WHO operational handbook on tuberculosis

Module 3: Diagnosis

Rapid diagnostics for tuberculosis detection

Third edition

World Health Organization

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WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition

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Abbreviations and acronyms

AFB	acid-fast bacilli
AHD	advanced HIV disease
AIDS	acquired immunodeficiency syndrome
ART	antiretroviral therapy
CC	critical concentration
CI	confidence interval
CSF	cerebrospinal fluid
DNA	deoxyribonucleic acid
DR-TB	drug-resistant tuberculosis
DST	drug susceptibility testing
EQA	external quality assessment
FIND	Foundation for Innovative New Diagnostics
FL-LPA	line probe assay for first-line drugs
GDG	guideline development group
GLI	Global Laboratory Initiative
HIV	human immunodeficiency virus
Hr-TB	isoniazid-resistant, rifampicin-susceptible TB
IT	information technology
LAM	lipoarabinomannan
LAMP	loop-mediated isothermal amplification
LF-LAM	lateral flow lipoarabinomannan assay
LoD	limit of detection
LPA	line probe assay
MDR/RR-TB	multidrug- or rifampicin-resistant tuberculosis
MDR-TB	multidrug-resistant tuberculosis
MGIT	Mycobacterial Growth Indicator Tube
MIC	minimal inhibitory concentration
MoH	ministry of health
MTBC	*Mycobacterium tuberculosis* complex
mWRD	molecular WHO-recommended rapid diagnostic test
NAAT	nucleic acid amplification test
NGS	next-generation sequencing
NTP	national TB programme
NTRL	national TB reference laboratory
PCR	polymerase chain reaction

PLHIV	people living with HIV/AIDS
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	line probe assay for second-line drugs
SOP	standard operating procedure
SRL	supranational reference laboratory
TB	tuberculosis
TWG	technical working group
USA	United States of America
WGS	whole-genome sequencing
WHO	World Health Organization
WHO/GTB	WHO Global TB Programme
WHO PQ	WHO Prequalification Unit
WRD	WHO-recommended rapid diagnostic test
XDR-TB	extensively drug-resistant tuberculosis

Abbreviations of TB agents

AMK	amikacin
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
MFX	moxifloxacin
Pa	pretomanid
PZA	pyrazinamide
REZ	rifampicin (R), ethambutol (E) and pyrazinamide (Z)
RIF	rifampicin
STR	streptomycin

1. Introduction

1.1 Background

Globally, tuberculosis (TB) continues to be a significant public health problem, with an estimated 10.6 million people developing TB in 2022 and 7.5 million reported as being newly diagnosed *(1)*. The gap between the numbers estimated and reported is large, and it worsened during the coronavirus disease (COVID-19) pandemic *(2).* However, there has been a major recovery after the 2 years of disruptions related to COVID-19. Drug-resistant TB (DR-TB) is another area of concern, particularly multidrug- or rifampicin-resistant TB (MDR/RR-TB), which is TB disease caused by *Mycobacterium tuberculosis* complex (MTBC) with resistance to rifampicin (RIF) and isoniazid (INH) or only to RIF. The relative gap was even larger for MDR/RR-TB in 2022, with an estimated 410,00 new cases of MDR/RR-TB, of which only about two in five were known to have been diagnosed and enrolled on treatment *(1)*. In addition, an estimated 1.1 million people had TB disease caused by MTBC with resistance to INH and susceptibility to RIF (referred to as Hr-TB), which is difficult to detect and is therefore largely undetected *(3)*.

The effective management of TB relies on the rapid diagnosis of TB, rapid detection of drug resistance and prompt initiation of an effective treatment regimen. Thus, there is a need for access to fast and accurate detection tests, and rapid and accurate drug susceptibility testing (DST) for all people with TB. In 2023, the first ever WHO standard on universal access to rapid TB diagnostics was issued; it emphasizes the need to undertake a comprehensive approach to diagnostics while following the care cascade and closing all gaps *(4)*. Furthermore, it highlights the need for universal DST: all persons with TB should have initial rapid DST for the detection of resistance to RIF; rapid testing for INH and FQs as required; and follow-on testing for all other drugs that might be included in their treatment regimen ideally performed before treatment is started. However, the initiation of treatment should not be delayed while waiting for DST results; also, efforts to build laboratory capacity (especially DST) should not slow the detection and enrolment of people with DR-TB in care and treatment programmes.

The World Health Organization's (WHO's) global strategy for TB prevention, care and control for 2015–2035 – known as the End TB Strategy *(5)* – calls for the early diagnosis of TB and universal access to DST. To meet the End TB Strategy targets, molecular WHO-recommended rapid diagnostic tests (mWRDs) should be made available to all individuals with signs or symptoms of TB. Individuals with bacteriologically confirmed TB should receive testing for resistance to RIF, those with RR-TB should receive testing for resistance to FQs and those with pre-XDR-TB should receive testing for resistance to bedaquiline and linezolid *(6)*.[1] In 2022, about 73% of

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

people with bacteriologically confirmed TB were tested for RIF resistance and of these 50% were tested for FQ resistance. Recent WHO guidelines stress the importance of DST before treatment, especially for the medicines for which mWRDs are available (e.g. FQs, INH and RIF) but the start of treatment should not be delayed *(7)*.

Furthermore, as described in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (8)*, 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.

Over recent decades, considerable effort has gone into building the laboratory, clinical and programmatic capacity to prevent, detect and treat TB and DR-TB. Many tools and guidance documents have been developed, including guidelines for the detection and treatment of MDR/RR-TB and Hr-TB; rapid tests to detect resistance to RIF, INH, FQs, ethionamide (ETO) and amikacin (AMK); model diagnostic testing algorithms; and guidance for scaling up laboratory capacities to combat DR-TB.

Recently, WHO approved the use of targeted next-generation sequencing (NGS) as a follow-on test for the detection of drug resistance *(7)*. Targeted NGS tests couple the amplification of selected genes with NGS technology to detect resistance to many drugs with a single test. Because targeted NGS tests can interrogate entire genes to identify specific mutations associated with resistance, such tests may provide improved accuracy compared with other WHO-recommended molecular tests. The recommendations include the use of this technology for the rapid detection of resistance to three of the drugs used in the BPaLM regimen (i.e. bedaquiline [BDQ], linezolid [LZD] and moxifloxacin [MFX]); therefore, this updated handbook includes further implementation considerations and algorithms using this technology. The fourth BPaLM drug – pretomanid (Pa) – has recently had criteria established for DST, and these details are also included in this document. 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 *(9)* and for which there are reliable phenotypic DST methods (e.g. BDQ, LZD, Pa, cycloserine [CS], clofazimine [CFZ] and delamanid [DLM]). The addition of a new molecular test for pyrazinamide [PZA] testing in the latest guidelines should facilitate testing for this drug. Also, countries should expand their capacity to monitor the culture conversion of people being treated for DR-TB.

An increasing number of novel tools serve similar purposes; hence, WHO has introduced recommendations that are class based, whereby, instead of evaluating and approving individual products, WHO will recommend a class that represents a group of products with similar characteristics and performances. This approach is expected to increase competitiveness in price, quality and services. The change was introduced in December 2020, with a guideline development group (GDG) recommending three new classes of nucleic acid amplification tests (NAATs).

Linked to this change to class-based recommendations was a joint announcement by the Global TB Programme at WHO (WHO/GTB) and the WHO Prequalification Unit (WHO PQ) on the pathways to a WHO endorsement for new in vitro diagnostics for TB *(10)*. All products will continue to be reviewed by WHO/GTB initially, to determine whether a product warrants a new class or falls within an existing class. In the latter case, the product can proceed to WHO PQ, where both the product and the processes used to manufacture it will be assessed to ensure the test meets performance and quality standards. If a new class is warranted, the product will be assessed as a "first in class" through a GDG process before proceeding to WHO PQ.

1.2 About this guide

This guide was developed to provide practical guidance for the implementation of WHO policies on recommended TB diagnostic tests and algorithms. The guide:

- describes the WHO-recommended tests for detecting TB and DR-TB and the most recent WHO policy guidance for their use, and the processes and steps needed for implementing a diagnostic test for routine use within the TB diagnostic network (Section 2) – important additions in this update are the recommendations for the use of targeted NGS as a follow-on test for detection of drug resistance, newly defined critical concentrations (CCs) for phenotypic DST of Pa and CS, and the updated technical manual for culture-based phenotypic DST;
- describes the steps that need to be taken to implement a new diagnostic tool (Section 3); and
- outlines TB diagnostic model algorithms that incorporate the most recent WHO recommendations for detecting and treating TB and DR-TB *(9)*, and considerations for the implementation of a new algorithm (Section 4).

The 3rd edition is an update of the previous operational handbook *(11)* with relevant chapters updated. A significant change that necessitated the update is the new recommendations on the use of targeted next generation sequencing for the detection of drug resistance *(7)* and this handbook includes operational considerations for implementation. WHO has developed an online e-learning course that complements 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 *(12)*.

This guide is not intended to be a comprehensive manual, nor does it repeat information provided by other guidance documents such as those listed in Annex 4; rather, the guide provides references and links to original resources.

The most up-to-date WHO policy guidance on TB diagnostics and laboratory strengthening can be found in the most recent edition of the consolidated guidelines on this topic *(13)*. Guidance on the implementation of diagnostic testing is also available on the website of the Global Laboratory Initiative (GLI) of the Stop TB Partnership *(14)*.

1.3 Target audience

The target audience for this guide includes ministry of health (MoH) officials, donors, implementing partners, programme managers, laboratory managers, clinicians and other key stakeholders engaged in TB laboratory strengthening or programme support.

2. Diagnostic tests with WHO recommendations

This section provides brief descriptions of WHO-recommended technologies for the detection of TB and DR-TB, summarizes WHO recommendations for such technologies and refers to the *WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection (7)* for a thorough discussion of the technologies and recommendations.

The guidelines updated in 2021 *(7)* added three new classes of NAAT technologies, as shown in Table 2.1. The most recent update added a fourth new class of technology – targeted NGS.

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

Technology class	Products included in the evaluation
	Xpert® MTB/RIF and Xpert® MTB/RIF Ultra (Cepheid)[a]
	Truenat™ (Molbio)[a]
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid	Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) BD MAX™ MDR-TB (Becton Dickinson) cobas® MTB and cobas MTB-RIF/INH (Roche) FluoroType® MTBDR and FluoroType® MTB (Hain Lifescience/Bruker)
	TB-LAMP (Eiken)[a]
Antigen detection in a lateral flow format (biomarker-based detection)	Alere Determine™ TB LAM Ag (Alere)
Low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents	Xpert® MTB/XDR (Cepheid)
LPAs	GenoType® MTBDRplus v1 and v2, and GenoType® MTBDRsl (Hain Lifescience/Bruker) Genoscholar™ NTM+MDRTB II and Genoscholar™ PZA-TB II (Nipro)
Targeted NGS	Deeplex® Myc-TB test (Genoscreen) AmPORE TB test (Oxford Nanopore Diagnostics) TBseq® test (Hangzhou ShengTing Medical Technology Co)

LPA: line probe assay; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; TB: tuberculosis.

[a] These recommendations are currently product specific but will be changed to class-based to align with the other recommendations.

The change from product-specific recommendations (e.g. Xpert MTB/RIF or Truenat MTB) to class-based recommendations (e.g. low complexity automated NAATs) was introduced in 2020. We are currently in a transitionary period, during which the previous product-based recommendations will be integrated into the new classes.

The WHO-recommended tests have also been reorganized in this document to clearly delineate their intended use, as per the recommendations. The new organizational structure is as follows:

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

The initial tests for the diagnosis of TB are broadly grouped as WRDs; these are defined as diagnostic tests that employ molecular- or biomarker-based techniques for the diagnosis of TB *(15)*. The newer, rapid and sensitive molecular tests recommended for the initial detection of MTBC and drug resistance are designated as mWRDs; they include Xpert MTB/RIF Ultra and Xpert MTB/RIF (Cepheid, Sunnyvale, United States of America [USA]), Truenat MTB, MTB Plus and MTB-RIF Dx tests (Molbio Diagnostics, Goa, India) and loop-mediated isothermal amplification (TB-LAMP; Eiken Chemical, Tokyo, Japan).

Also included as mWRDs are the new class of NAATs; that is, the automated moderate complexity NAATs, which detect not only MTBC and RIF resistance but also INH resistance. The four products evaluated and included in this class are Abbott RealTi*m*e MTB and MTB RIF/INH assays (Abbott Laboratories, Abbott Park, USA), the BD MAX MDR-TB assay (Becton, Dickinson and Company, Franklin Lakes, USA), the Hain FluoroType MTBDR assay (Bruker/Hain Lifescience, Nehren, Germany), and the Roche cobas MTB and MTB-RIF/INH assays (Hoffmann-La Roche, Basel, Switzerland).

In addition, the biomarker-based lateral flow lipoarabinomannan assay (LF-LAM) test (Alere Determine TB LAM Ag, USA) is recommended to assist in diagnosing TB in selected groups of people living with HIV/AIDS (PLHIV) presumed to also have TB. A positive LF-LAM result is considered to be bacteriological confirmation of TB in these people *(15)*, and this test is also included as a WRD. A negative result does not rule out TB; therefore, LF-LAM should be implemented in parallel with mWRD testing among PLHIV.

WHO has reviewed and recommended each of these tests, and has developed recommendations for their use. In all settings, WHO recommends that rapid techniques be used as the initial diagnostic test to detect MTBC and RIF resistance, to minimize delays in starting appropriate treatment. Follow-on testing for resistance to INH and FQ is important.

Follow-on tests to detect drug resistance include:

- line probe assays (LPAs) for detection of resistance to RIF and INH (GenoType MTBDRplus, Bruker/Hain Lifescience, Nehren, Germany; NTM+MDRTB Detection Kit, Nipro Corporation, Osaka, Japan), and to FQs and second-line injectable agents (GenoType MTBDRsl, Bruker/Hain Lifescience, Nehren, Germany);
- low complexity automated NAATs for the detection of resistance to INH, FQs, ETO and AMK (first in class: Xpert MTB/XDR [Cepheid, Sunnyvale, USA]);

- high complexity reverse hybridization NAAT for the detection of resistance to PZA (first in class: Genoscholar PZA-TB II [Nipro Corporation, Osaka, Japan]); and
- targeted NGS tests, which couple amplification of selected genes with NGS technology to detect mutations associated with resistance to many anti-TB medicines with a single test (first in class: Deeplex® Myc-TB [GenoScreen] for RIF, INH, PZA, ethambutol [EMB], FQ, BDQ, LZD, CFZ, AMK and streptomycin [STR]; AmPORE TB [Oxford Nanopore Technologies] for RIF, INH, FQ, LZD, AMK and STR; and TBseq® [ShengTing Biotech] for EMB).

2.1 Conventional diagnostic tests for the diagnosis of TB

In many high TB burden settings, sputum-smear microscopy remains the primary diagnostic technique for evaluating individuals presenting with the signs and symptoms of TB. However, sputum-smear microscopy is a relatively insensitive test, with a limit of detection (LoD) of 5000–10 000 bacilli per millilitre of sputum. Furthermore, sputum-smear microscopy cannot distinguish drug-susceptible strains from drug-resistant strains. WHO recommends that TB programmes transition to replacing microscopy as the initial diagnostic test with mWRDs that detect MTBC.

The current gold standard method for the bacteriological confirmation of TB is culture using commercially available liquid media. However, culture is not used as a primary diagnostic test in many high-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). Nonetheless, conventional microscopy and culture remain necessary to monitor a person's response to treatment. Culture is still important in the diagnosis of paediatric and extrapulmonary TB from paucibacillary samples, and in the differential diagnosis of non-tuberculous mycobacteria (NTM) infection.

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

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 (Web Annex C). Reliable phenotypic DST methods are available for RIF, INH, FQs, PZA, BDQ, LZD, AMK, STR CFZ, DLM, Pa and CS. Phenotypic DST is not recommended for EMB, ETO, prothionamide, para-aminosalicylic acid, imipenem-cilastatin and meropenem.

2.2 Initial tests for diagnosis of TB with drug-resistance detection

2.2.1 Xpert MTB/RIF assay

The Xpert MTB/RIF assay is a cartridge-based automated test that uses realtime polymerase chain reaction (PCR) on the GeneXpert platform to identify MTBC and mutations associated with RIF resistance directly from sputum specimens in less than 2 hours. WHO recommends using the Xpert MTB/RIF test in the following situations (*7*):

- In adults with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and detection of RIF resistance rather than smear microscopy, culture and phenotypic DST.
- In children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used in sputum, gastric aspirate, nasopharyngeal aspirate or stool specimens as the initial diagnostic test for TB and detection of RIF resistance detection, rather than smear microscopy or culture and phenotypic DST.
- In adults and children with signs and symptoms of TB meningitis, Xpert MTB/RIF should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for TB meningitis rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF may be used in lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine specimens as the initial diagnostic test for the corresponding form of extrapulmonary TB rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF should be used for detection of RIF resistance rather than culture and phenotypic DST.
- In HIV-positive adults and children with signs and symptoms of disseminated TB, Xpert MTB/RIF may be used in blood, as a diagnostic test for disseminated TB.
- In children with signs and symptoms of pulmonary TB in settings with pretest probability below 5% and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF in sputum, gastric fluid, nasopharyngeal aspirate or stool specimens may *not* be used.
- In children with signs and symptoms of pulmonary TB in settings with pretest probability of 5% or more and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF (for a total of two tests) in sputum, gastric fluid, nasopharyngeal aspirate and stool specimens *may* be used.

Note: The Xpert MTB/RIF has been superseded by the Xpert MTB/RIF Ultra. The product has been discontinued globally and will not be available in 2024 except in a few selected countries (e.g. India and the USA).

2.2.2 Xpert MTB/RIF Ultra assay

The Xpert MTB/RIF Ultra assay (hereafter called Xpert Ultra) uses the same GeneXpert platform as the Xpert MTB/RIF test, and was developed to improve the sensitivity and reliability of detection of MTBC and RIF resistance. To address sensitivity, Xpert Ultra uses two multicopy amplification targets (IS6110 and IS1081) and a larger PCR chamber; thus, Xpert Ultra has a lower LoD than

Xpert MTB/RIF (16 colony forming units [cfu]/mL and 131 cfu/mL, respectively). At very low bacterial loads, Xpert Ultra can give a "trace" result, which is not based on amplification of the *rpoB* target and therefore does not give results for RIF susceptibility or resistance. An additional improvement in the Xpert Ultra is that the analysis is based on melting temperature (Tm), which allows for better differentiation of resistance-conferring mutations. Planning the transition to the Xpert Ultra requires special consideration and a GLI document is available to assist in this process *(10)*. WHO recommends using the Xpert Ultra test in the following situations *(7)*:

- In adults with signs and symptoms of pulmonary TB without a prior history of TB or with a remote history of TB treatment (>5 years since end of treatment), Xpert Ultra should be used as the initial diagnostic test for TB and for detection of RIF resistance rather than smear microscopy or culture and phenotypic DST.
- In adults with signs and symptoms of pulmonary TB and a prior history of TB with an end of treatment within the past 5 years, Xpert Ultra may be used as the initial diagnostic test for TB and for detection of RIF resistance rather than smear microscopy or culture and phenotypic DST.
- In children with signs and symptoms of pulmonary TB, Xpert Ultra should be used as the initial diagnostic test for TB rather than smear microscopy or culture in sputum or nasopharyngeal aspirates.
- In adults and children with signs and symptoms of TB meningitis, Xpert Ultra should be used in CSF as an initial diagnostic test for TB meningitis rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra may be used in lymph node aspirate and lymph node biopsy as the initial diagnostic test for the detection of lymph node TB, rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra should be used for detection of RIF resistance rather than culture and phenotypic DST.
- In adults with signs and symptoms of pulmonary TB who have an Xpert Ultra trace positive result on the initial test, repeated testing with Ultra may *not* be used.
- In children with signs and symptoms of pulmonary TB in settings with a pretest probability of less than 5% and an Xpert Ultra negative result on the initial test, repeated testing with Xpert Ultra in sputum or nasopharyngeal aspirate specimens may *not* be used.
- In children with signs and symptoms of pulmonary TB in settings with a pretest probability of 5% or more and an Xpert Ultra negative result on the first initial test, a repeat of one Xpert Ultra test (for a total of two tests) in sputum and nasopharyngeal aspirate specimens may be used.

2.2.3 Truenat MTB, MTB Plus and MTB-RIF Dx assays

The Truenat MTB and MTB Plus assays use chip-based realtime micro-PCR for the semiquantitative detection of MTBC directly from sputum specimens and can report results in under an hour. The assays use automated, battery-operated devices to extract, amplify and detect specific genomic DNA loci.

The assays are designed to be operated in peripheral laboratories with minimal infrastructure and minimally trained technicians, although micropipetting skills are required. A practical guide is available to assist implementers considering these tests *(16)*.

If a positive result is obtained with the MTB or MTB Plus assay, an aliquot of extracted DNA is run on the Truenat MTB-RIF Dx assay to detect mutations associated with RIF resistance. WHO recommends using Truenat MTB, MTB Plus and MTB-RIF Dx tests in the following situations *(7)*:

- In adults and children with signs and symptoms of pulmonary TB, the Truenat MTB or MTB Plus may be used as an initial diagnostic test for TB rather than smear microscopy or culture.
- In adults and children with signs and symptoms of pulmonary TB and a Truenat MTB or MTB Plus positive result, Truenat MTB-RIF Dx may be used as an initial test for RIF resistance rather than culture and phenotypic DST.

Notes:

- These recommendations apply to the use of the test with sputum specimens from PLHIV, based on extrapolation of the data on test performance with smear-negative sputum specimens.
- These recommendations apply to the use of the test with sputum specimens from children, based on extrapolation of the data from adults, although the test is expected to be less sensitive in children.

2.2.4 Moderate complexity automated NAATs

The moderate complexity automated NAATs class of tests includes rapid and accurate tests for the detection of pulmonary TB from respiratory samples. Overall pooled sensitivity for TB detection was 93.0% (95% confidence interval [CI]: 90.9–94.7%) and specificity 97.7% (95% CI: 95.6–98.8%) (Tables 3.1–3.4 in Section 3). Moderate complexity automated NAATs are also able to 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 large population density or high TB prevalence. However, this class of tests is primarily for laboratory settings, and will require a reliable and rapid system for sample referral and result reporting. Moderate complexity automated NAATs may already be used programmatically for other diseases (e.g. severe acute respiratory syndrome coronavirus 2 [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 *(17)*.

WHO has made the following recommendation *(7)*:

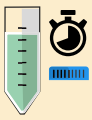
- In people with signs and symptoms of pulmonary TB, moderate complexity automated NAATs may be used on respiratory samples for detection of pulmonary TB, RIF resistance and INH resistance, rather than culture and phenotypic DST.

Notes:

- 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 PLHIV (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.

Fig. 2.1. Summary of testing procedures for the newly endorsed moderate complexity automated NAATs

	Abbott RealTime MTB and MTB RIF/INH	**BD MAX MDR-TB**	**Bruker-Hain FluoroType MTBDR**	**Roche cobas MTB and MTB-RIF/INH**
Maximum # of samples per run	N = 94	N = 24	N = 94	N = 94
Specimen reception to results out, measured for 24 samples	**7 hrs**	**4.6 hrs**	**4.5 hrs**	**5.5 hrs**
Inactivation 	3:1 IR ↓ Invert several times ↓ Vortex 20/30 sec	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 sec
Incubation 	1 h ↓ Vortex 20/30 sec at 20 min	25 min	30 min	1 h
DNA extraction, amplification & detection 	DNA extraction 4 h 22 min m2000 sp ↓ Seal plate and transfer to instrument ↓ Amplification and detection 2 h 30 min	3 h 41 min run time to results	DNA extraction 2 h GXT 96 ↓ Seal plate and transfer to instrument ↓ Amplification and detection 1 h 41 min	Sonicate 1 min per sample ↓ Centrifuge 60 sec ↓ 2 h 30 min run time to results

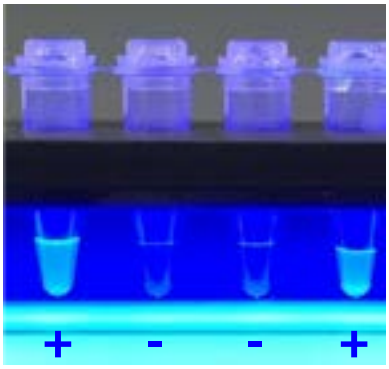
DNA: deoxyribonucleic acid; NAAT: nucleic acid amplification test.

Source: Sengstake & Rigouts (2020) *(18)*.

2.3 Initial tests for diagnosis of TB without drug-resistance detection

2.3.1 TB-LAMP assay

The TB-LAMP assay is designed to detect MTBC directly from sputum specimens. This is a manual assay that provides results in less than 1 hour, does not require sophisticated instrumentation and can be used at the peripheral health centre level, given biosafety requirements similar to those for sputum-smear microscopy. TB-LAMP does not detect resistance to anti-TB drugs. For the detection of TB in adults with signs and symptoms consistent with pulmonary TB, TB-LAMP has demonstrated a sensitivity of 78% (95% credible interval [CrI]: 71–83%) and a specificity of 98% (95% CrI: 96–99%) as compared with a microbiological reference standard.



