WHO operational handbook on tuberculosis

Module 3: Diagnosis

World Health Organization

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Contents

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Abbreviations and acronyms

AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
BCG	bacille Calmette-Guérin (vaccine)
CC	critical concentration
CFP-10	culture filtrate protein 10
CI	confidence interval
CLIA	chemiluminescence immunoassay
CSF	cerebrospinal fluid
CXR	chest radiography (X-ray)
DNA	deoxyribonucleic acid
DR-TB	drug-resistant tuberculosis
DST	drug susceptibility testing
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot
EQA	external quality assessment
ESAT-6	early secretory antigenic target-6 kDa
FIND	Foundation for Innovative New Diagnostics
FL-LPA	first-line drug line probe assay
GDG	guideline development group
GLI	Global Laboratory Initiative
HC-rNAAT	high-complexity reverse hybridization nucleic acid amplification test
GTB	Global Programme on Tuberculosis & Lung Health
HIV	human immunodeficiency virus
Hr-TB	isoniazid-resistant, rifampicin-susceptible tuberculosis
IGRA	interferon-gamma release assay
IT	information technology
LAM	lipoarabinomannan
LAMP	loop-mediated isothermal amplification
LC-aNAAT	low-complexity automated nucleic acid amplification test
LC-mNAAT	low-complexity manual nucleic acid amplification test
LF-LAM	lateral flow urine lipoarabinomannan assay
LMIC	low- and middle-income countries
LoD	limit of detection
LPA	line probe assay
MC-aNAAT	moderate-complexity automated nucleic acid amplification test

MDR/RR-TB	multidrug- or rifampicin-resistant tuberculosis
MDR-TB	multidrug-resistant tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
MIC	minimal inhibitory concentration
mRNA	messenger ribonucleic acid
Mtb	Mycobacterium tuberculosis
MTBC	Mycobacterium tuberculosis complex
mWRD	molecular WHO-recommended rapid diagnostic test
NAAT	nucleic acid amplification test
NGS	next-generation sequencing
NTM	non-tuberculous mycobacteria
NTP	national TB programme
NTRL	national TB reference laboratory
POC	point of care
PPD	purified protein derivative
PPDS	purified protein derivative (standard)
PPV	positive predictive value
QA	quality assurance
QC	quality control
RRDR	rifampicin-resistance determining region
RR-TB	rifampicin-resistant tuberculosis
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SL-LPA	second-line drug line probe assay
SOP	standard operating procedure
SRL	supranational reference laboratory
TB	tuberculosis
TBST	Mycobacterium tuberculosis antigen-based skin test
TPT	tuberculosis preventive treatment
TST	tuberculin skin test
TWG	technical working group
WGS	whole-genome sequencing
WHO	World Health Organization
WHO PQ	WHO prequalification
WRD	WHO-recommended rapid diagnostic test
XDR-TB	extensively drug-resistant tuberculosis

Abbreviations of anti-TB medicines and treatment regimens

AMK	amikacin
BDLC	bedaquiline (B), delamanid (D), linezolid (L) and clofazimine (C)
BDLLfxC	bedaquiline (B), delamanid (D), linezolid (L), levofloxacin (Lfx) and clofazimine (C)
BDQ	bedaquiline
BPaL	bedaquiline (B), pretomanid (Pa) and linezolid (L)
BPaLM	bedaquiline (B), pretomanid (Pa), linezolid (L) and moxifloxacin (M)
CFZ	clofazimine
Cs	cycloserine
DLM	delamanid
EMB	ethambutol
ETO	ethionamide
FQ	fluoroquinolone (e.g. levofloxacin or moxifloxacin)
HREZ	isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z)
INH	isoniazid
LFX	levofloxacin
LZD	linezolid
MPM	meropenem
MFX	moxifloxacin
Pa	pretomanid
PZA	pyrazinamide
REZ	rifampicin (R), ethambutol (E) and pyrazinamide (Z)
RIF	rifampicin
STR	streptomycin
TRD	terizidone

1. Introduction

1.1 Background

Globally, tuberculosis (TB) continues to be a significant public health problem. It has been estimated that about a quarter of the world's population was infected with *Mycobacterium tuberculosis* (*Mtb*) bacteria *(1)*. In 2023, an estimated 10.8 million infected individuals went on to develop TB disease, while 8.2 million were diagnosed and reported to the World Health Organization (WHO) *(2)*. Of those 8.2 million, an estimated 1 million people developed drug-resistant TB (DR-TB) – specifically, the form of DR-TB that is resistant to the first-line antibiotic isoniazid (INH) but susceptible to rifampicin (RIF) (i.e. Hr-TB) – and an additional 410 000 people developed TB disease resistant to both INH and RIF (i.e. multidrug-resistant TB [MDR-TB]), or to RIF alone (i.e. RIF-resistant TB [RR-TB]) *(3)*. Only two in five of the people estimated to have MDR-TB or RR-TB (MDR/RR-TB) were found and initiated on treatment, and only 55% of RR-TB patients received follow-on testing for resistance to the fluoroquinolones (FQs) as essential for diagnosis of pre-extensively drug-resistant TB (pre-XDR-TB). In addition, although the total number of deaths caused by TB fell from 1.32 million in 2022 to 1.25 million in 2023, the disease still probably overtook coronavirus disease (COVID-19) as the world's leading cause of death due to a single infectious agent *(2)*.

In support of addressing these gaps, WHO's global strategy for TB prevention, care and control for 2015–2035 – known as the End TB Strategy *(4)* – calls for the early detection of TB infection, diagnosis of TB and universal access to drug susceptibility testing (DST). To meet the End TB Strategy targets:

- individuals living with TB infection who are at higher risk of progression to active TB should receive TB infection testing to identify those who will benefit most from TB preventive treatment (TPT);
- WHO-recommended rapid diagnostic tests (WRDs) should be made available to all individuals with signs or symptoms of, or who screen positive for, TB;
- individuals with bacteriologically confirmed TB should receive testing for resistance to RIF;
- people with RR-TB should receive testing for resistance to the FQs; and
- individuals with pre-XDR-TB should receive testing for resistance to bedaquiline (BDQ) and linezolid (LZD), and to all other drugs that might be included in their treatment regimen *(3)*.[1]

Section 2 of this handbook outlines TB tests, with WHO recommendations for the detection of TB infection, disease and drug resistance; **Section 3** outlines considerations for the implementation of these tests.

[1] The original End TB Strategy called for the testing of all people with RR-TB for susceptibility to second-line injectable agents (kanamycin, capreomycin and amikacin). However, WHO currently recommends that injectable medicines be phased out of all treatment regimens as a priority and replaced by BDQ; this makes rapid DST for amikacin unnecessary.

In addition, the first WHO standard on universal access to rapid TB diagnostics was issued in 2023 *(3)*. The standard emphasizes the need to undertake a comprehensive approach to diagnostics while following the care cascade and closing all gaps; for example, availability and access to quality-assured testing and DST, testing capacity, rapid turnaround time for results and monitoring of indicators. Furthermore, the framework of indicators and targets for laboratory strengthening under the End TB Strategy *(4)* highlights that all national TB programmes (NTPs) should prioritize the development of a network of TB laboratories that use modern methods of diagnosis (e.g. molecular methods and liquid culture), have efficient referral systems, use electronic data and diagnostics connectivity, use standard operating procedures (SOPs) and appropriate quality assurance (QA) processes, adhere to biosafety principles for all testing and have sufficient human resources. These priorities should be comprehensively addressed in national strategic plans and should be adequately funded. Practical considerations for implementing and strengthening these essential testing programme elements are further detailed in **Section 3** and **Section 4**, respectively.

In addition to universal access to TB diagnostic testing services, both the WHO standard and recent guidelines on TB diagnosis continue to highlight the need for universal DST, especially for the medicines for which mWRDs are available (ideally, performed before treatment is started while not delaying treatment initiation when waiting for results) *(5)*. WHO recently approved the use of targeted next-generation sequencing (NGS) as a follow-on technology for the detection of drug resistance (**Section 2**) *(6)*. Targeted NGS solutions couple the amplification of selected genes with NGS technology, and can detect resistance to many drugs using a single processed sputum sample *(6)*. Because these tests can interrogate entire genes to identify specific mutations associated with resistance, targeted NGS may be more accurate than other mWRDs. In addition to detection of resistance to first-line TB drugs, current recommendations support the use of this technology for the rapid detection of resistance to three of the drugs used in the BPaLM regimen (i.e. BDQ, LZD and moxifloxacin [MFX]); therefore, this handbook includes further implementation considerations and algorithms using this technology. Of note, the fourth BPaL/M drug – pretomanid (Pa) – has recently had criteria established for DST, and these details are also included in this document (**Web Annex B**). Based on current treatment recommendations, countries embarking on interventions to detect and treat DR-TB should, in addition, establish laboratory capacity to perform culture-based phenotypic DST for drugs that are recommended for use in MDR-TB regimens *(7)* and for which there are reliable phenotypic DST methods (e.g. BDQ, LZD, Pa, cycloserine [Cs], clofazimine [CFZ] and delamanid [DLM]). Lastly, countries should expand their capacity to monitor the culture conversion of people being treated for DR-TB.

1.2 Advances in TB diagnostic test development, assessment and WHO recommendations

Over recent decades, considerable effort has gone into building the laboratory, clinical and programmatic capacity to prevent, detect and treat TB infection, TB disease and DR-TB. Many tools and guidance documents have been developed, including the recently consolidated and updated guidelines for the treatment of TB *(8)*; blood-based tests for TB infection that incorporate chemiluminescent methods for result detection; urine-based biomarker

point-of-care (POC) tests for TB detection among people living with HIV; low-complexity tests that can use stool samples from children to detect TB and resistance to RIF; low-complexity tests that laboratories with basic infrastructure can use to detect resistance to RIF, INH, FQs, ethionamide (ETO) and amikacin (AMK); genomic sequencing technologies that incorporate resistance interpretation analyses that consider advances in our understanding of the mechanisms of drug resistance; and consolidated model diagnostic testing algorithms and guidance for implementing testing programmes that are tailored to the purpose of testing and characteristics of the patient population (e.g. age, HIV status and risk of drug resistance).

Until 2024, the operational guidance that supported advancing WHO policy on testing for TB infection, diagnosis and drug resistance was presented separately (in operational handbooks on tests for TB infection, and on tests for TB diagnosis and drug resistance). This handbook is the first to combine and update this operational guidance into a single reference document. In addition, an increasing number of novel TB tests may be used for the purposes listed above; hence, the WHO Global Programme on Tuberculosis & Lung Health (WHO/GTB) has adopted "class-based recommendations" that apply to TB testing products with similar characteristics and performances. In addition, WHO prequalification (PQ) has been established to assess whether each testing product within a class, and the process used to manufacture that product, meet performance and quality standards *(9)*. This approach to TB test assessment, recommendation and PQ is expected to increase competitiveness in price, quality and services.

New TB testing products will continue to be reviewed for class determination by WHO/GTB, by comparing their characteristics with those of the existing diagnostic classes. If the characteristics differ sufficiently to warrant a new class, the product will be assessed as "first in class" using a standardized, Pathway A, WHO/GTB guideline development process, supported by a guideline development group (GDG). If the characteristics are sufficiently similar to an existing class, the product will be characterized as "within class" and may be referred directly to PQ. If a WHO PQ process has not yet been established for a given class, then WHO/GTB will oversee an interim within-class assessment of evidence with the support of the Technical Advisory Group (TAG) on TB Diagnostics and Laboratory Strengthening. Further details on class determination and product assessments are available in the 2025 publication, *WHO consolidated guidelines on tuberculosis: module 3: diagnosis (5)*.

In 2024 and early 2025, WHO oversaw both first-in-class and within-class assessments. Through the first-in-class assessment, two new classes of low-complexity nucleic acid amplification tests were established. Both classes include testing products previously endorsed by WHO for the detection of TB with and without RIF resistance (i.e. Xpert® MTB/RIF Ultra, Molbio Truenat® MTB Plus and MTB-RIF Dx, and TB-LAMP) (**Section 2**). There are also new recommendations on concurrent testing of respiratory and non-respiratory samples among adults and adolescents with HIV, children with HIV, and children without HIV or with unknown HIV status. These populations experience significant burdens of TB, increased risk of TB-associated morbidity and mortality, and challenges with sputum production; in addition, they may produce sputa with low and variable amounts of bacteria. Hence, concurrent testing may increase patient access to testing services through the included use of easy-to-collect urine and stool samples, while improving the accuracy of TB detection by using more than one sample and test.

Practical considerations for implementation of the concurrent testing recommendations are presented in **Section 3** and are reflected in the revised model diagnostic algorithms and decision pathways given in **Section 6**.

In addition, the 2025 within-class assessment evaluated new interferon-gamma release assays (IGRAs) for the detection of TB infection, and new and updated targeted NGS solutions for the detection of DR-TB. WHO recommendations for the class of IGRAs now apply to two new tests (SD Biosensor STANDARD E TB-Feron ELISA and Diasorin/QIAGEN LIAISON QFT-PLUS CLIA), which expand the list of WHO-recommended tests for TB infection and introduce the chemiluminescent detection method for IGRA testing for the first time. The recommendations for the class of targeted NGS apply to one updated solution (Oxford Nanopore Technologies AmPORE-TB) that can now be used to detect resistance to a wider range of TB drugs. Policy statements on the performance of the new technologies are summarized in the relevant portions of **Section 2**, new within-class products are highlighted in the full list of TB diagnostic classes and testing technologies in **Table 2.1**, and the TAG meeting and evidence review reports are provided in **Web Annex D**.

The full list of current TB testing recommendations is presented, with policy details, in the 2025 publication, *WHO consolidated guidelines on tuberculosis: module 3: diagnosis (5)* and in **Table 1.1**.

Table 1.1. Consolidated list of recommendations on TB diagnostics

NEW

1. For adults and adolescents with signs or symptoms of TB or who screened positive[2] for pulmonary TB, low-complexity **automated** NAATs should be used on respiratory samples as initial diagnostic tests for TB, rather than smear microscopy or culture.

   (Strong recommendation, high certainty of evidence)

NEW

2. For people with bacteriologically confirmed TB,[3] low-complexity **automated** NAATs should be used on respiratory samples as initial tests for detection of resistance to rifampicin, rather than culture-based DST.

   (Strong recommendation, high certainty of evidence)

NEW

3. For people with signs and symptoms of TB meningitis, low-complexity **automated** NAATs on cerebrospinal fluid should be used for the initial diagnosis of TB meningitis, rather than smear microscopy or culture.

   (Strong recommendation, moderate certainty of evidence)

[2] Having a positive result of a test, examination or other procedure is used to distinguish people who are highly likely to have TB disease from people who are highly unlikely to have TB. At present, WHO recommends the following as screening tests: chest radiography (chest X-ray; CXR) with or without computer-aided detection (CAD), C-reactive protein in people living with HIV, and molecular WHO-recommended rapid diagnostic test for TB (mWRD).

[3] A bacteriologically confirmed TB case is one from whom a biological specimen is positive by smear microscopy, culture or WRD (such as Xpert MTB/RIF). All such cases should be notified, regardless of whether TB treatment has started *(10)*.

NEW

4. For people with signs and symptoms of extrapulmonary TB, low-complexity **automated** NAATs on lymph node tissue aspirate, pleural tissue, pleural fluid, synovial fluid, peritoneal fluid or pericardial fluid should be used for the initial diagnosis of TB, rather than smear microscopy or culture.

   (Strong recommendation, low certainty of evidence for synovial fluid and pericardial fluid; very low certainty of evidence for lymph node tissue aspirate, pleural tissue, pleural fluid and peritoneal fluid)

NEW

5. For people with signs and symptoms of pulmonary TB, moderate-complexity **automated** NAATs may be used on respiratory samples for detection of pulmonary TB, and of rifampicin and isoniazid resistance, rather than culture and phenotypic DST.

   (Conditional recommendation, moderate certainty of evidence)

NEW

6. For adults and adolescents with signs or symptoms or who screen positive for pulmonary TB, low-complexity **manual** NAATs should be used on respiratory samples as initial diagnostic tests for TB, rather than smear microscopy or culture.

   (Strong recommendation, high certainty of evidence)

7. For adults and adolescents with HIV who have signs or symptoms of TB, screen positive for TB, are seriously ill or have advanced HIV disease, concurrent testing using low-complexity automated NAATs on respiratory samples and LF-LAM on urine samples should be used as the initial diagnostic strategy for diagnosing TB, rather than low-complexity automated NAATs on respiratory samples alone.

   (Strong recommendation, low certainty of evidence)

NEW

8. For children who are HIV-negative or have an unknown HIV status, who have signs or symptoms or screen positive for pulmonary TB, concurrent testing using low-complexity automated NAATs on respiratory and stool samples should be used as the initial diagnostic strategy for diagnosing TB, rather than low-complexity **automated** NAATs on respiratory or stool samples alone.

   (Strong recommendation, low certainty of evidence)

NEW

9. For children with HIV who have signs or symptoms or screen positive for pulmonary TB, concurrent testing using low-complexity **automated** NAATs on respiratory and stool samples and LF-LAM on urine may be used as the initial diagnostic strategy for diagnosing TB, rather than low-complexity automated NAATs on respiratory or stool samples alone.

   (Conditional recommendation, low certainty of evidence)

10. For people with bacteriologically confirmed pulmonary TB, low-complexity automated NAATs may be used on sputum for the initial detection of resistance to isoniazid and fluoroquinolones, rather than culture-based phenotypic DST.

    (Conditional recommendation, moderate certainty of evidence)

11. For people with bacteriologically confirmed pulmonary TB and resistance to rifampicin, low-complexity automated NAATs may be used on sputum for the initial detection of resistance to ethionamide, rather than DNA sequencing of the *inhA* promoter.

    (Conditional recommendation, very low certainty of evidence)

12. For people with bacteriologically confirmed pulmonary TB and resistance to rifampicin, low-complexity automated NAATs may be used on sputum for the initial detection of resistance to amikacin, rather than culture-based phenotypic DST.

    (Conditional recommendation, low certainty of evidence)

13. For people with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test instead of phenotypic culture-based DST, to detect resistance to rifampicin and isoniazid.

    (Conditional recommendation, moderate certainty of evidence)

14. For people with confirmed MDR/RR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones.

    (Conditional recommendation, moderate certainty of evidence)

15. For people with confirmed MDR/RR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to the SLIDs.

    (Conditional recommendation, low certainty of evidence)

16. For people with bacteriologically confirmed TB, high-complexity reverse hybridization-based NAATs may be used on *Mtb* culture isolates for detection of pyrazinamide resistance, rather than culture-based phenotypic DST.

    (Conditional recommendation, very low certainty of evidence)

17. For people with bacteriologically confirmed pulmonary TB disease, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol, rather than culture-based phenotypic DST.

    (Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide] and low [rifampicin, fluoroquinolones and ethambutol])

18. For people with bacteriologically confirmed rifampicin-resistant pulmonary TB disease, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin, rather than culture-based phenotypic DST.

    (Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate [ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin] and very low [amikacin])

19. *Mycobacterium tuberculosis* antigen-based skin tests may be used to test for TB infection.

    (Conditional recommendation, very low certainty of evidence)

20. Either a tuberculin skin test or an interferon-gamma release assay can be used to test for TB infection.

    (Strong recommendation, very low certainty of evidence)

21. Interferon-gamma release assays (IGRAs) and tuberculin skin tests (TSTs) should not be used in low- and middle-income countries for the diagnosis of pulmonary or extrapulmonary TB or for the diagnostic work-up of adults (including people living with HIV) with suspected active TB.

(Strong recommendation)

DNA: deoxyribonucleic acid; DST: drug susceptibility testing; HIV: human immunodeficiency virus; LF-LAM: lateral flow urine lipoarabinomannan assay; LPA: line probe assay; MDR/RR-TB: multidrug-resistant or rifampicin-resistant TB; *Mtb*: *Mycobacterium tuberculosis*; MTBC: *Mycobacterium tuberculosis* complex; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; SL-LPA: second-line line probe assay; SLID: second-line injectable drug; TB: tuberculosis; WHO: World Health Organization.

1.3 Scope of this operational handbook

This handbook was developed to provide practical guidance for the implementation of WHO policies on recommended TB diagnostic tests and algorithms; it describes:

- the WHO-recommended tests for detecting TB and DR-TB (**Section 2**), the most recent WHO policy guidance for their use, and consideration for implementing diagnostics (**Section 3**), and their placement in a network (**Section 4**);
- the WHO-recommended tests for TB infection, the most recent WHO policy guidance for their use, and considerations for who should be tested, why, and how under programmatic settings (**Section 2** and **Section 3**);
- the programmatic steps that need to be taken to implement and scale up a new diagnostic tool (**Section 5**); and
- TB diagnostic model algorithms that incorporate the most recent WHO recommendations for detection and clinically managing TB, DR-TB, and TB infection, as well as considerations for new algorithm implementation (**Section 6**).

The handbook is not intended to be a comprehensive manual, nor does it repeat information provided by other guidance documents. For those interested in learning more or reviewing TB diagnostic content, WHO has an online e-learning course *(11)* that will complement the content of the operational handbook. The self-paced course covers all the major topics in this handbook and includes videos covering the different tests *(11)*. Guidance on the implementation of diagnostic testing is also available on the website of the Global Laboratory Initiative (GLI) of the Stop TB Partnership for which WHO/GTB serves as the Secretariat *(12)*.

1.4 Target audience

The target audience for this handbook includes ministry of health officials, donors, implementing partners, programme managers, laboratory managers, clinicians, civil society and community organizations and other key stakeholders engaged in TB laboratory strengthening or programme support.

This handbook is intended for use by TB and HIV programmes, and by other experts involved in planning and implementing new or expanded programmes for TB detection or infection testing. It is also intended for people involved in training, monitoring and supervision, and

for providers performing these tests. The annexes provide additional details, resources and educational materials for providers and patients.

1.5 New in this version: a summary of changes

This operational handbook consolidates, for the first time, WHO's consolidated guidelines on rapid diagnostics for TB detection *(6)* and tests for TB infection *(13)*. Also, the contents have been revised to support the most recent, evidence-based WHO policy guidance that is described in the 2025 publication, *WHO consolidated guidelines on tuberculosis: module 3: diagnosis (5)*. A high-level summary of the changes made between the third and fourth versions of this handbook is presented in **Table 1.2**.

Table 1.2. Summary of changes in this *WHO operational handbook on tuberculosis. Module 3: diagnosis*

Description of change

This fourth version of the operational handbook:
• for the first time, consolidates operational handbooks on tests for TB diagnosis and tests for TB infection into a single document, with harmonization of content;
• introduces a summary table listing all current WHO recommendations for the diagnosis of TB infection and disease, and the detection of drug resistance (**Table 1.1**);
• has additional tables to define each class of diagnostics (**Section 2**);
• includes two new classes of TB diagnostic tests used for the initial detection of TB, with and without detection of RIF resistance (low-complexity automated NAATs [LC-aNAATs] and low-complexity manual NAATs [LC-mNAATs]), summarizing content for within-class tests Xpert® MTB/RIF, Xpert MTB/RIF Ultra, Truenat® MTB Plus and Truenat MTB-RIF Dx (LC-aNAAT), and for TB-LAMP (LC-mNAAT) tests (**Sections 2–4**);
• includes new and updated testing products in the IGRA and targeted NGS classes of diagnostics (**Section 2**), based on a January 2025 WHO product assessment (the findings of which are summarized in meeting reports in **Web Annex D**);
• includes operational considerations for the new WHO recommendations on concurrent testing among people (of all ages) living with HIV, and among children without HIV or with unknown HIV status (**Section 3**, Algorithm 1);
• includes a new subsection on QA specific to TB testing (**Section 3**);
• provides revisions to the model diagnostic algorithms (**Section 6**):
  ▪ Algorithm 1 is now presented in two parts:
  – the first part outlines sample collection and initial testing pathways for different populations, depending on their HIV status and age (reflecting new concurrent testing recommendations for people living with HIV and children, including the use of respiratory samples and stool on low complexity nucleic acid amplification tests and urine on the lateral flow urine lipoarabinomannan assay [LF-LAM]);
  – the second part guides interpretation of test results pathways, including those for both molecular and biomarker-based tests;
  ▪ Algorithm 2 is specific to DST in settings with and without access to targeted NGS;
  ▪ Algorithm 3 addresses follow-on drug-resistance testing and interpretation for people with RIF-susceptible TB who are at risk of resistance to other anti-TB drugs; and
  ▪ Algorithm 4 addresses testing for TB infection and interpretation of results.
• Content from the previous "Annex 5" has been moved into the References section of the main document.

2. TB tests with WHO recommendations

This section provides brief descriptions of WHO-recommended technologies for the detection of TB, DR-TB and TB infection. It also summarizes WHO recommendations for such technologies; these recommendations are more thoroughly discussed in the latest versions of the *WHO consolidated guidelines on tuberculosis: module 3: diagnosis (5)* and the *WHO consolidated guidelines on tuberculosis: module 1: prevention (14)*.

In 2020, WHO changed from product-specific TB testing recommendations to class-based recommendations, with products grouped into classes based on their:

- purpose of use (e.g. detection of *Mtb* or drug resistance);
- principle of action;
- infrastructure and human resource requirements;
- complexity (i.e. of the testing procedure and associated instrumentation);
- reporting method (automated versus manual); and
- intended setting of use (e.g. reference, peripheral or low-complexity laboratory; POC or near POC).

Since that time, nine classes of TB tests have been established, encompassing more than 25 recommended products.

The full list of classes and their products are organized by their intended use and presented in **Table 2.1** as:

- initial tests for diagnosis of TB:
  - with drug-resistance detection;
  - without drug-resistance detection;
- follow-on drug-resistance tests after bacteriological confirmation of TB; and
- tests for TB infection.

Table 2.1. Classes of TB testing technologies and products included in current guidelines

Purpose of classes	Technology class	Included products[a]
Initial tests for TB diagnosis with drug-resistance detection	**NEW:** Low-complexity automated nucleic acid amplification tests (LC-aNAATs) for detection of TB and resistance to RIF	Xpert® MTB/RIF Ultra (Cepheid)
		Truenat® MTB Plus and Truenat MTB-RIF Dx (Molbio)
	Moderate-complexity automated nucleic acid amplification tests (MC-aNAATs) for detection of TB, and resistance to RIF and INH	RealTi*me*® MTB and RealTi*me* MTB RIF/INH (Abbott)
		BD MAX™ MDR-TB (Becton Dickinson)
		cobas® MTB and cobas MTB-RIF/INH (Roche)
		FluoroType® MTB and FluoroType MTBDR (Bruker-Hain)
Initial tests for TB diagnosis without drug-resistance detection	**NEW:** Low-complexity manual nucleic acid amplification tests (LC-mNAATs) for detection of TB	Loopamp™ MTBC Detection Kit (TB-LAMP) (Eiken Chemical)
	Antigen detection in a lateral flow format (biomarker-based detection; LF-LAM) for detection of TB	Determine™ TB LAM Ag (Alere/Abbott)
Follow-on tests for detection of TB drug resistance	Low-complexity automated nucleic acid amplification tests (LC-aNAATs) for detection of resistance to INH and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid)
	Line probe assays (LPAs) – for detection of TB drug resistance	GenoType® MTBDRplus v2 (Bruker-Hain)
		GenoType MTBDRsl (Bruker-Hain)
		Genoscholar™ NTM+MDRTB II (Nipro)
	High complexity reverse hybridization based NAATs (HC-rhNAAT)	Genoscholar PZA-TB II (Nipro)

Purpose of classes	Technology class	Included products[a]
	Targeted next-generation sequencing (NGS) for detection of resistance to first-line and second-line anti-TB agents[b]	Deeplex® Myc-TB (GenoScreen/Illumina)
		****UPDATED** AmPORE-TB® (Oxford Nanopore Technologies)
		TBseq® (Shengting Medical Technology Co)
Tests for TB infection	*Mycobacterium tuberculosis (Mtb)* antigen-based skin tests (TBSTs)	Diaskintest® (Generium)
		SIILTIBCY® (Serum Institute of India)
		C-TST (Anhui Zhifei Longcom
	Interferon-gamma release assays (IGRAs)	QuantiFERON-TB® Gold Plus (QFT-Plus) (QIAGEN)
		T-SPOT®.TB (T-Spot) (Oxford Immunotec)Revvity)
		TB-IGRA (Wantai BioPharm)
		****NEW** STANDARD E TB-Feron (ELISA) (SD Biosensor)
		****NEW** LIAISON® QFT-PLUS (CLIA) (QIAGEN/ Diasorin)
	Tuberculin skin tests	Tuberculin purified protein derivative (PPD) products

NAAT: nucleic acid amplification test; NGS: next-generation sequencing; TB: tuberculosis.

Drugs: INH: isoniazid; RIF: rifampicin.

[a] Products recommended by WHO/GTB will also undergo WHO PQ assessment.

[b] Each targeted NGS end-to-end solution is recommended for the detection of resistance to specific anti-TB agents; lists can be found in the targeted NGS section below and in **Table 2.8**.

WHO has reviewed each of these tests and solutions and has developed recommendations for their use. In all settings, WHO recommends that rapid diagnostic tests should be used for initial diagnosis of TB and detection of RIF resistance, to minimize delays in starting treatment.

The initial tests for TB diagnosis are broadly grouped as WRDs; these are defined as diagnostic tests that directly target mycobacterial DNA (molecular) or cell components (biomarkers) to aid in the diagnosis of TB *(5)*. The newer, sensitive molecular tests recommended for the initial detection of *Mtb* complex (MTBC) – with and without initial drug-resistance detection – are mWRDs and are organized into three classes (*5*):

- the low-complexity automated NAATs (LC-aNAATs) for the detection of MTBC and RIF resistance;
- the moderate-complexity automated NAATs (MC-aNAATs) for the detection of MTBC and RIF and INH resistance; and
- the low-complexity manual NAATs (LC-mNAATs) for the detection of MTBC alone.

Table 2.1 lists the mWRDs that meet the criteria for the respective classes; **Table 2.2** provides further details on each test's manufacturer, instrumentation, infrastructure requirements, targets, times and limits of detection. Further product-specific details may also be found in the GLI mWRD selection manual *(15)*. Of note, policy guidance establishing these LC-mNAAT and LC-aNAAT classes and updating recommendations for use of the included tests was recently updated; hence, **Section 3** and **Section 4** of this handbook have been updated to reflect the latest changes *(5)*.

In addition to the mWRDs, one biomarker-based LF-LAM WRD – Determine TB LAM Ag – is recommended for diagnosis of TB in people living with HIV. A positive LF-LAM result is considered to be bacteriological confirmation of TB *(10)*. However, a negative result does not rule out TB; therefore, LF-LAM is recommended for concurrent use with mWRD testing among people living with HIV. Policy guidance on the LF-LAM testing was recently updated and is detailed in the *WHO consolidated guidelines on tuberculosis: module 3: diagnosis (5)*, while operational considerations are provided in **Section 3** and **Section 4** of this handbook. After the initial detection of TB, three classes of follow-on tests are recommended to detect resistance to first-line or second-line anti-TB drugs (or both): LC-aNAATs, line probe assays (LPAs) and targeted NGS end-to-end solutions, see **Table 2.1** and **Table 2.2**. A summary of the aspects of the tests included in the classes is found in **Table 2.6**; information sheets specific to the targeted NGS solutions are provided in **Web Annex A**.

To detect when a person is infected with *Mtb* and may be most likely to benefit from receiving TPT, WHO recommends three classes of TB infection tests: TBSTs, IGRAs and TST. All three classes include tests that target factors associated with a person's immune response to infection – they do not directly target components of *Mtb*. Hence, tests for TB infection require a competent immune response to produce valid results, cannot distinguish TB infection from TB disease, and cannot predict who will progress from TB infection to TB disease. Further information on the recommendations for use of these tests can be found in the *WHO consolidated guidelines on tuberculosis: module 1: prevention (14).* More information concerning operational considerations on testing for TB infection is outlined in **Section 2.5** below.

2.1 Conventional tests for the diagnosis of TB

In many high TB burden settings, sputum smear microscopy remains the primary diagnostic technique for evaluating individuals who present with the signs and symptoms of TB, or screen positive for TB. However, sputum smear microscopy is an insensitive test, with a limit of detection (LoD) of 5000–10 000 bacilli/mL of sputum *(16)*. Furthermore, sputum smear microscopy cannot distinguish drug-susceptible strains from drug-resistant strains. WHO continues to recommend that NTPs replace microscopy as the initial diagnostic test with WRDs that directly detect MTBC.

The current gold standard method for bacteriological confirmation of TB is culture using commercially available liquid media. However, culture is not used as a primary diagnostic test in many high TB burden countries because of the cost, the infrastructure requirements (biosafety level 3 [BSL-3] or TB containment laboratory) and the long time required to generate results (1–3 weeks for a positive result and up to 6 weeks for a negative result). Nevertheless, culture is important for the diagnosis of paediatric and extrapulmonary TB from paucibacillary samples, and in the differential diagnosis of non-tuberculous mycobacteria (NTM) infection. Additionally, both sputum smear microscopy and culture remain necessary to monitor a person's response to treatment.

The culture process can result in the growth of many different *Mycobacterium* species. As such, laboratory confirmation of TB requires testing of the recovered mycobacteria using a species identification test (e.g. Capilia™ TB-Neo, Tauns Laboratories, Numazu, Japan; Bioline™ TB Ag MPT64 Rapid Test, Abbott, Gyeonggi-do, Republic of Korea; or TBcID, Becton Dickinson Microbiology Systems, Sparks, United States of America [USA]) to definitively identify MTBC. Species identification is particularly important before initiating phenotypic DST.

Table 2.2. Summary of initial tests for TB diagnosis

Class	Test	Manufacturer	Instrument(s)	Gene target(s)	DST	Claimed LoD	Sample type recommended	Time to results	WHO recommendations	Key infrastructure requirements	Reagent storage, shelf life	Number of tests per 8-hour shift
Low-complexity automated NAAT	Truenat MTB Plus	Molbio	Truelab Uno, Duo, Quattro	*nrdZ*, IS6110	No	30 CFU/mL	Sputum, tracheal aspirate, bronchoalveolar lavage, nasopharyngeal aspirate and gastric aspirate	60 min	Initial TB and use for concurrent testing of PLHIV	Battery operated, power to charge, operating temperature 15–40 °C	2–30 °C, 2 years	7–9 per reaction chamber
	Truenat MTB-RIF Dx	Molbio	Truelab Uno, Duo and Quattro	*rpoB*	RIF	N/A	Sputum, tracheal aspirate, bronchoalveolar lavage, nasopharyngeal aspirate and gastric aspirate	60 min	Reflex testing for RIF resistance after performing the MTB Plus test	Battery operated, power to charge, operating temperature 15–40 °C	2–30 °C, 2 years	7–9 per reaction chamber
	Xpert MTB/RIF Ultra	Cepheid	GeneXpert I, II, IV, XVI and Infinity 6- or 10-colour module	IS6110, IS1081, *rpoB*	RIF	16 CFU/mL	Sputum, tracheal aspirate, bronchoalveolar lavage, nasopharyngeal aspirate, gastric aspirate, stool, CSF, lymph node tissue aspirates, pleural tissue, pleural fluid, synovial fluid, peritoneal fluid and pericardial fluid	90 min	Initial detection of TB and RIF resistance, and use for concurrent testing of children and PLHIV	Stable power, operating temperature ≤30 °C	2–28 °C, 9 months	3–4 per module
Moderate-complexity automated NAAT	RealTi*m*e MTB	Abbott	*m*2000rt	IS6110, PAB	No	17 CFU/mL	Sputum, tracheal aspirate and bronchoalveolar lavage	7 h	Initial detection of TB	Stable power, free-standing instrument, weight >300 kg	–15 °C to –25 °C, 18 months	94
	RealTi*m*e MTB RIF/INH	Abbott	m2000rt	*rpoB*, *katG*, *inhA*	RIF, INH	60 CFU/mL	Sputum, tracheal aspirate and bronchoalveolar lavage	7 h	Reflex testing for RIF and INH resistance after performing the RealTi*m*e MTB test	Stable power, free-standing instrument, weight >300 kg	–15 °C to –25 °C, 12 months	94
	BD MAX MDR-TB	Becton Dickinson	BD MAX	*IS6110*, *IS1081*, *rpoB*, *katG*, *inhA*	RIF, INH	20 CFU/mL	Sputum, tracheal aspirate and bronchoalveolar lavage	<4 h	Initial detection of MTBC and RIF and INH resistance	Stable power	2–28 °C, 9 months	24

Class	Test	Manufacturer	Instrument(s)	Gene target(s)	DST	Claimed LoD	Sample type recommended	Time to results	WHO recommendations	Key infrastructure requirements	Reagent storage, shelf life	Number of tests per 8-hour shift
	FluoroType MTB	Bruker-Hain	FluoroCycler 12, FluoroCycler XT	IS6110	No	15 CFU/mL	Sputum, tracheal aspirate and bronchoalveolar lavage	2.5 h	Initial diagnosis of TB disease	Stable power	–15 °C to –25 °C, on request	Up to 94
	FluoroType MTBDR	Bruker-Hain	FluoroCycler 12, FluoroCycler XT	*rpoB*, *katG*, *inhA*	RIF, INH	20 CFU/mL	Sputum, tracheal aspirate and bronchoalveolar lavage	2.5 h	Follow-on testing for RIF and INH resistance detection after performing the FluoroType MTB test	Stable power	–15 °C to –25 °C, on request	Up to 94
	cobas MTB	Roche	cobas 5800, 6800, 8800	*16S rRNA*, *esx*	No	9 CFU/mL	Sputum, tracheal aspirate and bronchoalveolar lavage	3.5 h	Initial diagnosis of TB disease	Stable power, free-standing instrument, weight >600 kg	2–8 °C, 18 months	94
	cobas MTB-RIF/ INH	Roche	cobas 5800, 6800, 8800	*rpoB*, *katG*, *inhA*	rifampicin, isoniazid	180 CFU/ mL	Sputum, tracheal aspirate and bronchoalveolar lavage	3.5 h	Follow-on testing for RIF and INH resistance detection after performing the cobas MTB test	Stable power, free-standing instrument, weight >600 kg	2–8 °C, 16 months	94
Low-complexity manual NAAT	TB-LAMP	Eiken Chemical	HumaLoop T	IS6110, *gyrB*	No	N/A	Sputum, tracheal aspirate, bronchoalveolar lavage and gastric aspirate	90 min	Initial diagnosis of TB disease	Battery operated, power to charge, operating temperature 15–40 °C	2–30 °C, 14 months	70
Lateral flow lipo-arabino-mannan detection	Determine TB LAM Ag	Abbott	N/A	LF-LAM antigen detection	No	N/A	Urine	25 min	Initial diagnosis of TB disease among PLHIV	No testing infrastructure; private urine collection infrastructure	2–30 °C, 18 months	N/A

CFU: colony-forming unit; CSF: cerebrospinal fluid; DST: drug susceptibility testing; HIV: human immunodeficiency virus; LF-LAM: lateral flow urine lipoarabinomannan assay; LoD: limit of detection; MTBC: *Mycobacterium tuberculosis* complex; N/A: not available; NAAT: nucleic acid amplification test; PLHIV: people living with HIV; TB: tuberculosis.

Drugs: INH: isoniazid; RIF: rifampicin.

2.2 Initial tests for diagnosis of TB with drug resistance detection

2.2.1 Low-complexity automated NAATs

The class of LC-aNAATs is defined in **Table 2.3**. The number of tests meeting the criteria for this class is expected to grow over the coming years; this will encourage a competitive environment for better product maintenance and support, more options for varying settings and lower prices. New products that meet the class definition according to a WHO/GTB rapid assessment are referred for WHO PQ *(17)*. The list of products currently under evaluation can be reviewed on the WHO PQ website. Products that are successfully WHO prequalified are listed in the *WHO list of prequalified in vitro diagnostic products*, and are added as within-class products by WHO/GTB *(18)*.

Table 2.3. Definition of the class of LC-aNAATs

Purpose		Detection of TB and RIF resistance
Principle of action		Nucleic acid amplification testing
Complexity	Reagents	Most reagents are enclosed in a disposable sealed container to which a clinical specimen is added; the disposable sealed container does not have special storage requirements
	Skills	Basic technical skills (e.g. basic pipetting, precision not critical)
	Pipetting	Either no, or only one, pipetting step in the process
	Testing procedure	May require an initial manual specimen treatment step before transferring the specimen into the disposable sealed container for automated processing Automated DNA extraction Automated real-time PCR
Type of test result reporting		Automated
Setting of use		Basic laboratory (no special infrastructure needed)

DNA: deoxyribonucleic acid; LC-aNAAT: low-complexity automated nucleic acid amplification test; PCR: polymerase chain reaction; TB: tuberculosis.

Drug: RIF: rifampicin.

WHO recommends the use of the LC-aNAAT class for detecting pulmonary TB and extrapulmonary TB in adults, children (defined as those aged <10 years), and people (of all ages) living with HIV (**Box 2.1**). For adult pulmonary TB, the summary sensitivity was 90.4% (95% confidence interval [CI]: 88.0–92.4) and the summary specificity was 94.9% (95% CI: 93.0–96.3). The two tests that meet the criteria for inclusion in the class – Xpert MTB/RIF Ultra (Xpert Ultra) and Truenat MTB Plus with MTB-RIF Dx – also detect resistance to RIF. For Xpert Ultra, this is achieved by integrating the detection of MTBC and mutations in the RIF-resistance

determining region (RRDR) in one cartridge. For the Truenat test, reflex testing with the MTB-RIF Dx assay should follow a positive MTB Plus result. The class summary sensitivity for the detection of RIF resistance is 95.1% (95% CI: 83.1–98.7) and the summary specificity is 98.1% (95% CI: 97.0–98.7).

When using LC-aNAATs to diagnose TB among children, concurrent testing of one respiratory specimen (i.e. sputum, gastric aspirate or nasopharyngeal aspirate) and one stool sample is recommended. If a child is HIV-positive, the concurrent testing strategy should also include LF-LAM testing on a urine sample (see **Section 3.4**). Concurrent testing of a respiratory sample (using LC-aNAAT) and urine (using LF-LAM) is also recommended for adults and adolescents living with HIV (see **Section 3.4**).

LC-aNAATs are also recommended for the detection of TB meningitis using cerebrospinal fluid (CSF), and extrapulmonary TB using lymph node aspirates, pleural tissue, pleural fluid, synovial aspirates, peritoneal fluid or pericardial fluid. It is important to stress that the bacillary load of extrapulmonary samples is often low; therefore, samples with sufficient volumes for testing should be collected and concentrated, where possible, to reach a detectable level of bacterial DNA.

When implementing these tests, users should be aware that the instruments required to run the tests have different requirements for laboratory infrastructure. For example, the GeneXpert instrument requires a dust-free environment, a stable electricity supply, and a maximum temperature of 30 °C, whereas the Trueprep and Truelab instruments can be battery operated and are less sensitive to the surrounding temperature (see **Table 2.2**). Some LC-aNAAT instrumentation may also be used to test for other diseases (e.g. severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], HIV, and hepatitis B and C), which can facilitate one-stop-shop services for patients and cost-sharing measures between disease programmes.

Staff running the tests need to have a basic understanding of the principles of molecular testing and know how to operate a computer. Low-complexity tests may have different pipetting requirements; for example, Xpert MTB/RIF Ultra does not require precision pipetting, whereas Molbio Truenat MTB Plus requires precision pipetting of a small volume of liquid. Maintenance and annual calibration are required; thus, manufacturers or countries often train "super users" to oversee or perform a basic service and annual calibration. Service contracts with manufacturers are also available, some of which bundle instrument service and maintenance costs into a transparent per-test unit cost.

Box 2.1. WHO recommendations on the use of LC-aNAATs *(6)*

For adults and adolescents with signs or symptoms of TB or who screened positive for pulmonary TB, low-complexity automated NAATs should be used on respiratory samples as initial diagnostic tests for TB, rather than smear microscopy or culture.

(Strong recommendation, high certainty of evidence)

Remarks:

- Respiratory samples refer to sputum (expectorated or induced), tracheal aspirate or bronchoalveolar lavage.
- Person screened positive: person in whom screening test has yielded a positive result.
- For children, see the recommendation below.
- For adults, adolescents, and children living with HIV, refer to the recommendations on current testing in **Box 2.4**.
- For children with HIV, refer to the section on concurrent use of TB diagnostic tests in children.

For people with bacteriologically confirmed TB, low-complexity automated NAATs should be used on respiratory samples as initial tests for detection of resistance to rifampicin, rather than culture-based DST.

(Strong recommendation, high certainty of evidence)

Remarks:

- This recommendation applies to all people living with HIV.
- The recommendation was extrapolated to children based on the generalization of data from adults and very limited data from children.
- The recommendation was extrapolated to extrapulmonary TB based on the generalization of data from adults.

For children who are HIV-negative or have an unknown HIV status, who have signs or symptoms or screen positive for pulmonary TB, concurrent testing using low-complexity automated NAATs on respiratory and stool samples should be used as the initial diagnostic strategy for diagnosing TB, rather than low-complexity automated NAATs on respiratory or stool samples alone.

(Strong recommendation, low certainty of evidence)

Remark:

- This recommendation prioritizes concurrent testing of two different sample types over the use of a single molecular test for diagnosis of TB in children.

For people with signs and symptoms of TB meningitis, low-complexity automated NAATs on cerebrospinal fluid should be used for the initial diagnosis of TB meningitis, rather than smear microscopy or culture.

(Strong recommendation, moderate certainty of evidence)

Remark:

- ➔ This recommendation applies to all patients with signs and symptoms of TB meningitis, including people living with HIV and children.

For people with signs and symptoms of extrapulmonary TB, low-complexity automated NAATs on lymph node tissue aspirate, pleural tissue, pleural fluid, synovial fluid, peritoneal fluid or pericardial fluid should be used for the initial diagnosis of TB, rather than smear microscopy or culture.

(Strong recommendation, low certainty of evidence for synovial fluid and pericardial fluid; very low certainty of evidence for lymph node tissue aspirate, pleural tissue, pleural fluid and peritoneal fluid)

Remarks:

- ➔ This recommendation applies to all patients with signs and symptoms of the respective form of extrapulmonary TB, including people living with HIV and children.
- ➔ Data on LC-aNAATs performance on pericardial fluid, urine and blood were limited or inconsistent.

2.2.2 Moderate-complexity automated NAATs

The MC-aNAATs are defined in **Table 2.4**.

Table 2.4. Definition of the class of MC-aNAATs

Purpose		Detection of TB and resistance to RIF and INH
Principle of action		Nucleic acid amplification testing
Complexity	Reagents	Reagents are available within standardized kits and may have temperature requirements for storage; the sample is added automatically or manually to a disposable sealed container for testing
	Skills	Moderate technical skills (e.g. multiple sample or reagent handling steps, precision pipetting may be required, molecular workflows may be required)
	Pipetting	One or more non-precision or precision pipetting steps required by the procedure
	Testing procedure	May require multiple specimen treatment steps before transferring the specimen into a sealed test container for automated processing Automated or manual DNA extraction Automated real-time PCR
Type of test result reporting		Automated
Setting of use		Laboratory (special infrastructure may be required)

DNA: deoxyribonucleic acid; MC-aNAAT: moderate-complexity automated nucleic acid amplification test; PCR: polymerase chain reaction; TB: tuberculosis.

Drugs: INH: isoniazid; RIF: rifampicin.

The class of MC-aNAATs includes rapid and accurate tests for the detection of pulmonary TB from respiratory samples (**Box 2.2**). Overall pooled sensitivity for TB detection was 93.0% (95% CI: 90.9–94.7%) and specificity was 97.7% (95% CI: 95.6–98.8%) (**Table 3.1**, **Table 3.2** and **Table 3.3** in **Section 3**). MC-aNAATs can simultaneously detect resistance to both RIF and INH and are less complex to perform than phenotypic DST and LPAs. After the sample preparation step, the tests are largely automated. Overall pooled sensitivity for detection of RIF resistance was 96.7% (95% CI: 93.1–98.4%) and specificity was 98.9% (95% CI: 97.5–99.5%). **Fig. 2.1** illustrates the procedures for each test. Overall pooled sensitivity for detection of INH resistance was 86.4% (95% CI: 82.8–89.3%) and specificity was 99.2% (95% CI: 98.1–99.7%).

These assays offer high throughput testing and are suitable for high workload settings, so could potentially be used in areas with a high population density or TB prevalence. However, the class is primarily for laboratory settings and will require a reliable and rapid system for sample referral and result reporting. MC-aNAATs may already be used programmatically for other diseases (e.g. SARS-CoV-2, HIV, and hepatitis B and C), which could potentially facilitate implementation of TB testing on shared platforms. Information sheets summarizing the individual technologies

in this class are available in **Web Annex A**. A detailed comparison of the different products is also available *(15)*.

Box 2.2. WHO recommendation on MC-aNAATs *(6)*

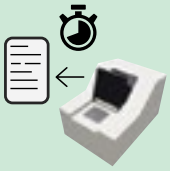
For people with signs and symptoms of pulmonary TB, moderate-complexity automated NAATs may be used on respiratory samples for detection of pulmonary TB, and of rifampicin and isoniazid resistance, rather than culture and phenotypic DST.

(Conditional recommendation, moderate certainty of evidence)

Remarks**:**

- ➔ This recommendation is based on evidence of diagnostic accuracy in respiratory samples of adults with signs and symptoms of pulmonary TB.
- ➔ The recommendation applies to people living with HIV (studies included a varying proportion of such people). Performance on smear-negative samples was reviewed but was only available for TB detection, not for resistance to RIF and INH. Data stratified by HIV status were not available.
- ➔ The recommendation applies to adolescents and children based on the generalization of data from adults. An increased rate of indeterminate results may be found with paucibacillary TB disease in children.
- ➔ Extrapolation for use in people with extrapulmonary TB and testing on non-sputum samples was not considered because data on diagnostic accuracy of technologies in the class for non-sputum samples were limited.
- ➔ The recommendation applies to adolescents and children based on the generalization of data from adults. An increased rate of indeterminate results may be found with paucibacillary TB disease in children.
- ➔ Extrapolation for use in people with extrapulmonary TB and testing on non-sputum samples was not considered because data on diagnostic accuracy of technologies in the class for non-sputum samples were limited.

