which may mean that staff prefer to work in other laboratory areas.

The issue of insufficient staffing is serious and will only be resolved if government health programmes provide better wages, well-defined paths for career progression and safe working environments. Proper planning for building human resource capacity should be included in a country's NSP. Consultants involved in strategic plan development should encourage programmes to improve human resource capacity and implement strategies to retain technically qualified staff. Without sufficient laboratory staff who are adequately trained, motivated, skilled, readily available, well distributed and supported, national TB control targets will not be met.

2.7.1 Human resource capacity and development

As networks are built or expanded and technical capacity increased, human resources should also be expanded. When a laboratory is being developed or technologies are being implemented, it is important to assess the human resource situation and the current and predicted workload. As noted in Section 1.2.6, there are limitations on daily workload for laboratory testing. For example, it is recommended that microscopists performing Ziehl–Neelsen staining read only 25 smears per day. In laboratories where multiple methods are performed, staff may be assigned to one type of test, or they may perform parts of different test procedures (e.g. decontamination for culture and reading of smears). Alternatively, technicians can perform a variety of tasks throughout the day or week. Having a routine schedule of activities with defined roles and responsibilities for staff will improve the quality and efficiency of the work. To have efficient testing and reach recommended turnaround times, laboratories must have enough staff to perform the work. Laboratories also need support staff for preparing materials for testing, waste management, housekeeping and facilities maintenance, and data recording and reporting. Proper time management is crucial to timely results.

Following a proper assessment of the human resources situation, areas of weakness can be addressed, such as the absence of consistent routine training programmes or the need for systems to manage human resources.

2.7.2 Training programmes

For many countries, laboratory services at all levels are limited by a human resources crisis. At the peripheral level, the shortage of laboratory technicians forces countries to train a new cadre of individuals with little or no formal education. For AFB microscopy and even Xpert MTB/RIF testing, individuals with no formal background are often trained “on the job”. In these scenarios, training programmes must be well thought out and must include competency assessment at the end of training, supported by some combination of regular review of quality indicators (broken down by operator), routine supervision and proficiency testing to monitor performance.

In other settings, formal training for laboratory technicians (or technologists) may require a 2- or 3-year certification, diploma or university degree. With the increased focus on skills for culture methods and DST, more attention needs to be given to the curriculum and requirements of laboratory technology, to ensure that graduates have the competencies required for increasingly specialized work.

One major human resource deficiency is the lack of programmes for laboratory managers and leaders. Management of laboratory personnel requires highly skilled laboratory scientists who understand the complexity and details associated with each testing platform and with quality systems, while also having the skills to manage people. In many high-resource countries, a laboratory director requires a doctorate; however, in many resource-limited countries, most laboratory managers, even at the national level, do not have a graduate degree or any specific management training. In addition, many postgraduate qualifications focus on research, with little or no training in laboratory management. Laboratory management and network management are underestimated capacities that require mentoring and training, to develop the next generation of leaders who will implement new technologies and programmes. Therefore, it is important to facilitate technical assistance in a manner that will transfer knowledge and build internal national capacity, to allow programmes to gain independence and become sustainable. Some organizations and institutions offer postgraduate training opportunities and in-service training focused on laboratory management.

It may be useful to assess and review current country level training programmes for laboratory staff and managers. The process should include assessment of the availability and quality of training provided, procedures for the evaluation of competency and proficiency, and refresher training. Proper documentation of trainings conducted and feedback from participants are also important.

A primary responsibility of management is to maintain and upgrade staff training programmes as new staff are hired and testing programmes are expanded. Consultants may be required to assist with building effective and routine mechanisms for training, to ensure continuity of testing and maintain a high level of performance.

When offering training, a consultant should help the laboratory or programme to incorporate the training into their current system, to encourage knowledge transfer and internal capacity-building. Often, this is performed by training-of-trainers programmes that specifically train a cadre of personnel to lead the implementation

and training for new technologies or methods throughout the network. Training curriculum development is a critical component of a consultant's work; it should involve country officials and designated NRL staff to guide the development according to the country situation, programme strategies or national guidelines. The primary purpose of training from an external consultant is to build capacity and provide modes for sustainability.

It is essential that appropriate individuals are selected to attend training courses. Technical staff who will perform tests should be trained on new techniques, whereas staff who will oversee or supervise implementation may be selected to attend programmatic level trainings or workshops. Those organizing training courses should work closely with laboratory or facility management and programme managers to ensure that the purpose of the training is well communicated, and that staff can be appropriately selected. Various factors need to be considered when planning trainings, including location (on-site versus regional or central trainings), transportation costs, accommodation and per diems, facilitators (local and external), and the content and format of the training.

2.7.3 Roles and responsibilities

Laboratories should have competency-based job descriptions for each position within the laboratory, encompassing specific requirements for education, experience, theoretical and practical background, and demonstrated skills. In addition, each individual should have a personal job description that outlines work activities, employer expectations, how competency will be assessed and in-service training requirements. Establishing clear roles and responsibilities for all staff members alleviates confusion and promotes a systematic strategy for daily work.

2.7.4 Leveraging resources

In countries with a weak TB laboratory network, it is critical to optimize all available technical resources. All countries should cast a wide net to identify the best technical base possible, both inside and outside their NTP and MoH. For TB diagnostics and related clinical services, expertise can be greatly increased by collaborating with national academic and research laboratories in both the public and private sectors. The nature and scope of such partnerships must be established from the beginning, with formally defined roles and responsibilities. It is also important to establish links with international laboratory networks. Ideally, each NRL should be connected to a WHO SRL, from which it receives training and to which the NRL is accountable in terms of technical proficiency.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Assist with programmes that enhance human resource capacity-building
- Assist with training programmes for laboratory technicians on methods and protocols
- Assess competency and proficiency of staff
- Help to establish a guideline for human resource development for laboratories
- Assist with the development of incentive programmes

Resources for Section 2.7

Assessment of human resources and time needed to implement the DOTS strategy for TB control in health facilities: survey instrument and guide to implementation. Geneva: World Health Organization; 2008 (<https://apps.who.int/iris/handle/10665/69774>).

Planning the development of human resources for health for implementation of the Stop TB Strategy: a handbook. Geneva: World Health Organization; 2009 (<https://apps.who.int/iris/handle/10665/44051>).

Bergström, Karin, Tuberculosis Coalition for Technical Assistance. Task Force Training. Checklist for review of the human resource development component of national plans to control tuberculosis. Geneva: World Health Organization; 2005 (<https://apps.who.int/iris/handle/10665/69050>).

2.8 Linking laboratory services to TB care and treatment

A laboratory test is just one part of the diagnostic cascade that starts with a clinician evaluating a patient and ordering a test, and continues through the receipt and interpretation of the results and initiation of appropriate TB treatment. Delays in any of the steps—from specimen collection to transport to the laboratory, to the laboratory testing to reporting the results back to the clinician, to the clinician receiving and acting on the laboratory results—can reduce the clinical and public health impact of a laboratory test.

Too often, improvements in laboratory services or the introduction of a new laboratory test focuses only on improving testing in the laboratory (i.e. the examination phase). Efforts must also be made to improve the pre-examination (i.e. pre-analytic) and post-examination (i.e. post-analytic) phases of testing (see Fig. 2.8 in Section 2.4.1). Furthermore, the clinical–laboratory interface must be strengthened to improve the linkage to care. Laboratory staff be trained in conducting the entire diagnostic testing process; in addition, health care workers must be trained in selecting people to be tested, ordering the most appropriate tests, collecting and shipping samples to the laboratory, and receiving, interpreting and acting upon results.

Effectively addressing the gaps in the diagnostic cascade requires a comprehensive approach that includes identifying the gaps in the diagnostic process; systematic evaluation of technologies, diagnostic networks, test quality, linkage to care-related challenges, and barriers to patient and programmatic impact; and development and

introduction of innovative solutions and models to address barriers and reduce TB-associated morbidity and mortality.

To strengthen the entire diagnostic cascade, a systems approach should be used that identifies gaps in the diagnostic cascade, emphasizes access to quality-assured laboratory services, and uses quality management principles to ensure prompt and reliable flow of specimens and information. A systems approach can dramatically reduce the time from ordering a test to making a treatment decision; it can also increase access to laboratory services for all patients.

Clinical and public health impact is increased when timely and accurate diagnosis of TB is quickly followed by appropriate, quality treatment and care – the diagnosis alone will not cure the patient, nor will it prevent further transmission of TB within the community.

The steps necessary for linking diagnosis and treatment include:

- reporting the results back to the health care professional who ordered the test and (in some countries) to the patient – because TB laboratory testing is not usually completed at the point of care while the person being evaluated for TB waits, the laboratory needs to ensure that a mechanism is in place to report the results to the required recipients:
 - in some cases, the mechanism will be a paper form that is transported back to the provider; in other cases, it will be a telephone call or text message;
 - LIMS and electronic reporting systems can facilitate reporting of results;
 - the laboratory should ensure that the results are actually received by the intended recipient;
- immediately reporting positive TB results to the health care professional who ordered the test;
- notifying positive TB results to the appropriate TB programme staff or office;
- registering the person with positive TB results for treatment;
- having the person with positive TB results begin appropriate treatment;
- completing additional laboratory tests at the initial laboratory, or sending a portion of a specimen or a second specimen to another laboratory for confirmatory testing, DST or other testing when indicated; and
- monitoring the treatment response through routine collection and testing of patient specimens as per the national guidelines, and timely reporting of results back to the provider.

There are separate registers at the laboratory and treatment site, which should be reviewed regularly to ensure that people with positive TB results are registered for and started on treatment. Treatment registers should be reviewed to ensure that follow-up laboratory testing is completed and recorded to monitor the treatment response.

The diagnostics connectivity solutions described in Section 2.6 can facilitate the automatic transmission of electronic data and can improve linkage to care and patient management. For example, test results can be automatically integrated into LIMS or electronic registers, reducing staff time and the chance of transcription errors, and greatly facilitating monitoring and evaluation processes. A text message could be sent to a patient informing them when their test results are ready and instructing them to visit the clinician to receive the results; this may reduce loss to follow-up. As soon as test results become available they can automatically and instantly be sent to a clinician's phone or email, SMS printer or other clinical results reporting mechanism, allowing faster patient follow-up and improving linkage to care. Access to specialty care (e.g. MDR-TB treatment) can be facilitated by including automatic transmission of the detection of a patient with RR-TB to the local MDR-TB treatment focal point.

Coordination between the TB laboratory services and the NTP and treatment facilities is essential at all levels, to ensure that all diagnosed cases are treated, and all treated cases are bacteriologically monitored to confirm they are cured. Coordination can be monitored through routine reporting, routine meetings or other communication between the laboratories and the programme.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Support sensitization of clinicians to new diagnostic tools, the importance of referral of specimens for testing and the interpretation of results
- Participate in joint laboratory–clinical planning and review meetings
- Participate in revisions to laboratory and TB registers for new diagnostics
- Support assessment and improvement of the diagnostic cascade

Resources for Section 2.8

International standards for tuberculosis care, 3rd edition. The Hague: TB CARE I; 2014 (https://www.currytbcenter.ucsf.edu/sites/default/files/istc_3rded.pdf).

Shinnick TM, Iademarco MF, Ridderhof JC. National plan for reliable tuberculosis laboratory services using a systems approach. Recommendations from CDC and the Association of Public Health Laboratories Task Force on Tuberculosis Laboratory Services. *MMWR Recomm Rep*. 2005;54(Rr-6):1–12 (<https://pubmed.ncbi.nlm.nih.gov/15829862/>).

GLI quick guide to TB diagnostics connectivity solutions. Geneva: Global Laboratory Initiative; 2016 (<https://stoptb.org/wg/gli/gat.asp>).

2.9 Strengthening the role of private laboratories in NTPs

Private sector laboratories play an important role in TB services in many countries. People with signs and symptoms of TB often first access diagnostic services in the private sector. Also, private laboratories are often better resourced (with more funding and staff) and may have testing capacity that exceeds that of the public sector laboratory network. It is therefore critical that private sector TB laboratory services be linked to the NTP and the NRL at several points in the diagnostic and treatment path. The nature of such collaborations will be agreed on between the NTP and private laboratories, but may include the following areas:

- **TB diagnostic reporting and treatment follow-up:** Private sector laboratories should be required to report results that identify new TB patients and DST results to the NTP. In turn, NTPs should make national laboratory request forms and registries available to private laboratories and be part of the referral and feedback mechanisms, to ensure that all TB cases are promptly registered with the NTP and linked to appropriate treatment.
- **TB laboratory testing:** Private sector laboratories should be advised to follow WHO laboratory policies and use WHO-recommended tests. For example, they should be encouraged NOT to use serological methods or IGRAs to diagnose active TB. Also, private laboratories should adhere to WHO-recommended and internationally recommended biosafety policies and procedures. When available, established specimen transportation and referral mechanisms used by the NTP should also be accessible to private laboratories.
- **Training and supervision:** Private sector laboratories should be included in national training workshops and should be provided with NRL-developed SOPs and other guidelines. Private laboratories should also be included in national and subnational supervision schedules, and have in place mechanisms for performance monitoring and feedback.
- **Supply management and equipment validation and maintenance:** Where necessary and possible, private laboratories should have access to quality-assured reagents and supplies, either free from the NTP or through access to approved procurement agents and distributors. Private laboratories should also benefit from equipment validation and maintenance agreements for TB diagnostic instruments that are recommended by the NTP or NRL.
- **QA and management:** Private laboratories should be required to participate in an EQA programme, which may include site visits, panel proficiency testing and blinded rechecking of results.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Advise on engagement of private sector laboratories with the NTP
- Participate in planning and implementation of projects aimed at engaging private laboratories in improving quality of services (e.g. through enrolling laboratories in an EQA programme)
- Advise on engagement with and coordination of private providers towards meeting NTP goals

Resources for Section 2.9

International standards for tuberculosis care, 3rd edition. The Hague: TB CARE I; 2014 (https://www.currytbcenter.ucsf.edu/sites/default/files/istc_3rded.pdf).