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WHO has made the following recommendations *(7)*:

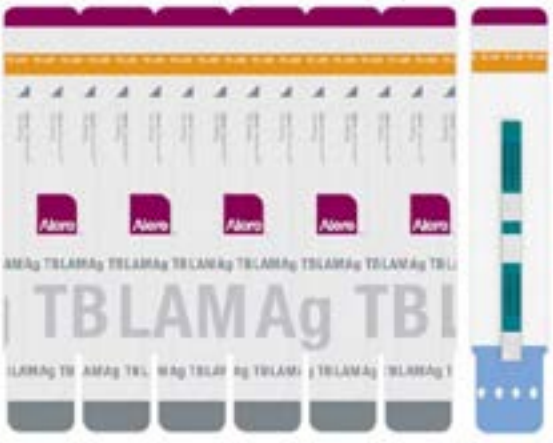
- TB-LAMP may be used as a replacement test for sputum-smear microscopy for diagnosing pulmonary TB in adults with signs and symptoms consistent with TB.
- TB-LAMP may be used as a follow-on test to smear microscopy in adults with signs and symptoms consistent with pulmonary TB, especially when further testing of sputum-smear-negative specimens is necessary.

Notes:

- Because TB-LAMP does not provide any information on RIF resistance, TB-LAMP should not replace the use of rapid molecular tests that detect both MTBC and RIF resistance, especially among populations at risk of MDR-TB.
- TB-LAMP should also not replace the use of rapid molecular tests that have a higher sensitivity for the detection of TB among PLHIV who have signs and symptoms consistent with TB.

2.3.2 Urine LF-LAM

The urine LF-LAM is an immunocapture assay based on the detection of the mycobacterial LAM antigen in urine; it is a potential point-of-care test for certain populations being evaluated for TB. Although the assay lacks sensitivity, it can be used as a fast, bedside, rule-in test for HIV-positive individuals, especially in urgent cases where a rapid TB diagnosis is critical for the person's survival. The Alere Determine TB LAM Ag is currently the only commercially available urine LF-LAM test endorsed by WHO. The detection of mycobacterial LAM antigen in



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urine does not provide any information on drug resistance. A document is available addressing practical considerations for the implementation of the LF-LAM *(19)*.

WHO recommends using urine LF-LAM in the following situations *(7)*:

- In inpatient settings, WHO recommends using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:
  - with signs and symptoms of TB (pulmonary or extrapulmonary);
  - with advanced HIV disease;
  - who are seriously ill; or
  - who have a CD4 cell count of less than 200 cells/mm^3, irrespective of signs and symptoms of TB.
- In outpatient settings, WHO suggests using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children who:
  - have signs and symptoms of TB (pulmonary or extrapulmonary);
  - are seriously ill; or
  - have a CD4 cell count of less than 100 cells/mm^3, irrespective of signs and symptoms of TB.
- In outpatient settings, WHO recommends against using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:
  - who have not been assessed for TB symptoms; or
  - without TB symptoms and with an unknown CD4 cell count or with a CD4 cell count greater than 100 cells/mm^3.

Notes:

- For their initial diagnostic test, anyone with signs and symptoms of pulmonary TB who is capable of producing sputum should have at least one sputum specimen submitted for an mWRD assay. 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 triage test.

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

2.4.1 Low complexity automated NAATs for detection of resistance to INH and second-line anti-TB drugs

The first-in-class product for low complexity automated NAATs for detection of resistance to INH and second-line anti-TB drugs is the Xpert MTB/XDR Assay (Cepheid, Sunnyvale, USA). This test uses a cartridge designed for the GeneXpert instrument to detect resistance to INH, FQs, ETO and second-line injectable drugs (AMK, kanamycin and capreomycin). However, unlike Xpert MTB/RIF and Xpert MTB/RIF Ultra, which are performed on a GeneXpert instrument that can detect six colours, the new test requires a 10-colour GeneXpert instrument. 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 MTB/RIF and Ultra cartridge use on GeneXpert 6-colour instruments are also valid for their use on GeneXpert 10-colour instruments *(20)*.

The low complexity automated NAAT 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 intermediate and even peripheral laboratories. The Xpert MTB/XDR test provides results in less than 90 minutes, leading to faster time to results than the current standard of care (i.e. 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 directly on clinical specimens. Web Annex A includes an information sheet summarizing this test.

WHO recommends the use of low complexity automated NAATs for detection of resistance to INH and second-line anti-TB drugs in the following situations *(7)*:

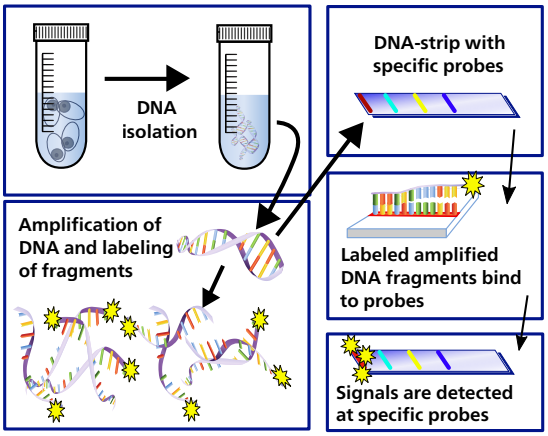
- In people with bacteriologically confirmed pulmonary TB, low complexity automated NAATs in sputum may be used for initial detection of resistance to INH and FQs, rather than culture-based phenotypic DST.
- In people with bacteriologically confirmed pulmonary TB and resistance to RIF, low complexity automated NAATs in sputum may be used for initial detection of resistance to ETO, rather than DNA sequencing of the *inhA* promoter.
- In people with bacteriologically confirmed pulmonary TB and resistance to RIF, low complexity automated NAATs in sputum may be used for initial detection of resistance to AMK, rather than culture-based phenotypic DST.

Notes:

- These 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 for adolescents and children based on the generalization of data from adults.
- The recommendations apply to PLHIV (studies included a varying proportion of such people). 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 recommendations exist for similar technologies for use on non-sputum samples (e.g. Xpert MTB/RIF and Xpert Ultra).

2.4.2 LPAs

LPAs are a family of DNA strip-based tests that detect mutations associated with drug resistance. They do this either directly, through binding DNA amplification products (amplicons) to probes targeting the most commonly occurring mutations (MUT probes), or indirectly, inferred by the lack of binding the amplicons to the corresponding wild-type probes.



First-line LPAs

First-line LPAs (FL-LPAs) such as GenoType MTBDR*plus* and NTM+MDRTB Detection Kit allow the detection of resistance to RIF and INH. WHO recommends using FL-LPAs in the following situations *(7)*:

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

Notes:

- These recommendations apply to the use of FL-LPAs for testing smear-positive sputum specimens (direct testing) and cultured isolates of MTBC (indirect testing) from both pulmonary and extrapulmonary sites.
- Conventional culture-based phenotypic DST for INH may still be used to evaluate a person with TB when the LPA result does not detect INH resistance. This is particularly important for populations with a high pretest probability of resistance to INH.
- FL-LPAs are not recommended for the direct testing of sputum-smear-negative specimens for the detection of MTBC.

Second-line LPAs

Second-line LPAs (SL-LPAs) such as the GenoType MTBDR*sl* test allow the detection of resistance to FQs and AMK. WHO recommends using SL-LPAs in the following situations *(7)*:

- For a person with confirmed MDR/RR-TB, an SL-LPA may be used as the initial test, instead of phenotypic DST, to detect resistance to FQs and AMK.

Notes:

- This recommendation applies to the use of SL-LPA for testing sputum specimens, irrespective of the smear status, and cultured isolates of MTBC from both pulmonary and extrapulmonary sites.
- Culture-based phenotypic DST may be useful in evaluating people with negative SL-LPA results, particularly in populations with a high pretest probability for resistance to FQs or AMK.
- SL-LPA tests are also useful for detecting FQ resistance before starting therapy for Hr-TB.

2.4.3 High complexity reverse hybridization NAAT

The first-in-class product for this class 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 a large number of hybridization probes to cover the full *pncA* gene (>700 base pairs [bp]). Reading the hybridization results on the crowded strips with a total of 48 probes requires careful attention to avoid errors. However, it 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%) *(7)*. The hybridization can be performed on the TwinCubator instruments (Hain Lifescience, Germany) that are used for LPAs *(18)*. An information sheet summarizing high complexity reverse hybridization NAATs is available in Web Annex A.

WHO recommends the use of high complexity reverse hybridization NAATs in the following situations *(7)*:

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

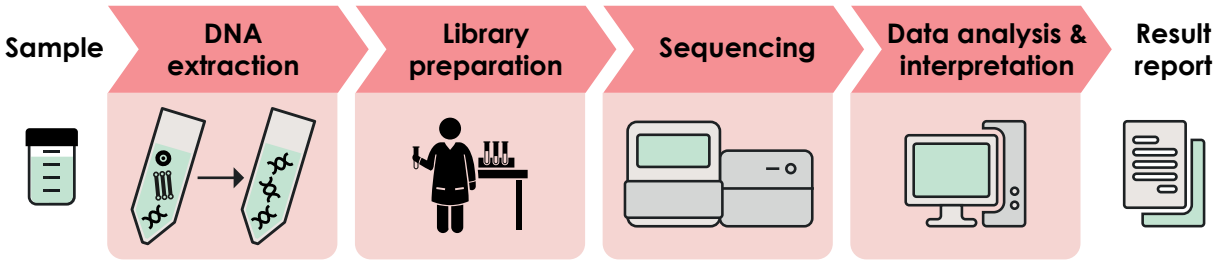
Note:

- 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 tests

Targeted NGS tests couple amplification of selected genes with NGS technology to detect resistance to many drugs with a single test. Because targeted NGS tests can interrogate entire genes to identify specific mutations associated with resistance, targeted NGS tests may provide improved accuracy compared with other WRDs. In addition, new targeted NGS tests can detect resistance to new and repurposed drugs not currently included in any other molecular assays. Thus, targeted NGS tests offer great potential to provide comprehensive resistance detection matched to modern treatment regimens *(21)*.

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



DNA: deoxyribonucleic acid; NGS: next-generation sequencing.

The class of targeted NGS tests was defined as tests that use massively parallel sequencing to detect resistance to TB drugs, starting from a processed clinical sample and ending with an end-user report that relates detected *M. tuberculosis* mutations to the presence (or absence) of drug resistance, based on the interpretation of a standard catalogue of mutations (Fig. 2.2). The first-in-class products include Deeplex® Myc-TB (GenoScreen) for RIF, INH, PZA, EMB, FQ, BDQ, LZD, CFZ, AMK and STR; AmPORE TB (Oxford Nanopore Technologies) for RIF, INH, FQ, LZD, AMK and STR; and TBseq® (ShengTing Biotech) for EMB. Where a product has not yet met the requirements for a specific drug (i.e. the drug is not currently listed), further improvements to the product and a review of the evidence will be necessary before that drug can be put into clinical use.

Among people with bacteriologically confirmed pulmonary TB, the targeted NGS test performances (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, moxifloxacin (MFX) and EMB; more than 93% for RIF and levofloxacin (LFX); and 88% for PZA. 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 9% (LFX and MFX) 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 between low and moderate for test accuracy.

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, 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.

WHO recommends the use of targeted NGS tests as follow-on tests to detect drug resistance in the following situations *(7)*:

- In people with bacteriologically confirmed pulmonary TB disease, targeted NGS tests may be used on respiratory samples to diagnose resistance to RIF, INH, FQ, PZA and EMB rather than phenotypic DST.
- In people with bacteriologically confirmed RIF-resistant pulmonary TB disease, targeted NGS tests may be used on respiratory samples to diagnose resistance to INH, FQ, BDQ, LZD, CFZ, PZA, EMB, AMK and STR rather than phenotypic DST.

Notes:

- The targeted NGS tests do not replace existing rapid tests that are more accessible and easier to perform for detecting resistance to RIF, INH and FQ. However, if targeted NGS tests can be performed rapidly, they can be considered as an alternative initial option for prioritized populations. Those who will benefit most from these tests are individuals requiring rapid and comprehensive DST, where there is limited access to phenotypic DST.

- The usefulness of the targeted NGS tests depends on the information provided in the WHO catalogue of mutations *(4)*, which allows for interpretation of resistance data. Regular updating of that catalogue – incorporating additional genetic targets, including new drugs (e.g. Pa) – is needed to enhance the sensitivity and specificity of targeted NGS tests.
- An implementation consideration is that the indeterminate rate of the currently available targeted NGS tests ranged from 9% to 21%, and was higher in samples with low bacillary load. Priority should be given to testing samples with a high bacillary load, as determined by initial bacteriological tests (e.g. semiquantitative grading: high or medium, or smear-positive). In situations where the bacillary load is low (e.g. semiquantitative grading: low, very low or trace, or smear-negative), the recommendations still hold, while acknowledging the higher rates of indeterminate results. Annex 3 provides additional resources guiding the implementation of targeted NGS.

2.5 Tests WHO recommends against using

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 serologic 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, which may have an adverse impact on people's health *(22)*.

WHO recommendations are specific for intended uses and, sometimes, even a recommended test is not recommended to be used for a specific purpose. For example, NAATs (e.g. Xpert MTB/RIF, Xpert Ultra or Truenat) are not recommended for use in monitoring the response to treatment. Also, in outpatient settings, WHO recommends against using LF-LAM to assist in the diagnosis of active TB in:

- HIV-positive adults, adolescents and children without first assessing TB symptoms;
- HIV-positive people without TB symptoms and with an unknown CD4 cell count, or without TB symptoms and a CD4 cell count greater than or equal to 100 cells/mm^3; and
- HIV-negative individuals.

Similarly, WHO recommends that, in low- and middle-income countries, interferon-gamma-release assays may be used to aid in the detection of latent TB infection. However, such tests should not be used for the diagnosis of pulmonary or extrapulmonary TB, or for the diagnostic work-up of adults (including HIV-positive individuals) suspected of active TB.

2.6 Phenotypic and genotypic DST

2.6.1 Phenotypic DST

Treatment of TB has undergone significant changes over recent years, with new drugs and regimens recommended; hence, the definitions for DR-TB have been revised accordingly. The updated pre-extensively drug-resistant TB (pre-XDR-TB) definition is "TB caused by *M. tuberculosis* strains that fulfil the definition of MDR/RR-TB and are also resistant to any

FQ", and the updated XDR-TB definition 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)" *(23)*. 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.

In 2018, WHO updated the CCs used in phenotypic DST to include the new and repurposed drugs for existing methods *(24).* In 2023, the CCs for Pa and cycloserine were established (Web Annex B). The updated manual on culture-based DST for medicines used in the treatment of TB is included at Web Annex C. The updated DST manual describes the WHO recommendations for phenotypic and genotypic DST for anti-TB medicines. Also, the manual provides CCs to be used in solid and liquid media for phenotypic DST of medicines used to treat drug-susceptible TB and DR-TB, sources of pure powders for phenotypic DST, detailed methods for preparing drug-containing media, interpretation and reporting of results, and quality control (QC).

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 available from Becton Dickinson, and are expected to be listed in the Global Drug Facility (GDF) catalogue in 2024. In addition, the BDQ pure drug substance for use in phenotypic DST is provided free through the US National Institutes of Health (NIH) HIV Reagent Program *(25)*; however, courier costs need to be covered. An information note that explains the request process is also available *(26)*. In 2024, the HIV Reagent Program will also provide DLM and Pa. LZD powder is available from Sigma (PZ0014–5MG) or Cayman Chemical (CAS 65800–03–3). The updated DST manual in Web Annex C provides specific details on the sources of the drug powders used for phenotypic DST.

CCs for the rifamycins and INH were also reviewed, and findings were published in 2021 (*27*). The CCs for INH have remained the same, whereas those for RIF in MGIT and 7H10 have been revised downwards, from 1.0 mg/L to 0.5 mg/L. These revisions are expected to reduce but not eliminate discordance between genotypic and phenotypic DST results, and laboratories are advised to implement these changes immediately. For MGIT, this can be achieved by reconstituting the lyophilized RIF from the BD SIRE kit in 8 mL (instead of 4 mL) of sterile distilled or deionized water. The sources of other pure drug substances for DST are described in Table 5 in Web Annex C.

The latest CCs for all drugs are listed in Tables 2.2 and 2.3; they were adapted from Tables 2 and 3 of Web Annex C.

Table 2.2. CCs 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; INH: isoniazid; LPA: line probe assay; MGIT: Mycobacterial Growth Indicator Tube; MTBC: *Mycobacterium tuberculosis* complex; NAAT: nucleic acid amplification test; PZA: pyrazinamide; TB: tuberculosis.

[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 rifampicin resistance using the BACTEC MGIT 960 system has limitations and 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 rifampicin resistance.

[c] People with MTBC isolates that are resistant at the CC may be effectively treated with high-dose isoniazid. 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. To date, no clinical breakpoint concentration has been established for INH.

[d] All phenotypic DST methods for ethambutol produce inconsistent results. Phenotypic DST is not recommended.

[e] BACTEC MGIT 960 liquid culture method for PZA susceptibility testing is reportedly associated with a high rate of false positive resistance results. Careful inoculum preparation is essential for performing PZA testing reliably. The Genoscholar PZA-TB LPA is the only high complexity hybridization NAAT recommended for PZA. 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.

Source: adapted from Table 2 in Web Annex C.

Table 2.3. CCs and clinical breakpoints for medicines recommended for the treatment of MDR/RR-TB (adapted from Table 3 in Web Annex C)

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/Tad[ic]	–	–	–	16
Group C	Ethambutol[d]	E	2.0	5.0	7.5	5.0
	Delamanid[e]	DLM	–	–	0.016	0.06
	Pyrazinamide[f]	PZA	–	–	–	100.0
	Imipenem-cilastatin Meropenem	IMP/CLN MPM	– –	– –	– –	– –
	Amikacin (Streptomycin)[g]	AMK (STR)	30.0 4.0	2.0 2.0	– 2.0	1.0 1.0
	Ethionamide Prothionamide	ETO PTO	40.0 40.0	5.0 –	10.0 –	5.0 2.5
	Para-aminosalicylic acid	PAS	–	–	–	–

Group	Medicine	Abbreviation	CCs (µg/mL) for DST by medium			
			Löwenstein–Jensen	Middlebrook 7H10	Middlebrook 7H11	BACTEC MGIT liquid culture
Other	Pretomanid	Pa				0.5 2[h]

ATU: area of technical uncertainty; 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.

[a] LFX and MFX CCs for LJ 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 terizidone resistance.

[d] DST not reliable and reproducible. 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.

2.6.2 Genotypic DST

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 these obstacles. Currently, available WHO-recommended rapid molecular drug susceptibility tests (Section 2.4) can be used to detect specific mutations known to confer phenotypic resistance. Although genotypic DST has advanced over the past decade, the spectrum of drugs covered is limited and does not cover the new and repurposed drug. However, rapid molecular tests for resistance to RIF, INH and FQ are feasible to implement in decentralized settings; such tests can deliver rapid results to inform initial treatment regimen selection while awaiting follow-on DST for other anti-TB drugs.

New and rapid next-generation technologies are thus needed to cover all priority drugs and to be accessible at the peripheral level, to expedite appropriate therapy and improve patient outcomes. WHO has developed a target product profile (TPP) to guide research and development to address this need *(28)*.

DNA sequencing using NGS technologies is a promising method for the rapid detection of mutations associated with drug resistance for many anti-TB drugs *(29)*. NGS-based DST could reduce the need for phenotypic DST for patient-care decisions, and it may be particularly useful for drugs for which phenotypic DST is unreliable or in 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. WGS is used on cultured MTBC isolates whereas targeted NGS can be used directly on sputum specimens *(30)*. In Section 2.4.4, the targeted NGS tests for the detection of resistance to anti-TB medicines recently recommended by WHO are described 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.

One important issue identified when using NGS tests was the lack of a standardized and robust single reference source for the interpretation of mutations. To address this need, WHO developed guidance and released the second edition of the catalogue of mutations in MTBC and their association with resistance, in 2023 *(4)*. The catalogue provides a reference standard for the interpretation of mutations conferring resistance to all first-line and a variety of second-line drugs. The report summarizes the analysis of over 52 000 isolates with matched data on WGS and phenotypic DST from 67 countries for 13 anti-TB medicines. The catalogue provides information on more than 30 000 mutations, along with their frequencies within the dataset. The mutations are categorized into groups: those associated with resistance, those not associated with resistance, and those with uncertain significance (a significant number of mutations are in this category). Additionally, the catalogue provides details about the methods employed and important findings related to each drug. Future regular updates of the catalogue are planned.

Importantly, a transition to rapid molecular testing does not eliminate the need for phenotypic DST because the method is still needed for conducting DST for drugs for which a molecular tool is not available or for which resistance has not been clearly associated with specific mutations, conducting DST to guide drug dosing determinations, monitoring the response to TB treatment, and investigating discordant results from diagnostic testing or DST. In particular, phenotypic DST is needed for testing some of the new and repurposed drugs used for treatment; especially important in this context are BDQ, LZD and Pa.

Table 2.4 presents an overview of the WHO-recommended diagnostic approaches, reference methods and clinical interpretation for anti-TB medicines.

Table 2.4. WHO-recommended diagnostic approaches, reference methods and clinical interpretation for anti-TB medicines

Drug		Initial diagnostic test	Phenotypic DST	Reference Method	Comment
First-line anti-TB drugs	Rifampicin	WRDs LPAs tNGS	MGIT may not be reliable for isolates with borderline resistance mutations	DNA sequencing of the entire *rpoB* gene	Any mutation (excluding silent mutations) observed in the 81bp RRDR[a] hotspot region of the *rpoB* gene are known or assumed to be associated with rifampicin resistance. In a few cases, mutations in the *rpoB* gene outside the RRDR region are associated with rifampicin resistance, these would require DR-TB treatment Consider conducting sequencing if the suspicion of resistance is high.
	Isoniazid	Moderate complexity automated NAATs Low complexity automated NAATs LPAs tNGS	Reliable and reproducible when testing the CC in all media.	MGIT	If specific inhA promoter mutations are detected (and in the absence of any katG mutations), increasing the dose of isoniazid is likely to be effective. Low and moderate complexity NAATs and LPA for RIF and INH detection are preferred to guide patient selection for the (H)RZE-Lfx regimen. Rifampicin resistance should be excluded before starting the Hr-TB regimen and FQ resistance should be excluded as soon as possible. Consider conducting pDST if the suspicion of resistance is high.
Group A second-line anti-TB drugs	Levofloxacin	Moderate complexity automated NAATs Low complexity automated NAATs LPAs tNGS	Reliable and reproducible when testing the CC in LJ, 7H10 and MGIT media	MGIT	Strains with known or assumed resistance mutations should be considered to be resistant. Most strains without mutations should be susceptible. However, a strain with no mutations detected by NAATs/LPAs may still be resistant. Consider conducting pDST if the suspicion of resistance is high.
	Moxifloxacin (Critical concentration)	Low complexity automated NAATs LPAs tNGS	Reliable and reproducible when testing the CC in LJ, 7H10, 7H11 and MGIT media.	MGIT	A strain of TB with no mutations detected by LPA or Xpert MTB/XDR may still be resistant. Confirm with pDST both the CC and CB concentrations.