Fig. 2.1. Summary of testing procedures for the Bruker Hain FluoroType MTB and MTBDR

	Abbott RealTime MTB and MTB RIF/INH	BD MAX MDR-TB	Bruker-Hain FluoroType MTB and MTBDR	Roche cobas MTB and MTB-RIF/INH
Maximum number of samples per run	94	24	94	94
Specimen reception to results out, measured for 24 samples	**7.5 h MTB 3 h RIF/INH**	**4 h**	**3 h MTB 3 h MTBDR**	**3.5 h MTB 3.5 h RIF/INH**
Inactivation 	3:1 IR ↓ Invert several times ↓ Vortex 20/30 s	2:1 BD Max STR ↓ Shake 10 times ↓ Pre-incubate 5 min ↓ Shake 10 times	2:1 Liquefaction ↓ Vortex	2:1 Cobas MIS ↓ Vortex 30/60 s
Incubation 	1 h ↓ Vortex 20/30 sec at 20 min	25 min	30 min	1 h
DNA extraction, amplification and detection 	DNA extraction 4 h 22 min m2000 sp ↓ Seal plate and transfer to instrument ↓ Amplification and detection 2 h 30 min	3.5 h run time to results	DNA extraction 2 h GXT 96 ↓ Seal plate and transfer to instrument ↓ Amplification and detection 1.5 h	Sonicate 1 min per sample ↓ Centrifuge 60 s ↓ 2 h 30 min run time to results

DNA: deoxyribonucleic acid; MC-aNAAT: moderate-complexity automated nucleic acid amplification test.
Source: Sengstake & Rigouts (2020) *(19)*.

2.3 Initial tests for diagnosis of TB without drug resistance detection

2.3.1 Low-complexity manual NAATs

The class of LC-mNAATs is defined in **Table 2.5**. Only one test met the criteria for the class: the Loopamp™ MTBC detection kit from Eiken Chemical Company in Tokyo, Japan. The test detects MTBC but not drug resistance, and it is useful in decentralized settings with limited laboratory infrastructure. New products that are successfully added to the class by WHO PQ can be found in the *WHO list of prequalified in vitro diagnostic products (18)*.

Table 2.5. Definition of the class of LC-mNAATs

Purpose		Detection of TB
Principle of action		Nucleic acid amplification testing
Complexity	Reagents	Reagents are enclosed in multiple disposable sealed containers not requiring special storage requirements
	Skills	Basic technical skills (e.g. basic pipetting, precision not critical)
	Pipetting	Multiple pipetting steps (maximum of 10) from processed sample to result generation
	Testing procedure	At least four distinct steps: Specimen treatment step before transferring the specimen into the disposable sealed container DNA extraction PCR amplification Results visualization
Type of test result reporting		Automated or manual
Setting of use		Basic laboratory (no special infrastructure needed)

DNA: deoxyribonucleic acid; LC-mNAAT: low-complexity manual nucleic acid amplification test; PCR: polymerase chain reaction; TB: tuberculosis.

The LC-mNAATs are accurate in detecting pulmonary TB on respiratory samples in adults, children and people living with HIV (**Box 2.3**). Currently, such tests are only recommended for use with sputum (all ages) and gastric aspirate (children) because evidence on other sample types is lacking (see the section on further research in the consolidated guidelines *(5)*). The manual TB-LAMP assay is designed to detect MTBC directly from sputum specimens in about 90 minutes, does not require sophisticated instrumentation and can be used at the peripheral laboratory level, with the biosafety requirements being similar to those for sputum smear microscopy. For the detection of TB in adults with signs and symptoms consistent with pulmonary TB, LC-mNAAT demonstrated a sensitivity of 84% (95% credible interval [CrI]: 78–89%) and a specificity of 96% (95% CrI: 94–97%) on sputum, as compared with a microbiological reference standard.

Box 2.3. WHO recommendation on LC-mNAATs *(6)*

For adults and adolescents with signs or symptoms of or who screen positive for pulmonary TB, low-complexity manual NAATs should be used on respiratory samples as initial diagnostic tests for TB, rather than smear microscopy or culture.

(Strong recommendation, high certainty of evidence)

Remarks:

- ➔ This recommendation applies to all people living with HIV with the caveat of low or moderate certainty. The drop in sensitivity will increase with more advanced forms of HIV disease.
- ➔ This recommendation is extrapolated for use in children on respiratory samples (including induced sputum and nasogastric aspirate) based on the generalization of data from adults and very limited data in children, acknowledging the difficulties of collecting sputum specimens from children.
- ➔ The use of the test on paediatric stool samples was very limited and for nasogastric aspirate there were no data. The group did not extrapolate the recommendation for use on stool or nasogastric aspirate. If this is the only test available and used, it should be accompanied by culture testing on appropriate sample types or should be upward referred for further investigation.
- ➔ No recommendation was made on extrapulmonary TB owing to insufficient data.
- ➔ The test does not provide information on RIF resistance; hence, follow-up testing is required.

2.3.2 Urine LF-LAM

The urine LF-LAM is an immunocapture lateral flow assay based on the detection of LAM (lipoarabinomannan), which is a component of the mycobacterial cell wall. It is a POC test for the initial diagnosis of TB among people living with HIV. Although the assay lacks sensitivity, it can be used as a fast, rule-in test for HIV-positive individuals that can be used in the community, a health facility or at the bedside, especially in urgent cases where a rapid TB diagnosis is critical for the person's survival. The Determine TB LAM Ag assay is currently the only commercially available urine LF-LAM endorsed by WHO. The presence of LAM in the urine is an indication of the presence of mycobacteria; hence, WHO has defined a positive LF-LAM result as a bacteriologically confirmed TB case. However, the detection of mycobacterial LAM antigen in urine does not provide any information on drug resistance. A document addressing practical considerations for the implementation of the LF-LAM is available *(20)*.

NEW The recommendations have been updated to include adults and adolescents living with HIV in all settings (inpatient and outpatient, irrespective of CD4 cell count) and children with HIV *(5)*, as detailed in **Box 2.4**.

Box 2.4. WHO recommendations on concurrent use of tests in people living with HIV *(5)*

For adults and adolescents with HIV who have signs or symptoms of TB, screen positive for TB, are seriously ill or have advanced HIV disease, concurrent testing using low-complexity automated NAATs on respiratory samples and LF-LAM on urine samples should be used as the initial diagnostic strategy for diagnosing TB rather than low-complexity automated NAATs on respiratory samples alone.

(Strong recommendation, low certainty of evidence)

Remarks:

- Serious illness in people living with HIV is defined based on any of the following symptoms: respiratory rate ≥30 breaths per minute, temperature ≥39 °C, heart rate ≥120 beats per minute or unable to walk unaided.
- Advanced HIV disease is defined in people living with HIV who have a CD4 cell count of <200 cells/mm3 or presenting with a WHO Stage 3/4 AIDS-defining illness.
- This concurrent testing recommendation supersedes prior guidance on using LF-LAM for people living with HIV and the use of a single molecular test for diagnosis of TB in this group.

For children with HIV who have signs or symptoms or screen positive for pulmonary TB, concurrent testing using low-complexity automated NAATs on respiratory and stool samples and LF-LAM on urine samples may be used as the initial diagnostic strategy for diagnosing TB, rather than low-complexity automated NAATs on respiratory or stool samples alone.

(Conditional recommendation, low certainty of evidence)

Remarks:

- This recommendation prioritizes concurrent testing over the use of molecular testing and LF-LAM in isolation for diagnosis of TB in children with HIV.
- Use of LC-aNAATs on isolated specimens was also evaluated. The findings supported the use of LC-aNAATs for initial diagnostic testing for TB in HIV-positive children with signs or symptoms or who screen positive for pulmonary TB, using sputum, gastric aspirate, stool or nasopharyngeal aspirate, rather than smear or culture.

Notes:

- For their initial diagnostic test, anyone with signs and symptoms of pulmonary TB who can produce sputum should have a sputum specimen submitted for concurrent testing with an LC-aNAAT. This includes children and adolescents living with HIV who can provide a sputum sample. LF-LAM results (test time 25 minutes) are likely to be available before mWRD results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.
- LF-LAM may be used to assist in the diagnosis of TB but it should not be used as a screening test.

2.4 Follow-on diagnostic tests for detection of additional drug resistance

The availability of follow-on tests to detect additional resistance to anti-TB drugs is becoming increasingly important for making informed decisions on treatment regimens. WHO has recommended four classes: LC-aNAAT for detection of resistance to INH and second-line anti-TB drugs, LPAs, high-complexity reverse hybridization NAATs (HC-rhNAATs) and targeted NGS. A summary of the tests included in the different classes is shown in **Table 2.6**.

Table 2.6. Comparison of follow-on tests for the detection of drug resistance

Class of test	Test	Manufacturer	Platform	Gene target(s)	DST	Time to results	Recommendations	Infrastructure requirements	Batch size	Number of tests per 8-hour shift
LC-aNAATs	Xpert MTB/XDR	Cepheid	GeneXpert I, II, IV, XVI, requires 10-colour module	*inhA*, *katG*, *fabG1*, *oxyR-ahpC*, *gyrA*, *gyrB*, *Rrs*, *eis*	INH, ETO, FQ and AMK	90 min	Follow-on detection of resistance to INH, ETO, FQ and second-line injectable drugs	Stable electricity, ambient temperature not above 25° C	Single sample per test	3–4 per module
LPAs	GenoType MTBDR*plus*	Bruker-Hain		*rpoB*, *katG*, *inhA*,	RIF, INH	1–2 days	Follow-on detection of resistance to RIF and INH	Thermocycler, incubation solution, stable electricity, ambient temperature, clean room for DNA amplification steps	Single test, 12 or 48	Depends on laboratory capacity
	GenoType MTBDR*sl*	Bruker-Hain		*gyrA*, *gyrB*, *rrs*, *eis*	FQ, AMK	1–2 days	Follow-on detection of resistance to FQ and AMK	Thermocycler, incubation solution, stable electricity, ambient temperature, clean room for DNA amplification steps	Single test, 12 or 48	Depends on laboratory capacity
	Genoscholar NTM+MDRTB II	Nipro		*rpoB*, *katG*, *inhA*	RIF, INH	1–2 days	Follow-on detection of resistance to RIF and INH	Thermocycler, incubation solution, stable electricity, ambient temperature, clean room for DNA amplification steps	Single test, 12 or 48	Depends on laboratory capacity
HC-rNAAT	Genoscholar PZA-TB	Nipro		*pncA*	PZA	1–2 days	Follow-on detection of resistance to PZA	Thermocycler, incubation solution, stable electricity, ambient temperature, clean room for DNA amplification steps	Single test, 12 or 48	Depends on laboratory capacity

Class of test	Test	Manufacturer	Platform	Gene target(s)	DST	Time to results	Recommendations	Infrastructure requirements	Batch size	Number of tests per 8-hour shift
Targeted NGS	Deeplex Myc-TB	Genoscreen	Illumina iSeq, MiniSeq, MiSeq etc.	*	RIF, INH, PZA, EMB, FQ, BDQ, LZD, CFZ, AMK and STR	48+ h	Follow-on detection of resistance to INH, FQ, BDQ, LZD, CFZ, PZA, EMB, AMK and STR in drug-susceptible TB or MDR/RR-TB patients	Thermocycler, sequencer, powerful computing system for data analysis, high-speed internet connection	13 up to 1200 samples depending on the instrument used	Depends on laboratory capacity
	AmPORE-TB	Oxford Nanopore Technologies	MinION	**	RIF, INH, PZA, FQ, BDQ, LZD, CFZ, AMK and STR	5 h	Follow-on detection of resistance to RIF, INH, FQ, LZD, AMK and STR in MDR/RR-TB patients	Thermocycler, sequencer, powerful computer for data analysis, high-speed internet connection	22 samples excluding controls per flow cell	Depends on laboratory capacity
	Tbseq	Shen Ting Biotech	Illumina iSeq, MiniSeq, MiSeq etc.	embB, embA	EMB	12 h	Follow-on detection of resistance to EMB in MDR/RR-TB patients	Thermocycler, sequencer, powerful computer for data analysis, high-speed internet connection	13 up to 1200 samples depending on the instrument used	Depends on laboratory capacity

DNA: deoxyribonucleic acid; DST: drug susceptibility testing; HC-rNAAT: high-complexity reverse hybridization NAAT; LC-aNAAT: low-complexity automated NAAT; LPA: line probe assay; MDR/RR-TB: multidrug- or rifampicin-resistant TB; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; TB: tuberculosis.

* RIF (*rpoB*), INH (*inhA*, *fabG1*, *katG*, *ahpC*), PZA (*pncA)*, EMB (*embB*), FQ *(gyrA*, *gyrB*), BDQ/CFZ (*rv0678*), LZD (*rrl*, *rplC*), AMK (*rrs*) and STR (*rrs*, *gidB*, *rpsL*).

** RIF (*rpoB*), INH (*fabG1*, *inhA*, *katG*), PZA (*pncA*), FQ (*gyrA*, *gyrB*), BDQ (*atpE*, *rv0678*, *atpE)*, LZD (*rrl*, *rplC*), CFZ (*fbiA*, *fbiB*, *fbiC*, *fgd1*, *rv0678*), AMK *(rrs*) and STR (*gid*, *rpsL*, *rrs*).

Drugs: AMK: amikacin; BDQ: bedaquiline; CFZ: clofazimine; EMB: ethambutol; ETO: ethionamide; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.

2.4.1 LC-aNAATs for detection of resistance to INH and second-line anti-TB drugs

The first-in-class product for LC-aNAATs for detection of resistance to INH and second-line anti-TB drugs was the Xpert MTB/XDR Assay (Cepheid, Sunnyvale, USA); the class is defined in the consolidated guidelines *(5)* and in **Table 2.3**. This test uses a cartridge designed for GeneXpert instruments to detect resistance to INH, FQs, ETO and second-line injectable drugs (e.g. AMK). In contrast to Xpert MTB/RIF and Xpert Ultra, which are performed on a GeneXpert instrument that can detect six colours, the XDR test requires a 10-colour GeneXpert module. There is an option to combine the 6- and 10-colour systems through a common computer, or to replace one 6-colour module in an instrument with a 10-colour module. The current WHO recommendations for Xpert Ultra cartridge use on GeneXpert 6-colour instruments are also valid for their use on GeneXpert 10-colour instruments *(21)* (**Box 2.5**).

Box 2.5. WHO recommendations on LC-aNAATs for detection of resistance to INH and second-line anti-TB drugs

For people with bacteriologically confirmed pulmonary TB, low-complexity automated NAATs may be used on sputum for the initial detection of resistance to isoniazid and fluoroquinolones, rather than culture-based phenotypic DST.

(Conditional recommendation, moderate certainty of evidence)

For people with bacteriologically confirmed pulmonary TB and resistance to rifampicin, low-complexity automated NAATs may be used on sputum for the initial detection of resistance to ethionamide, rather than DNA sequencing of the inhA promoter.

(Conditional recommendation, very low certainty of evidence)

For people with bacteriologically confirmed pulmonary TB and resistance to rifampicin, low-complexity automated NAATs may be used on sputum for the initial detection of resistance to amikacin, rather than culture-based phenotypic DST.

(Conditional recommendation, low certainty of evidence)

Remarks:

- ➔ The recommendations are based on the evidence of diagnostic accuracy in sputum of adults with bacteriologically confirmed pulmonary TB, with or without RIF resistance.
- ➔ The recommendations are extrapolated to adolescents and children, based on the generalization of data from adults.

- ➔ The recommendations apply to people living with HIV (studies included a varying proportion of such individuals); data stratified by HIV status were not available.
- ➔ The recommendations are extrapolated to people with extrapulmonary TB, and testing of non-sputum samples was considered appropriate, which affects the certainty. The panel did not evaluate test accuracy in non-sputum samples directly, including in children; however, extrapolation was considered appropriate given that WHO has recommendations for similar technologies for use on non-sputum samples (e.g. Xpert MTB/RIF and Xpert Ultra).
- ➔ Recommendations for detection of resistance to AMK and ETO are only relevant for people who have bacteriologically confirmed pulmonary TB and resistance to RIF.

The LC-aNAAT is intended for use as a follow-on test in specimens determined to be MTBC-positive; it offers the chance to improve access to rapid DST in peripheral laboratories. In addition, the test provides results in less than 90 minutes, meaning that the time to results is faster than with LPAs or culture-based phenotypic DST. This NAAT requires the same infrastructure and training of technicians as the other Xpert tests.

The overall pooled sensitivity for detection of INH resistance was 94% (95% CI: 89–97%) and specificity was 98% (95% CI: 95–99%) (**Table 3.3**). Overall pooled sensitivity for detection of FQ resistance was 93% (95% CI: 88–96%) and specificity was 98% (95% CI: 94–99%) (**Table 3.4**). Thus, Xpert MTB/XDR could be used as a reflex test to complement existing technologies that only test for RIF resistance, allowing the rapid and accurate detection of resistance to INH and FQs in cases of RIF-susceptible TB; and of resistance to FQ, INH, ETO and AMK in cases of RR-TB. The package insert includes the use of the test on culture isolates. However, the primary purpose of this test is to achieve rapid and early detection of resistance, and recommendations are for use of the test directly on clinical specimens. **Web Annex A** includes an information sheet summarizing the procedure, and providing operational and implementation considerations for this test.

2.4.2 Line probe assays

LPAs are a family of hybridization tests based on DNA strips that detect the presence or absence of mutations associated with drug resistance. The assays do this directly, through binding DNA amplification products (amplicons) to probes targeting the most commonly occurring mutations (MUT probes), or indirectly, by inferring resistance through the binding of a wild-type probe to a wild-type target sequence. The definition of the class is found in **Table 2.7**.

Table 2.7. Definition of the class criteria for LPAs

Purpose		Detection of resistance to first-line and/or second-line TB drugs
Principle of action		DNA-based reverse hybridization, or line probe, assays
Complexity	Reagents	Reagents are available within standardized kits and may have temperature requirements for storage
	Skills	Advanced technical skills (i.e. multiple sample or reagent handling steps, precision pipetting, molecular workflows may be required)
	Pipetting	Multiple precision pipetting steps required by the procedure
	Testing procedure	May require multiple specimen treatment steps before transferring the specimen into a sealed container for multistep testing Manual or automated DNA extraction Manual or automated real-time PCR Instrument-based reverse hybridization
Type of test result reporting		Manual
Setting of use		Molecular laboratory (special infrastructure and separate spaces for different parts of the testing procedure are required)

First-line drug LPAs

First-line drug LPAs (FL-LPAs) such as the GenoType MTBDR*plus* test and the NTM+MDRTB Detection Kit allow the detection of resistance to RIF and INH. WHO recommends using FL-LPAs in the situations shown in **Box 2.6**.

Box 2.6. WHO recommendation on FL-LPAs *(6)*

For people with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to rifampicin and isoniazid.

(Conditional recommendation, moderate certainty of evidence)

Remarks:

- ➔ This recommendation applies to the use of LPAs for testing sputum smear-positive specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.
- ➔ LPAs are not recommended for the direct testing of sputum smear-negative specimens.
- ➔ This recommendation applies to the detection of MTBC and the diagnosis of MDR-TB, but acknowledges that the accuracy of detecting resistance to RIF and INH differs and, hence, that the accuracy of a diagnosis of MDR-TB is reduced overall.
- ➔ This recommendation does not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.
- ➔ Conventional culture-based DST for INH may still be used to evaluate patients when the LPA result does not detect INH resistance. This is particularly important for populations with a high pretest probability of resistance to INH.
- ➔ These recommendations apply to the use of LPA in children based on the generalization of data from adults.

Second-line drug LPAs

Second-line drug LPAs (SL-LPAs) such as the GenoType MTBDR*sl* test allow the detection of resistance to FQs and AMK (**Box 2.7**).

Box 2.7. WHO recommendations on SL-LPAs *(6)*

For people with confirmed MDR/RR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones.

(Conditional recommendation, moderate certainty of evidence)

For people with confirmed MDR/RR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to the SLIDs.

(Conditional recommendation, low certainty of evidence)

Remarks:

- ➔ These recommendations apply to the use of SL-LPA for testing sputum specimens (direct testing) and cultured isolates of *Mtb* (indirect testing) from both pulmonary and extrapulmonary sites. Direct testing on sputum specimens allows for the earlier initiation of appropriate treatment.
- ➔ These recommendations apply to the direct testing of sputum specimens from MDR/RR-TB, irrespective of the smear status, while acknowledging that the indeterminate rate is higher when testing sputum smear-negative specimens than with sputum smear-positive specimens.
- ➔ These recommendations do not eliminate the need for conventional phenotypic DST capacity, which will be necessary to confirm resistance to other drugs and to monitor the emergence of additional drug resistance.
- ➔ Conventional phenotypic DST can still be used in the evaluation of patients with negative SL-LPA results, particularly in populations with a high pretest probability for resistance to FQs or second-line injectable drugs (SLIDs) (or both).
- ➔ These recommendations apply to the use of SL-LPA in children with confirmed MDR/RR-TB, based on the generalization of data from adults.
- ➔ Resistance-conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to ofloxacin and levofloxacin.
- ➔ Resistance-conferring mutations detected by SL-LPA are highly correlated with phenotypic resistance to SLIDs.
- ➔ Given the high specificity for detecting resistance to FQ and SLIDs, the positive results of SL-LPA could be used to guide the implementation of appropriate infection control precautions.

2.4.3 High-complexity reverse hybridization NAATs

The first-in-class product for the class of HC-rNAATs is the Genoscholar PZA-TB (Nipro, Osaka, Japan) for the detection of resistance to PZA. The Genoscholar PZA-TB test is based on the same principle as the FL-LPA and SL-LPA but requires the use of many hybridization probes to cover the full *pncA* gene (>700 base pairs [bp]). Reading the hybridization results on the strips with a total of 48 probes requires careful attention; however, the test provides faster results than phenotypic DST and is based on molecular detection. The overall pooled sensitivity for the detection of PZA resistance was 81.2% (95% CI: 75.4–85.8%) and the pooled specificity was 97.8% (95% CI: 96.5–98.6%) *(22)*. The hybridization can be performed on the TwinCubator instruments (Bruker-Hain, Germany) that are used for LPAs *(19)*. An information sheet summarizing HC-rNAATs is available in **Web Annex A**. The WHO recommendation on HC-rNAATs is given in **Box 2.8**.

Box 2.8. WHO recommendation on HC-rNAATs *(6)*

For people with bacteriologically confirmed TB, high-complexity reverse hybridization-based NAATs may be used on *Mtb* culture isolates for detection of pyrazinamide resistance, rather than culture-based phenotypic DST.

(Conditional recommendation, very low certainty of evidence)

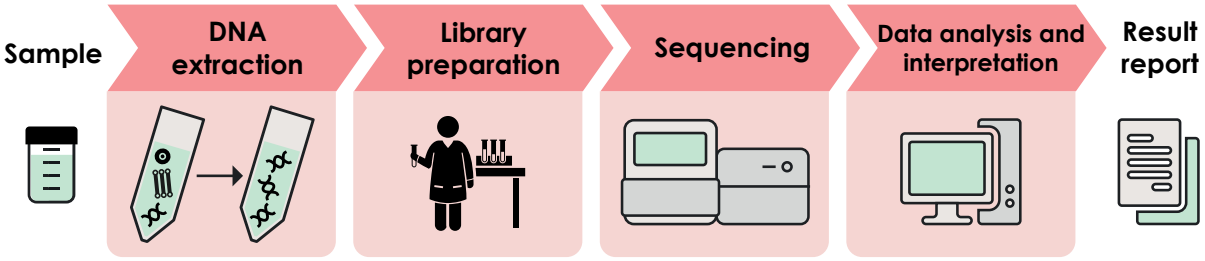
Remarks:

➔ The recommendation only applies to culture isolates; thus, this test is appropriate for use only where culture facilities are available.

2.4.4 Targeted NGS end-to-end solutions

The class of targeted NGS tests was defined as workflows that use massively parallel sequencing to detect resistance to TB drugs, starting from a processed clinical sample and concluding with an end-user report that relates detected *Mtb* mutations to the presence (or absence) of drug resistance, based on the interpretation of the WHO catalogue of mutations *(23)*. Solutions that involve end-to-end targeted NGS tests couple DNA extraction and amplification of selected genes with NGS technology to detect resistance to many drugs with a single test, as shown in **Fig. 2.2**. Because targeted NGS tests can interrogate entire genes to identify specific mutations associated with resistance, these tests may provide improved accuracy compared with other WRDs. In addition, targeted NGS tests can detect resistance to new and repurposed drugs not currently included in any other molecular assays. Thus, these tests offer great potential to provide comprehensive resistance detection matched to modern treatment regimens *(24)*.

Fig. 2.2. Process of testing, from sample to result report, for targeted NGS



DNA: deoxyribonucleic acid; NGS: next-generation sequencing

In 2023, WHO recommended the use of the class of targeted NGS to detect drug resistance for clinical decision-making, and included three solutions as first-in-class technologies. In January 2025, the WHO TAG assessed new and updated targeted NGS solutions that met class criteria. Based on the evidence available and outcomes of the evidence assessment, no new technologies were added to the targeted NGS class. However, the resistance interpretation software for the AmPORE-TB solution had been updated, and this product was found to meet class-based performance criteria for detection of resistance to three additional drugs (PZA, BDQ and CFZ). The current list of targeted NGS solutions and the drugs for which WHO recommends their use for resistance detection are given in **Table 2.8**.

Table 2.8. List of targeted NGS solutions and drugs for which WHO recommends their use

Targeted NGS end-to-end solution	TB drugs for which detection of resistance is recommended									
	RIF	INH	PZA	EMB	FQ	BDQ	LZD	CFZ	AMK	STR
Deeplex Myc-TB (GenoScreen)	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
AmPORE-TB (ONT)	✓	✓	✓		✓	✓	✓	✓	✓	✓
TBSeq (Shengting Medical Technology Co Ltd)				✓						

NGS: next-generation sequencing; TB: tuberculosis; WHO: World Health Organization.
Drugs: AMK: amikacin; BDQ: bedaquiline; CFZ: clofazimine; EMB: ethambutol; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.

The WHO policy statement on the outcomes of the TAG meeting is summarized in **Box 2.9**. Further considerations for the implementation of targeted NGS solutions are outlined in **Annex 3**; these considerations are supported by the WHO manual on the use of NGS for the surveillance of DR-TB *(25)*.

Box 2.9. *WHO policy statement on targeted NGS solutions for detection of DR-TB, 2025*

Recently, new and updated targeted NGS solutions for TB drug resistance testing have become available. WHO solicited information on these technologies directly from manufacturers and through a public call for information. In addition, a systematic review of published and unpublished literature was conducted. All available evidence was assessed by a TAG in January 2025. Products that had sufficient independent evidence for consideration were AmPORE-TB (Oxford Nanopore Technologies) and DeepChek Assay 13-Plex KB Drug Susceptibility Testing (ABL Diagnostics SA).

Following TAG review and advice, WHO makes the following policy statements:

1. The performance of the AmPORE-TB (ONT) end-to-end solution is comparable to that of other WHO-recommended targeted NGS solutions for the detection of resistance to RIF, INH, FQs and PZA among people with bacteriologically confirmed pulmonary TB.
2. The performance of the AmPORE-TB (ONT) end-to-end solution is comparable to that of WHO-recommended targeted NGS solutions for the detection of resistance to INH, FQs, BDQ, LZD, CFZ, PZA, AMK and STR among people with RR-TB.
3. The performance of the DeepChek Assay 13-Plex KB Drug Susceptibility Testing (ABL Diagnostics SA) solution could not be adequately compared to that of WHO-recommended targeted NGS solutions for the detection of drug resistance among people with bacteriologically confirmed pulmonary TB, with or without RIF resistance.
4. Current WHO recommendations for the use of targeted NGS are valid for the AmPORE-TB (ONT) solution, which now meets class-based performance criteria for detection of resistance to three additional drugs (PZA, BDQ and CFZ).

Remarks:

- ➔ AmPORE-TB performance was not comparable to that of the tNGS class for detection of resistance to ethambutol, so a statement on resistance detection for this drug was not made in either population.
- ➔ The decision on AmPORE-TB regarding streptomycin was not unanimous due to concerns about the relatively lower sensitivity compared with class performance criteria. However, considering this is a last resort drug, and pDST results take longer to become available, it was considered acceptable for inclusion.
- ➔ The decision on DeepCheck comparability was based on the high sample and drug-specific failure rates that occurred across the entire range of Xpert MTB/RIF semiquantitative categories when compared with those for the class of tNGS solutions. The review team and TAG were unable to assess whether the indeterminate rates were due to site-specific differences. Notably the three evaluation sites used different DNA extraction methods; however, the impact of the methodology difference could not be individually assessed for impact apart from other factors that varied between study sites. WHO welcomes a future submission from the manufacturer with more independent evidence on the performance of the end-to-end solution. If multiple extraction methods are used, data on the extraction method performance and equivalence may be required.

- The analysis software for tNGS end-to-end solutions is anticipated to be updated with new versions of the WHO Mutations Catalogue; these updates are expected to improve solution performance for detection of resistance over time but will have to be evaluated.

Implementation considerations

When implementing targeted NGS, programmes need to consider factors such as disease burden, placement, costs, skills of staff and the diagnostic network. In addition to the implementation considerations the GDG made in 2024 when targeted NGS was recommended for use *(5)*, the TAG listed additional factors during its meeting in January 2025:

- High-speed internet is required for software solution functionality to support analysis and automated reporting.
- NGS generates large amounts of data that require planning for and the availability of appropriate storage capacity.
- Countries should ensure that targeted NGS testing is included within an EQA programme for sequencing that covers all relevant targets of interest.

The guideline also notes that **further research** is needed to close the knowledge gaps on targeted NGS.

Where a product has not yet met the requirements for a specific drug (i.e. the drug is not currently recommended in **Table 2.8**), further improvements to the product and a review of the evidence will be necessary before DST results for the specific drug can be used clinically. The full report on the TAG review is given in **Web Annex D**.

NEW The TAG report outlines plans for how targeted NGS diagnostic class performance will be updated as individual product performance improves over time.

WHO provides two recommendations on the use of targeted NGS (**Box 2.10**). Among people with bacteriologically confirmed pulmonary TB, the targeted NGS test performances (**Table 3.3**, **Table 3.4** and **Table 3.5** in **Section 3**) were determined to be accurate for all drugs included in the assessment, with pooled sensitivity of at least 95% for INH, MFX and ethambutol (EMB); more than 93% for RIF and levofloxacin (LFX); and 88% for PZA.[4] The pooled specificity was at least 96% for all drugs. The reference standard was phenotypic DST for INH, LFX and MFX, and a combination of phenotypic DST and whole-genome sequencing (WGS) for RIF, PZA and EMB. The indeterminate rate ranged from 7% (AMK) to 18% (PZA) but was highest in samples with low or very low bacterial loads, which may have implications for implementation; therefore, priority should be given to samples with a higher bacillary load. The overall certainty of the evidence ranged from low to moderate for test accuracy.

[4] Pooled sensitivity and specificity for targeted NGS were calculated on the first-in-class tests when the class recommendation was made; values may be updated in the future if expected improvements in performance eventuate. Considerations on performance value updates for the targeted NGS class can be found in the TAG meeting report in **Web Annex D**.

Among people with bacteriologically confirmed RIF-resistant pulmonary TB, the targeted NGS test performances (**Table 3.6** in **Section 3**) were determined to be accurate for INH, LFX, MFX, streptomycin (STR) and EMB (pooled sensitivity ≥95%), and acceptable for BDQ (68%), LZD (69%), CFZ (70%), AMK (87%) and PZA (90%). The specificity was at least 95% for all drugs except STR (75%). The reference standard was phenotypic DST for all drugs except EMB and PZA, where a combination of phenotypic DST and WGS was used. The indeterminate rate ranged from 9% (LFX and MFX) to 21% (EMB) and depended on the bacterial load. For test accuracy, the overall certainty of the evidence ranged from low to high.

Box 2.10. WHO recommendations on targeted NGS *(6)*

For people with bacteriologically confirmed pulmonary TB disease, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol, rather than culture-based phenotypic drug susceptibility testing.

(Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide], low [rifampicin, fluoroquinolones and ethambutol])

Remarks:

- ➔ Priority should be given to those at higher risk of resistance to first-line treatment medications, including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or experience treatment failure;
  - have previously had TB treatment;
  - are in contact with a person known to have resistance to TB drugs; or
  - reside in settings or belong to subgroups where there is a high probability of resistance to either RIF, INH or FQ (used in new shorter regimens), or where there is a high prevalence of *Mtb* strains harbouring mutations not detected by other rapid molecular tests.
- ➔ This recommendation is conditional because of the lack of data on health benefits, the variable certainty of evidence on diagnostic accuracy, and the fact that accuracy is suboptimal for certain drugs. In addition, because this is a new technology that has not yet been widely implemented, there is still limited and variable evidence on costs, cost–effectiveness and feasibility of implementation.

For people with bacteriologically confirmed rifampicin-resistant pulmonary TB disease, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin, rather than culture-based phenotypic drug susceptibility testing.

(Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate [ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin], very low [amikacin])

Remarks:

➔ Priority should be given to those at a higher risk of resistance to medications used for the treatment of RR-TB, including individuals who:

- continue to be smear or culture positive after 2 months or more of treatment or have experienced treatment failure;
- have previously had TB treatment, including with the new and repurposed drugs;
- are in contact with a person known to have resistance to TB drugs, including the new and repurposed drugs; or
- have pre-XDR-TB with resistance to FQ.

➔ As above, this recommendation is conditional because of the lack of data on health benefits, the variable certainty of evidence on diagnostic accuracy, the fact that accuracy is suboptimal for certain drugs, and limited and variable evidence on costs, cost–effectiveness and feasibility of implementation.

All products recommended by WHO are automatically eligible to be included in the WHO essential diagnostic list. The WHO recommendations on diagnostics are based on clinical research evidence; they do not include quality assessments of the products or the manufacturing process involved. Before introducing any new products, countries should ensure that those products fulfil local or internationally recognized regulatory requirements.

2.5 Phenotypic and genotypic drug resistance testing methods

2.5.1 Phenotypic DST

Treatment of TB has undergone significant changes over recent years, with new drugs and regimens being recommended; hence, the definitions for DR-TB have been revised accordingly. The updated definitions are as follows *(26)*:

- pre-XDR-TB is "TB caused by *M. tuberculosis* strains that fulfil the definition of MDR/RR-TB and that are also resistant to any FQ"; and
- XDR-TB is "TB caused by *M. tuberculosis* strains that fulfil the definition of MDR/RR-TB and that are also resistant to any FQ and at least one additional Group A drug (i.e. BDQ or LZD)".

These changes have important implications for Member States, particularly for the scaling-up of the detection of resistance to FQs and BDQ. In addition, there is an increasing demand for DST for other new and repurposed drugs.

Indirect phenotypic DST on solid (LJ, 7H10 agar, 7H11 agar) and liquid media (7H9 broth, BACTEC Mycobacterial Growth Indicator Tube [MGIT] system) is reliable and reproducible, and it remains the reference standard for many anti-TB medicines *(27)*. The DST manual in **Web Annex C** collates the WHO recommendations for phenotypic and genotypic DST, and includes the critical concentrations for Pa and Cs that were established in 2023 (**Box 2.11**). Reliable phenotypic DST methods are available for RIF, INH, FQs, PZA, BDQ, LZD, AMK, STR CFZ, DLM, Pa and Cs. Phenotypic DST is currently not recommended for EMB, ETO, prothionamide, *para*-aminosalicylic acid (PAS), imipenem-cilastatin and meropenem (MPM). The manual also provides information on sources of pure powders for phenotypic DST, detailed methods for preparing drug-containing media, interpretation and reporting of results, and quality control (QC).

Box 2.11. WHO policy statement on the assessment of critical concentrations for pretomanid and cycloserine, 2023

Pa and Cs are used to treat individuals with DR-TB, but there was no established phenotypic DST method or interpretive criteria to define resistance. To address this gap, the first step was to establish CCs for these drugs, informed by epidemiological cut-off values and by pharmacokinetic (PK), pharmacodynamic (PD) and clinical outcome data where available.

WHO initiated a systematic search and analysis of the available evidence, which was then assessed by the TAG.

Following review of the evidence and advice from the TAG, WHO made the following policy statements:

1. Two test concentrations (0.5 and 2.0 mg/L) should be used for pretomanid DST, using the MGIT method with the following interpretation:

- ➔ no growth at 0.5 mg/L = susceptible;
- ➔ growth at 0.5 mg/L and no growth at 2.0 mg/L = susceptible (with a comment added to the laboratory report stating there is an interpretive uncertainty of this result and close patient follow-up is required); and
- ➔ growth at 2.0 mg/L = resistant.

2. A critical concentration of 16 mg/L should be used for Cs DST using the MGIT method.

Remarks:

- ➔ *Mtb* lineage 1 isolates frequently have minimum inhibitory concentrations (MICs) within the range 0.5–2.0 mg/L.
- ➔ For results within this range, there are interpretive difficulties, and susceptibility cannot be guaranteed.
- ➔ The combination regimens of BPaLM or BPaL appear to retain clinical efficacy even when the MIC for Pa falls within the range 0.5–2.0 mg/L (low availability of clinical data).
- ➔ WHO will review this recommendation when further clinical evidence of the efficacy of Pa-containing regimens for isolates with MICs within the range 0.5–2.0 mg/L becomes available.

Implementation considerations

Implementation considerations for Pa

- Preferably, the two concentrations should be tested simultaneously. Sequential testing on the same isolate can be considered based on the local frequency of L1 isolates or isolates with MICs of 0.5 mg/L or more.
- For isolates with an MIC within the range 0.5–2.0 mg/L, recommendations are:
  - continue a BPaL/M regimen; and
  - continue follow-up – if there is a poor bacteriological or clinical response, consider a change in treatment regimen *(28)*.

Implementation considerations for Cs

- The critical concentration for Cs may be used as a surrogate for terizidone (TRD) resistance.
- Given the known heat instability of Cs, stock solutions should be stored at between –80 °C and –60 °C (not at –20 °C) for up to 1 year, and they should not be refrozen following use.

Further research

Further research for Pa and Cs

Further research topics for Pa and Cs overall include:

- further investigation and characterization of new and known molecular mechanisms of resistance for Pa and Cs, and resolution of uncertainty in the annotation of genes associated with resistance;
- further investigation and establishment of the breakpoints for DST for Pa and Cs with media other than MGIT, including broth microdilution;
- therapeutic drug monitoring tests to guide approaches to dose adjustment in patients experiencing toxicity; and
- temporal trends in MIC through routine surveillance.

Further research for Pa

Further research topics for Pa specifically include:

- population representative sampling to understand the geographical distribution of L1 disaggregated by RIF status;
- operational research evaluating clinical outcomes of individuals with L1 and isolates with MICs of 0.5 mg/L or more among those on BPaL, BPaLM and other investigational regimens;
- operational studies, research studies and routine surveillance on resistance disaggregated by lineage (strongly desired: matched phenotypic and sequencing data); and
- PK/PD studies on L1 and isolates with MICs of 0.5 mg/L or more to inform future DST criteria.

The full TAG report can be found in **Web Annex B**.

The new definition of XDR-TB requires DST results for FQs, BDQ and LZD; thus, testing for resistance to BDQ and LZD has become a priority, particularly testing for resistance to BDQ. Phenotypic DST for BDQ and LZD can be performed using either the MGIT or Middlebrook 7H11 media. Lyophilized vials containing BDQ powder for use with MGIT are manufactured by Becton Dickinson and are available in the Global Drug Facility catalogue *(29)*. In addition, the BDQ pure drug substance for use in phenotypic DST is provided free through the BEI Resources of the US National Institutes of Health (NIH) *(30)*; however, courier costs need to be covered. An information note that explains the request process is also available *(31)*. The BEI Resources also provides DLM and Pa. LZD powder is available from Sigma (PZ0014–5MG) or Cayman Chemical (CAS 65800–03–3). The DST manual in **Web Annex C** provides specific details on the sources of the drug powders used for phenotypic DST.

It is essential to monitor all new batches of drug solutions to detect challenges with quality or performance as early as possible. It may not be enough to test only one reference strain at the CC because of possible variations in the isolates. A more robust way to control for variations is to include multiple dilutions above and below the CC, to ensure that the reference strain used is behaving as expected.

The latest CCs for all drugs are listed in **Table 2.9** and **Table 2.10**; they were adapted from Tables 1 and 2 of **Web Annex C**.

Table 2.9. Critical concentrations for first-line medicines recommended for the treatment of drug-susceptible TB

Medicine	Abbreviation	CCs (µg/mL) for DST by medium[a]			
		Löwenstein–Jensen	Middlebrook 7H10	Middlebrook 7H11	BACTEC MGIT liquid culture
Rifampicin	RIF	40.0	0.5	1.0	0.5[b]
Isoniazid[c]	INH	0.2	0.2	0.2	0.1
Ethambutol[d]	EMB	2.0	5.0	7.5	5.0
Pyrazinamide[e]	PZA	–	–	–	100
Moxifloxacin	MFX (CC)	1.0	0.5	0.5	0.25

CC: critical concentration; DNA: deoxyribonucleic acid; DST: drug susceptibility testing; LPA: line probe assay; MGIT: Mycobacterial Growth Indicator Tube; MTBC: *Mycobacterium tuberculosis* complex; HC-rNAAT: reverse hybridization nucleic acid amplification test; TB: tuberculosis.

Drugs: EMB: ethambutol; INH: isoniazid; PZA: pyrazinamide; RIF: rifampicin.

[a] The use of the indirect proportion method is recommended. Other methods using solid media (e.g. the resistance ratio or absolute concentration) have not been adequately validated for anti-TB agents.

[b] The detection of RIF resistance using the BACTEC MGIT 960 system has limitations and it cannot detect clinically significant resistance in certain isolates. The detection of resistance-conferring mutations in the entire *rpoB* gene using DNA sequencing may be the most reliable method for the detection of RIF resistance.

[c] People with MTBC isolates that are resistant at the CC may be effectively treated with high-dose INH. Formerly, a higher concentration of INH (0.4 µg/mL in MGIT) was used to identify strains that may be effectively treated with a higher drug dose; however, molecular patterns of INH resistance may be more reliable for predicting patient outcomes than phenotypic DST.

[d] All phenotypic DST methods for EMB produce inconsistent results. The Genoscholar PZA-TB LPA is the only HC-rNAAT recommended for PZA.

[e] The detection of resistance-conferring mutations in the *pncA* gene using DNA sequencing may be the most reliable method for the detection of PZA resistance, although there is emerging evidence of non-*pncA* mutational resistance to PZA.

Table 2.10. Critical concentrations and clinical breakpoints for medicines recommended for the treatment of MDR/RR-TB

Group	Medicine	Abbreviation	CCs (µg/mL) for DST by medium			
			Löwenstein–Jensen	Middlebrook 7H10	Middlebrook 7H11	BACTEC MGIT liquid culture
Group A	Levofloxacin (CC)	LFX[a]	2.0	1.0	–	1.0
	Moxifloxacin (CC)	MFX[a]	1.0	0.5	0.5	0.25
	Moxifloxacin (CB)[b]	–	–	2.0	–	1.0
	Bedaquiline	BDQ	–	–	0.25	1.0
	Linezolid	LZD	–	1.0	1.0	1.0
Group B	Clofazimine	CFZ	–	–	–	1.0
	Cycloserine/ terizidone	CS/TRD	–	–	–	16.0
Group C	Ethambutol[d]	EMB	2.0	5.0	7.5	5.0
	Delamanid[e]	DLM	–	–	0.016	0.06
	Pyrazinamide[f]	PZA	–	–	–	100.0
	Imipenem-cilastatin	IMP/CLN	–	–	–	–
	Meropenem	MPM	–	–	–	–
	Amikacin	AMK	30.0	2.0	–	1.0
	(streptomycin)[g]	(STR)	4.0	2.0	2.0	1.0
	Ethionamide	ETO	40.0	5.0		5.0
	Prothionamide	PTO	40.0	–	–	2.5

Group	Medicine	Abbreviation	CCs (µg/mL) for DST by medium			
			Löwenstein–Jensen	Middlebrook 7H10	Middlebrook 7H11	BACTEC MGIT liquid culture
	Para-aminosalicylic acid	PAS	–	–	–	–
Other	Pretomanid	Pa	–	–	–	0.5[h] 2.0[h]

CB: clinical breakpoint; CC: critical concentration; DST: drug susceptibility testing; LJ: Löwenstein–Jensen media; MDR/RR-TB: multidrug- or rifampicin-resistant tuberculosis; MGIT: Mycobacterial Growth Indicator Tube.

Drugs: AMK: amikacin; CS: cycloserine; DLM: delamanid; LFX: levofloxacin; MFX: moxifloxacin; PZA: pyrazinamide; STR: streptomycin; TRD: terizidone.

[a] LFX and MFX CCs for LJ were established despite very limited data.

[b] CB concentration for 7H10 and MGIT apply to high-dose MFX (i.e. 800 mg daily).

[c] The CC for CS may be used as a surrogate for Tad resistance.

[d] DST is not reliable and reproducible; hence, DST is not recommended.

[e] DLM should be stored away from light and heat, as per the manufacturer's materials safety data sheet.

[f] PZA is only counted as an effective agent when DST results confirm susceptibility in a quality-assured laboratory.

[g] AMK and STR are only to be considered in case of rescue regimens or individualized treatment, and only if DST results confirm susceptibility.

[h] No growth at 0.5 = susceptible; growth at 0.5 and no growth at 2.0 = susceptible, but with a comment on uncertainty; growth at 2.0 = resistant.

Source: adapted from Table 3 in **Web Annex C**.

2.5.2 Whole-genome sequencing

Phenotypic DST remains the reference standard for most anti-TB compounds; however, this method is slow, and it requires specialized infrastructure and highly skilled staff. Genotypic DST (also referred to as molecular DST) holds promise to overcome some of the obstacles of phenotypic DST, and complements phenotypic DST as a reference standard when testing for RIF, EMB and PZA resistance. Currently, the available mWRDs for DST can be used to detect specific mutations known to confer phenotypic resistance. Rapid molecular tests for resistance to RIF, INH and FQ can be implemented in decentralized settings; such tests can deliver rapid results to inform the selection of the initial treatment regimen while awaiting follow-on DST for other anti-TB drugs.

DNA sequencing using NGS technologies is a method for the detection of mutations associated with drug resistance for many anti-TB drugs *(32)*. NGS-based DST could reduce the need for phenotypic DST for patient-care decisions; it may be particularly useful for drugs for which phenotypic DST is unreliable or for settings that do not have the capacity to perform phenotypic DST.

NGS refers to techniques that rely on the sequencing of multiple DNA fragments in parallel, followed by bioinformatics analyses to assemble the sequences. The technologies can be used to determine the nucleotide sequence of an entire genome (i.e. WGS) or part of a genome (i.e. targeted NGS) in a single sequencing run. WGS and targeted NGS are recommended for use in the surveillance of DR-TB and for DST.

WGS is a powerful tool for detecting mutations and it serves as a reference test for confirming mutations; however, it depends on having a culture isolate and is impacted by delays in growing the organism *(33)*. The WHO mutation catalogue *(23)* provides a standardized methodology for the analytical pipeline for analysis of NGS results and interpretation of mutations. The tools can be used both for WGS and targeted NGS.

Phenotypic DST remains important for classifying resistance, particularly for new drugs where resistance is not fully elucidated. As more phenotypic DST data are matched with WGS data in the catalogue, the performance of the catalogue and the utility of WGS for new drugs will improve. Contributors are encouraged to upload data to the TB sequencing portal *(34)*.

Use of WGS and phenotypic DST is now the reference standard for RIF and PZA owing to the limitations of phenotypic DST alone for these drugs.

Section 2.4.4 describes the targeted NGS tests for the detection of resistance to anti-TB medicines recently recommended by WHO for use directly on clinical samples. These tests can detect mutations associated with resistance to RIF, INH, PZA, EMB, FQ, BDQ, LZD, CFZ, AMK and STR.

Table 2.11 presents an overview of the WHO-recommended diagnostic approaches, reference methods and clinical interpretation for anti-TB medicines.

Table 2.11. WHO-recommended diagnostic approaches, reference methods and clinical interpretation for anti-TB medicines

Drug		Genotypic DST	Phenotypic DST	Reference method	Comment
First-line anti-TB drugs	Rifampicin	WRDs LPAs Targeted NGS	MGIT may not be reliable for isolates with borderline resistance mutations	MGIT and DNA sequencing of the entire *rpoB* gene	Any mutations (excluding silent mutations) observed in the 81bp RRDR[a] hotspot region of the *rpoB* gene are known or assumed to be associated with RIF resistance. In a few cases, mutations in the *rpoB* gene outside the RRDR are associated with RIF resistance, these would require DR-TB treatment. Sequencing should be considered if the suspicion of resistance is high.
	Isoniazid	Moderate-complexity automated NAATs Low-complexity automated NAATs LPAs Targeted NGS	Reliable and reproducible when testing the CC in all media	MGIT	If specific *inhA* promoter mutations are detected in the absence of any *katG* mutations, increasing the dose of INH is likely to be effective. Low- and moderate-complexity NAATs and LPAs for RIF and INH detection are preferred to guide patient selection for the (H)RZE-LFX regimen. RIF resistance should be excluded before starting the Hr-TB regimen and FQ resistance should be excluded as soon as possible. Targeted NGS (for faster results) or phenotypic DST should be considered if the suspicion of resistance is high and the initial rapid test is susceptible.