Uplekar M, Lönnroth K. Engaging all health care providers in TB control: guidance on implementing public–private mix approaches. Geneva: World Health Organization; 2006 (<https://apps.who.int/iris/handle/10665/69240>).

A practical handbook for national TB laboratory strategic plan development. The Hague: TB CARE I; 2014 (https://stoptb.org/wg/gli/assets/documents/Lab_Strategic_Handbook.pdf).

2.10 Strategic planning for national TB laboratory networks

2.10.1 Laboratory strategic planning

It is important for national TB laboratory services to look at future needs for diagnosis and patient monitoring, to develop goals and long-term plans to improve quality, build capacity and expand services. Therefore, NTPs and MoHs should work together with NRLs to devise a long-term strategy with a supportive budget. As the need for diagnosing and managing drug resistance increases, the demand for services will climb. At present, most networks have limited capacity to ensure quality DST and they face challenges in providing access to services for all those in need.

NRLs and networks are critical to ensuring that patients receive appropriate diagnosis, care and treatment. They must also conduct routine surveillance activities to assess changes in the epidemic and measure the impact of the NTP on national public health. Therefore, these laboratories need to have a strategic plan that will ensure the delivery of high-quality services. A strategic plan describes an organization's direction and outlines the activities that need to be undertaken to successfully implement the plan over a fixed period. In a dynamic environment where planning for the future is difficult, 2-year to 3-year plans have been recommended. Traditionally, strategic plans are written for a 5-year period, synchronized with current funding mechanisms, recognizing that needs may change with fluctuations in the epidemic or with the implementation of new technologies that may become essential to TB control. The strategic plan considers current and future influences, both internal and external, that may affect the laboratory's activities.

The importance of having strategic plans for laboratories was emphasized in the Maputo declaration on strengthening of laboratory systems (1), which recommended that a strategic plan for national laboratories be part of national health plans.

Specifically, the Maputo declaration “call[s] on national governments with support of their donors and partners in resource-limited settings to develop national strategic laboratory plans that integrate laboratory support for the major diseases of public health importance including HIV, tuberculosis, and malaria”.

Additionally, the Global Fund considers strategic planning to be an integral component of effective TB control. Developing an NSP for the NTP is considered fundamental to the effective organization and management of TB care and control activities. Consequently, success in obtaining funding may depend on having a strategic plan for the NRL and its associated network. To assist countries in developing or improving their NSPs, WHO’s Global TB Programme is developing a framework of key components that can be used to guide countries in creating or improving their strategic plans¹ (2).

GLI partners have developed and endorsed the publication *A practical handbook for national TB laboratory strategic plan development*² (3), which provides important information and guidance on the steps necessary to write a complete and comprehensive plan with a projected budget (See Fig.2.11). Laboratory strategic plans allow the NTP to earmark funding for projected laboratory activities and developments in Global Fund funding proposals.

2.10.2 Laboratory strategic plan development

When assisting an NRL to create or improve its strategic plan, it is best to first determine whether a laboratory strategic plan is already in place under either the NTP or the MoH’s programme for national laboratory services. This helps to ensure that all plans are integrated with one another and are complementary rather than overlapping.

A starting point for creating a strategic plan is to formulate a vision statement. This statement is used to define the role of the NRL and TB laboratory services. The vision statement is followed by a mission statement, which more specifically describes the roles and activities of the laboratory and its network, and identifies the customers.

Basic steps involved in laboratory strategic plan designs are:

- define a vision and mission;
- perform a situational analysis;
- identify desired outcomes;
- prioritize strategy and activities;
- identify indicators and targets;
- establish a monitoring platform; and
- outline a workplan and budget.

An initial step in creating a relevant strategic plan for a laboratory is to perform a gap analysis, to compare the laboratory’s current performance with the desired performance. A gap analysis can be conducted in steps, to determine which resources

¹ See <https://apps.who.int/iris/rest/bitstreams/872635/retrieve>.

² See https://stoptb.org/wg/gli/assets/documents/Lab_Strategic_Handbook.pdf.

are needed to develop a national network of TB laboratories that will provide diagnostic testing services.

Before performing a gap analysis, an assessment must be conducted using the national testing algorithm, to determine which laboratory services are needed. The assessment should also evaluate which elements of the national laboratory network should be improved or created. Once the assessment is complete, the steps required to undertake a gap analysis are as follows:

- using the plan for the laboratory network, determine the number, location and type (or level) of existing laboratories and whether additional laboratories are needed;
- determine the number and location of laboratory personnel in each job classification, and whether and how many additional personnel are needed;
- learn which, if any, funds are available for additional employees (including salaries, benefits and training), supplies, equipment, and designing and building new laboratories, if relevant; and
- determine the feasibility of obtaining administrative authorization for adding new employees or contract workers if funding can be made available.

A gap analysis is most often performed using a SWOT analysis (i.e. analysing strengths, weaknesses, opportunities and threats) – an analytical tool that helps to identify internal factors (i.e. strengths and weaknesses) and external factors (i.e. opportunities and threats) relevant to the laboratory. Once factors that may affect a laboratory's performance have been identified in the SWOT analysis, they can be used to outline goals and objectives for the strategic plan and activities for the operational plan.

Once the current situation has been assessed, specific outcomes or objectives required to achieve the overall goals can be set; for example:

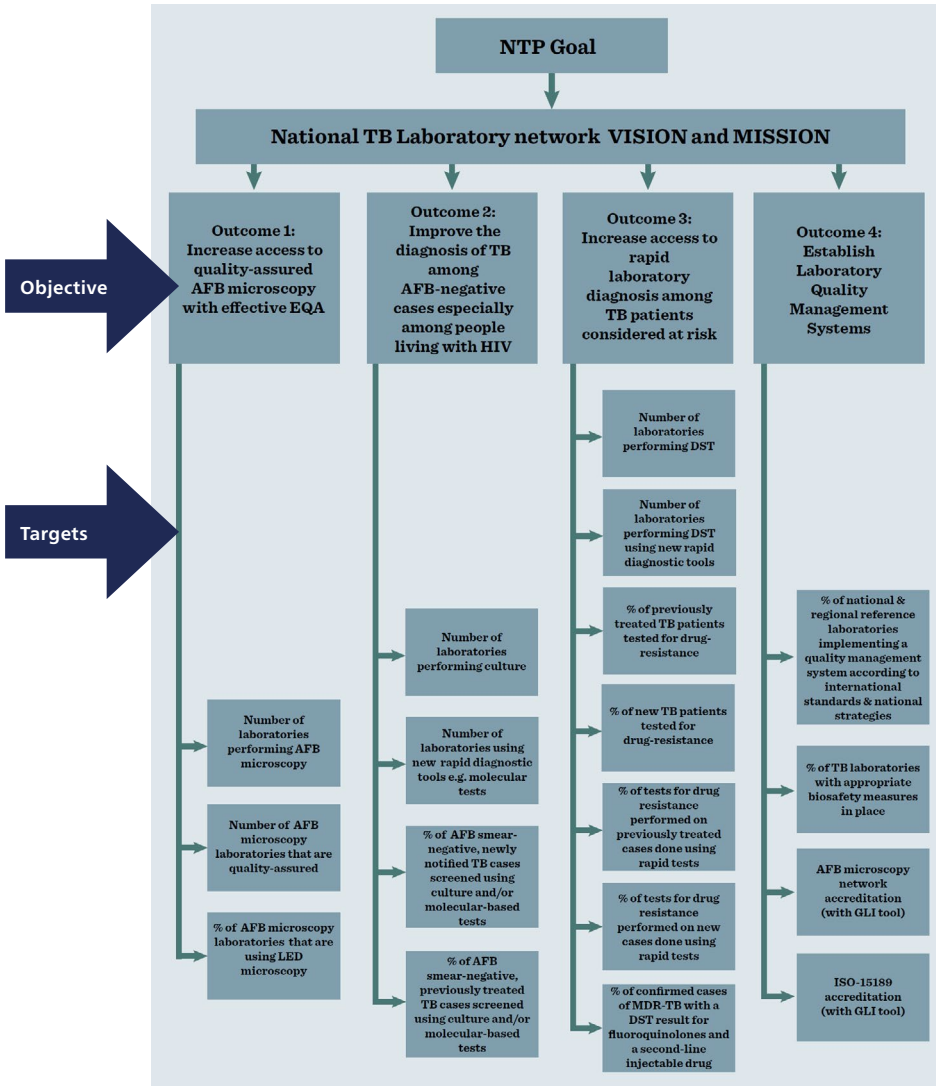
- **Objective 1:** Increase access to quality-assured AFB microscopy with effective EQA.
- **Objective 2:** Improve the diagnosis of TB for AFB-negative cases, especially among PLHIV.
- **Objective 3:** Increase access to rapid laboratory testing among TB patients considered to be at risk for MDR-TB or XDR-TB.
- **Objective 4:** Establish laboratory QMS.

Under each of these objectives, the strategic plan would list measurable targets. Activities required to achieve these targets would then be included in a multiyear workplan and budget. A more detailed explanation of this process is given in *A practical handbook for national TB laboratory strategic plan development*¹ (3).

All strategic plans should be followed by an operational plan that defines how the strategic plan will be implemented. Generally, operational plans are more detailed than strategic plans and cover a shorter time frame (usually, they are prepared annually).

¹ See https://stoptb.org/wg/gli/assets/documents/Lab_Strategic_Handbook.pdf.

Fig. 2.11 Laboratory strategic plan framework



AFB: acid fast bacilli; DST: drug-susceptibility testing; EQA: external quality assurance; GLI: Global Laboratory Initiative; HIV: human immunodeficiency virus; LED: light-emitting diode; MDR-TB: multidrug-resistant TB; NTP: national TB programme; PLHIV: people living with HIV; QMS: quality management system; TB: tuberculosis.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Participate as a member of the technical working group for planning
- Coordination of or participation in subgroups or task teams responsible for strategic planning
- Providing information to technical working groups or subgroups on partner-specific activities and budgets for inclusion in strategic planning
- Leading or participating in strategic planning workshops

References for Section 2.10

(Key resource and suggested reading highlighted in bold font)

1. The Maputo declaration on strengthening of laboratory systems. Geneva: World Health Organization; 2008 (<https://www.who.int/publications/m/item/the-maputo-declaration-on-strengthening-of-laboratory-systems>).
2. World Health Organization & WHO Collaborating Centre for TB and Lung Diseases, Fondazione S. Maugeri, Care & Research. Toolkit to develop a national strategic plan for TB control: prevention, care and control: methodology on how to develop a national strategic plan. Geneva: World Health Organization; 2014 (<https://apps.who.int/iris/handle/10665/153811>).
3. A practical handbook for national TB laboratory strategic plan development (updated) (<https://www.challenge.tb.org/library/lab>). This is a package that contains the handbook, a facilitators' manual, participants' manual and dynamic budget Microsoft Excel sheets.

2.11 Funding TB laboratories and services

In many high TB burden countries, there is often little or no separate budget for TB laboratory services within the overall MoH budget. If a budget exists, it typically covers basic reagent costs and staff. Most of the other costs (and some basic reagent and staff costs) are covered by external funding. The primary source of external funding for TB laboratories in high TB burden countries is through the Global Fund. However, as countries move into higher income brackets, they are no longer eligible for Global Fund grants; hence, they need to develop strategies to advocate for and receive appropriate funds for TB laboratories through domestic resources.

2.11.1 Preparing applications to the Global Fund

To develop the concept note that must be used when applying for grants under the Global Fund's new funding mechanism, countries should have a strategic plan for their national TB laboratories, either incorporated into an NSP or as a stand-alone document. The plan should describe:

- the capacity of different levels of the laboratory network;
- gaps in capacity that the funds will be used to remedy; and
- a clear description of the burdens of TB, MDR-TB and HIV-associated TB.

2. Key technical areas for guidance

Requests for funding from the Global Fund may include budgets for:

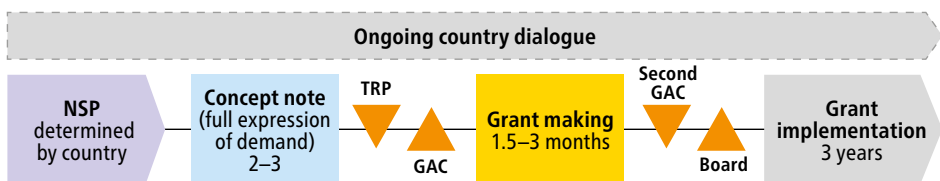
- building or renovating facilities;
- purchasing equipment and supplies (including maintenance contracts);
- hiring, training and supervising staff;
- developing and implementing QA and QMS; and
- requesting external technical assistance.