Drug		Initial diagnostic test	Phenotypic DST	Reference Method	Comment
Group A second-line anti-TB drugs	Moxifloxacin (Clinical breakpoint concentration)	Low complexity automated NAATs LPAs tNGS Certain mutations detected by NAATs, tNGS or LPA result in very high MICs for which even high-dose moxifloxacin is not effective.	Clinical breakpoint concentration (CB) for 7H10 and MGIT apply to high-dose moxifloxacin (i.e. 800 mg daily).	MGIT	Moxifloxacin- even at high dose- is unlikely to be effective if resistant at the CB concentration or if certain high confidence mutations associated with high MICs are detected
	Bedaquiline	tNGS	CCs established for testing in 7H11 and MGIT media.	MGIT	Ideally, perform pDST at the time of treatment initiation. If baseline DST is not performed, perform DST with the first strain isolated from patients sample during treatment monitoring.[a]
	Linezolid	tNGS	CCs established for testing in 7H10, 7H11 and MGIT media.	MGIT	Ideally, perform pDST at the time of treatment initiation. If baseline DST is not performed, perform DST with the first strain isolated from patients sample during treatment monitoring.[a]
Group B second-line anti-TB drugs	Clofazimine	tNGS	CC established for testing MGIT media only.	MGIT	Ideally, perform pDST at the time of treatment initiation. If baseline DST is not performed, perform DST with the first strain isolated from patients sample during treatment monitoring.[a]
	Cycloserine Terizidone	No rapid method currently exists for the detection resistance	CCs have been established for cycloserine on MGIT media only	MGIT	Ideally, perform pDST at the time of treatment initiation. If baseline DST is not performed, perform DST with the first strain isolated from patients sample during treatment monitoring.[a]

Drug		Initial diagnostic test	Phenotypic DST	Reference Method	Comment
C second-line anti-TB drugs	Ethambutol	No rapid method currently exists for the detection resistance	DST not reliable and reproducible	N/A	Both gDST and pDST are not reliable. Ethambutol, if used, cannot be counted as an effective drug in an MDR/RR-TB treatment regimen
	Delamanid	No rapid method currently exists for the detection of resistance	CCs established for testing in 7H11 and MGIT media.	MGIT	Ideally, perform pDST at the time of treatment initiation. If baseline DST is not performed, perform DST with the first strain isolated from patients during treatment monitoring.[a]
	Pyrazinamide	tNGS or LPAs	DST method standardised in the MGIT. False resistant results can be detected if DST inoculum not properly prepared	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. Do not include PZA if resistance is detected or if used do not count as an effective agent
	Amikacin (or Streptomycin)	Low complexity automated NAATs tNGS (for Amikacin and Streptomycin) -LPAs[b] (for Amikacin)	CCs 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 planned to be included in the DR-TB regimen A strain with no mutations in the *rrs* and *eis* genes detected by genotypic assays may still be resistant to AMK. Confirm with pDST. If streptomycin is used, perform pDST at the time of treatment initiation if possible.
	Imipenem-cilastin Meropenem	No rapid method currently exists for the detection resistance	CCs have not been established for any DST media	N/A	DST is not recommended as both imipenem and meropenem are highly unstable in liquid media

Drug		Initial diagnostic test	Phenotypic DST	Reference Method	Comment
C second-line anti-TB drugs	Ethionamide Prothionamide	Mutations in the promotor region of the *inh*A gene: LPAs, and Moderate complexity automated NAATs and Low complexity automated NAATs	DST not reliable and reproducible	DNA sequencing of the *inhA* promotor region and *ethA* and *ethR* genes.	Do not include thioamides if resistance associated mutations are detected
	Para-aminosalicylic acid	No rapid method currently exists for the detection resistance	CCs have not been established for any DST media	N/A	DST is not recommended
Other	*Pretomanid*	No rapid method currently exists for the detection of resistance	CCs have been established for MGIT media only	MGIT	Ideally, perform pDST at the time of treatment initiation. If baseline DST is not performed, perform DST with the first strain isolated from patients sample during treatment monitoring.[a]

AMK: amikacin; ATU: area of technical uncertainty; CB: clinical breakpoint; CC: critical concentration; DNA: deoxyribonucleic acid; DR-TB: drug-resistant TB; DST: drug susceptibility testing; EMB: ethambutol; FQ: fluoroquinolone; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; HREZ: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z); INH: isoniazid; LFX: levofloxacin; LJ: Löwenstein–Jensen media; LPA: line probe assay; MFX: moxifloxacin; MGIT: Mycobacterial Growth Indicator Tube; MIC: minimal inhibitory concentration; n.a: not available; NAAT: nucleic acid amplification test; NGS: next-generation sequencing; PZA: pyrazinamide; RIF: rifampicin; RRDR: rifampicin-resistance-determining region; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis; WGS: whole-genome sequencing.

[a] 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.

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

Source: adapted or reproduced from Table 4 in Web Annex C.

3. Implementing a new diagnostic test

3.1 Placement of diagnostic tests in the tiered laboratory network

Diagnostic tests that are implemented 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 that 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.

In many resource-limited or high-burden settings, TB laboratory networks have a pyramidal structure, as shown in Fig. 3.1. 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 (or more than one in large countries) central laboratory (Level III) at the provincial, state or national level. Each level or tier has specific requirements for infrastructure and biosafety, defined by the activities and diagnostic methods being performed in the laboratories.

3.1.1 Peripheral level

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

- The LF-LAM is an instrument-free, point-of-care test that delivers results within 15–20 minutes and is suitable for use in the clinic. Current recommendations are limited to use among PLHIV with preset criteria. Thus, antiretroviral therapy (ART) initiation sites or similar care centres for PLHIV would be examples of appropriate placement sites. The LF-LAM is a complementary test to be used with other tests, particularly because it lacks drug-resistance detection, and this should also be considered.
- Acid-fast bacilli (AFB) smear microscopy is widely used. Existing smear microscopy sites are suitable for placement of Xpert MTB/RIF and Ultra, as well as Truenat MTB and MTB Plus because the same population is served and the infrastructure requirements are similar.

- These mWRDs offer the advantage of higher sensitivity for TB detection and detection of RIF resistance. The Truenat MTB can run on battery for periods of time and is thus useful where the electricity supply is unstable; also, it can operate at higher temperatures (up to 40 ºC) *(16)*.
- TB-LAMP is also suitable for placement at the peripheral level. It is less automated and somewhat more complex to perform than other tests (e.g. Xpert MTB/RIF or Truenat MTB) but is cheaper than other mWRDs and can replace microscopy for initial TB testing. However, because it does not detect RIF resistance, an alternative test should be considered in populations at high risk of MDR-TB, particularly where follow-on testing for RIF resistance is not accessible or available.
- The low complexity automated NAATs that detect FQ and INH resistance are also technologically suited to the peripheral level. However, these tests are used as follow-on tests to the primary tests for TB detection (and RIF resistance detection). An important factor to consider, especially since these tests require a different instrument to that presently used for Xpert assays, is that the number of tests needed over a certain period will be much lower than the number for initial TB diagnosis. Furthermore, since the test has important value in providing rapid results on FQ, ETO and AMK resistance for management of MDR/RR-TB, consideration should be given to placing these tests at sites where MDR/RR-TB treatment is delivered.

3.1.2 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 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 or can be more sustainable where there are shortages of skilled staff to capacitate and maintain a large, quality-assured peripheral level network.

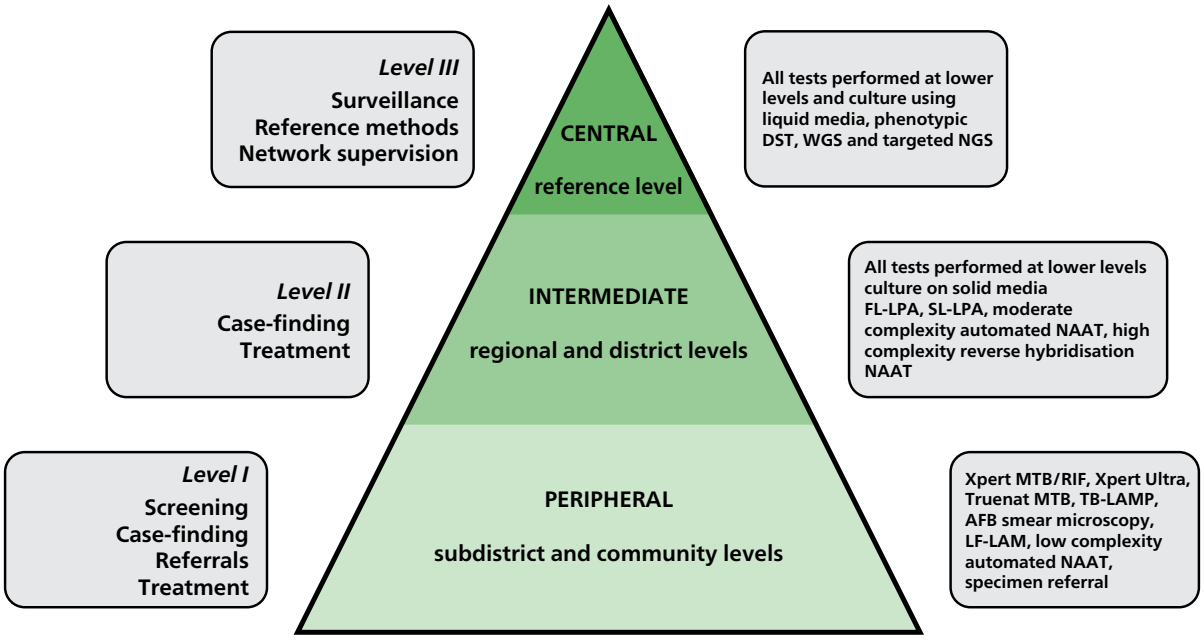
- The new moderate complexity automated NAATs are suitable at this level. These tests require laboratory infrastructure that varies in size from just under 1 m wide (94 × 75.4 × 72.4 cm) to instruments over 4 m wide (429 × 216 × 129 cm). The class of technologies has varied throughput, from performing 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.

3.1.3 Central level

At the central level (Level III), testing that requires highly advanced skills, infrastructure and biosafety precautions is offered. 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.

- FL-LPA, SL-LPA and targeted NGS tests are all applicable at this level. The moderate complexity automated NAATs with high throughput could also be considered at this level. In addition, the class of high complexity reverse hybridization NAAT for PZA is suited to this level and can make use of existing LPA infrastructure.
- 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 and are most suitable for centralized laboratories equipped with specialized skills and infrastructure (including information technology [IT] and data storage).

Fig. 3.1. Organization of a TB diagnostic network



AFB: acid-fast bacilli; DST: drug susceptibility testing; FL: first-line; LAMP: loop-mediated isothermal amplification; LF-LAM: lateral flow lipoarabinomannan assay; LPA: line probe assay; NAAT: nucleic acid amplification test; SL: second-line; TB: tuberculosis.

3.1.4 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 demand based rather than population based 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 epidemiologic 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. 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. As an example, primary TB testing of people presumed to have TB will be relevant across all health facilities where individuals are screened for TB. In contrast, 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.

The *Framework of indicators and targets for laboratory strengthening under the End TB Strategy* can serve as a guide for implementing and monitoring improvements to TB testing and TB diagnostic networks *(8)*.

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

- resources available for implementation;
- infrastructural requirements;
- biosafety requirements;
- specimen types and collection procedures;
- projected testing volumes;
- 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;
- trained human resources (HR) capacity;
- links to other laboratories for further testing;
- specimen referral and result reporting systems; and
- possibility of integration with testing, specimen referral and reporting systems for other diseases.

Well-designed specimen referral systems underpin a strong diagnostics network and 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 (31)* and the *GLI specimen referral toolkit (32)* are useful sources of information for designing, implementing and monitoring systems for referring specimens and reporting results.

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. Table 3.1 provides examples of population-level projections of the results of testing with the various mWRDs 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 – rapid diagnostics for tuberculosis detection, 2021 update (13)* for each test. Tables 3.2–3.4 provide those same parameters for detection of resistance to RIF, INH and FQs, respectively. Tables 3.5–3.6 provide those same parameters for detection of resistance to first-line and second-line anti-TB agents using targeted NGS 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, countries 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. False negative results may lead to missed opportunities to treat TB. 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.

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 diagnostic opportunities. To improve TB case detection and identify individuals suitable for TB preventive treatment, WHO has updated the TB screening guidelines *(33)*. Several modalities are recommended for screening: symptom screening, chest X-ray and mWRDs. For screening PLHIV, recommended screening modalities include the classical four-symptom screen, chest X-ray, and an mWRD or positive C-reactive protein test (>5 mg/L).

Chest X-ray as a screening or triage 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 the cost of the radiography would need to be lower than the cost of the test *(33)*. This approach is likely to improve the pretest probability for TB; therefore, it should improve the predictive value of the molecular test and reduce false positive results, especially in populations with a low prevalence of TB. For example, the addition of chest X-ray as a screening tool to an algorithm in which all individuals with an abnormal chest X-ray receive mWRD was calculated to increase the positive predictive value 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, in a population with a TB prevalence of 1% *(33)*.

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)	Studies (participants)	Certainty of evidence	2.5% prevalence	10% prevalence	30% prevalence
Xpert MTB/RIF	Se: 0.85 (0.82–0.88)	70 (10 409)	High	TP: 21 / FN: 4	TP: 85 / FN: 15	TP: 255 / FN: 45
	Sp: 0.98 (0.97–0.98)	70 (26 828)	High	TN: 965 / FP: 10	TN: 891 / FP: 9	TN: 693 / FP: 7
Xpert MTB/RIF Ultra	Se: 0.90 (0.84–0.94)	6 (960)	High	TP: 22 / FN: 3	TP: 90 / FN: 10	TP: 269 / FN: 31
	Sp: 0.96 (0.93–0.98)	6 (1694)	High	TN: 932 / FP: 43	TN: 860 / FP: 40	TN: 669 / FP: 31
MC-aNAAT	Se: 0.93 (0.91–0.95)	29 (4767)	Moderate	TP: 23 / FN: 2	TP: 93 / FN: 7	TP: 279 / FN: 21
	Sp: 0.98 (0.96–0.99)	29 (9085)	High	TN: 953 / FP: 22	TN: 879 / FP: 21	TN: 684 / FP: 16
Truenat MTB[a]	Se: 0.73 (0.68–0.78)	1 (258)	Moderate	TP: 18 / FN: 7	TP: 73 / FN: 27	TP: 220 / FN: 80
	Sp: 0.98 (0.97–0.99)	1 (1122)	High	TN: 957 / FP: 18	TN: 884 / FP: 16	TN: 687 / FP: 13
Truenat MTB Plus[a]	Se: 0.80 (0.75–0.84)	1 (261)	Moderate	TP: 20 / FN: 5	TP: 80 / FN: 20	TP: 239 / FN: 61
	Sp: 0.96 (0.95–0.97)	1 (1087)	High	TN: 940 / FP: 25	TN: 868 / FP: 32	TN: 675 / FP: 25
TB-LAMP	Se: 0.78 (0.71–0.83)	7 (1810)	Very low	TP: 20 / FN: 5	TP: 78 / FN: 22	TP: 234 / FN: 66
	Sp: 0.98 (0.96–0.99)	7 (1810)	Very low	TN: 955 / FP: 20	TN: 882 / FP: 18	TN: 686 / FP: 14

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

[a] When used in a microscopy laboratory. When tested in reference laboratories, the sensitivities of Truenat MTB and Truenat MTB Plus were 0.84 and 0.87, respectively, and specificities were 0.97 and 0.95, respectively.

Table 3.2. Performance of molecular tests for the detection of rifampicin 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 rifampicin resistance out of 1000)

Intervention	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	2% prevalence	10% prevalence	15% prevalence
Xpert MTB/RIF	Se: 0.96 0.94–0.97)	48 (1775)	High	TP: 19 / FN: 1	TP: 96 / FN: 4	TP: 144 / FN: 6
	Sp: 0.98 (0.98–0.99)	48 (6245)	High	TN: 960 / FP: 20	TN: 882 / FP: 18	TN: 833 / FP: 17
Xpert MTB/RIF Ultra	Se: 0.94 (0.87–0.97)	5 (240)	High	TP: 19 / FN: 1	TP: 94 / FN: 6	TP: 141 / FN: 9
	Sp: 0.99 (0.98–1.00)	5 (690)	High	TN: 970 / FP: 10	TN: 891 / FP: 9	TN: 842 / FP: 8
Truenat MTB-RIF Dx	Se: 0.84 (0.72–0.92)	1 (51)	Very Low	TP: 17 / FN: 3	TP: 84 / FN: 16	TP: 126 / FN: 24
	Sp: 0.97 (0.95–0.99)	1 (258)	Moderate	TN: 951 / FP: 29	TN: 873 / FP: 27	TN: 825 / FP: 25
MC-aNAAT	Se: 0.97 (0.93–0.98)	18 (702)	Moderate	TP: 19 / FN: 1	TP: 97 / FN: 3	TP: 146 / FN: 4
	Sp: 0.99 (0.97–0.99)	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.

[a] The rifampicin resistance detection by Xpert MTB/RIF, Ultra, Truenat MTB-RIF Dx and MC-aNAAT occurs only in case TB is detected; that is why suggested prevalence reflects rifampicin resistance in people newly detected with TB.

Table 3.3. Performance of molecular tests for the detection of isoniazid 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 isoniazid resistance out of 1000)

Intervention	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	2% prevalence	10% prevalence	15% prevalence
MC-aNAAT	Se: 0.86 (0.83–0.89)	18 (854)	Moderate	TP: 17 / FN: 3	TP: 86 / FN: 14	TP: 129 / FN: 21
	Sp: 0.99 (0.98–1.00)	18 (1904)	High	TN: 970 / FP: 10	TN: 891 / FP: 9	TN: 842 / FP: 8
LC-aNAAT	Se: 0.94 (0.89–0.97)	3 (994)	Moderate	TP: 19 / FN: 1	TP: 94 / FN: 6	TP: 141 / FN: 9
	Sp: 0.98 (0.95–0.99)	3 (611)	Moderate	TN: 960 / FP: 20	TN: 882 / FP: 18	TN: 833 / FP: 17
Targeted NGS	Se: 95.8 (0.93–99)	12 (1440)	Moderate	TP: 19 / FN: 1	TP: 96 / FN: 4	TP: 144 / FN: 6
	Sp: 97.0 (0.95–0.99)	12 (517)	Moderate	TN: 951 / FP: 29	TN: 873 / FP: 27	TN: 825 / FP: 25
FL-LPA by direct testing of SS+ samples	Se: 0.89 (0.86–0.92)	46 (3576)	Moderate	TP: 18 / FN: 2	TP: 89 / FN: 11	TP: 134 / FN: 16
	Sp: 0.98 (0.97–0.99)	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/MC-aNAAT: low/moderate complexity automated nucleic acid amplification test; NGS: next-generation sequencing; Se: sensitivity; Sp: specificity; SS+: sputum-smear-positive; TB: tuberculosis; TN: true negative; TP: true positive.

[a] The isoniazid resistance detection by MC-aNAAT occurs only in cases where TB is detected. That is why 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 fluoroquinolone 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 fluoroquinolone resistance out of 1000)

Intervention	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	1% prevalence	5% prevalence	10% prevalence
LC-aNAAT	Se: 0.93 (0.88–0.96)	3 (384)	High	TP: 9 / FN: 1	TP: 47 / FN: 3	TP: 93 / FN: 7
	Sp: 0.98 (0.94–0.99)	3 (953)	Moderate	TN: 973 / FP: 17	TN: 934 / FP: 16	TN: 885 / FP: 15
Targeted NGS (MFX)	Se: 95.6 (0.92–0.99)	6 (652)	Moderate	TP: 10 / FN: 0	TP: 48 / FN: 2	TP: 96 / FN: 4
	Sp: 96.3 (0.93–0.99)	8 (921)	Moderate	TN: 950 / FP: 40	TN: 912 / FP: 38	TN: 864 / FP: 36
Targeted NGS (LFX)	Se: 94.2 (0.884–0.99)	6 (654)	Low	TP: 9 / FN: 1	TP: 47 / FN: 3	TP: 94 / FN: 6
	Sp: 96.2 (0.93–0.99)	7 (913)	Moderate	TN: 950 / FP: 40	TN: 912 / FP: 38	TN: 864 / FP: 36
SL-LPA by direct testing of SS+ samples	Se: 0.86 (0.75–0.93)	9 (519)	Moderate	TP: 9 / FN: 1	TP: 43 / FN: 7	TP: 86 / FN: 14
	Sp: 0.99 (0.97–0.99)	9 (1252)	High	TN: 980 / FP: 10	TN: 937 / FP: 13	TN: 887 / FP: 13

CI: confidence interval; FN: false negative; FP: false positive; FQ: fluoroquinolone; LC-aNAAT: low complexity automated nucleic acid amplification test; LFX: levofloxacin; MFX: moxifloxacin; 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.

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 hybridization NAAT	Pyrazinamide	8,50,90%	Se: 81.2 (75.4–85.8) Sp: 97.8 (96.5–98.6)	7 (214) 7 (750)	Very Low Low	TP: 65 / FN: 15 TN: 900 / FP: 20	TP: 406 / FN: 94 TN: 489 / FP: 11	TP: 731 / FN: 169 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 rifampicin-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)	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
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

Drug	Resistance prevalence	Test accuracy % (95% CI)	Studies (participants)	Certainty of evidence	Lower prevalence cut-off	Middle prevalence cut-off	Higher prevalence cut-off
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; MDR/RR-TB: multidrug- or rifampicin-resistant TB; NGS: next-generation sequencing; Se: sensitivity; Sp: specificity; TB: tuberculosis; TN: true negative; TP: true positive.

3.3 Epidemiologic 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:

- TB cases resistant to RIF, INH and FQs;
- PLHIV;
- TB that is extrapulmonary;
- TB among children;
- those with severe 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.

3.4 Multidisease platform considerations

Health needs are diverse, and programmes are expected to provide a range of diagnostics to assist health workers in managing patients. The diagnosis of TB often begins with symptom screening, which is not specific to TB, given that cough and fever overlap with COVID-19 and other respiratory infections. Additionally, people with TB may be coinfected with HIV, particularly in sub-Saharan Africa, 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, justifying the need for multidisease testing using the same equipment. An information note describing considerations for multidisease testing is available *(34)*.

All currently recommended molecular diagnostics for the initial diagnosis of TB have a SARS-CoV-2 test available on the same platform as the TB test, although some may not have received regulatory approval for such use. Several platforms are widely used in diagnosing and managing PLHIV, whereas others are used for antimicrobial resistance detection of bacterial pathogens. NGS platforms can 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 low- and middle-income countries, 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. The decision to choose a particular test and brand would also need to consider the instruments available in a particular setting and the capacity to add TB testing. If multidisease testing on an instrument is planned, then it would be best to employ platforms that use random access approaches (e.g. GeneXpert) or allow different types of tests to be performed on the same batch (e.g. cobas, BD MAX or targeted NGS).

Multidisease testing has the advantage of shared financial costs for equipment purchasing and maintenance, as well as human resources. Efficiencies could also be achieved where multidisease testing results in optimal use of equipment and batch sizes; however, if it is not well planned (e.g. if a well-functioning collaboration between vertical disease programmes is not

ensured), such testing could have the opposite effect. Equitable access and shared prioritization of testing are important to ensure that people with a particular disease are not disadvantaged. For example, the use of targeted NGS for clinical decision-making should be prioritized over its use for surveillance. The overall laboratory budgets should ensure fair distribution based on burden of disease and need.

Multidisease testing is mainly useful in settings where only a small number of tests are undertaken by individual programmes (e.g. the likely small numbers of people with DR-TB on a day-to-day basis that may require targeted NGS). In this scenario, prioritizing such samples in a run and "filling" the batch with SARS-CoV-2 or other surveillance samples may improve cost efficiency. In contrast, multidisease testing will be less relevant where there are large needs for TB and HIV testing and infrastructure is installed to meet the demand. Nonetheless, the burden of disease and testing volumes change over time; hence, the use of equipment should be monitored and programmes may need to adapt.

3.5 Steps and processes for implementing a new diagnostic test

Box 3.1 Key steps in implementing a new diagnostic test

- ➔ Establish a technical working group to lead the process
- ➔ Define the intended use of the new test, and update diagnostic algorithms
- ➔ Develop a realistic costed implementation plan and budget for ongoing costs
- ➔ Procure and install equipment in safe, functional testing sites
- ➔ Ensure a reliable supply of quality-assured reagents and consumables
- ➔ Develop SOPs and clinical protocols
- ➔ Implement a comprehensive QA programme
- ➔ Implement training, mentoring and competency assessment programmes
- ➔ Monitor and evaluate the implementation and impact of the new test

As an initial step in implementing a new diagnostic test, countries should review WHO policies, guidance and reports, as well as any available implementation guide from WHO, the GLI, the Foundation for Innovative New Diagnostics (FIND) and implementing partners. Particular attention should be paid to WHO policies and recommendations for the use of the test, the test's limitations and the interpretation of test results.

The key steps in implementing a new test are listed in Box 3.1. Critical early steps include defining the intended use of the new test, developing a costed implementation plan, building the infrastructure (instruments and facilities) and developing the human resources needed for the new test.

As described in Annex 3 and in *The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in Mycobacterium tuberculosis complex: technical guide (30)*, implementation of targeted NGS tests follows the same basic steps described below, with some additional considerations (e.g. cost of the equipment, complexity of the assay, and need for highly skilled laboratory workers and bioinformatics resources).

Within Section 3.5, the key steps for implementing a diagnostic test are organized into 10 main areas:

- Area 1 – Policies, budgeting and planning (Section 3.5.1)
- Area 2 – Regulatory issues (Section 3.5.2)
- Area 3 – Equipment (Section 3.5.3)
- Area 4 – Supply chain (Section 3.5.4)
- Area 5 – Procedures (Section 3.5.5)
- Area 6 – Digital data (Section 3.5.6)
- Area 7 – Quality assurance, control and assessment (Section 3.5.7)
- Area 8 – Recording and reporting (Section 3.5.8)
- Area 9 – Human resource training and competency assessment (Section 3.5.9)
- Area 10 – Monitoring and evaluation (Section 3.5.10).

The rest of this section discusses the steps in each of these areas.

3.5.1 Area 1 – Policies, budgeting and planning

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

A TWG comprising representatives from all key stakeholders should be established, to guide the implementation process of the new diagnostic tests and technologies. It is important to include representatives from other disease programmes if multidisease testing is planned. The establishment of the TWG should be led by the MoH, NTP and national TB reference laboratory (NTRL). The TWG should be mandated to advise the MoH, NTP and NTRL on test implementation; develop action plans; oversee the test's implementation; and assess the impact and success of the test's introduction. Representatives from the following key stakeholders may be invited to participate:

- MoH, NTP, NTRL(s) and regional laboratories;
- 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;
- specimen transport systems logisticians for centralized or regional testing (TB and non-TB);
- community 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 and reports, as well as any available implementation guides from WHO, GLI, 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, the test's limitations 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. 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 lead a review of existing national diagnostic algorithms, taking into consideration the needs of people with TB, clinical needs, country epidemiology, existing testing algorithms, sample referral systems and other operational considerations, and make recommendations to the MoH and NTP. Section 4 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. The assessment 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 *(31)*, and a checklist for implementing targeted NGS tests can be found in the NGS technical guide *(30)*.