Drug		Genotypic DST	Phenotypic DST	Reference method	Comment
Group A second-line anti-TB drugs	Levofloxacin	Low-complexity automated NAATs LPAs Targeted NGS	Reliable and reproducible when testing the CC in LJ, 7H10 and MGIT media[a]	MGIT	Strains with known or assumed resistance mutations should be classified as resistant. Most strains without mutations should be classified as susceptible; however, a strain with no mutations detected by NAATs or LPAs may still be resistant. Targeted NGS (for faster results) or phenotypic DST should be considered if the suspicion of resistance is high.
	Moxifloxacin (CC)	Low-complexity automated NAATs LPAs Targeted NGS	Reliable and reproducible when testing the CC in LJ, 7H10, 7H11 and MGIT media[a]	MGIT	A strain of TB with no mutations detected by LPA or Xpert MTB/XDR may still be resistant. Targeted NGS (for faster results) or phenotypic DST should be used to confirm both the CC and CB concentrations.
	Moxifloxacin (CB)	Low-complexity automated NAATs LPAs Targeted NGS	CB for 7H10 and MGIT apply to high-dose moxifloxacin (i.e. 800 mg daily)	MGIT	MFX, even at high doses, is unlikely to be effective if resistant at the CB concentration or if certain high-confidence mutations associated with high MICs are detected.

Drug		Genotypic DST	Phenotypic DST	Reference method	Comment
Group A second-line anti-TB drugs	Bedaquiline	Targeted NGS	CCs established for testing in 7H11 and MGIT media	MGIT	Ideally, targeted NGS (for faster results) or phenotypic DST should be performed at the time of treatment initiation. If baseline DST is not performed, DST should be performed with the first strain isolated from the patient's sample during treatment monitoring.[b]
	Linezolid	Targeted NGS	CCs established for testing in 7H10, 7H11 and MGIT media	MGIT	Ideally, targeted NGS (for faster results) or phenotypic DST should be performed at the time of treatment initiation. If baseline DST is not performed, DST should be performed with the first strain isolated from the patient's sample during treatment monitoring.[b]
Group B second-line anti-TB drugs	Clofazimine	Targeted NGS	CC established for testing MGIT media only	MGIT	Ideally, targeted NGS (for faster results) or phenotypic DST should be performed at the time of treatment initiation. If baseline DST is not performed, DST should be performed with the first strain isolated from the patient's sample during treatment monitoring.[b]
	Cycloserine	Currently, there is no rapid method for the detection of resistance	CCs have been established for CS on MGIT media only	MGIT	Ideally, phenotypic DST should be performed at the time of treatment initiation. If baseline DST is not performed, DST should be performed with the first strain isolated from the patient's sample during treatment monitoring.[b]
	Terizidone				

Drug		Genotypic DST	Phenotypic DST	Reference method	Comment
Group C second-line anti-TB drugs	Ethambutol	Currently, there is no rapid method for the detection of resistance to EMB	DST is not reliable and reproducible	MGIT and DNA sequencing of the *embB* gene	Both genotypic DST and phenotypic DST are not reliable. If EMB is used in an MDR/RR-TB treatment regimen, it cannot be counted as an effective drug in that regimen. Phenotypic DST can result in poor reproducibility for specific mutations; hence, testing in combination with phenotypic DST and DNA sequencing is preferred.
	Delamanid	No rapid method currently exists for the detection of resistance	CCs established for testing in 7H11 and MGIT media	MGIT	Ideally, phenotypic DST should be performed at the time of treatment initiation. If baseline DST is not performed, DST should be performed with the first strain isolated from the patient's sample during treatment monitoring.[a]
	Pyrazinamide	Targeted NGS LPAs	The DST method is standardized in the MGIT False resistant results can be detected if the DST inoculum is not properly prepared	MGIT and DNA sequencing of the *pncA* gene	In a quality-assured laboratory, a susceptible DST result for PZA can be used to guide the inclusion of PZA in a DR-TB treatment regimen. If resistance is detected, PZA should not be included; however, if it is used, it should not be counted as an effective agent.

Drug		Genotypic DST	Phenotypic DST	Reference method	Comment
Group C second-line anti-TB drugs	Amikacin (or streptomycin)	Low-complexity automated NAATs Targeted NGS (for AMK and STR) LPAs[b] (for AMK)	CCs have been established for testing in LJ, Middlebrook and MGIT media	MGIT	Injectable agents are no longer part of the routine DR-TB regimen. However, testing should be completed if there are plans to use AMK or STR in an individualized DR-TB regimen. A strain with no mutations in the *rrs* and *eis* genes detected by genotypic assays may still be resistant to AMK; this should be confirmed with phenotypic DST[c]. If STR is used, phenotypic DST should be performed at the time of treatment initiation if possible.
	Imipenem-cilastatin Meropenem	Currently, there is no rapid method for the detection of resistance	CCs have not been established for any DST media	N/A	DST is not recommended because both IMP and MPM are highly unstable in liquid media.
	Ethionamide	Low-complexity automated NAATs Moderate-complexity automated NAATs LPAs Targeted NGS	DST not reliable and reproducible	DNA sequencing of the *inhA* promotor region and *ethA* and *ethR* genes	The thioamides (ETO and PTO) should not be included if resistance-associated mutations are detected.
	Para-aminosalicylic acid	Currently, there is no rapid method for the detection of resistance	CCs have not been established for any DST media	N/A	DST is not currently recommended.

Drug		Genotypic DST	Phenotypic DST	Reference method	Comment
Other	Pretomanid	Currently, there is no rapid method for the detection of resistance	CCs have been established for MGIT media only	MGIT	Ideally, phenotypic DST should be performed at the time of treatment initiation. If baseline DST is not performed, DST should be performed with the first strain isolated from the patient's sample during treatment monitoring.[c]

bp: base pair; CB: clinical breakpoint; CC: critical concentration; DNA: deoxyribonucleic acid; DR-TB: drug-resistant TB; DST: drug susceptibility testing; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; LJ: Löwenstein–Jensen media; LPA: line probe assay; MDR/RR-TB: multidrug- or rifampicin-resistant TB; MGIT: Mycobacterial Growth Indicator Tube; MIC: minimal inhibitory concentration; N/A: not available; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; RRDR: rifampicin-resistance determining region; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis; WGS: whole-genome sequencing; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

Drugs: AMK: amikacin; CS: cycloserine; EMB: ethambutol; ETO: ethionamide; FQ: fluoroquinolone; HREZ: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z); IMP: imipenem; INH: isoniazid; LFX: levofloxacin; MPM: meropenem; MFX: moxifloxacin; PTO: prothionamide; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.

[a] LFX and MFX CCs for LJ were established despite limited data.

[b] Phenotypic DST should be performed for strains isolated from people during treatment monitoring. If resistance is detected, strains should be stored and WGS should be performed, if possible, to collect data on mutations associated with resistance.

[c] SL-LPAs do not cover the relevant region of the *rrs* gene or other genes associated with resistance to STR.

Source: adapted from Table 3 in **Web Annex C**.

2.6 Tests for TB infection

TB infection is a state of persistent immune response to *Mtb* antigens with no evidence of clinically manifest TB disease *(35)*. People with TB infection have no signs or symptoms of TB disease, are not infectious, have normal or stable images on chest X-ray (CXR), and have negative microbiological tests (if such tests are performed). It is estimated that about 25% of the world's population has been infected with *Mtb (1)*, of whom 5–10% will develop TB disease over their lifetime *(36)*. This risk is much higher in those with certain epidemiological factors (e.g. recent contact with bacteriologically confirmed pulmonary TB), social characteristics (low socioeconomic status or malnutrition) and demographical characteristics (e.g. very young children or the elderly) or clinical conditions that compromise the immune system (e.g. HIV infection, diabetes or immunosuppressive medications) *(37–39)*.

Among people with such risk factors, TPT can provide important individual and public health benefits. However, implementing TPT raises various challenges in terms of programme prioritization, reluctance of health workers to treat people who are asymptomatic, medication adherence, availability of appropriate drugs and formulations, costs, demands on health systems and the individuals concerned, and access to free screening and testing for TB infection. TPT can induce adverse drug reactions (although these are rarely serious) in people who are generally healthy. Hence, TPT is recommended only for groups who are at high risk of developing TB disease, in whom the benefits of TPT clearly outweigh the risks.

The risk of TB disease is higher in those who have a positive test for TB infection than in those with the same risk factors but a negative test for TB infection. In addition, it is useful to test for TB infection because individuals who test positive are more likely to benefit from TPT than those who test negative; thus, TPT will be of greatest individual and public health benefit if it is directed by an assessment of risk alongside the results of testing for TB infection. Hence, the extension of TPT creates a need to expand the capacity to test for TB infection. However, the absence of TB infection testing should not prevent the use of TPT, particularly for TB contacts aged below 5 years and people living with HIV *(41)*.

WHO recommends three classes of tests for TB infection: TST, TBSTs and IGRAs (**Box 2.12**). These tests are described in more detail in the subsections below.

Box 2.12. WHO recommendations on tests for TB infection *(6)*

Mycobacterium tuberculosis antigen-based skin tests may be used to test for TB infection.

(Conditional recommendation, very low certainty of evidence)

Either a tuberculin skin test or an interferon-gamma release assay can be used to test for TB infection.

(Strong recommendation, very low certainty of evidence)

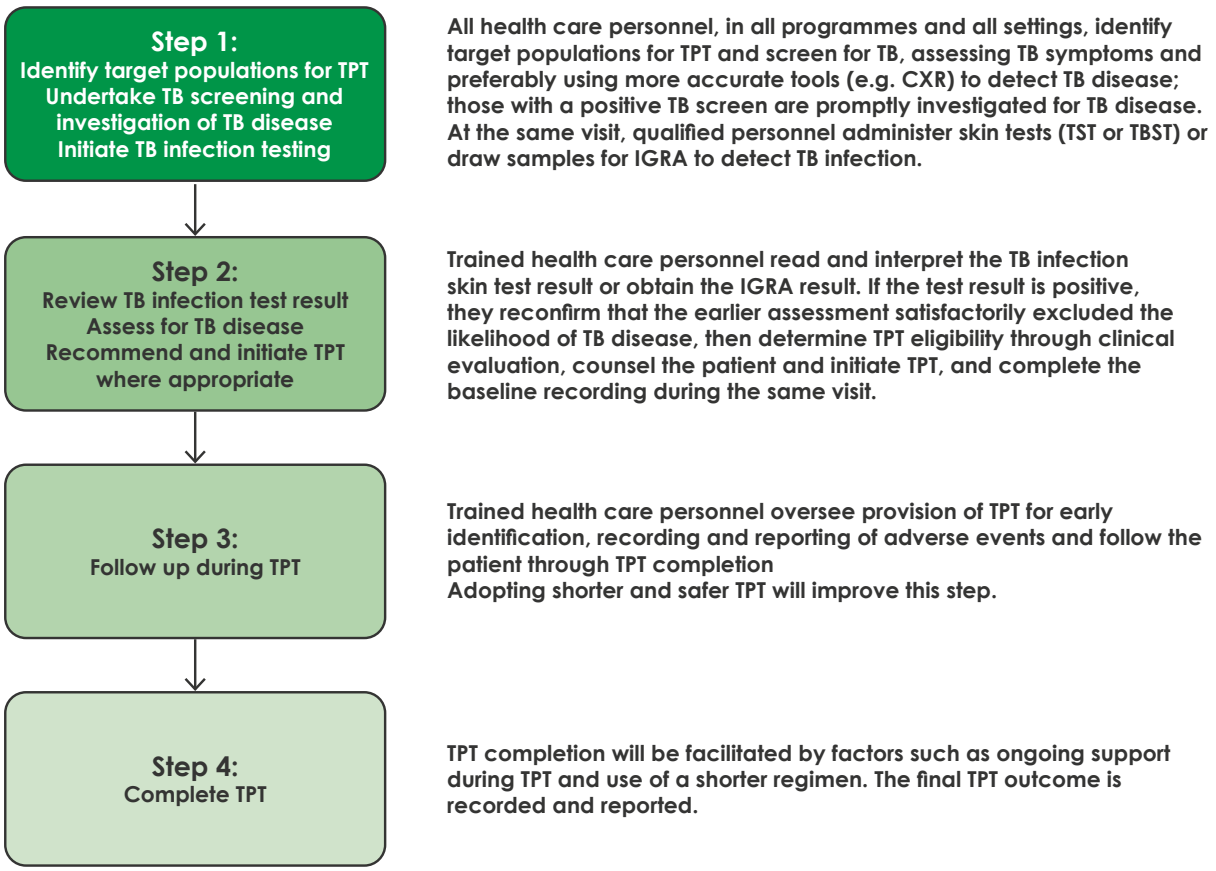
Remark:

➔ Testing for TB infection is not a precondition for initiating TPT among and children aged under 5 years who are household contacts of people with active TB. Nevertheless, testing for TB infection increases the certainty that individuals will benefit from TPT.

2.6.1 The TB infection cascade of care

Identifying, testing, evaluating and treating people with TB infection is a multistep process that has been termed the "TB infection cascade of care" *(40)*. A systematic review showed that these losses resulted in less than 20% of those eligible for TPT completing their course of medication *(40)*. Importantly, under field conditions, 70–80% of the losses occurred before the initiation of TPT. Various practical and programmatic challenges limit access to testing for TB infection. The impact of these challenges on the cascade of care must be considered at local level, and steps taken to resolve identified challenges. A simplified four-step process is shown in **Fig. 2.3**.

Fig. 2.3. Simplified four-step person-centred TB infection cascade of care



CXR: chest X-ray; IGRA: interferon-gamma release assay; TB: tuberculosis; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TPT: TB preventive treatment; TST: tuberculin skin test.

Who should be tested for TB infection?

The decision to test an individual for TB infection implies an intention to offer TPT. Therefore, testing for TB infection should be reserved for populations in whom the risk of developing TB disease is high and who will benefit the most from TPT. Decisions to start TPT should always consider the risk of adverse drug events, in addition to TB symptoms and the results of the test for TB infection. **Box 2.13** summarizes the groups WHO recommends should receive TPT, with testing not mandatory for Groups 1–5. More detail can be found in Module 1 of the WHO operational handbook on TB *(41)*.

Box 2.13. Groups that WHO recommends should receive TPT *(6)*

People living with HIV

1. Adults and adolescents living with HIV who are unlikely to have TB disease on an appropriate clinical evaluation or according to national guidelines should receive TPT as part of a comprehensive package of HIV care. Treatment should also be given to those on antiretroviral treatment, to pregnant women and to those who have previously been treated for TB, irrespective of the degree of immunosuppression and even if testing for TB infection is unavailable.
2. Infants aged below 12 months living with HIV who are in contact with a person with TB and who are unlikely to have TB disease on an appropriate clinical evaluation or according to national guidelines should receive TPT.
3. Children aged 12 months or more living with HIV who are considered unlikely to have TB disease on an appropriate clinical evaluation or according to national guidelines should be offered TPT as part of a comprehensive package of HIV prevention and care if they live in a setting with high TB transmission, regardless of contact with TB.
4. All children living with HIV who have successfully completed treatment for TB disease may receive TPT.

Household contacts of people with TB (regardless of HIV status)

5. Children aged below 5 years who are household contacts of people with bacteriologically confirmed pulmonary TB and who are found not to have TB disease on an appropriate clinical evaluation or according to national guidelines should be given TPT even if testing for TB infection is unavailable.
6. Children aged 5 years or more, adolescents and adults who are household contacts of people with bacteriologically confirmed pulmonary TB who are found not to have TB disease on an appropriate clinical evaluation or according to national guidelines may be given TPT.

Other people at risk of TB

7. People who are initiating treatment with anti-tumour necrosis factor (anti-TNF), receiving dialysis, preparing for an organ or haematological transplant, or who have silicosis, should be systematically tested and treated for TB infection.
8. Systematic testing and treatment for TB infection may be considered for prisoners, health workers, immigrants from countries with a high TB burden, homeless people and people who use drugs.

2.6.2 TB infection skin test using tuberculin

The standardized preparation of tuberculin from *Mtb*, termed "purified protein derivative (standard)" (PPDS), was first produced in 1941 by Florence Seibert *(42)*; since then, all production of tuberculin material has used the same methods and testing against this standard. All commercially available tuberculin material, other than the "next-generation" skin test described later, are manufactured to produce PPD material that is bioequivalent to this standard PPDS.

PPDS contains a mix of antigens, including some that are specific to *Mtb*, but also many that are found in NTM and BCG. Hence, false positive reactions to PPDS have been described in people with NTM disease or with sensitization to NTM antigens *(43)*, and in people who have received BCG vaccination, particularly if they received BCG more than once or after infancy *(44)*.

Testing with PPDS is safe. Although severe local reactions with blistering can be seen in 2–3% of people, these are true positive reactions that are self-limited and heal spontaneously. Allergic reactions with generalized rash occur in less than 1% of people *(45)*, and anaphylaxis occurs in only one person per million *(46)*. Based on decades of experience, TST with PPDS is considered safe in pregnant and lactating women *(47)*. A detailed step-by-step description on how to perform the TST test is provided in **Annex 4**.

2.6.3 TB infection skin tests using Mtb-specific antigens

To make the in vivo skin testing more specific, various test manufacturers have developed skin tests with recombinant *Mtb* antigens. The *Mtb*-specific ESAT-6 and CFP-10 were used either as individual proteins or in one fusion protein combining the two antigens. WHO has assessed three tests that all showed acceptable performance and could be recommended for use. All tests are based on the same principle as the TST; that is, an intradermal injection of the recombinant antigens is made and induration is measured after 48–72 hours. A summary of the different tests recommended by WHO is found in **Table 2.12**. Of note, as of early 2025, the Cy-TB TBST was available through the Stop TB Global Drug Facility for only $1.50 USD per test; supporting low unit-cost implementation of TB infection testing programmes. Further details on the tests, procedures and interpretation of test results for TST and the three TBSTs are available in **Annex 4**.

Table 2.12. Comparison of *Mtb*-specific infection skin tests

	Cy-Tb *(48)*	Diaskintest *(49)*	C-TST *(50)*
Manufacturer	Serum Institute of India	Generium	Anhui Zhifei Longcom
Recombinant antigen and organism used for production	Individual recombinant ESAT-6 and CFP-10 *Lactobacillus lactis*	Fusion recombinant protein of ESAT-6 and CFP-10 *Escherichia coli*	Fusion recombinant protein of ESAT-6 and CFP-10 *Escherichia coli*
Amount of antigen in one dose	0.05 µg of each recombinant protein	0.2 µg of the fusion protein	5 U
Additional ingredients	Disodium hydrogen phosphate dihydrate, potassium dihydrogen orthophosphate, potassium chloride, sodium chloride, polysorbate 20 and phenol	Disodium phosphate dihydrate, sodium chloride, potassium dihydrogen phosphate, polysorbate 80 and phenol	Disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, phenol and polysorbate 80
Pregnancy and lactation	No evidence of adverse effects on pregnant or breastfeeding women and their children recorded on a limited cohort (<500 women)	No data available	No data available
Contraindications	Allergy to products from *Lactobacillus lactis*	• Acute and chronic (in the exacerbation period) infections, except for TB (possible or presumptive) • Somatic and other diseases during exacerbation • Common skin diseases • Allergic conditions • Epilepsy • Hypersensitivity to the active substance or to any of the excipients in the product	• Those aged under 6 months or above 65 years, relative contraindication, because no data regarding safety or accuracy available in these age ranges • Patients with acute infectious diseases (e.g. measles, pertussis, influenza or pneumonia), acute meningitis with ocular involvements, acute otitis media and extensive skin diseases

CFP-10: culture filtrate protein 10; ESAT-6: early secretory antigenic target-6 kDa; *Mtb*: *Mycobacterium tuberculosis*; TB: tuberculosis.

2.6.4 Interferon-gamma release assays

IGRAs are in vitro blood tests that measure interferon-gamma released by circulating lymphocytes in whole blood during overnight incubation with exposure to *Mtb*-specific antigens (enzyme-linked immunosorbent assay [ELISA] based) or the number of T-lymphocytes producing interferon-gamma (enzyme-linked immunosorbent spot [ELISPOT] based). In 2011, WHO issued its first recommendations on the use of IGRAs for the diagnosis of TB infection; three tests were included in this class. Following the advice of the TAG held in January 2025, WHO updated the list of tests that meet the class criteria, supported by policy statements on their use (**Box 2.14**). The tests included in the class for which the WHO recommendations apply to are:

- Qiagen's QuantiFERON-TB Gold Plus;
- Oxford Immunotec T-SPOT®.TB;
- Beijing Wantai's TB-IGRA;

NEW

- SD Biosensor's STANDARD E TB-Feron ELISA; and
- Diasorin's LIAISON QFT-Plus (CLIA).

QuantiFERON Gold and QuantiFERON Gold In-Tube were previously recommended by WHO; however, they have been discontinued by the manufacturer.

Box 2.14. WHO policy statement on IGRAs, 2025

Recently, new and updated IGRAs for TB infection testing have become available. WHO solicited information on these technologies directly from manufacturers and through a public call for information in 2024. The following IGRAs had sufficient independent evidence for consideration:

- ➔ STANDARD E TB-Feron (ELISA) – SD Biosensor;
- ➔ ASACIR.TB (ELISA) – Haikou VTI Biological Institute;
- ➔ ichroma IGRA-TB (FIA) – Boditech Med Inc.;
- ➔ LIAISON QuantiFERON Gold PLUS (QFT-Plus; CLIA) – Diasorin;
- ➔ Wantai TB-IGRA CLIA – Wantai Biological Pharmacy Enterprise Co; and
- ➔ AdvanSure i3 TB-IGRA (CLIA) – Invitros (formerly owned by LG Chem Ltd.).

To evaluate these tests and determine whether one or more of them could be included under the existing WHO recommendations for IGRAs, WHO convened a TAG on TB Diagnostics and Laboratory Strengthening, which met on 20–24 January 2025 in Geneva, Switzerland.

Following the TAG's review of the available evidence and provision of advice, WHO makes the following policy statements:

1. The performance of the STANDARD E TB-Feron ELISA and LIAISON QFT-PLUS (CLIA) is comparable to that of WHO-recommended IGRAs for the detection of TB infection. Current WHO recommendations for the use of IGRAs are also valid for Beijing Wantai's TB-IGRA (ELISA) and Qiagen QuantiFERON-TB Gold Plus.

2. The performance of the ASACIR.TB, ichroma IGRA-TB (FIA), Wantai TB-IGRA (CLIA), and AvanSureI3 TB-IGRA (CLIA) could not be adequately compared with that of WHO-recommended IGRAs for the detection of TB infection.
3. Current WHO recommendations for the use of IGRAs are also valid for SD Biosensor's STANDARD E TB-Feron ELISA and Diasorin's LIAISON QFT-PLUS (CLIA).

In addition to the implementation considerations the GDG made in 2011 when IGRAs were recommended for use *(5)*, the TAG listed additional factors during its meeting in January 2025, as outlined below.

Remarks:

- ➔ Due to the lack of sufficient data for four of the tests: ASACIR.TB (ELISA), ichroma IGRA-TB (FIA), AdvanSure i3 TB-IGRA (CLIA) and Wantai TB-IGRA (CLIA), the TAG could not adequately compare their performance to that of current WHO recommended IGRAs; this does not imply any concerns with the tests themselves but rather a lack of sufficient independent data to make a recommendation.. WHO welcomes future submissions by the manufacturers when additional independent data are available.
- ➔ The primary analysis compared paired results of the index test with a reference test; it included a difference in sensitivity and specificity that was considered helpful for this comparative evaluation, particularly when the prevalence of TB infection varied by study population.
- ➔ The comparative evaluations did not assess test performance in subgroups (e.g. people living with HIV, children and other immunocompromised populations); however, data from these groups were included where available.
- ➔ The studies for the LIAISON QFT-PLUS were from low-burden TB settings; comparative evaluation data from high-burden settings were noted as desirable to diversify the evidence on test performance and inform uptake of new testing technologies.
- ➔ The study for the Wantai TB-IGRA (CLIA) was from a single, large, high-burden country (China).
- ➔ No data on predictive accuracy for development of active TB were available for any of the tests, highlighting a continued gap in research.
- ➔ A high risk of bias was observed for all studies, irrespective of the index test evaluated.
- ➔ The advantages and disadvantages of blood-based IGRAs compared with skin-based TB infection tests apply to all index tests.
- ➔ Indeterminate result rates need to be considered because these have implications for patient and health system costs.

Implementation considerations

- ➔ The requirements for centralized laboratory infrastructure, equipment, staff skills and training, and multiday time-to-result are expected to be similar among the QFT-Plus, LIAISON and STANDARD E TB-Feron assays.

- The LIAISON testing procedure is more automated than other IGRAs, and it provides opportunities to use the same analyser to test for other diseases and conditions; however, programmes should consider the cost implications associated with the instrumentation, equipment and reagents. Manufacturer-reported costs may vary by test and setting. Negotiation through the Global Drug Facility is needed to provide standardized pricing and a catalogue listing for each index test.
- WHO recommendations on diagnostics are based on clinical research evidence – they do not include quality assessments of the products, batch-to-batch variations or manufacturing processes. In advance of the availability of WHO prequalification processes for IGRAs, countries should ensure that new products fulfil local or internationally recognized regulatory requirements before implementation, such as those from the United States Food and Drug Administration.
- Processes for implementation of new tests apply – the following are example areas for consideration during updates: registration, supply chain, laboratory and clinical trainings, diagnostic algorithms, SOPs, QA programmes, service and maintenance programmes, monitoring and evaluation, result reporting and information systems.
- For all TB infection tests, including IGRAs, programmes should monitor impact on TPT.

Further research

Studies should be designed to limit the risk of bias and concerns about applicability, in line with QUADAS-2, a revised tool for the quality assessment of diagnostic tests *(51)*. There is a need for:

- evaluation of the recommended tests in more diverse geographical and epidemiological settings, including high-burden settings;
- evaluation of the recommended tests in subpopulations, where these were not originally included (e.g. people living with HIV, children and other immunocompromised individuals);
- evaluation of reproducibility of testing for TB infection;
- more accurate quantification of direct and indirect costs for all index tests, ideally using time and motion studies;
- evaluation of cost and cost–effectiveness for all index tests; and
- evaluation of feasibility, applicability, equity, and end-user values and preferences for all index tests.

Further aspects on IGRA implementation and research please are given in the WHO guidelines *(5)*. The full TAG and evidence reports are found in **Web Annex D**.

NEW The TAG report includes updated information on evidence generation for future TAG assessments of within-class IGRAs.

The five tests can be grouped into three types of IGRA detection methods:

- ELISAs (QFT-Plus, Wantai TB-IGRA, STANDARD E TB-Feron)
- ELISPOT (T-SPOT.TB) (*52*)
- CLIAs (LIAISON QFT-PLUS)

In contrast to TST and TBST, the WHO-recommended IGRAs require a well-equipped laboratory and trained laboratory technicians. Similarly to the recommended TBSTs, IGRAs are based on the lymphocyte response to *Mtb*-specific antigens (ESAT-6 and CFP-10), meaning that results are not affected by prior BCG vaccination. However, challenges with phlebotomy in young children limit the applicability of IGRAs, with skin tests being the alternative. A comparison of the different IGRA tests is given in **Table 2.13** (which covers all tests) and in **Web Annex D** (which covers only new within-class products). The guidance provided should facilitate the procurement and uptake of the recommended technologies and improve patient care.

All products recommended by WHO are automatically eligible to be included in the WHO essential diagnostic list. The WHO recommendations on diagnostics are based on clinical research evidence; they do not include quality assessments of the products or the manufacturing process involved. Before introducing any new products, countries should ensure that those products fulfil local or internationally recognized regulatory requirements.

Table 2.13. Comparison of IGRA tests

	Manufacturer	Antigen	Key workflow consideration	Incubation time	Required equipment	Staff requirements
QuantiFERON® -TB Gold Plus (ELISA)	QIAGEN	ESAT-6 and CFP-10 simulating peptides	Third-party ELISA reader required	16–24 hours	Incubator Centrifuge Plate washer and agitator ELISA reader	Phlebotomist, technician trained to perform ELISA
Wantai TB-IGRA (ELISA)	Beijing Wantai Biological Pharmacy Enterprise Co Ltd	Recombinant fusion protein of CFP-10 and ESAT-6	Third-party ELISA reader required	20–24 hours	Incubator Centrifuge Plate washer and agitator ELISA reader	Phlebotomist, technician trained to perform ELISA
T-SPOT.TB (ELISPOT)	Oxford Immunotec	ESAT-6 and CFP-10 simulating peptides	Separation of blood cells should be performed in a Level II biosafety cabinet	16–20 hours	Lymphocyte counter Humidified incubator with a 5% CO_2 Centrifuge Plate washer and agitator ELISPOT reader	Phlebotomist, technician trained to perform ELISPOT
STANDARD E TB-Feron (ELISA)	SD Biosensor	Recombinant proteins of ESAT-6, CFP-10 and TB7.7	Third-party ELISA reader required	16–20 hours	Incubator Centrifuge Plate washer and agitator ELISA reader	Phlebotomist, technician trained to perform ELISA
LIAISON QFT-PLUS (CLIA)	Diasorin and Qiagen	Peptide cocktail of ESAT-6 and CFP-10	LIAISON CLIA analyser can be used for other diseases as well	16–20 hours	Incubator Centrifuge LIAISON CLIA analyser	Phlebotomist, technician trained in CLIA techniques

CFP-10: culture filtrate protein 10; CLIA: chemiluminescence immunoassay; ELISA: enzyme-linked immunosorbent assay; ELISPOT: enzyme-linked immunosorbent spot; ESAT-6: early secretory antigenic target-6 kDa; IGRA: interferon-gamma release assay.

2.7 Tests WHO recommends against using or recommends limited usage

Based on reviews of available data, WHO has recommended against using tests that do not provide reliable information for diagnosing TB. In 2011, WHO recommended that commercial serological tests should not be used for the diagnosis of pulmonary and extrapulmonary TB because the commercial serodiagnostic tests available at that time provided inconsistent and imprecise findings, there was no evidence that using those commercial serological assays improved patient outcomes, and the tests generated high proportions of false positive and false negative results that may have an adverse impact on people's health *(53)*.

WHO recommendations are specific for intended uses; sometimes, even a test that is recommended is not recommended to be used for a specific purpose. For example, the various classes of NAATs (LC-aNAAT, LC-mNAAT and MC-aNAAT) are not recommended for use in monitoring the response to treatment.

WHO's recommendation is that tests for TB infection may be used in low- and middle-income countries (LMIC) to aid in the detection of TB infection. However, such tests should not be used for the diagnosis of pulmonary or extrapulmonary TB (**Box 2.15**), or for the diagnostic work-up[5] of adults (including HIV-positive individuals) suspected of having active TB.

Box 2.15. WHO recommendations on the use of the TST and IGRAs for the diagnosis of TB disease *(6)*

Interferon-gamma release assays (IGRAs) (and tuberculin skin tests ([TSTs]) should not be used in low- and middle-income countries for the diagnosis of pulmonary or extrapulmonary TB or for the diagnostic work-up of adults (including people living with HIV) with suspected active TB.

(Strong recommendation)

[5] A diagnostic work-up involves gathering information about a patient's health, performing tests and making a diagnosis.

3. Strategies and considerations for diagnostic testing

3.1 Epidemiological considerations

In selecting a diagnostic test to implement, it is important to consider the characteristics (i.e. risk factors) of the population being served. These characteristics should be derived from population-based studies, if available, and should include the proportion of:

- people with TB resistant to RIF, INH and FQs;
- people living with HIV;
- people with extrapulmonary TB;
- children with TB;
- people with acute illness requiring rapid diagnosis; and
- people who are hospitalized versus those who are ambulatory.

Understanding the proportion resistant to a newly introduced drug (e.g. BDQ) is particularly important during the initial stages of using the drug, when treatment capacity may expand more rapidly than DST capacity.

3.2 Pretest probability and test accuracy considerations

The predictive values of a test vary depending on the prevalence of TB in the population being tested. The prevalence of TB in a country is best estimated through a national TB prevalence survey. Countries should conduct prevalence surveys about every 10 years. If a survey has not been conducted recently, WHO provides estimates of prevalence in its annual global TB report *(2)*. These estimates are based on the number of notified TB cases submitted each year by Member States. However, the number of notified cases is not a good proxy for the actual number of people who develop TB disease. Both underreporting of diagnosed TB (especially in the private sector) and underdiagnosis (especially in countries with geographical or financial barriers to seeking and accessing health care) will affect the reported numbers and thus the estimates. Also, figures provided by WHO are national, and regional variations in prevalence may warrant the use of different tests in different regions.

Table 3.1 provides examples of population-level projections of the results of testing with automated NAATs (low complexity and moderate complexity) and manual NAATs (low complexity) in settings with different levels of TB prevalence, based on pooled sensitivity and specificity estimates that were extracted from the *WHO consolidated guidelines on tuberculosis:*

module 3: diagnosis (5). **Table 3.2**, **Table 3.3** and **Table 3.4** provide those same parameters for detection of resistance to RIF, INH and FQs, respectively. **Table 3.5** and **Table 3.6** provide the parameters for detection of resistance to first-line and second-line anti-TB agents using targeted NGS end-to-end solutions and tests. The sensitivity of the test may be lower when used for active case-finding in a population-screening context because such people would be less ill and have a lower bacillary burden. In choosing a test to implement, each country will need to consider the possible trade-offs between higher or lower sensitivity and higher or lower specificity, based on the prevalence of TB in their country (**Box 3.1**). False negative results may lead to missed opportunities to treat TB, whereas false positive results may lead to the overtreatment of people who do not have TB. In some settings, countries may need to conduct additional modelling work to support decisions on implementation strategies, based on the trade-offs between sensitivity and specificity in their settings.

In addition to geographical variability, differences in the approach to screening for TB disease may also affect the predictive value of TB testing. Usually, a decision to undertake a diagnostic work-up of an individual for TB begins with assessing symptoms and signs of TB disease. However, many individuals with culture-positive TB may not have symptoms or may consider the symptoms too insignificant to report, leading to missed opportunities for diagnosis. To improve TB case detection and identify individuals suitable for TPT, WHO has updated the TB screening guidelines *(54)*. Several modalities are recommended for screening all people for TB, including the four-symptom screen, CXR (**Box 3.2**) and mWRDs; for screening of people living with HIV, additional testing for positive C-reactive protein testing (>5 mg/L) may be considered.

Box 3.1. Selection of tests based on prevalence, sensitivity and specificity

Based on the prevalence and the sensitivity and specificity of a test, a two-by-two table can easily be drawn (see below). With an example cohort (e.g. 1000 individuals) the number of true and false positive and negative test results can be calculated.

		Reference test		
		Positive	**Negative**	
Index test	Positive	True positive (TP)	False positive (FP)	**Positive predictive value** = TP/ (TP + FP)
	Negative	False negative (FN)	True negative (TN)	**Negative predictive value** = TN/ (FN + TN)
		Sensitivity = TP/ (TP + FN)	**Specificity** = TN/ (FP + TN)	

Example 1

In this example, the prevalence is estimated to be 2.5% in an outpatient setting in a high-burden country. In a cohort of 1000 individuals there will be 25 people with TB and 975 people without TB. The test in this example has a sensitivity of 90% and a specificity of 96%. Of the 25 people who are TB positive, 22 people with TB are estimated to be detected (i.e. 25 × 0.90 = 22). These are the true positive (TP) results. The three people who are TB positive but were undetected are the false negative (FN) results. Thus, in this cohort, 975 people will not have TB (i.e. 1000 – 25 = 975). The specificity of 96% results in 936 people being true negative (TN) (i.e. 975 × 0.96 = 936). The results for the remaining 39 people (i.e. 975–936 = 39) will be false positive (FP). In summary, of the 62 people diagnosed with TB, only 23 will be TP (i.e. 23 [TP] + 39 [FP] = 62), giving a positive predictive value (PPV) of 37%.

Example 2

In this example, the prevalence is estimated to be 20% in a TB hospital where many clients present with TB symptoms. In the cohort of 1000 individuals, there will be 200 people with TB. The test has a sensitivity of 73% and a specificity of 98%. Of the 200 people with TB, 146 will be detected (TP) and 54 will not be detected (FN). Of the 800 people without TB, 784 will have negative results (TN) and 16 will have positive results (FP).

Depending on the context of testing (e.g. testing symptomatic individuals presenting at clinics or large-scale testing of people in communities), the prevalence of TB disease in the population will vary and will result in different numbers of true and false positive and negative test results.

Box 3.2. Effect of screening with CXR

CXR as a screening tool can identify individuals to be tested with an initial molecular test. Thus, it can reduce the number of individuals tested and the associated costs (but only if the cost of the radiography is lower than the cost of the test). This approach is likely to improve the pretest probability for TB; therefore, it should improve the predictive value of the molecular test and reduce FP results, especially in populations with a low prevalence of TB. For example, in a population with a TB prevalence of 1%, the addition of CXR as a screening tool to an algorithm in which all individuals with an abnormal CXR receive mWRD was calculated to increase the PPV of the mWRD from 56.8% to 78.5%, and the prevalent cases detected from 69% to 80%, compared with testing with an mWRD, irrespective of symptoms.

Table 3.1. Performance of mWRDs for the detection of TB in adults with signs and symptoms being evaluated for pulmonary TB compared with a microbiological reference standard (absolute number of TP, FP, TN or FN, under a given prevalence out of 1000)

Intervention	Test accuracy % (95% CI)[a]	Studies (participants)	Certainty of evidence	2.5% prevalence	10% prevalence	30% prevalence
LC-aNAATs[b]	Se: 90.4 (88.0–92.4)	34 (*3636*)	High	TP: 23 / FN: 2	TP: 90 / FN: 10	TP: 271 / FN: 29
	Sp: 94.9 (93.0–96.3)	34 (11 204)	High	TN: 925 / FP: 50	TN: 854 / FP: 46	TN: 664 / FP: 36
MC-aNAAT	Se: 93.0 (90.9–94.7)	29 (*4767*)	Moderate	TP: 23 / FN: 2	TP: 93 / FN: 7	TP: 279 / FN: 21
	Sp: 97.7 (95.6–98.8)	29 (*9085*)	High	TN: 953 / FP: 22	TN: 879 / FP: 21	TN: 684 / FP: 16
LC-mNAATs	Se: 84 (78–89)	26 (*4108*)	High	TP: 21 / FN: 4	TP: 84 / FN: 16	TP: 252 / FN: 48
	Sp: 96 (94–97)	26 (14 189)	High	TN: 936 / FP: 39	TN: 864 / FP: 36	TN: 672 / FP: 28

CI: confidence interval; FN: false negative; FP: false positive; MC-aNAAT: moderate-complexity automated nucleic acid amplification test; mWRD: molecular WHO-recommended rapid diagnostic test; Se: sensitivity; Sp: specificity; TB: tuberculosis; TN: true negative; TP: true positive; WHO: World Health Organization.

[a] Test accuracy obtained from the WHO consolidated guideline on TB diagnostics *(5)*.

[b] When used in a microscopy laboratory. When tested in reference laboratories, the sensitivities of Truenat MTB and Truenat MTB Plus were 84 and 87, respectively, and specificities were 97 and 95, respectively.

Table 3.2. Performance of molecular tests for the detection of RIF resistance in adults with signs and symptoms being evaluated for pulmonary TB[a] compared with a microbiological reference standard (absolute number of TP, FP, TN or FN, under a given prevalence of RIF resistance out of 1000)

Intervention	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	2% prevalence	10% prevalence	15% prevalence
LC-aNAAT	Se: 95.1 (83.1–98.7)	11 (*487*)	High	TP: 19 / FN: 1	TP: 95 / FN: 5	TP: 143/ FN: 7
	Sp: 98.1 (97.0–98.7)	11 (*2053*)	High	TN: 961 / FP: 19	TN: 883 / FP: 17	TN: 834/ FP: 16
MC-aNAAT	Se: 96.7 (93.1–98.4)	18 (*702*)	Moderate	TP: 19 / FN: 1	TP: 97 / FN: 3	TP: 146 / FN: 4
	Sp: 98.9 (97.5–99.5)	18 (*2172*)	High	TN: 970 / FP: 10	TN: 891 / FP: 9	TN: 842 / FP: 8

CI: confidence interval; FN: false negative; FP: false positive; MC-aNAAT: moderate-complexity automated nucleic acid amplification test; Se: sensitivity; Sp: specificity; TB: tuberculosis; TN: true negative; TP: true positive.

Drug: RIF: rifampicin.

[a] The detection of RIF resistance by Xpert Ultra, Truenat MTB-RIF Dx and MC-aNAAT occurs only in cases where TB is detected; hence, suggested prevalence reflects RIF resistance in people newly detected with TB.

Table 3.3. Performance of molecular tests for the detection of INH resistance in adults with detected pulmonary TB[a] compared with a microbiological reference standard (absolute number of TP, FP, TN or FN, under a given prevalence of INH resistance out of 1000)

Intervention	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	2% prevalence	10% prevalence	15% prevalence
MC-aNAAT	Se: 86.4 (82.8–89.3)	18 (*854*)	Moderate	TP: 17 / FN: 3	TP: 86 / FN: 14	TP: 129 / FN: 21
	Sp: 99.2 (98.1–99.7)	18 (*1904*)	High	TN: 970 / FP: 10	TN: 891 / FP: 9	TN: 842 / FP: 8
LC-aNAAT	Se: 94.2 (89.3–97.0)	3 (*994*)	Moderate	TP: 19 / FN: 1	TP: 94 / FN: 6	TP: 141 / FN: 9
	Sp: 98.0 (95.2–99.2)	3 (*611*)	Moderate	TN: 960 / FP: 20	TN: 882 / FP: 18	TN: 833 / FP: 17
Targeted NGS	Se: 96 (93–99)	12 (*1440*)	Moderate	TP: 19 / FN: 1	TP: 96 / FN: 4	TP: 144 / FN: 6
	Sp: 97 (95–99)	12 (*517*)	Moderate	TN: 951 / FP: 29	TN: 873 / FP: 27	TN: 825 / FP: 25
FL-LPA by direct testing of SS+ samples	Se: 89 (86–92)	46 (*3576*)	Moderate	TP: 18 / FN: 2	TP: 89 / FN: 11	TP: 134 / FN: 16
	Sp: 98 (97–99)	46 (*6896*)	Moderate	TN: 960 / FP: 20	TN: 882 / FP: 18	TN: 833 / FP: 17

CI: confidence interval; FL-LPA: line probe assay for first-line drugs; FN: false negative; FP: false positive; INH: isoniazid; LC-aNAAT: low-complexity automated NAAT; MC-aNAAT: moderate-complexity automated NAAT; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; Se: sensitivity; Sp: specificity; SS+: sputum smear positive; TB: tuberculosis; TN: true negative; TP: true positive.

Drug: INH: isoniazid.

[a] The detection of INH resistance by MC-aNAAT occurs only in cases where TB is detected; hence, suggested prevalence, reflecting INH resistance in people newly detected with TB, also applies to this technology class.

Table 3.4. Performance of molecular tests for the detection of FQ resistance in adults with detected pulmonary TB compared with a microbiological reference standard (absolute number of TP, FP, TN or FN, under a given prevalence of FQ resistance out of 1000)

Intervention	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	1% prevalence	5% prevalence	10% prevalence
LC-aNAAT	Se: 93 (88–96)	3 (*384*)	High	TP: 9 / FN: 1	TP: 47 / FN: 3	TP: 93 / FN: 7
	Sp: 98 (94–99)	3 (*953*)	Moderate	TN: 973 / FP: 17	TN: 934 / FP: 16	TN: 885 / FP: 15
Targeted NGS (MFX)	Se: 96 (92–99)	6 (*652*)	Moderate	TP: 10 / FN: 0	TP: 48 / FN: 2	TP: 96 / FN: 4
	Sp: 96 (93–99)	8 (*921*)	Moderate	TN: 950 / FP: 40	TN: 912 / FP: 38	TN: 864 / FP: 36
Targeted NGS (LFX)	Se: 94 (88–99)	6 (*654*)	Low	TP: 9 / FN: 1	TP: 47 / FN: 3	TP: 94 / FN: 6
	Sp: 96 (93–99)	7 (*913*)	Moderate	TN: 950 / FP: 40	TN: 912 / FP: 38	TN: 864 / FP: 36
SL-LPA by direct testing of SS+ samples	Se: 86 (75–93)	9 (*519*)	Moderate	TP: 9 / FN: 1	TP: 43 / FN: 7	TP: 86 / FN: 14
	Sp: 99 (97–99)	9 (*1252*)	High	TN: 980 / FP: 10	TN: 937 / FP: 13	TN: 887 / FP: 13

CI: confidence interval; FN: false negative; FP: false positive; LC-aNAAT: low-complexity automated nucleic acid amplification test; NGS: next-generation sequencing; Se: sensitivity; SL-LPA: line probe assay for second-line drugs; Sp: specificity; SS+: sputum smear positive; TB: tuberculosis; TN: true negative; TP: true positive.

Drugs: FQ: fluoroquinolone; LFX: levofloxacin; MFX: moxifloxacin.

Table 3.5. Performance of molecular tests for the detection of resistance to other first-line anti-TB medicines in adults with detected pulmonary TB compared with a microbiological reference standard (absolute number of TP, FP, TN or FN, under a given prevalence of resistance out of 1000)

Intervention	Drug	Resistance prevalence	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	Lower prevalence cut off	Middle prevalence cut off	Higher prevalence cut off
High-complexity reverse hybridization NAAT	Pyrazinamide	8,50,90%	Se: 81.2 (75.4–85.8)	7 (*214*)	Very low	TP: 65 / FN: 15	TP: 406 / FN: 94	TP: 731 / FN: 169
			Sp: 97.8 (96.5–98.6)	7 (*750*)	Low	TN: 900 / FP: 20	TN: 489 / FP: 11	TN: 98 / FP: 2
Targeted NGS	Pyrazinamide	1,3,10%	Se: 88.4 (85.2–91.7)	3 (*346*)	Moderate	TP: 9 / FN: 1	TP: 26 / FN: 4	TP: 88 / FN: 12
			Sp: 98.5 (97.1–100)	3 (*269*)	Moderate	TN: 980 / FP: 10	TN: 960 / FP: 10	TN: 891 / FP: 91
Targeted NGS	Ethambutol	1,3,10%	Se: 95.8 (94.0–97.6)	4 (*432*)	Low	TP: 10 / FN: 0	TP: 29 / FN: 1	TP: 96 / FN: 4
			Sp: 99.3 (98.2–100)	4 (*268*)	Moderate	TN: 980 / FP: 10	TN: 960 / FP: 10	TN: 891 / FP: 9

CI: confidence interval; FN: false negative; FP: false positive; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; Se: sensitivity; Sp: specificity; TB: tuberculosis; TN: true negative; TP: true positive.

Table 3.6. Performance of targeted NGS for the detection of resistance to anti-TB medicines used to treat MDR/RR-TB in adults with bacteriologically confirmed RIF-resistant pulmonary TB compared with a microbiological reference standard (absolute number of TP, FP, TN or FN, under a given prevalence of resistance out of 1000)

Drug	Resistance prevalence	Test accuracy % (95% CI)[a]	Studies (participants)	Certainty of evidence	Lower prevalence cut off	Middle prevalence cut off	Higher prevalence cut off
Isoniazid	60,75,90%	Se: 96.5 (93.8–99.2)	12 (*1440*)	High	TP: 576 / FN: 24	TP: 720 / FN: 30	TP: 864 / FN: 36
		Sp: 95.8 (91.8–99.8)	12 (*517*)	High	TN: 384 / FP: 16	TN: 240 / FP: 10	TN: 96 / FP: 4
Levofloxacin	10,30,50%	Se: 95.8 (90.4–100)	6 (*654*)	Moderate	TP: 96 / FN: 4	TP: 288 / FN: 12	TP: 480 / FN: 20
		Sp: 96.0 (93.1–98.9)	7 (*913*)	High	TN: 864 / FP: 36	TN: 672 / FP: 28	TN: 480 / FP: 20
Moxifloxacin	10,30,50%	Se: 96.5 (93.6–99.5)	6 (*652*)	High	TP: 97 / FN: 3	TP: 291 / FN: 9	TP: 485 / FN: 15
		Sp: 95.2 (91.0–99.4)	8 (*921*)	High	TN: 855 / FP: 45	TN: 665 / FP: 35	TN: 475 / FP: 25
Pyrazinamide	30,50,90%	Se: 90.0 (86.8–93.2)	3 (*346*)	High	TP: 270 / FN: 30	TP: 450 / FN: 50	TP: 810 / FN: 90
		Sp: 98.6 (96.8–100)	3 (*269*)	High	TN: 693 / FP: 7	TN: 495 / FP: 5	TN: 99 / FP: 1
Bedaquiline	1,3,5%	Se: 67.9 (42.6–93.2)	3 (*31*)	Low	TP: 7 / FN: 3	TP: 20 / FN: 10	TP: 34 / FN: 16
		Sp: 97.0 (94.3–99.7)	4 (*519*)	High	TN: 960 / FP: 30	TN: 941 / FP: 29	TN: 922 / FP: 28

Drug	Resistance prevalence	Test accuracy % (95% CI)[a]	Studies (participants)	Certainty of evidence	Lower prevalence cut off	Middle prevalence cut off	Higher prevalence cut off
Linezolid	1,3,5%	Se: 68.9 (38.7–99.1)	4 (*31*)	Low	TP: 7 / FN: 3	TP: 21 / FN: 9	TP: 34 / FN: 16
		Sp: 99.8 (99.6–100)	6 (*1093*)	High	TN: 990 / FP: 0	TN: 970 / FP: 0	TN: 950 / FP: 0
Clofazimine	1,3,5%	Se: 70.4 (34.6–100)	4 (*36*)	Low	TP: 7 / FN: 3	TP: 21 / FN: 9	TP: 35 / FN: 15
		Sp: 96.3 (93.2–99.3)	6 (*789*)	High	TN: 950 / FP: 40	TN: 931 / FP: 39	TN: 912 / FP: 38
Amikacin	5,10,15%	Se: 87.4 (74.5–100)	5 (*115*)	Very low	TP: 44 / FN: 6	TP: 87 / FN: 13	TP: 131 / FN: 19
		Sp: 99.0 (98.4–99.6)	8 (*1003*)	Moderate	TN: 941 / FP: 9	TN: 891 / FP: 9	TN: 842 / FP: 8
Ethambutol	10,30,50%	Se: 96.7 (95.0–98.4)	4 (*431*)	Moderate	TP: 97 / FN: 3	TP: 291 / FN: 9	TP: 485 / FN: 15
		Sp: 98.4 (96.1–100)	4 (*123*)	Moderate	TN: 882 / FP: 18	TN: 686 / FP: 14	TN: 490 / FP: 10
Streptomycin	10,30,50%	Se: 98.1 (96.1–100)	5 (*493*)	High	TP: 98 / FN: 2	TP: 294 / FN: 6	TP: 490 / FN: 10
		Sp: 75.0 (59.5–90.5)	5 (*250*)	Low	TN: 675 / FP: 225	TN: 525 / FP: 175	TN: 375 / FP: 125

CI: confidence interval; FN: false negative; FP: false positive; GDG: guideline development group; MDR/RR-TB: multidrug- or rifampicin-resistant TB; NGS: next-generation sequencing; Se: sensitivity; Sp: specificity; TB: tuberculosis; TN: true negative; TP: true positive.