In preparing applications for support from the Global Fund, the following issues should be considered:

- the epidemiological situation;
- the diagnostic algorithms used for different risk groups;
- laboratories' infrastructure needs, including needs for implementing biosafety measures;
- the need to purchase additional equipment, and the potential for maintaining the equipment;
- whether the required referral mechanisms for specimens exist; and
- whether there are links to external partners who can provide technical assistance.

The publication *A practical handbook for national TB laboratory strategic plan development (updated)*¹ contains dynamic budget Microsoft Excel sheets to help countries develop plans and budgets for TB laboratory services at national and subnational level within the framework provided by the Global Plan to Stop TB and the End TB Strategy. These plans can be used as the basis for resource mobilization from national governments and donor agencies. Details of the Global Fund's new funding process are available online² and are illustrated in Fig. 2.12.

Fig. 2.12 New funding model for the Global Fund



NSP – National strategic plan, TRP – Technical review panel, GAC – Grant approvals committee

GAC: grant approvals committee; Global Fund: Global Fund to Fight AIDS, Tuberculosis and Malaria; TRP: technical review panel.

¹ See <https://www.challengeTB.org/library/lab>.

² See https://www.theglobalfund.org/media/1467/replenishment_2013newfundingmodel_report_en.pdf.

KEY ACTIVITY AREAS FOR TECHNICAL SUPPORT

- Participate in planning and budgeting for TB laboratory activities as part of the NTP strategic planning process
- Participate in developing joint concept notes for the Global Fund and applications for other funding

Resources for Section 2.11

A practical handbook for national TB laboratory strategic plan development (updated). <https://www.challengetb.org/library/lab>. This is a package that contains the handbook, a facilitators' manual, participants' manual and dynamic budget Microsoft Excel sheets.

The End TB Strategy – global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2016

(<https://www.who.int/teams/global-tuberculosis-programme/the-end-tb-strategy>).

Global strategy and targets for tuberculosis prevention, care and control after 2015 (WHA67.1).

Geneva: World Health Assembly; 2014 (<https://apps.who.int/iris/handle/10665/162760>).

Implementing the End TB Strategy: the essentials. Geneva: World Health Organization; 2015

(<https://apps.who.int/iris/handle/10665/206499>).

Global tuberculosis report 2016. Geneva: World Health Organization; 2016

(<https://apps.who.int/iris/handle/10665/250441>).

3. Providing technical assistance

3.1 Types of technical assistance

Technical assistance can encompass a wide variety of activities, including:

- capacity-building through training and mentoring;
- specialized training programmes for new diagnostics;
- producing guidance on policy and programme development;
- writing an operations manual for the NRL;
- developing SOPs;
- laboratory strategic planning;
- undertaking Global Fund programme reviews;
- implementing accreditation and laboratory QMS;
- implementing new technologies;
- developing routine surveillance practices;
- assisting with survey planning and capacity-building;
- implementing LIMS;
- implementing diagnostics connectivity;
- strengthening the diagnostic cascade and linkage to care;
- mentoring of laboratory management;
- undertaking gap analysis and assessments;
- developing biosafety;
- managing the supply chain management;
- building specimen referral strategies; and
- undertaking operational research activities.

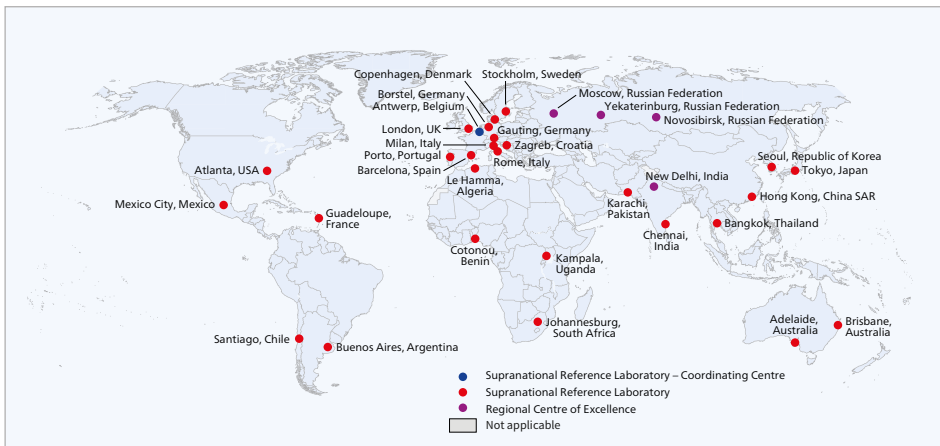
Technical assistance for these activities can come from a local country consultant, a leading institute or partner organization, or an international professional. The duration of the consultancy can depend on the tasks outlined in the national workplan, the extent of skill development or capacity-building to be accomplished, and the current capacity of the country's skilled human resources. All three determine which activities require short-term technical assistance and which will require longer term assistance. Short-term assistance can vary from a one-time visit (1–3 weeks) to multiple visits over the course of a year. Requests for longer term assistance may

require that the consultant reside in the country for several weeks or months, or even an entire year. SRLs may be able to recommend consultants.

3.2 Role of the TB Supranational Reference Laboratory Network

As a key partner in strengthening the capacity and quality of TB diagnostic testing in many countries, the WHO TB Supranational Reference Laboratory Network (SRLN), shown in Fig. 3.1, comprises 37 laboratories that provide a benchmark for proficiency testing. The SRLN can also provide long-term technical assistance under the framework of collaborative agreements.

Fig. 3.1 WHO TB SRLN



SRLN: Supranational Reference Laboratory Network; TB: tuberculosis; WHO: World Health Organization.

Improved coordination of technical assistance provided by the SRLs remains a key priority for the network. Individual SRLs vary in terms of capacity, competencies and available funding; hence, it is important that SRLs, donors and technical partners collaborate closely in the context of a MoH-led national (TB) laboratory strategic plan, to leverage complementary skill sets and mandates to meet a country’s needs for technical assistance and capacity-building. To facilitate this communication and coordination, individuals and organizations providing technical assistance to TB laboratories should request information from the NRL on the SRL providing support to the country, and should contact that SRL to discuss ways to harmonize the approaches used and support provided. A complete listing of current SRLs, including contact information, is available online.¹

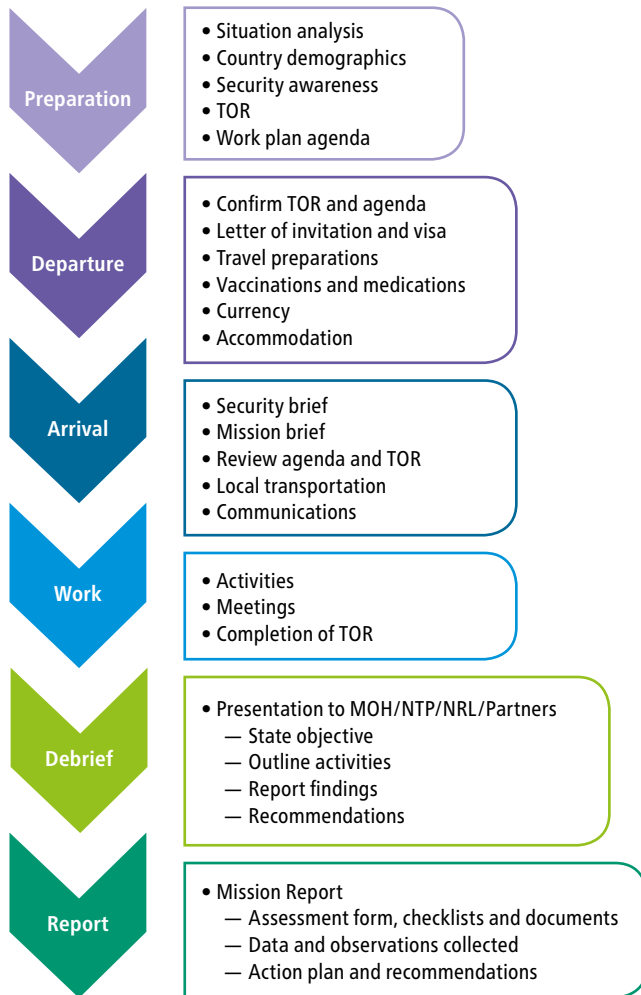
¹ See <https://sites.google.com/site/srtblaboratories/>.

The SRLN has also adopted a reporting system that uses standardized forms to assess laboratories and laboratory networks, and a standardized form for reporting on visits to countries made by SRLs.¹

3.3 Processes for technical assistance

The processes involved in technical assistance provided by internationally based consultants are outlined in Fig. 3.2. Technical assistance provided from consultants based in the country (i.e. local technical assistants) follow a similar process, with

Fig. 3.2 Processes involved in providing technical assistance



MoH: ministry of health; NRL: national reference laboratory; NTP: national TB programme; TOR: terms of reference.

¹ See <https://sites.google.com/view/srln/about-us>.

the exception of the various activities concerning preparation and travel. Country-based consultants from partner organizations will already be familiar with the various aspects of the country and its national programme, including the directions for implementation and programme developments involved in laboratories. Local consultants also often have well-formed relationships with the MoH, NTP and NRL, and often they can provide more efficient and cost-effective support. However, the use of a local consultant should not mean that the work should be overly informal. Local consultants still require formal terms of reference (TOR), workplans or agendas for their activities, to offer the necessary assistance. Consultants should prepare debriefings, final reports and recommendations, since these are important for the advancement of laboratory development. The next sections describe critical aspects of the technical assistance process for both international and local technical support.

3.3.1 Preparation

Preparing for a technical assistance visit is complex, because technical, professional and practical issues must be considered. This applies both to short-term assistance and to longer term work in the country. To plan technical activities effectively, it is important to prepare for the visit by becoming familiar with background information on the organization, the functioning of the laboratory network and the epidemiological profile of TB in the country. Close collaboration with local authorities is essential to acquire relevant information and data. It is important to review various reports and previous assessments to gain a comprehensive understanding of the current laboratory situation.

Situational analysis – desk review

Before going to a country, a consultant should acquire the necessary information regarding the current status of the TB laboratory network and diagnostic services presently in use. This information can be provided by the supporting SRL, by reviewing documents from previous programme reviews or missions, or by reviewing the NTP guidelines and other national documents. However, the most accurate information is often through direct communications with the NTP, the NRL or the lead affiliation for the national laboratory services, depending on the organizational scheme established for the individual country.

As part of the desk review, it is important to consider that levels of laboratory development and diagnostic services can vary tremendously, depending on the country context and the local situation. For example, not all countries have an NRL or a functional network for systematic TB diagnosis. Many national programme services typically offer microscopy examination as the initial test for diagnosis; however, some countries or regions within countries still rely on basic clinical examination and chest X-ray as the primary case-finding strategies. On the other hand, countries with highly evolved laboratory networks may have implemented rapid molecular testing methods for TB case detection, which also provides information on drug resistance. Advanced services will be found at the provincial, regional or central level laboratories that have the required infrastructure. More traditional modes of TB screening are generally used at levels closer to the patient, but effective systems of specimen transport can allow rural clinics access to advanced testing. These links are necessary to expand coverage and increase case finding, and therefore play a significant role in national TB control.

The process of performing a situational review of a country's TB diagnostic services requires an understanding of the current TB situation. Questions that may help guide this review are shown in Table 3.1.

Resources for this information include:

- WHO global TB reports;
- Global Fund or WHO programme reviews;
- regional Green Light Committee mission reports;
- national TB guidelines;
- national epidemiological assessments;
- surveillance reports;
- national TB laboratory guidelines or quality manual;
- NSPs;
- laboratory strategic plans;
- SRL reports; and
- annual NRL or NTP reports.