For the prospective testing site, detailed assessments of the laboratory's readiness with respect to physical facilities, staffing and infrastructure will be needed. Because laboratory-acquired TB infection is a well-recognized risk for laboratory workers, undertaking a risk assessment for conducting the new test in the prospective site is critical, to ensure that the required biosafety requirements are in place before the new test is implemented *(35)*.

For implementing targeted NGS-based DST, particular attention must be paid to the availability of highly skilled staff that can meticulously perform the library preparation steps, and to the laboratory's capacity to analyse and store large amounts of data. IT requirements include:

- adequate computing resources, including the instrument computer and computers for data analysis;
- sufficient data storage capacity (local or cloud-based services), including external storage for data backups;
- appropriate data security measures;
- access to data analysis tools;
- staff trained in the use of the analytic tools for NGS data;
- IT resources and expertise for the installation and ongoing maintenance of hardware, software and networks; and
- a network and high-speed internet connection; and data connectivity solutions to link NGS data to other relevant patient data.

Step 1.6 – Develop a costed operational plan for phased implementation

The final step in this area is to develop a detailed, costed, prioritized action plan for phased implementation, with targets and timeline. Often, implementation of a new test must overcome potential obstacles such as cost of instruments, ancillary equipment and consumables; requirements for improving or establishing the necessary laboratory and network infrastructure (e.g. a specimen transport system); the need for specialized, skilled and well-trained staff; the need for expert technical assistance; maintenance of confidentiality of patient information; and the need to establish a QA system.

Successful implementation of the plan will require financial and human resource commitments from the MoH 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.

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 *(30)*.

3.5.2 Area 2 – Regulatory issues

Step 2.1 – Determine importation requirements

Step 2.2 – Conduct country validation and verification studies, as required

Step 2.3 – Complete national regulatory processes

Step 2.1 – Determine importation requirements

National authorities should be consulted to determine relevant processes to be followed for importation. Countries should work closely with manufacturers and authorized providers of equipment and consumables, to determine importation and registration requirements, and to initiate country verifications, if required.

Step 2.2 – Conduct country validation and verification studies, as required

Validation includes conducting large-scale evaluation studies to measure the performance of the test if there is any possibility that country-specific factors (e.g. prevalence of different mutations or microorganism strains) may cause performance to deviate substantially from the manufacturer's results or other evaluation studies. Validation 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 *(36)*.

Verification includes small-scale method evaluation studies in cases where commercial tests are used according to the manufacturer's intended use. 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 *(37)*. 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, laboratories that are implementing the method do not need to repeat such large-scale studies. Instead, implementing laboratories should conduct small-scale verification studies to demonstrate that the laboratory 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. Countries must make their own determination on the need for verification, based on national guidelines and accreditation requirements.

For example, when planning a targeted NGS verification study *(38)*, the following should be considered:

- A detailed protocol is required, outlining the number and types of samples to be tested and defining the criteria that must be met to demonstrate that the laboratory can achieve the targeted performance characteristics. Samples should be chosen to assess the performance of the entire NGS process, from DNA isolation to interpretation of results.

- If targeted NGS is to be implemented in several laboratories, a more extensive verification study may be done at the NTRL (e.g. 30–50 samples that cover all drugs to be verified and contain a balance of susceptible and resistant isolates), with limited verification studies done at the other laboratories, for cost saving and efficiency.
- For verification, a mix of samples should be selected that will give results at test thresholds (e.g. a mix of positive and negative results) and will give a variety of semiquantitative results. Samples for verification could be leftover sputum or frozen sputum samples with known results, stored clinical isolates or proficiency testing panels. Countries should select a variety of strains for verification, based on their local epidemiology.
- Reproducibility and repeatability could be assessed by testing three reference samples, three to five times each.
- A verification report should be compiled, the observed performance parameters compared with the published performance and a determination of acceptance made.

Step 2.3 – Complete national regulatory processes

Countries 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 to submit the application and provide any required supplementary evidence.

3.5.3 Area 3 – Equipment

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

Step 3.2 – Instrument verification and maintenance

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 the diagnostic test. In general, 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 *(34)*. 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 moderate complexity automated NAATs may require infrastructure to be modified to accommodate the instrumentation. Potential setup 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 (17)*. Guidance on selecting NGS equipment is available elsewhere *(30).*

Step 3.2 – Instrument verification and maintenance

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.

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. Countries should take advantage of any available extended warranties or service contracts to ensure continued functioning of the instruments.

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, existing testing sites should be assessed regularly for safety and operational functionality.

A functional testing site requires testing instruments 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 biologic waste to be disposed of safely and in accordance with regulations. Failure to provide a functional and safe work environment can affect the quality and reliability of testing.

3.5.4 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 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 laboratory staff have input into defining the specifications for reagents, consumables and equipment; and streamlining of 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.

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. The shelf life of NGS reagents is normally short; hence, robust planning is required to avoid kits expiring, with resulting high costs for testing.

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. Preferably, new-lot testing of commercially available test kits is 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.

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;
- laboratory testing;
- data analysis, security and confidentiality (see Area 6);
- process controls (internal QC) and external quality assessment (see Area 7);
- recording and reporting of results (see Area 8); and
- waste management.

A well-defined, comprehensive set of SOPs that addresses all aspects of the laboratory testing processes – from sample collection to reporting of results – will be essential; 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 in 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 use of standardized educational materials developed by the NTP.

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 a low or unexpectedly high testing rate may need additional training and sensitization.

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 tests

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

Many of the latest testing platforms offer the opportunity to use digital data. The implementation plan should consider software and hardware requirements, to take advantage of digital data. "Diagnostics 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 *(39)*. 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, which allows 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, to assist with surveillance of disease trends or resistance patterns and rates, and to enhance 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. An SOP for regularly backing up data (e.g. to an external drive) is essential, as is an 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 software should be installed and kept up to date. Access restriction should be in place 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 tests

This step briefly summarizes data storage requirements, data analytic tools and data-sharing protocols needed for targeted NGS tests. Technical assistance from an IT expert or department

may be needed. A checklist to assess IT and data readiness is included in the site readiness checklist in Annex 3 of the WHO implementation manual *(30).*

Plan for data storage and computing

Adequate secure data storage is essential for successful implementation of targeted NGS. Each run of targeted NGS can generate thousands of megabytes of data (see Annex 9 of the WHO implementation manual *(30)*). For example, when "benchtop" NGS platforms are used, 7.5–15 gigabytes of data are generated during a 24–48 hour run. A sequencing run may include as many as 24–48 samples for WGS or 96 samples for targeted NGS.

Options for data storage include computer hard drives or external hard drives (a 1 terabyte hard drive may be able to store NGS files for up to 1500 samples); high-capacity flash drives (also known as memory sticks or thumb drives); a scalable, local computing cluster; and cloud-based systems (e.g. Illumina BaseSpace, Google Cloud and Amazon Drive).

Select data analytic tools

In recent years, many commercial and public data analytic pipelines have become available that make the analysis of MTBC NGS data accessible to both nonexperts and bioinformatic experts *(29)*. Factors to be considered in selecting the tool or tools that are best suited for the intended use of the NGS data include compatibility with the data output by the NGS instrument, the type of information produced, the computational resources and bioinformatics expertise needed at the local site, and the time required to complete the analysis. The targeted NGS solutions included in the latest recommendations include proprietary software that analyses the user uploaded sequencing read and exports it in the form of interpretable results.

Develop procedures for data sharing and ensuring confidentiality

Because NGS generates sequences of whatever DNA is present in a sample, laboratories must be particularly careful with sequence data generated from human clinical samples (e.g. sputum specimens, which may contain considerable amounts of human DNA). Currently, two features of the NGS procedures for TB mitigate this concern: the process of isolating bacteria by culture should eliminate the risk of sequencing any human DNA; and the amplification or capture step used in the processing of clinical specimens for targeted NGS serves to minimize the relative amount of human DNA that is sequenced. Furthermore, most bioinformatics pipelines include steps to discard any sequence reads that map to the human genome before sharing TB sequencing data.

Area 7 – Quality assurance, control and assessment

Step 7.1 – Implement a comprehensive QA programme

Step 7.2 – Establish and monitor QCs

Step 7.3 – Develop an external quality assessment programme

Step 7.4 – Monitor and analyse quality indicators

Step 7.1 – Implement a comprehensive QA programme

A comprehensive QA or quality management programme 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;
- external quality assessment (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 (36)*. This section describes QC, EQA and quality indicator monitoring.

Step 7.2 – Establish and monitor QCs

QC monitors activities related to the analytical phase of testing, with the goal of detecting errors due to test failure, environmental conditions or operator performance before results are reported. Internal QC typically involves examining control materials or known substances at the same time and in the same manner as patient specimens, to monitor the accuracy and precision of the analytical process. If QC results are not acceptable (e.g. positive results are obtained on negative controls), patient results must *not* be reported.

Because of the complexity of the targeted NGS workflow and the need for multiple reagent kits and processes, it is particularly important to conduct quality checks after each of the main steps in the process. The following should be assessed:

- *specimens* – the source, quantity and quality of the source sample (e.g. sputum specimen);
- *DNA extraction* – the quality and quantity of the extracted DNA;
- *library preparation* – the quality and quantity of the generated library;
- *sequencing* – the quality of the run and base calling;
- *sequence assembly and analysis* – the proportion of coverage, depth of coverage and quality scores of the mapping; and
- *variant calling* – the variant call quality score, strand bias and allele frequencies.

Step 7.3 – Develop an EQA programme

An EQA programme includes quality and performance indicator monitoring, proficiency testing, re-checking or making comparisons between laboratories, regular on-site supportive supervision and timely feedback, corrective actions and follow-up. On-site 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.

The governance structure of an EQA programme at the national and supervisory levels is likely to vary by country. In many countries, implementation of national policies and procedures is coordinated at the central level by the MoH, NTP or NTRL. In some settings, particularly in large countries, these activities may be decentralized to the regional level. Commonly, the central level provides policies, guidance and tools for standardized QA activities, whereas the regional and district levels operationalize and supervise the QA activities and monitor adherence to the procedures. In turn, data collected at the testing sites are reviewed regionally and centrally, and are used to inform and update policies and procedures.

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 AFB smear microscopy.

On-site supervisory visits

On-site 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. On-site 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 on-site supervisory programme requires substantial planning and resources (both financial and human).

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 *(36)*, quality indicators specific to the new diagnostic 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 *(30)*. The indicators should be collected using a standardized format and analysed on a monthly or quarterly basis, disaggregated according to tests.

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.

3.5.8 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 form (i.e. specimen examination request form) 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. 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 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 align 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 tests because these tests 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 quality criteria (e.g. at least 10 successful reads per base across the gene of interest) be met for results to be reported.

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 NGS-based DST results *(40)*. The consensus forms are intended to be used to report NGS-based DST results to clinicians for use in patient-care decisions, but may also be useful guides for developing forms for reporting information for a DR-TB surveillance system.

Step 8.2 – Review and revise laboratory and clinical registers

Current laboratory and clinical registers that are based on the WHO reporting framework *(15)* 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 news tests in the software package.

3.5.9 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 the staff involved in 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, and 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 manufacturer-supplied on-site training

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.

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 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.

3.5.10 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.

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 as well as 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.

4. Model algorithms

Effective and efficient TB diagnostic algorithms are key components of a diagnostic cascade that is designed to ensure that people with TB are diagnosed accurately and rapidly, and are 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 and DR-TB. The model algorithms should be adapted to the local situation.

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

- **Algorithm 1** relies on mWRDs 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).
- **Algorithm 2** incorporates the most recent WHO recommendations for the use of the LF-LAM as an aid in the diagnosis of TB in PLHIV; it is most relevant to settings with a high prevalence of HIV. However, Algorithm 2 applies to any PLHIV who meet the testing criteria, regardless of the underlying prevalence of HIV in that setting.
- **Algorithm 3** and **Algorithm 4** are for follow-up testing, after TB is diagnosed, to detect additional drug resistance:
  - Algorithms 3a and 3b are used when the purpose is to detect resistance to second-line drugs in people with RR-TB; and
  - Algorithm 4 is used when the purpose is to detect resistance in individuals with RIF-susceptible TB at risk of having DR-TB and those with Hr-TB. Molecular testing is preferred and may use any existing WHO-recommended rapid test. Targeted NGS tests are the preferred test for Algorithm 4 because these tests can detect mutations associated with resistance to many anti-TB medicines, and are rapid molecular tests for people at high risk of having DR-TB (e.g. people in whom therapy is failing).

Algorithms 3 and 4 are appropriate for all settings; however, the resource requirements for follow-up testing may differ widely between settings with a high burden of DR-TB and settings with a low burden of DR-TB.

Each algorithm is accompanied by explanatory notes and a decision pathway that provides a detailed description of the various decisions in the algorithm. In general, the algorithms do not show a specific test; rather, they provide a flow of the expected outcomes of the test that are common across the algorithms (e.g. "TB detected") and the follow-up action required.

Although the algorithms are presented separately, they are interlinked and cascade from one to the other. This is illustrated in the overview (Fig. 4.1), which lists the various diagnostic tests currently recommended within each algorithm. Algorithm 2 is complementary to Algorithm 1, and currently it includes only a single product option intended as an additional test for PLHIV.

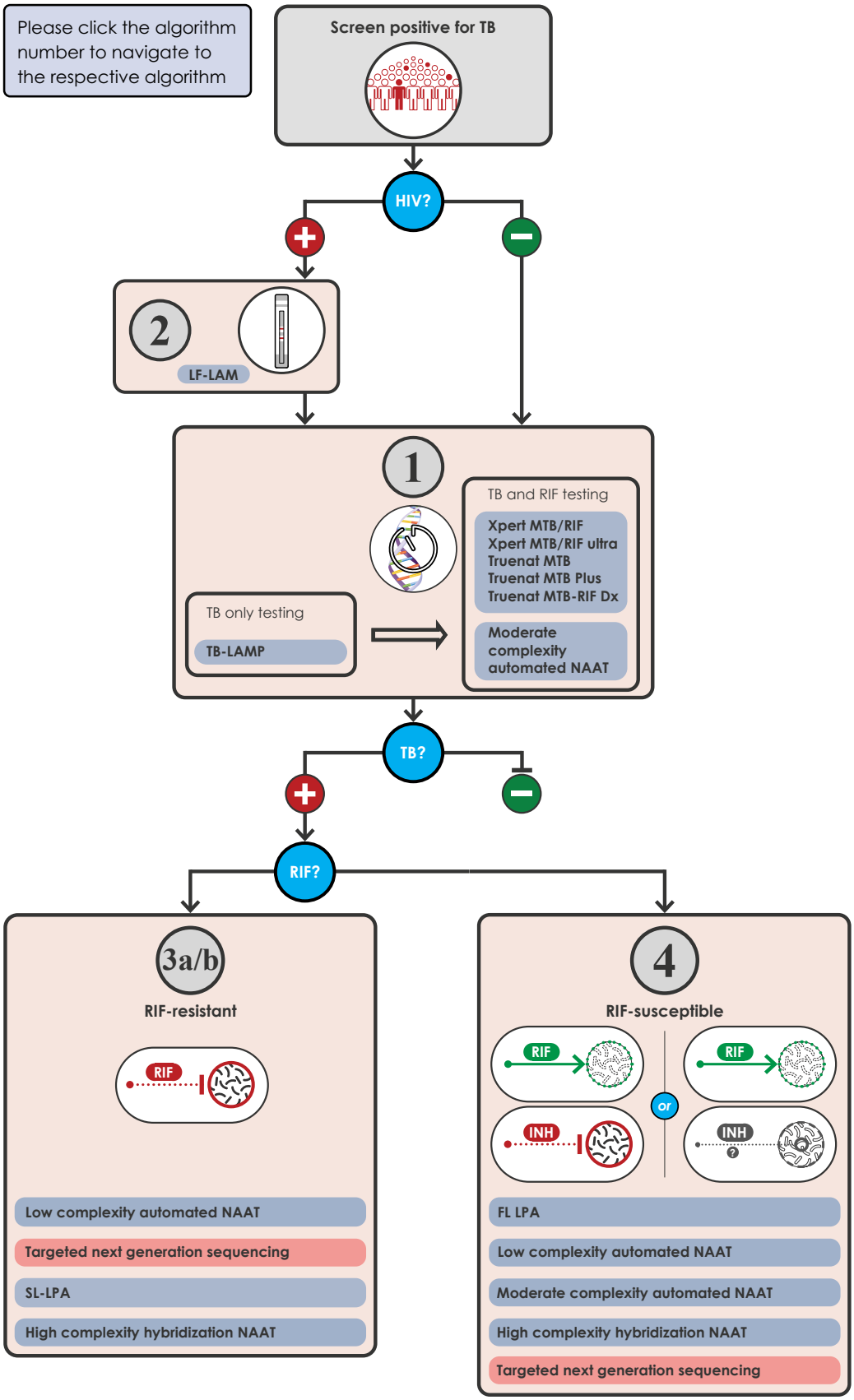
The diagnostic pathway begins with a person identified as a presumptive TB person through assessment of signs and symptoms or screened through another approach. WHO has released updated recommendations on TB screening, and readers are encouraged to consult the latest guidance *(33)*. People presumed to have TB may not always present with symptoms that match the latest screening guideline recommendations but still have an increased probability for TB disease requiring diagnostic testing. The modalities for screening, beyond the four-symptom screen, now include chest X-ray, an mWRD used as a screening tool or C-reactive protein in PLHIV. The addition of mWRD for screening of selected at-risk populations and settings goes beyond its primary use as an initial line diagnostic tool and the different uses should not be confused. 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 would have large financial and operational implications, and should be carefully considered.

People who are referred for diagnostic evaluation after a positive screen for TB using modalities other than an mWRD should go through a clinical evaluation, including other relevant investigations if available (e.g. chest X-ray) and follow Algorithm 1 to reach a bacteriologically confirmed diagnosis. Among those who are referred for diagnostic evaluation after a positive screen for TB using an mWRD *(33)* – that is. used in a screening rather than a diagnostic context – the pretest probability is an important consideration in addition to the clinical picture when deciding to repeat the mWRD or proceed with treatment. Due to the high specificity even when used as a screening test (99%), a positive test is likely to be a true positive when the pretest probability is high.

In the scenario recommending mWRD for screening all PLHIV who are medical inpatients and where the prevalence of TB is at least 10%, the likelihood of the mWRD being a true positive is high and treatment should be considered if the clinical picture is in keeping with a diagnosis of TB. However, the detection of TB DNA does not necessarily indicate that the person has active TB. This may occur in a person with a history of prior TB treatment (<5 years) and is particularly true for the more sensitive molecular tests (Xpert Ultra); culture would aid in interpretation. Furthermore, in PLHIV who require hospital admission, more than one infection may be present, and such people should be fully investigated.

In the scenario where an mWRD is used as a screening tool in a community with a prevalence of 0.5%, the positive predictive value would only be 39.5%, despite having a specificity of 99%, meaning that more than half of the positive mWRDs may be false positive. In such a situation, a repeat mWRD is warranted but should follow a clinical evaluation, and other investigations should be considered. Treatment should be based on the totality of evidence for the particular person.

Fig. 4.1. Integrated pathway of the diagnostic algorithms[a]



FL-LPA: line probe assay for first-line drugs; INH: isoniazid; LF-LAM: lateral flow lipoarabinomannan assay; NAAT: nucleic acid amplification test; RIF: rifampicin; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis.

[a] Text with grey background: currently recommended tests, text with orange background: newly recommended tests. Numbers on grey background refer to the model algorithms.

Algorithm 1 is the starting point for the diagnostic pathway for most people suspected of having TB. The number of tests recommended for this purpose has increased from five to nine with the latest addition of the moderate complexity automated NAAT class. This is a great improvement compared with the past, when smear microscopy was the only option. Member States can now make choices that best fit their circumstances, with the ultimate objective being to serve patient needs. The initial test options can be split into those that provide a TB diagnosis only (TB-LAMP) and those that also provide at least detection of RIF resistance (simultaneously or as a two-step process): Xpert MTB/RIF, Xpert Ultra, Truenat MTB, Truenat MTB Plus, Truenat MTB-RIF Dx and moderate complexity automated NAATs. RIF resistance detection is recommended as a target for universal DST. Among the tests that detect RIF resistance, a subgroup provides this as a two-step procedure, separating TB detection and detection of RIF resistance. This second step could also be used as a follow-on test to initial tests that do not offer RIF resistance detection, such as TB-LAMP.

The test options for Algorithm 1 vary in complexity. The Xpert MTB/RIF, Xpert Ultra, Truenat MTB and Truenat MTB Plus all require basic pipetting skills and are easy to decentralize but have limited throughput with the commonly used instruments. In contrast, although some of the moderate complexity automated NAATs have minimal hands-on time, they have large infrastructure requirements; also, most of these tests provide higher throughput and are suited to established laboratories with reliable sample referral networks, and they detect resistance to INH in addition to RIF. 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 using 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.

Algorithm 2 may be the starting point for some people, primarily for symptomatic PLHIV who would benefit from a rapid point-of-care test to diagnose TB. It is recommended that testing using Algorithm 1 and Algorithm 2 is done in parallel.

Algorithms 3 and 4 follow on from Algorithm 1, with the split being based on the RIF result.

Algorithm 3 is for those with confirmed RR-TB, and is aimed at providing additional rapid DST for FQs and BDQ.

- Algorithm 3a relies on the use of the recently recommended targeted NGS tests as the primary diagnostic tool for all people with RR-TB. It focuses on the detection of BDQ, FQ and LZD resistance, to address key aspects of recently recommended BPaL and BPaLM regimens as well as other shorter regimens for the treatment of MDR/RR-TB.
- Algorithm 3b uses existing tools complemented with the use of targeted NGS (where available) to provide rapid and comprehensive DST to drugs in prioritized groups. This algorithm focuses on the rapid detection of FQ resistance for two reasons: rapid early diagnosis of FQ resistance among people with MDR/RR-TB is important for selecting treatment regimens, and low to moderate complexity molecular tests to detect FQ resistance are readily available in many countries. The options for detecting resistance to FQs are low complexity automated NAAT (e.g. Xpert MTB/XDR), FL-LPA and SL-LPA, and the recently recommended targeted NGS-based tests.

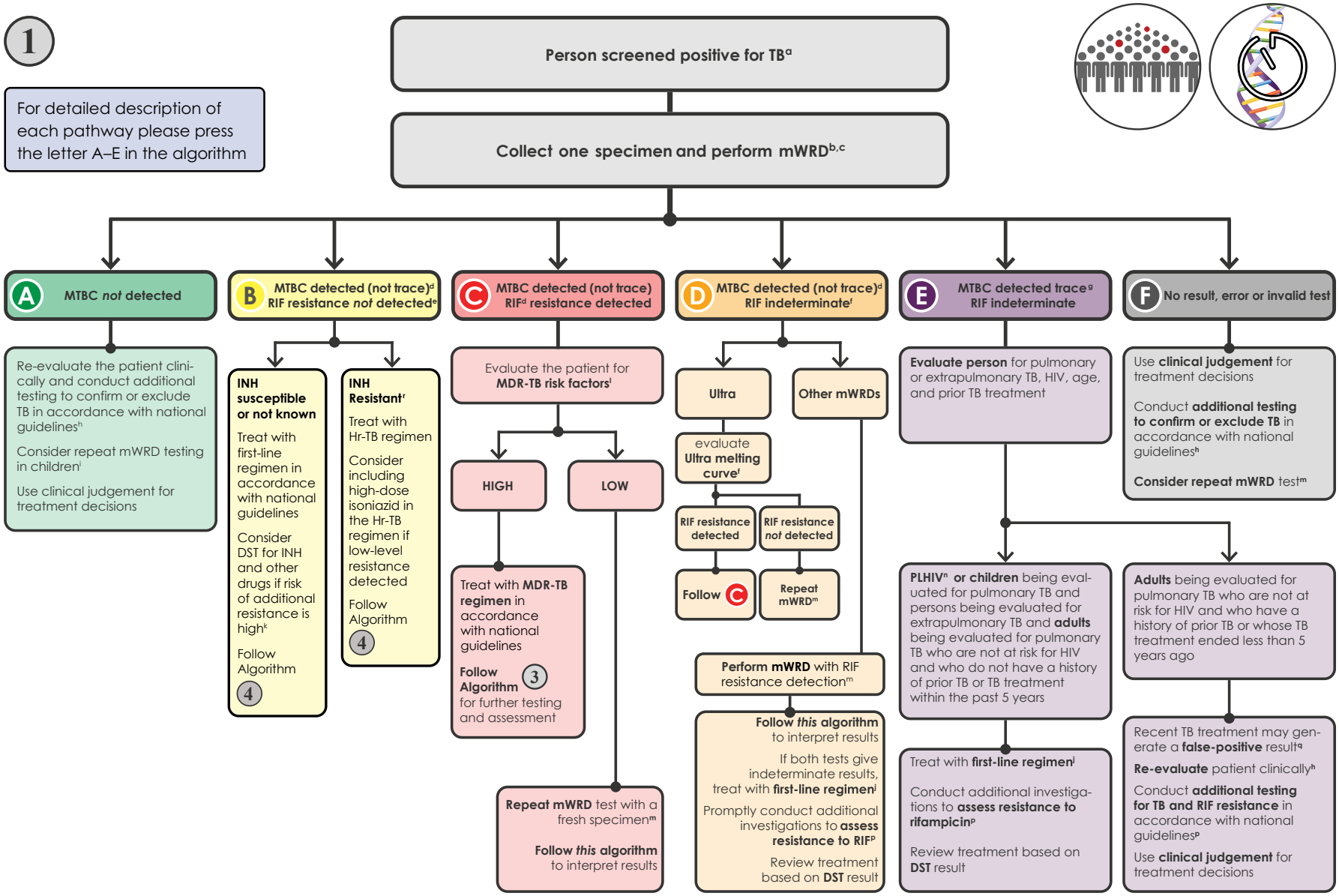
Algorithm 4 is a follow-on algorithm when the purpose is to detect resistance in individuals with RIF-susceptible TB at risk of having DR-TB, and in individuals with Hr-TB. Molecular testing is preferred and may use any existing WHO-recommended rapid test. Algorithm 4 also takes advantage of the ability of targeted NGS tests to detect mutations associated with resistance to many anti-TB medicines to provide rapid molecular tests for people at high risk of having DR-TB (e.g. people in whom therapy is failing). Currently, INH resistance detection is not widely implemented at initial diagnosis, but this will change with the adoption of moderate complexity automated NAATs that also detect INH resistance as initial tests. The adoption of low complexity automated NAATs that detect INH resistance for follow-on testing of RIF-susceptible TB could also increase the detection of INH resistance. It is estimated that the incidence of Hr-TB is greater than that of RR-TB, and both types of TB require modification of the treatment regimen. People with Hr-TB would need an FQ added to the therapy. In scenarios where the only diagnostic tests available are those for RIF resistance (e.g. Xpert MTB/RIF and Truenat MTB), follow-on testing in settings or subpopulations with high Hr-TB burden using the low complexity automated NAAT for INH resistance detection will be appropriate, being simpler and cheaper than performing the FL-LPA or targeted NGS test.