Drug: RIF: rifampicin.

[a] The sensitivity and specificity presented are the pooled values calculated for the recommendations of the class of targeted NGS carried out for the GDG in 2024. In the next edition of this handbook, the pooled sensitivity and specificity will be updated to include values for additional recommended solutions.

3.3 Planning for and implementing quality-assured TB testing services

As part of a wider quality management system (QMS) for testing services, quality assurance (QA) is defined as a set of planned and systematic activities to ensure that organizational quality requirements are met; this enables laboratories to maintain accuracy, reliability and reproducibility of results. Essential QA activities include training and competency assessments for staff, equipment verification and maintenance, method validation, lot testing for reagents, routine QC procedures, EQA such as proficiency testing, monitoring of quality indicators and continuous quality improvement actions. Cyclical feedback and corrective actions are crucial for sustaining and strengthening QA processes. These essential activities enhance confidence among clinicians and patients who rely on diagnostic services to guide decisions about care and treatment, which can in turn enhance demand for, and the impact of, clinical tests for patient management.

Key ongoing QA challenges in resource-limited settings include inconsistent practices, lack of systematic collection or monitoring of laboratory indicators, insufficient resource allocation for QC and EQA, and variability in the quality and timeliness of training and competency assessment provided to testing and clinical staff.

To address these challenges, programmes should review and budget for QA needs early in the processes for test consideration, selection and placement. **Annex 1** provides budgeting considerations for TB tests, and the GLI planning and budgeting tool *(55)* for projecting test-related costs, including required and recommended test-specific cost considerations. In addition, the GLI has produced a dashboard of EQA panels and programmes for TB tests (with details and contact information for providers), to help programmes select and budget for EQA service provision *(56)*. Lastly, close collaboration between testing and clinical programmes, as well as between TB and other disease programmes where relevant, should be sought out and maintained during test implementation to help inform QA activities related to test selection, placement, training, usage and indicator measurement. Further practical considerations for QA of specific tests can be found at the Stop TB Partnership GLI website *(12)*, for which WHO serves as the Secretariat.

3.4 Concurrent testing to improve case detection in children and in people (of all ages) living with HIV

The use of an mWRD as the initial test to diagnose TB greatly increases the sensitivity of the diagnostic process compared with the use of sputum smear microscopy *(57)*. However, certain subpopulations will not fully benefit from the high sensitivity of the molecular tests. For example, people living with HIV, especially those who are seriously ill, are known to have difficulties producing sputum; similarly, children, especially those aged below 5 years, often cannot easily produce sputum. In addition, if a person living with HIV or a young child can produce sputum, it will frequently have low or variable amounts of mycobacteria. These issues mean that it can be difficult to confirm TB disease bacteriologically; inclusion of alternate specimen types that can be collected in non-invasive ways could overcome some of these challenges, improve bacteriological confirmation of disease and speed time to diagnosis.

To assess whether testing of multiple specimen types with different tests – referred to by WHO as "concurrent testing" – enhances diagnostic accuracy. Therefore, WHO commissioned a systematic review in 2024, in which a GDG evaluated evidence for three risk groups: adult and adolescent people living with HIV, children aged below 10 years without HIV or with unknown HIV status, and children living with HIV. The tests for which data were available included LC-aNAAT (used on respiratory and stool samples), and the urine LF-LAM for HIV-positive individuals. The GDG assessed the following combinations:

- adults and adolescents with HIV – LC-aNAAT on one respiratory sample and urine LF-LAM;
- children – LC-aNAAT on one respiratory and one stool sample; and
- children living with HIV – LC-aNAAT on one respiratory and one stool sample, and urine LF-LAM.

For all three combinations, the GDG concluded that concurrent testing improved testing accuracy compared with use of a single test, with moderate cost requirements and possible increases in testing workload. Based on the evidence, the GDG issued a strong recommendation to test concurrently with urine LF-LAM and LC-aNAAT on a respiratory sample for adults and adolescents living with HIV; and with LC-aNAAT on a respiratory and a stool sample for children without HIV or with unknown HIV status. However, for children living with HIV, the recommendation to test concurrently with urine LF-LAM and LC-aNAAT on a respiratory and a stool sample is conditional, owing to the low certainty of the evidence and the increased number of false positive results. The recommendation for use of LC-aNAATs on respiratory samples in the concurrent testing strategies applies to both Xpert MTB/RIF Ultra and Truenat MTB Plus and MTB-Rif Dx, whereas concurrent testing of stool samples is limited to the use of Xpert MTB/RIF Ultra, given insufficient evidence on the use of Truenat tests with this sample type at the time of evidence assessment *(5)*.

The recent update of the consolidated guidelines includes these new recommendations on concurrent testing for children and for people (of all ages) living with HIV (see Table 1.1 in the guidelines *(5)*). The idea of concurrent testing is that all tests should be performed as soon as possible. People eligible for testing should be provided with information to help them understand this approach, including the need for more than one sample type and the need for any follow-up. Where possible, all sample types required for the concurrent testing strategy should be collected during the initial visit, and promptly tested or sent for analysis. Staffing and training needs should be assessed to ensure they are adequate for collection of quality samples, sample referral, and any changes or volume increase associated with onsite testing. Similarly, sites collecting urine should be equipped with private collection areas that have handwashing facilities, sterile collection containers, and clear procedures on the sanitary collection of midstream urine samples, to minimize contamination.

A positive result on either test is a positive result overall; it is sufficient to confirm TB diagnosis and should trigger a decision to treat, to reduce delays. Although conducting multiple tests for anyone who is eligible can increase the sensitivity of the diagnostic process, the approach must be balanced against reduced specificity and programmatic implications (e.g. higher test costs and access to multiple sample types and tests). It is important to monitor and prevent patient loss to follow-up while awaiting a second diagnostic test or drug susceptibility test, or test results.

LF-LAM is a POC test that can be performed onsite without delay and can provide results within 30 minutes, whereas results from LC-aNAATs require more time (≥90 minutes) for onsite testing (or longer when samples require referral for offsite testing). For example, concurrent testing in one setting involves an adult person living with HIV being tested onsite with LF-LAM while a sputum sample is transported to a site with LC-aNAAT. If the onsite LF-LAM yields a positive result, treatment can begin immediately and can later be adjusted based on the RIF result from the LC-aNAAT. If LF-LAM is unavailable, treatment decisions will rely on LC-aNAAT results alone; however, efforts should be made to ensure that LF-LAM testing is done.

If a test included in the concurrent testing strategy is not available at a site, this should not hinder the use of existing diagnostic tools. For example, if a site has LC-mNAAT (i.e. TB-LAMP) available onsite – a test not included in this recommendation – and also has LF-LAM, both tests should be conducted as soon as possible. A positive result from either test warrants treatment initiation. An additional sample should be referred for testing with LC-aNAAT, to determine the individual's RIF resistance status; this should also be done in cases where both tests are negative.

For children who are HIV-negative or whose HIV status is unknown, one stool sample and one respiratory sample should be tested on LC-aNAAT according to the concurrent testing strategy. Stool can be collected at peripheral sites and, if there is also capacity to collect respiratory samples from children, the samples can be tested with LC-aNAAT concurrently. If testing capacity is not available onsite, samples should be transported to a site with LC-aNAAT capacity. For children living with HIV, a urine sample should be collected in addition to the respiratory and stool samples. Testing with LF-LAM should be conducted onsite if the test is available, regardless of whether LC-aNAAT is available, and a positive result should trigger a treatment decision while awaiting LC-aNAAT results.

In situations where collection of quality respiratory samples is not possible, it may be necessary to refer a child to a facility that has the capacity to perform the specialized procedures of collecting respiratory samples from children. If a stool sample is available and the LC-aNAAT result is negative, additional respiratory sampling should be undertaken (e.g. induced sputum, nasopharyngeal or gastric aspirate) for LC-aNAAT testing at a higher level facility, to maximize opportunities for bacteriological confirmation of disease and determine RIF resistance status. If a stool sample is available and the result is positive, and RIF resistance results are available, treatment should be initiated; in such cases, collection of a respiratory sample is not necessary.

Owing to the limited sensitivity of the available diagnostic assays in children, clinical diagnosis continues to play a critical role in the diagnosis of TB. The aim of the concurrent testing strategy in this population is to increase testing sensitivity, but diagnosis should not be delayed because of the unavailability of a sample type or test, or the availability of negative test results alone. Delaying diagnosis leads to higher rates of loss to follow-up and additional burdens on clients. Therefore, laboratory-based investigations in children should not happen in isolation but rather should be embedded as part of the clinical evaluation process. Clinical diagnoses are usually based on suggestive signs and symptoms and a history of TB contact, with or without chest radiography. The *WHO operational handbook on tuberculosis: module 5: management of tuberculosis in children and adolescents* contains example integrated treatment decision algorithms aimed at encouraging health workers at the primary care level to make an evidence-based decision to start TB treatment in children aged below 10 years *(58)*.

3.5 Testing for TB infection

3.5.1 What are the advantages and disadvantages of testing for TB infection?

Testing for TB infection will be beneficial for both individuals and programmes if it identifies people who will benefit most from TPT. For programmes, investments in the capacity to test for TB infection will be justified if this results in greater efficacy and efficiency in the use of resources to provide TPT, increased acceptance and enhanced coverage. Investments are needed to cover not only the cost of drugs but also the human resources for medical evaluation, TPT initiation and follow-up. Testing for TB infection will also reduce unnecessary expenditure on medication or adverse events experienced by those receiving unnecessary treatment. Hence, testing for TB infection before TPT is a valuable way to increase the TPT benefit–risk ratio.

The use of TPT reduces the risk of developing TB disease among people living with HIV, particularly in those who are TST positive. The updated systematic review undertaken during the development of the WHO guidelines on programmatic management of TPT in 2018 clearly demonstrated the benefits of systematic testing and treatment of TB infection among people living with HIV in terms of prevalence of TB infection, risk of progression to TB disease and incidence of TB disease when compared with the general population.

Household contacts were found to be at a substantially higher risk for progression to TB disease than the general population. The highest risk progression to active disease was among contacts who were aged below 5 years; hence, a strong recommendation to start TPT – irrespective of the availability of testing for TB infection – was issued. In addition, TPT was conditionally recommended for household contacts in other age groups, following assessment of harms versus benefits. Among household contacts aged above 5 years, testing for TB infection before TPT initiation may be desirable, although treatment was considered justifiable even without testing *(59)*.

Among other risk groups, the evidence of benefits from systematic testing for TB infection and TPT varied. The benefits clearly exceeded the risks among people starting anti-TNF treatment, receiving dialysis, preparing for an organ or haematological transplant, or having silicosis. In other risk groups, the risk versus benefit was less clear. Therefore, prioritization of target groups for systematic testing and TPT based on individual risk and the local and national context was acceptable to people with TB infection and to key stakeholders, including clinicians, nurses and programme managers.

In summary, TPT will provide the greatest individual health benefits if given to people with clinical or epidemiological characteristics that increase the risk of TB disease and who also test positive for TB infection (not including contacts of TB patients who are aged <5 years and people living with HIV, for whom testing is not a prerequisite for TPT).

The most important disadvantage of testing for TB infection is the potential for significant delays between initial identification of someone at risk of developing TB disease and the initiation of TPT. In contacts, particularly young children *(59)* and people living with HIV, TB disease can develop rapidly after exposure and TB infection. In all contacts, the highest risk

period for progression to TB disease is in the first 6 months after exposure *(38,39)*. Hence, prompt initiation of TPT is crucial to prevent TB disease. Testing for TB infection may contribute to substantial delays, owing either to a lack of trained personnel to administer the test or read the skin test result, or to delays in laboratory processing and communication of IGRA test results. The results of IGRA testing should be available within 24–36 hours (although there may be additional delays due to sample transport and batch testing) and within 72 hours for TST or TBST; thus, testing for TB infection should not delay the initiation of TPT by more than 3 days after the initial identification.

The second potential challenge with testing is the greater burden on patients, including discomfort, fear of injections or blood collection, and the need for more visits before starting TPT (with associated potential patient costs, time, delays and resulting losses from the cascade of care). However, effective organization of health services can minimize cascade of care losses related to testing *(42,60)*, both in high-income countries and in LMIC.

False negative and indeterminate TB infection tests are a third potential challenge *(61)*. Such test outcomes are more frequent among immunocompromised individuals. However, the high relative risks of developing TB disease in people with positive TB infection tests compared with those with negative tests suggests that false negative results are not major determinants of outcomes that are important to patients. Additionally, some people at risk (e.g. older contacts) may test negative but become infected later, or show infection shortly after the test; in such cases, not giving TPT would be a missed opportunity to protect people.

3.5.2 When is testing for TB infection not advised?

This section sets out the various situations in which testing for TB infection is not advised.

Prior positive TB infection tests

If a prior positive test for TB infection or TB treatment is documented, then repeat testing for TB infection will not be useful and should not be done. Depending on the circumstances, the individual may be referred for further medical evaluation. However, if a prior positive result is self-reported and not documented, it is recommended to repeat the test, because studies have documented highly inaccurate self-reporting of prior skin test results *(62)*.

Concomitant or recent vaccines or viral illnesses

Testing for TB infection may result in false negatives in individuals with certain viral illnesses (e.g. measles) or live virus vaccination (e.g. measles or mumps) within the preceding 30 days *(63)*. This has been described with TST, but a similar effect with all TB infection tests is biologically plausible. Hence, it may be appropriate to delay the test for TB infection for 30 days after infection or vaccination. Alternatively, a negative test for TB infection may be repeated after 30 days.

A common question in recent years has been the impact of COVID-19 infection or vaccination on testing for TB infection. To date, no studies of TST or IGRA results after COVID-19 vaccination have been published. Given what is known about the immunological response to COVID-19 mRNA vaccination, such vaccination would not be expected to change TST or IGRA results *(64)*. However, given that test results could (at least theoretically) be modified by immunization, it

may be prudent to test before the vaccine or postpone testing for a few weeks after the vaccine where possible *(65)*.

Clinical work-up of adults to diagnose TB disease or monitoring of the response to treatment

TB infection tests should not be used for the diagnosis of pulmonary or extrapulmonary TB, nor should they be used for the diagnostic work-up of adults (including people living with HIV) with presumed TB disease. TB infection tests should not be used for screening, or to monitor the response to treatment for TB disease or TB infection.

History of TST or TBST allergic reactions (but IGRAs may be used)

Skin testing is not advisable in people with a history of allergic reaction to TST or TBST. Allergic reactions to TST (PPD or equivalent), such as a generalized rash that occurs within the first 24 hours, are seen in less than 1% of recipients *(46)*. If this has been well documented in the past, then it is best to avoid repeating the test with the same tuberculin material. Currently, it is unclear whether use of an alternative tuberculin material would be safe. Anaphylaxis in response to tuberculin skin testing is extremely rare (1 per million) *(47)*; however, if there is well-documented anaphylaxis in response to TST, then TB infection skin testing should not be performed, even with TBST, until further safety information is available.

Loss of consciousness after TST administration due to a vasovagal reaction (simple fainting) is far more common than anaphylaxis.

Challenges with blood collection in young children when using IGRAs (but TBST may be used)

IGRA should not be used to test for TB infection in children aged 6 months to 2 years, because of insufficient data and the challenges of phlebotomy in this age group. Instead, TST or TBST may be used. Testing household contacts aged below 5 years is not a prerequisite for providing TPT.

3.6 Multidisease testing considerations

Health needs are diverse, and programmes are expected to provide a range of diagnostics to assist health workers in managing patients as effectively and efficiently as possible. The diagnosis of TB often begins with assessing symptoms; this is not specific to TB, given that cough and fever overlap with COVID-19 and other respiratory infections. Additionally, people with TB may also have HIV, and services for both diseases are usually provided at the same levels of care. The relative diagnostic volumes are also quite heterogeneous, and they can be low for a specific disease or on a specific day at peripheral health centres. Multidisease testing can maximize the use of limited testing instruments and other resources; an information note describing considerations for multidisease testing is available *(59)*.

All currently recommended molecular diagnostics for the initial diagnosis of TB have a SARS-CoV-2 test available on the same instrument as the TB test, although some may not have received regulatory approval for such use. Several platforms are also widely used in diagnosing and managing TB in people living with HIV, whereas others are used for diagnosis of other viral pathogens (e.g. hepatitis C virus and human papillomavirus) or antimicrobial resistance

detection among bacterial pathogens. Additionally, one of the recommended IGRAs also runs on a platform that allows testing a range of other conditions; NGS platforms can also be used to sequence any nucleic acids present in a sample. The response to the COVID-19 pandemic led to NGS capacity being established in many countries, including LMIC, for surveillance. Such capacity and expertise may be available and could be used to facilitate the rapid uptake of targeted NGS-based DST for TB. If multidisease testing on an instrument is planned, then it may be best to employ platforms that use random access approaches (e.g. GeneXpert or Truenat) or allow different types of tests to be performed in the same batch or at the same time (e.g. BD MAX or targeted NGS) so that patient results are not delayed.

4. Placement of diagnostic tests in the tiered laboratory network

The desired outcomes from diagnostic tests that are implemented are that the test should:

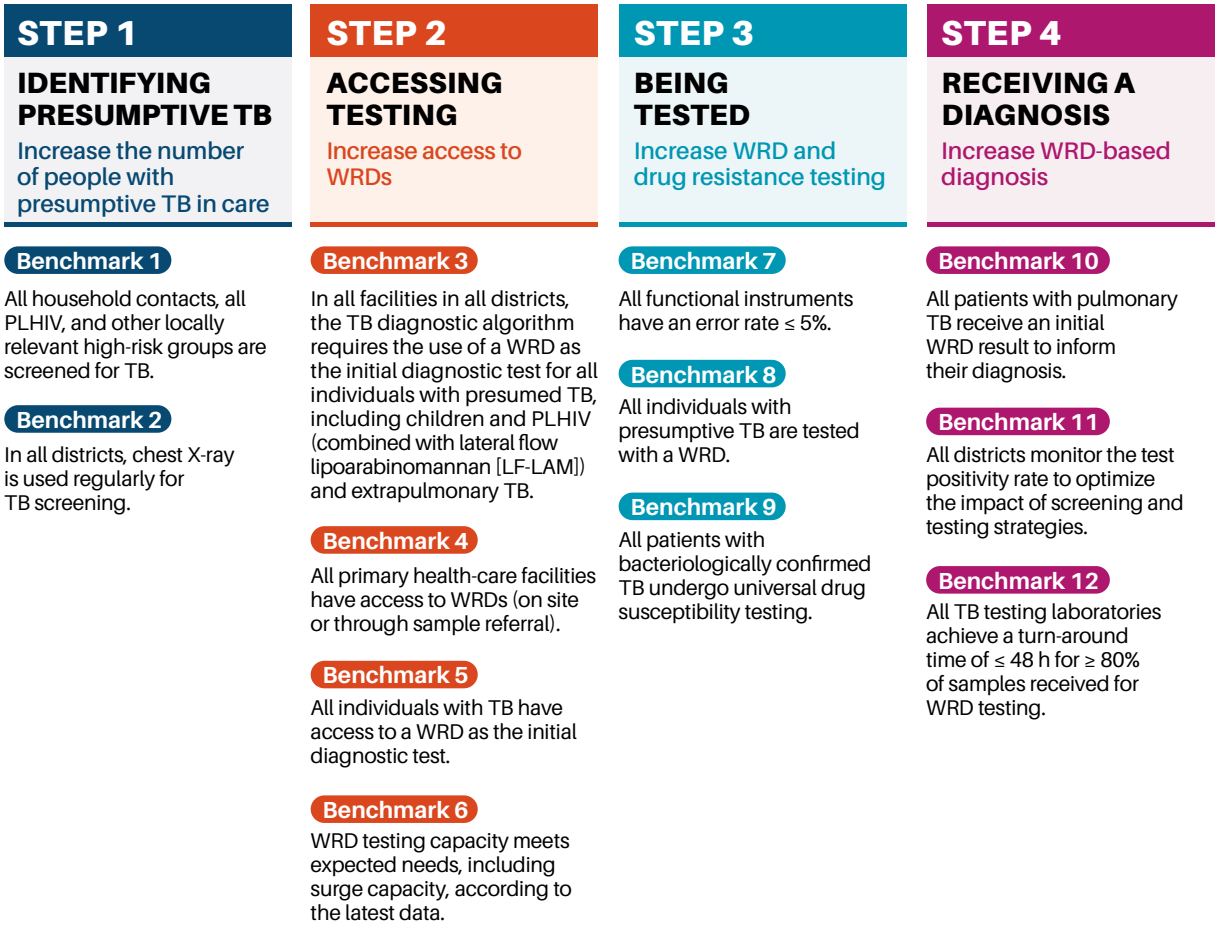
- provide accurate results;
- provide timely results to impact clinical decision-making;
- be justified based on need; and
- be quality assured, reliable and reproducible.

The decision on where to place a specific test is an important one because it can lead to success or failure in achieving these desired outcomes. Also, a diagnostic test should not be seen in isolation from the broader ecosystem of tests (TB and non-TB) used to deliver results for clinical management. To support these decisions, WHO and the GLI have published a manual for selection of mWRDs, for programme reference *(15)*.

The *WHO standard: universal access to rapid tuberculosis diagnostics (3)* can serve as a guide for implementing and monitoring improvements to TB testing and diagnostic networks, to achieve universal access. The standard comprises 12 benchmarks across four steps (**Fig. 4.1**), requiring a holistic approach to diagnostic delivery that comprises laboratories, health facilities and individual patients.

In many resource-limited or high-burden settings, TB laboratory networks have a pyramidal structure, as shown in **Fig. 4.2**. This structure has the largest number of laboratories at the peripheral level (Level I); a moderate number of intermediate laboratories (Level II), usually located in mid-sized population centres and health facilities; and a single central laboratory (Level III) – or more than one in large countries – at the provincial, state or national level. Each level or tier has specific requirements for infrastructure and biosafety, defined by a risk assessment, as outlined in the WHO biosafety manual for TB laboratories *(60)*.

Fig. 4.1. The 12 benchmarks constituting the WHO standard are divided into four steps along the diagnostic cascade



LF-LAM: lateral flow urine lipoarabinomannan assay; PLHIV: people living with human immunodeficiency virus; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

4.1 Peripheral level

At the peripheral level (Level I), laboratories offer a range of basic diagnostic tests, with the focus being on providing initial testing to rapidly detect TB (and RIF resistance):

- The LF-LAM is the only instrument-free, POC, lateral flow TB test that delivers results within 25–30 minutes and is suitable for use in the clinic. Thus, antiretroviral therapy initiation sites or similar care centres for people living with HIV would be examples of appropriate placement sites. The test is recommended for use concurrently with an LC-aNAAT among adults, adolescents and children living with HIV. Therefore, the collection of sputum and stool must also be performed, and the samples sent for testing with an LC-aNAAT.
- LC-aNAATs are suitable for decentralized laboratory placement, offering the advantage of sensitive TB detection with simultaneous or reflex-based drug-resistance testing at the same site. Prior or existing smear microscopy sites are suitable for LC-aNAAT placement because the population served is the same and the infrastructure requirements are similar.
  - Battery operated LC-aNAATs (e.g. Truenat assays) may be useful at sites where the power supply is unstable *(61)*.

  - LC-aNAATs for follow-on testing of samples from patients confirmed as having TB and RIF resistance may also be done onsite to speed up the availability of resistance results for INH, FQ, ETO and AMK without sample referral. However, the current within-class test, Xpert MTB/XDR, requires a different type of GeneXpert module (10-colour) than the Xpert Ultra test (6-colour). Given that the number of tests needed over a certain period will be much lower than the number for initial TB diagnosis, programmes can decide to replace one or more 6-colour module(s) with one or more 10-colour module(s) in existing GeneXpert instruments, to avoid replacing fully functional instruments. Furthermore, because the test has important value in providing rapid results for the management of MDR/RR-TB, sites where drug resistance is more prevalent or treatment is delivered may be prioritized for Xpert MTB/XDR testing.
- The LC-mNAAT (TB-LAMP) is also suitable for placement at the peripheral laboratory level. This class is less automated and has more testing steps than LC-aNAATs, but is also cheaper than LC-aNAATs and provides results where power supplies may vary or can be boosted with solar panels *(62)*. However, because it does not detect RIF resistance, an alternative test or a sample referral mechanism should be considered in populations who are at high risk of MDR-TB and in settings with moderate-to-high or unknown pretest probability of DR-TB, particularly where follow-on testing for RIF resistance is not accessible or available.
- TB infection skin tests can be used in peripheral settings owing to their simple procedures. Taking the incubation period and the need for a follow-up visit into consideration, it is preferable to provide access to the test as close to the target population as possible. It is important to ensure that storage possibilities (2–8 °C) are available for the TST or TBST, and that staff are adequately trained in administering the test and reading the results.

4.2 Intermediate level

At the intermediate level (Level II), technologies requiring more sophisticated infrastructure, expertise or biosafety precautions are offered. An important aspect of laboratories at this level is the need for reliable and rapid sample transport networks to transfer samples from peripheral laboratories to the intermediate laboratory, and from the intermediate laboratory to the central laboratory. Combining an efficient specimen referral system with centralized testing can be a cost-effective approach where the burden is low; it can also be more sustainable where there are shortages of skilled staff to capacitate and maintain a large, quality-assured peripheral-level network:

- The new MC-aNAATs are suitable at this level. These tests require laboratory infrastructure that can accommodate instruments that range in size from just under 1 m wide (94 × 75.4 × 72.4 cm) to over 4 m wide (429 × 216 × 129 cm). The throughput of this class of technologies varies, from performing up to 24 samples (multidisease) in one run to 96 samples (single disease) per run. Thus, depending on the specific product and setting, these tests could potentially be positioned at Level II or Level III.
- Culture on liquid or solid media, or FL-LPA or SL-LPA (or both) using sputum specimens may also be applicable at this level, but such tests are gradually being superseded by more automated and rapid alternatives.

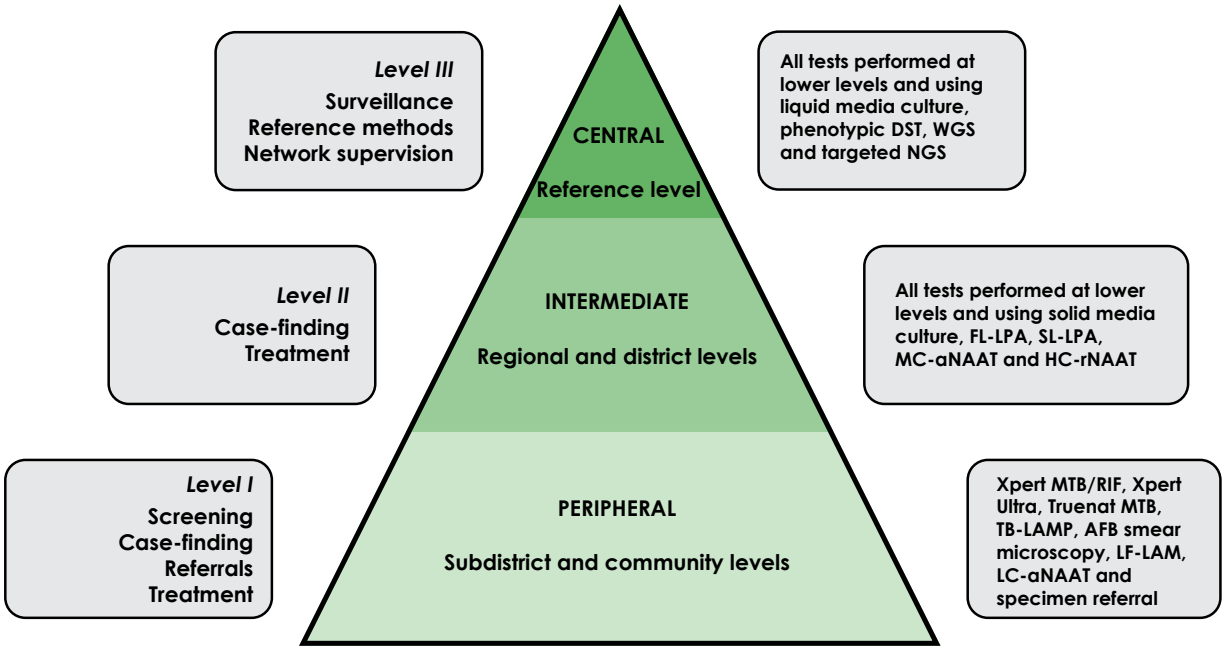
- The IGRAs are also suitable for this level of the health system. The test procedure includes multiple steps; also, an ELISA reader or a CLIA analyser that is maintained and calibrated is required to generate results.

4.3 Central level

The central level (Level III) offers testing that requires highly advanced skills, infrastructure and biosafety precautions. An important expectation at this level is to provide testing to resolve discordant results, troubleshooting, training support to other levels, QA and monitoring, and surveillance.

- HC-rNAATs, including the FL-LPA, SL-LPA and PZA-LPA (**Table 2.1**) tests, are all applicable at this level. The MC-aNAATs with high throughput could also be considered at this level, especially in urban settings where the workload is high.
- Culture and phenotypic DST using solid or liquid media should be available at this level. At a minimum, phenotypic DST for the new and repurposed drugs should be available.
- Establishing capacity for sequencing (targeted or WGS) is becoming increasingly important. Targeted NGS tests are high-complexity tests in their current format; hence, they are most suitable for centralized laboratories equipped with specialized skills and infrastructure, including information technology (IT) and data storage.

Fig. 4.2. Organization of a TB diagnostic testing network



AFB: acid-fast bacilli; DST: drug susceptibility testing; FL: first-line; HC-rNAAT: high-complexity reverse hybridization NAAT; LC-aNAAT: low-complexity automated NAAT; LC-mNAAT: low-complexity manual NAAT; LF-LAM: lateral flow urine lipoarabinomannan assay; LPA: line probe assay; MC-aNAAT: moderate-complexity automated NAAT; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; SL: second-line; TB: tuberculosis; WGS: whole-genome sequencing.

4.4 Structure of network and testing packages

The structure of the network and the testing packages available at each level should be tailored to meet the needs of the community and the local epidemiology of TB. When considering placement of a diagnostic test, targets to be considered should be based on demand rather than population, and should include:

- the volume of testing at a laboratory, which is likely to vary between dense urban settings and sparse rural communities;
- a strategy for providing optimal access to quality testing, either by increasing the number of sites providing a test or by transporting specimens to high-volume testing centres through an efficient specimen referral system – the strategy of choice will be determined by geography, infrastructure for transporting of specimens and result reporting, and epidemiological situation; and
- interlinking of the different levels; for example, the results of an initial test (e.g. RIF resistance detected) may trigger a follow-on test (e.g. testing for FQ resistance), which may not be available at the same level of the health system.

Although the levels described are useful conceptually, in practice they may overlap considerably, and careful logistical planning by mapping the current network of health facilities, population densities, the testing burden across the different facilities, transport infrastructure and the available laboratory network will aid placement (e.g. primary TB testing of people presumed to have TB will be relevant across all health facilities where individuals are screened for TB, whereas people with RR-TB may be managed at selected sites, and placement of testing for FQ and BDQ resistance may only be needed in selected laboratories that serve those sites).

Several considerations should guide the placement of a new diagnostic test within the existing laboratory network structure, including:

- anticipated workload, in terms of:
  - projected testing volumes;
  - availability of trained staff;
- possibility of integration with testing, specimen referral and reporting systems for other diseases; and
- resources available, such as:
  - infrastructural requirements (laboratory and storage space, power supply and temperature control);
  - biosafety requirements;
  - specimen types and collection procedures;
  - requirement for rapid diagnosis in people who are severely ill;
  - minimum number of tests needed to maintain expertise and optimal use of instruments;
  - current and planned testing algorithms;
  - links to other laboratories for further testing;
  - specimen referral and result reporting systems; and

Well-designed specimen referral systems underpin a strong diagnostics network. Such systems can help to:

- optimize access to services, and 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.

The *GLI guide to TB specimen referral systems and integrated networks (63)* and the *GLI specimen referral toolkit (64)* are useful sources of information for designing, implementing and monitoring systems for referring specimens and reporting results.

5. Steps and processes for implementing a new diagnostic test

The key steps for implementing a new diagnostic test are organized into 10 main areas. Details for each area are described in this chapter.

- Area 1 – Policies, budgeting and planning (**Section 5.1**)
- Area 2 – Regulatory issues (**Section 5.2**)
- Area 3 – Equipment (**Section 5.3**)
- Area 4 – Supply chain (**Section 5.4**)
- Area 5 – Procedures (**Section 5.5**)
- Area 6 – Digital data (**Section 5.6**)
- Area 7 – Quality assurance, control and assessment (**Section 5.7**)
- Area 8 – Recording and reporting (**Section 5.8**)
- Area 9 – Human resource training and competency assessment (**Section 5.9**)
- Area 10 – Monitoring and evaluation (**Section 5.10**).

5.1 Area 1 – Policies, budgeting and planning

Step 1.1 – Establish a technical working group (TWG) with defined roles and responsibilities

Step 1.2 – Review WHO policies and available technical and implementation guides

Step 1.3 – Define immediate and future purposes of the test

Step 1.4 – Update national diagnostic algorithm and guidelines

Step 1.5 – Perform a situational analysis, including biosafety

Step 1.6 – Develop a costed operational plan for phased implementation

Step 1.1 – Establish a TWG with defined roles and responsibilities

A TWG comprising representatives from all key stakeholders should be established to guide the implementation process. It is important to include representatives from other disease programmes or the national laboratory directorate, particularly if multidisease testing is planned. The establishment of the TWG should be led by the ministry of health, NTP and

national TB reference laboratory (NTRL). The TWG should be mandated to advise the ministry of health, NTP and NTRL on national policy and test implementation; develop action plans and implementation tools (e.g. testing algorithms, SOPs and protocols); and oversee the test's implementation. The TWG should also monitor the quality of implementation; suggest steps to address gaps in implementation across the cascade of care; and assess the impact and success of the test's introduction. Representatives from the following key stakeholders may be invited to participate:

- ministry of health, NTP, national programmes (e.g. HIV/AIDS programmes and noncommunicable disease programmes), NTRL(s) and regional laboratories;
- multisectoral representatives for the implementation of tests for TB infection (e.g. correctional services and mining);
- research institutes or other organizations with experience using the new diagnostic test;
- implementing partners, including those outside of TB;
- peripheral laboratories and clinical facilities that will participate in the testing;
- regulatory bodies;
- data management or IT experts and managers who can enable the interoperability of electronic systems;
- specimen transport system logisticians for centralized or regional testing (TB and non-TB);
- other technical and implementing partners (in addition to representatives of TB and HIV programmes) and other United Nations agencies;
- community and civil society representatives; and
- clinical staff.

A suitably qualified individual should lead the team; for example, a national TB laboratory officer or laboratory focal person from the NTP or NTRL. An integral component of the planning process should be defining the roles and responsibilities of members of the implementation team, and those of external partners and donors.

Step 1.2 – Review WHO policies and available technical and implementation guides

The TWG members should familiarize themselves with the contents of the relevant WHO policies, guidance, handbooks and reports, as well as any available implementation guides from WHO, GLI, the Foundation for Innovative New Diagnostics (FIND) and implementing partners. Particular attention should be paid to WHO policies and recommendations on using the test to aid in the diagnosis of TB or detection of drug resistance, who to test, how to conduct the test, the limitations of the test and interpretation of test results.

Step 1.3 – Define immediate and future purposes of the test

Programmes must clearly define the purpose, scope and intended use of the new diagnostic test because that will affect many aspects of the implementation plan, including resources. For example, the laboratory system or network needed to provide timely results for patient-care decisions is quite different from that needed to conduct a once-a-year drug-resistance survey.

Step 1.4 – Update national diagnostic algorithm and guidelines

The TWG should undertake a review of existing national diagnostic algorithms, taking into consideration the needs of people with TB, clinical needs, the country's epidemiology, existing testing algorithms, sample referral systems and other operational considerations; it should also make recommendations to the ministry of health and NTP. **Section 6** provides details on model algorithms for the use of WHO-recommended tests in detail.

The TWG should also lead a review of guidelines for the use of the new diagnostic test results in patient-care decisions. Clinical guidelines should provide clear guidance to clinicians, nurses and health care professionals on the intended use of the new diagnostic test; outline target populations; explain how to order the test; and explain how to interpret, use and communicate test results.

Step 1.5 – Perform a situational analysis, including biosafety

To inform plans for implementing the new diagnostic test, a situational analysis of the existing laboratory network and capacities should be conducted. For most tests, key elements to be assessed include regulatory requirements; laboratory and network infrastructure; existing sample transportation system; staff skills, expertise and experience; IT capabilities and infrastructure; diagnostics connectivity; availability and adequacy of SOPs; supply chain; financial resources; and QA systems and resources. The assessment should inform selection of testing sites; it should also determine needs for revision of training, recording and reporting forms, and tools for monitoring and evaluation. Of particular relevance is the specimen referral system; a checklist for evaluating such a system can be found in the relevant GLI publication *(63)*, and a checklist for implementing targeted NGS tests can be found in the NGS technical guide *(33)*.

Step 1.6 – Develop a costed operational plan for phased implementation

The final step in this area is to develop a detailed, costed and prioritized action plan for phased implementation, with (coverage) targets and timeline. Often, implementation of a new test involves startup costs associated with the procurement and installation of instruments, ancillary equipment and consumables; requirements for improving or establishing the necessary laboratory and network infrastructure (e.g. a specimen transport system); specialized, skilled and well-trained staff; expert technical assistance; maintenance of confidentiality of patient information; and establishment of a QA system.

Successful implementation of the plan will require financial and human resource commitments from the ministry of health or NTP, with possible support from implementing partners. A budget should be developed to address activities in collaboration with key partners. Budget considerations are summarized in **Annex 1**.

As an example, because of the high cost of targeted NGS-based DST (i.e. initial equipment and running costs), a carefully worked out plan covering all costs of implementation and operation will be needed. Testing volumes and batch sizes will impact cost and the turnaround times of NGS-based DST; they are described in detail elsewhere *(33)*.

5.2 Area 2 – Regulatory issues

Step 2.1 – Complete national regulatory processes

Step 2.2 – Determine importation requirements

Step 2.3 – Conduct country validation and verification studies, as required

Step 2.1 – Complete national regulatory processes

For new technologies, the ministry of health should work closely with the relevant government authorities, manufacturers and authorized service providers to meet the requirements of the national regulatory authority. Sufficient time must be allowed for review of the application and any supplementary evidence. Regulatory approval is generally provided for a specific test produced by a specific manufacturer, not for the class of the test. Approval of more than one test may improve access to tests and allow countries to choose between different tests based on test performance, as well as cost–effectiveness, feasibility and other operational aspects.

As an example, the regulatory pathway for approval of TBST will probably be the same as the one for TST materials. In many settings, because TB infection skin test material is injected, it is considered to be a medicine or a vaccine; therefore, the time to approval may be longer, with greater requirements for data (especially for safety), and the procedures for regulatory approval need to be initiated well in advance.

Step 2.2 – Determine importation requirements

National authorities should be consulted to determine relevant processes to be followed for importation of equipment and supplies for new tests. Early in the implementation process, countries should work closely with manufacturers and authorized providers of equipment and consumables to determine importation and registration as well as transportation and storage requirements, and to initiate country verifications, if required.

Step 2.3 – Conduct country validation and verification studies, as required

Validation studies are typically large-scale evaluations that measure the performance of the test, to determine whether it performs as expected under a setting's implementation conditions or whether country-specific factors (e.g. prevalence of different mutations or microorganism strains) cause performance to deviate substantially from the manufacturer's results or other evaluation studies. Validation studies are an essential part of the WHO review process and the development of recommendations for the use of a new test. Once large-scale validation studies have been published and a test's target performance characteristics have been established, countries that are implementing the method do not need to repeat such large-scale studies. Repeat validation should only be conducted if registration or importation regulations require it, because the efforts may use valuable limited testing resources and delay the implementation of the test.

Instead, implementing laboratories should conduct small-scale verification studies to demonstrate that they can achieve the same performance characteristics that were obtained

during the validation studies when using the test as described in those validation studies, and establish that the method is suitable for its intended use in the population being tested. This usually involves testing a well-characterized panel of known positive and negative samples (in a blinded fashion), in line with requirements for national or international accreditation schemes. In addition, these studies may include prospectively testing the current gold standard and the new test in parallel on clinical specimens. Verification is also required before commencing testing of clinical specimens in cases where laboratories perform non-standard or modified methods, use tests outside their intended scope (e.g. on specimens for which the test has not been validated), or use methods developed in-house. These studies, in addition to testing a well-characterized panel of known positive and negative samples, may include prospectively testing the current gold standard and the new test in parallel on clinical specimens *(65)*, or testing it in situations where a method has changed when compared with a previously completed validation (e.g. new versions) *(66)*.

Countries must make their own determination on the need for validation or verification studies, based on national regulatory policies, guidelines and accreditation requirements.

5.3 Area 3 – Equipment

Step 3.1 – Select, procure, install and set up equipment

Step 3.2 – Verify and maintain instruments

Step 3.3 – Assess site readiness and ensure a safe and functional testing site

Step 3.1 – Select, procure, install and set up equipment

An essential step in the implementation process is selecting appropriate instruments that fit the needs of the clinical or microbiological laboratory, and can be used to perform the new diagnostic test. The most suitable instrument for a country will depend on the intended use of, and demand for, the diagnostic test. It is important to choose an instrument that is widely available and has good supply distribution and support from the manufacturer. In addition to the testing instrument, some tests will require the use of specialized ancillary instruments.

To bring cost efficiency to testing services, a priority should be to consider the integration of TB testing with existing platforms in locations where integrated testing is feasible *(59)*. In settings where TB diagnostic services are standalone and there is a high workload for TB testing, dedicated instruments may be preferred.

Whichever instrument is selected, expert set-up will generally be required, with the manufacturer's engineers or authorized service providers performing the installation. Some of the MC-aNAATs may require infrastructure to be modified to accommodate the instruments. Potential set-up complexities include power supply and backup options, electrical and network connections, environmental conditions for the laboratory (e.g. maximum temperature), biosafety and ventilation requirements, computing hardware and software, a maintenance plan (e.g. weekly, monthly or pre-run checks), equipment warranty and necessary training.

Guidance for selecting which mWRD to implement has been published in the *Manual for selection of molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis (15)*. Guidance on selecting NGS equipment is available elsewhere *(33).*

For TB infection skin tests, a standalone or medical-grade refrigerator for storing consumables (tuberculin or TB antigens), vaccines and medications must be available, as must backup generators or power supply system. Tuberculin vials should be stored at 2–8 °C (35–46 °F), protected from light, in the original packaging and separated from other similar vials (to avoid confusion). For IGRAs, material for blood sample collection is necessary.

Step 3.2 – Verify and maintain instruments

All instruments must be documented as being "fit for purpose" through verification with known positive and negative materials before starting to test clinical specimens. Instrument verification is conducted at installation, after service or calibration, or after moving instruments.

To ensure quality, reproducibility and reliability, maintenance and calibration must be performed regularly. The frequency of calibration and its intervals vary for each instrument. To decide on the frequency of calibration, the manufacturer's recommendations should be followed at a minimum. However, where it is suspected that the level of accuracy of the equipment is declining, those responsible should be able to discern the problem and take the initiative to perform calibration.

Laboratory equipment calibration requires an association between measurements of a scale or accuracy that have been made or set with the equipment to be tested, and similar measurements that have been made with the standard (i.e. equipment with known or assigned accuracy). Standards vary among countries, depending on the type of industry. Manufacturers designate their measurement criterion; they also recommend the frequency and level of calibration, depending on how often the device is used and the specific application.

Many tests rely on precision instruments that require regular preventive maintenance, and ad hoc servicing and maintenance. The end-user should perform regular preventive maintenance to ensure good performance of the instrument. Suppliers or authorized service providers should perform on-request maintenance, as necessary. To ensure continued functioning of the instruments, countries should take advantage of all-inclusive and transparent maintenance contracts; these contracts should have clear service and maintenance terms and associated costs.

Step 3.3 – Assess site readiness and ensure a safe and functional testing site

The NTP or NTRL usually determines which sites will conduct diagnostic testing, based on factors such as TB epidemiology, geographical considerations, testing workload, availability of qualified staff, efficiency of referral networks and access to services for people being tested. Each testing site should be evaluated for readiness using a standardized checklist before testing of clinical specimens at the site begins. In addition, testing sites should be assessed regularly for safety and operational functionality.

A functional testing site requires the instruments used for testing to be properly positioned in a clean, secure and suitable location. Most instruments will require an uninterrupted supply

of power, and appropriate working and storage conditions (e.g. humidity and temperature controlled). A safe environment requires WHO biosafety recommendations for conducting the diagnostic test to be followed in appropriate containment facilities with adequate ventilation; it also requires appropriate personal protective equipment to be used, and biological waste to be disposed of safely and in accordance with regulations. Failure to provide a functional and safe work environment can affect the health and well-being of testers, and the quality and reliability of testing.

5.4 Area 4 – Supply chain

Step 4.1 - Review forecasting, ordering and distribution procedures

Step 4.2 - Develop procedures to monitor reagent quality and shelf life

Step 4.1 – Review forecasting, ordering and distribution procedures

Uninterrupted availability of reagents and disposables at the testing site is essential to ensure that technical capacity is built in the early stages of implementation (avoiding long delays between training and the availability of reagents and disposables), and to ensure consistent service during routine use. The following measures will be required to ensure uninterrupted supply of reagents and disposables:

- ensuring that qualified staff contribute to defining the specifications for reagents, consumables and equipment;
- streamlining importation and in-country distribution procedures to ensure sufficient shelf life of reagents and consumables, once they reach testing sites;
- careful monitoring of consumption rates, tracking of reagent-specific shelf lives and forecasting to avoid expirations or stock-outs;
- careful planning to ensure that sites have received training, and that equipment has been installed ahead of shipment of reagents;
- ongoing monitoring of all procurement and supply chain steps, to ensure that delays are minimized and that sites receive correct reagents as per the planned schedule; and
- regular reassessment of purchasing and distribution strategies, to ensure that they are responsive to needs and the current situation.

There has been a global shortage of tuberculin in recent years, with a direct impact on TB infection screening in countries that rely on TST *(67)*. Tuberculin is not listed among the supplies in the Global Drug Facility list; this can hamper the acquisition and distribution of prequalified material. This situation is being addressed for TBST consumables; increased availability of TBST may help to overcome some of these challenges in the future.

Step 4.2 – Develop procedures to monitor reagent quality and shelf life

The shelf life of reagents and their required storage conditions must be considered when designing a procurement and distribution system. Laboratory managers should routinely monitor reagent quality and shelf life to ensure that high-quality test results are generated. Also, the laboratory must establish SOPs for handling the reagents and chemicals used, to

ensure both quality and safety. For example, the shelf life of NGS reagents is normally short; hence, robust planning is required to avoid kits expiring, with resulting high costs for testing. For tuberculin, once the vial has been opened, the material should be used within 30 days; thus, the date of opening should be clearly written on the label of the vial. The vial should be stored immediately at the end of the working day, at the prescribed temperature. Any tuberculin remaining after 30 days must be discarded. The duration varies for TBST; hence, manufacturer specifications should be followed.

New-lot testing, also known as lot-to-lot verification, should be performed on new batches of reagents or test kits. Such testing usually involves testing a sample of the new materials and comparing the results to an existing lot of materials with known performance. Where possible, new-lot testing of commercially available test kits should be performed at the central (e.g. NTRL) or regional level, thereby ensuring that kits with test failures are not distributed. At the testing site, new-lot testing is needed for reagents prepared at that site; it may also be needed to monitor conditions during transport and storage of test kits within the country. For QC, WHO recommends using positive and negative controls when testing new batches of reagents.

5.5 Area 5 – Procedures

Step 5.1 – Develop SOPs

Step 5.2 – Update clinical procedures and strengthen the clinical–laboratory interface

Step 5.1 – Develop SOPs

Based on the intended use or uses of the diagnostic test, procedures must be defined, selected, developed or customized for:

- identifying people for whom the test should be performed;
- collecting, processing, storing and transporting specimens to the testing laboratory;
- testing;
- tuberculin injection and induration reading for TB infection skin tests (**Annex 4**);
- data analysis, security and confidentiality (see Area 6);
- process controls (internal QC) and EQA (see Area 7);
- recording and reporting of results (see Area 8); and
- waste management.

It is essential to have a well-defined, comprehensive set of SOPs that addresses all aspects of the laboratory testing processes, from sample collection to reporting of results; in part, because errors at any step can have a significant impact on the quality of testing. Some SOPs will rely on the manufacturer's protocols included with commercial kits whereas others will need to be developed. SOPs must be made readily available for staff and must be updated regularly.

Step 5.2 – Update clinical procedures and strengthen the clinical–laboratory interface

A comprehensive plan to implement a new diagnostic test must address all relevant parts of the diagnostic cascade, not just what happens in the laboratory. In addition to laboratory-related SOPs, clear clinical protocols and guidance will be needed for selecting people to be tested, ordering tests, interpreting test results, reporting and making patient-care decisions. Before the introduction of a new diagnostic test or any changes to an existing test, all clinical staff involved in diagnosis and patient management must be informed about the planned changes, and relevant training must be conducted. Information must also be shared with clinical staff at all referral sites through staff training opportunities and through the use of standardized educational materials developed by the NTP. Enhanced clinical trainings should be considered when new tests or testing strategies differ from existing assays in terms of performance or rates of discordant results (e.g. LF-LAM and targeted NGS testing versus LC-aNAAT and phenotypic DST assays).