The extent of the review will depend on the scope of work outlined in the TOR for the consultancy. Some consultancies are based purely on bench work activities (e.g. technical training or mentoring during the implementation of new diagnostics), whereas others could include assisting with policy development, strategic planning activities or performing a programme review. The focus of the technical assistance may be on developments for a single laboratory or may cover the entire network. Information that may be useful to have before starting consultancy includes the following:

- recent TB epidemiology;
- structure of the organization (e.g. MoH, NTP and NRL); consultants must aim to fully understand where management of TB laboratory services falls within the MoH and how activities are coordinated with the NTP;
- existing network capacity and services;
- annual workload data;
- national algorithms and guidelines;
- any formal laboratory manuals or strategic plans;
- current capacity-building activities;
- a list of partners involved in laboratory development;
- information on referral mechanisms;
- information on data management practices;
- methods of routine surveillance activities;
- available funding mechanisms to support laboratory strengthening and capacity-building;

Table 3.1 Questions that may guide a situational review of TB diagnostic services

Questions?	Information
What is the country population?	Country population with demographic data from recent census
What is the current TB situation or epidemiology?	Notification, incidence and prevalence data for TB, MDR-TB, XDR-TB, HIV/TB, extrapulmonary TB and paediatric TB
What is the country landscape?	Geographic information; national structure or how the country is divided, current socioeconomic situation; regions or states with hotspots for TB, HIV, DR-TB or other pertinent health issues (e.g. chronic illnesses such as diabetes and undernutrition)
What are the primary risk groups for TB in this population?	PLHIV, children, immigrants, cross-border workers, prisoners, people with diabetes, contacts, other vulnerable people, etc.
How is the TB programme organized in the MoH?	Within public health, infectious diseases or independent
What treatment guidelines are already available?	TB DOTS, TB/HIV, PMDT, paediatric TB, extrapulmonary TB and TB diabetes
Who are the existing donors and partners of the NTP?	Examples include US CDC, USAID, Unitaid, CIDA, MSF, WHO, the Union, KNCV and MSH
How are laboratory services organized?	Public and private services As an arm of the national laboratory or as stand-alone TB services
How is the current TB laboratory network structured?	Hierarchy under NRL or under another institute Minimal structure
What are the existing laboratory services for TB?	Microscopy, culture, culture and DST, molecular diagnostics (Xpert, Truenat MTB, MC-aNAAT, LPA and TB-LAMP), other
What is the current diagnostic coverage?	Microscopy centres WRD laboratories in country and their capacity Culture and DST laboratories LPA laboratories in country (first-line and second-line)
What is the current testing algorithm?	Priority risk groups and flow of testing with recommended line of treatment
What is the annual workload for testing?	Total test performed and average throughput per diagnostic per facility for microscopy, culture, Xpert, TB-LAMP, LPA, phenotypic DST or other
Is there a national laboratory strategic plan in place?	Design for laboratory expansion and capacity-building over the next 3–5 years
Have national TB laboratory guidelines been established?	Outlines the national TB laboratory network structure, diagnostic services provided, biosafety guidelines, waste management practices, QA measures, etc.

CIDA: Canadian International Development Agency; DOTS: directly observed treatment short course; DR-TB: drug-resistant TB; DST: drug-susceptibility testing; HIV: human immunodeficiency virus; KNCV: KNCV Tuberculosis Foundation; LAMP: loop-mediated isothermal amplification; LPA: line-probe assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant TB; MoH: ministry of health; MSF: Médecins Sans Frontières; MSH: Management Sciences for Health; NRL: national reference laboratory; NTP: national TB programme; PLHIV: people living with HIV; PMDT: programmatic management of drug-resistant TB; QA: quality assurance; TB: tuberculosis; Union: International Union Against Tuberculosis and Lung Disease; US CDC: United States Centers for Disease Control and Prevention; USAID: United States Agency for International Development; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test; XDR-TB: extensively drug-resistant TB.

3. Providing technical assistance

- current laboratory training or human resource development programmes;
- procurement and supply chain management practices;
- biosafety regulations and health surveillance measures already in place;
- facilities and equipment management programmes available; and
- QA practices.

A complete desk review will provide the necessary background and understanding before travel, but may also be requested as a deliverable as part of the mission. Once all the relevant information has been collected from the desk review, the consultant will be able to provide the necessary technical assistance contracted under the official TOR.

TOR

Consultancy activities to support the NTP and NRL will invariably include different TOR covering policy, technical or programmatic issues. It is important to be realistic about what can be achieved during the chosen time frame. Clear TOR should be established before each technical visit. These terms should be tailored to the type of work required and the objectives of the mission, and all TOR should include the goal of establishing links with local and international partners participating in TB laboratory strengthening efforts. The TOR should be specific, well-defined and in line with the NTP's strategy for TB control. The desk review will help to ensure that the TOR are aligned with a broader vision of the health system context and development trends, to provide sustainable interventions.

Defining the TOR may include the following steps:

- communicating with officials from the NTP, NRL, MoH, other appropriate government bodies, and the WHO office or partner office in country facilitating the consultancy;
- communicating with the donors and partners working to implement TB-related laboratory interventions within the country;
- defining the mission objectives and outlining specific tasks with a daily schedule of activities to achieve these objectives;
- determining the appropriate duration required to perform all activities and establishing a start date and completion date (if multiple visits are required to fulfil the stated goals, then defined dates for consecutive interventions with specific milestones or outcomes should be outlined);
- determining dates for intermediate and final deliverables;
- establishing distribution lists for deliverables (e.g. final reports or assessments); and
- scheduling arrival and departure briefings with all relevant parties.

Country demographics

Before travelling to a country, it is important to understand that country's demographics. The consultant should do some background reading on the culture, history, socioeconomics, population dynamics and current political situation of the host country. By having some understanding of the local landscape, the consultant will be prepared for various situations and discussions that may arise with clients or local colleagues, and will have a clear understanding of the current local challenges or the issues of the day. Understanding political and social realities allows the consultant to develop workplans that take into account national holidays, and political activities or social events that might pose a risk. Knowing the economic status and population dynamics will prepare the consultant for observing severe poverty, wide social disparity or caste systems, infrastructure limitations (e.g. limited or unavailable power, water or sanitation facilities), or systems rendered dysfunctional because of rapid economic development or growth (e.g. transportation). Understanding specifics about the culture, traditions and religion(s) of a country helps to prevent inappropriate behaviours or actions. It is also important that the consultant consider travel locations outside of the capital city in their work agenda, to assess security or health risks in those areas. Before starting their travel, the consultant should review travel warnings posted on government or embassy websites, and review WHO alerts for recent outbreaks or residual pockets of emerging diseases that could be a health risk (e.g. dengue, Ebola or Marburg viruses). Areas where there is unrest, violence or war should be avoided and not included in the scope of work. Vaccinations or prophylaxis for endemic diseases are also recommended; further information can be found on websites of organizations that provide travel advice, such as the US CDC. Climate, seasonal changes and terrain in the regions where travel is planned need to be understood, to be fully prepared with appropriate clothing, shoes and other personal items.

3.3.2 Departure for an international technical assistance mission

The following steps should be taken before departing for an international assignment:

- finalize the TOR;
- draw up a working agenda with the MoH, NTP and NRL, including dates, places and details of people travelling;
- outline travel routes if travelling outside of the capital city, to assess personal risk;
- inform the WHO's country office of the visit (if necessary);
- acquire a letter of invitation from the MoH;
- acquire a visa;
- obtain appropriate vaccinations and other medications needed for travel;
- obtain currency to pay for transportation if needed upon arrival (money can typically be exchanged at hotels and banks or via ATMs);
- ensure arrangements for transportation and accommodation, including:
 - flight;

3. Providing technical assistance

- transfer from and to the airport or point of arrival;
- safe local transportation for work travel;
- hotel reservations (it is wise to ask what the best form of payment is for the hotel before arrival, because some rural hotels do not take credit cards);
- internet access, which should be available at the hotel or work office;
- local telephone or SIM card, which should be provided; and
- confirm language requirements and arrange for translation to be provided if needed.

3.3.3 Arrival for an international technical assistance mission

After arriving, the consultant should:

- receive a security and country briefing from WHO or the host;
- be briefed by the NTP and other parties involved;
- review and confirm the TOR with the NTP and other relevant parties;
- review the proposed activities and expected outcomes, and revise if needed;
- clarify whether the NTP has any specific concerns about the mission;
- if the duration of the technical visit is longer than a few weeks, schedule meetings to report on progress with targeted outcomes or milestones; and
- establish proper lines of communication with the host, NTP and other interested parties.

3.3.4 Work

During the technical assistance visit, it is critical to involve NTP and NRL representatives in the work of the mission as much as possible; ideally, by conducting joint site visits and activities. If this is not possible, at a minimum the NTP and NRL must be briefed before and after all activities. In most settings, formal written approval must be obtained before site visits. Certain sites may require that the consultant brief local health directors or hospital or laboratory administrators on the objectives and proposed outcomes of the interventions both before and after accomplishing the work. Again, during these official meetings, lines of communication must be kept open and local protocols observed. When working on-site, it is important to involve key staff as well as some junior staff, to build internal capacity. This is an opportunity for exchange and sharing of knowledge; engaging with local staff helps ensure that the work will continue after the consultant has left. The primary goal for a technical assistance visit is to complete the TOR; in doing so, the consultant must provide quality work and strive to build local capacity while achieving that goal.

When a consultant is developing TB laboratory services, managers of NTPs and national laboratory services should be actively involved throughout the process. It is particularly important to obtain support from individuals who have direct knowledge of and experience within the current system. Such individuals may include staff working in public and private TB laboratories; consultants from WHO's regional office or SRLN;

and personnel from the NRL, local research institutes and academic institutions specializing in infectious diseases or surveillance epidemiology. It is also important to engage all country partners and consultants from nongovernmental organizations (NGOs) that are actively involved in supporting programme development.

3.3.5 Debrief

At the end of the technical assistance mission, consultants should:

- summarize their findings and prepare a list of important recommendations in collaboration with the MoH and NTP; these should be shared at a debriefing meeting with the relevant stakeholders before the consultants depart;
- ensure that the recommendations are consistent with the TOR for the mission; if they are not, then the report should explain why they varied;
- ensure that there is evidence for the recommendations being made;
- seek clarification of any issues that are unclear before departing;
- ensure that contact information needed for providing follow-up is correct; and
- secure the list of parties who are to receive the final report and relevant data or documents acquired during the mission.

3.3.6 Final report

It is essential that consultants write a mission report. The aim of the report is to clearly and concisely present information and facts (not opinions), using a consistent and appropriate format. The author or authors must ensure that the report presents information clearly to all readers. It is best to use short paragraphs, supported by appropriate illustrations where necessary (e.g. tables or graphs), and to include numbered headings and subheadings. This section offers some general guidance on writing a mission report, but the most important element is to follow the TOR agreed to before the visit.

The report outline should be as follows (the main sections are discussed below):

- cover page;
- list of abbreviations;
- executive summary;
- background epidemiological information, context in which the NTP operates and background supporting the particular mission;
- purpose of the mission with primary objectives;
- a summary of each laboratory activity conducted, with observations and data;
- findings and observations;
- specific recommendations to support current developments and progress for the NRL and network related to the mission;
- conclusions;

- acknowledgement of the work of those who contributed to the mission;
- annexes, which may include:
 - materials prepared before the mission;
 - relevant data, checklists or documents acquired during the mission; and
 - the itinerary of the mission and the final TOR, with an explanation for any deviations from the TOR.

Executive summary

The executive summary should be about one page long and should summarize the purpose, objectives, TOR, deliverables, activities, findings, recommendations and conclusions of the mission. It should not contain any technical details.

Background information

The background information section of the report should include a description of:

- the local epidemiology (TB, HIV and MDR-TB);
- country-specific priorities for case detection;
- a brief summary of local treatment policies and guidelines;
- a description of the local TB laboratory organization, network and capacity;
- the situational overview of human resources for laboratories;
- the financial resources available for laboratory support; and
- a list of partners involved in supporting laboratory activities.

Mission purpose and objectives

The purpose of the mission should be clear and concise. Objectives should be focused, with direct outcomes that support desired deliverables. The activities planned should link back to the primary objectives and complete the tasks outlined in the TOR. The TOR should be provided as an annex.

Summary of activities

The activities undertaken should be described in a logical sequence to demonstrate the systematic approach used during the visit. The itinerary or agenda for the activities should be provided as an annex. The report should include the sites visited, the type of work performed at each site, and a brief summary of observations and data.

Findings

The findings are the core of the report. They should include all data, compiled results from checklists, graphs or tables, detailed descriptions of observed practices and specific challenges or deficiencies. The text should be concise and accurate, particularly when describing challenges or deficiencies. Positive aspects or outcomes should be presented first, leading in to difficult or sensitive findings. Findings should be simplified into tables, charts or graphs. Photographs can also be helpful to illustrate

a problem or demonstrate a successful programme or intervention. Only information that is entirely relevant to the objectives and the mission TOR should be included; however, if there is a serious observation outside the scope of the work, then it must be addressed.

This section should have a simple structure, to make it easier for the reader to follow the information and absorb important findings. The use of subtitles for different categories will allow readers to quickly find specific information.

Recommendations

The findings should be summarized at the end of the visit and a list of the most important recommendations should be shared at a debriefing meeting with the relevant stakeholders before the consultant departs. These recommendations should be reiterated in the final report. Recommendations should clearly indicate whom they are addressing.

The consultant's visit may include working at several sites, in which case it is important to clarify to whom the action or recommendation is addressed. Most of the recommendations will be addressed to the MoH, NTP or NRL, unless the network system is divided according to regional or state governance, in which case recommendations may be directed accordingly. If the assistance is part of the implementation of a project, the recommendations could also be directed to the project director. If a partner organization is involved and is the focal point for the project, then that organization should also be included.

Recommendations should be consistent with and appropriate to the TOR, based on evidence and clearly derived from the report's findings. Recommendations are often presented as concise bullet points.

Conclusions

The conclusions should provide a clear interpretation of the author's evidence and findings. It should be brief and cover the main priorities, with only relevant information included. Next steps or ways forward should be included, to provide direction for future interventions.

Annexes

The annexes are the place to add bulk data or supplementary documentation to help the reader to understand the report. The annexes include information such as:

- the final TOR;
- the itinerary and meeting schedule;
- bulk data and results;
- checklists or tools used during the mission; and
- added documents, photos, workplans, protocols, etc.

Resources and suggested reading for Section 3.3

Framework for conducting reviews of tuberculosis programmes. Geneva: World Health Organization; 2014 (<https://apps.who.int/iris/handle/10665/127943>).