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

Algorithm 1 is the preferred algorithm for testing to support the diagnosis of TB in individuals being evaluated for pulmonary and extrapulmonary TB, and to achieve universal DST. In this algorithm, mWRDs are used as the initial diagnostic test to detect TB, RIF resistance (except if TB-LAMP is used) and – in the case of using moderate complexity automated NAATs – INH resistance (i.e. this algorithm meets the goals of the End TB Strategy for the use of mWRDs and universal DST). If TB-LAMP is used, follow-on testing for RIF resistance is needed. This algorithm is designed to be used with any of the mWRDs for detection of MTBC (Xpert MTB/RIF, Xpert Ultra, Truenat MTB, Truenat MTB Plus and TB-LAMP, moderate complexity automated NAAT), although the algorithm may need to be modified, depending on which mWRD is used and in which population. For example, in a setting with a high MDR-TB burden, it would be preferable to use an mWRD that detects MTBC and RIF resistance initially (e.g. Xpert MTB/RIF, Truenat MTB then Truenat MTB-RIF Dx or moderate complexity automated NAAT) rather than one that detects only MTBC (e.g. TB-LAMP). In a setting with a well-functioning referral system and a high risk of Hr-TB, a moderate complexity automated NAAT may be preferred as the initial test because of the ability to test for INH and RIF resistance simultaneously.

This algorithm is feasible when the mWRD testing can be conducted on site or can be accessed through a reliable referral system with short turnaround times.

Fig. 4.2. Algorithm 1: Molecular WRD as the initial diagnostic test for TB



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

[a] People screened positive for TB include adults and children with signs or symptoms suggestive of TB, with a chest X-ray showing abnormalities suggestive of TB, a positive mWRD used as a screening tool or positive C-reactive protein test (>5 mg/L) in PLHIV. 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 3 or 4. This algorithm may also be followed for the diagnosis of extrapulmonary TB using CSF, lymph node and other tissue specimens. However, mWRDs that are recommended for use in the diagnosis of extrapulmonary TB investigations are currently limited to Xpert MTB/RIF and Xpert Ultra.

[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] mWRDs or classes appropriate for this algorithm include Xpert MTB/RIF, Xpert Ultra, Truenat MTB, Truenat MTB Plus, MC-aNAAT and TB-LAMP.

[d] "MTBC detected (not trace)" includes MTBC detected as high, medium, low or very low. These categories apply to the Xpert MTB/RIF and Xpert Ultra tests. Results of the Truenat MTB and MTB Plus tests, MC-aNAAT and the TB-LAMP test also fall into the category of "MTBC detected (not trace)". The MC-aNAAT provides additional resistance detection for isoniazid and leads to additional considerations in **Box B**.

[e] Determination of RIF resistance occurs simultaneously in the Xpert MTB/RIF, Xpert Ultra and MC-aNAAT tests. A second test is needed to determine RIF resistance in the Truenat MTB or MTB Plus test, using the same DNA isolated for the Truenat MTB tests (Truenat MTB-RIF Dx test) and in the TB-LAMP test, which requires a fresh specimen to be collected and a molecular or phenotypic DST to be conducted. In the case of MC-aNAAT, INH resistance detection would also occur simultaneously with RIF detection.

[f] 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 that RIF indeterminate results obtained with the Xpert Ultra test (especially those with high and medium semiquantitative results) may be due to large deletions or multiple mutations that confer RIF resistance. Analysis of the Ultra melt curves can detect such resistance-conferring mutations. In some cases, culture and DST, sequencing or alternative mWRD will be needed to confirm or exclude RIF resistance. Indeterminate results for the other mWRDs are usually related to very low numbers of bacilli in the sample. When using an mWRD test without detection of RIF resistance (e.g. TB-LAMP), further testing for RIF resistance is required using an mWRD able to detect resistance.

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

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

[i] In children with signs and symptoms of pulmonary TB in settings with a pretest probability of 5% or more, and an Xpert MTB/RIF or Xpert Ultra negative result on the initial test, repeat testing with Xpert MTB/RIF or Ultra (for a total of two tests) in sputum or nasopharyngeal aspirate. Furthermore, repeated testing with Xpert MTB/RIF may be used only in gastric fluid, and stool specimens. No data were available to assess the performance of Xpert Ultra in gastric fluid and stool specimens. Programmes are encouraged to use Xpert Ultra in gastric fluid and stool specimens under operational research conditions. The mWRD should be repeated at the same testing site with a fresh specimen, with the result of the repeat test interpreted as shown in this algorithm. The result of the second test is the result that should be used for clinical decisions.

[j] 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. 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.

[k] 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 mono- or poly-resistance) in this setting. Where a result for INH resistance is "not detected" (e.g. MC-aNAAT), and the pretest probability for Hr-TB is high, phenotypic DST for INH should be performed.

[l] 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.

[m] 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.

[n] 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.

[o] People should be promptly initiated on an MDR-TB regimen in accordance with national guidelines. Algorithm 3 should be followed for additional testing for any person with RR-TB.

[p] 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.

[q] 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 Xpert MTB/RIF "MTBC detected low or very low") may be positive, not because of active TB but because of the presence of non-viable bacilli. Clinical decisions must be made based on all available information and clinical judgement.

[r] 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 RIF/EMB/PZA (REZ) and levofloxacin. For practical purposes, HREZ fixed-dose combination tablets may be used instead of REZ. Consider including high-dose INH in the Hr-TB regimen if low-level resistance is detected (*inhA* mutation only). Follow Algorithm 4.

4.1.1 Decision pathway for Algorithm 1 – mWRD as the initial diagnostic test for TB

Tests

The mWRDs appropriate for this algorithm include the Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, Truenat MTB Plus and TB-LAMP tests and moderate complexity automated NAATs.

- "Xpert MTB test" designates either the Xpert MTB/RIF test or the Xpert MTB/RIF Ultra (hereafter referred to as "Xpert Ultra") test. The individual tests are named when describing test-specific features. The Xpert Ultra test has an additional semiquantitative category called "trace", caused by small amounts of bacterial DNA.
- The Truenat MTB and MTB Plus assays use the same results categories as the Xpert MTB/RIF assay, and the decision pathway for the Truenat tests is the same as that for the Xpert MTB/RIF test.
- Moderate complexity automated NAATs include a number of tests for detection of MTBC as well as RIF and INH resistance. Currently, tests from four manufacturers have been reviewed and recommended, but only for use on respiratory samples:
  - Abbott RealTi*m*e MTB and Abbott RealTi*m*e MTB RIF/INH (Abbott);
  - FluoroType MTBDR and FluoroType MTB (Bruker/Hain Lifescience);
  - BD MAX MDR-TB (Becton Dickinson); and
  - cobas MTB and cobas MTB-RIF/INH (Roche).
- Simultaneous detection of MTBC and RIF resistance applies to the Xpert MTB/RIF, Xpert Ultra and moderate complexity automated NAAT tests. The Truenat MTB or MTB Plus test requires subsequent testing with Truenat MTB-RIF Dx. Some moderate complexity automated NAATs (Abbott RealTi*m*e MTB and cobas MTB) have the option of simultaneous or manual reflex testing for resistance to RIF or INH (or both). For the two-step procedures, the same DNA sample that was isolated for the initial test is used.
- The TB-LAMP test only detects MTBC and it requires a fresh specimen to be collected for subsequent testing with an alternative molecular test or phenotypic DST to detect RIF resistance.

General considerations

WHO recommends the use of an mWRD (Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, Truenat MTB Plus, Truenat MTB-RIF Dx, TB-LAMP or moderate complexity automated NAAT) as the initial diagnostic test, rather than microscopy or culture, for all individuals with signs and symptoms of TB. This includes all newly presenting symptomatic individuals; it may also include people who are on treatment or have been previously treated, if the person is 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 to replacing microscopy as the initial diagnostic test with mWRDs that show a higher sensitivity for the diagnosis of TB as well as simultaneous detection of resistance to RIF (and, for moderate complexity automated NAATs, INH as well).

This algorithm is designed to be used with any of the mWRDs for the detection of MTBC, although the algorithm may require minor modification based on which mWRD is used and in which population. The description and WHO recommendations for each test are presented in Section 2. Only special considerations as they relate to the algorithms are provided here.

- The Xpert MTB/RIF and Xpert Ultra tests have specific recommendations for extrapulmonary TB and children being evaluated.
  - These tests are recommended for use with CSF (preferred sample for TB meningitis), lymph node aspirates and lymph node biopsies. In addition, the Xpert MTB/RIF test is recommended for pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine, as an initial diagnostic test for the corresponding extrapulmonary TB disease. Blood may also be used as a specimen for HIV-positive adults and children with signs and symptoms of disseminated TB. Sample-specific optimization steps may be needed to achieve optimal results, especially for paucibacillary samples.
  - Minor variations for use of these tests with children with signs and symptoms of pulmonary TB include the following:
    - Xpert MTB/RIF can be used as the initial diagnostic test for pulmonary TB with sputum, gastric aspirate, nasopharyngeal aspirate or stool samples, whereas Xpert Ultra is recommended for use with sputum and nasopharyngeal aspirate specimens; and
    - in settings with a pretest probability of 5% or more and an Xpert MTB/RIF or Ultra negative result on the initial test, repeated testing with Xpert MTB/RIF or Ultra with the same or different specimen types (for a total of two tests) may be used; otherwise, repeat testing is not recommended.
- The Truenat MTB and MTB Plus test considerations include the following:
  - There is uncertainty about use of this test in PLHIV, because insufficient data were available on the performance of these tests in PLHIV. The indirect data on test performance in people who were smear-negative were used to extrapolate the recommendation to use in PLHIV.
  - In children, sufficient data were available to recommend the use of these tests with sputum samples only. There were no data on how these tests performed with other specimens.
  - The performance of these test for the detection of extrapulmonary TB is unknown.
- The TB-LAMP test is recommended as a replacement test for sputum-smear microscopy; it would be suitable for use in settings that have a low prevalence of HIV and MDR-TB. Considerations for the use of this test include the following:
  - In populations with a high burden of MDR-TB, TB-LAMP should not replace the use of rapid molecular tests that detect RIF resistance (e.g. Xpert MTB/RIF), because TB-LAMP does not provide any information on RIF resistance.
  - In populations with a high prevalence of HIV, TB-LAMP should not replace the use of rapid molecular tests that have a higher sensitivity for detection of TB (e.g. Xpert Ultra).
  - Considerations for the moderate complexity automated NAATs include the following:
  - The recommendations apply to PLHIV. Performance on smear-negative samples was reviewed but was only available for TB detection, not for RIF and INH resistance. Data stratified by HIV status were not available.
  - The recommendations apply to adolescents and children based on the generalization of data from adults. An increased rate of false negative or indeterminate results may be found with paucibacillary TB disease in children.

– Extrapolation to 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 mWRDs are not recommended as tests for monitoring treatment, because the presence of dead bacilli may generate a positive result. Instead, microscopy and culture should be used for monitoring, in accordance with national guidelines and WHO recommendations.

Algorithm 1 describes the collection of one initial specimen to be used for mWRD testing and the collection of additional specimens as needed. For operational issues, programmes may consider collecting two specimens (e.g. spot and morning sputum samples, or two spot specimens) from each person routinely, 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 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 tissue biopsy samples are difficult or impossible to obtain repeatedly), 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 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 "MTB 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".

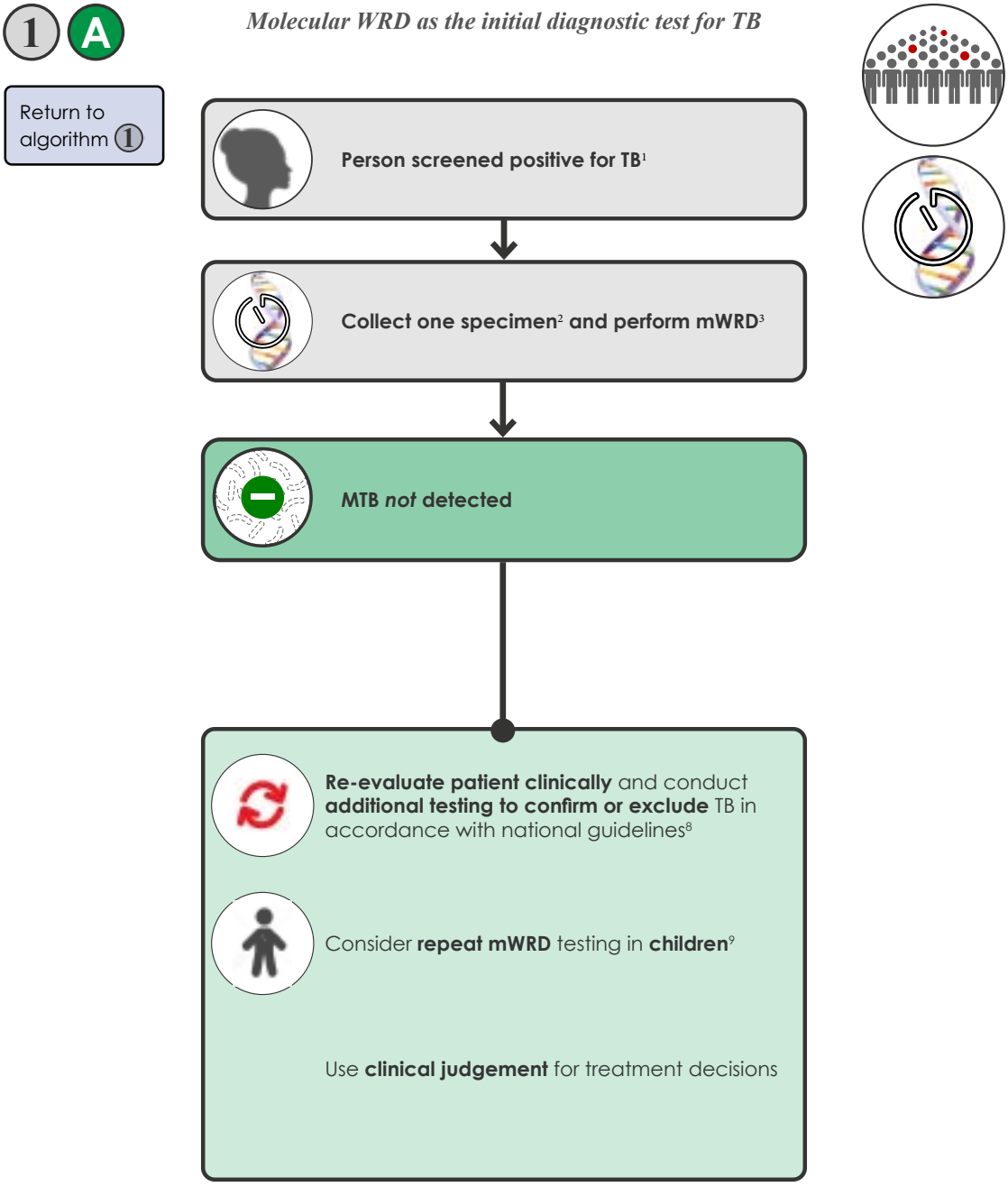
- Each of the semiquantitative categories of MTBC detected, including "trace", is considered as bacteriological confirmation of TB.
- Xpert Ultra "trace": for PLHIV and children who are being evaluated for pulmonary TB, and for individuals being evaluated for extrapulmonary TB, the "MTBC detected trace" result is considered as bacteriological confirmation of TB.
- In HIV-negative, symptomatic adults with a recent history of TB treatment (i.e. completed <5 years ago), Xpert Ultra "trace" results (and occasionally other molecular 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, the 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. Xpert MTB/RIF and Truenat MTB-RIF Dx) it may be more appropriate to state that resistance is **inferred** rather than detected.

The use of an mWRD to detect resistance to RIF or INH (or both) does not eliminate the need for conventional culture-based phenotypic DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.

Decision pathway

1. Collect a good-quality specimen and transport it to the testing laboratory. Conduct the mWRD. For individuals being evaluated for pulmonary TB, the following specimens may be used: induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage or aspirates, nasopharyngeal aspirates and stool samples. (For information on which specimens may be used with which mWRD, see Section 2.2 above or individual WHO policy statements.)
2. If the mWRD result is "MTB not detected" (1)(A), re-evaluate the person and conduct additional testing in accordance with national guidelines.
   a. Further investigations for TB may include chest X-ray, additional clinical assessments, additional mWRD testing or culture and clinical response following treatment with broad-spectrum antimicrobial agents (FQs should *not* be used).
   b. In children with signs and symptoms of pulmonary TB in settings with a high pretest probability (>5%) and a negative Xpert MTB result on the first initial test, repeat the Xpert MTB test for a total of two tests. The tests may use the same specimen types or different specimen types (e.g. one sputum specimen and one nasopharyngeal aspirate sample).
   c. The performance of the other mWRDs in repeat testing is not known.
   d. Consider the possibility of clinically defined TB (i.e. TB without bacteriological confirmation). Use clinical judgement for treatment decisions.



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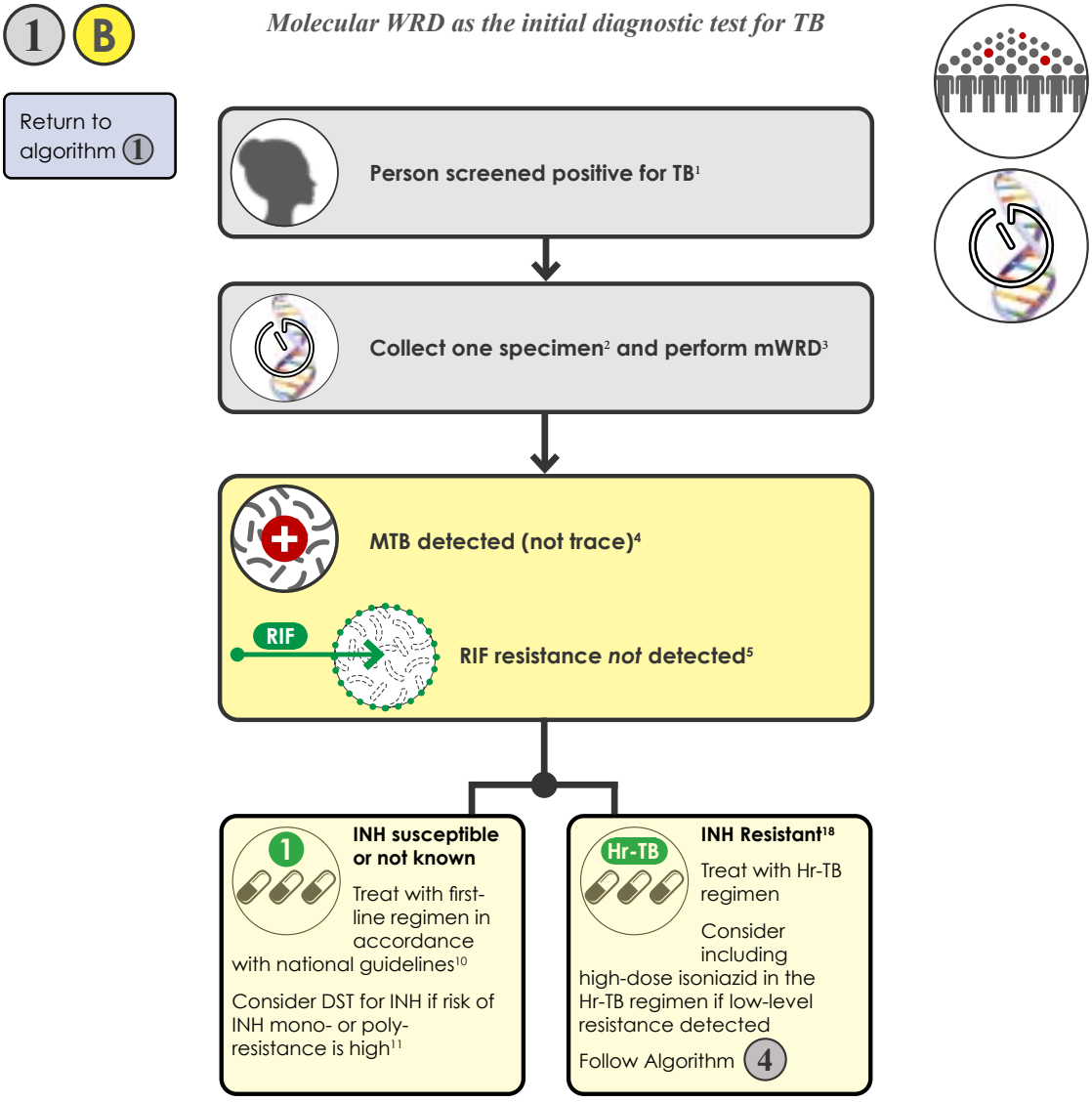
3. If the mWRD result is "MTBC detected, RIF resistance not detected" and "INH resistance not detected" or INH results are unknown: (1)(B)
   a. Initiate the person on an appropriate regimen using first-line TB drugs in accordance with national guidelines.
   b. Request additional DST in the following cases:
      i. Molecular or phenotypic DST for INH is indicated particularly (see Algorithm 4 for follow-up testing):
         - if the person has been treated 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 poly-resistance, not MDR-TB) in this setting.

      If the INH resistance is "not detected" by the moderate complexity automated NAAT 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 current molecular tests.
      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 "susceptibility". Sometimes, these anomalous results may be due to sample labelling errors and a repeat test may resolve the issue. False RIF-susceptible Xpert MTB results can occur but are uncommon (1–5% of RIF-resistant TB cases tested) and the level of such results depends on the epidemiologic settings. In contrast, phenotypic DST for RIF, especially using liquid culture, is associated with a higher proportion of false-susceptible results *(41)*. The updated critical concentration for RIF which should be used will reduce, but not eliminate, this issue. Sequencing should be performed when available, and should cover not only the RIF-resistance-determining region (RRDR) but regions outside as well (e.g. codons 170 and 491).
   c. If additional molecular or phenotypic testing is performed:
      i. The molecular and phenotypic testing may be performed in different laboratories. Perform these tests in parallel – do not wait for the results of one test before initiating another test.
      ii. The molecular and phenotypic DST may be performed using the specimen (direct DST) or using bacteria recovered by culture (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. A rapid molecular test is preferred using WHO-recommended tests. Mutation interpretation can also be found in the WHO catalogue of mutations *(4)*. DNA sequencing has proven useful in many cases but WHO has not yet evaluated it for clinical use.
      iv. Culture-based phenotypic DST for INH and RIF requires 3–8 weeks to produce a result. Phenotypic DST may be useful for evaluating people with a susceptible molecular result, particularly in populations with a high pretest probability for resistance to INH.