The rate of ordering of the new test must be monitored, to ensure that the test is being used by the clinical staff at all sites offering the test. Clinical staff at sites with an unexpectedly low or high testing rate may need additional training and sensitization.

5.6 Area 6 – Digital data

Step 6.1 – Develop the use of digital data and diagnostics connectivity

Step 6.2 – Develop procedures for data backup, security and confidentiality

Step 6.3 – Develop data requirements for targeted NGS

Step 6.1 – Develop the use of digital data and diagnostics connectivity

Many of the latest testing instruments offer the opportunity to use digital data. The implementation plan should consider software and hardware requirements, to take advantage of digital data. "Diagnostic connectivity" refers to the ability to connect diagnostic test devices that produce results in a digital format, in such a way as to transmit data reliably to a variety of users *(68)*. 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, providing an overview of the facilities, devices and commodities in the network. Software can track consumption and inventory to avoid stock-outs and expiring supplies. It can also identify commodity lots or specific instruments with poor performance or abnormal error rates for QA purposes, and provide a pre-emptive service to avoid instrument failure. This approach is a highly cost-effective way to ensure that a diagnostic device network functions properly; it is also useful for reporting and connecting with treatment sites.

Data, results and information updates can also be transmitted automatically to:

- clinicians and patients, allowing for faster patient follow-up;

- laboratory information management systems or electronic registers, reducing staff time and the chance of transcription errors, and greatly facilitating monitoring and evaluation processes; and
- the NTP, assisting with surveillance of disease trends or resistance patterns and rates, and enhancing the capacity of the NTP to generate the data needed for performance indicators of the End TB Strategy.

Step 6.2 – Develop procedures for data backup, security and confidentiality

With any electronic data system, there is a risk of losing testing data. A SOP for regularly backing up data (e.g. to an external drive) is essential, as is a SOP for data retrieval. Also needed are policies and procedures to ensure the security of laboratory data and confidentiality of patient data, in line with national and international regulations. Antivirus and antipiracy software should be installed and kept up to date. Antihacking mechanisms should be in place, as should access restriction to safeguard confidentiality, protect personal information and prevent data breaches by unauthorized users. Data access and governance policies should be developed and enforced.

Step 6.3 – Data requirements for targeted NGS

Data storage requirements, data analytical tools and data-sharing protocols needed for targeted NGS are more complex and require a comprehensive strategy, possibly with technical assistance from an IT expert or department. A checklist to assess IT and data readiness is included in the site readiness checklist in Annex 3 of the WHO implementation manual *(33).*

5.7 Area 7 – Quality assurance, control and assessment

Step 7.1 – Implement a comprehensive QA system

Step 7.2 – Establish and monitor QC

Step 7.3 – Develop an EQA programme

Step 7.4 – Monitor and analyse quality indicators

Step 7.1 – Implement a comprehensive QA system

A comprehensive QMS is needed to ensure the accuracy, reliability and reproducibility of test results. Essential elements of a QA system include:

- SOPs, training and competency assessment (Area 9);
- instrument verification and maintenance (Area 3);
- method validation or verification (Area 2);
- lot-to-lot testing (Area 4);
- internal QC;
- EQA; and

- quality indicator monitoring and continuous quality improvement.

A comprehensive discussion of the essential elements of a QA system can be found in the *Practical manual on TB laboratory strengthening, 2022 update (65)*. This section describes QC, EQA and quality indicator monitoring. Additionally, a variety of QA manuals are available for multiple WHO-recommended tests through the GLI *(12)*.

Step 7.2 – Establish and monitor QC

Internal QC monitors the activities related to the analytical phase of testing, with the goal of detecting non-conforming events (e.g. errors due to test failure, environmental conditions or operator performance) before results are reported. Internal QC typically involves examining control materials or known substances that are included in the assay at the same time as patient specimens, to monitor the quality of all steps of testing, from specimen preparation to result reporting. If QC results are not acceptable (e.g. positive results are obtained on negative controls), patient results must *not* be reported, and a root cause investigation and corrective action plan should be put in place and monitored to resolve the challenge.

QC is important for all tests, irrespective of their placement or complexity. Programmes should ensure availability of QC programmes across all levels of the testing network. Because of the complexity of the targeted NGS workflow and the need for multiple reagent kits and processes, it is important to conduct quality checks after each of the main steps in the process. Further information is given in *The use of next-generation sequencing for the surveillance of drug-resistant tuberculosis: an implementation manual (33)*.

Step 7.3 – Develop an EQA programme

An EQA programme includes quality and performance indicator monitoring, external QC or proficiency testing, re-checking or making comparisons between laboratories, regular onsite supportive supervision and timely feedback, corrective actions and follow-up. Onsite supervision should be prioritized at poorly performing sites identified through proficiency testing, monthly monitoring of performance indicators or site assessments. Failure to enrol in a comprehensive EQA programme is a missed opportunity to identify and correct problems that affect the quality of testing. Where EQA programmes are available, their use should be considered during test selection, planning and budgeting processes before tests are introduced.

The GLI has developed an EQA dashboard that lists available EQA panel and programme providers *(56)*. The products are not endorsed by GLI; however, the dashboard is a resource where countries can find suitable panels or programmes *(56)*.

Proficiency testing

For many laboratory tests, the EQA programme includes proficiency testing to determine the quality of the results generated at the testing site. Proficiency testing compares testing site results with a reference result to determine comparability. The purpose of such testing is to identify sites with serious testing deficiencies, target support to the most poorly performing sites and evaluate the proficiency of users following training.

Re-checking of samples

Comparisons between laboratories can also be used as an external assessment of quality. This usually involves the retesting of samples at a higher level laboratory. Many TB laboratories are familiar with this approach because blinded re-checking is a routine method of EQA for acid-fast bacilli (AFB) smear microscopy.

Onsite supervisory visits

Onsite supervisory visits are especially critical during the early stages of implementing a new test because they provide motivation and support to staff. Supervisory visits are opportunities to provide refresher training, mentoring, troubleshooting advice and technical updates. Onsite assessments should be documented using standardized checklists, to ensure consistency and completeness of information, enable monitoring of trends, and allow follow-up on recommendations and corrective actions. An onsite supervisory programme requires substantial planning and resources (both financial and human).

Although not performed in laboratories, skin tests for TB infection need continuous internal QC and EQA. In many countries, certification of such tests is required after complex training and control, but a follow-up quality assessment is not in place. Mobile health (mHealth) technologies have been developed to allow quality assessment and QC of the performance of health care personnel even in remote areas *(69)*.

For TST and TBST, resources include a guide for reviewers performing quantitative assessment of the skin test for TB infection *(70)*, and instructions for health care personnel on how to take mTST photos *(71)* (**Annex 4**).

Step 7.4 – Monitor and analyse quality indicators

Routine monitoring of quality indicators, also known as performance indicators, is a critical element of assuring the quality of any diagnostic test. In addition to the general laboratory quality indicators recommended in the 2022 update to the practical manual on TB laboratory strengthening *(65)*, quality indicators specific to the new diagnostic test should be adapted from international guidelines or developed from scratch. Quality indicators for NGS-based DST have been developed and are described in the WHO implementation manual *(33)*. The indicators should be collected using a standardized format and analysed on a monthly or quarterly basis, disaggregated according to test.

Programmes should establish a baseline for all indicators. Targets should be set for all indicators monitored, and any unexplained change in quality indicators (e.g. an increase in error rates or a change in MTBC positivity) 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 between sites.

The continuous quality improvement process is a cyclical, continuous, data-driven approach to improving the quality of diagnostic testing. The process relies on a cycle of monitoring quality indicators, planning interventions to correct or improve performance, and implementing the interventions. Quality indicators should be reviewed by the laboratory manager and must always be linked to corrective actions if any unexpected results or trends are observed. Critical

to the process is documentation of corrective actions, and subsequent improvement and normalization of laboratory indicators following the corrective actions.

5.8 Area 8 – Recording and reporting

Step 8.1 – Review and revise request for examination and reporting forms

Step 8.2 – Review and revise laboratory and clinical registers

Step 8.1 – Review and revise request for examination and reporting forms

It may be necessary to revise the country's current test requisition forms (i.e. specimen examination request forms) to accommodate a new diagnostic test. Countries should determine whether an update of the examination forms is needed, considering the cost and time required for such a revision. If a system is not already in place, countries should establish a numbering system to identify repeat samples from the same person, to monitor the proportion and performance of repeat tests.

Given that patient data (e.g. on treatment status) are critical for the correct interpretation of test results, programmes should ensure that the test request form captures such information. In many countries, request forms already contain fields for such data; however, sometimes data may either not be entered in some of these fields or be entered inconsistently. Refresher training for clinical and laboratory staff should be conducted, to ensure that forms are filled out correctly and completed properly.

The forms used for reporting test results must balance the need to convey the test information while also conveying the information that is essential to allow a clinician to interpret the results and act promptly on them. An easy-to-read format is important because there is likely to be a wide range of expertise among the clinicians interpreting test results. Also, to avoid confusion, results should only be reported for medicines that are being used in-country and in alignment with national guidelines. For example, WHO recommends against the use of AMK, except for people with XDR-TB; hence, AMK results should only be reported when relevant for clinical decisions.

An easy-to-read format is particularly important for targeted NGS solutions because they generate a large amount of data. At a minimum, the reporting form should capture the unique patient identifier; also, for each drug analysed, the report form and database should include information on the genes analysed, any mutations detected, and the corresponding confidence grading and resistance profile for each drug. It is recommended that reporting of results should depend on quality criteria (e.g. at least 20 successful reads per base across the gene of interest) being met *(72)*.

A global consortium previously led a consensus process to standardize language for reporting of NGS-based DST results and generate a generic reporting form for MTBC based on NGS DST results *(73)*. The consensus forms are intended to be used to report NGS-based DST results to clinicians for use in patient-care decisions, but they may also be useful guides for developing forms for reporting information for a DR-TB surveillance system.

Reporting results of tests for TB infection, medical evaluation, CXR and other testing should also be streamlined, and ideally it should be digitized to facilitate rapid referral and analysis. The *Prevent TB* mobile application and digital platform is expected to facilitate systematic recording and reporting, as well as monitoring of data across the cascade of care for TB infection *(74)*. Where paper forms are used, it is suggested that patients always be given a copy of the results.

Step 8.2 – Review and revise laboratory and clinical registers

Current laboratory and clinical registers that are based on the WHO reporting framework *(10)* may need to be modified to record the results of the diagnostic test being implemented. Forms for laboratory records may also need to be modified. Countries should implement a standardized approach for recording test results in laboratory and clinical registers, and should use that approach consistently across all testing and clinical sites. Countries with electronic laboratory information management systems may need to include new tests in the software package.

5.9 Area 9 – Human resource training and competency assessment

Step 9.1 – Develop terms of reference and position descriptions

Step 9.2 – Develop and implement a training curriculum and strategy

Step 9.3 – Assess and document the competence of staff

Step 9.4 – Provide for post-training mentoring and support

Step 9.1 – Develop terms of reference and position descriptions

The successful implementation of a diagnostic test will depend on the expertise, training and experience of the laboratory personnel involved in testing. This will be particularly important for the more technically complex testing methods such as targeted NGS tests. Position descriptions – with clearly defined roles and responsibilities, and required competencies and skills – will be needed for staff undertaking diagnostic testing.

Step 9.2 – Develop and implement a training curriculum and strategy

Training and competency assessment are critical for generating quality-assured test results; they should be offered for the different levels of personnel (e.g. managers, senior technologists, technicians and laboratory assistants). Implementing a diagnostic test requires training beyond the steps required to carry out the test, and the onsite training supplied by manufacturers following installation is often too short to cover QA activities. The testing site manager must ensure that test users are trained in the operation and maintenance of the test instrument, correct performance of the test and associated QA activities.

Clinician training or sensitization must be done in parallel with training of laboratory staff, to ensure that all clinicians involved in the screening and care of people with TB understand the benefits and limitations of the new test and are sensitized to the new testing algorithm, test

requisition process, specimen requirements, specimen referral procedures and interpretation of results.

Clinical staff responsible for implementing TPT require training on how to perform and interpret TB infection tests if these are used. Also, it is crucial to train nurses for injection and reading of induration. Simple protocols for training *(75)*, and using mobile technologies for training and QC of skin testing *(69)*, may facilitate the widespread use of these tests, even in remote settings. Practical training material is available in several languages; for example, for injection *(76)* and reading of results *(77)*.

Educational material developed in the local language for health care personnel and patients is useful *(78)*; examples are available online *(79, 80)*.

Step 9.3 – Assess and document the competence of staff

Competency assessments should be performed, using a standardized template, after training and periodically (e.g. annually) thereafter. They should include assessment of the knowledge and skills for performing each of the tasks involved in a diagnostic test. Assessments should be conducted by an experienced test user or trainer, and should include observation of the person being assessed as that person independently conducts each of the required tasks. Proficiency testing panels may be used for competency assessments. The results of competency testing should be recorded in personnel files.

Step 9.4 – Provide for post-training mentoring and support

Post-training mentoring and support that builds on the initial training is useful. It will help to ensure success in the implementation of a diagnostic test and enable the programme to keep abreast of the latest advances in this rapidly evolving field. A support programme will also facilitate troubleshooting during the implementation of any new technology.

5.10 Area 10 – Monitoring and evaluation

Step 10.1 – Monitor implementation of the diagnostic test

Step 10.2 – Monitor and evaluate impact of the diagnostic test

Step 10.1 – Monitor implementation of the diagnostic test

During the initial planning phase, countries should establish a set of key indicators and milestones that can be used to monitor the implementation process. Once the testing services have been launched, use of the services should be tracked. The quantity and quality of testing and reporting must be monitored including, for example, the number of tests performed, number of indeterminate results and number with positive results. Additional training and sensitization may be needed for staff at sites with an unexpectedly low or high testing or indeterminate rate, or very high frequencies of positive or negative test results.

Step 10.2 – Monitor and evaluate impact of the diagnostic test

A framework for monitoring and evaluation of the impact of a diagnostic test is essential to inform decision-making. Often, the objective of new or improved TB diagnostic tests is to improve the laboratory confirmation of TB or the detection of drug resistance. Indicators to assess the impact of test objectives should be developed. For each such indicator, programmes should define the purpose, target, data elements and data sources; they should also stipulate how the indicator is to be calculated, process indicators and corresponding data elements that contribute to the main indicator.

In-depth analyses of the process indicators may be useful as follow-up investigations, to elucidate the test's contribution to patient outcomes and to identify opportunities for interventions towards increasing impact.

6. Model algorithms

Effective and efficient TB diagnostic algorithms are key components of a diagnostic cascade that is designed to ensure that people with TB disease or TB infection are diagnosed accurately and rapidly, and promptly placed on appropriate therapy. In turn, that therapy should improve patient outcomes, reduce transmission and avoid development of drug resistance. This section presents a set of four model algorithms that incorporate the goals of the End TB Strategy and the most recent WHO recommendations for the diagnosis and treatment of TB, DR-TB and TB infection. The model algorithms should be adapted to the local situation.

When adapting a diagnostic algorithm to local settings, it is important to consider the characteristics of the population being served. The four algorithms are as follows:

- **Algorithm 1** is the general algorithm that relies on WRDs, such as low- or moderate-complexity NAATs, as the initial diagnostic tests and is appropriate for all settings, although the choice of which mWRD to use may differ in a setting with a high prevalence of MDR/RR-TB (e.g. a test that detects MTBC and RIF with or without INH resistance may be needed), HIV (e.g. a more sensitive test may be needed) or Hr-TB (e.g. a test that detects MTBC, RIF and INH resistance simultaneously will be needed). The algorithm considers the new recommendations on concurrent testing (including use of LF-LAM) for specified risk groups as the first step before results interpretation and management.
- **Algorithm 2** and **Algorithm 3** are for follow-up testing, after TB is diagnosed, to detect additional drug resistance:
  - **Algorithms 2a** and **2b** are used when the purpose is to detect resistance to second-line drugs in people with RR-TB; the choice depends on the availability of targeted NGS.
  - **Algorithm 3** is used when the purpose is to detect resistance in individuals with RIF-susceptible TB who are at risk of having DR-TB and those with Hr-TB. Molecular testing is preferred, but any existing WRD may be used. Targeted NGS is a valuable addition for Algorithm 2 and 3 because these tests can detect mutations associated with resistance to many anti-TB medicines; also, these molecular tests are useful for people at high risk of having DR-TB (e.g. people in whom therapy is failing).
- **Algorithm 4** is the model algorithm for diagnosing TB infection performed based on the recommendations in the *WHO consolidated guidelines for tuberculosis: module 2: screening (81)*. Testing for TB infection should only be conducted after ruling out TB disease.

Algorithms 2 and 3 are appropriate for all settings; however, the resource requirements for follow-up testing may differ widely between settings with a high or low burden of DR-TB.

Each algorithm is accompanied by explanatory notes and a decision pathway that provides a detailed description of considerations for how test results may be used to guide patient care.

In general, the algorithms are not necessarily test-specific; rather, they present classes of tests that share expected result categories (e.g. "TB detected") and their follow-up actions.

TB screening strategies

The diagnosis of TB begins with a person identified as presumed to have TB disease through assessment of signs and symptoms, or screened using another approach. WHO has released updated recommendations on TB screening, and readers are encouraged to consult the latest guidance *(54, 81)*. People presumed to have TB may not always present with symptoms that match the latest screening recommendations, but they still have an increased probability of TB disease that requires diagnostic testing. The modalities for screening, beyond the four-symptom screen, now include CXR, an mWRD used as a screening tool or C-reactive protein in people living with HIV. The addition of mWRD for screening of selected at-risk populations and settings goes beyond its primary use as an initial diagnostic tool; the different uses should not be confused and are further described elsewhere *(54)*. However, priority should be given to ensuring universal access to mWRDs as a diagnostic test for TB and DR-TB before extending its use to screening. Furthermore, the use of mWRDs as a screening test should be considered carefully because it would have large financial and operational implications (e.g. are there enough tests and testing capacity to use mWRDs for screening, in addition to what is needed to conduct all testing for the initial diagnosis and detection of RIF resistance). The methods for screening for TB are described in detail in the latest operational handbook on screening *(54)*. It is important to consider prevalence and pretest probability when choosing a screening strategy, especially in scenarios where mWRDs are used in a screening context rather than for diagnostic testing.

6.1 Implementing a new diagnostic algorithm

Modifications to diagnostic algorithms must be put in place only after a formal evaluation, review and approval by officials within the ministry of health, NTP and NTRL. Often, nationally appointed thematic working groups are used to evaluate new technologies and develop implementation plans, which typically include revising current algorithms. These working groups comprise local ministry officials, implementing partners, civil society and professionals (laboratory and medical), who will decide the optimal use and placement of the new technology within the current network structure. The following points should be considered when designing or reviewing algorithms for testing at different levels of the laboratory network:

- the specific diagnostic tests in use or being considered for use;
- whether the tests are recommended by WHO and, if so, for what purposes;
- the ability to collect the specimens required for the test;
- what additional testing is recommended to follow up the results of the new tests;
- the current and planned capacity of the country's laboratories, laboratory infrastructure and availability of competent personnel to conduct the tests;
- the adequacy of systems for specimen collection and transport;
- the capacity of clinical services to offer diagnosis and treatment;
- the drugs used for the treatment of TB and DR-TB in the country; and
- the characteristics of the population (i.e. the groups at risk) being served, which should be derived from population-based studies (if available), including the proportion of people

with DR-TB, HIV-associated TB and extrapulmonary TB, as well as the proportion of TB among children.

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

6.2 The cascade of the four model algorithms

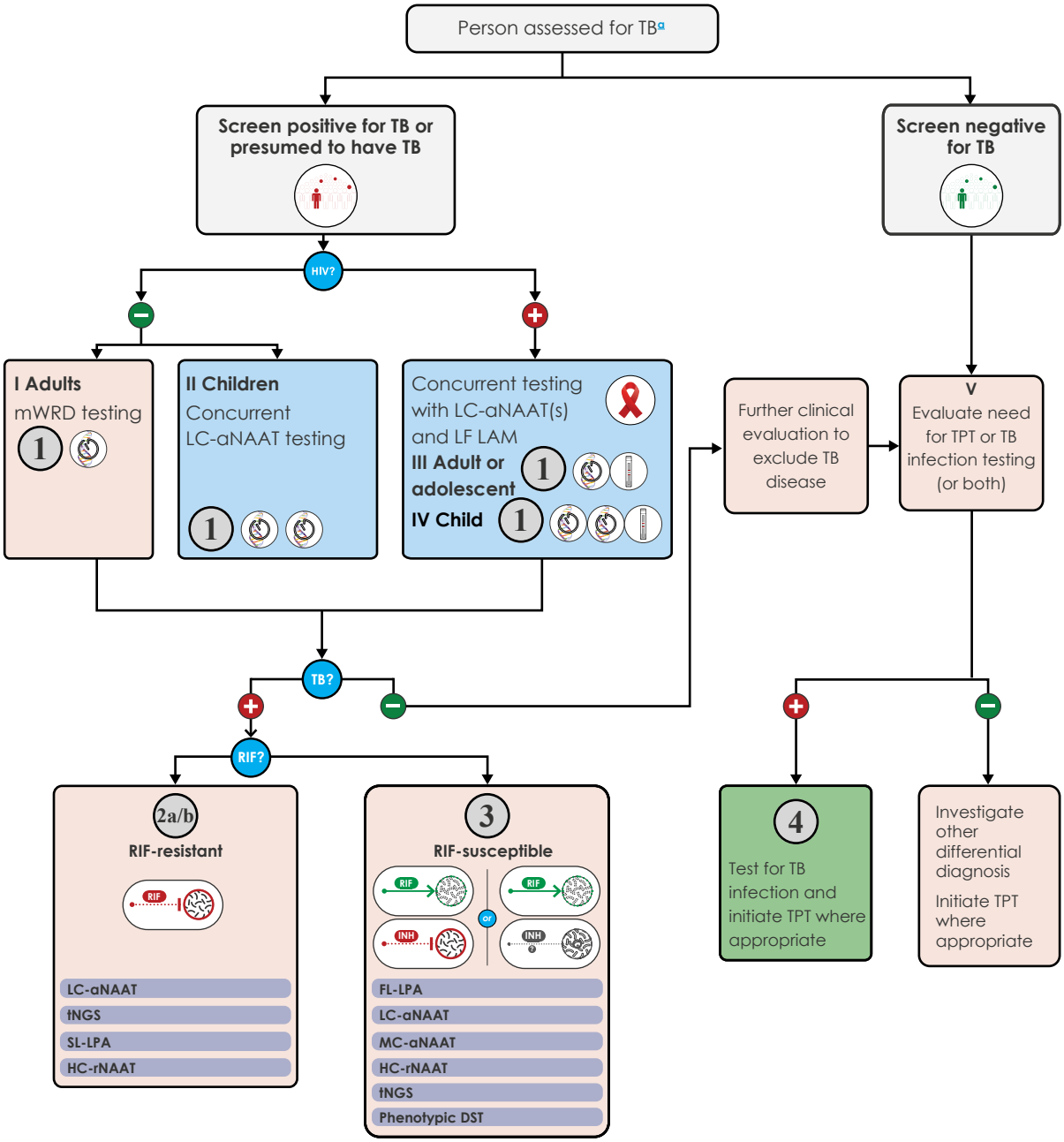
Although the algorithms are presented separately, they are interlinked and cascade from one to another. This is illustrated in the overview in **Fig. 6.1**, which describes how the different algorithms cascade from the starting point of a person being evaluated for TB. If the person screens positive, or is presumed to have TB, samples are collected for diagnostic and RIF-resistance testing according to Algorithm 1. The number and type of recommended samples and tests varies according to the person's age and HIV status (as shown in **Fig. 6.1: I-IV**). The result of the initial test or tests will guide further actions (**Fig. 6.1**).

For people who test positive for TB and in whom RIF resistance is detected, further work-up is needed. Algorithms 2a and 2b provide guidance on the additional testing required to detect resistance to FQs and other second-line drugs. People with TB that is susceptible to RIF may require further testing for INH resistance and second-line drugs; Algorithm 3 provides guidance on suitable tests.

People who screen negative for TB or in whom TB disease has been ruled out through testing should be clinically evaluated. Clinical evaluation is especially important for children, who experience lower rates of bacteriological confirmation of TB owing to low and variable amounts of mycobacteria in their samples. Further practical guidance on the clinical management of childhood TB and example treatment decision algorithms can be found in the *WHO operational handbook on tuberculosis: module 5: management of TB in children and adolescents (58)*.

People in whom TB is excluded are then assessed for the need for TPT or testing for TB infection (or both) (**Fig. 6.1: V**). Algorithm 4 shows the steps needed to perform testing for TB infection. As explained in **Section 2.5.1**, identifying, testing, evaluating and treating people with TB infection is a multistep process that has been termed the TB infection cascade of care *(40)*. Those that do not need TPT or TB infection testing undergo evaluations for differential diagnoses.

Fig. 6.1. The cascade of the four diagnostic algorithms



DST: drug susceptibility testing; FL-LPA: line probe assay for first-line drugs; HC-rNAAT: high-complexity reverse hybridization NAAT; LC-aNAAT: low-complexity automated NAAT; LF-LAM: lateral flow urine lipoarabinomannan assay; MC-aNAAT: moderate-complexity automated NAAT; mWRD: molecular WHO-recommended rapid diagnostic test; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis; TPT: TB preventive treatment; WHO: World Health Organization.

Drugs: INH: isoniazid; RIF: rifampicin.

[a] Definitions on who should undergo TB screening are listed in the *WHO consolidated guidelines on tuberculosis: module 2: screening: systematic screening for tuberculosis disease (81)*.

6.3 Algorithm 1 – WRDs as initial diagnostic tests for TB

Algorithm 1 is the starting point for the diagnostic pathway, and in this context the recommended classes of WRDs include both molecular tests (LC-mNAAT, LC-aNAAT and MC-aNAAT) and a biomarker-based test (LF-LAM) (**Fig. 6.2**). Member States can choose the mWRD that best fits their circumstances, with the ultimate objective being to serve patient needs and ensure universal access to diagnostic and RIF-resistance testing. The mWRD classes provide different types of results:

- TB diagnosis only, using an LC-mNAAT;
- TB diagnosis and detection of RIF resistance (simultaneously or as a two-step process) using an LC-aNAAT; or
- TB diagnosis and detection of RIF and INH resistance (simultaneously or as a two-step process) using an MC-aNAAT.

Where an LC-mNAAT is used, follow-on testing for RIF resistance may be needed.

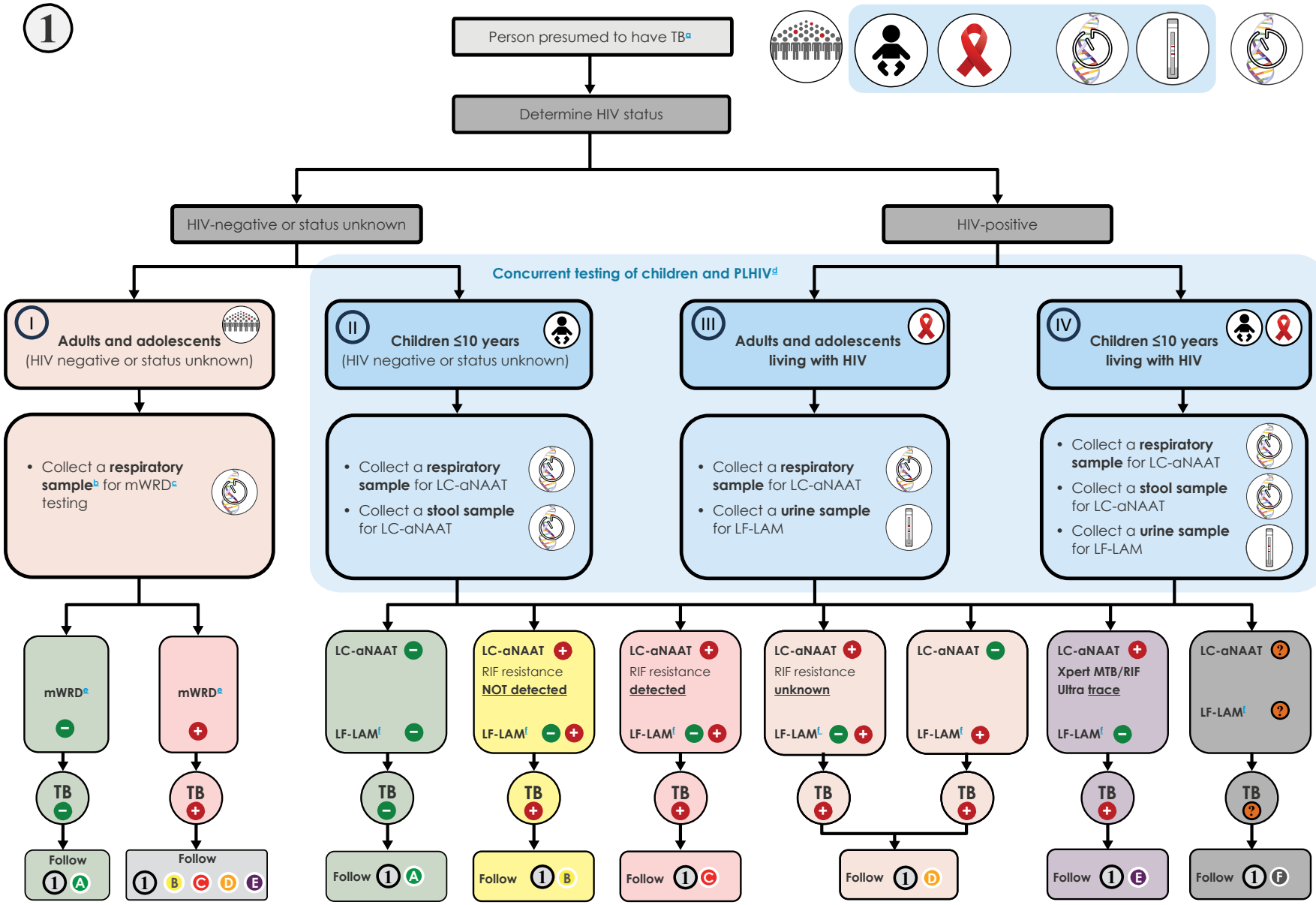
LF-LAM provides a result for TB diagnosis only (without RIF resistance). Recommendations for the urine LF-LAM have been expanded to apply to all individuals infected with HIV, regardless of setting and CD4 count; also, the test has been integrated with LC-aNAATs in the recently recommended concurrent testing strategy for adults, adolescents and children living with HIV. The recommendations on concurrent testing do not include the use of LC-mNAATs or MC-aNAATs.

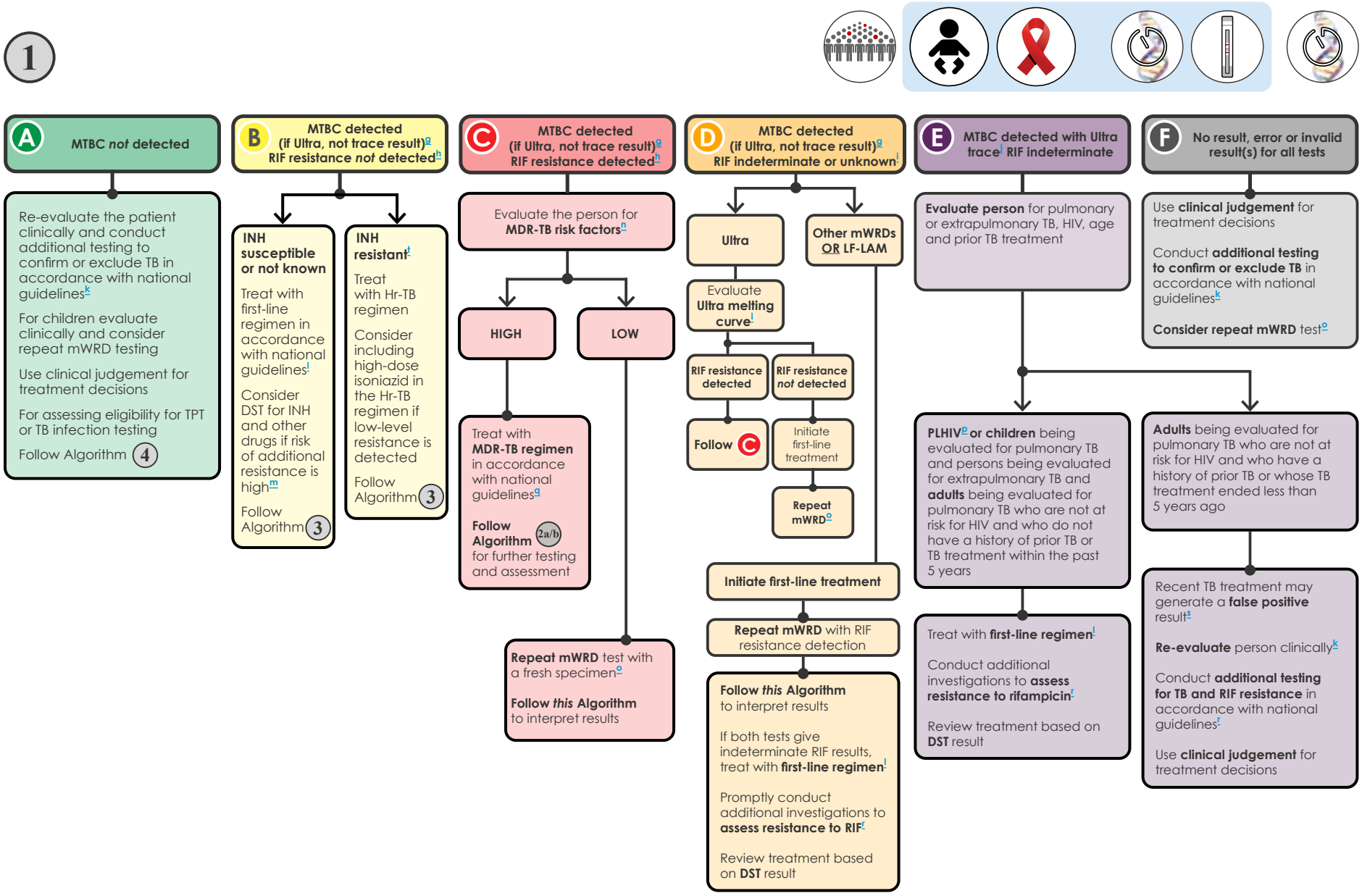
The test options for Algorithm 1 vary in complexity. LF-LAM is the only POC test for the initial detection of TB that does not require special skills for testing or laboratory infrastructure. The LC-aNAATs require basic pipetting skills and are easy to decentralize to basic laboratories; however, they have limited throughput with the commonly used instruments. In contrast, some MC-aNAATs require more hands-on time and have large infrastructure requirements; most of them provide high throughput and are best suited to established laboratories with reliable sample referral networks. In practice, test needs and associated choices are likely to vary, depending on the setting within a country or province. Consideration should be given towards hybrid models that use a combination of tests from different manufacturers; this has the added advantage of providing a safety mechanism in the event of an expected problem with a supplier.

It is feasible to implement Algorithm 1 when the mWRD testing can be conducted onsite or can be accessed through a reliable referral system with short turnaround times.

Fig. 6.2. Algorithm 1: mWRD as the initial diagnostic test for TB

Footnotes are interactive




1
A
MTBC not detected
Re-evaluate the patient clinically and conduct additional testing to confirm or exclude TB in accordance with national guidelines[k]
For children evaluate clinically and consider repeat mWRD testing
Use clinical judgement for treatment decisions
For assessing eligibility for TPT or TB infection testing
Follow Algorithm 4
B
MTBC detected (if Ultra, not trace result)[g] RIF resistance not detected[h]
INH susceptible or not known
Treat with first-line regimen in accordance with national guidelines[l]
Consider DST for INH and other drugs if risk of additional resistance is high[m]
Follow Algorithm 3
INH resistant[l]
Treat with Hr-TB regimen
Consider including high-dose isoniazid in the Hr-TB regimen if low-level resistance is detected
Follow Algorithm 3
C
MTBC detected (if Ultra, not trace result)[g] RIF resistance detected[h]
Evaluate the person for MDR-TB risk factors[n]
HIGH
Treat with MDR-TB regimen in accordance with national guidelines[q]
Follow Algorithm 2a/b for further testing and assessment
LOW
Repeat mWRD test with a fresh specimen[o]
Follow this Algorithm to interpret results
D
MTBC detected (if Ultra, not trace result)[g] RIF indeterminate or unknown[i]
Ultra
Evaluate Ultra melting curve[i]
RIF resistance detected
Follow C
RIF resistance not detected
Initiate first-line treatment
Repeat mWRD[o]
Other mWRDs OR LF-LAM
Initiate first-line treatment
Repeat mWRD with RIF resistance detection
Follow this Algorithm to interpret results
If both tests give indeterminate RIF results, treat with first-line regimen[l]
Promptly conduct additional investigations to assess resistance to RIF[r]
Review treatment based on DST result
E
MTBC detected with Ultra trace[j] RIF indeterminate
Evaluate person for pulmonary or extrapulmonary TB, HIV, age and prior TB treatment
PLHIV[p] or children being evaluated for pulmonary TB and persons being evaluated for extrapulmonary TB and adults being evaluated for pulmonary TB who are not at risk for HIV and who do not have a history of prior TB or TB treatment within the past 5 years
Treat with first-line regimen[l]
Conduct additional investigations to assess resistance to rifampicin[r]
Review treatment based on DST result
Adults being evaluated for pulmonary TB who are not at risk for HIV and who have a history of prior TB or whose TB treatment ended less than 5 years ago
Recent TB treatment may generate a false positive result[s]
Re-evaluate person clinically[k]
Conduct additional testing for TB and RIF resistance in accordance with national guidelines[r]
Use clinical judgement for treatment decisions
F
No result, error or invalid result(s) for all tests
Use clinical judgement for treatment decisions
Conduct additional testing to confirm or exclude TB in accordance with national guidelines[k]
Consider repeat mWRD test[o]

CSF: cerebrospinal fluid; CXR: chest X-ray; DNA: deoxyribonucleic acid; DST: drug susceptibility testing; HIV: human immunodeficiency virus; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; LAMP: loop-mediated isothermal amplification; LC-aNAAT: low-complexity automated NAAT; LC-mNAAT: low-complexity manual NAAT; LF-LAM: lateral flow urine lipoarabinomannan assay; LPA: line probe assay; MC-aNAAT: moderate-complexity automated NAAT; MC-mNAAT: moderate-complexity manual NAAT; MDR-TB: multidrug-resistant TB; MTB: *Mycobacterium tuberculosis*; MTBC: *Mycobacterium tuberculosis* complex; mWRD: molecular WHO-recommended rapid diagnostic test; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; PLHIV: people living with HIV; RR-TB: rifampicin-resistant TB; TB: tuberculosis; TPT: TB preventive treatment; WHO: World Health Organization.

Drugs and regimens: INH: isoniazid; HREZ: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z); REZ: rifampicin (R), ethambutol (E) and pyrazinamide (Z); RIF: rifampicin.

[a] Persons presumed to have TB include those with signs and symptoms of TB and those who screen positive for TB. They include adults and children with signs or symptoms suggestive of TB, with a CXR showing abnormalities suggestive of TB, a positive mWRD used as a screening tool or positive C-reactive protein test (>5 mg/L) in PLHIV. A person with a positive mWRD used as a screening tool and a low pretest probability should be clinically assessed and, if deemed to be a person presumed to have TB, 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 person should be managed based on the result of the test and, if relevant, should continue on to Algorithm 2 or 3. 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 Ultra.

[b] 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).

[c] mWRD includes LC-aNAAT, LC-mNAAT and MC-aNAAT for the general population.

[d] The concurrent testing strategy was only assessed for LC-aNAAT in combination with LF-LAM owing to lack of evidence for LC-mNAAT and MC-aNAAT. A laboratory equipped only with LC-mNAAT or MC-aNAAT can also use these tools to diagnosis both of PLHIV and children because both classes are recommended for initial diagnosis of TB of these groups. Additional testing may be required to detect RIF resistance if LC-mNAAT was used. This strategy is not covered by the concurrent testing recommendations but is a way to increase sensitivity in the diagnostic cascade without unnecessary delays. An initial positive result on any of these tests, LC-mNAAT, MC-aNAAT or LF-LAM should lead to treatment initiation.

[e] Only the result from the single mWRD is available for this group.

[f] LF-LAM should not be used on people who are HIV-negative.

[g] "MTBC detected (if Ultra, not trace results)" includes MTBC detected as high, medium, low or very low. These categories apply to the 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 (if Ultra, not trace results)". The MC-aNAAT provides additional resistance detection for INH and leads to additional considerations in pathway B.

[h] Determination of RIF resistance occurs simultaneously in the Xpert Ultra test and the MC-aNAAT. A reflex test is needed to determine RIF resistance for Truenat testing, using the same DNA isolated for the Truenat MTB test. The TB-LAMP test 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 also occurs simultaneously with RIF detection.

[i] The interpretation and follow-up testing for MTBC detected and RIF indeterminate or unknown for the Xpert Ultra test differs from the interpretation of results for other mWRDs. MTBC detected with 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 Xpert 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. When using an mWRD without detection of RIF resistance (e.g. TB-LAMP), further testing for RIF resistance is required preferably using an mWRD able to detect resistance.

[j] "MTBC detected trace" applies only to the Xpert Ultra test.

[k] Further investigations for TB may include CXR, additional clinical assessments, repeat mWRD testing, culture or clinical response following treatment with broad-spectrum antimicrobial agents.

[l] People should be initiated on a first-line regimen according to national guidelines, unless the person is at very high risk of having MDR-TB. Such people should be further investigated and initiated on an MDR-TB regimen if pertinent. 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.

[m] A sample may be sent for molecular or phenotypic DST if there is a high prevalence of INH or other drug resistance and RIF susceptible (i.e. INH monoresistance or polyresistance) 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 for INH should be performed.

[n] People at high risk for MDR-TB include people who were treated previously, including those who had been lost to follow-up, relapsed or experienced treatment failure; 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.

[o] The mWRD with RIF testing should be performed at the same testing site with a fresh specimen, and the result of the second test should be interpreted as shown in this algorithm. The RIF result from the second test is the result that should be used for clinical decisions.

[p] 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.

[q] People should be promptly initiated on an MDR-TB regimen in accordance with national guidelines. Algorithm 2 should be followed for additional testing for any person with RR-TB.

[r] Phenotypic (culture and DST) and molecular (e.g. alternate mWRDs, LPAs and targeted NGS) methods are available for evaluating drug resistance. Rapid molecular methods are preferred.

[s] In people 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 "MTBC detected low or very low") may be false 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.

[t] People diagnosed using an MC-aNAAT and whose result is RIF resistance not detected, and INH resistance detected should be treated for Hr-TB with 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 3.

6.3.1 Decision pathway for Algorithm 1 – WRD as the initial diagnostic test for TB

General considerations

WHO recommends the use of an mWRD from one of the LC-mNAAT, LC-aNAAT and MC-aNAAT classes as an initial diagnostic test for TB (rather than microscopy or culture) for all adult and adolescent HIV-negative individuals with signs and symptoms of TB. WHO also recommends the concurrent use of urine LF-LAM and an LC-aNAAT to diagnose TB among adults, adolescents and children living with HIV (see **Section 3.4**). Details on the different tests included in the different classes are given in **Section 2**. The target populations include:

- newly presenting individuals with symptoms of TB (cough of any duration, fever, haemoptysis, night sweats and weight loss);
- individuals who have screened positive for TB by an alternative method (e.g. CXR or C-reactive protein) and require confirmation; and
- individuals who are on treatment or have been previously treated, or people being evaluated for possible RR-TB or Hr-TB (e.g. non-converters at the end of the intensive phase of treatment despite treatment adherence) or for a new or continuing episode of TB (e.g. relapse cases or people treated previously, including those who had been lost to follow-up).

TB programmes should transition from using microscopy as the initial diagnostic test to using mWRDs. The mWRDs show a higher sensitivity for the diagnosis of TB; also, they can simultaneously detect resistance to RIF and, in cases of MC-aNAAT use, resistance to INH.

For monitoring treatment, mWRDs are not recommended, because the tests do not discriminate between live and dead bacilli and thus may generate false positive results. Instead, microscopy and culture should be used for monitoring treatment, in accordance with national guidelines and WHO recommendations. More information on the need to develop more rapid and sensitive tools for TB treatment monitoring can be found in the 2023 *WHO target product profiles for tests for tuberculosis treatment monitoring and optimization (82)*.

Algorithm 1 describes the collection of one or more quality initial specimens to be used for mWRD testing and the collection of additional specimens as needed (information on which specimens can be used with which WRD is given in **Section 2** above and in individual WHO policy recommendations):

- for adults and adolescents, specimens that may be used are induced or expectorated sputum (preferred), tracheal aspirate or bronchoalveolar lavage; in addition, urine should be used for LF-LAM testing among those who are HIV-positive;
- for children, additional specimens include gastric aspirates, nasopharyngeal aspirate and stool; in addition, urine may be used for LF-LAM testing among those who are HIV-positive; and
- for people being evaluated for extrapulmonary TB, specimens that may be used are cerebrospinal fluid, lymph node tissue aspirate, pleural tissue, pleural fluid, synovial fluid, peritoneal fluid or pericardial fluid.

For LF-LAM, midstream urine should be collected in a sterile, standard urine specimen cup at a facility with private urine collection and handwashing areas. Whenever feasible, fresh samples should be tested, ideally immediately after collection. If immediate testing is not possible, urine should be stored according to the LF-LAM manufacturer instructions for use *(24)*. LF-LAM testing is appropriate for all persons living with HIV being evaluated for pulmonary or extrapulmonary TB, regardless of the overall prevalence of HIV in the setting. The LF-LAM test result (<30 minutes) is likely to be available before the mWRD result and, if positive, is considered as bacteriological confirmation of TB.While LF-LAM does not differentiate between various species of the genus Mycobacterium, in areas with a high prevalence of TB, the LAM antigen detected in a clinical sample is likely to be attributed to MTBC.

For operational issues, programmes may consider routinely collecting two specimens (e.g. spot and morning sputum samples, or two spot specimens) from each HIV-negative person or child, instead of only collecting a second specimen when additional testing is needed. If two specimens are collected, the first should be tested promptly using the first available mWRD. The second specimen may be used for the additional testing described in the algorithm (e.g. repeat mWRD testing for failed tests or follow-on resistance testing, or for smear microscopy or culture as a baseline for treatment monitoring).

If only one specimen can be collected (e.g. if it is difficult or impossible to obtain tissue biopsy samples), the TB diagnostic algorithm should be modified to prioritize testing with the mWRD. If additional TB testing is warranted, one option is to consider using any portion of the sample remaining after the mWRD for other tests (e.g. culture, histology, LPA and DST). Alternatively, the sample could be processed for culture in an appropriately equipped laboratory, and the same sediment could be used for the mWRD, culture and other tests. Clinical decisions should be made based on clinical judgement and the results of available laboratory tests.

With respect to the detection of MTBC, mWRD results are typically reported as "MTBC not detected", "MTBC detected", "no result", "error" or "invalid". Within the "MTBC detected" result group, some mWRDs provide semiquantitative results (high, medium, low or very low). The Xpert Ultra test has an additional semiquantitative category called "trace".

- When Xpert Ultra "trace" is used for people living with HIV and children who are being evaluated for pulmonary TB, and for individuals being evaluated for extrapulmonary TB, the "MTBC detected trace" result is considered as bacteriological confirmation of TB.
- In adults who are HIV-negative and symptomatic, with a recent history of TB treatment (i.e. completed <5 years ago), Xpert Ultra "trace" results (and occasionally other mWRD "MTBC detected very low") may be positive not because of active TB but because of the presence of non-viable bacilli. Clinical decisions must be made on all available information and clinical judgement.

With respect to the detection of RIF and INH resistance, mWRDs report the results as RIF or INH "resistance detected", "not detected" or "indeterminate". For the assays for which resistance detection relies on the absence of binding of wild-type reporter probes to amplicons (e.g. Truenat MTB-RIF Dx) it may be advisable to state that resistance is inferred rather than detected.

When two mWRDs are performed (in the context of the concurrent testing strategy), and the results of the two tests are discordant for RIF status, possible errors should be investigated (e.g.

wrongly labelled samples or machine error) and the resistance profile of contacts reviewed. If the investigation in inconclusive, the resistant results should be used; alternatively, the test could be repeated with the resistant sample type before a DR-TB regimen is prescribed.

The use of an mWRD to detect resistance to RIF or INH (or both) may not eliminate the need for follow-up DST.