Laboratory assessment tool. Lyon, France: WHO Lyon Office for National Epidemic Preparedness and Response; 2012 (<https://apps.who.int/iris/handle/10665/70874>).

Tuberculosis laboratory network assessment. Washington, DC: TB CARE I; No date (https://challengetb.org/publications/tools/lab/Laboratory_Assessment_Form.pdf).

Global tuberculosis report 2020. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/336069>).

Annexes



Annex 1. WHO-recommended testing for TB

The tests included in this table are up-to-date as of the publication of this handbook.

Procedure and use	Test	Comments
Conventional tests used as the initial diagnostic test in people being evaluated for pulmonary and extrapulmonary TB		
AFB-smear microscopy to detect MTBC for diagnosis of TB or for monitoring therapy ^a	Conventional light microscopy with ZN staining	LED fluorescence microscopy is about 10% more sensitive and the observation time is significantly shorter than for conventional light microscopy
	Conventional fluorescence microscopy	WHO recommends replacing light microscopy with LED fluorescence microscopy
	LED fluorescence microscopy	Direct smear microscopy may be done in a low-risk-level TB laboratory. ^b Processing of samples for concentrated smear microscopy should be done in a moderate-risk-level TB laboratory Test TAT ^c is 15–30 minutes
Culture to detect MTBC for diagnosis of TB or for monitoring therapy or for isolating MTBC for DST ^d	Löwenstein–Jensen medium (egg-based)	Acceptable rate of contamination is 3–5% Processing of samples for culture should be done in a moderate-risk-level TB laboratory
	Middlebrook 7H10 or 7H11 media (agar based)	Test TAT is 3–8 weeks. Samples should not be declared culture negative until 8 weeks of incubation
	Liquid media ^e (e.g. BACTEC TM MGIT TM 960 TB System [Becton Dickinson Microbiology Systems, Sparks, MD])	Acceptable rate of contamination is 8–10% Processing of samples for culture should be done in a moderate-risk-level TB laboratory Test TAT is 1–6 weeks. Samples should not be declared culture negative until 6 weeks of incubation
Immunochromatographic assay for species identification of bacteria recovered from solid or liquid cultures ^d	Capilia TB-Neo [®] (Tauns Laboratories, Numazu, Japan)	Used with cultures Species identification tests are used to determine the species of any isolated mycobacteria
	TB Ag MPT64 Rapid Test [®] (SD Bioline, Kyonggi-do, South Korea)	Processing of cultures should be done in a high-risk-level TB laboratory
	TBcID [®] (Becton Dickinson Microbiology Systems, Sparks, MD)	Test TAT is 15 minutes

Rapid tests used as the initial diagnostic test in people being evaluated for pulmonary TB to detect MTBC without drug resistance detection	
NAAT to detect MTBC ^f	<p>Loopamp MTBC detection kit (Eiken Chemical Company Ltd, Japan)</p> <p>Recommended for use with sputum and BAL specimens</p> <p>Suitable for use in a peripheral facility (e.g. microscopy centre)</p> <p>Should not replace the use of mWRDs that detect TB and drug resistance</p> <p>May be done in a low-risk-level TB laboratory</p> <p>Test TAT is 90 minutes</p>
Rapid antigen detection test for TB ^g	<p>LF-LAM assay (e.g. Alere Determine™ Urine TB LAM Ag test [Alere Inc, Waltham, USA]).</p> <p>Recommended for use with urine specimens</p> <p>Suitable for use at the point of care and has minimal infrastructure or biosafety requirements</p> <p>Recommended for use for HIV-positive people to aid in the diagnosis of both pulmonary and extrapulmonary TB⁵</p> <p>Test TAT is 30 minutes</p>
Rapid molecular tests used as the initial diagnostic test in people being evaluated for pulmonary TB to detect MTBC and resistance to rifampicin	
Automated NAAT to detect MTBC and resistance to RIF ^f	<p>Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA)</p> <p>Xpert MTB/RIF Ultra assay (Cepheid, Sunnyvale, CA, USA)</p> <p>Truenat MTB, MTB Plus and MTB-RIF Dx (Molbio Diagnostics, Goa, India)</p> <p>Recommended for use with pulmonary and selected extrapulmonary specimens</p> <p>Suitable for all levels of the health system with adequate infrastructure</p> <p>May be done in a low-risk-level TB laboratory</p> <p>Test TAT is 2 hours (MTB/RIF) and 90 minutes (Ultra)</p> <p>Recommended for use with sputum and BAL specimens</p> <p>Suitable for use in a peripheral facility (e.g. microscopy centre)</p> <p>Battery-operated instruments are available</p> <p>May be done in a low-risk-level TB laboratory</p> <p>Test TAT is 1 hour (detection test) plus 1 hour (resistance test)</p>

Rapid molecular tests used as the initial diagnostic test in people being evaluated for pulmonary TB to detect MTBC and resistance to rifampicin and isoniazid	
<p>MC-aNAAT to detect MTBC and resistance to RIF and INH^f</p>	<p>RealTime MTB and MTB RIF/INH assays (Abbott Laboratories, Abbott Park, USA)</p> <p>BD MAX MDR-TB assay (Becton Dickinson, Franklin Lakes, USA)</p> <p>FluoroType MTB and MTBDR assay (Bruker/Hain Lifescience, Nehren, Germany)</p> <p>cobas MTB and MTB-RIF/INH assays (Hoffmann-La Roche, Basel, Switzerland)</p> <p>Recommended for use with sputum and BAL specimens</p> <p>Suitable for use in intermediate and central reference laboratories because of infrastructure requirements, complexity of the installing, operating and maintaining the instruments, and technical skills required</p> <p>May be done in a low-risk-level TB laboratory</p> <p>RealTime tests: Test TAT is 7 hours (detection test) plus 3.5 hours (resistance test)</p> <p>BD MAX test: Test TAT is 4.5 hours</p> <p>FluoroType tests: Test TAT is 2.5 hours</p> <p>cobas tests: Test TAT is 5.5 hours (detection test) plus 3.5 hours (resistance test)</p>
Conventional diagnostic tests used to detect resistance to anti-TB drugs	
<p>Phenotypic DST (indirect method)^{e,h}</p> <p>DST for at least RIF, INH and FQ is needed and strongly encouraged for Group A drugs used for MDR-TB treatment</p>	<p>Indirect DST is used with cultured isolates</p> <p>Suitable for use in intermediate or central laboratories or advanced intermediate laboratories because of requirements for infrastructure, biosafety and technical skills</p> <p>Processing of cultures should be done in a high-risk-level TB laboratory</p> <p>Recommended critical concentrations are described in the <i>WHO operational handbook on tuberculosis Module 3ⁱ</i></p> <p>Solid media: Test TAT is 3–4 weeks plus time required for culture</p> <p>Liquid media: Test TAT is 1–3 weeks plus time required for culture</p>
Rapid molecular tests used to detect resistance to anti-TB drugs in people with bacteriologically confirmed pulmonary TB	
<p>FL-LPA: reverse hybridization assay to detect resistance to INH and RIF^f</p>	<p>GenoType[®] MTBDRplus (Hain Lifescience, Nehren, Germany)</p> <p>NTM+MDRTB Detection Kit (NIPRO Corporation, Osaka, Japan)</p> <p>Recommended for use with culture isolates and AFB smear-positive specimens</p> <p>Suitable for use in intermediate laboratories because of requirements for infrastructure, biosafety and technical skills</p> <p>Processing of sputum specimens should be done in a moderate-risk-level TB laboratory</p> <p>Processing of cultures should be done in a high-risk-level TB laboratory</p> <p>Test TAT is 1–2 days</p>

SL-LPA: ^f reverse hybridization assay to detect resistance to FQs and AMK ^f	GenoType [®] MTBDRsl test (Hain Lifescience, Nehren, Germany)	Recommended for use with culture isolates and sputum specimens Suitable for use in intermediate or central laboratories because of requirements for infrastructure, biosafety and technical skills Processing of sputum specimens should be done in a moderate-risk-level TB laboratory Processing of cultures should be done in a high-risk-level TB laboratory Test TAT is 1–2 days
LC-aNAAT to detect resistance to INH and second-line anti-TB drugs (FQ, ETO, AMK) ^f	Xpert MTB/XDR test (Cepheid, Sunnyvale, CA, USA). Requires a 10-colour GeneXpert instrument	Recommended for use with sputum specimens Suitable for all levels of the health system with adequate infrastructure May be done in a low-risk-level TB laboratory Test TAT is 90 minutes
HC-rNAAT to detect PZA resistance ^f	Genoscholar PZA-TB II test (NIPRO Corporation, Osaka, Japan)	Recommended for use with culture isolates Suitable for use in intermediate or central reference laboratories because of requirements for infrastructure, biosafety and technical skills Processing of cultures should be done in a high-risk-level TB laboratory Test TAT is 1–2 days plus time required for culture
Immunologic tests to detect TB infection		
Tests for TB infection ⁱ	TST	There is no strong evidence that one test should be preferred over the other in terms of predicting progression from TB infection to TB disease These tests should only be used in certain populations at risk of having TB infection Neither TSTs nor IGRAs should be used in persons having a low risk of TB infection Neither TSTs nor IGRAs should be used to aid in the diagnosis of active TB IGRAs should follow biosafety precautions for blood-borne pathogens IGRAs: Test TAT is 1–2 days TSTs are read 2–3 days after placement
	IGRA <ul style="list-style-type: none"> • QuantiFERON[®]-TB Gold In-Tube or Gold Plus (QIAGEN GmbH, Hilden, Germany) • T-SPOT.TB (Oxford Immunotec Ltd, Abingdon, United Kingdom) • WANTAI TB-IGRA (Beijing Wantai Biological Pharmacy Enterprise Co., Beijing, China) 	

- AFB: acid-fast bacilli; AMK: amikacin; BAL: bronchoalveolar lavage; DST: drug-susceptibility testing; ETO: ethionamide; FL-LPA: first-line line-probe assay; FQ: fluoroquinolone; GLI: Global Laboratory Initiative; HC-rNAAT: high complexity reverse hybridization nucleic acid amplification test; HIV: human immunodeficiency virus; IGRA: interferon-gamma release assay; INH: isoniazid; LC-aNAAT: low complexity automated nucleic acid amplification test; LED: light emitting diode; LF-LAM: lateral flow lipoarabinomannan; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant tuberculosis; MTBC: *Mycobacterium tuberculosis* complex bacteria; mWRD: molecular WHO-recommended rapid diagnostic test; NAAT: nucleic acid amplification test; PZA: pyrazinamide; RIF: rifampicin; SL-LPA: second-line line-probe assay (to detect resistance to AMK); TAT: test turnaround time; TB: tuberculosis; TST: tuberculin skin test; United Kingdom: United Kingdom of Great Britain and Northern Ireland; USA: United States of America; WHO: World Health Organization; ZN: Ziehl-Neelsen.
- a See *Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook* (<https://www.stoptb.org/wg/gli/gat.asp>) (1).
- b Refers to the relative risk of conducting the procedure (low, moderate or high) as defined in the WHO *Tuberculosis laboratory biosafety manual* (<https://apps.who.int/iris/handle/10665/77949>) (2) and *GLI tuberculosis laboratory safety handbook* (<https://www.stoptb.org/wg/gli/gat.asp>) (3).
- c Test turnaround time refers to the time required for conducting the test. Laboratory turnaround time, from receipt of a specimen at the laboratory to issuing a laboratory test result, may be longer depending of the arrival of specimen in the laboratory, testing schedules and batching of samples for testing. The turnaround time from specimen collection to receipt of the result by the clinician may be much longer, depending on several factors including speed of referral of specimens to the laboratory and delivery of results to the clinician.
- d See *GLI mycobacteriology laboratory manual* (<https://www.stoptb.org/wg/gli/gat.asp>) (4).
- e WHO has conditionally recommended selected noncommercial liquid culture systems for detecting MTBC and for detecting rifampicin resistance as an interim solution, pending the development of genotypic or automated liquid culture and DST capacity (5). These methods include microscopic observation of drug susceptibility (MODS), nitrate reductase assay (NRA) and colorimetric redox indicator (CRI). They are suitable for use at central level or reference laboratories and require highly trained personnel. However, their use is not intended to replace conventional culture and DST.
- f See *WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update* (<https://apps.who.int/iris/handle/10665/342369>) (6).
- g See *Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV* (<http://www.stoptb.org/wg/gli/gat.asp>) (7).
- h See *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis* (<https://apps.who.int/iris/handle/10665/275469>) (8).
- i See *WHO consolidated guidelines on tuberculosis Module 1: Prevention – tuberculosis preventive treatment* (<https://apps.who.int/iris/handle/10665/331170>) (9).

References for Annex 1

- 1 Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).
- 2 Tuberculosis laboratory biosafety manual (WHO/HTM/TB/2012.11). Geneva: World Health Organization; 2012 (<https://apps.who.int/iris/handle/10665/77949>).
- 3 GLI laboratory safety handbook. Geneva: Global Laboratory Initiative; 2019 (<https://stoptb.org/wg/gli/gat.asp>).
- 4 GLI mycobacteriology laboratory manual. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).
- 5 Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis: policy statement. Geneva: World Health Organization; 2011 (<https://apps.who.int/iris/handle/10665/44601>).