For footnotes please see page 65

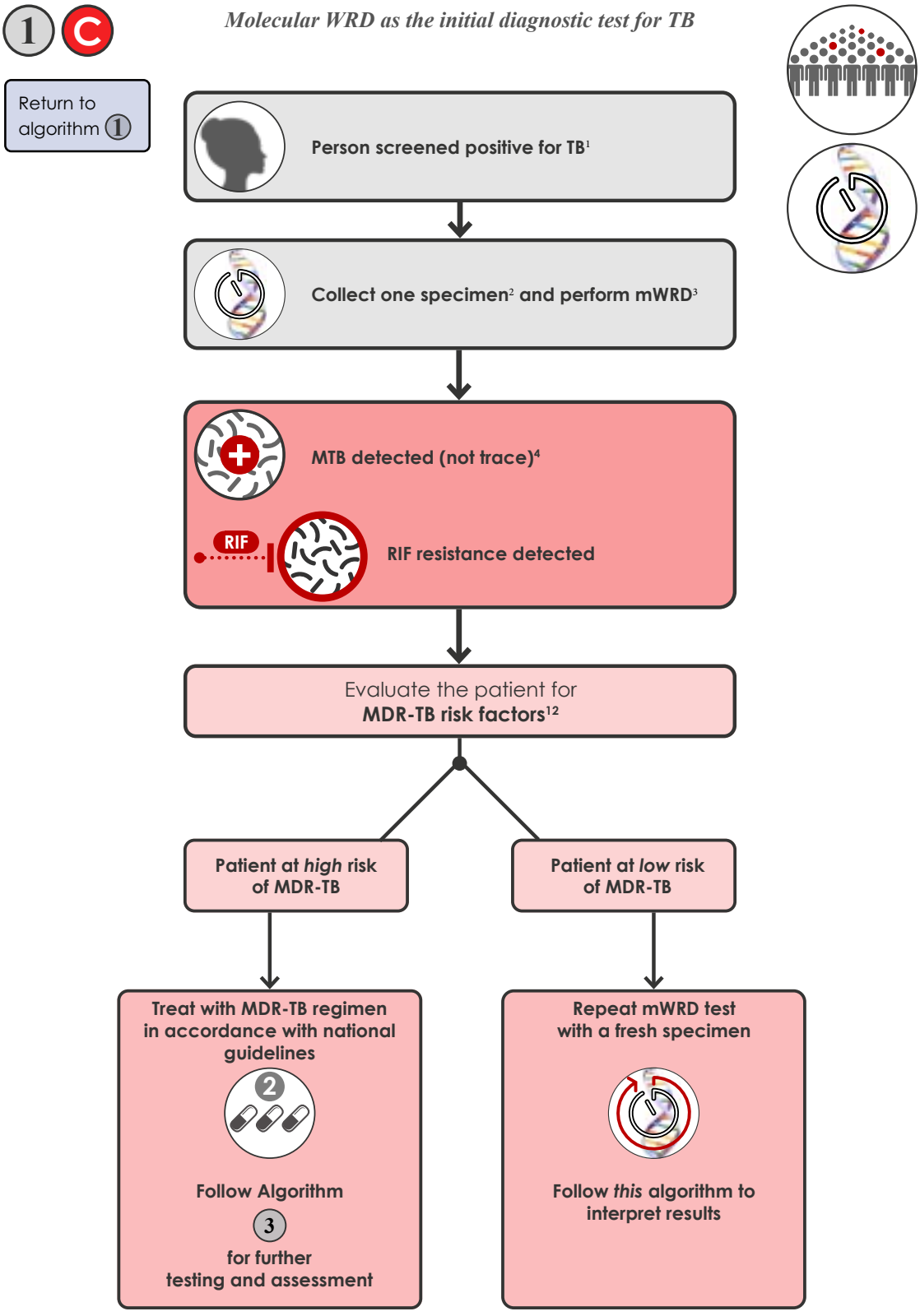
4. If the mWRD test result is "MTBC detected, RIF resistance not detected and INH resistance detected" (currently only applicable to the moderate complexity automated NAATs):
   a. Initiate the person on an appropriate Hr-TB regimen in accordance with national guidelines. The WHO recommendation for people with Hr-TB is treatment with RIF, ethambutol (EMB), PZA and levofloxacin (LFX) for a duration of 6 months *(9)*.
   b. Follow Algorithm 4 for Hr-TB:
      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 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 *(27)*.
5. 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 burden countries, every person with TB is considered to be at high risk of having MDR-TB.

a. If the person is at high risk of having MDR-TB and the test result shows RIF resistance, initiate them on a regimen for MDR/RR-TB in accordance with national guidelines. Follow Algorithm 3 for additional testing.
b. If the person is not at high risk of having MDR-TB, repeat 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 having a low bacillary load, which can be inferred from the semiquantitative readings (e.g. low and very low), have been associated with increased false resistance in some settings *(42–44)*.
    i. If the second test also indicates RIF resistance, initiate an MDR/RR-TB regimen in accordance with national guidelines and WHO recommendations, and follow Algorithm 3 for additional testing.
    ii. If the mWRD result for the second sample is "MTBC detected, RIF resistance not detected", initiate treatment 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 repeat tested if the response to first-line treatment is poor. If an INH resistance or susceptibility result is available, interpret the result and follow-up as described in Algorithm 4.
    iii. In the event that the mWRD result for the second sample is "MTBC detected, RIF resistance is inconclusive", 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. In the event that a moderate complexity automated NAAT was performed and INH results are also available, this could be useful to provide certainty. INH resistance is associated with RIF resistance, and the finding of INH resistance should prompt further investigation to exclude RIF resistance.
c. For all people with RR-TB or MDR-TB, conduct additional investigations 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 a low complexity automated NAAT for the detection of FQ resistance provides a rapid and accurate peripheral level solution that can be performed directly on specimens. Phenotypic (culture and DST) and molecular (e.g. SL-LPA and DNA sequencing) methods are available

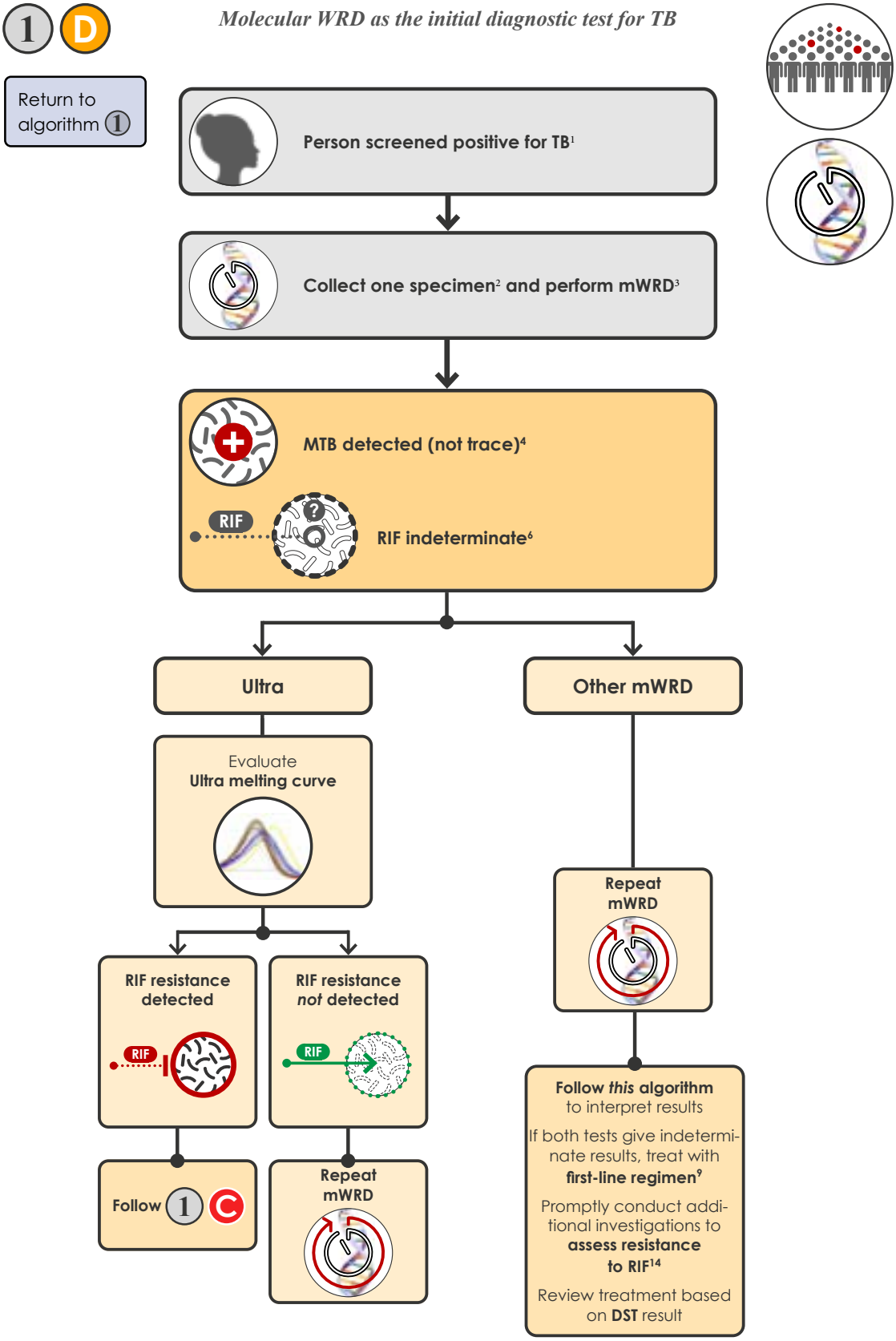
to evaluate drug resistance beyond RIF and INH. Rapid molecular methods are preferred. However, for resistance detection to some of the new and repurposed drugs, phenotypic DST is the only currently available option; thus, two separate specimens may be required.

i. MDR/RR-TB regimens rely on the use of either FQs (submit a sample for molecular testing for FQ resistance – see Algorithm 3a) or BDQ (submit a sample for molecular testing for BDQ resistance – see Algorithm 3b).
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, do not delay treatment initiation 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.



For footnotes please see page 65

6. If the mWRD gives a result of "MTBC detected, RIF indeterminate" (1)(D), the person will require further investigation. The interpretation and follow-up testing for Xpert Ultra differs from that for other mWRDs. With any of the mWRDs, the initial result of "MTBC detected" should be considered as bacteriological confirmation of TB. 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, they should be initiated on an MDR-TB regimen). 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", follow Step 3. If the result is "MTBC detected, RIF resistance detected", follow Step 5.
        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 (non-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 mutations that pose a challenge with mutation analysis software *(45)*.
        iii. The 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 probes and melt curve profile *(45)*.
            1. 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, follow Steps 6.1 and 6.2.
            2. 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, follow Step 7 for additional testing.
            3. 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.



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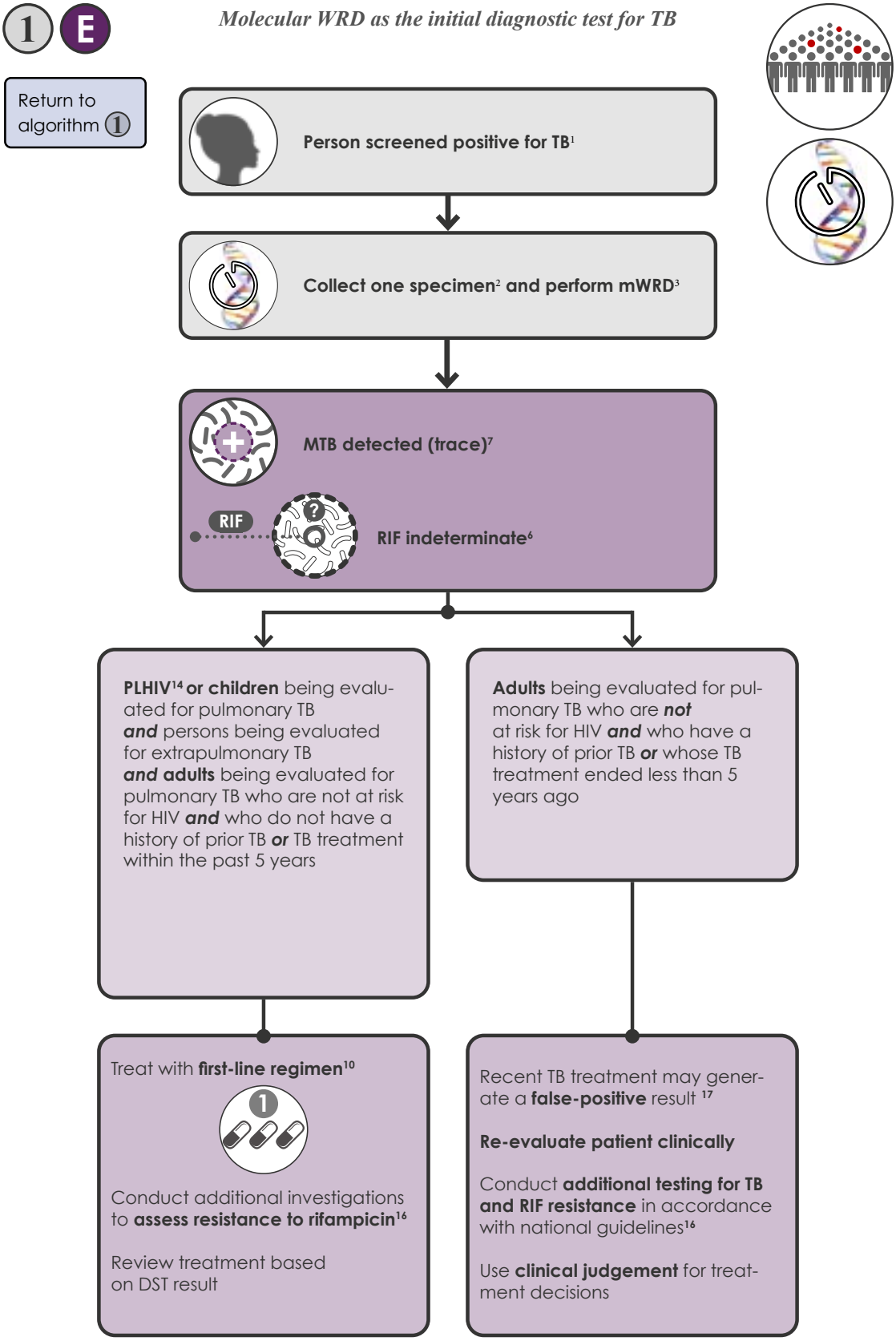
7. If the Xpert Ultra test result is "MTBC detected trace" (1)(E), additional considerations are needed. However, WHO suggests *not* repeating Xpert Ultra testing in adults who have an initial Xpert Ultra trace result to confirm the result.
    a. Review the clinical characteristics to determine the person's age, HIV-infection status and history of TB treatment, and determine whether the samples are pulmonary or extrapulmonary.
        i. PLHIV include individuals 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, perform HIV testing in accordance with national guidelines.
        ii. Children are defined as those aged under 15 years.
        iii. 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 test result is highest immediately after completing treatment, and slowly declines with time *(46, 47)*. 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; such people require careful clinical evaluation.
        iv. Xpert Ultra is recommended for use with CSF, lymph nodes and tissue specimens. Data are limited for the test's performance with other extrapulmonary samples.
        v. 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.



For footnotes please see page 65

b. For certain populations – PLHIV and children who are being evaluated for pulmonary TB; for individuals being evaluated for extrapulmonary TB using CSF, lymph nodes and tissue specimens; and for adults being evaluated for pulmonary TB, who are not at risk for HIV and who do not have a history of prior TB treatment within the past 5 years:
   i. Consider the MTBC detected trace result obtained with the first specimen as bacteriological confirmation of TB (i.e. a true positive result) and use for clinical decisions.
   ii. Initiate the person 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, initiate the person on an MDR-TB regimen).
   iii. Undertake additional investigations (e.g. culture and DST) to confirm or exclude resistance to RIF.

c. For adults being evaluated for pulmonary TB, who are not at risk of HIV and have a history of TB treatment in the past 5 years:
   i. For adults with a history of recent TB treatment or unknown treatment history, consider the possibility of the Xpert Ultra trace result being a false positive result because of the presence of non-viable bacilli.
   ii. Clinically re-evaluate the person and conduct additional testing (including liquid culture) in accordance with national guidelines. Consider the possibility of TB caused by reactivation, relapse or reinfection.
   iii. In initiating treatment, consider the clinical presentation and context of the person. Make clinical decisions based on all available information and clinical judgement.
   iv. Further investigations for TB may include chest X-ray, additional clinical assessments and clinical response following treatment with broad-spectrum antimicrobial agents (FQs should not be used).
      1. 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.
      2. Culture and phenotypic DST may be of benefit to detect TB and drug resistance. The trace result provides no information on RIF resistance.

8. If the mWRD does not give a result, 1 F or gives a result of "error" or "invalid", repeat the mWRD at the same testing site with a second specimen. Return to algorithm 1

Interpretation of discordant results

This algorithm relies on testing of a sample with an mWRD to detect MTBC and assess susceptibility to RIF. Discordance in resistance to INH is described in Algorithm 4. 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. 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. Treatment decision should be based on the culture result. If the person started treatment based on clinical judgement, continue treatment. Record the person 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 *(48).* Their sensitivity is lower in PLHIV, 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, evaluate the response to therapy.
3. mWRD result "MTBC detected, RIF resistance detected"; RIF susceptible by phenotypic DST:
   a. Use the mWRD result 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). People infected with strains carrying these mutations often fail treatment with RIF-based first-line regimens *(41).*
   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 *(49)* or banding pattern on the FL-LPA can aid in determining of inferring the specific mutation (e.g. borderline resistant or silent).
   e. False RIF-resistant results have been observed with the Xpert MTB/RIF G4 cartridge when the MTBC detected result was "very low" and associated with probe binding delay *(42)*. Follow-up action may include mWRD testing of the culture.
   f. Follow-up actions may include DNA sequencing, confirmatory testing on other 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 Xpert MTB/RIF test in various epidemiologic settings. Mutations in the region of the *rpoB* gene sampled by the Xpert MTB tests have been shown to account for 95–99% of RIF resistance. The remainder of RIF resistance arises from mutations outside the sampled region, which produce an Xpert MTB result of "RIF resistance not detected". In settings with a prevalent clone that harbours a mutation outside the RRDR, for example Eswatini *(50)*, this may be more common; however, this has not been identified as a major concern in other settings *(51)*. 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 (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 PLHIV and children who are being evaluated for pulmonary TB, or when extrapulmonary specimens (CSF, lymph nodes and tissue specimens) are tested, the benefits of the increased sensitivity for the detection of MTBC (i.e. true positives) outweighs 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. Consider the possibility that the culture result was a false negative result.

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. Consider the possibility that the culture result was a false negative result, 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 culture (preferably using liquid culture).

ii. For adults with a history of recent TB treatment:

1. Consider the possibility that the Xpert Ultra "MTBC detected trace" result was a false positive result because of the presence of non-viable bacilli. A culture-negative result is consistent with this possibility.
2. If these people 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.

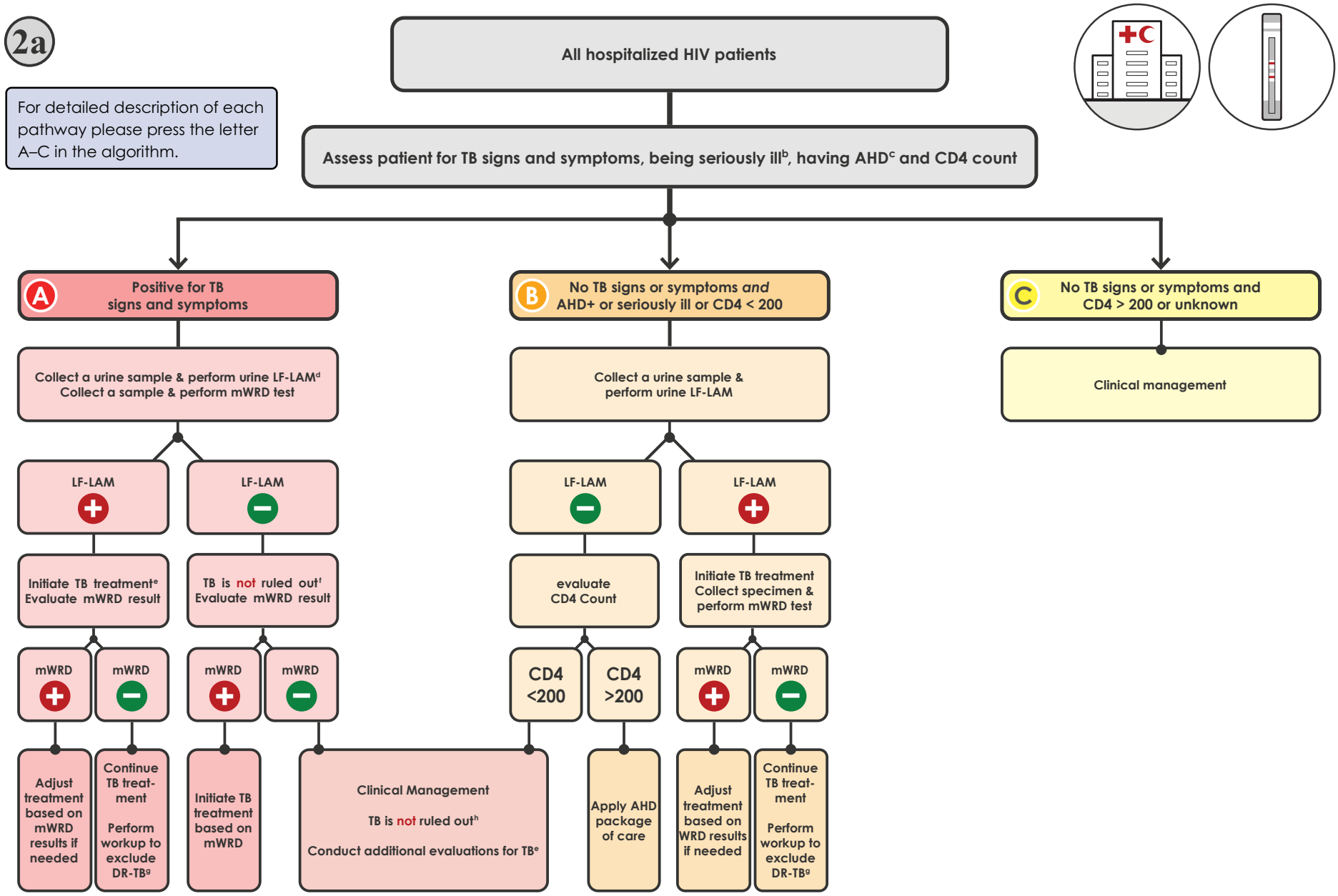
4.2 Algorithm 2 – LF-LAM testing to aid in the diagnosis of TB among PLHIV

Algorithm 2 is the preferred algorithm for testing to support the diagnosis of TB in PLHIV. It is appropriate for use in settings with a high burden of HIV and for use with individual PLHIV who meet the testing criteria, regardless of the overall HIV burden. The algorithm emphasizes the use of LF-LAM to quickly identify people needing TB treatment; it also emphasizes that all individuals with signs and symptoms of TB should receive a rapid mWRD (Algorithm 1). LF-LAM results (test time <15 minutes) are likely to be available before mWRD results, and treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.

The currently available LF-LAMs have sufficient sensitivity and specificity to aid in the diagnosis of TB among individuals coinfected with HIV, but have suboptimal sensitivity and specificity in those who are HIV-negative. Hence, this algorithm emphasizes the use of the LF-LAM test as a diagnostic test in all PLHIV with signs and symptoms of TB, as well as in other specific scenarios (described below) for the diagnosis of TB among PLHIV *(52).* The ease of use of the LF-LAM test makes it suitable for implementation outside of the laboratory – for example, in clinics (especially in those that see critically ill PLHIV) – for rapid diagnosis of TB and treatment initiation in urgent cases of suspected TB among PLHIV. Algorithm 2a is used for PLHIV being evaluated for TB (pulmonary or extrapulmonary) in an inpatient setting. The updated WHO screening guidelines now recommend adult and adolescent inpatients with HIV in medical wards where the TB prevalence is more than 10% should be tested systematically for TB disease with an mWRD, as described in Algorithm 1 *(9)*. This is in addition to the recommendations on the use of LF-LAM among inpatient PLHIV *(49)*. Algorithm 2b is used for PLHIV being evaluated for TB (pulmonary or extrapulmonary) in an outpatient setting or clinic.

These algorithms are appropriate for both low and high MDR-TB or Hr-TB burden settings. The choice of which molecular test to use may be different in a low or high MDR-TB or Hr-TB burden setting, as discussed under Algorithm 1. For example, in a setting with a high burden of MDR-TB, it would be preferable to use an mWRD that detects MTBC and RIF resistance simultaneously (e.g. Xpert MTB/RIF), rather than an mWRD that uses a two-step process (e.g. Truenat MTB) to detect RIF resistance.

Fig. 4.3. Algorithm 2a: LF-LAM to aid in the diagnosis of TB among PLHIV[a] in inpatient settings



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

[a] PLHIV include people who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all people with unknown HIV status, HIV testing should be performed in accordance with national guidelines. PLHIV with TB may also present with signs and symptoms of extrapulmonary TB, including lymphadenopathy, meningitis or other atypical presentations that warrant evaluation.

[b] "Seriously ill" is defined based on four danger signs: respiratory rate >30 per minute, temperature >39 °C, heart rate >120 beats per minute and unable to walk unaided.

[c] For adults, adolescents and children aged >5 years, AHD is defined as CD4 cell count <200 cells/mL^3, or WHO stage 3 or 4 event at presentation for care. All children aged <5 years are considered as having AHD.