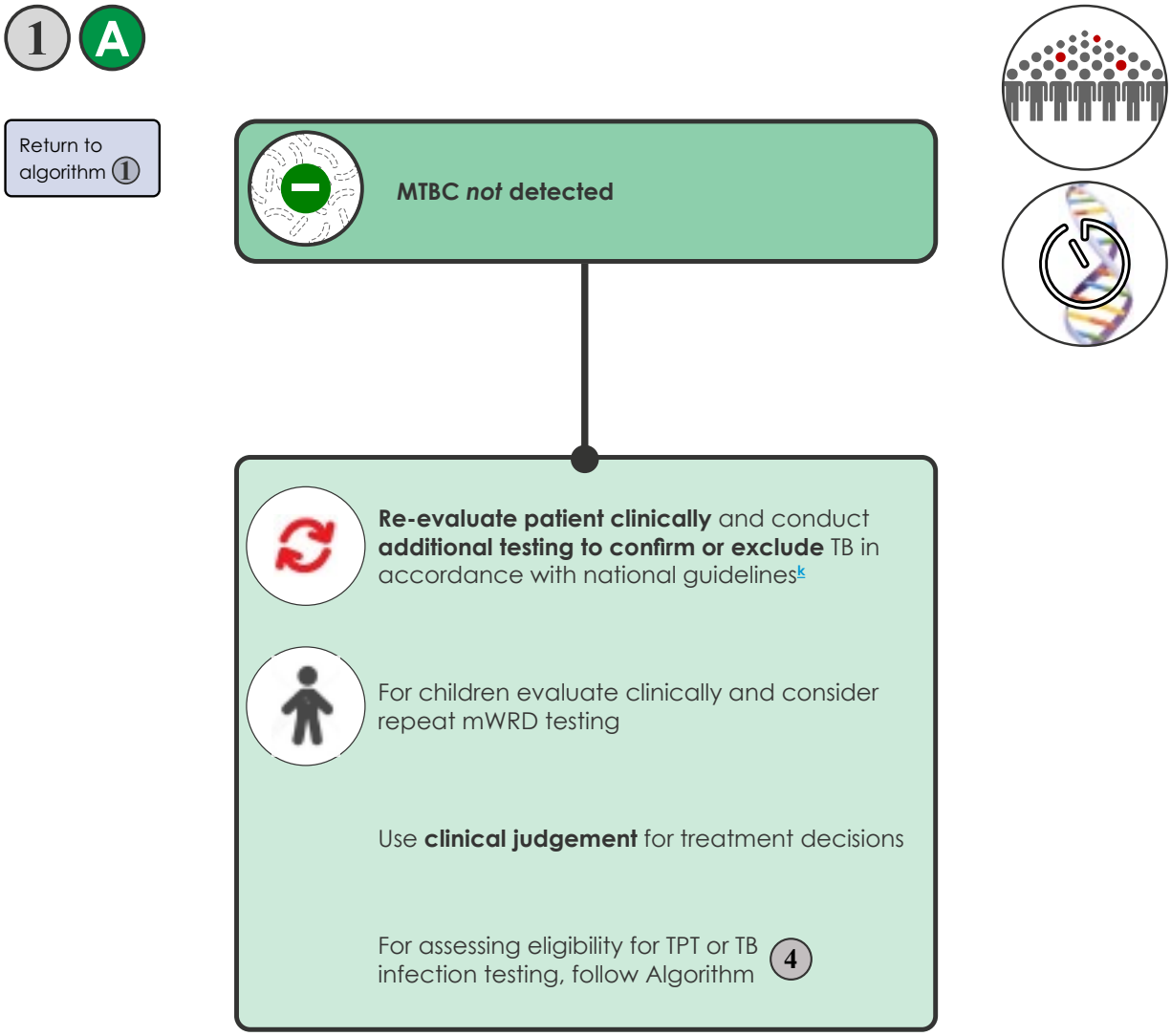
Decision pathway

The results of the test or tests should be used to guide next steps and inform treatment decisions. A description of pathway selection is given below, followed by the considerations and actions for each of the WRD pathways.

- If all tests are negative, Pathway 1A applies.
- If any test is positive, the test results guide selection of Pathways B to E:
  - Pathway 1B: MTBC detected, and RIF susceptibility confirmed;[6]
  - Pathway 1C: MTBC detected and RIF resistance detected;
  - Pathway 1D: MTBC detected (if Ultra, not trace result) and RIF resistance unknown or indeterminate; and
  - Pathway 1E: MTBC detected with trace result, RIF indeterminate.
- If all tests yield no results, invalid results or errors, Pathway 1F applies.

When conducting concurrent testing of multiple samples and tests for people living with HIV and children, Pathway 1A applies if all tests are negative, whereas Pathways 1B to 1E apply if any test is positive. Pathway 1B applies when all resistance tests indicate RIF susceptibility, whereas Pathway 1C applies if any test indicates RIF resistance. For patients who test positive for TB with LF-LAM or LC-mNAAT but without the initial availability of a RIF-resistance result and without access to LC-aNAAT, an additional sample should be collected and transported for testing with an LC-aNAAT offsite. Pathway 1D may be followed until the RIF-resistance result is available.

[6] RIF susceptibility should be confirmed (not unknown) using WRDs.

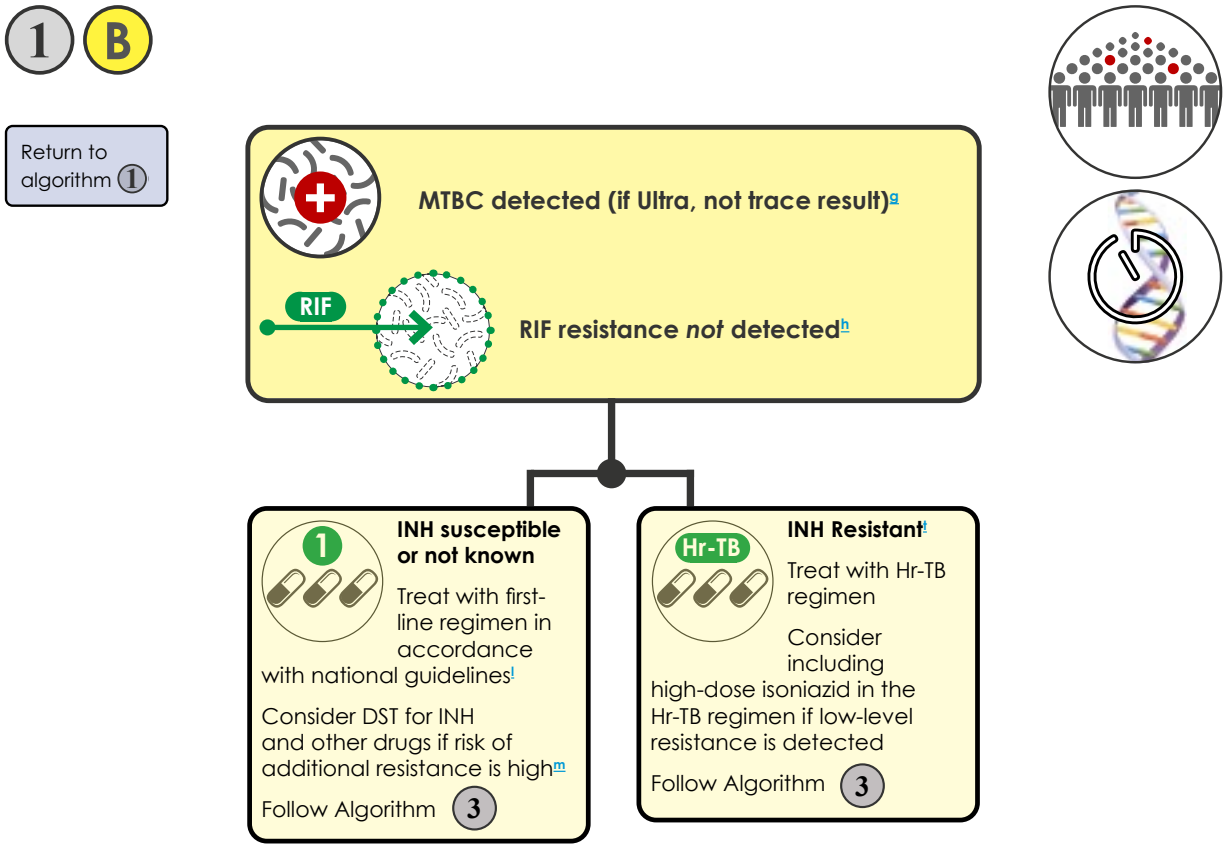


MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostic test; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

For figure notes, please see page 112.

1. If the mWRD result is "MTB not detected" (1)(A), the person should be re-evaluated, and additional testing undertaken in accordance with national guidelines.
   a. For the concurrent testing, all tests included in the strategy must be negative to follow this pathway, including LF-LAM tests among persons living with HIV.
   b. Further investigations for TB may include CXR, additional clinical assessments, additional mWRD testing, or culture and clinical response following treatment with broad-spectrum antimicrobial agents (FQs should not be used).
   c. For children, a careful clinical assessment is necessary to rule out TB, as detailed in the *WHO operational handbook on tuberculosis: module 5: management of tuberculosis in children and adolescents (58)*.
   d. For persons living with HIV, treatment for *Pneumocystis* pneumonia should be considered. If there is clinical worsening or no improvement after 3–5 days of treatment, further investigations for TB and other diseases may be re-initiated and, if the person is seriously ill with danger signs, presumptive TB treatment should be initiated.

e. For persons living with HIV with advanced HIV disease, the AHD package of care should be applied irrespective of the negative test results. All children living with HIV aged under 5 years are considered as having AHD.
f. As always, clinical judgement should be used for treatment decisions, including considering the possibility of clinically defined TB (i.e. TB without bacteriological confirmation).



DST: drug susceptibility testing; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostic test; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

Drugs: INH: isoniazid; RIF: rifampicin.

For figure notes, please see page 112.

2. If the mWRD result is "MTBC detected, RIF resistance not detected" and "INH resistance not detected" or INH results are unknown: 1 B
   a. For children, this applies when either or all of the LC-aNAATs are "MTBC detected, RIF resistance not detected". If one LC-aNAAT shows "MTBC detected, RIF resistance not detected" and one LC-aNAAT shows "MTBC detected, RIF indeterminate", the test should be repeated on the specimen type that initially tested as RIF indeterminate and the final valid result should be used for decision-making.
   b. For people living with HIV, this applies regardless of the LF-LAM result.
   c. The person should be initiated on an appropriate regimen using first-line TB drugs in accordance with national guidelines.
   d. Additional DST should be requested in the following cases:
      i. Molecular or phenotypic DST for INH is indicated:

- if the person has been treated previously with INH or is a contact of a person known to have Hr-TB; or
- if there is high prevalence of INH resistance that is not associated with RIF resistance (i.e. Hr-TB or polyresistance, not MDR-TB) in this setting;

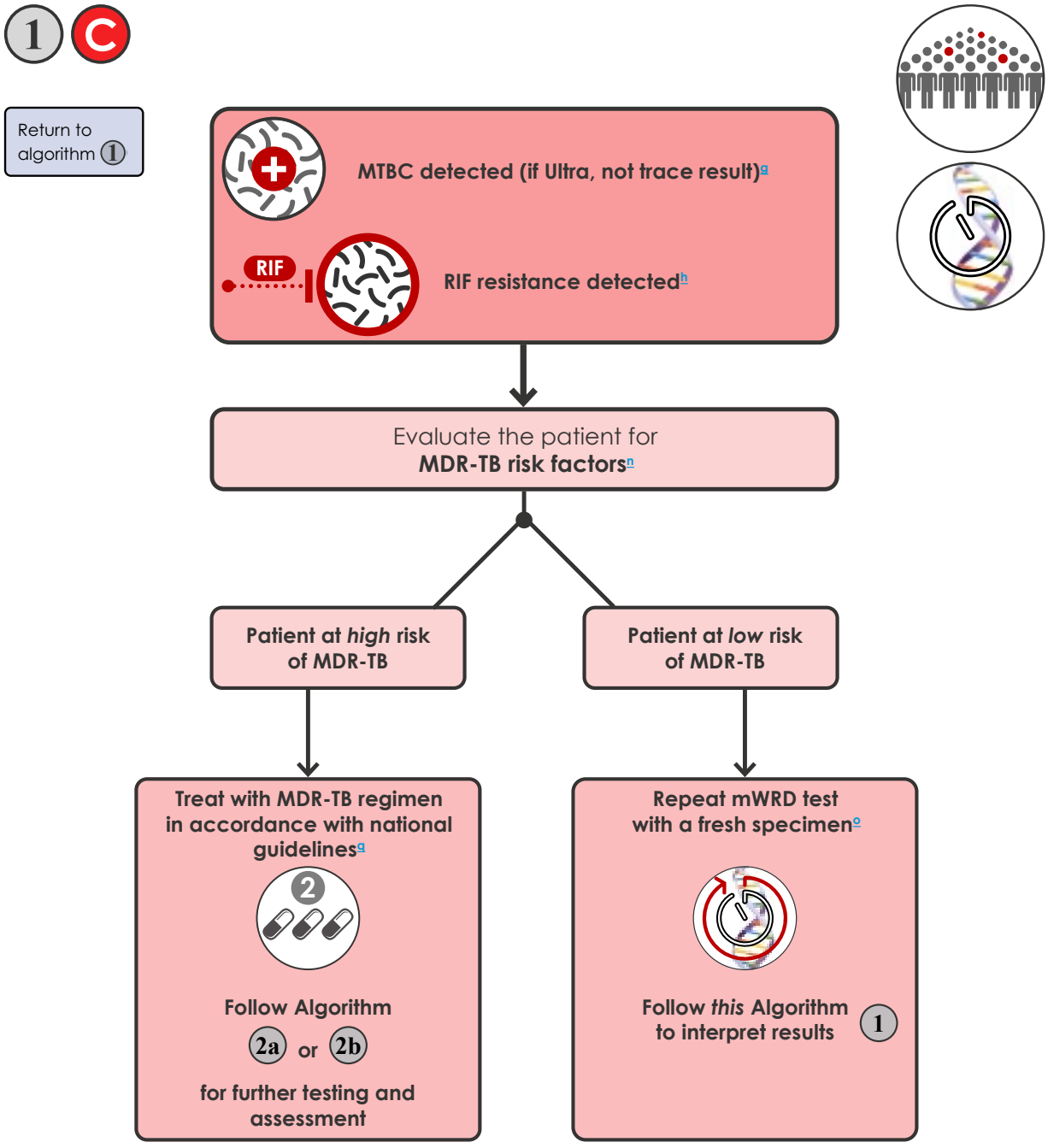
If the INH resistance is "not detected" by an MC-aNAAT and the person has a high risk of Hr-TB, phenotypic DST for INH should be performed because 6–14% of INH resistance can be missed by MC-aNAATs.

ii. Molecular or phenotypic DST for RIF resistance may be requested if the person is at risk of having RR-TB despite the initial mWRD result showing "resistance not detected". Sometimes, these anomalous results may be due to sample handling errors and a repeat test may resolve the issue. False RIF-susceptible Ultra results can occur but are uncommon (1–5% of RIF-resistant TB cases tested) and the level of such results depends on the epidemiological settings. In contrast, phenotypic DST for RIF, especially using liquid culture, is associated with a higher proportion of false susceptible results *(83)*. The updated critical concentration for RIF should be used to reduce, but not eliminate, this issue. Sequencing should be performed when available, and should cover codons 170–491 of the *rpoB* gene (including those outside of the rifampicin resistance determining region, or RRDR).

e. If additional molecular or phenotypic testing is performed:
   i. Testing done in different laboratories should be done in parallel – it is important to not wait for the results of one test before initiating another test.
   ii. Molecular and phenotypic DST may be performed using the specimen (direct DST) or using a culture isolate (indirect DST). Direct DST is preferred for molecular testing, whereas indirect DST may be preferred for phenotypic DST because of technical issues related to producing an appropriate inoculum and loss to contamination.
   iii. An mWRD is preferred. Mutation interpretation can also be found in the WHO catalogue of mutations *(23)*. Targeted NGS is an accurate method for DST that can produce results faster than culture-based DST. Targeted NGS shows a sensitivity of almost 90% or above for detection of resistance to INH, LFX, MXF, PZA, AMK, ETB and STR, and about 70% for BDQ, LZD and CFZ for RIF-resistant samples.

3. Culture-based phenotypic DST for INH and RIF often require 3–8 weeks to produce results, but may be useful for evaluating people with a susceptible molecular result, particularly in populations with a high pretest probability for resistance to INH. If the mWRD result is "MTBC detected, RIF resistance not detected and INH resistance detected" (currently only applicable to the MC-aNAAT):
   a. The person should be initiated on an appropriate Hr-TB regimen in accordance with national guidelines. The WHO recommendation for people with Hr-TB is treatment with RIF, EMB, PZA and LFX for a duration of 6 months *(7)*.
   b. For individuals with Hr-TB, Algorithm 3 should be followed:
      i. Additional DST for RIF may be required in settings where RIF-resistant mutations outside the RRDR are common. The decision on the choice between phenotypic testing or sequencing will depend on the availability of sequencing and the type of mutation expected. In places where the *rpoB* I491F mutation is common, sequencing is

preferred because phenotypic DST, even with the lower CC, will still miss many resistant infections; in other cases (e.g. V170F) phenotypic testing is appropriate *(84)*.



MDR-TB: multidrug-resistant TB; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostic test; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

Drug: RIF: rifampicin.

For figure notes, please see page 112.

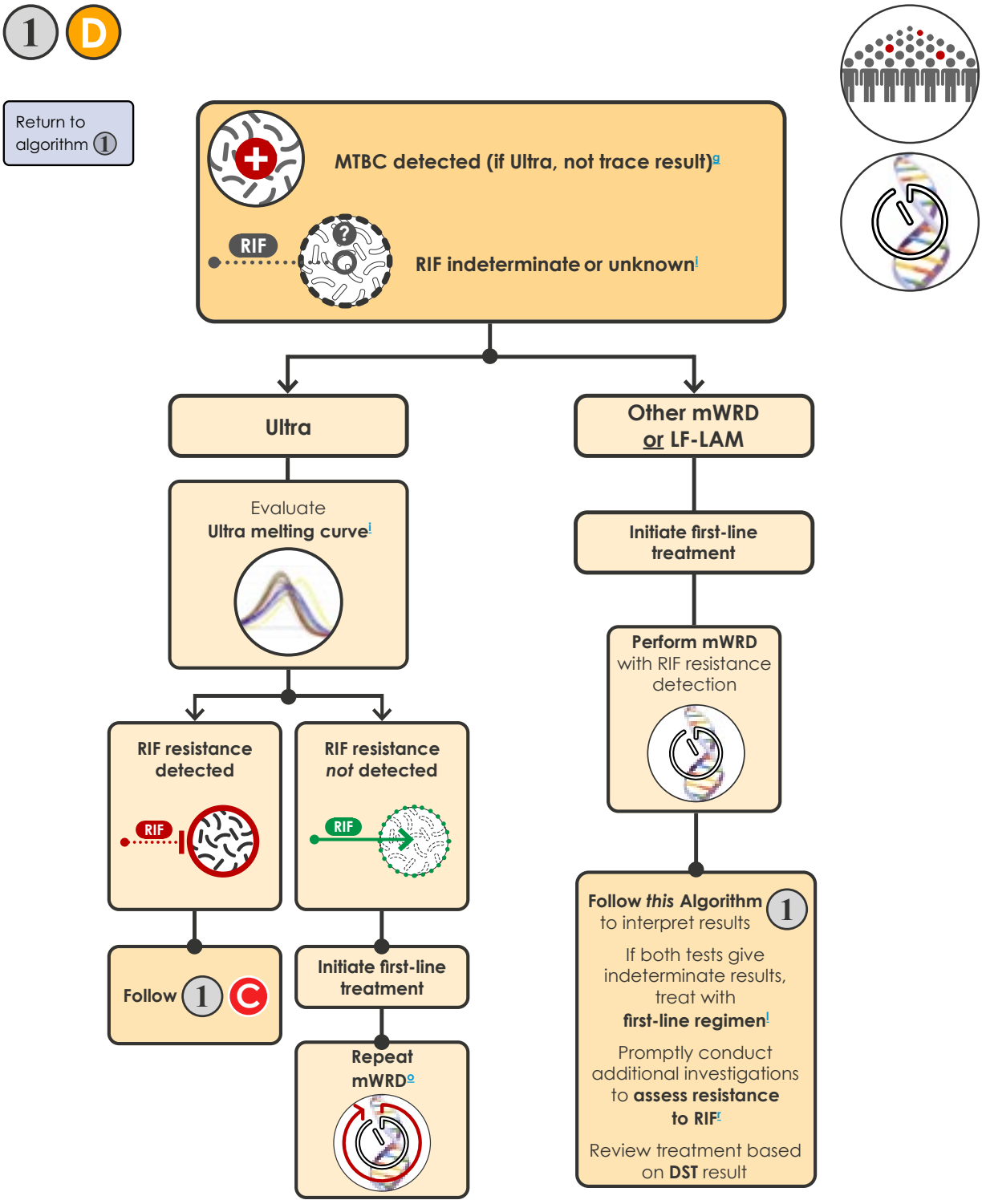
4. If the mWRD result is "MTBC detected, RIF resistance detected" 1 C, an MDR-TB risk assessment is needed, irrespective of the INH result. People at high risk for MDR-TB include people who have been previously treated (e.g. those who had been lost to follow-up, relapsed or experienced a failed treatment regimen); non-converters (e.g. people who were smear positive at that end of the intensive phase of treatment for drug-susceptible TB);

contacts of people with MDR-TB; and any other groups at risk for MDR-TB identified in the country. In high MDR-TB (1)(C) burden countries, every person with TB is considered to be at high risk of having MDR-TB.

a. For the concurrent testing strategy, applies when at least one LC-aNAAT is "MTBC detected, RIF resistance detected". The LF-LAM results need not be taken into consideration in this case.
b. If the person is at high risk of having MDR-TB, they should be initiated on a regimen for MDR/RR-TB in accordance with national guidelines, and Algorithm 2a or 2b should be followed for additional testing.
c. If the person is not at high risk of having MDR-TB, the test should be repeated using an mWRD with a second sample. To aid interpretation, the initial instrument output for the result can be reviewed when available. Probe binding delay and samples that have low bacillary loads (e.g. Xpert Ultra semiquantitative low and very low categories) have been associated with increased false resistance in some settings *(85–87)*.
    i. If the second test also indicates RIF resistance, an MDR/RR-TB regimen should be initiated in accordance with national guidelines and WHO recommendations, and Algorithm 2a or 2b should be followed for additional testing.
    ii. If the mWRD result for the second sample is "MTBC detected, RIF resistance not detected", treatment should be initiated with a first-line regimen in accordance with national guidelines. In most situations, false positive RIF-resistant results due to technical performance of the assay are rare; however, such results may occur because of laboratory or clerical errors. It is assumed that the repeat test will be performed with more caution, that the result of the second test will be correct, and that the result of the first test may have been due to a laboratory or clerical error. Mixed infections in high-burden settings could also explain such discordance; therefore, people should be closely followed up and tested again if the response to first-line treatment is poor. If an INH resistance or susceptibility result is available, the result should be interpreted and followed up as described in Algorithm 3.
    iii. If the mWRD result for the second sample is "MTBC detected, RIF resistance indeterminate", the person will require further investigation. A possible mixed infection may explain such a scenario. History of prior treatment and TB contact history should be reassessed. The decision to manage a person as having Hr-TB or MDR/RR-TB will need to be based on further investigation that includes phenotypic DST to RIF and INH and, where available, DNA sequencing. A third mWRD should be performed to decide on the initial therapy; the person should be closely followed up while awaiting the final definitive results, and the appropriate algorithm should be followed.
    iv. If an MC-aNAAT was performed and INH results are also available, this could be useful to provide certainty. The finding of INH resistance should prompt further investigation to exclude RIF resistance.
d. For all people with MDR/RR-TB, additional investigations should be conducted to assess resistance to the drugs being used in the treatment regimen. Rapid detection of FQ resistance is essential in determining the regimen to be selected. The recent addition of an LC-aNAAT for the detection of FQ resistance provides a rapid and accurate peripheral-level solution that can be performed directly on specimens. The sample preparation for the Xpert MTB/XDR test is identical to the Xpert MTB/RIF Ultra test. If more than 2 mL

of that reaction mix (the minimum volume to add to the Xpert cartridge) used for the Xpert Ultra test remains, this can be used directly for the Xpert MTB/XDR test if the mix has been stored at 2–8 ˚C for less than 4 hours.

e. Phenotypic methods (culture and DST) and molecular methods (e.g. LC-aNAAT, SL-LPA and targeted NGS) are available to evaluate drug resistance beyond RIF and INH. Rapid molecular methods are preferred. However, for resistance detection to some of the new and repurposed drugs, targeted NGS should be used where available, otherwise phenotypic DST should be performed if it is the only available option; thus, two separate specimens may be required.
    i. MDR/RR-TB regimens rely on the use of BDQ with or without an FQ (a sample should be submitted for targeted NGS or molecular testing for FQ resistance plus phenotypic DST – see Algorithm 2a or 2b).
    ii. Ideally, a specimen from each person should be submitted for DST for each of the drugs used in the regimen for which there is a reliable testing method. However, treatment initiation should not be delayed while waiting for DST results (e.g. phenotypic DST can take weeks or even months to provide results).
    iii. Any positive culture recovered during treatment monitoring that is suggestive of treatment failure should undergo DST for the drugs used in the treatment regimen.

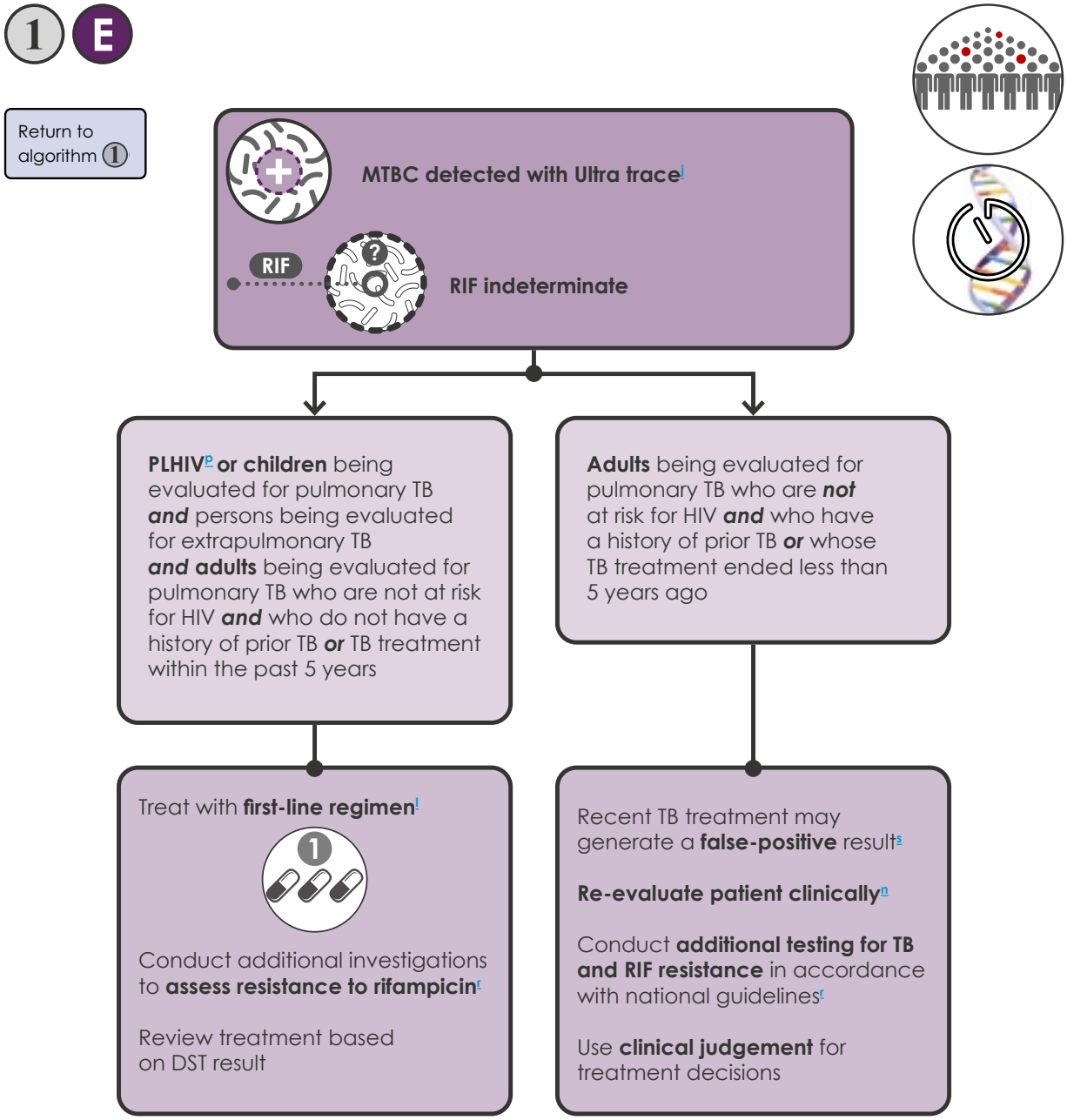


DST: drug susceptibility testing; LF-LAM: lateral flow urine lipoarabinomannan assay; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostic test; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

Drug: RIF: rifampicin.

For figure notes, please see page 112.

5. If the mWRD gives a result of "MTBC detected, RIF indeterminate" (1)(D), the person will require further investigation. This also applies to concurrent testing with LC-aNAAT and LF-LAM (for people living with HIV) when only the LF-LAM is positive. The interpretation and follow-up testing for Xpert Ultra differs from that for other mWRDs. With any of the mWRDs and for LF-LAM, the initial result of "MTBC detected" (or "Positive" for LF-LAM) should be considered bacteriological confirmation of TB. The person should be initiated on an appropriate regimen using first-line TB drugs in accordance with national guidelines. If the person is at high risk of having MDR-TB, the next step is either to initiate MDR-TB treatment based on local guidelines or to initiate first-line treatment and refer to the local MDR treatment committee for a final decision. In most settings, for the purpose of making treatment decisions, a history of prior TB treatment is not sufficient to indicate that the person is at high risk of having MDR-TB.
    a. For most mWRDs, an "MTBC detected, RIF resistance indeterminate" result is generally caused by a paucibacillary TB load in the sample; in such cases, retesting a fresh specimen is recommended.
        i. If the result of the second mWRD is "MTBC detected, RIF resistance not detected", Step 3 should be followed. If the result is "MTBC detected, RIF resistance detected", Step 5 should be followed.
        ii. In some cases, testing a second sample, which might also contain very few bacteria, may generate a result of "MTBC detected, RIF indeterminate" or "MTB not detected". In these situations, additional investigations such as culture and phenotypic DST or molecular testing of the isolate or sequencing may be needed to confirm or exclude resistance to RIF, because the indeterminate result provides no information on resistance. "MTBC detected (not trace), RIF indeterminate" results obtained with the Xpert Ultra test (especially those with high or medium semiquantitative results) may be due to the presence of large deletions or multiple mutations in the RRDR, or to mutations that pose a challenge with mutation analysis software *(88)*.
        iii. The Xpert Ultra melt curves from "MTBC detected (non-trace), RIF indeterminate" samples should be reviewed (preferably by an advanced Xpert user or supervisor), including a review of the amplification of the target sequences and melt curve profile *(88)*.
            - Melt curves that suggest the presence of a large deletion or multiple mutations in the RRDR should be interpreted as "RIF resistance detected". In such cases, Steps 4.b and 4.c should be followed.
            - If the melt curve is not consistent with the presence of a large deletion or multiple mutations in the RRDR, the result is interpreted as "indeterminate". In such cases, Step 5a.ii should be followed for additional testing.
            - If the semiquantitative result is high or medium, FL-LPA or DNA sequencing may be useful.
    b. Culture and phenotypic DST, FL-LPA or DNA sequencing may be performed for follow-up testing, to confirm or exclude RIF resistance.



DST: drug susceptibility testing; HIV: human immunodeficiency virus; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostic test; PLHIV: people living with HIV; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

Drug: RIF: rifampicin.

For figure notes, please see page 112.

6. If the Xpert Ultra test result is "MTBC detected trace" 1E, additional considerations are needed. If concurrent testing with LF-LAM is conducted and the LF-LAM test provides a positive result (i.e. for people living with HIV, children and children living with HIV), an Xpert Ultra "MTBC detected trace" result is considered positive and the person should be initiated on first-line treatment according to WHO and national guidelines.
7. WHO suggests not repeating Xpert Ultra testing in adults who have an initial Xpert Ultra trace result to confirm the result, but instead doing the following.

a. The person's clinical characteristics should be reviewed to determine their age, HIV infection status and history of TB treatment, and determine whether the samples are pulmonary or extrapulmonary.
   i. People may be considered HIV positive if they have tested positive for HIV infection, or have an unknown HIV status but 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 in accordance with national guidelines.
   ii. Individuals with a history of recent TB treatment include those who successfully completed a course of therapy within the past 5 years. The likelihood of a false positive mWRD result is highest immediately after completing treatment, and slowly declines with time *(89, 90)*. Those who initiated but did not complete therapy and those who failed therapy should be considered as being at high risk of having MDR-TB; careful clinical evaluation is required in such cases.
   iii. A trace positive result in an extrapulmonary sample is considered a true positive and should be treated with first-line treatment.
   iv. Health workers must endeavour to obtain a reliable history of TB treatment, recognizing that some people may not communicate their treatment history because of stigma or, in the case of migrants, concern over legal status.

b. For certain populations (e.g. people living with HIV and children who are being evaluated for pulmonary TB; individuals being evaluated for extrapulmonary TB using CSF, lymph nodes and tissue specimens; and adults being evaluated for pulmonary TB, who are not at risk for HIV and who do not have a history of TB treatment in the past 5 years) the following steps should be taken:
   i. The MTBC detected trace result obtained with the first specimen should be considered as bacteriological confirmation of TB (i.e. a true positive result) and used for clinical decisions.
   ii. The person should be initiated on an appropriate regimen using first-line TB drugs, in accordance with national guidelines, unless the person is at high risk of having MDR-TB (in which case, the person should be initiated on an MDR-TB regimen).
   iii. Additional investigations (e.g. culture and DST) should be undertaken to confirm or exclude resistance to RIF.

c. For adults who are being evaluated for pulmonary TB, who are not at risk of HIV and have a history of TB treatment in the past 5 years, the following steps should be taken:
   i. For adults with a history of recent TB treatment or unknown treatment history, the possibility of the Xpert Ultra trace result being a false positive result should be considered because of the possible presence of non-viable bacilli.
   ii. The person should be re-evaluated and additional testing conducted (including liquid culture) in accordance with national guidelines. The possibility of TB caused by reactivation, relapse or reinfection should be considered.
   iii. In initiating treatment, the clinical presentation and context of the person should be considered, and clinical decisions should be made based on all available information and clinical judgement.

iv. Further investigations for TB may include CXR, additional clinical assessments and clinical response following treatment with broad-spectrum antimicrobial agents (FQs should not be used).
    - Repeat Xpert Ultra testing is of uncertain benefit. A recent WHO GDG recommended against repeat Xpert Ultra testing for individuals with an initial Xpert Ultra trace result for the detection of MTBC.
    - Culture and phenotypic DST may be of benefit for detecting TB and drug resistance. The trace result provides no information on RIF resistance.
    - Further investigations for TB in children may consider chest x-ray and additional clinical assessments according to the WHO guidelines on management of TB in children (*56*).

8. If the mWRD does not give a result (1)(F), or gives a result of "error" or "invalid", the mWRD should be repeated at the same testing site with a second specimen.

Interpretation of discordant results

This algorithm relies on testing of a sample with an mWRD (with or without LF-LAM) to detect MTBC and assess susceptibility to RIF. Discordance in resistance to INH is described in Algorithm 3. On occasion, follow-up testing is recommended to ensure that clinical decisions are well informed. However, discordant results may occur, usually when comparing culture-based results with molecular results. Each discordant result will need to be investigated on a case-by-case basis. General considerations are outlined below.

1. For an mWRD result "MTBC detected other than trace", culture negative (see Point 5 for trace):
    a. The mWRD result and clinical judgement should be used to guide the treatment decision, pending additional testing.
    b. The mWRD result should be considered as bacteriological confirmation of TB if the sample was collected from a person who was not recently receiving treatment with anti-TB drugs. Cultures from individuals with pulmonary TB may be negative for several reasons, including that the person is being treated for TB (effective treatment rapidly renders MTBC non-viable), transport or processing problems have inactivated the tubercle bacilli, cultures have been lost to contamination, the testing volume was inadequate, or a laboratory or clerical error occurred.
    c. Follow-up actions may include re-evaluating the person for TB, reassessing the possibility of prior or current treatment with anti-TB drugs (including FQ use), evaluating response to therapy, and evaluating the possibility of laboratory or clerical error.
2. mWRD result "MTB not detected", culture positive:
    a. The treatment decision should be based on the culture result. If the person started treatment based on clinical judgement, treatment should be continued and the person recorded as having bacteriologically confirmed TB.
    b. The culture-positive result should be considered as bacteriological confirmation of TB because culture is the current gold standard for the laboratory confirmation of TB. Using a sputum specimen, WRDs have a pooled sensitivity of 83–90% for detecting pulmonary TB compared with culture *(91)*. Their sensitivity is lower in people living with HIV, children and other specimen types such as CSF.

c. False positive cultures can result from a variety of causes, such as cross-contamination in the laboratory (e.g. from inappropriate specimen processing) or sample labelling problems. In well-functioning laboratories, such errors are rare.
d. Follow-up actions may include re-evaluating the person for TB, conducting additional testing using mWRDs, culturing additional samples, and evaluating the possibility of laboratory or clerical error. If the person was initiated on anti-TB therapy based on clinical judgement, the response to therapy should be evaluated.

3. mWRD result "MTBC detected, RIF resistance detected"; RIF susceptible by phenotypic DST:
   a. The mWRD result should be used to guide treatment decisions pending additional testing.
   b. Borderline resistant mutations are known to generate this discordant result, particularly in the BACTEC MGIT system (i.e. a false susceptible phenotypic result). In people infected with strains carrying these mutations treatment with RIF-based first-line regimens often fails *(83)*.
   c. In some settings with a low prevalence of MDR-TB, silent mutations have been observed that generate a false resistant mWRD result, but these are rare.
   d. A review of the probe melting temperatures when available *(92)* or banding pattern on the FL-LPA can aid in determining or inferring the specific mutation (e.g. borderline resistant or silent).
   e. Follow-up actions may include DNA sequencing, confirmatory testing on another mWRD testing platform, phenotypic DST using solid media, and evaluation of the possibility of laboratory or clerical error.
4. mWRD result "MTBC detected, RIF resistance not detected"; RIF resistant by phenotypic DST:
   a. The treatment regimen should be modified based on the results of the phenotypic DST.
   b. False RIF-susceptible mWRD results are rare but have been observed in 1–5% of RIF-resistant TB cases tested with the Ultra test in various epidemiological settings. Mutations in the RRDR of the *rpoB* gene have been shown to account for 95–99% of RIF resistance. The remainder of RIF resistance arises from mutations outside RRDR, which produce an Xpert MTB/RIF result of "RIF resistance not detected". In settings with a prevalent clone that harbours a mutation outside the RRDR – for example, Eswatini *(93)* –this may be more common; however, this has not been identified as a major concern in other settings *(94)*. Surveillance to monitor emergence of such clones over time should be considered.
   c. Follow-up actions may include DNA sequencing, repeating the phenotypic DST, and evaluating the possibility of laboratory or clerical error.
5. Xpert Ultra "MTBC detected trace", culture negative – the interpretation of this result must consider the person's characteristics, the specimen type and whether the person had been previously treated for TB:
   - Cultures may be negative for several reasons, including the person being treated for TB or treated with FQs, transport or processing problems that inactivated the tubercle bacilli, culture contamination or inadequate testing volume, or laboratory or clerical error.
   - The small numbers of bacilli in a sample that generates an "MTBC detected trace" result may be due to active TB disease, laboratory cross-contamination, recent exposure to (or infection with) tubercle bacilli (i.e. incipient TB), and current or past treatment for TB.

- The FIND multicentre study revealed that many of the samples that generated results of "MTBC detected trace" and culture negative were from individuals who had completed therapy within the past 4–5 years, presumably because of the presence of small numbers of non-viable or non-replicating bacilli. Thus, "MTBC detected trace" results must be interpreted within the context of prior treatment.

a. For people living with HIV and children who are being evaluated for pulmonary TB, or when extrapulmonary specimens are tested, the benefits of the increased sensitivity for the detection of MTBC (i.e. true positives) outweigh the potential harm of decreased specificity (i.e. false positives).
   i. The "MTBC detected trace" result is considered as bacteriological confirmation of TB (i.e. true positive results) and such people should have been initiated on therapy based on the Xpert Ultra result. The possibility that the culture result was a false negative result should be considered.
   ii. Follow-up actions may include assessing the response to therapy (culture results are often not available for weeks after specimen collection), reassessing the possibility of prior or current treatment with anti-TB drugs (including FQ use), and evaluating the possibility of laboratory or clerical error.

b. For adults being evaluated for pulmonary TB who are not at risk of HIV, the balance of benefit and potential harm varies, based on whether the person had been treated previously for TB because of decreased specificity (i.e. false positives).
   i. For individuals in whom a history of current or prior TB treatment can be reliably excluded:
      1. Although the "MTBC detected trace" results should be considered as bacteriological confirmation of TB (i.e. true positive results), any clinical decision (e.g. to treat for TB) should be made based on all available laboratory, clinical and radiological information, and clinical judgement.
      2. The possibility that the culture result was a false negative result should be considered if the samples were collected from a person who was not receiving treatment with anti-TB drugs, because of the paucibacillary nature of the sample. Follow-up actions for people placed on anti-TB therapy may include re-evaluating the person for TB, assessing the response to therapy, reassessing the possibility of prior or current treatment with anti-TB drugs (including FQ use), repeating Xpert Ultra testing, evaluating the possibility of laboratory or clerical error, and repeating the culture (preferably using liquid culture).
   ii. For adults with a history of recent TB treatment:
      1. The possibility that the Xpert Ultra "MTBC detected trace" result was a false positive result should be considered because of the presence of non-viable bacilli. A culture-negative result is consistent with this possibility.
      2. If such adults had been initiated on anti-TB therapy based on clinical judgement, follow-up actions may include assessing the response to therapy, conducting additional testing in accordance with national guidelines, repeating culture (preferably using liquid culture), and evaluating the possibility of laboratory or clerical error.

6. mWRD positive, urine LF-LAM negative:
    a. An mWRD may give a different result than the urine LF-LAM because the tests have different sensitivities and measure different analytes.
    b. An mWRD test results of 'MTBC Detected' is considered as bacteriologically confirmation of TB. Treatment decisions may be determined following algorithm decision pathways 1B-1E.
7. Urine LF-LAM positive, mWRD negative:
    a. The LF-LAM test may give a different result than an mWRD or culture. This is not unexpected because the tests have different sensitivities and measure different analytes.
    b. A positive LF-LAM result is considered is considered as bacteriological confirmation of TB. Treatment decisions should rely on clinical judgement and all available information, including (but not limited to) chest x-ray findings (if available) and any other available bacteriological results.
    c. When LF-LAM results are consistently positive, without positive LC-aNAAT results, investigation of the quality of testing and local epidemiology of non-tuberculosis mycobacteria and extrapulmonary TB in the tested population is warranted to understand the difference.

6.4 Algorithm 2 – DST for second-line drugs for people with MDR/RR-TB

Algorithm 2a and 2b are used for further evaluation of people with MDR/RR-TB. In its most recent recommendations *(7)*, WHO strongly encourages DST in people with MDR/RR-TB, although that should not delay treatment initiation. DST should be performed as soon as possible and even if results are not available at the start of the selected regimen (preferably BPaLM or BDLLfxC), the results should be used to adjust it. Two of the key medicines in these regimens are BDQ and FQ. Two WHO-recommended molecular tests to detect mutations associated with BDQ resistance belong to the class of targeted NGS (Deeplex® Myc-TB from GenoScreen and AmPORE-TB from Oxford Nanopore Diagnostics). Algorithm 2a relies on testing using the targeted NGS test to detect mutations associated with resistance to BDQ, FQ, LZD and other medicines used in the recommended regimens. Because of the limited availability of targeted NGS tests at this time, a second algorithm (Algorithm 2b) is included that relies on the detection of mutations associated with FQ resistance using mWRDs (LC-aNAAT and SL-LPA) and phenotypic DST for BDQ and other drugs. WHO stresses the need to scale up laboratory phenotypic DST capacity for medicines for which there are accurate and reproducible phenotypic methods, including BDQ, LZD, Pa, CS, CFZ and DLM.

6.4.1 Decision pathway for Algorithm 2 – DST for second-line drugs for people with MDR/RR-TB

General considerations

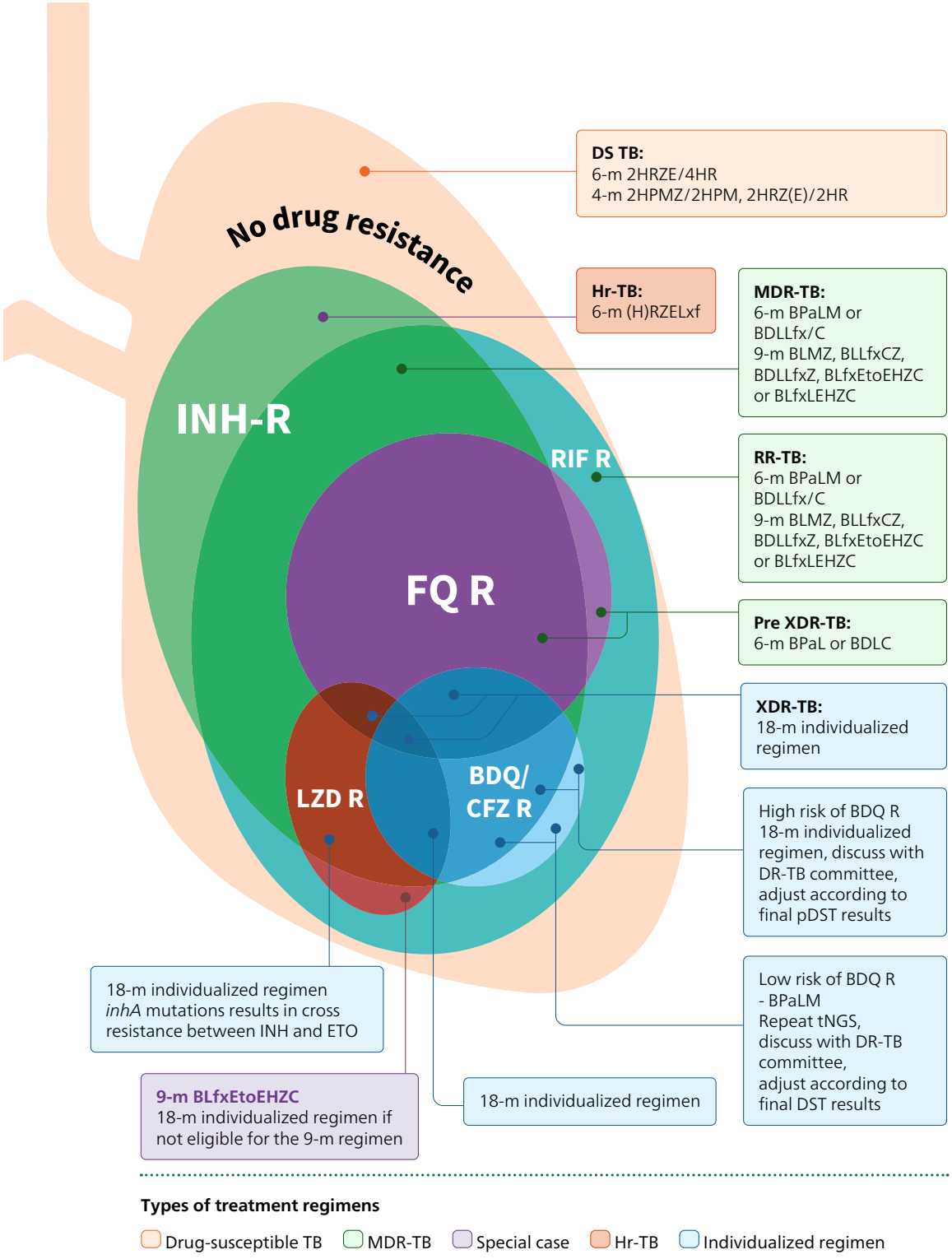
Seven BDQ-containing 6- or 9-month regimens are recommended for the treatment of MDR/RR-TB *(7, 95)*:

- Two all-oral 6-month regimens: BPaLM (for individuals aged 14 years and older) composed of BDQ, Pa and LZD, with MFX, which is dropped in case FQ resistance is detected; and BDLLfxC composed of BDQ, DLM, LZD, LFX and CFZ, for which LFX is dropped if FQ resistance is detected, CFZ is dropped if FQ susceptibility is confirmed, and both are retained if FQ status cannot be determined. This latter regimen is suitable for use in children and pregnant and lactating women because Pa is not currently recommended for use in these groups.
- Five all-oral regimens of 9 months comprising different combinations of BDQ, LFX or MFX, LZD, CFZ, DLM and PZA (BLfxEtoEHZC, BLfxLEHZC, BLMZ, BLLfxCZ and BDLLfxZ).

Individualized all-oral longer regimens, designed using the WHO priority grouping of medicines, may still be used for people with MDR/RR-TB and FQ resistance who do not meet the eligibility criteria for the 6-month and 9-month regimens.

Fig. 6.3 provides an overview of the treatment regimens recommended by WHO. Several factors matter when a regimen is selected, as detailed in WHO guidelines *(7, 95)*. Common to all MDR/RR-TB regimens is that BDQ is the central drug. An individual with a confirmed resistance to BDQ should be switched to an individualized regimen and drugs should be selected based on targeted NGS results and adjusted based on phenotypic DST results.