- 6 WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (<https://apps.who.int/iris/handle/10665/342369>).
- 7 Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV. Geneva: Global Laboratory Initiative; 2019 (<https://stoptb.org/wg/gli/gat.asp>).
- 8 Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva: World Health Organization; 2018 (<https://apps.who.int/iris/handle/10665/275469>).
- 9 WHO consolidated guidelines on tuberculosis Module 1: Prevention – tuberculosis preventive treatment. Geneva: World Health Organization; 2020 (<https://apps.who.int/iris/handle/10665/331170>).

Annex 2. Test-specific quality indicators

This annex provides recommended quality indicators for each WHO-approved method and the general quality indicators listed in Table 8 of the main document. Data for the quality indicators should be collected and analysed on a monthly basis. Targets provided in the tables below are intended as a guide; they will vary based on factors such as local situation and patient population tested.¹ Laboratories should monitor indicators and establish baseline performance and acceptable ranges. The indicators should be reviewed by the laboratory manager; any deviations from baseline or results outside the acceptable range should be investigated and corrective actions taken. Documentation of corrective actions and subsequent improvement and normalization of laboratory indicators following the corrective actions are critical components of quality assurance.

In addition to the test-specific quality indicators, the general quality indicators shown in Table A1 should be collected for all tests, analysed on a monthly basis and disaggregated according to tests. These indicators are provided as a guide, and laboratories should review and set locally appropriate targets.

Table A1. General quality indicators

Indicator	Target
Number of tests performed, by type of test	–
Service interruptions	No interruptions
Stock-outs	No stock-outs leading to service interruption
Equipment down time	No equipment downtime leading to service interruption
Turnaround time	90% of results meet test-specific turnaround time
Test statistics (quality indicator) report	100% of reports completed by the defined due date
EQA results	>90% of EQA panels are passed
QC results	>90% of QC results meet expected criteria
Specimen rejection	<1% of specimens rejected
Customer satisfaction	>80% of surveyed customers are satisfied
Technician productivity	Setting specific; report average number of tests performed per month per technician

EQA: external quality assurance; QC: quality control.

¹ Targets are setting specific. Laboratories should monitor indicators and establish local targets and acceptable ranges. Deviations from expected values should be investigated.

The quality indicators recommended for acid-fast bacilli (AFB) smear microscopy, shown in Table A2, should be disaggregated by type of microscopy (light, fluorescence) where more than one method is employed.

Table A2. Quality indicators for smear microscopy

Indicator ^a	Description	Target
Smear positivity rate for diagnostic smears (new and relapse cases)	Total number AFB-positive diagnostic smears/ total number of diagnostic smears	Typically, 10%
Proportion of low-grade AFB-positive smears among diagnostic smears (new and relapse cases)	Number of scanty and 1+ diagnostic smears/ total number of diagnostic smears	30–50%
Smear positivity rate for follow-up smears	Number of AFB-positive follow-up smears/ total number of follow-up smears	5–10%
Laboratory turnaround time	Time between receipt of specimen for smear microscopy at the laboratory and result reporting (mean, range and 90th percentile)	24–48 hours

AFB: acid-fast bacilli.

^a See *Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook* (<https://stoptb.org/wg/gli/gat.asp>) (1).

Quality indicators should be disaggregated by type of culture medium if more than one type is used. For laboratories processing a range of specimen types for *Mycobacterium tuberculosis* complex (MTBC) culture further disaggregation is recommended.

Table A3. Quality indicators for culture

Indicator ^a	Description	Target
Number and proportion of diagnostic specimens (new and relapse) that were culture positive (MTBC and NTM combined)	Number of diagnostic specimens that were culture positive for MTBC or NTM/number of diagnostic specimens processed for culture	15–20%
Number and proportion of diagnostic specimens (new and relapse) that were MTBC positive	Number of diagnostic specimens culture that were positive for MTBC/number of diagnostic specimens processed for culture	10–15%
Number and proportion of diagnostic AFB smear-positive specimens (new and relapse) that were culture positive for MTBC	Number of AFB smear-positive specimens that were culture positive for MTBC/number of smear-positive diagnostic specimens processed for culture	95–98% (liquid) 85–90% (solid)
Number and proportion of diagnostic AFB smear-negative specimens that were culture positive for MTBC	Number of AFB smear-negative specimens that were culture positive for MTBC/number of smear-negative diagnostic specimens processed for culture	20–30%
Number and proportion of contaminated cultures leading to uninterpretable results ^b	Number of inoculated culture tubes or plates discarded due to contamination/total number of inoculated tubes or plates inoculated for culture	3–5% (solid) 8–10% (liquid)

Table A3. Continued

Laboratory turnaround time	Time between receipt of specimen for culture at the laboratory and result reporting (mean, range and 90th percentile)	Solid: 3 weeks for smear-positive samples and 4–8 weeks for smear-negative samples Liquid culture: 8–10 days for smear-positive samples and 2–6 weeks for smear-negative samples
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AFB: acid fast bacilli; MTBC: *Mycobacterium tuberculosis* complex; NTM: non-tuberculous mycobacteria.

^a See *MGIT procedure manual* (https://www.finddx.org/wp-content/uploads/2016/02/mgit_manual_nov2006.pdf) (2) and *GLI mycobacteriology laboratory manual* (<https://stoptb.org/wg/gli/gat.asp>) (3).

^b For solid culture, some results may be interpretable in the presence of low-level contamination. Some laboratories may also reprocess contaminated cultures and the results of the repeat testing may be reportable.

Secondary quality indicators (e.g. the number and proportion of unusual drug resistance patterns), shown in Table A4, may be collected on a less frequent basis (e.g. quarterly). Critical concentrations used for phenotypic drug-susceptibility testing (DST) are found in Table 2.2 of the *WHO operational handbook on tuberculosis Module 3* (4) and in other publications (5–7).

Table A4. Quality indicators for phenotypic DST

Indicator ^a	Description	Target
Number and proportion of isolates with monoresistance and multidrug resistance to all combinations of drugs tested (e.g. isoniazid monoresistance, rifampicin monoresistance, MDR)	Number of isolates resistant to single or multiple drug combination/total number of isolates tested Stratify by each drug tested	Dependent on population tested and drug resistance prevalence and patterns
Number and proportion of isolates inoculated for DST that were discarded due to contamination	Number of isolates discarded due to contamination/total number of isolates inoculated for DST	<3%
Number and proportion of isolates inoculated for DST that were uninterpretable due to lack of growth of control (drug-free) tubes/plates	Number of isolates discarded due to lack of growth on drug-free media/total number of isolates inoculated for DST	<3%
Laboratory turnaround time	Time between inoculation of DST and result reporting (mean, range and 90th percentile)	Solid media: 3–4 weeks Liquid media: 2–3 weeks
	Total DST turnaround time including time for primary culture to produce inoculum	Solid media: 8–16 weeks Liquid media: 4–6 weeks

DST: drug-susceptibility testing; MDR: multidrug resistance.

^a See *MGIT procedure manual* (https://www.finddx.org/wp-content/uploads/2016/02/mgit_manual_nov2006.pdf) (2), *GLI mycobacteriology laboratory manual* (<https://stoptb.org/wg/gli/gat.asp>) (3) and *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis* (<https://apps.who.int/iris/handle/10665/275469>) (5).

In line-probe assay (LPA) testing, first-line LPAs (FL-LPAs) and second-line LPAs (SL-LPAs) are monitored similarly, except that FL-LPAs are used to assess resistance to isoniazid and rifampicin, whereas SL-LPAs are used to assess resistance to FQ and amikacin. A critical component of monitoring good performance of LPA testing is noticing when the indicators fall outside expected values. For example, positive results obtained on negative controls will require investigation regarding concerns for cross contamination.

If LPA testing is performed both directly from clinical specimens and from isolates, quality indicators should be disaggregated according to sample. Additional secondary indicators, including breakdown of mutations (e.g. *inhA* and *katG*) and unusual banding patterns, may be collected on a less frequent basis (e.g. quarterly).

Table A5. Quality indicators for FL-LPAs and SL-LPAs

Indicator ^a	Description	Target
FL-LPAs only		
Number and proportion of MTBC detected, RIF-R detected	Number of MTBC detected, RIF-R detected/number of MTBC detected	Dependent on population tested and RIF-R prevalence
Number and proportion of MTBC detected, INH-R detected	Number of MTBC detected, INH-R detected/number of MTBC detected	Dependent on population tested and INH-R prevalence
Number and proportion of MTBC detected, MDR detected	Number of MTBC detected, MDR detected/number of MTBC detected	Dependent on population tested and MDR-TB prevalence
Number and proportion of patients with RIF-R detected tested for resistance to FQ on-site or by referral	Number of patients with RIF-R detected tested for resistance to FQ on-site or by referral/number of MTBC detected, RIF-R detected	100%
FL-LPAs and SL-LPAs		
Number of tests performed per month		
Number and proportion of samples with MTBC detected	Number of samples with MTBC detected/number of samples tested	Dependent on population tested and TB prevalence
Number and proportion of MTBC detected, resistance not detected	Number of MTBC detected, resistance not detected/number of MTBC detected	Dependent on population tested and drug resistance prevalence
Number and proportion of MTBC detected, resistance indeterminate	Number of MTBC detected, resistance indeterminate/number of MTBC detected	<5%
Number and % of MTBC not detected	Number of MTBC not detected/number of samples tested	Dependent on population tested and TB prevalence
Number and proportion with results that cannot be interpreted	Number with uninterpretable results/number of samples tested	<5%
Laboratory turnaround time	Time between receipt of specimen for LPA at the laboratory and result reporting (mean, range and 90th percentile); for indirect LPA, add the culture turnaround time for total turnaround time	1–2 days (longer if batching of tests)

Table A5. Continued

SL-LPAs only		
Number and proportion of MTBC detected, FQ-R detected	Number of MTBC detected, FQ-R detected/number of MTBC detected	Dependent on population tested and FQ-R prevalence
Number and proportion of MTBC detected, AMK-R detected	Number of MTBC detected, AMK-R detected/number of MTBC detected	Dependent on population tested and AMK-R prevalence

AMK-R: amikacin resistance; FL-LPA: first-line line-probe assay; FQ: fluoroquinolone; FQ-R: fluoroquinolone resistance; INH-R: isoniazid resistance; LPA: line-probe assay; MDR: multidrug resistance; MDR-TB: multidrug-resistant tuberculosis; MTBC: *Mycobacterium tuberculosis* complex; RIF-R: rifampicin resistance; SL-LPA: second-line line-probe assay; TB: tuberculosis.

^a See *GLI training package on DST by phenotypic and molecular methods* (<https://stoptb.org/wg/gli/trainingpackages.asp>) and *GLI guide for the interpretation and reporting of line probe assays* (<https://stoptb.org/wg/gli/gat.asp>) (8).

The Xpert MTB/RIF test and Xpert Ultra test are monitored in a similar way (as shown in Table A6), except that the number and proportion of “trace” results are only assessed for the Xpert Ultra test. Where possible, countries should collect disaggregated data according to the population group tested (e.g. HIV-positive, multidrug-resistant tuberculosis [MDR-TB] risk and extrapulmonary TB).

If the quality indicator for error rates exceeds the target value, it should be further disaggregated to identify common error codes, to assist with corrective and preventive actions. The GeneXpert platform produces electronic data; therefore, a data connectivity solution should be established to allow remote monitoring of quality indicators. More information on the advantages of remote monitoring can be found in Section 2.6.1.

The quality indicators for the Truenat tests, shown in Table A7, are modelled on the indicators for the Xpert MTB/RIF test. An unexpectedly high frequency of errors may indicate that retraining of technicians is required or that the instruments require servicing. The Truelab analyser manual includes a table of possible errors and their interpretations.

The indicators listed in Table A8 are recommended for TB loop-mediated isothermal amplification (TB-LAMP) testing; they should be collected and analysed on a monthly basis, in addition to the general quality indicators. Where possible, countries should collect disaggregated data according to the population group tested (e.g. HIV-positive or extrapulmonary TB).

Table A6. Quality indicators for Xpert MTB/RIF and Xpert Ultra

Indicator ^a	Description	Target
Number of tests performed per month	–	–
Number and proportion of specimens with MTBC detected	Number of specimens with MTBC detected/total number of specimens tested	Dependent on population tested and TB prevalence
Number and proportion of MTBC detected trace (for Ultra test)	Number of specimens with MTBC detected trace/total number of specimens tested	Dependent on population tested and TB prevalence
Number and proportion of specimens with MTBC detected, RIF-R not detected	Number of specimens with MTBC detected, RIF-R not detected/total number of specimens with MTBC detected	Dependent on population tested and RIF-R prevalence
Number and proportion of specimens with MTBC detected, RIF-R detected	Number of specimens with MTBC detected, RIF-R detected/total number of specimens with MTBC detected	Dependent on population tested and RIF-R prevalence
Number and proportion of specimens with MTBC detected, RIF indeterminate	Number of specimens with MTBC detected, RIF indeterminate/total number of specimens tested	Dependent on population tested and RIF-R prevalence
Number and proportion of specimens with MTBC not detected	Number of specimens with MTBC not detected/total number of specimens tested	Dependent on population tested and TB prevalence
Number and proportion of specimens with errors	Number of specimens with errors/total number of specimens tested	<3%
Number and proportion of specimens with invalid results	Number of specimens with invalid results/total number of specimens tested	<1%
Number and proportion of specimens with no results	Number of specimens with no results/total number of specimens tested	<1%
Laboratory turnaround time	Time between receipt of specimen for Xpert at the laboratory and result reported	2–24 hours
Number and % of patients with RIF-R detected tested for resistance to FQ on-site or by referral	Number of patients with RIF-R detected tested for resistance to FQ on-site or by referral/number of MTBC detected, RIF-R detected	100%

FQ: fluoroquinolone; MTBC: Mycobacterium tuberculosis complex; RIF: rifampicin; RIF-R: rifampicin resistance; TB: tuberculosis.