[d] The LF-LAM test and mWRD should be done in parallel. The results of the LF-LAM (test time <15 minutes) are likely to be available before the mWRD results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.

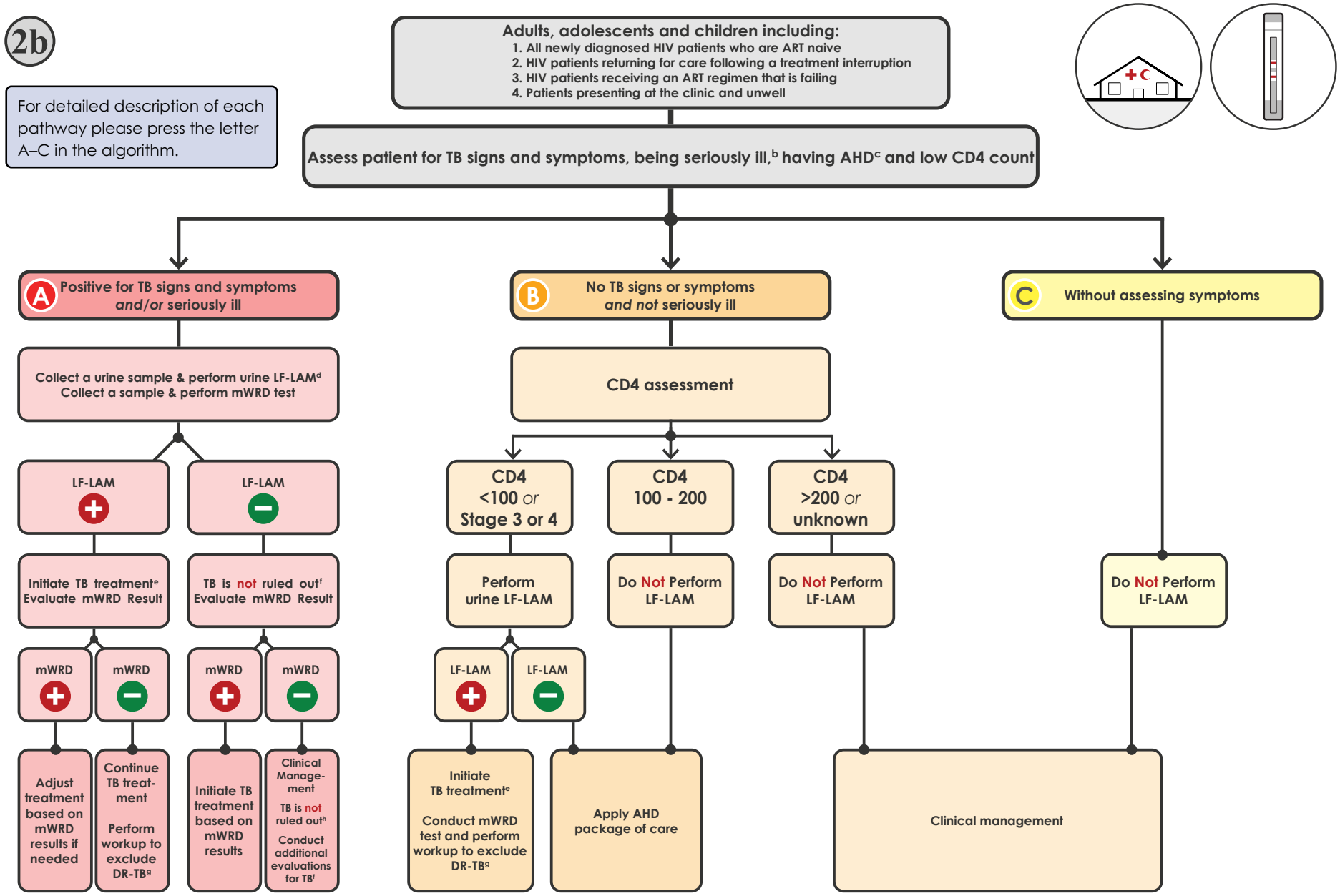
[e] People should be initiated on a first-line regimen according to national guidelines, unless they are at very high risk of having MDR-TB (In whichh case, they should be initiated on an MDR-TB regimen).

[f] Negative LF-LAM results do not rule out TB in people with symptoms. The mWRD result should be evaluated when it becomes available for treatment decisions. See Algorithm 1 for interpretation of mWRD results.

[g] Phenotypic (culture and DST) and molecular (e.g. mWRDs, LPAs and DNA sequencing) methods are available to evaluate drug resistance. Rapid molecular methods (e.g. Xpert MTB or Truenat MTB-RIF Dx or MC-aNAAT tests) are preferred.

[h] Negative mWRD and LF-LAM results do not rule out TB in people with symptoms; In such cases, additional clinical evaluations for TB should be carried out. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, and additional mWRD testing or culture. Initiation of treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (FQs should not be used) and for *Pneumocystis* pneumonia should be considered. The clinical response should be evaluated after 3–5 days of treatment.

Fig. 4.4. Algorithm 2b: LF-LAM testing to aid in the diagnosis of TB among PLHIV[a] in clinic and outpatient settings



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

[a] PLHIV include people who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all people with unknown HIV status, HIV testing should be performed in accordance with national guidelines. PLHIV with TB may also present with signs and symptoms of extrapulmonary TB, including lymphadenopathy, meningitis or other atypical presentations warranting evaluation.

[b] "Seriously ill" is defined based on four danger signs: respiratory rate >30 per minute, temperature >39 °C, heart rate >120 beats per minute and unable to walk unaided.

[c] For adults, adolescents and children aged >5 years, AHD is defined as CD4 cell count <200 cells/mL^3 or WHO stage 3 or 4 event at presentation for care. All children aged <5 years are considered as having AHD.

[d] The LF-LAM test and mWRD should be done in parallel. The results of the LF-LAM (test time <15 minutes) are likely to be available before mWRD results, and treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.

[e] 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 (In which case, the person should be initiated on an MDR-TB regimen). Treatment regimens should be modified as needed based on the results of the mWRD testing.

[f] LF-LAM negative results do not rule out TB in people with symptoms. The result of the mWRD should be evaluated when it becomes available (see Algorithm 1 for interpretation of mWRD results).

[g] Phenotypic (culture and DST) and molecular (e.g. LPAs, DNA sequencing and high-throughput assays) methods are available to evaluate drug resistance. Rapid molecular methods (e.g. Xpert MTB or Truenat MTB-Truenat MTB Plus or MC-aNAATs) are preferred.

[h] The mWRD negative and LF-LAM negative results do not rule out TB in people with symptoms. Additional clinical evaluations for TB should be conducted. Further investigations for TB may include chest X-ray, additional clinical assessments, and additional mWRD testing or culture. Initiation of treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (not FQs) and those for *Pneumocystis* pneumonia should be considered. The clinical response should be evaluated after 3–5 days of treatment.

4.2.1 Decision pathway for Algorithm 2 – LF-LAM testing to aid in the diagnosis of TB among PLHIV

General considerations for Algorithm 2a and Algorithm 2b

- The LF-LAM is a point-of-care test that may be implemented outside the laboratory (e.g. at the bedside in clinics that see critically ill PLHIV) for rapid diagnosis of TB and treatment initiation.
- The algorithms may be used for PLHIV being evaluated for pulmonary or extrapulmonary TB.
- The algorithms are appropriate for all PLHIV who meet the testing requirements, regardless of the overall prevalence of HIV in the setting.
- The algorithms are appropriate for both low and high MDR-TB or Hr-TB burden settings. The choice of which mWRD to use may be different in a low- or high-burden MDR-TB setting.
- The algorithms emphasize the use of the LF-LAM test in addition to an mWRD in all PLHIV with signs and symptoms of TB, and in:
  - inpatient settings, for HIV-positive adults, adolescents and children with advanced HIV disease or who are seriously ill, or PLHIV with a CD4 cell count of less than 200 cells/mm^3, irrespective of signs and symptoms of TB; and
  - outpatient settings, for HIV-positive adults, adolescents and children who are seriously ill or PLHIV with a CD4 cell count of less than 100 cells/mm^3, irrespective of signs and symptoms of TB.
- WHO recommends against using LF-LAM to:
  - assist in the diagnosis of active TB in HIV-positive adults, adolescents and children without TB symptoms and an unknown CD4 cell count, or a CD4 cell count greater than 100 cells/mm^3 in outpatient settings; and
  - assist in the diagnosis of TB in HIV-negative individuals, because of suboptimal sensitivity and specificity in HIV-negative people.
- Anyone who has signs and symptoms of pulmonary TB and who is capable of producing sputum should have at least one specimen submitted for mWRD testing. This also includes children and adolescents living with HIV who are able to provide a sputum sample (see Algorithm 1).

Decision pathway for Algorithm 2a – LF-LAM testing to aid in the diagnosis of TB among PLHIV in inpatient settings

1. Evaluate the hospitalized person for TB, determine HIV status and assess the presence of danger signs for being seriously ill. In PLHIV who are not seriously ill, consider measuring CD4 cell counts, to assess eligibility for testing with the LF-LAM.
   a. Individuals to be evaluated for TB include hospitalized HIV-positive adults, adolescents and children with signs or symptoms suggestive of TB (pulmonary or extrapulmonary) or with a chest X-ray with abnormalities suggestive of TB, or hospitalized people who have advanced HIV disease (AHD), are seriously ill or have CD4 counts of less than 200 cells/mm^3, regardless of TB signs and symptoms.
   b. PLHIV include individuals 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, perform HIV testing in accordance with national guidelines. For all

adults living with HIV/AIDS, regardless of CD4 cell count or clinical stage, recommend ART and consider initiating co-trimoxazole preventive therapy.

c. "Seriously ill" is defined as presenting with any one of the following danger signs: respiratory rate >30 per minute, temperature >39 °C, heart rate >120 beats per minute or unable to walk unaided.

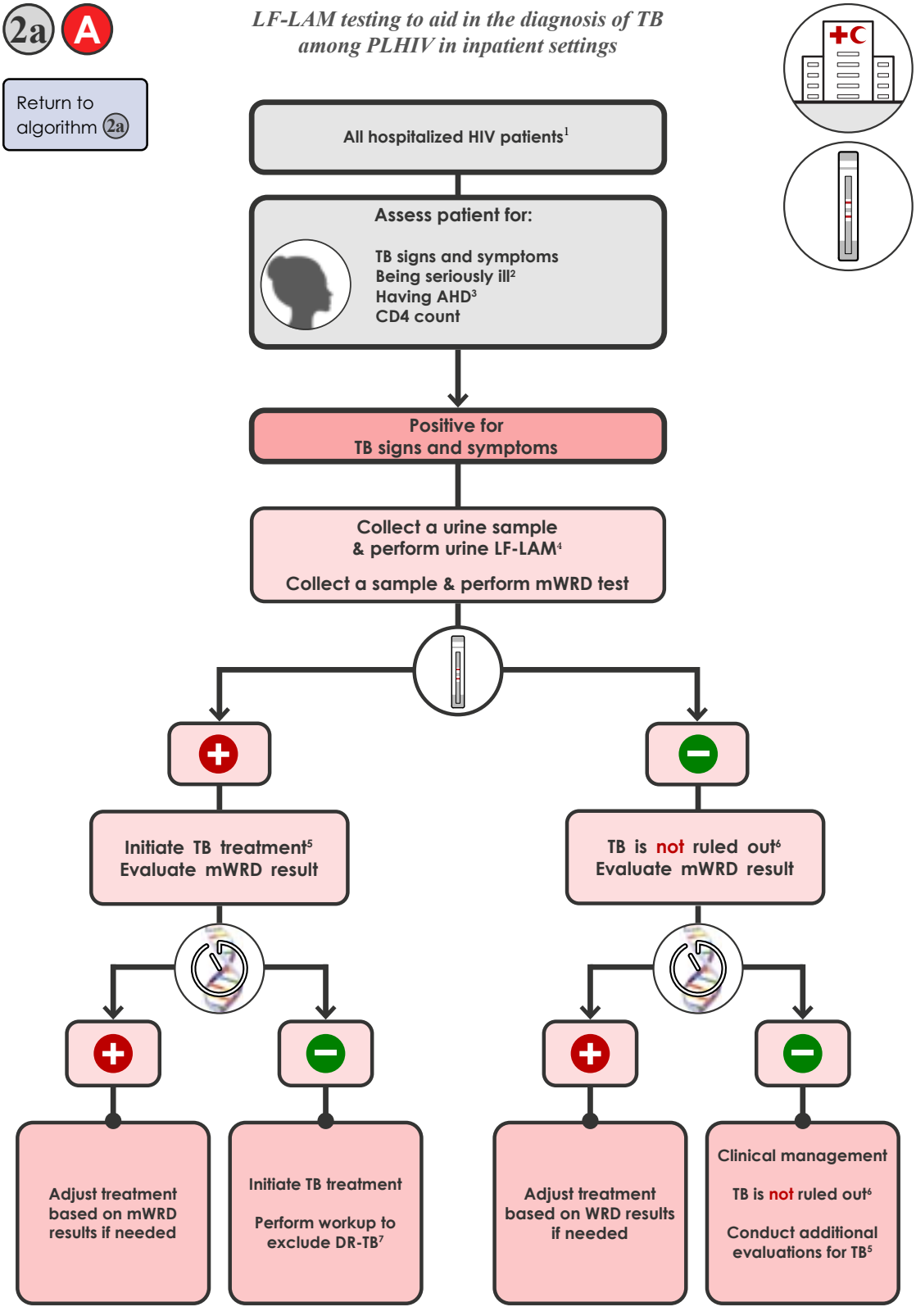
d. For adults, adolescents and children aged more than 5 years, AHD is defined as CD4 cell count <200 cells/mm^3 or WHO stage 3 or 4 event at presentation for care. All children aged under 5 years are considered as having AHD.

2. For hospitalized PLHIV being evaluated for TB 2a A, who are positive for signs and symptoms of TB:

   a. Collect a urine specimen and conduct the LF-LAM **and** collect a specimen and conduct mWRD testing. If the mWRD is available on site, perform the mWRD testing in parallel to the LF-LAM testing.

      i. For individuals being evaluated for pulmonary TB, the following samples may be used for the mWRD: induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage or aspirate. Additional sample types suitable for use with Xpert MTB/RIF and Xpert Ultra include nasopharyngeal aspirate and for Xpert MTB/RIF includes stool samples.

      ii. For individuals being evaluated for extrapulmonary TB, the Xpert MTB test is recommended for use with CSF (preferred sample for TB meningitis), lymph node aspirates and lymph node biopsies. In addition, Xpert MTB/RIF is recommended for pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine as an initial diagnostic test for the corresponding extrapulmonary TB. Blood may also be used as a specimen for Xpert MTB/RIF for HIV-positive adults and children with signs and symptoms of disseminated TB. Other tests for use in extrapulmonary TB include the Xpert Ultra.

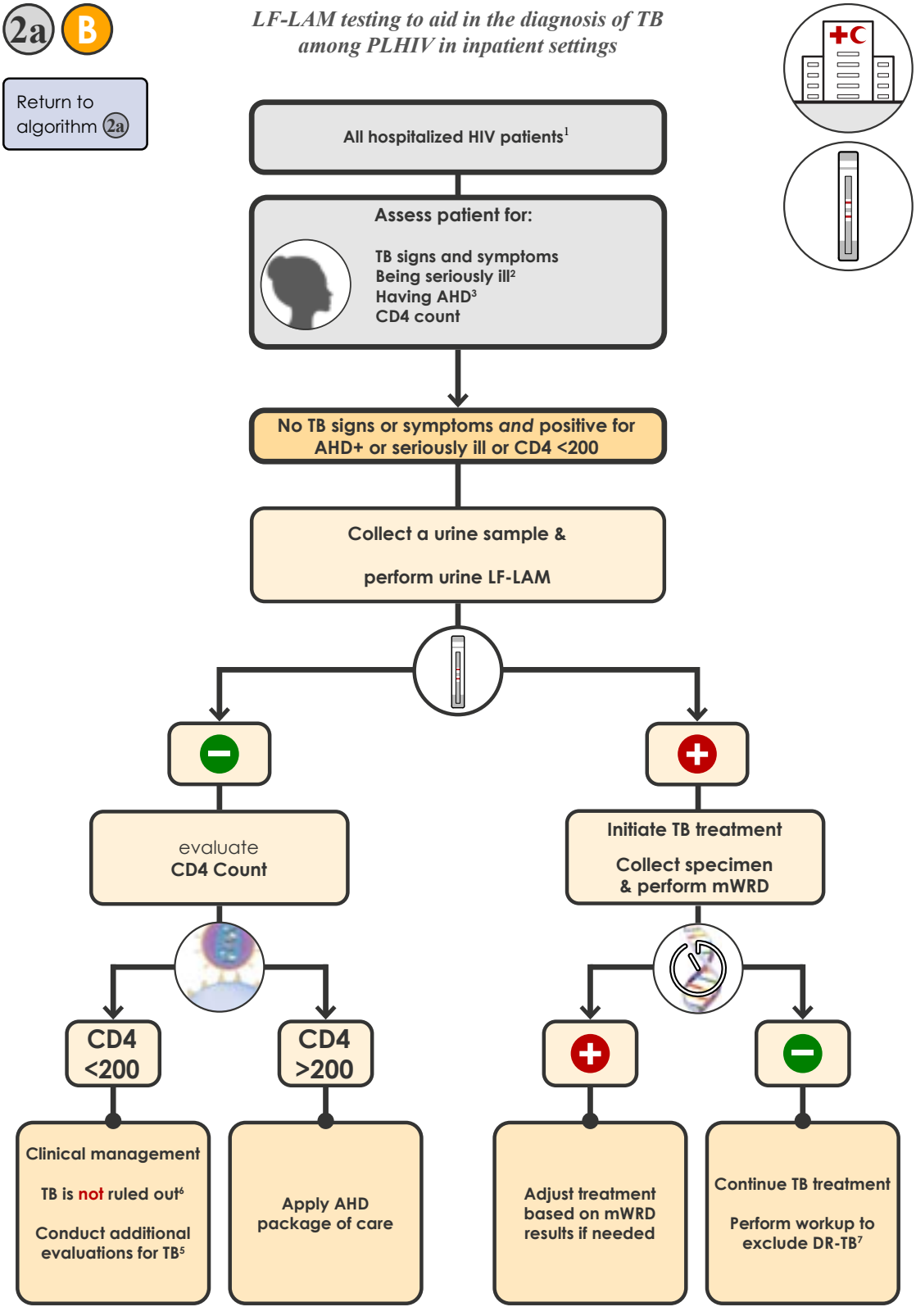


For footnotes please see page 87

b. The LF-LAM result (test time <15 minutes) is likely to be available before the mWRD result and should be interpreted in the context of clinical judgement, chest X-ray findings (if available) and any available bacteriological results.

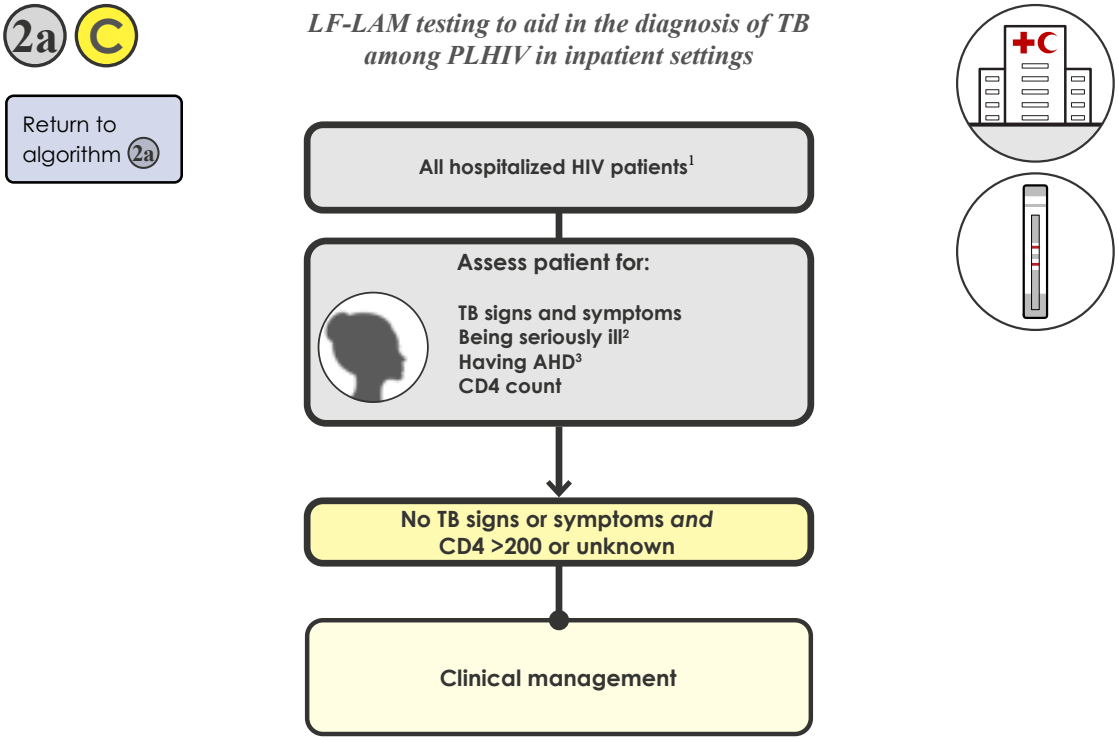
c. All people with TB meeting the testing requirements who have a positive LF-LAM result should be initiated on TB treatment immediately, while awaiting results of the mWRD. Follow Algorithm 1 for the selection and interpretation of mWRD results, which include resistance detection, and modify therapy as needed.
d. TB is not ruled out if the LF-LAM test result is negative. Evaluate the results of the mWRD, and follow Algorithm 1 for interpretation of results and follow-up testing.
e. Treat all people with TB with an mWRD result of "MTBC detected" for TB (see Algorithm 1), regardless of LF-LAM result.
f. TB is not ruled out if both the LF-LAM result and mWRD results are negative (or if no mWRD is performed). Re-evaluate the person and conduct additional testing in accordance with national guidelines. Further investigations for TB may include chest X-ray, additional clinical assessments or culture. Conduct additional clinical evaluations for TB, such as initiating treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (but do not use FQs). Consider treatment for *Pneumocystis* pneumonia. Evaluate clinical response after 3–5 days of treatment.
    i. If there is clinical worsening or no improvement after 3–5 days of treatment, initiate further investigations for TB and other diseases and, if the person is seriously ill with danger signs, start presumptive TB treatment.
    ii. If there is clinical improvement, reassess for TB and other HIV-related diseases.
        1. Consider that clinical improvement may occur if the person has TB and a bacterial infection (i.e. clinical improvement does not necessarily rule out TB).
        2. If there is high clinical suspicion of TB (i.e. clinical history and physical exam, history of previous TB that can be reactivated and chest X-ray suggestive of TB), use clinical judgement as to whether to initiate TB treatment.
    iii. All people with TB should complete the course of treatment for bacterial or *Pneumocystis* infections.

3. For hospitalized PLHIV being evaluated for TB who do not have signs or symptoms of TB but have AHD *or* are seriously ill *or* have CD4 <200 cells/mm^3 (2a)(B):
    a. Collect a urine specimen and conduct the LF-LAM.
    b. If the LF-LAM is negative and the CD4 count is <200 cells/mm^3, re-evaluate the person and conduct additional testing in accordance with national guidelines (see Step 2f).
    c. If the LF-LAM is negative and the CD4 count is >200 cells/mm^3, apply an AHD package of care.
    d. If the LF-LAM is positive, initiate TB treatment based on this result and clinical judgement. Collect a specimen and conduct an mWRD to assess the possibility of rifampicin resistance.
        i. If the mWRD result is "MTBC detected", follow Algorithm 1 for interpretation, testing and treatment recommendations.
        ii. If the mWRD result is "MTBC not detected", continue treating the person for TB and conduct additional laboratory testing (e.g. culture and phenotypic DST) to assess drug resistance.



For footnotes please see page 87

4. For hospitalized PLHIV without signs or symptoms of TB and whose CD4 is 200 cells/mm^3 or above (or is unknown), 2a C do not conduct an LF-LAM test. The latest WHO TB screening guidelines, however, recommend that all hospitalized PLHIV irrespective of signs and symptoms should be routinely tested using an mWRD for TB.



For footnotes please see page 87

Decision pathway for Algorithm 2b – LF-LAM testing to aid in the diagnosis of TB among PLHIV in clinic and outpatient settings

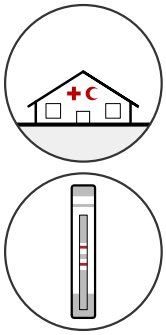
1. Evaluate the person for TB, determine HIV status, and assess the presence of AHD and danger signs for being seriously ill. In PLHIV who are not seriously ill, also consider measuring CD4 cell counts, to assess eligibility for testing with the LF-LAM:
   a. Individuals to be evaluated for TB include HIV-positive adults, adolescents and children, including all people with TB who are newly diagnosed with HIV and are ART naive, PLHIV returning for care following an interruption of treatment, PLHIV receiving an ART regimen that is failing, and people presenting at the clinic and unwell.
   b. PLHIV include individuals 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, perform HIV testing in accordance with national guidelines.
   c. "Seriously" ill is defined as presenting with any one of the following danger signs: respiratory rate >30 per minute, temperature >39 °C, heart rate >120 beats per minute or unable to walk unaided.
   d. For adults, adolescents and children aged more than 5 years, AHD is defined as CD4 cell count <200 cells/mm^3 or WHO stage 3 or 4 event at presentation for care. All children aged under 5 years are considered as having AHD.