Fig. 6.3. Treatment option overview

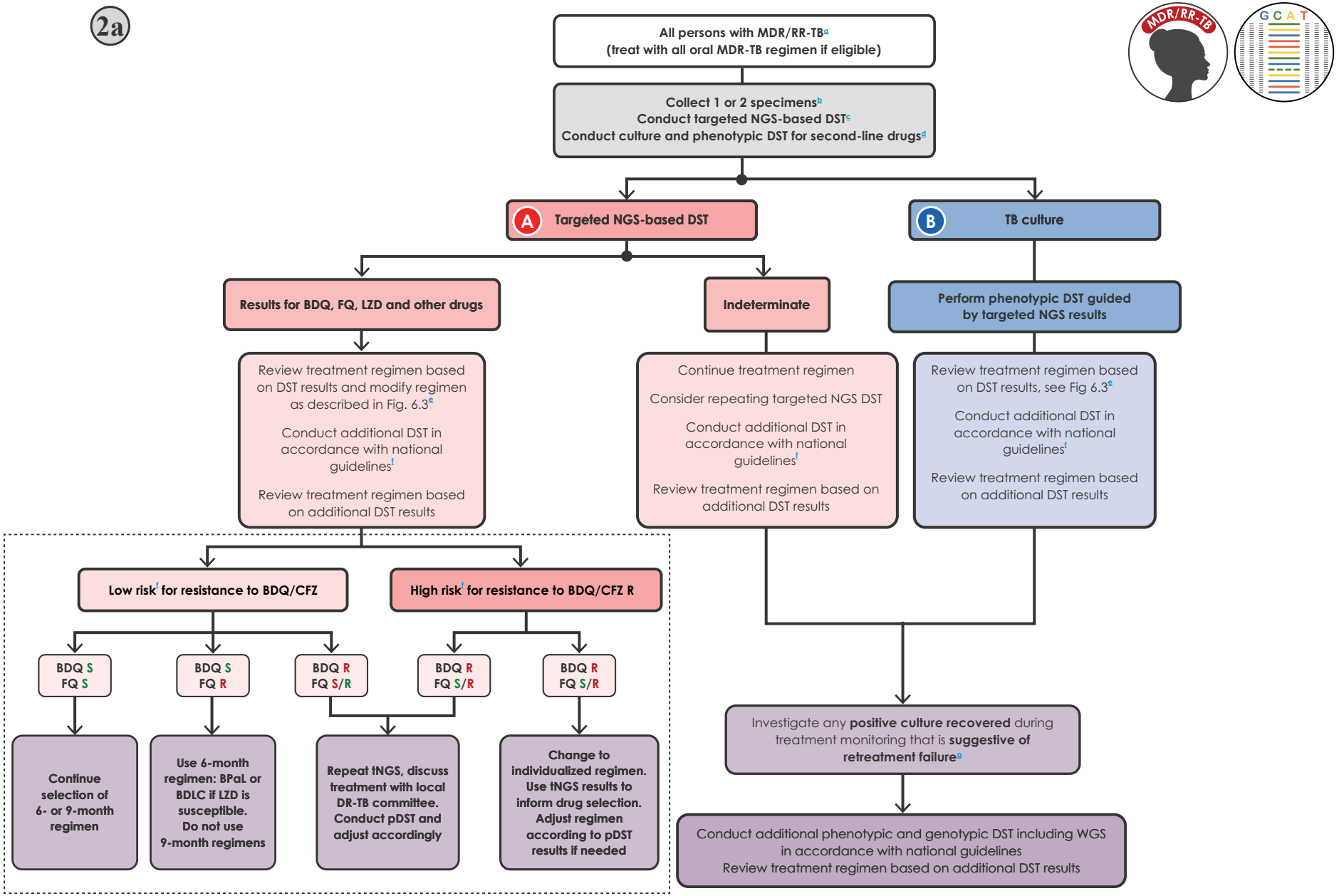


DR-TB: drug-resistant TB; DS-TB: drug-susceptible TB; DST: drug susceptibility testing; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; MDR-TB: multidrug-resistant TB; pDST: phenotypic DST; R: resistant; RR-TB: rifampicin-resistant TB; TB: tuberculosis; tNGS: targeted next-generation sequencing.

Drugs and regimens: BDLC: bedaquiline (B), delamanid (D), linezolid (L) and clofazimine (C); BDQ: bedaquiline; BPaL: bedaquiline (B), pretomanid (Pa) and linezolid (L); CFZ: clofazimine; ETO: ethionamide; FQ: fluoroquinolone; HPM: isoniazid (H), rifapentine (P) and moxifloxacin (M); HPMZ: isoniazid (H), rifapentine (P), moxifloxacin (M) and pyrazinamide (Z); HR: isoniazid (H) and rifampicin (R); HRZE: isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E); INH: isoniazid; Lfx: levofloxacin; LZD: linezolid.

- WHO guidelines stress the importance of DST, especially for medicines for which molecular tests are available; however, it is important that the initiation of treatment is not delayed while waiting for DST results. The selected regimen should be adjusted if needed, based on DST results that may become available after initiation of treatment.
  - WHO recommends molecular tests for the detection of mutations associated with resistance to FQs (LC-aNAAT, SL-LPA and targeted NGS tests) and mutations associated with BDQ resistance (targeted NGS tests). Targeted NGS tests can also detect mutations associated with resistance to some of the other drugs included in MDR-TB regimens (e.g. LZD, CFZ and PZA).
  - WHO recommends a molecular test for PZA resistance detection belonging to the class "HC-rNAAT". Its use is limited to culture isolates. Alternatively, *pncA* sequencing should be performed when available.
- In settings where targeted NGS is not available, phenotypic DST should be used.
  - In a quality-assured laboratory, with careful attention to inoculum preparation, a susceptible phenotypic DST result using MGIT for PZA can be used to guide the inclusion of PZA in a DR-TB treatment regimen (**Web Annex C**).
  - Reliable phenotypic DST methods are available for RIF, INH, FQs, BDQ, CFZ, Pa, CS, LZD, AMK and DLM. Testing algorithms that rely on culture and phenotypic DST are described in the relevant WHO policy framework *(96)* and technical manual (**Web Annex C**). Member States should ensure that there is capacity for DST for drugs used for treatment and for which reliable testing is available.
  - No reliable phenotypic DST methods are available for EMB, ETO/prothionamide, or imipenem-cilastatin/MPM; hence, results should not be used for clinical decision-making.
  - If phenotypic DST to second-line drugs is not available in-country, specimens or isolates may be shipped to an external laboratory for testing (e.g. a WHO supranational reference laboratory [SRL]). Material transfer agreements and import or export permits may be needed.
  - Currently, the availability of phenotypic DST for BDQ and LZD is limited in many settings, and resistance levels are likely to be low. There is, however, increasing evidence that BDQ resistance occurs even in unexposed people at a level of 1.4–3.4% *(97)*. BDQ is a core drug for DR-TB treatment and is included in the revised definition of XDR-TB. Thus, it is essential to build testing capacity to test this and other drugs used in treatment (e.g. LZD, Pa, CS, CFZ and DLM). If resistance is suspected during treatment and DST is not available, the strains should be referred to a TB SRL for further testing.

Fig. 6.4. Algorithm 2a: DST for MDR/RR-TB using targeted NGS



DR-TB: drug-resistant TB; DST: drug susceptibility testing; MDR/RR-TB: multidrug- or rifampicin-resistant TB; MDR-TB: multidrug-resistant TB; NGS: next-generation sequencing; pDST: phenotypic DST; RR-TB: rifampicin-resistant TB; SRL: supranational reference laboratory; TB: tuberculosis; tNGS: targeted next-generation sequencing; WGS: whole-genome sequencing; WHO: World Health Organization.

Drugs and regimens: BDQ: bedaquiline; BPaL/M: bedaquiline (B), pretomanid (Pa) and linezolid (L), and moxifloxacin (M); CFZ: clofazimine; CS: cycloserine; DLM: delamanid; EMB: ethambutol; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; Pa: pretonamid; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.

[a] Patients should be promptly initiated on an MDR/RR-TB regimen in accordance with national guidelines and WHO recommendations. A shorter all-oral bedaquiline-containing treatment regimen (BPaL/M or BDLLfxC) is the preferred option for eligible people with MDR/RR-TB.

[b] 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.

[c] WHO recommends performing DST using rapid molecular tests among all people diagnosed with TB, although this testing should not delay the start of treatment. Currently, targeted NGS tests can provide results for BDQ, FQ, LZD, INH, PZA, EMB, CFZ, AMK, STR and RIF.

[d] 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 are available for RIF, INH, FQs, PZA, BDQ, CFZ, Pa, CS, LZD, AMK and DLM. The initiation of treatment should not be delayed while awaiting the results of the phenotypic DST.

[e] More details on individualized regimens can be found in the *WHO consolidated guidelines on tuberculosis: module 4: treatment (7)*.

[f] Low risk for BDQ/CFZ resistance is when there is no prior BDQ/CFZ exposure, and the prevalence of resistance to BDQ/CFZ is less than 5% in the population (people with RR-TB on national or subnational level where reliable estimates are available), and there is no history of contact with a person known to have BDQ/CFZ-resistant TB. If any of these conditions are not met the risk is considered high.

[g] If resistance to an individual drug (e.g. BDQ) is suspected and DST for this drug is not available in the country, laboratories will need to have mechanisms to store the isolate and ship it to a WHO SRL for DST.

Decision pathway for Algorithm 2a – Testing for BDQ and FQ resistance (Fig. 6.4)

1. The person should be promptly initiated on an MDR-TB regimen in accordance with national guidelines. The most recent WHO recommendations include two different all-oral 6-month BDQ-containing regimens *(7, 95)*. In addition, five all-oral 9-month regimens are recommended by WHO and can be used if neither of the 6-month regimens are suitable *(7, 95)*.

2. If molecular and phenotypic testing are performed in the same laboratory, collecting 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. Sputum specimens or isolates should be transported to the appropriate testing laboratory, if necessary.

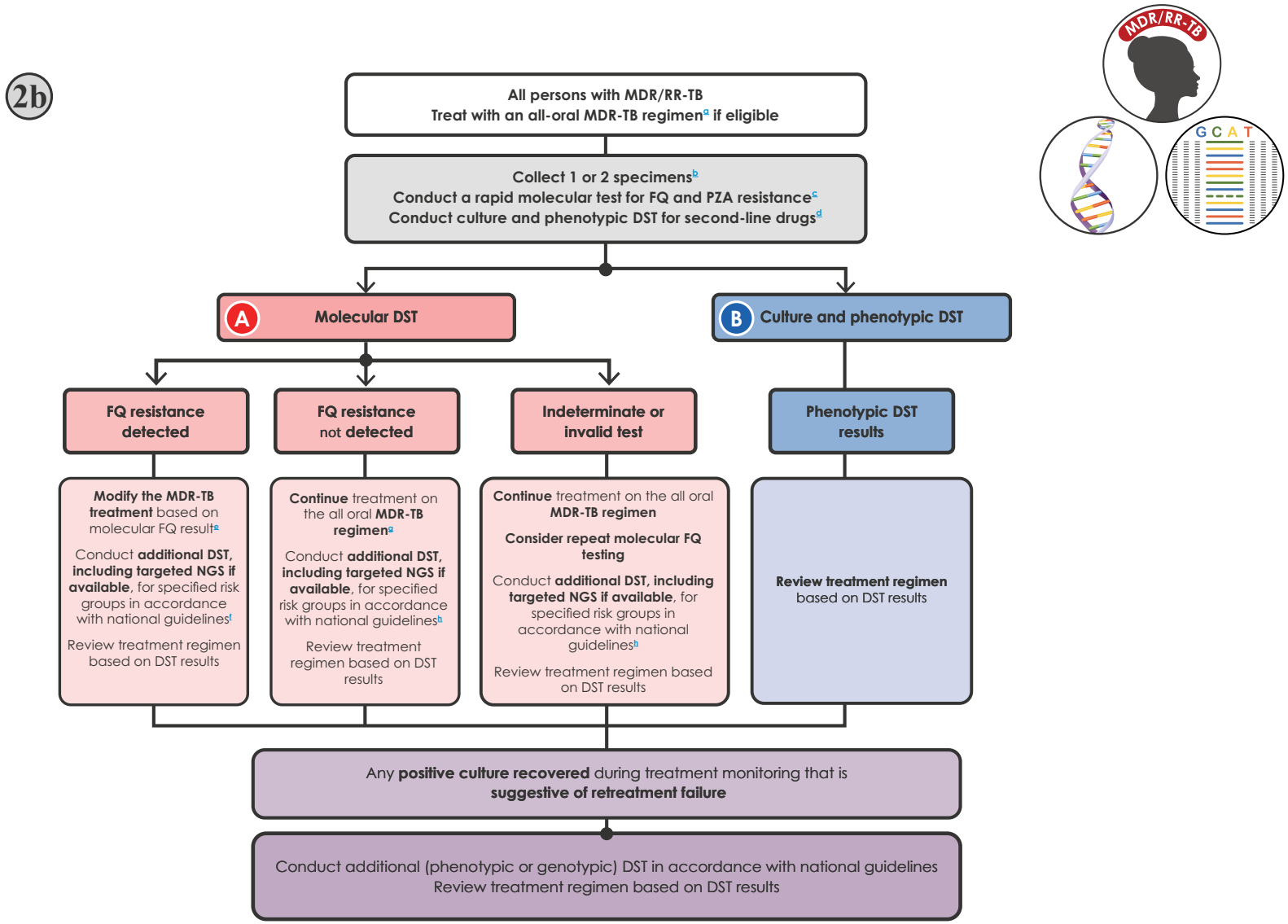
3. Targeted NGS testing should be conducted to detect mutations associated with resistance to BDQ and other medicines, and culture and DST should be undertaken for drugs not included in the targeted NGS solution used, in parallel 2a A.

4. If the targeted NGS test result is indeterminate, the test can be repeated with a fresh sample in cases where the bacterial load is expected to give a definitive result (smear positive or high or medium grade on mWRD); treatment decisions should be based on clinical assessment, the epidemiological situation and the results of phenotypic DST. The results of the targeted NGS test should be used to modify treatment if appropriate, and to select the drugs included in the regimen requiring phenotypic DST when the culture is positive 2a B. Pa and DLM are not covered by any targeted NGS solutions and require phenotypic DST.

5. When selecting or designing the treatment regimen, the results of the DST for all drugs should be taken into consideration (**Fig. 6.3**). Further details on regimens are given in Module 4 of the WHO consolidated guidelines *(7)*.

6. BDQ is a core drug of all 6-month and 9-month MDR/RR-TB regimens, and the interpretation of the targeted NGS results has important implications for treatment choices. It is necessary to evaluate the pretest probability of BDQ resistance before making clinical decisions, because of the suboptimal performance of the targeted NGS test for detecting BDQ resistance (sensitivity: 67.9%, 95% CI: 42.6–93.2%; specificity: 97.0%, 95% CI: 94.3–99.7%). A low risk for BDQ/CFZ resistance is when there is no prior BDQ/CFZ exposure, AND the prevalence of resistance to BDQ/CFZ is less than 5% in the population, AND there is no history of contact with a person known to have BDQ/CFZ-resistant TB. If any of these conditions are NOT met the risk is considered high.
    a. If the risk of BDQ resistance is low and the targeted NGS test does not detect mutations associated with resistance to BDQ, the negative predictive value will be high, and the result is likely to be true. In cases of FQ resistance, one of two 6-month regimens should be used but the FQ should be dropped (i.e. BPaL or BDLC [BDQ, DLM, LZD and CFZ] should be used, assuming that tests show susceptibility to LZD). The 9-month regimens recommended by WHO should not be used. In cases of susceptibility to FQ, the selected 6-month or 9-month regimen should be continued, or one of the other 6-month or 9-month regimens should be adapted based on the full targeted NGS profile. The decision to perform phenotypic DST for BDQ for this group will be context dependent, taking into account the prevalence of resistance, DST capacity and the expected number missed by targeted NGS. Ideally, where resources are available, phenotypic DST should be done for all samples. The regimen should be modified as appropriate if resistance to other medicines in the regimen is detected.
    b. In situations with low pretest probability of BDQ resistance and where the targeted NGS test detects one or more mutations associated with resistance to BDQ, the targeted NGS should be repeated, the treatment decision discussed with the local DR-TB committee, phenotypic DST performed and adjustments made accordingly. Note: *Rv0678* mutations detected and associated with BDQ resistance usually result in cross-resistant to CFZ because they share a common mechanism; furthermore, the accuracy of targeted NGS for CFZ is similar to that for BDQ.
    c. In a situation with a high pretest probability of BDQ/CFZ resistance, where the targeted NGS test does not detect mutations associated with resistance to BDQ, the targeted NGS should be repeated and treatment discussed with the local DR-TB committee based on risk factors, to make a decision on treatment while awaiting phenotypic DST results. The number of true susceptible cases will be much higher than the number of false susceptible cases.
    d. If the risk of BDQ resistance is high and the targeted NGS test detects one or more mutations associated with resistance to BDQ, the person should be given an individualized regimen that is based on targeted NGS and adjusted according to phenotypic DST results. As noted above, *Rv0678* mutations detected and associated with BDQ resistance usually result in cross-resistant to CFZ because they share a common mechanism; furthermore, the accuracy of targeted NGS for CFZ is similar to that for BDQ.
    e. People should be closely monitored, and additional DST performed on any culture isolated at month 2 or later during treatment.

Fig. 6.5. Algorithm 2b: DST for people with MDR/RR-TB (limited or no targeted NGS capacity)



DR-TB: drug-resistant TB; DST: drug susceptibility testing; MDR/RR-TB: multidrug- or rifampicin-resistant TB; MDR-TB: multidrug-resistant TB; mWRD: molecular WHO-recommended rapid diagnostic test; NGS: next-generation sequencing; SL-LPA: line probe assay for second-line drugs; SRL: supranational reference laboratory; TB: tuberculosis; WHO: World Health Organization.

Drugs and regimens:

AMK: amikacin; BDLC: bedaquiline (B), delamanid (D), linezolid (L) and clofazimine (C); BDQ: bedaquiline; BDLLfx/C: bedaquiline (B), delamanid (D), linezolid (L), levofloxacin (Lfx) and clofazimine (C); BPaL: bedaquiline (B), pretomanid (Pa) and linezolid (L); BPaLM: bedaquiline (B), pretomanid (Pa), linezolid (L) and moxifloxacin (M); CFZ: clofazimine; CS: cycloserine; DLM: delamanid; EMB: ethambutol; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; Pa: pretonamid; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.

[a] People suspected of having TB should be promptly initiated on an MDR-TB regimen in accordance with national guidelines and WHO recommendations. An all-oral BDQ-containing treatment regimen (BPaLM or BDLLfx/C) is the preferred option for eligible people with MDR/RR-TB.

[b] 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.

[c] WHO recommends getting the rapid DST results before the start of treatment, although this testing should not delay the start of treatment. The mWRDs for detecting FQ resistance include Xpert MTB/XDR and SL-LPAs and the Genoscholar PZA-TB II test for detecting PZA resistance. Also, targeted NGS tests can provide results for BDQ, FQ, LZD, INH, PZA, EMB, CFZ, AMK, STR and RIF.

[d] 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 are available for RIF, INH, FQs, PZA, BDQ, CFZ, Pa, CS, LZD, AMK and DLM. The initiation of treatment should not be delayed while awaiting the results of the phenotypic DST.

[e] For more information regarding modifications of the treatment regimen, see the *WHO consolidated guidelines on tuberculosis: module 4: treatment (7)*. BPaL or BDLC may be used for people with FQ-resistant MDR-TB *(7)*. See **Fig. 6.3**.

[f] For FQ-resistant MDR/RR-TB, a specimen should be collected and submitted for phenotypic DST to the WHO Group A (BDQ, Pa and LZD) and B drugs, if not already being done as described in Note 4 below. If targeted NGS tests are available, a sample should be submitted for testing for resistance to additional medicines for specified risk groups in accordance with national guidelines.

[g] In settings with a high underlying prevalence of resistance to FQs or for people considered at high risk of FQ resistance, a specimen should be referred for culture and phenotypic DST for FQs.

[h] If resistance to an individual drug (e.g. BDQ) is suspected and DST for these drugs is not available in the country, laboratories should establish mechanisms to store the isolate and ship it to a WHO SRL for DST.

Decision pathway for Algorithm 2b – Testing for FQ resistance (Fig. 6.5)

1. The person should be promptly initiated on an MDR-TB regimen in accordance with national guidelines (see "General considerations" above).
2. If molecular and phenotypic testing are performed in the same laboratory, collecting one specimen may be sufficient. If testing is performed in two laboratories, two specimens should be collected and the molecular and phenotypic testing should be conducted in parallel. Sputum specimens or isolates should be transported to the appropriate testing laboratory, if necessary.
3. LC-aNAAT or SL-LPA should be conducted to detect mutations associated with FQ resistance. Targeted NGS tests can also detect mutations associated with resistance to FQ. If a targeted NGS test is available, Algorithm 2a should be followed for interpretation of results and follow-up actions.
4. If the LC-aNAAT or SL-LPA detects one or more mutations associated with resistance to FQs and:
   a. the individual is on a BPaL/M regimen, MFX should be discontinued and BPaL treatment continued while awaiting the results of the phenotypic DST;

b. the individual is on a BDLLfx/C, the LFX should be discontinued and BDLC continued (if the individual is FQ susceptible, C should be dropped from the regimen);
c. the individual is on a 9-month all-oral regimen, the person should be moved to an individualized longer regimen, designed using the WHO priority grouping of medicines *(7)*:
    - the first-in-class LC-aNAAT (Xpert MTB-XDR) provides results for INH, FQs, ETO and AMK, and can be used to inform individualized regimen selection;
    - a specimen should be collected and submitted for phenotypic DST to the WHO Group A, B and C drugs (e.g. for BDQ, Pa and LZD), if phenotypic DST is not already being done as described in Step 6; and
    - DST for MFX should be performed at the clinical breakpoint to determine the potential use of high-dose (800 mg) MFX for treatment *(7)* (**Web Annex C**).

5. If the LC-aNAAT or SL-LPA is negative for mutations associated with resistance to FQs (i.e. susceptible) and:
    a. the individual is on a BPaL/M regimen, treatment should continue without modifications while awaiting the results of the phenotypic DST;
    b. the individual is on a BDLLfx/C regimen, then C should be discontinued but BDLLfx continued while awaiting the results of the phenotypic DST;
    c. the individual is on one of the five 9-month regimens, treatment should continue without modification while awaiting the results of the phenotypic DST (Step 6); and
    d. in settings with a high underlying prevalence of resistance to FQs or for people considered at high risk of resistance, a specimen should be referred for culture and phenotypic DST for FQs, because the sensitivity of the LC-aNAAT and SL-LPA to detect mutations associated with FQ resistance is about 93% and 86%, respectively; the phenotypic DST should include testing for resistance to the FQs used in the country, and it should also include testing at the clinical breakpoint to inform individualized drug selection; the regimen should be modified as necessary, based on the phenotypic DST results.
6. Culture and phenotypic DST should be performed for each of the drugs included in the treatment regimen for which accurate and reproducible methods are available. For the preferred regimens, phenotypic DST methods that are reliable when performed in a quality-assured laboratory are available for BDQ, LZD, Pa, CS, FQs, CFZ, PZA and INH (**Web Annex C**). A WHO-recommended molecular test for PZA resistance detection is available (HC-rNAAT) but its use is currently limited to culture isolates.
    a. If the isolate is susceptible to all drugs, the person should be continued on the preferred MDR-TB regimen.
    b. If resistance to a drug is detected, **Fig. 6.3** should be used to guide treatment modification. Given that results for phenotypic DST are slow, the person's response should be reassessed when these results become available. The decision to change from a shorter to the longer MDR-TB regimen should consider the phenotypic DST result and clinical response. Monthly monitoring is important, and the person should be closely followed up.
7. For all people with TB, it is important to ensure that treatment monitoring includes the collection of samples for culturing, as described in the WHO consolidated guidelines *(7)*. Any positive culture suggestive of treatment failure should undergo phenotypic DST and WGS where available, with interpretation based on the WHO catalogue of mutations *(23)*. The regimen should be modified as necessary, based on the results.

a. WHO recommends that all people with TB being treated with an MDR-TB regimen be monitored for treatment response using sputum culture and sputum smear microscopy. It is desirable for sputum culture to be repeated at monthly intervals.
b. Although the risk of treatment failure increases with each additional month without bacteriological conversion, no discrete cut-off point has been defined that could serve as a reliable marker of a failing regimen. The choice of cut-off point will depend on the clinician's desire to minimize the risk of failure; in particular, to limit the risk of prolonging a failing regimen.

6.5 Algorithm 3 – Follow-on testing for individuals with RIF-susceptible TB at risk of resistance to other drugs

Algorithm 3 is a follow-on algorithm, the purpose of which is to detect resistance in individuals with RIF-susceptible TB who are at risk of having DR-TB and individuals with Hr-TB (Fig. 6.6). People at high risk for having DR-TB include those who have prior drug exposure; reside in settings where the probability of resistance to RIF, INH or FQs is high (≥5%), or belong to subgroups where the probability of such resistance is high; or have a history of contact with a person known to have DR-TB. Individuals not responding to first-line treatment include those who continue to be smear or culture positive after 2 months or more of treatment, and those who experience treatment failure.

Decentralized molecular testing is preferred, and can make use of any of the existing WHO-recommended tests that detect resistance to INH and FQ. However, the ability of targeted NGS tests to detect mutations associated with resistance to many anti-TB medicines could be particularly useful for people at high risk of having DR-TB (e.g. people in whom therapy has failed).

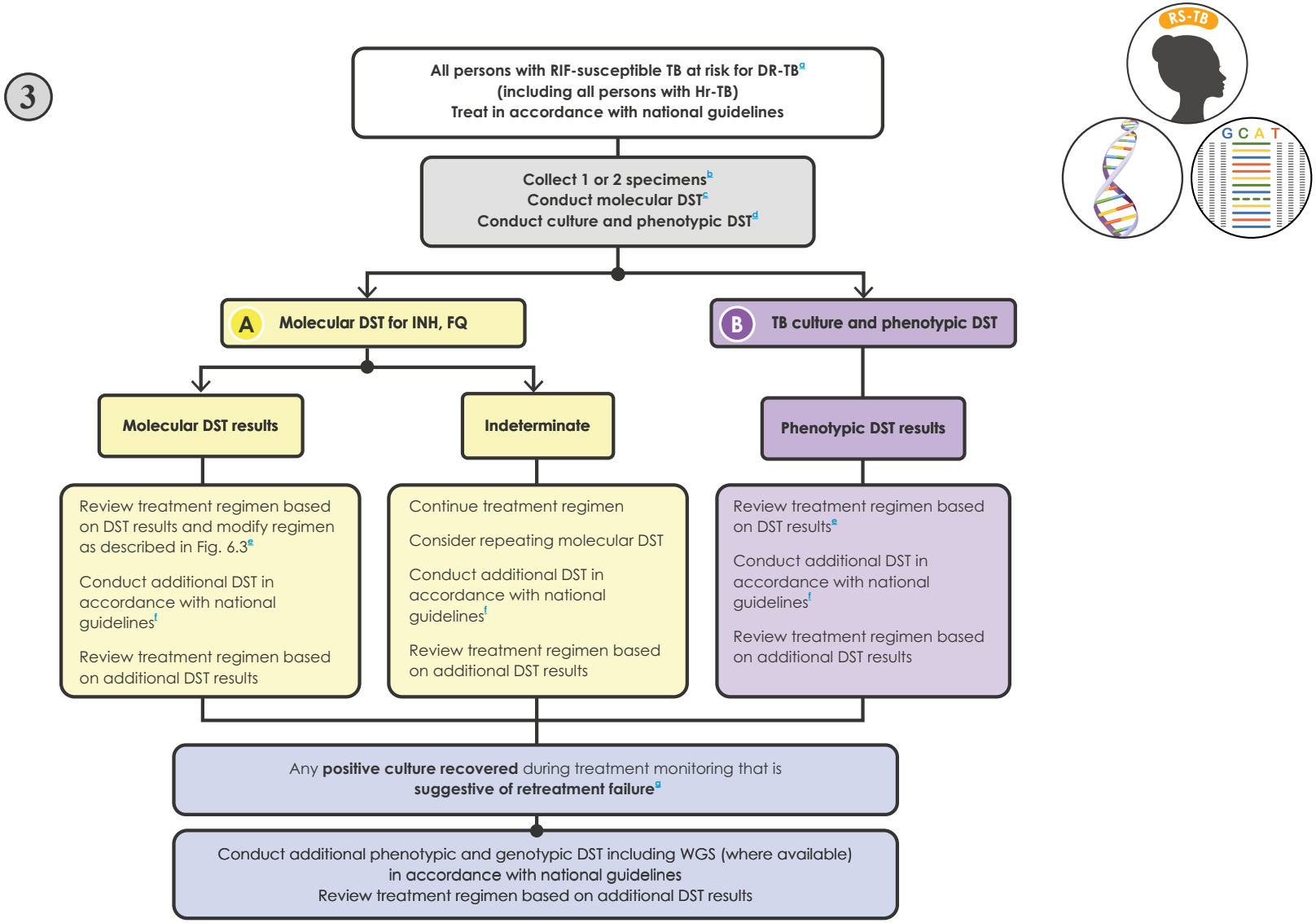
6.5.1 General considerations

- WHO guidelines stress the importance of DST before treatment, especially for medicines for which genotypic DST is available.
- People with TB that is RIF susceptible, INH susceptible or unknown should be started on a first-line regimen for drug-susceptible TB *(7)*.
- Globally, Hr-TB prevalence is 7.4% (95% CI: 6.5–8.4%) in new cases and 11.4% (95% CI: 9.4–13.4%) in people who were treated previously *(98)*. The prevalence in some settings can exceed 25% *(98)*. Contacts of a person known to have Hr-TB are also at increased risk. The prevalence of any INH resistance is particularly high in some parts of the WHO European Region and Western Pacific Region.
- Hr-TB is currently undetected in many settings but is clinically important. Compared with people with drug-susceptible TB, people 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%) *(98)*.
- The successful treatment of Hr-TB, prevention of the spread of Hr-TB and acquisition of resistance to additional drugs such as RIF rely on rapidly detecting people with Hr-TB and

placing them on effective treatment regimens. The LC-aNAATs for follow-up detection of INH resistance can be valuable tools because they are easy to use and can be implemented in the lower levels of the health system.

- The recommended Hr-TB treatment regimen is RIF, EMB, PZA and LFX for 6 months *(7, 99)*.
- Targeted NGS tests report results for many medicines not used for treatment of drug-susceptible TB (e.g. BDQ, LZD, CFZ, AMK and STR). These results should not be released for people with RIF-susceptible TB; however, if the results are released, it should be made clear that these medicines are only to be used for individualized regimens in specialized circumstances.
- Reliable phenotypic DST methods are available for RIF, INH, FQs, BDQ, CFZ, Pa, CS, LZD, AMK and DLM. Testing algorithms that rely on culture and phenotypic DST are described in the WHO policy framework *(96)* and technical manual (**Web Annex C**). Member States should ensure there is capacity for DST for drugs that are used for treatment and for which reliable testing is available.
- No reliable phenotypic DST methods are available for EMB, ETO/prothionamide, or imipenem-cilastatin/MPM; hence, results for these medicines should not be used for clinical decision-making.
- Initiation of treatment should not be delayed while waiting for the results of DST.

Fig. 6.6. Algorithm 3: Follow-on testing for people with RIF-susceptible TB at risk of resistance to other drugs



DR-TB: drug-resistant TB; DST: drug susceptibility testing; FL-LPA: line probe assay for first-line drugs; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; MC-aNAAT: moderate-complexity automated nucleic acid amplification test; mWRD: molecular WHO-recommended rapid diagnostic test; NGS: next-generation sequencing; SL-LPA: line probe assay for second-line drugs; SRL: supranational reference laboratory; TB: tuberculosis; WHO: World Health Organization.

Drugs: AMK: amikacin; BDQ: bedaquiline; CFZ: clofazimine; CS: cycloserine; DLM: delamanid; EMB: ethambutol; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; Pa: pretomanid; PZA: pyrazinamide; RIF: rifampicin; STR: streptomycin.

[a] People diagnosed with TB should be promptly initiated on a regimen for drug-susceptible TB or Hr-TB in accordance with national guidelines and WHO recommendations *(7, 100)*. People at high risk for having DR-TB include those who have prior drug exposure; reside in settings where the probability of resistance to RIF, INH or FQs is high (≥5%), or belong to subgroups in which the probability of such resistance is high; or have a history of contact with a person with known DR-TB or not responding to first-line treatment, including those who continue to be smear or culture positive after 2 months or more of treatment or experience treatment failure.

[b] 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.

[c] WHO recommends getting the rapid DST results before the start of treatment, although this testing should not delay the start of treatment. Rapid mWRDs for detecting FQ resistance include Xpert MTB/XDR and SL-LPAs; for detecting INH resistance include Xpert MTB/XDR, FL-LPAs and MC-aNAATs, and for detecting PZA resistance include the Genoscholar PZA-TB II test. Targeted NGS tests can provide results for BDQ, FQ, LZD, INH, PZA, EMB, CFZ, AMK, STR and RIF.

[d] 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 are available for RIF, INH, PZA, FQs, BDQ, CFZ, Pa, CS, LZD, AMK and DLM (**Web Annex C**). The initiation of treatment should not be delayed while awaiting the results of the phenotypic DST.

[e] For more information regarding modified treatment regimens, see the WHO consolidated guidelines on treatment of DR-TB *(7)* and drug-susceptible TB *(100)*.

[f] For DR-TB, a specimen should be collected and submitted for phenotypic DST, if not already being done as described in Note 4. If targeted NGS tests are available, a sample should be submitted for testing for resistance to additional medicines for specified risk groups in accordance with national guidelines.

[g] If resistance to an individual drug is suspected and DST for these drugs is not available in the country, laboratories should establish mechanisms to store the isolate and ship it to a WHO SRL for DST.

Decision pathway for Algorithm 3 – Follow-on testing for individuals with RIF-susceptible TB at risk of resistance to other drugs

1. The person should be promptly initiated on a regimen for the treatment of RIF-susceptible TB in accordance with national guidelines *(100)*. Individuals with Hr-TB should be started on an Hr-TB regimen *(99, 100)*.
2. If molecular and phenotypic testing are performed in the same laboratory, collecting 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. Sputum specimens or isolates should be transported to the appropriate testing laboratory, if necessary.
3. Molecular testing and culture should be performed in parallel.
    a. If targeted NGS tests are used:
        i. The treatment should be modified if appropriate, and phenotypic DST performed when the culture is positive based on **Table 6.1**. Note: the results from sequencing produce information on multiple drugs simultaneously; however, for simplicity, **Table 6.1** takes a single-drug approach for interpreting targeted NGS test results, although all results should be taken into consideration when designing a treatment regimen.

ii. If the targeted NGS test result is indeterminate, the test should be repeated with a fresh sample, and treatment decisions should be based on clinical assessments, the epidemiological situation and the results of phenotypic DST.

b. If a WHO-recommended follow-on molecular test other than a targeted NGS test is used:

i. For a person with RR-TB detected by either a molecular (e.g. Xpert Ultra or Truenat) or phenotypic DST, but for whom no results are available for INH and who is at high risk for Hr-TB, the process should start at Step 1 below.

ii. For a person who had an initial TB test that included RIF and INH results (e.g. an MC-a NAAT was used) in Algorithm 1, the process should start at Step 4 below.

1. A good-quality specimen should be collected and transported to the testing laboratory for molecular or phenotypic testing for INH resistance:
   - Testing could follow a two-step process: detection of INH resistance followed by detection of FQ resistance. The two-step process is applicable when an MC-aNAAT or FL-LPA is used for Hr-TB detection, followed by the LC-aNAAT or SL-LPA for detection of FQ resistance. A single-step option is now available using the first-in-class LC-aNAAT, which detects both INH and FQ resistance simultaneously.
   - Phenotypic DST may be required for INH resistance determination because of the sensitivity; depending on the test used, it may miss about 15% of resistant samples (**Table 3.3**). Phenotypic DST will be relevant when the person is at high risk for Hr-TB. If both molecular and phenotypic tests are performed, the tests should be initiated in parallel; it is important to not wait for the results of one test before initiating the other test.
   - Culture-based phenotypic DST for INH requires 3–8 weeks to produce a result. Phenotypic DST may be useful for evaluating people with results from an mWRD showing susceptibility to INH, particularly in populations with a high pretest probability for resistance to INH.

Table 6.1. Treatment modifications and follow-on DST for Hr-TB based on results from targeted NGS

Drug	Targeted NGS result	Is phenotypic DST to a specific drug required?	Action: treatment modification
RIF	Resistant	No	Change to MDR/RR-TB regimen, taking into account any resistances detected to other medicines – see Algorithm 2a
	Susceptible	No	Continue with the first-line regimen, including RIF if no other resistance is detected; otherwise, use an individualized regimen

Drug	Targeted NGS result	Is phenotypic DST to a specific drug required?	Action: treatment modification
INH	Resistant	No	Change to an Hr-TB regimen if no other resistance is detected
			If resistant to FQs or PZA is detected, change to an individualized treatment
	Susceptible	Yes	Continue with the first-line regimen if no other resistance is detected; otherwise, individualize the regimen
EMB	Resistant	No	If resistance is detected during the continuation phase, continue RH
			If resistance is detected during the intensive phase, clinically assess (considering other results) and closely monitor
	Susceptible	No	Continue with the first-line regimen if no other resistance is detected; otherwise, individualize the regimen
PZA	Resistant	No	If resistance is detected during the continuation phase, continue RH
			If resistance is detected during the intensive phase, clinically assess (considering other results) and closely monitor
	Susceptible	No	Continue with the first-line regimen if no other resistance is detected; otherwise, individualize the regimen
FQ	Resistant	No	If the person is on an FQ-containing 4-month regimen (e.g. HPMZ), change to HRZE if no other resistance is detected; otherwise, individualize the regimen
			If the person is on an Hr-TB regimen, discontinue the FQ and individualize treatment based on clinical assessment
	Susceptible	No	FQs should be used only when appropriate (4-month first-line regimen or Hr-TB regimen)

DST: drug susceptibility testing; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; MDR/RR-TB: multidrug- or rifampicin-resistant TB; NGS: next-generation sequencing; TB: tuberculosis.

Drugs and regimens: EMB: ethambutol; FQ: fluoroquinolone; HPMZ: isoniazid (H), rifapentine (P), moxifloxacin (M) and pyrazinamide (Z); HRZE: isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E); INH: isoniazid; PZA: pyrazinamide; RH: rifampicin (R) and isoniazid (H); RIF: rifampicin.

2. If INH resistance is not detected, treatment should be continued with a first-line regimen in accordance with national guidelines:
   - Additional DST should be conducted in accordance with national guidelines.
   - Consideration should be given to requesting additional molecular or phenotypic DST for resistance to INH if the person is thought to be at risk of having Hr-TB, despite the mWRD result.
3. If INH resistance is detected:
   a. The LC-aNAAT will provide simultaneous detection of resistance to INH and FQ. If FQ resistance is not detected, Steps 3b and 5 should be followed; if FQ resistance is detected, Steps 3d(ii) and 5 should be followed; and if the FQ result is unknown or unsuccessful, Step 3c should be followed.
   b. Treatment with an Hr-TB regimen should be initiated *(7)*:
      i. There is no clear evidence showing that adding INH at the usual doses benefits or harms people. For the convenience of the people being treated and for ease of administration, the four-drug INH/RIF/EMB/PZA (HREZ) fixed-dose combination tablets may be used to deliver the Hr-TB regimen, alongside LFX.
      ii. Emerging evidence suggests that people infected with strains with only *inhA* promoter mutations and corresponding modest increases in minimal inhibitory concentration (MIC) may benefit from high-dose INH therapy. Thus, additional INH – up to a maximum dose of 15 mg/kg per day – may be considered for use with the Hr-TB regimen for such isolates. The added value of INH in the regimen, even when used at the higher dose, declines as MICs increase further.
   c. A specimen should be referred from a person with laboratory-confirmed Hr-TB for molecular (e.g. LC-aNAAT or SL-LPA) or phenotypic DST for FQs and PZA. Note: if the Xpert MTB/XDR test was used in Step 1, the FQ result will already be available and Step 6d applies.
      i. Rapid molecular testing for FQ resistance is preferred. When used for direct testing of sputum specimens, the LC-aNAAT and SL-LPA detect 93% and 86% of people with FQ resistance, respectively (**Table 3.4**):
         - LC-aNAATs provide rapid results and are suitable for use at the peripheral level. The first-in-class test, Xpert MTB/XDR, reports low-level FQ resistance when the mutations *gyrA A90V*, *gyrA S91P* and *gyrA D94A* are detected from the probe melting temperature *(92)*. Phenotypic DST at the clinical breakpoint for MFX should be performed to confirm the potential value of high-dose MFX treatment for such people.
         - The diagnostic accuracy of SL-LPA is similar when it is performed directly on sputum or cultured isolates. SL-LPA can be used with smear-positive or smear-negative specimens, although the indeterminate rate will be higher when testing smear-negative specimens.
         - Despite the good specificity and sensitivity of LC-aNAATs and SL-LPA for the detection of FQ resistance, phenotypic DST is required to completely exclude resistance to individual FQs. In particular, phenotypic DST may be needed in settings with a high pretest probability for resistance to FQ, to exclude resistance when the SL-LPA does not detect mutations associated with resistance.

d. FQ resistance results should be reviewed:
    i. If FQ resistance is not detected, treatment should be continued with the LFX-containing Hr-TB regimen.
    ii. If FQ resistance is detected:
        - Use of LFX should be discontinued and instead a 6-month regimen of (INH)/ RIF/EMB/ PZA should be used; that is, 6(H)REZ, where the "(H)" indicates that the INH is optional, or an individualized Hr-TB regimen.
        - A specimen should be referred for PZA DST if reliable PZA DST is available in the country. Options include the HC-rNAAT, phenotypic DST in the MGIT system and *pncA* sequencing. Further details are given in the WHO technical manual for drug susceptibility testing of medicines used in TB treatment (**Web Annex C**).
        - If PZA resistance is not detected, or if PZA DST is not available, therapy should be continued with the regimen that was designed based on the previous DST results.
        - If PZA resistance is detected, it may be necessary to design individualized treatment regimens, especially if resistance to both FQs and PZA is detected.

4. If the INH result cannot be interpreted or is invalid, the LC-aNAAT or MC-aNAAT or FL-LPA should be repeated with a fresh specimen. Consideration should be given to conducting culture and molecular or phenotypic DST for INH on the isolate, if the person is considered to be at risk of having Hr-TB.
5. For all people with TB, treatment monitoring should include collection of samples for culturing, as described in WHO guidelines. Any positive culture suggestive of treatment failure should undergo phenotypic and molecular DST, if available. At a minimum, DST should include testing for resistance to INH and RIF for people on first-line regimens, and for RIF, FQs and PZA (if available) for people on Hr-TB regimens. The treatment regimen should be modified as necessary, based on the results of the DST.

6.6 Discordant results

Interpretation of discordant results

When more than one test is performed on an individual, the results may be discordant, which can lead to diagnostic dilemmas. It is important as a first step to exclude possible administrative errors (specimen mislabelling or switching) by reviewing the tests conducted at or around the same time. Reviewing the patient history is also useful because that may highlight a long history with a possibility that an individual may be harbouring more than one strain of *Mtb*. Technical issues could also be the cause – although the tests are expected to perform similarly, they are not identical; in addition, sample-to-sample variability complicated the issue. Each discordant result will need to be investigated on a case-by-case basis. A few examples are outlined below.

1. Where the mWRD (e.g. Xpert Ultra) result is "MTBC detected", and the follow-on FL-LPA result is "MTB not detected" or "uninterpretable":

a. The mWRDs recommended for detection of TB have a lower LoD than the FL-LPA; thus, FL-LPA may fail to detect TB in mWRD-positive samples that contain few bacilli. For example, it is estimated that only about 80% of specimens with "MTBC detected" by Xpert MTB/RIF will generate an FL-LPA result that can be interpreted.
b. The initial mWRD result should be used to guide treatment decisions, pending additional testing.
c. Follow-up actions may include submitting a specimen for culture and a molecular or phenotypic testing of the recovered isolate, and evaluating the possibility of laboratory or clerical error.

2. Where the initial mWRD result is "MTBC detected, RIF resistance not detected" and the sample is RIF resistant by FL-LPA:
   a. Treatment decisions should be based on the FL-LPA result (i.e. based on the worst-case scenario).
   b. This result is expected to be rare because both assays interrogate the same region of the *rpoB* gene. There have been reports of mWRD RIF-susceptible and FL-LPA RIF-resistant discordances, but the data are too sparse to assess how often this occurs.
   c. FL-LPA is more sensitive for identifying RIF resistance than most mWRDs in heteroresistant populations (i.e. mixtures of susceptible and resistant bacteria). The test includes hybridization probes specific to both the common mutated and the wild-type sequences in the bacterial genome. If the Xpert Ultra is used, a review of the probe melting temperature curves may help to identify heteroresistant populations (e.g. dual peak).
   d. Follow-up actions may include DNA sequencing, conducting phenotypic DST, and evaluating the possibility of laboratory or clerical error.
3. Where the MC-aNAAT result is "MTBC detected, RIF resistance not detected, INH resistance detected" but the result is "INH susceptible" by LC-aNAAT:
   a. This result is expected to be rare because both assays interrogate the same region of the *katG* and *inhA* genes.
   b. A more likely reason is the existence of heteroresistant populations (i.e. mixtures of susceptible and resistant bacteria), especially in high-burden settings where the force of infection is high. A review of the LC-aNAAT probe melting temperatures *(92)* may identify such a possibility (e.g. dual peak).
   c. Follow-up actions may include DNA sequencing, conducting phenotypic DST, and evaluating the possibility of laboratory or clerical error.
   d. The risk of Hr-TB should be reassessed; if the reason is found not to be a high risk of Hr-TB or administration errors (e.g. mislabelling), treatment decisions should cover the worst-case scenario and be based on the MC-aNAAT result.
4. Where the MC-aNAAT result is "MTBC detected, RIF resistance not detected, INH resistance not detected" and the sample is INH resistant by LC-aNAAT:
   a. Treatment decisions should be based on the LC-aNAAT result (i.e. treat based on the worst-case scenario).
   b. This result is expected to be rare because both assays interrogate the same region of the *katG* and *inhA* genes. However, the LC-aNAAT is more sensitive for INH detection because it includes additional gene targets (*fabG1* and *oxyR-ahpC* intergenic regions).

c. The existence of heteroresistant populations (i.e. mixtures of susceptible and resistant bacteria) is another possible reason, especially in high-transmission settings. A review of the LC-aNAAT probe melting temperatures may identify such a possibility (e.g. dual peak).
d. Follow-up actions may include DNA sequencing, conducting phenotypic DST, and evaluating the possibility of laboratory or clerical error.

5. Where targeted NGS detects resistance and other molecular tests show susceptibility (or vice versa):
   a. Results should be checked to see whether the mutation detected by targeted NGS was in a region not covered by the other molecular test. If that is the case, the targeted NGS should be considered to be the final result.
   b. Results should be checked to see whether the mutation is a synonymous mutation by targeted NGS. If that is the case, it would indicate that the other molecular test result is incorrect.
   c. Results should be checked to see whether targeted NGS detected heteroresistance. If that is the case, the most resistant profile should be used for clinical management. Targeted NGS is better at resolving heteroresistance than other molecular tests.

6.7 Algorithm 4 – Testing for TB infection

An integrated algorithm for TPT among contacts (aged <5 years), people living with HIV and other risk groups has been released by WHO as Module 1 of the guidelines *(17)*. For household contacts who are aged 5 years and older and who are not HIV-positive, testing for TB infection is advised as part of their care. Given that contacts are also at risk of TB disease, an integration of testing for TB infection with TB screening would be an important step to enhance implementation.

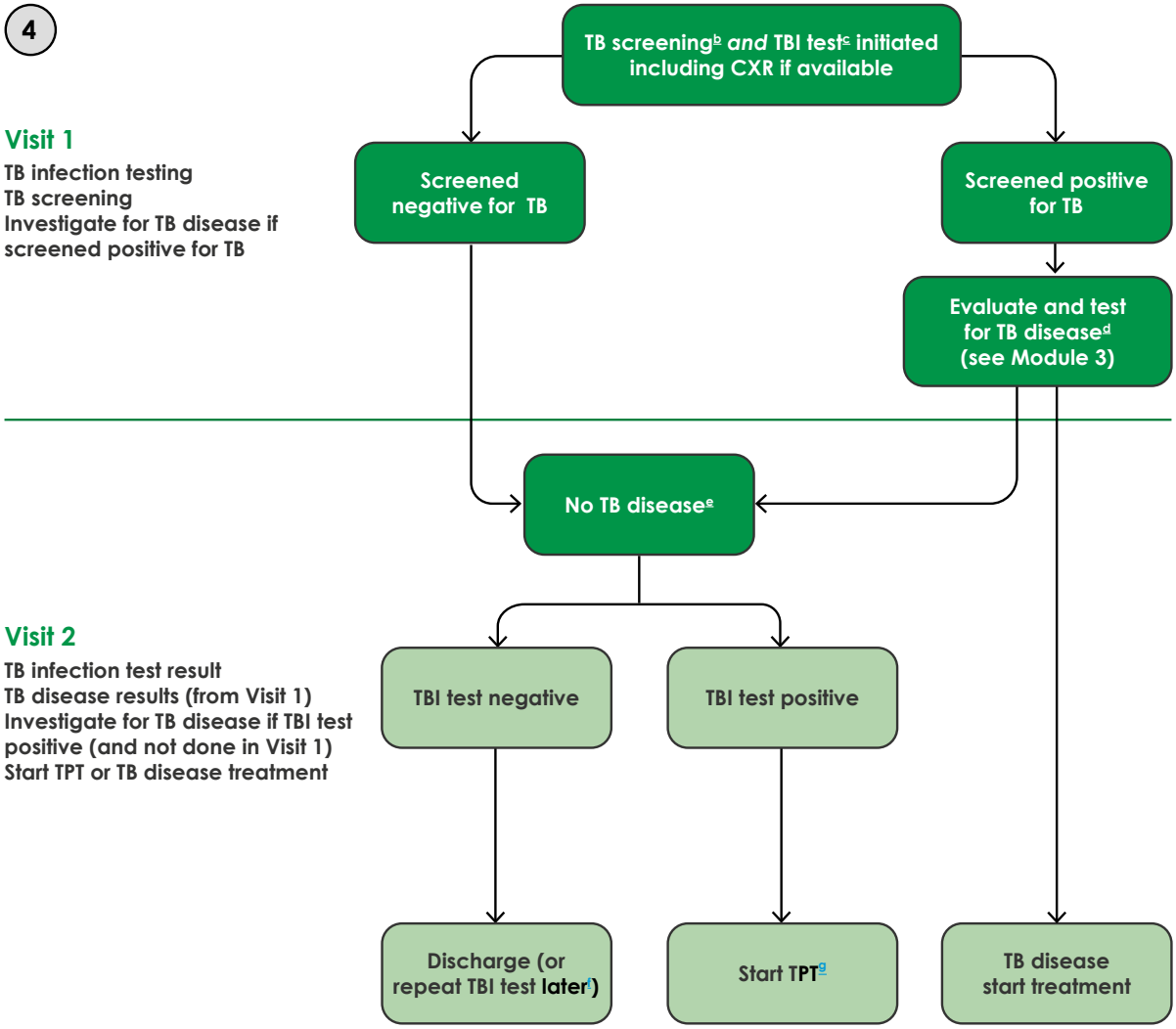
In the first visit, as soon as someone at risk of TB is identified, that person should undergo TB screening to detect people who should be tested for TB disease and should, at the same time, be tested for TB infection (**Fig. 6.7**). Children aged under 5 years and people living with HIV do not require testing for TB infection; for these groups, TPT can be initiated if TB disease can be ruled out. TB screening could be performed, for example, by assessing TB symptoms or using more sensitive WHO-recommended tools such as CXR, with or without computer-aided detection, mWRDs or C-reactive protein (the latter only applies to people living with HIV) *(55)*. Sequential screening and diagnostic testing (first for TB disease, then for TB infection) may incur substantial delays or losses in testing for TB infection, particularly if these tasks are performed by different health care personnel or in different locations. Hence, during the first health care encounter, it is preferable to combine screening for TB disease with testing for TB infection. People with symptoms that are suggestive of TB disease should undergo further evaluation as soon as possible, preferably on the same visit *(5)*. If onsite testing is available for TB disease and TB is confirmed, TB treatment should be promptly initiated, though this is unlikely in many settings.