^a See *Practical guide to implementing a quality assurance system for Xpert MTB/RIF testing* (<https://stoptb.org/wg/gli/gat.asp>) (9) and *GLI planning for country transition to Xpert MTB/RIF Ultra cartridges* (<https://stoptb.org/wg/gli/gat.asp>) (10).

Table A7. Quality indicators for Truenat tests

Indicator	Description	Target
Trueprep		
Number and proportion of specimens for which DNA extraction was unsuccessful	Number of specimens for which DNA could not be extracted/total number of specimens processed. Errors should be stratified by type, to enable troubleshooting	Initial test: <3% Repeat test: <1%
Truenat MTB or MTB Plus		
Number and proportion of specimens with MTBC detected	Number of specimens with MTBC detected/total number of specimens tested with successful results	Dependent on population tested and TB prevalence
Number and proportion of specimens with MTBC not detected	Number of specimens with MTBC not detected/total number of specimens tested with successful results	Dependent on population tested and TB prevalence
Number and proportion of specimens with unsuccessful results (errors, invalid, no results)	Number of specimens with unsuccessful results/total number of specimens tested. Errors should be stratified by type, to enable troubleshooting	Initial test: <10% Repeat test: <3%
Truenat MTB-RIF Dx		
Number and proportion of specimens with RIF-R not detected	Number of specimens with RIF-R not detected/total number of specimens tested with successful results	Dependent on population tested and RIF-R prevalence
Number and proportion of specimens with RIF-R detected	Number of specimens with RIF-R detected/total number of specimens tested with successful results	Dependent on population tested and RIF-R prevalence
Number and proportion of specimens with RIF indeterminate	Number of specimens with RIF indeterminate/total number of specimens tested for RIF-R	Dependent on population tested (e.g. proportion of patients with smear-negative TB)
Number and proportion of specimens with unsuccessful results (errors, invalid, no result)	Number of specimens with unsuccessful results/total number of specimens tested. Errors should be stratified by type, to enable troubleshooting	<3% for Truenat MTB or MTB Plus test Initial RIF-Dx test: <7% if reflexed from Truenat MTB Initial RIF-Dx test: <15% if reflexed from Truenat MTB Plus
Number and % of patients with RIF-R detected tested for FQ-R on-site or by referral	Number of patients with RIF-R detected tested for FQ-R on-site or by referral/number of MTBC detected, RIF-R detected	100%
Laboratory turnaround time ^b	Time between receipt of specimen for Truenat at the laboratory and result reporting (mean, range and 90th percentile)	2–24 hours

DNA: deoxyribonucleic acid; FQ-R: fluoroquinolone resistance; MTBC: *Mycobacterium tuberculosis* complex; RIF: rifampicin; RIF-R: rifampicin resistance; TB: tuberculosis.

^a See *Practical guide to implementation of Truenat tests* (<https://stoptb.org/wg/gli/gat.asp>) (11).

^b For troubleshooting, analyse turnaround time for the individual processes (e.g. time from specimen receipt to completion of DNA extraction using Trueprep).

Table A8. Quality indicators for TB-LAMP

Indicator ^a	Description	Target
Number of tests performed per month	–	–
Number and proportion of specimens with MTBC detected	Number of specimens with MTBC detected/total number of specimens tested	Dependent on population tested and TB and HIV prevalence
Number and proportion of specimens with invalid results or no results	Number of specimens with invalid or no results/total number of specimens tested	<1%
Laboratory turnaround time	Time between receipt of specimen for TB-LAMP at the laboratory and result reporting	2–24 hours

HIV: human immunodeficiency virus; LAMP: loop-mediated isothermal amplification; MTBC: *Mycobacterium tuberculosis* complex; TB: tuberculosis.

^a See *GLI practical guide to TB laboratory strengthening* (<https://stoptb.org/wg/gli/gat.asp>) (12).

The indicators listed in Table A9 are recommended for lateral flow lipoarabinomannan (LF-LAM) testing and should be collected and analysed on a monthly basis, in addition to the general quality indicators. Where possible, countries should collect disaggregated data according to the population group tested (e.g. pulmonary or extrapulmonary TB) and by inpatient and outpatient settings.

Table A9. Quality indicators for urine LF-LAM testing

Indicator	Description	Target
Number of tests performed per month	–	–
Number and proportion of specimens with LAM detected	Number of specimens with LAM detected/total number of specimens tested	Dependent on population tested and TB prevalence among HIV-positive patients
Number and proportion of specimens with invalid results or no results	Number of specimens with invalid or no results/total number of specimens tested	Not enough evidence for global guidance
Number and % of patients with LAM detected tested using an mWRD on-site or by referral	Number of patients with LAM detected tested using an mWRD on-site or by referral/ number of patients with LAM detected	100% of patients capable of producing a specimen for testing using an mWRD
Laboratory turnaround time	Time between receipt of specimen at the laboratory and result reporting	1–24 hours

HIV: human immunodeficiency virus; LAM: lipoarabinomannan; LF-LAM: lateral flow lipoarabinomannan assay; mWRD: molecular WHO-recommended rapid diagnostic test; TB: tuberculosis; WHO: World Health Organization.

^a See *Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV* (<https://stoptb.org/wg/gli/pgiqas.asp>) (13).

The indicators and targets shown in Table A10 are modelled after the Xpert MTB/RIF test; they may need to be adjusted as information on the use of moderate complexity automated nucleic acid amplification tests (MC-aNAATs) in routine diagnostic settings becomes available.

Table A10. Quality indicators for MC-aNAATs^a

Indicator	Description	Target
Number of tests performed per month	–	–
Number and proportion of samples with MTBC detected	Number of samples with MTBC detected/number of samples tested	Dependent on population tested and TB prevalence
Number and proportion of MTBC detected, resistance not detected	Number of MTBC detected, resistance not detected/number of MTBC detected	Dependent on population tested and country drug resistance prevalence
Number and proportion of MTBC detected, RIF-R detected	Number of MTBC detected, RIF-R detected/number of MTBC detected	Dependent on population tested and RIF-R prevalence
Number and proportion of MTBC detected, INH-R detected	Number of MTBC detected, INH-R detected/number of MTBC detected	Dependent on population tested and INH-R prevalence
Number and proportion of MTBC detected, MDR detected	Number of MTBC detected, MDR detected/number of MTBC detected	Dependent on population tested and MDR-TB prevalence
Number and proportion of MTBC detected, resistance indeterminate	Number of MTBC detected, resistance indeterminate/number of MTBC detected	<5%
Number and proportion of MTBC not detected	Number of MTBC not detected/number of samples tested	Dependent on population tested and TB prevalence
Number and proportion with uninterpretable results	Number with uninterpretable results/number of samples tested	<5%
Laboratory turnaround time	Time between receipt of specimen for LPA at the laboratory and result reporting (mean, range and 90th percentile)	1–2 days (longer if batching of tests)
Number and proportion of patients with RIF-R detected tested for resistance to FQ on-site or by referral	Number of patients with RIF-R detected tested for resistance to FQ on-site or by referral/number of MTBC detected, RIF-R detected	100%
Number and proportion of patients with INH-R detected tested for resistance to FQ on-site or by referral	Number of patients with INH-R detected tested for resistance to FQ on-site or by referral/number of MTBC detected, INH-R detected	Setting specific

FQ: fluoroquinolone; INH-R: isoniazid resistance; LPA: line-probe assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR: multidrug resistance; MDR-TB: multidrug-resistant tuberculosis; MTBC: *Mycobacterium tuberculosis* complex; RIF-R: rifampicin resistance; TB: tuberculosis; USA: United States of America; WHO: World Health Organization.

^a At the time of publication, WHO-recommended MC-aNAATs included RealTime MTB and MTB RIF/INH (Abbott Molecular, Des Plaines, IL, USA), BD MAX MDR-TB (Becton Dickinson, Sparks, MD, USA), FluoroType MTB and MTBDR (Bruker/Hain Lifescience, Nehren, Germany) and cobas MTB and MTB RIF/INH (Roche Molecular Diagnostics, Pleasanton, CA, USA).

The indicators and targets shown in Table A11 are modelled after the Xpert MTB/RIF test; they may need to be adjusted as information on the use of low complexity automated nucleic acid amplification tests (LC-aNAATs) in routine diagnostic settings becomes available.

Table A11. Quality indicators for LC-aNAATs^a

Indicator	Description	Target
Number of tests performed per month	–	–
Number and proportion of samples with MTBC detected	Number of samples with MTBC detected/number of samples tested	Dependent on population tested and TB prevalence
Number and proportion of MTBC detected, resistance not detected	Number of MTBC detected, resistance not detected/number of MTBC detected	Dependent on population tested and country drug resistance prevalence
Number and proportion of MTBC detected, FQ-R detected	Number of MTBC detected, FQ-R detected/number of MTBC detected	Dependent on population tested and FQ-R prevalence
Number and proportion of MTBC detected, INH-R detected	Number of MTBC detected, INH-R detected/number of MTBC detected	Dependent on population tested and INH-R prevalence
Number and proportion of MTBC detected, ETO-R detected	Number of MTBC detected, ETO-R detected/number of MTBC detected	Dependent on population tested and ETO-R prevalence
Number and proportion of MTBC detected, AMK-R detected	Number of MTBC detected, AMK-R detected/number of MTBC detected	Dependent on population tested and AMK-R prevalence
Number and proportion of MTBC detected, MDR detected	Number of MTBC detected, MDR detected/number of MTBC detected	Dependent on population tested and MDR-TB prevalence
Number and proportion of MTBC detected, resistance indeterminate	Number of MTBC detected, resistance indeterminate/number of MTBC detected	<5%
Number and proportion of MTBC not detected	Number of MTBC not detected/number of samples tested	Dependent on population tested and TB prevalence
Number and proportion of specimens with errors	Number of specimens with errors/total number of specimens tested	<3%
Number and proportion of specimens with invalid results	Number of specimens with invalid results/total number of specimens tested	<1%
Number and proportion of specimens with no results	Number of specimens with no results/total number of specimens tested	<1%
Laboratory turnaround time	Time between receipt of specimen for LPA at the laboratory and result reporting (mean, range and 90th percentile)	2–24 hours

AMK-R: amikacin resistance; ETO-R: ethionamide resistance; FQ-R: fluoroquinolone resistance; INH-R: isoniazid resistance; LC-aNAAT: low complexity automated nucleic acid amplification test; LPA: line-probe assay; MDR: multidrug resistance; MDR-TB: multidrug-resistant tuberculosis; MTBC: *Mycobacterium tuberculosis* complex; TB: tuberculosis; USA: United States of America; WHO: World Health Organization.

^a At the time of publication, WHO-recommended LC-aNAATs included the Xpert MTB/XDR test (Cepheid, Sunnyvale, CA, USA).

The indicators and targets shown in Table A12 are modelled after FL-LPAs and SL-LPAs; they may need to be adjusted as information on the use of high complexity reverse hybridization nucleic acid amplification tests (HC-rNAATs) in routine diagnostic settings becomes available.

Table A12. Quality indicators for HC-rNAATs^a

Indicator ^b	Description	Target
Number of tests performed per month	–	–
Number and proportion of samples with control band detected	Number of samples with control band detected/ number of samples tested	100%
Number and proportion of MTBC detected, PZA-R detected	Number of MTBC detected, PZA-R detected/number of MTBC detected	Dependent on population tested and PZA-R prevalence
Number and proportion of MTBC detected, resistance not detected	Number of MTBC detected, resistance not detected/ number of MTBC detected	Dependent on population tested and PZA-R prevalence
Number and proportion of MTBC detected, resistance indeterminate	Number of MTBC detected, resistance indeterminate/ number of MTBC detected	<5%
Number and proportion with uninterpretable results	Number with uninterpretable results/number of samples tested	<5%
Laboratory turnaround time	Time between receipt of specimen for LPA at the laboratory and result reporting (mean, range and 90th percentile); for indirect tests, add the culture turnaround time for the total turnaround time	1–2 days (longer if culturing required)

HC-rNAAT: high complexity reverse hybridization nucleic acid amplification test; LPA: line-probe assay; MTBC: *Mycobacterium tuberculosis* complex; PZA-R: pyrazinamide resistance; WHO: World Health Organization.

^a At the time of publication, WHO-recommended HC-rNAATs included the Genoscholar PZA-TB II test (NIPRO Corporation, Osaka, Japan).

For interferon gamma release assays (IGRAs), targets should be set for all indicators that are monitored, and any unexplained change in quality indicators (e.g. an increase in error rates or a change in positivity rate) should be documented and investigated. Targets may need to be adjusted as information on the use of these test in routine diagnostic settings becomes available.

Table A13. Quality indicators for IGRAs

Indicator	Description	Target
Number of samples received per month	–	–
Number of tests performed per month	–	–
Number and proportion of rejected samples ^a	Number of rejected samples/ total number of specimens received	<3%
Number and proportion of positive samples	Number of positive samples/ number of samples tested	Dependent on population tested
Number and proportion of samples with valid standard curves	Number of samples with valid standard curves/number of samples tested	<5%
Number and proportion of samples with indeterminate results	Number of samples with indeterminate results/number of samples tested	<5%
Number and proportion of specimens with errors ^b	Number of specimens with errors/total number of specimens tested	<3% (Xpert)
Number and proportion of specimens with invalid results	Number of specimens with invalid results/total number of specimens tested	<1% (Xpert)
Laboratory turnaround time ^c	Time between receipt of specimen for LPA at the laboratory and result reporting (mean, range and 90th percentile)	1–2 days

ELISA: enzyme-linked immunosorbent assay; IGRA: interferon gamma release assay; LPA: line-probe assay.

^a Stratify by reason rejected (e.g. insufficient volume, haemolyzed, incorrect collection tube or received past cutoff) to enable troubleshooting.

^b Stratify errors by type to enable troubleshooting.

^c For troubleshooting, analyse the time from blood collection to receipt in the laboratory, the time from blood collection to completion of the immune stimulation and the time for the interferon gamma detection step (e.g. ELISA).

References for Annex 2

- 1 Laboratory diagnosis of tuberculosis by sputum microscopy – the GLI handbook. Geneva: Global Laboratory Initiative; 2014 (<https://stoptb.org/wg/gli/gat.asp>).
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Annex 3. Quality assurance components for TB diagnostic tests

Test	Quality indicator monitoring ^a	Quality control	Proficiency testing	On-site supervision	Blinded re-checking	Source of training materials (reference)
Smear microscopy, light	Monthly	QC of in-house prepared stains Incoming QC of new batches of commercial stains One positive and one negative slide tested with each batch of slides stained and examined Cross-check results with a second reader before releasing report (on all or a portion of results)	Recommended at least once per year Provided by NICD South Africa, SRLs	Usually quarterly with NTP site visits for data collection	Recommended Slide sampling usually quarterly Re-staining of slides may be considered	<i>External quality assessment for AFB smear microscopy</i> (https://stoptb.org/wg/gli/assets/documents/External%20Quality%20Assessment%20for%20AFB%20Smear%20Microscopy.pdf) (1)
Smear microscopy, FM	Monthly	QC of in-house prepared stains Incoming QC of new batches of commercial stains One positive and one negative slide tested with each batch of slides stained and examined Cross-check results with a second reader before releasing report (on all or a portion of results)	Recommended at least once per year Provided by NICD South Africa, SRLs	Usually quarterly with NTP site visits for data collection	Recommended Slide sampling may be monthly or quarterly Re-staining of slides may be considered	<i>External quality assessment for AFB smear microscopy</i> (https://stoptb.org/wg/gli/assets/documents/External%20Quality%20Assessment%20for%20AFB%20Smear%20Microscopy.pdf) (1)

Test	Quality indicator monitoring ^a	Quality control	Proficiency testing	On-site supervision	Blinded re-checking	Source of training materials (reference)
Solid culture	Monthly	<p>QC of in-house prepared media and reagents</p> <p>Incoming QC of new batches of commercial media</p> <p>Process one well-characterized known positive sample (drug-susceptible MTBC) and one negative sample (decontamination solution, water or PBS) with each batch of specimens processed for culture</p> <p>Cross-check results with a second reader before releasing report (on all or a portion of results)</p>	<p>PT for culture is not recommended</p> <p>PT for identification may be done using MTBC and NTM isolates (provided by CAP)</p>	<p>For NRL, may be provided by SRLs or other partners providing technical assistance</p> <p>For other facilities, NRL or other experienced referral laboratory should provide at least annual site visits</p>	Not recommended	<p><i>Training package on culture in solid and liquid media</i> (https://istoptb.org/wg/gli/documents.asp?xpid=2) (2)</p>
Liquid culture	Monthly	<p>QC of in-house prepared reagents (e.g. decontamination solutions)</p> <p>Incoming QC of new batches of commercial media</p> <p>Process one well-characterized known positive sample (drug-susceptible MTBC) and one negative sample (decontamination solution, water or other bacteria) with each batch of specimens processed for culture</p> <p>Internal QC: Cross-check results with a second reader before releasing report (on all or a portion of results)</p>	<p>PT for culture is not recommended</p> <p>PT for identification may be done using TB and non-TB isolates (provided by CAP)</p>	<p>For NRL, may be provided by SRLs or other partners providing technical assistance</p> <p>For other facilities, NRL or other experienced referral laboratory should provide at least annual site visits</p>	Not recommended	<p><i>Training package on culture in solid and liquid media</i> (https://istoptb.org/wg/gli/documents.asp?xpid=2) (2)</p> <p><i>MGIT procedure manual</i> (https://www.finddx.org/wp-content/uploads/2016/02/mgit_manual_nov2006.pdf) (3)</p>

Annex 3. Quality assurance components for TB diagnostic tests

Species identification tests	Monthly	<p>Incoming QC of new batch</p> <p>Process positive culture controls included in batch, and add positive (MTBC) and negative (NTM – <i>M. avium</i>, <i>M. intracellulare</i>, or <i>M. kansasii</i>) samples to immunochromatographic assays</p> <p>Cross-check results with a second reader before releasing report (on all or a portion of results)</p>	Species identification is included in culture and DST PT	Provided as part of liquid culture supervision	Not recommended	<p><i>Training package on culture in solid and liquid media</i> (https://stoptb.org/wg/gli/documents.asp?xpan=2) (2)</p>
Culture-based DST for first-line drugs	Monthly	<p>QC of in-house prepared media and reagents</p> <p>Incoming QC of new batches of commercial media</p> <p>Process one well-characterized known positive sample (drug-susceptible MTBC) and one negative sample (decontamination solution, water or other bacteria) with each batch of specimens processed for culture</p> <p>Internal QC: Cross-check results with a second reader before releasing report (on all or a portion of results)</p>	<p>Recommended at least once per year</p> <p>Provided by SRLs, once per year</p> <p>Other providers available (e.g. UK NEQAS, NICD South Africa, CDC)</p>	<p>For NRL, may be provided by SRLs or other partners providing technical assistance</p> <p>NRL or experienced referral laboratories should undertake regular on-site supervision visits</p>	<p>Laboratory should establish formal link with SRL; SRLs may re-check a proportion of isolates for DST</p> <p>Expected levels of agreement for RIF and INH DST are >95%, and acceptable agreement for other drugs should be established</p>	<p><i>Training package on DST by phenotypic and molecular methods</i> (https://stoptb.org/wg/gli/trainingpackages.asp) (4)</p> <p><i>MGIT procedure manual</i> (https://www.finddx.org/wp-content/uploads/2016/02/mgit_manual_nov2006.pdf) (3)</p>

Test	Quality indicator monitoring ^a	Quality control	Proficiency testing	On-site supervision	Blinded re-checking	Source of training materials (reference)
Culture-based DST for second-line drugs	Monthly	QC of in-house prepared media and reagents Incoming QC of new batches of commercial media Process one well-characterized known drug-susceptible MTBC and a well-characterized drug-resistant MTBC Internal QC: Cross-check results with a second reader before releasing report (on all or a portion of results)	Recommended at least once per year Provided by SRLs	May be provided to NRL by SRL experienced in SL DST	Laboratory should establish formal link with SRLs; SRLs may re-check a proportion of isolates for SL DST Expected level of agreement for each drug should be established	Training package on DST by phenotypic and molecular methods (https://stoptb.org/wg/gli/trainingpackages.asp) (4)
Line-probe assays <ul style="list-style-type: none"> • FL-LPA for RIF and INH • SL-LPA, for FQs and AMK • HC-rNAAT for PZA 	Monthly	Incoming QC of new batches Process a positive control using an aliquot of a previously extracted DNA from a well-characterized drug-susceptible MTBC strain and a blank with PBS as sample (negative control) Include a negative PCR control in every batch using molecular grade water Internal QC: check each strip for the presence of the controls (must be present in ALL including negatives) to ensure quality of hybridization and PCR reagents Check strip from patient and positive control for the presence of the TB control band to ensure presence of MTBC Cross-check results with a second reader before releasing report (on all or a portion of results)	Recommended at least once per year Provided by SRLs	For NRL, may be provided by SRLs or other partners providing technical assistance NRL or experienced referral laboratories should undertake regular on-site supervision visits	Not recommended	Training package on LPA (MTBDRplus v2) (http://www.stoptb.org/wg/gli/documents.asp?xpaand=2) (5)

Annex 3. Quality assurance components for TB diagnostic tests

TB-LAMP	Monthly	Incoming QC of new batches Process a positive control using an aliquot of a previously extracted DNA from a well-characterized drug-susceptible MTBC and a blank with molecular grade water as sample (negative control) Cross-check results with a second reader before releasing report (on all or a portion of results)	Recommended at least once per year	NRL or experienced referral laboratories should undertake regular on-site supervision visits	Not recommended	<i>GLI practical guide to laboratory strengthening</i> (https://stoptb.org/wg/gli/gat.asp) (6)
Xpert MTB/RIF Xpert Ultra Xpert MTB/XDR	Monthly Remote monitoring using diagnostics connectivity is recommended	Incoming QC of new batch Internal QC: Cross-check results for transcription errors on manually reported results (on all or a portion of results)	Recommended at least once per year	NRL or experienced referral laboratories should undertake regular on-site supervision visits	Not recommended due to insufficient sample remaining after testing	<i>GLI Xpert MTB/RIF training package</i> (https://stoptb.org/wg/gli/trainingpackages.asp) <i>Practical guide to implementing a quality assurance system for Xpert MTB/RIF testing</i> (https://stoptb.org/wg/gli/pglqas.asp)
Truenat MTB, MTB Plus and MTB-RIF Dx	Monthly	Incoming QC of new batch Internal QC: Cross-check results for transcription errors on manually reported results (on all or a portion of results)	Recommended at least once per year	NRL or experienced referral laboratories should undertake regular on-site supervision visits	Not recommended due to insufficient sample remaining after testing	<i>Practical guide to implementation of Truenat tests</i> (https://stoptb.org/wg/gli/gat.asp) (7)
MC-aNAAT	Monthly	Incoming QC of new batch Internal QC: Cross-check results for transcription errors on manually reported results (on all or a portion of results) Process one well-characterized known positive sample and one negative sample with each batch of tests	Recommended at least once per year	NRL or experienced referral laboratories should undertake regular on-site supervision visits	Not recommended due to insufficient sample remaining after testing	Modelled after Xpert NTB/RIF QA components

Test	Quality indicator monitoring ^a	Quality control	Proficiency testing	On-site supervision	Blinded re-checking	Source of training materials (reference)
LF-LAM	Monthly	Incoming QC of new batch Internal QC: check each strip for the presence of the control bar; if the control bar does not turn purple/grey by assay completion, the test result is invalid Cross-check results with a second reader before releasing report (on all or a portion of results) Process one known positive sample and one negative sample weekly	Recommended at least once per year	NRL or experienced regional referral laboratories should undertake regular on-site supervision visits of test laboratories	Not recommended	http://www.alere.com/en/home/product-details/determine-tb-lam.html <i>Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV</i> (https://stop.tb.org/wg/gli/gat.asp) (8)
IGRAS	Monthly	Mitogen tube is positive control and Nil tube is negative control for immune stimulation Internal controls (standard curve) are conducted with each ELISA	Recommended at least once per year Provided by UK NEQAS, INSTAND e.V., and CAP	NRL or experienced regional referral laboratories should undertake regular on-site supervision visits	Not recommended	–

AMK: amikacin; CAP: College of American Pathologists; CDC: Centers for Disease Control and Prevention; DNA: deoxyribonucleic acid; DST: drug susceptibility testing; ELISA: enzyme-linked immunosorbent assay; FL-LPA: first-line line-probe assay; FM: fluorescence microscopy; FQ: fluoroquinolone; HC-rNAAT: high complexity reverse hybridization nucleic acid amplification test; IGRA: interferon gamma release assay; INH: isoniazid; LF-LAM: lateral flow lipoarabinomannan assay; LPA: line-probe assay; M.: *Mycobacterium*; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MTBC: *Mycobacterium tuberculosis* complex; NICD: National Institute for Communicable Diseases; NRL: national TB reference laboratory; NTM: non-tuberculous mycobacteria; NTP: national TB programme; PBS: phosphate-buffered saline; PCR: polymerase chain reaction; PT: proficiency testing; PZA: pyrazinamide; QA: quality assurance; QC: quality control; RIF: rifampicin; SL: second-line; SL-LPA: second-line line-probe assay; SRL: WHO TB Supranational Reference Laboratory; TB: tuberculosis; TB-LAMP: TB loop-mediated isothermal amplification; WHO: World Health Organization.

^a Refer to list of quality indicators in Annex 2.

References for Annex 3

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**For further information,
please contact:**

Global TB Programme
World Health Organization
20, Avenue Appia
CH-1211 Geneva 27
Switzerland
Web site: www.who.int/tb