The second visit may be optimized for the following: reading the TST or TBST or obtaining the IGRA result; reviewing the results of microbiological tests for TB disease if the person had a positive TB screen and submitted samples for TB testing; undertaking clinical evaluation to rule out TB or before starting TB treatment; and starting TPT or TB treatment. This second visit should

occur 48–72 hours after the test. If the test for TB infection is negative, the individual can be discharged; however, a repeat test for TB infection may be required if an initial test is negative, particularly in people with very recent exposure or concomitant viral infection. When the test for TB infection is positive in contacts who were asymptomatic initially, or who had symptoms but in whom TB disease was excluded, the person should be re-evaluated for immediate initiation of TPT (**Fig. 6.7**). Although TB infection tests may be positive in the presence of TB disease, their low accuracy means that these tests are not recommended for screening or as part of the diagnostic work-up of presumptive TB; this should be emphasized during the training of health workers.

It is proposed that tests for TB infection for eligible individuals be conducted early in the assessment of people at risk of TB (i.e. during the first visit) because this would help to reduce delays in starting the appropriate treatment; also, the test result may be helpful when deciding the best course of action in most of the people tested. If a person has no symptoms at screening, evaluation to exclude TB disease may still be warranted, and CXR increases the sensitivity in such situations. Ideally, all these activities should be done on the same day, so that TPT can be prescribed during the second visit as soon as test results are available. Because most people with TB symptoms are not expected to have TB disease, deferring the test for TB infection to a later stage (e.g. after further work-up) in such individuals may result in significant losses.

Fig. 6.7. Algorithm for person-centred TB infection care; integrated infection and disease assessment where testing for TB infection is available[a] and recommended



CXR: chest X-ray; IGRA: interferon-gamma release assay; PLHIV: people living with human immunodeficiency virus; TB: tuberculosis; TBI: TB infection; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TPT: TB preventive treatment; TST: tuberculin skin test; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

[a] Where testing is not available (or not required based on local circumstances), the algorithm in the WHO operational handbook on TB prevention *(41)* can be used.

[b] TB screening performed using four TB symptoms (cough, fever, night sweats and weight loss). The WHO-recommended screening tools with high accuracy are preferred; they include CXR (with or without computer-aided detection), and for persons living with HIV, mWRDs or C-reactive protein *(54)*.

[c] TB infection tests include TST, IGRAs and TBSTs.

[d] A thorough clinical assessment should be performed to exclude TB disease (including extrapulmonary TB) and should include CXR where available. A WRD should be used for diagnosis of TB disease where available *(101)*.

[e] Individuals screened using only symptoms initially should be carefully assessed for TB disease (including for extrapulmonary TB); the screening should include CXR where available, to rule out TB disease before initiating TPT.

[f] In circumstances where a test may be suspected to be false negative (e.g. concurrent viral infection), repeat testing may be considered after 30 days.

[g] Details on eligibility criteria and regimen choices can be found in the relevant WHO guidelines *(14)*.

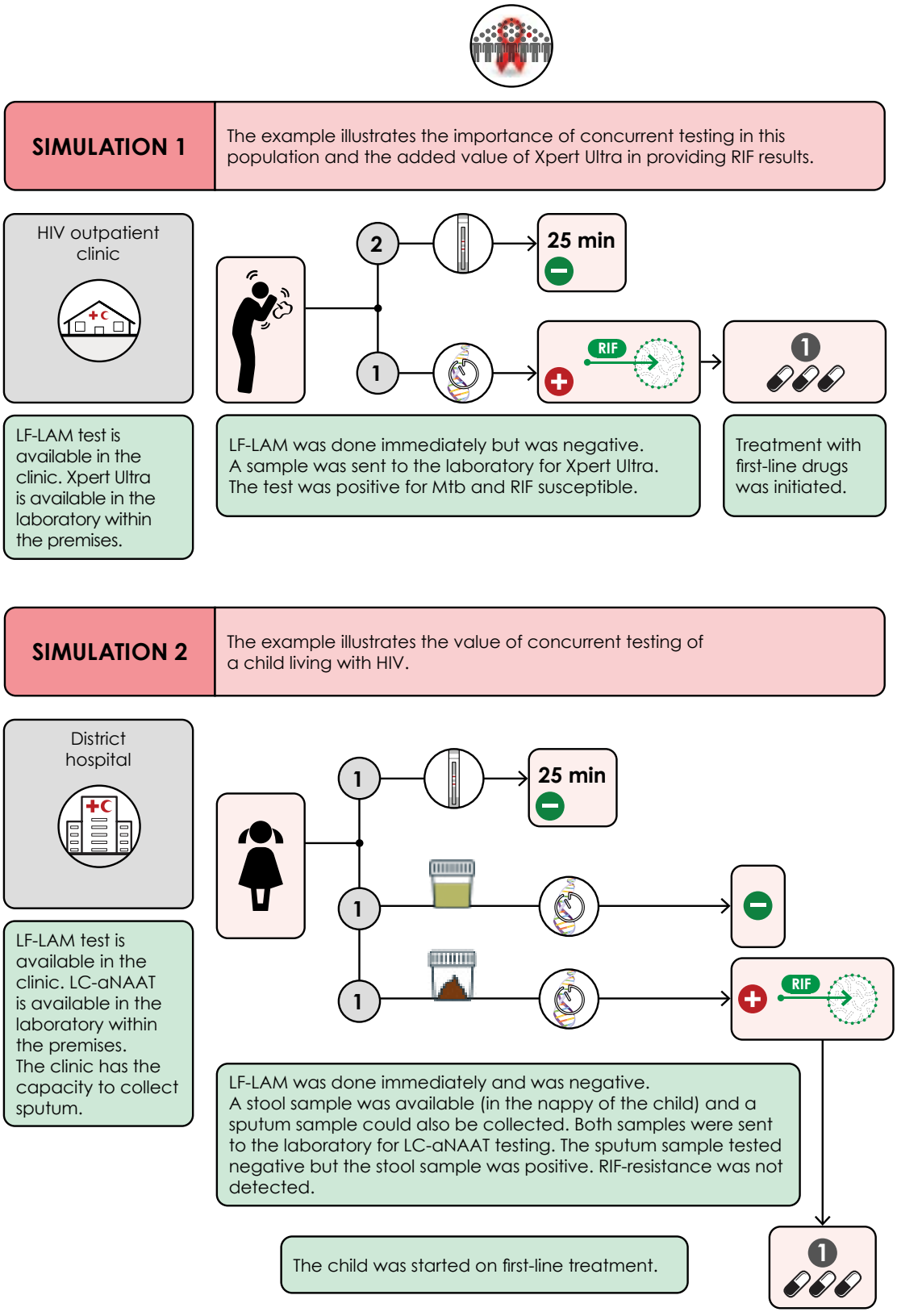
The programmatic decision to initiate testing for TB infection implies a commitment to start TPT rapidly where indicated. Hence, the programme should ensure that the referral pathways and medical services are well organized before launching testing for TB infection, and that the mechanisms for clinical assessment, start of medication and treatment support are all available (Section 2). An advantage of skin testing for TB infection (using TST or TBST) is the capacity to administer the test and read the result in 48 hours at the POC. The portable nature of the supplies and temperature-controlled reagents needed for the test mean that testing can be done at home or in remote communities. However, these advantages are lost if the subsequent evaluation to exclude TB disease and the provision of TPT are not equally accessible. Hence, a decision to expand access to testing for TB infection needs to be matched by efforts to expand access to the medical services needed for management of those with positive test results.

6.8 Illustrative algorithm combinations

To aid understanding of how the different algorithms interlink to provide a final diagnosis for a person, illustrative scenarios are presented in **Fig. 6.8**, **Fig. 6.9** and **Fig. 6.10**. Three scenarios are provided, with two simulated pathways in each. The scenarios are based on three epidemiological settings: high TB/HIV, high Hr-TB and high MDR/RR-TB. These examples are for illustrative purposes only – they do not represent a specific recommendation. There could be many alternative combinations that could achieve the same outcome; the choice to use one test instead of another would depend on factors such as availability, ease of use, in-country product support and cost.

Algorithms should be designed to use existing laboratory services and networks, so that specimens can be referred to the appropriate level for tests that are not available at peripheral-level laboratories.

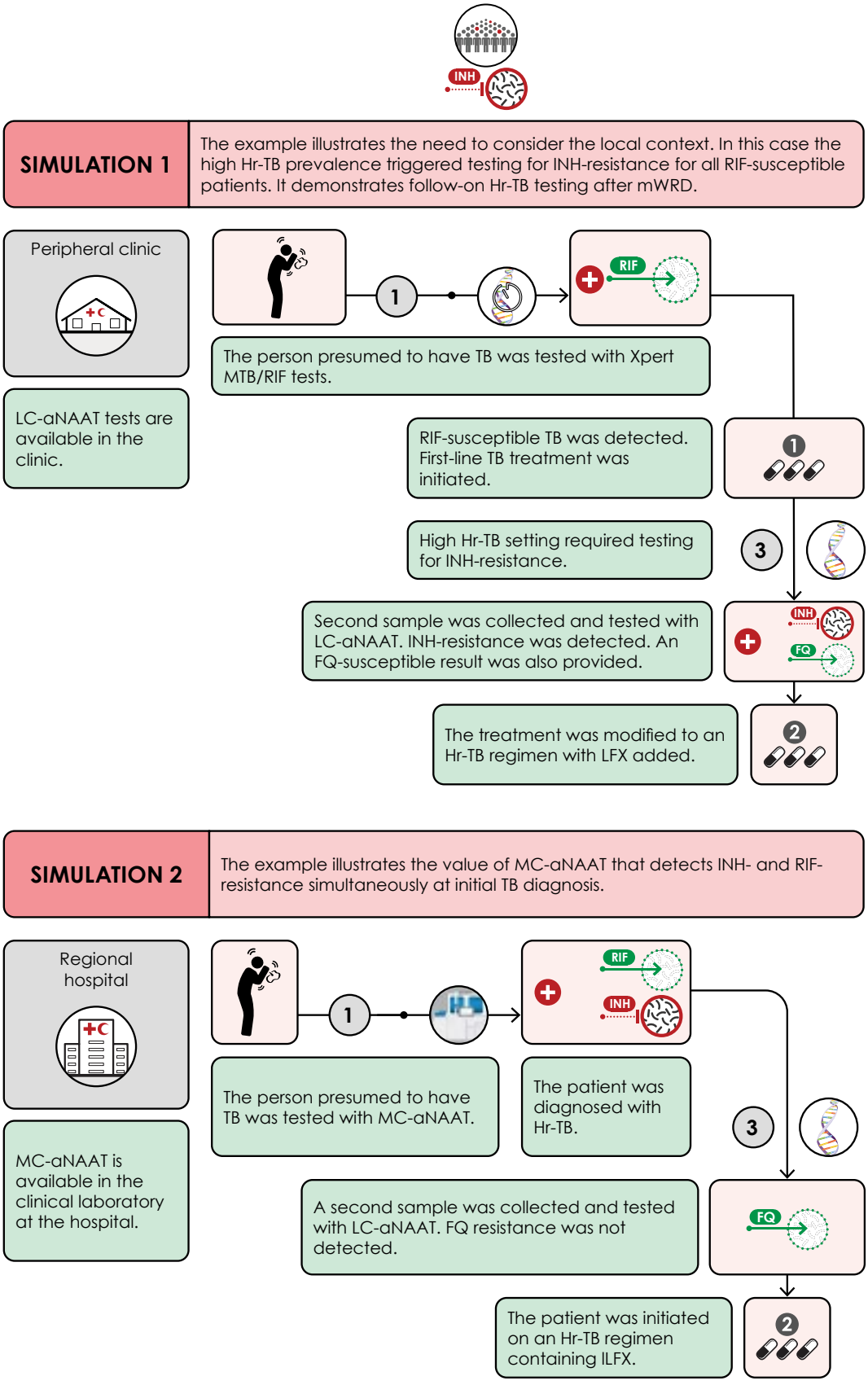
Fig. 6.8. Scenario 1: High TB/HIV setting



DST: drug susceptibility testing; HIV: human immunodeficiency virus; LC-aNAAT: low-complexity automated NAAT; LF-LAM: lateral flow urine lipoarabinomannan assay; MC-aNAAT: moderate-complexity automated nucleic acid amplification test; *Mtb*: *Mycobacterium tuberculosis*; TB: tuberculosis.

Drugs: RIF: rifampicin.

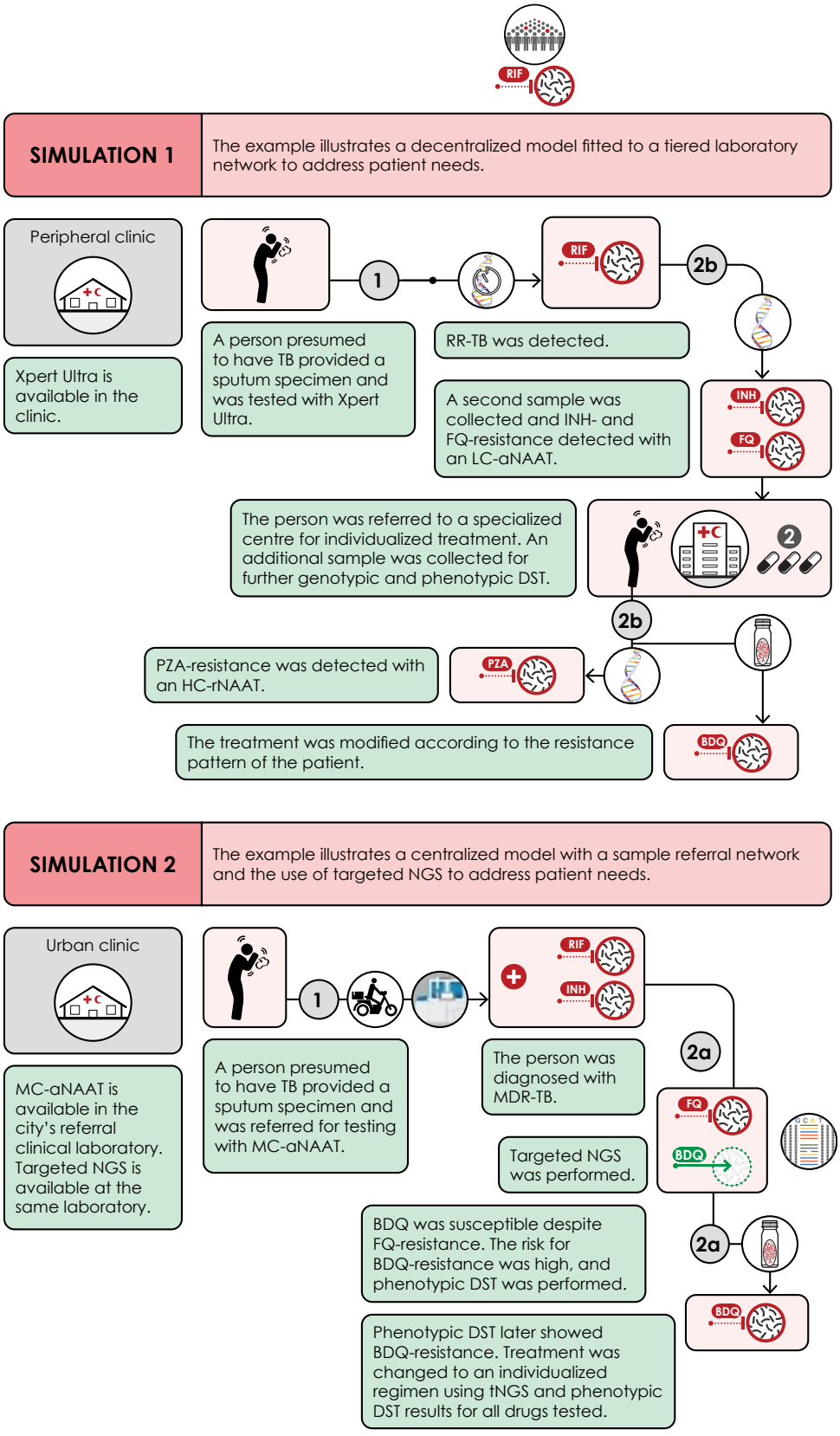
Fig. 6.9. Scenario 2: High Hr-TB setting



Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; LC-aNAAT: low-complexity automated NAAT; MC-aNAAT: moderate-complexity automated NAAT; mWRD: molecular WHO-recommended rapid diagnostic test; NAAT: nucleic acid amplification test; TB: tuberculosis; WHO: World Health Organization.

Drugs: FQ: fluoroquinolone; INH: isoniazid; LFX: levofloxacin; RIF: rifampicin.

Fig. 6.10. Scenario 3: High MDR/RR-TB setting



DST: drug susceptibility testing; HC-rNAAT: high-complexity reverse hybridization NAAT; LC-aNAAT: low-complexity automated NAAT; MC-aNAAT: moderate-complexity automated NAAT; MDR/RR-TB: multidrug- or rifampicin-resistant TB; MDR-TB: multidrug-resistant TB; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; SL-LPA: second-line line probe assay; TB: tuberculosis.

Drugs: BDQ: bedaquiline; FQ: fluoroquinolone; INH: isoniazid; PZA: pyrazinamide; RIF: rifampicin.

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Annex 1. Budgetary considerations for implementing a new diagnostic test

Equipment
Costs of assessing site readiness (travel and HR)
Costs of upgrading laboratory facilities and infrastructure (e.g. electricity and air conditioning) to ensure a safe and functional testing site
Costs to adhere to biosafety precautions, and biological and chemical waste disposal requirements
Costs of selecting, procuring and installing equipment: • purchase (or lease) of instruments and any necessary ancillary equipment • delivery and importation costs • installation by manufacturer or authorized service provider (e.g. per diems and travel) • training • instrument verification • extended warranty or service contract Costs of routine preventive maintenance Costs of annual maintenance or calibration
Supplies
Workshop for stakeholders involved in procurement, to strengthen the supply chain
Costs of maintaining centralized stores and costs of distribution
Material cost per test (e.g. test reagents, consumables, sample collection items and printing paper), and additional equipment costs such as requirements for additional equipment (e.g. printer, computer and printer cartridges), shipping and courier costs
Costs of new-lot testing
Procedures
Workshop and HR for the development of SOPs Printing and dissemination of revised SOPs
Development, printing and dissemination of revised clinical protocols and guidance for selecting people to be tested, ordering tests, interpreting test results and making decisions on patient care

Digital data
Purchase and implementation of a laboratory information management system, if applicable
Purchase and installation of a diagnostics connectivity solution, if applicable HR and training
Costs of data transmission (e.g. high-speed internet service)
Costs associated with providing and maintaining a remote monitoring system in-country
QA, control and assessment
Preparation and regular review of all testing and QA documents (e.g. SOPs and checklists) based on national requirements
Cost of conducting quality controls (e.g. testing known positives or negatives) Costs of HR for routinely collecting and analysing quality indicators
Costs of conducting on-site visits (e.g. travel, HR, and preparation of checklists and reports) Costs associated with hosting an on-site visit and preparation of documents
Costs associated with providing PT panels and overseeing PT, reporting results and corrective actions, and costs associated with testing PT panels at each site
Costs associated with retesting samples at a higher level laboratory (e.g. shipment of samples, testing and reporting), if applicable
Recording and reporting
Workshop and HR to update recording and reporting forms, registers, etc.
Preparation, printing and distribution of standardized forms (e.g. test request and results reporting) and logbooks
Training and competency assessment
Workshop and HR to update training packages for laboratory and clinical staff
Training-of-trainers workshop, participant and instructor travel, on-site training and sensitization meetings
Printing and distribution of updated training manuals and sensitization materials
Costs associated with facility and classroom-based training (e.g. travel, accommodation, printing materials, venue hire and catering)
Costs associated with annual competency testing of staff
Monitoring and evaluation
Meetings to update monitoring and evaluation system, and regular meetings to review impact of transition and re-planning
Monitoring and evaluation of refresher training Operational research study to measure clinical impact

Annual ongoing costs
Consumables and reagents for diagnostic testing
Costs associated with repeat testing and proficiency testing
Specimen referral and results reporting HR
Equipment calibration and servicing Diagnostics connectivity
QA
Successful implementation of the plan will require financial and human resource commitments from the ministry of health or national tuberculosis (TB) programme (NTP), with possible support from implementing partners. Consider integrating TB testing on existing multidisease platforms in locations where integrated testing is feasible, to share costs across disease programmes. A budget should be developed to address activities in collaboration with key partners, using the considerations outlined below. Technical assistance may be needed.
Budgetary considerations
Policies and planning
Workshop for stakeholder engagement and planning Costs of TWG meetings
Technical workshop for guideline and algorithm update Situational analysis costs (HR, travel and report writing)
Printing and distribution costs for revised guidelines and algorithms Development of a costed operational plan
External technical assistance costs, if needed
Regulatory
Regulatory submission costs, if applicable Local travel costs to regulatory authority Importation processes and costs
Verification study, if required (samples, reagents and HR)

HR: human resources; PT: proficiency testing; QA: quality assurance; SOP: standard operating procedure; TWG: technical working group.

Annex 2. Drug susceptibility testing methods and critical concentrations

Culture-based DST methods for certain anti-tuberculosis (anti-TB) medicines are reliable and reproducible, but these methods are time consuming, and require specific laboratory infrastructure, skilled staff and adherence to quality control. The WHO *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis (1)* describes the methods, media, sources of drug powders and critical concentrations for conducting drug susceptibility testing (DST) of *Mycobacterium tuberculosis* complex (MTBC isolates). Only indirect phenotypic DST procedures for anti-TB medicines are described in this document; they include Löwenstein–Jensen (LJ), 7H10 agar, 7H11 agar and 7H9 broth (BACTEC Mycobacterial Growth Indicator Tube [MGIT] instrument). The manual incorporates recent revisions to critical concentrations for rifampicin *(2)* as well as newly developed critical concentrations for pretomanid and cycloserine (Web Annex B).

Key topics in the manual are:

- biosafety;
- evidence basis for determining critical concentrations for DST;
- recommendations for DST for first-line anti-TB agents;
- recommendations for DST for second-line anti-TB agents;
- susceptibility testing for anti-TB agents using the proportion method on solid media (LJ medium, or Middlebrook 7H10 or 7H11 agar media):
  - anti-TB agents and critical concentrations for testing;
  - recommended drug powders and preparing solutions of anti-TB agents;
  - preparing the mycobacterial suspension;
  - diluting the suspension and inoculating the medium;
  - interpreting and reporting results;
  - quality control;
- susceptibility testing for anti-TB agents using liquid media (MGIT):
  - anti-TB agents and critical concentrations for testing;
  - recommended drug powders and preparing solutions of anti-TB agents;
  - preparing the mycobacterial inoculum;
  - diluting the suspension and inoculating the liquid medium;
  - interpreting and reporting results; and
  - quality control.

Recommended critical concentrations for testing anti-TB medicines are presented in **Tables 2.2** and **2.3** in **Section 2.6** of the main text. **Table A2.1** lists available pure powders and their sources.

Table A2.1. Availability of pure powders from GDF and other manufacturers

Drug	Description and ingredients	Manufacturer (Catalogue No.)	Quantity	GDF Catalogue No	GDF Quantity	Storage
Levofloxacin	>98% HPLC	Sigma- Aldrich (28266-IG-F)	1 g	106560	1 g	RT
Moxifloxacin BACTEC MGIT vial	Moxifloxacin Hydrochloride 249ug/vial	BD REF: 215404	6 vials of 249 µg each	106975	6 vials of 249 µg each	2–8°C
Bedaquiline	Bedaquiline fumarate 12 mg BDQ fumarate salt equivalent to 10 mg BDQ base	Available (free of charge)[a] through BEI Resources[b]	20 mg	Not applicable	Not applicable	RT
Bedaquiline BACTEC MGIT vial	Bedaquiline fumarate 170 µg (active) Ficoll (inactive). Contents of vial soluble in 2 mL DMSO)	BD REF: 215449	170 µg (2 vials)	106976	2 vials of 170 µg	2–8°C
Ethionamide	830 µg (active); Ficoll (inactive) Contents of vial soluble in 4 mL sterile, distilled water	BD REF: 215355	830 µg (6 vials)	Please refer to GDF catalogue or contact GDF or manufacturer for more information	Contact GDF or BD	2–8°C
	Pure substance	Sigma Aldrich (E6005)	5 g	106316	5g	2–8°C
Linezolid	Pure substance ≥ 98% activity	(1) Sigma (PZ0014–5MG); (PZ0014–25MG) (2) Cayman Chemical (CAS 165800–03–3)	5 mg / 25 mg 25mg	106653	25 mg	RT
Clofazimine	Pure substance	Sigma-Aldrich (C8895–1G)	1 g	106654	1 g	RT
Delamanid	Pure substance	Available through: BEI Resources[b]	20 mg	Not applicable	Not applicable	RT (protect from light and heat)
Amikacin	Amikacin disulfate salt potency: 674–786 µg per mg (as amikacin base)	Sigma-Aldrich (A1774–250MG)	250 mg	106318	5 g	2–8°C
		BD REF 215350	6 vials of 332 µg each	106586	6 vials of 332 µg each	2–8°C
Streptomycin	Streptomycin sulphate. Potency ≥720 µg per mg (as streptomycin base)	Sigma-Aldrich (D7253–5G)	5 g	106311	5 g	2–8°C
Pretomanid	Pure substance	Available through: BEI Resources[b]	40 mg	Not applicable	Not applicable	RT
		Sigma-Aldrich (SML1290)	10 or 50 mg	Please refer to GDF catalogue or contact GDF or manufacturer for more information	Please refer to GDF catalogue or contact GDF or manufacturer for more information	–20°C
Cycloserine	Pure-substance ≥ 98%	Sigma-Aldrich (C6880)	1 mg or 5 mg	Not available	Not available	See footnote[c]

RT: room temperature.

[a] Free of charge shipment when specifying carrier as JNJ.

[b] See **https://www.beiresources.org;** collection from the closest airport and customs clearance of the drug shipment is the responsibility of the receiving laboratory.

[c] Given the known heat instability of DCS, DCS powder should be stored as instructed by the manufacturer and stocks solutions in sterile distilled/deionised water should be stored at –70° C ± 10° C for no longer than 6 months (i.e. lower temperatures should not be used and vials should never be re-frozen).

References for Annex 2

1 Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva: World Health Organization; 2018 (https://www.who.int/publications/i/item/9789241514842).

2 Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis. Geneva: World Health Organization; 2018 (https://www.who.int/publications/i/item/WHO-CDS-TB-2018.5).

Annex 3. Implementation of next-generation sequencing technologies

The World Health Organization (WHO) recently published *The use of next-generation sequencing for the surveillance of drug-resistant tuberculosis: an implementation manual (1)* which provides practical guidance on planning and implementing next-generation sequencing (NGS) technology for characterization of *Mycobacterium tuberculosis* complex (MTBC) bacteria. In this manual, the focus is on the detection of mutations associated with drug resistance for the surveillance of drug resistance in tuberculosis (TB). The implementation guidance is also appropriate for the implementation of targeted next-generation sequencing (NGS) tests to detect mutations associated with drug resistance, to guide clinical decision-making for treatment of drug-resistant TB (DR-TB).

This implementation manual complements two other publications on TB:

- *The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex: technical guide (2)* – this document provides an overview of NGS methods and workflows, and a comprehensive review of the scientific evidence on characterization of the genetic basis of phenotypic drug resistance to major anti-TB medicines; and
- The second edition of the *Catalogue of mutations in Mycobacterium tuberculosis complex and their association with drug resistance (3)*.

The technical guide *(2)* offers a framework for making decisions about NGS-based drug susceptibility testing (DST), a roadmap for implementation, and practical guidance for country planning and implementation of NGS-based DST. The main steps for implementing targeted NGS for the detection of mutations associated with drug resistance in TB are the same as those described in Section 3.5 of the main text, with an emphasis on the nuances of the NGS technologies (e.g. NGS equipment, bioinformatics needs and reporting forms). The main steps are as follows:

1. Define the intended immediate and future use of NGS tests in the country, in line with the objectives of the country's national strategic plan (NSP) for TB. This will have important implications for the choice of technologies and equipment to use, the selection of a site or sites for conducting testing, specimen referral systems and target turnaround times for results.

2. Establish a technical working group to lead planning, including performing a readiness assessment, developing a costed operational plan with timelines and milestones, and overseeing compliance with relevant regulatory processes and procedures.
3. Based on the intended use of NGS in the country, select, procure and set up equipment in one or more safe, secure and functional testing sites.
4. Establish forecasting, ordering and distribution procedures to ensure a reliable and timely supply of quality-assured reagents and consumables.
5. Develop and deploy a well-defined, comprehensive set of standard operating procedures (SOPs) to address all aspects of the laboratory testing process, from sample collection to reporting of results. Provide clear decision-making guidance for the selection of people for NGS-based DST.
6. Secure adequate storage capacity and processes for backup and retrieval of the large amounts of data generated by NGS; select and implement relevant bioinformatic tools to analyse and interpret NGS data; and develop SOPs for data security, sharing and ensuring confidentiality.
7. Implement a comprehensive quality assurance (QA) programme that includes quality control (QC), performance indicator monitoring, proficiency testing, re-checking or interlaboratory comparisons, and regular onsite supportive supervision (with timely feedback, corrective actions and follow-up for each step of the process).
8. Update surveillance forms and registers to capture the relevant data on the person being treated and NGS, ideally through an electronic case-based recording and reporting system. Standardize the recording of NGS results in an easy-to-read format, to facilitate their interpretation.
9. Develop and implement training, mentoring and competency assessment programmes to ensure that the workforce is well trained and has the knowledge, skills and abilities to implement NGS.
10. Establish and monitor a set of indicators or milestones to assess the implementation process. Implement a framework for monitoring and evaluation (M&E) to assess the impact of NGS.

The manual also has 17 annexes to help inform the implementation process:

Annex 1: Template of a Gantt chart for an implementation roadmap

Annex 2: NGS implementation high level checklist

Annex 3: Checklists for situational analysis

Annex 4: Example of an NGS situational analysis – the South African experience Annex 5: Budgetary considerations for NGS implementation

Annex 6: List of commercially available NGS instruments Annex 7: Installation checklist and resources

Annex 8: List of essential equipment and reagents required for NGS

Annex 9: Estimated data storage needs based on anticipated NGS workload

Annex 10: Key quality indicators and quality control considerations for NGS workflows

Annex 11: ERLTB-NET Proficiency testing programme for TB NGS

Annex 12: Data and quality indicators for NGS-based DST Annex 13: Examples of NGS-based DST reporting forms

Annex 14: Example TOR for senior NGS scientist, molecular biologist and bioinformatics officer

Annex 15: Suggested agenda for NGS training programmes Annex 16: Competency assessment

Annex 17: Impact measures

References for Annex 3

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Annex 4. Skin tests for tuberculosis infection – detailed description

This annex provides step-by-step procedures for administering and reading two types of skin test for tuberculosis (TB) infection: the tuberculin skin test (TST) and *Mycobacterium tuberculosis* antigen-based skin tests (TBSTs). The photographs used in this annex were kindly provided by Dr Richard Menzies and colleagues.

A1 – Test administration

Step 1. TB Screening check

In an integrated and person-centred TB infection cascade of care, the first encounter with a provider will include initiation of both screening for TB disease and testing for TB infection. Just before administration of the TST or TBST, TB disease screening should be done; it should include a TB symptom check and preferably where available use more sensitive tools recommended by WHO such as chest X-ray (CXR, with or without computer-aided detection), WHO-recommended rapid diagnostics or C-reactive protein for people living with HIV. If a contact has clinical features that suggest TB disease, then that person should be referred immediately for further diagnostic evaluation, which should be done on the same day.

Where a contact has no symptoms or has only non-TB-related respiratory symptoms (e.g. a sore throat or rhinorrhoea, with a duration of only a few days), then TB infection testing can be initiated, and these symptoms can be re-evaluated 48–72 hours later. It is important to aim to minimize loss along the TB infection care cascade. The decision to immediately evaluate an individual with symptoms, or to perform TB infection testing and re-evaluate symptoms at the time of reading, is a matter of clinical judgement.

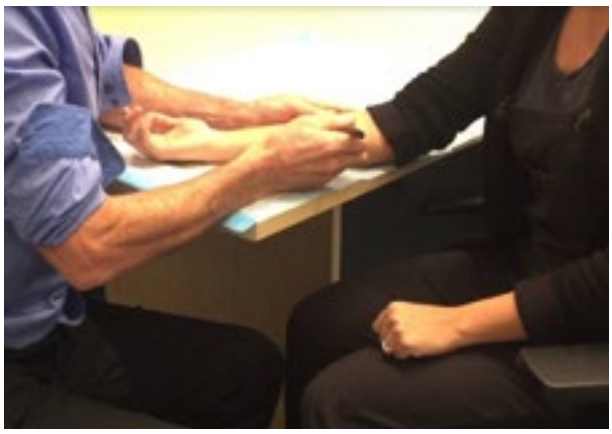
Step 2. Explain the administration of TST or TBST

Signed informed consent is not considered necessary for TB infection testing because this is part of routine care; however, the person being tested should understand the procedures and agree to them. In cases where a child is administered the test, consent by a parent or guardian is required and the presence of the parent or guardian may be necessary. The provider should take time to explain the test procedures and emphasize the need to return for test reading within 72 hours. The provider should verify that the person can return within this time frame for the test to be read; the provider should also respect the individual's confidentiality and privacy.

If an mHealth approach is being used to measure the size of swelling immediately after intradermal injection as a quality control (QC) procedure (mTST) *(1)* or similar) the provider should explain to the patient that photos will be taken of the injection site immediately after the injection.

Step 3. Prepare for the administration of TST or TBST

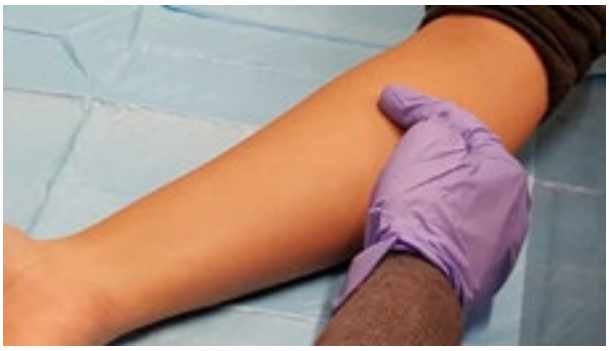
It is important that the person undergoing the TB infection test is comfortable while the test is being administered. The person should be seated with their arm supported on a flat surface, such as the corner of a table, facing the provider who will administer the TB infection test. Where possible, the TB infection test should be administered in a private room, with only the person being tested in attendance (family members are appropriate).



Preparing to perform the injection.
© Richard Menzies

Step 4. Select site for injection

TST and TBST tests are generally administered on the inner aspect of the forearm, about 10 cm below the elbow (middle to upper third of the forearm). The area to be injected should be free of recent cuts, burns or other injuries, and not affected by a rash or eczema. The area should also be free of scarring, particularly keloids. Skin disease or scars may interfere with the injection and proper reading of the result; also, they may cause greater discomfort from the test. The presence of tattoos is not a problem unless erythema also needs to be measured (for C-TST only). If there are such skin problems, then another site should be selected for injection.



Adminster the TST or TBST 10 cm below the elbow.
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Step 5. Prepare the syringe

Draw up the test dose in a 1 mL syringe that has markings to indicate each 0.1 mL (or smaller units). It is important to eliminate air from the syringe, to prevent accidentally injecting air into the patient and to ensure that the full test dose is administered. The syringe should be prepared just before the injection; older studies with standard purified protein derivative (PPDS) demonstrated reduced sensitivity if syringes were prepared more than 20 minutes before injection *(2)*. If the mTST QC programme or similar is in place, the smartphone or digital camera should be prepared, so that the provider is ready to take photos immediately after the injection.

Step 6. Clean the injection site

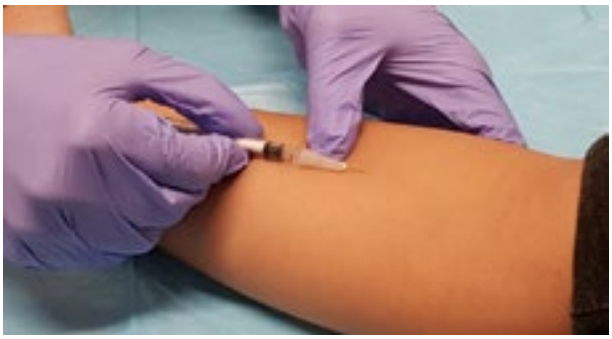
Opinion varies on how best to clean the injection site. Alcohol swabs may be used, but this may cause greater pain unless the alcohol is allowed time to dry completely (it is important not to blow on the site, because this will reinfect the area). Allowing the alcohol to dry completely is especially important when administering the test to a child. Cleaning the area with sterile saline or water is adequate and will cause less stinging if the liquid has not fully dried at the time of injection.

A topical anaesthetic should not be applied, because this can sensitize the skin. There have been case reports of induration resulting from topical anesthetics *(2)*, and that induration will be mistaken for a positive test at the time of reading.

Step 7. Administer the injection

All TB infection skin tests (TST or TBST) are administered using the Mantoux method of intradermal injection. This method of administration was first described for TST many decades ago *(3)*, and the manufacturers of the three TBSTs have all described this same method in their product monographs *(4–6)*. The material must not be injected subcutaneously because this makes it more difficult to measure the result and can even lead to false negative results.

A needle (25 gauge to 27 gauge) is placed on the syringe, which is then laid flat on the skin with the bevelled side upward. The provider then slides the needle under the skin (the tip of the needle is often visible even when it is intradermally). The material is then slowly injected (over 2–3 seconds). In cases where the test material runs out initially, the needle should be pushed in a little deeper. A small induration or papule or bleb ("weal") should form. This bleb should be at least 7 mm in diameter *(1)*. When the injection is finished, the needle should be withdrawn.



Perform an intradermal injection.
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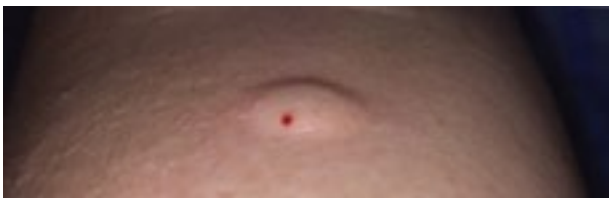
Step 8. After the injection

After the injection, there should be a small induration or bleb on the skin. If there is a little bleeding, this can be wiped away. There is no need to cover the injection site with a dressing or bandage, and no need to mark the spot (having a large circle or an X on the forearm may be stigmatizing, particularly for children).



A small induration should be seen after the injection.
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Step 9. Take photographs (for mHealth – if applicable)

If an mHealth QC programme is in place, then the provider should take several photographs of the injection site. The syringe should be placed with gradation markings visible, 2 cm away from the injection site (towards the elbow). Once the patient has left, the best three photographs should be sent to the supervisor.

Step 10. Provide post-TST care

The patient should remain seated in the clinic area for 10–15 minutes. This is for surveillance in case of allergy or anaphylaxis, which is rare (1 per million) (*25*), or vasovagal fainting, which is much more common than anaphylaxis and can result in injury from an unprotected fall. If an individual feels faint during or after injection, then ensure that the person is protected from falling and immediately record vital signs, particularly blood pressure and heart rate. If the heart rate is rapid and the blood pressure low, and particularly if there are any other signs of anaphylaxis, provide care for a potential anaphylactic reaction. If the heart rate is low (<60 beats per minute), then this is much more likely to be a simple vasovagal faint. Position the patient with their head down and use other manoeuvres effective for vasovagal reaction.

At the time of discharge, educate the patient about care of the injection site; in particular, not to scrub vigorously when washing or to scratch if there is significant itching. If there is any blistering or pain, advise the patient that they can use cold compresses and take nonsteroidal anti-inflammatories for relief.

Step 11. Arrange for the result to be read

Remind the patient of the need to return 48–72 hours later (24–72 hours for C-TST) for the result to be read and provide them with an appointment (with date and time) to come for the reading. Flexibility is important to accommodate patient schedules. Ideally, the reading should be scheduled after 48 hours, so that if the patient is unable to come, the reading can be rescheduled for the next day and still fall within the 72-hour maximum window for reading. The person tested should be aware that the reading is not valid if more than 72 hours have elapsed since administration.

Step 12. Record the result and document details

In the patient's medical record or on specific forms or registries, as appropriate, it is important to record the following details regarding the test administration:

- name of person administering the TST or TBST;
- date and time of administration;
- product used and lot number;
- date on which the product vial was opened (if vials are used over multiple days, based on manufacturer instructions);
- site of injection;
- whether a bleb was seen, and any bleeding or leakage of test fluid;
- any adverse event – if the patient had hypotension or loss of consciousness, it is crucial to document whether this was due to vasovagal reaction or anaphylaxis; and
- date and time of appointment for reading.

A2 – Reading of TST or TBST result

Step 1. Seeing the patient

Patients should be seen as quickly as possible after they arrive for the reading of their TST or TBST. Reading of the test takes less than 3 minutes on average *(7)*. If a person must wait a long time simply for the result to be read, this may discourage other contacts in the same household from coming forward for testing or reading, and may discourage the same person from having a re-test, if needed.

Step 2. Re-check symptoms

If the person had non-TB respiratory symptoms at the time the test was administered, then these symptoms should be re-checked at the time of reading. If the symptoms are less severe or have resolved, then this individual can be considered to have "no symptoms" in the algorithm. However, if symptoms have not improved – and particularly if the symptoms have worsened or are suggestive of TB (e.g. fever or night sweats) – the individual should be considered to have symptoms for investigation. In such cases, the result of the TST or TBST should be read and recorded, but the person should be referred promptly for medical evaluation, including chest X-ray (CXR) – if not already done – and other testing as appropriate, regardless of the result of the test for TB infection.

Step 3. Place and position of patient for reading

Reading should be done in a private room (wherever possible), out of view of all other individuals (although family members can attend, as appropriate). For optimal measurement, the patient should be seated with the arm supported.

Step 4. Inspection of injection site

The site of the injection for the TST or TBST should be carefully inspected. If there is blistering, skin breakdown or lymphadenitis these should be recorded because they are considered to be strong positive reactions for all TB infection skin tests.

Erythema or redness is an indicator of potential induration (if erythema is present, then induration may be present). However, erythema does not need to be measured for TST *(3)*, or for Cy-Tb *(6)*, and only needs to be measured for Diaskintest if there is no induration *(5)*. For C-TST, erythema should be demarcated and measured *(4)*.

Induration or firm swelling can be detected visually or by palpation.

If there is no redness and no visible or palpable induration, then the test is negative and the result is marked as "0 mm". Likewise, if there is erythema but no obvious swelling, and on light palpation there is no evidence of induration, then the induration can be considered negative and "0 mm" can be recorded. If any swelling or induration can be palpated, then this should be demarcated (see Step 5) and measured.

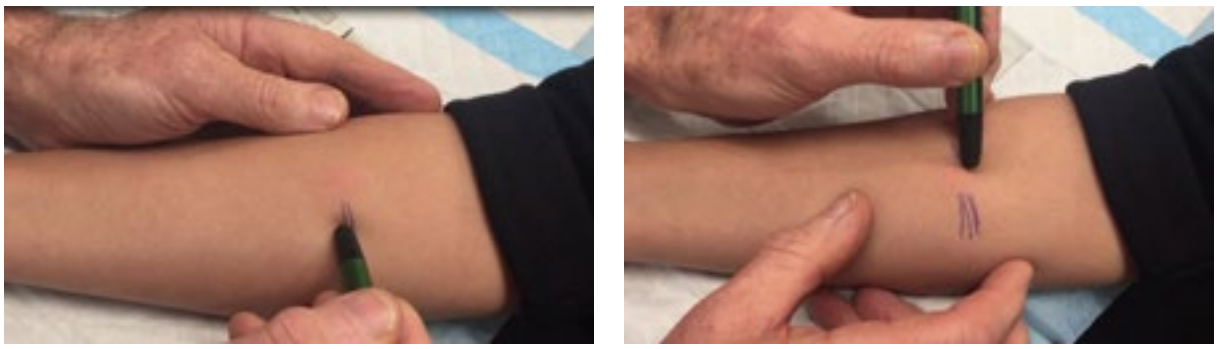
If an mHealth QC programme is in place, then photographs of the injection site should be taken.

Step 5. Demarcation of induration

For TST, Cy-Tb and Diaskintest, the induration is measured *(3, 5, 6)*. For C-TST, both induration and erythema are measured, and the largest of the two is used for clinical management *(4)*.

To demarcate the edges of the induration, the ballpoint pen method can be used *(8)*. With this method, a ballpoint pen tip is pushed gently against the skin at a 45-degree angle towards the site of the injection. If there is a firm and distinct induration, the ballpoint pen tip will consistently stop at the margins. This procedure is repeated several times from different directions around the injection site. If there is no visible or palpable induration, it is not necessary to use a ballpoint pen; the result can be marked as "0 mm".

Large reactions can be painful, and it is not necessary to insist on demarcating and measuring large painful indurations. These can be simply marked as "greater than 15 mm" or "greater than 20 mm" and any blistering or skin necrosis noted.

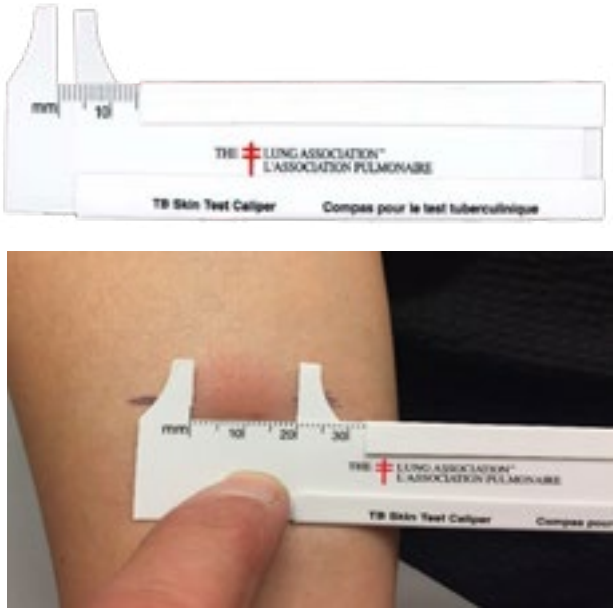


Demarcate the edges of the induration with a ballpoint pen several times from different directions.
© Richard Menzies

Step 6. Measurement

Once the edges of the induration (or, in the case of C-TST, the erythema) have been demarcated, then the diameter of the induration should be measured. For Cy-Tb and TST, the transverse diameter is measured and recorded in millimetres *(3, 6)*. For the C-TST, the transverse and longitudinal diameters of erythema and induration are measured, and the average of each is recorded *(4)*. The largest of these two will be taken as the clinically relevant information. For the Diaskintest, the largest diameter of the induration in any dimension is recorded *(5)*.

Measurement of the size of reaction is often subject to rounding error, because readers



Measure the induration between the demarcations.
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tend to group readings at 5, 10 and 15 mm. To avoid this error, it is a good practice to use machinist calipers or tailor calipers.

Step 7. Post-TST care

If the patient has blistering or skin breakdown, then it is important to prevent secondary infection. The area should be carefully cleaned and covered with a dry dressing. Patients should be instructed not to scratch the area.

Topical steroids should not be used, because these were shown to be ineffective in placebo-controlled trials *(9)*. Subcutaneous injection of steroids under the induration may be more effective, but conservative management with cold compresses and dry dressings to cover the site is usually sufficient and prescription of oral analgesics (aspirin or acetaminophen/paracetamol).

Step 8. Management of results

If the test is negative (as per Chapter 3 of the main text) and the patient is asymptomatic (or if symptoms are resolving), then the patient can be discharged. In some settings, close contacts will have a second TB infection test 8 weeks after the end of exposure if an initial test is negative – particularly in those with very recent exposure or concomitant viral infection. In this case, an appointment should be made for administration of the second test.

If the test is positive the person should be referred immediately for medical evaluation and CXR. In a well-organized person-centred care cascade, medical evaluation (including CXR) is available at the same site and on the same day. This minimizes delays between the first identification of a contact and the initiation of appropriate therapy for TB disease or infection.

Step 9. Recording and registration

The TST or TBST reading should be recorded in the patient's medical record or registries (or both); additional information may be recorded, depending on the setting and programme organization.

The following should be recorded:

- date and time of reading;
- product used and lot number;
- size of the induration in millimetres (transverse, maximal or average diameter, depending on the TST or TBST);
- for C-TST, the average diameter of erythema should also be recorded, and for Diaskintest, erythema (but only if there is no induration); for the TST and Cy-Tb, erythema does not need to be recorded;
- presence of blistering, skin necrosis, lymphangitis or lymphadenopathy; and
- for those with positive tests – disposition: date and time of follow-up for medical evaluation and CXR; for those with negative tests, date and time of follow-up appointment, if appropriate.

Certain articles provide useful general information on skin tests for TB infection *(10, 11)*.

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