2. For PLHIV being evaluated for TB who are positive for signs and symptoms, or who are seriously ill regardless of TB symptoms 2b A:
   a. Collect a urine specimen and conduct the LF-LAM **and** collect a specimen and conduct mWRD testing. If the mWRD is available on site, do the mWRD testing in parallel to the LF-LAM testing.
      i. For individuals being evaluated for pulmonary TB, the following samples may be used for the mWRD: induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage or aspirate. Additional sample types suitable for use with Xpert MTB/RIF and Xpert Ultra include nasopharyngeal aspirate and for Xpert MTB/RIF includes stool samples.
      ii. For individuals being evaluated for extrapulmonary TB, the Xpert MTB test is recommended for use with CSF (preferred sample for TB meningitis), lymph node aspirates, lymph node biopsies, pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine as an initial diagnostic test for the corresponding extrapulmonary TB. Blood may also be used as a specimen for HIV-positive adults and children with signs and symptoms of disseminated TB. Other tests for use in extrapulmonary TB include the Xpert Ultra.

2b A

Return to algorithm 2b

LF-LAM testing to aid in the diagnosis of TB among PLHIV in clinic and outpatient settings



Adults, adolescents and children including:

1. All newly diagnosed HIV patients that are ART naive
2. HIV patients returning for care following an interruption of treatment
3. HIV patients receiving an ART regimen that is failing
4. Patients presenting at the clinic and unwell

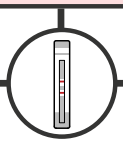
Assess patient for:

TB signs and symptoms
Being seriously ill[2]
Having AHD[3]
CD4 count

Positive for TB signs and symptoms

Collect a urine sample and perform urine LF-LAM

Collect a pample and perform mWRD



LF-LAM +

Initiate TB treatment[5]
Evaluate mWRD result

mWRD +

Adjust treatment based on mWRD results if needed

mWRD −

Continue TB treatment

Perform workup to exclude DR-TB[7]

LF-LAM −

TB is not ruled out[6]
Evaluate mWRD result

mWRD +

Adjust treatment based on mWRD results if needed

WRD −

Clinical management

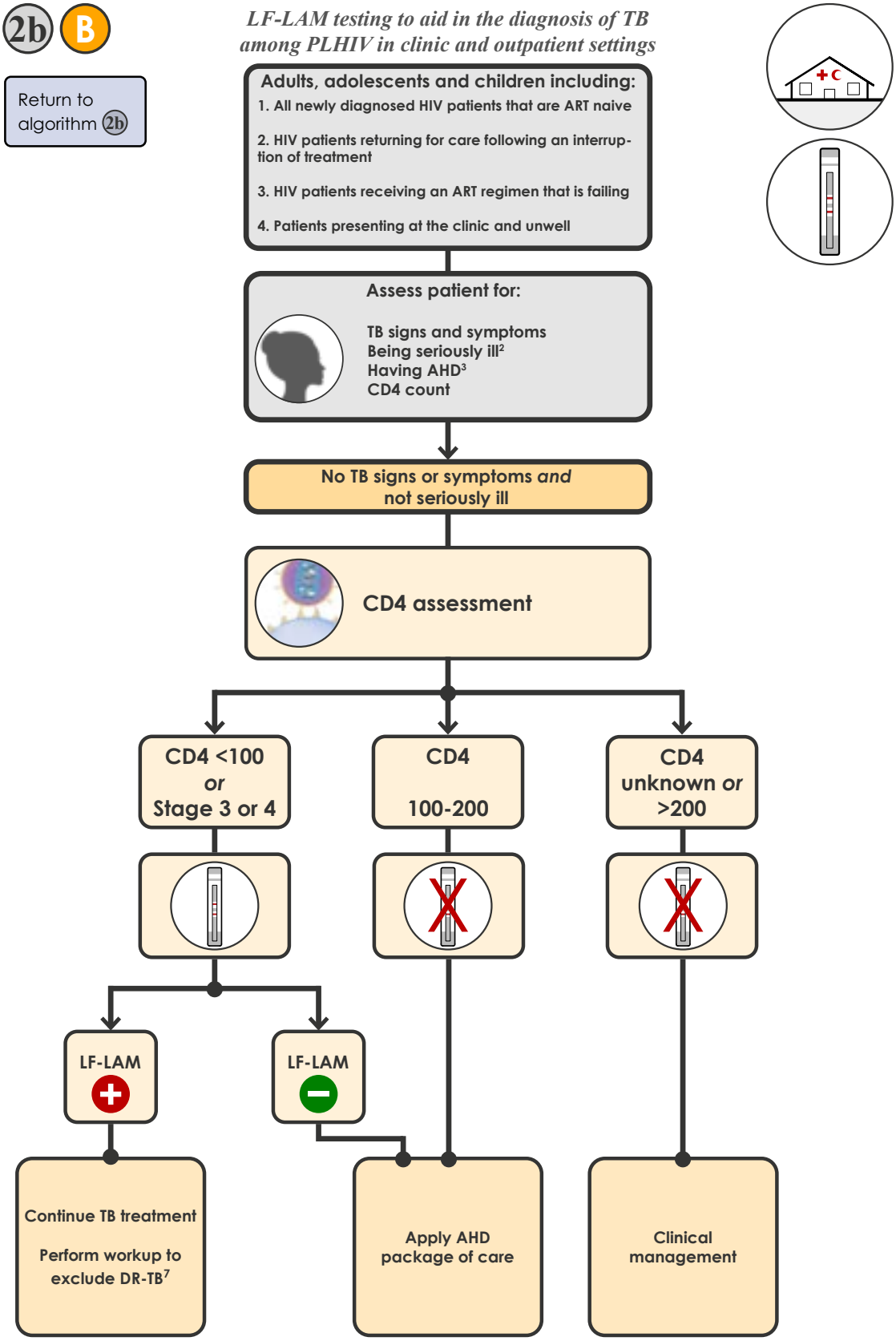
TB is not ruled out[6]

Conduct additional evaluations for TB[5]

For footnotes please see page 89

b. The LF-LAM result (test time <15 minutes) is likely to be available before the mWRD result, and it should be interpreted in the context of clinical judgement, chest X-ray findings (if available) and any available bacteriological results.
c. All people with TB meeting the testing requirements who have a positive LF-LAM result should be initiated on TB treatment immediately, while awaiting results of the mWRD. Follow Algorithm 1 for selection and interpretation of mWRD results which include resistance detection, and modify therapy as needed.
d. TB is not ruled out if the LF-LAM test result is negative. Evaluate the results of the mWRD, and follow Algorithm 1 for result interpretation and follow-up testing.
e. Treat all people with TB with an mWRD result of "MTBC detected" for TB (see Algorithm 1), regardless of LF-LAM result.
f. TB is not ruled out if both the LF-LAM and mWRD results are negative (or if no mWRD is performed). Re-evaluate the person and conduct additional testing in accordance with national guidelines. Further investigations for TB may include chest X-ray, additional clinical assessments or culture. Conduct additional clinical evaluations for TB, such as initiating treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (but do not use FQs). Consider treatment for *Pneumocystis* pneumonia. Evaluate clinical response after 3–5 days of treatment.
    i. If there is clinical worsening or no improvement after 3–5 days of treatment, initiate further investigations for TB and other diseases and, if the person is seriously ill with danger signs, start presumptive TB treatment.
    ii. If there is clinical improvement, reassess for TB and other HIV-related diseases.
        1. Consider that clinical improvement may occur if the person has TB and a bacterial infection (i.e. clinical improvement does not necessarily rule out TB).
        2. If there is high clinical suspicion of TB (i.e. clinical history and physical exam, history of previous TB that can be reactivated and chest X-ray suggestive of TB), use clinical judgement as to whether to initiate TB treatment.
    iii. All people with TB should complete the course of treatment for bacterial or *Pneumocystis* infections.

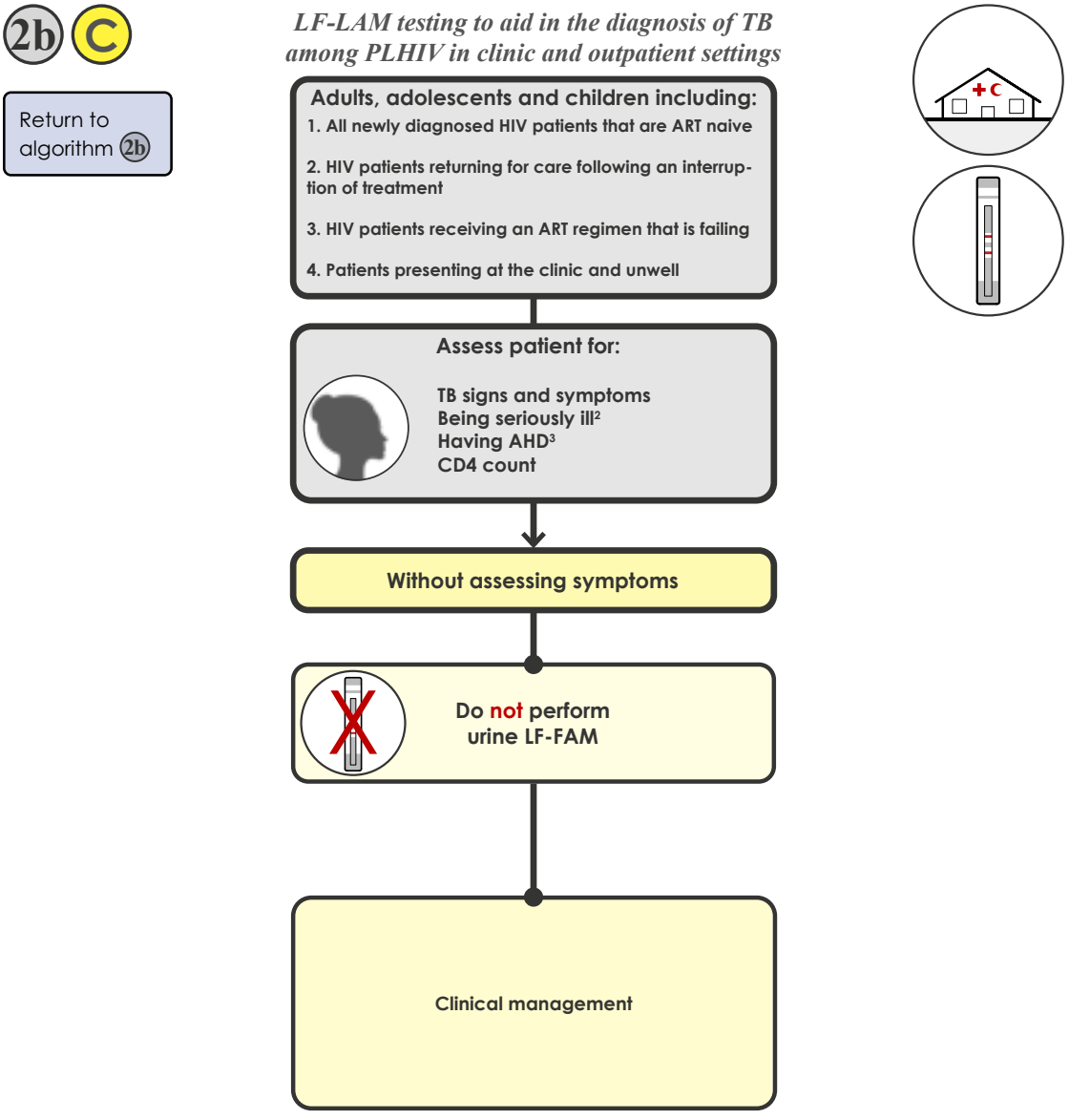
3. For PLHIV being evaluated for TB who do not have signs or symptoms of TB, or who are not seriously ill, determine their CD4 count and whether they have AHD 2b B.
    a. If the CD4 is <100 cells/mm^3 or the person presents with a WHO stage 3 or 4 event, collect a urine specimen and perform an LF-LAM.
        i. If the LF-LAM test is positive, initiate TB treatment immediately. Conduct additional studies to assess drug resistance. Rapid molecular methods are preferred (see Algorithm 1) and include drug-resistance detection.
        ii. If the LF-LAM test is negative, apply an AHD package of care.
    b. If CD4 is 100–200 cells/mm^3, DO NOT perform an LF-LAM; apply an AHD package of care.
    c. If the CD4 is >200 cells/mm^3 or unknown, DO NOT perform an LF-LAM; clinically manage the person.



For footnotes please see page 89

Considerations when using the LF-LAM test are as follows:

- Do not use the LF-LAM test to assist in the diagnosis of TB in populations other than those described in Algorithm 2a and Algorithm 2b, and do not use this test as a screening test for TB.
- LF-LAM is designed for use with urine samples. Do not use other samples (e.g. sputum, serum, CSF or other body fluids).
- LF-LAM does not differentiate between the various species of the genus *Mycobacterium.* However, in areas with a high prevalence of TB, the LAM antigen detected in a clinical sample is likely to be attributed to MTBC.
- The use of the LF-LAM does not eliminate the need for other diagnostic tests for TB, such as Xpert MTB, other mWRD or culture. These tests exceed the LF-LAM test in diagnostic accuracy; they also provide information on drug susceptibility. Whenever possible, a positive LF-LAM should be followed up with other tests such as mWRD or bacteriological culture and DST.
- Published studies reveal that the LF-LAM test may give a different result than an mWRD or culture (e.g. LF-LAM positive, mWRD result "MTBC not detected"). This is not unexpected because the tests have different sensitivities and measure different analytes. Treatment decisions should rely on clinical judgement and all available information.



For footnotes please see page 89

4.3 Algorithm 3 – DST for second-line drugs for people with RR-TB or MDR-TB

Algorithm 3 is used for further evaluation of people with RR-TB or MDR-TB. In its most recent recommendations *(9)*, WHO stresses the importance of DST before starting the preferred all-oral BDQ-containing MDR-TB regimen, especially for medicines for which mWRDs are available. Two of the key medicines in these regimens are BDQ and FQ. Currently, the only WHO-recommended molecular test to detect mutations associated with BDQ resistance is a targeted NGS test (Deeplex® Myc-TB from GenoScreen). Algorithm 3a 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 3b) is included that relies on the detection of mutations associated with FQ resistance using WHO-recommended molecular tests (a low complexity automated NAAT and SL-LPA). In addition to molecular testing, 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. As in any potentially life-saving situation, treatment for DR-TB should not be withheld from a person because of a lack of complete DST results.

4.3.1 Decision pathway for Algorithm 3 – DST for second-line drugs for people with RR-TB or MDR-TB

Tests

- Recently targeted NGS tests have been recommended that can detect mutations associated with resistance to BDQ, FQ, LZD, CFZ, PZA, RIF, INH, EMB, AMK and STR.
  - The use of targeted NGS tests to detect resistance does not eliminate the need for conventional phenotypic DST, which will be necessary for determining resistance to anti-TB agents that have not been assessed or where performance of resistance detection was suboptimal by the targeted NGS test, and for monitoring the emergence of additional drug resistance.
  - Targeted NGS tests are suitable for use at the central laboratory or NTRL level. They may also be used at the regional level if the appropriate infrastructure, human resources and QA systems are available. Implementation of targeted NGS testing depends on the availability of a reliable specimen transport system and an efficient mechanism for reporting results.
- A low complexity automated NAAT is recommended for the detection of resistance to FQs:
  - It can be used in PLHIV, children and people with extrapulmonary TB.
  - The first-in-class test, the Xpert MTB/XDR test, provides results in under 2 hours, requires minimal hands-on time, can be used at the peripheral level and provides results simultaneously for FQ, INH, ETO and AMK. This test requires a 10-colour GeneXpert instrument, unlike the current Xpert MTB/RIF and Xpert Ultra test, which use the 6-colour GeneXpert instruments. The new instruments can be linked to existing 6-colour GeneXpert systems through a common computer.
  - The use of low complexity automated NAATs to detect FQ resistance does not eliminate the need for conventional phenotypic DST, which will be necessary for determining resistance to other anti-TB agents and for monitoring the emergence of additional drug resistance.

- The SL-LPA is recommended for the detection of resistance to FQs and should be used where available:
  - The diagnostic accuracy of SL-LPA is similar when it is performed directly on sputum or on cultured isolates. SL-LPA can be used on smear-positive or smear-negative specimens, although a higher indeterminate rate will occur with smear-negative specimens.
  - SL-LPA is only recommended for use with sputum specimens or MTBC isolates. The laboratory testing of other specimen types should rely on culture and phenotypic DST.
  - SL-LPA is suitable for use at the central or NTRL level. It may also be used at the regional level if the appropriate infrastructure, human resources and QA systems are available. Implementation of SL-LPA testing depends on the availability of a reliable specimen transport system and an efficient mechanism for reporting results.
  - The use of SL-LPA to detect FQ resistance does not eliminate the need for conventional phenotypic DST, which will be necessary for determining resistance to other anti-TB agents and monitoring the emergence of additional drug resistance.
  - Culture-based phenotypic DST is still required and recommended for drugs that are used in RR/MDR-TB regimens and for which there are either no rapid molecular DST methods or where their performance is suboptimal (e.g. BDQ, LZD, Pa, CS, CFZ and DLM).

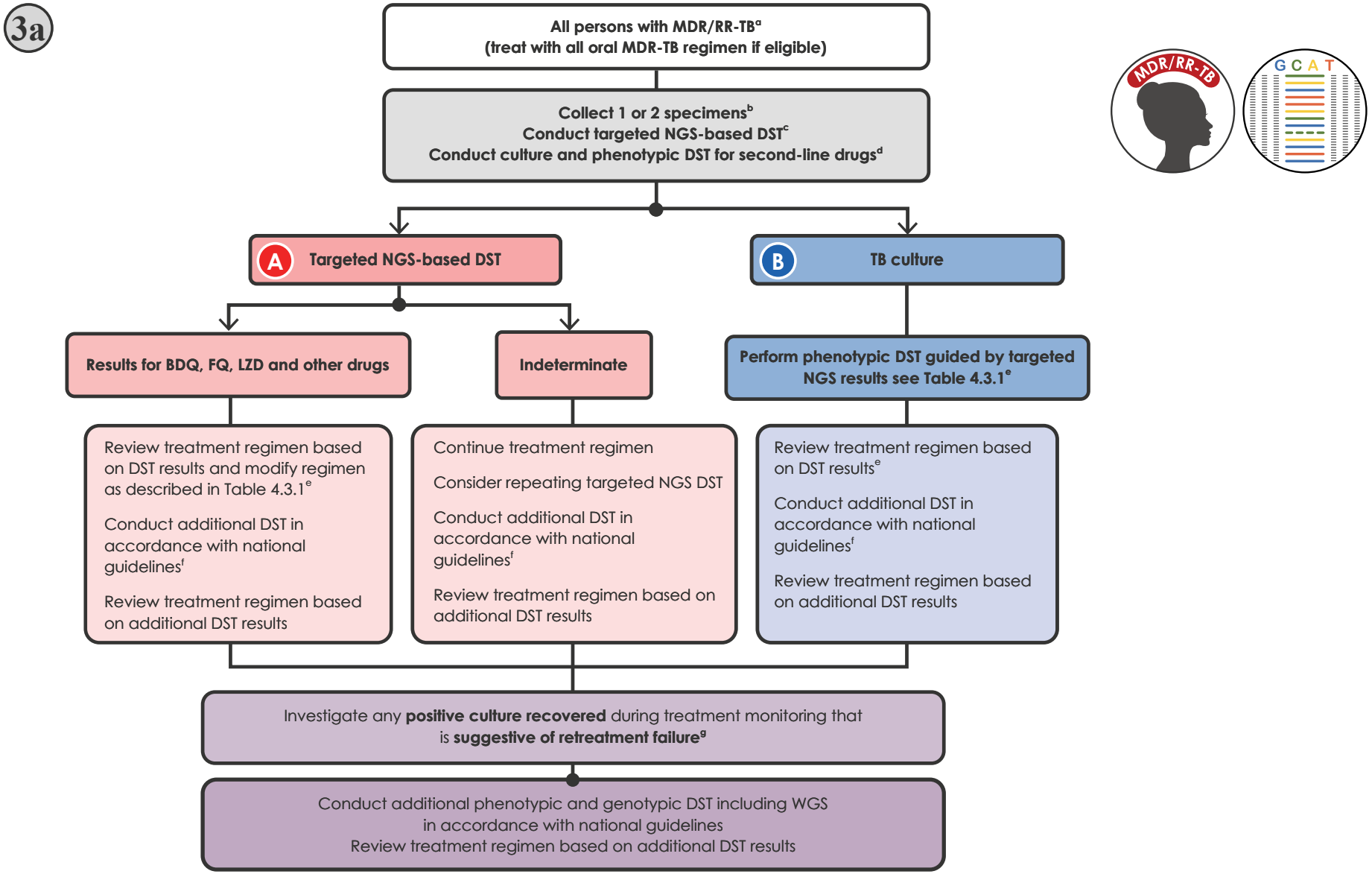
General considerations

- Two BDQ-containing regimens are recommended for the treatment of MDR/RR-TB *(9)*:
  - An all-oral 6-month regimen composed of BDQ, Pa and LZD, with or without MFX (BPaL or BPaLM) in people suffering from MDR/RR-TB or MDR/RR-TB with additional resistance to FQs (pre-XDR-TB).
  - An all-oral regimen of 9 months composed of BDQ, LFX or MFX, ETO, EMB, INH (high-dose), PZA and CFZ (4–6 months of BDQ-LFX-ETO-EMB-PZA-high-dose INH-CFZ / 5 months of LFX-CFZ-PZA-EMB) for individuals not eligible for BPaL or BPaLM.
  - Individualized all-oral longer regimens, designed using the WHO priority grouping of medicines, may still be used for people with MDR/RR-TB who do not meet the eligibility criteria for the BDQ-containing regimens.
- WHO guidelines stress the importance of DST before treatment, especially for medicines for which mWRDs are available.
  - WHO-recommended rapid molecular tests are available for the detection of mutations associated with resistance to FQs (a low complexity automated NAAT, FL-LPA, SL-LPA and targeted NGS tests) and mutations associated with BDQ resistance (targeted NGS tests). The recently recommended 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, PZA, AMK and STR).
  - WHO recommends a new molecular test for PZA resistance detection belonging to the class "high complexity reverse hybridization NAAT". Its use is limited to culture isolates. Alternatively, *pncA* sequencing should be performed when available. 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 *(53)* 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/meropenem; 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% *(54)*. BDQ is a core drug for DR-TB treatment and is included in the revised definition of XDR-TB. Thus, building testing capacity to test this and other drugs used in treatment (e.g. LZD, Pa, CS, CFZ and DLM) is essential. If resistance is suspected during treatment and DST is not available, the strains should be referred to a TB SRL for further testing.
- Initiation of treatment should not be delayed while waiting for the results of DST.

Fig. 4.5. New Algorithm 3a: DST for MDR/RR-TB using targeted NGS



AMK: amikacin; BDQ: bedaquiline; BPaLM: bedaquiline (B), pretomanid (Pa), linezolid (L) and moxifloxacin (M); CFZ: clofazimine; CS: cycloserine; DLM: delamanid; DR-TB: drug-resistant TB; DST: drug susceptibility testing; EMB: ethambutol; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; MDR-TB: multidrug-resistant TB; MDR/RR-TB: multidrug- or rifampicin-resistant TB; NGS: next-generation sequencing; Pa: pretomanid; PZA: pyrazinamide; RIF: rifampicin; RR-TB: rifampicin-resistant TB; SRL: supranational reference laboratory; STR: streptomycin; TB: tuberculosis; WHO: World Health Organization.

[a] People suspected of having TB should be promptly initiated on an MDR-TB regimen in accordance with national guidelines and WHO recommendations. A shorter all-oral BDQ-containing treatment regimen (BPaLM) 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 should be conducted in parallel.

[c] WHO recommends obtaining the rapid DST results before the start of treatment, 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] For more details regarding individualized regimens, see the 2022 update of the WHO consolidated guidelines on the treatment of DR-TB *(9)*.

[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 d. 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.

[g] If resistance to an individual drug (e.g. BDQ) is suspected and DST for that 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 3a – testing for BDQ and FQ resistance

1. Promptly initiate the person on an MDR-TB regimen in accordance with national guidelines. The most recent WHO recommendation is to use an all-oral 6-month regimen composed of BDQ, Pa, LZD and MFX (BPaLM) *(9)*. The use of an all-oral BDQ-containing treatment regimen of 9 months is now limited to those who are not eligible for the BPaLM or BPaL regimens (e.g. aged below 14 years, or pregnant or breastfeeding) *(9)*.
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, collect two specimens and conduct the molecular and phenotypic testing in parallel. Transport sputum specimens or isolates to the appropriate testing laboratory, if necessary.
3. Conduct targeted NGS testing to detect mutations associated with resistance to BDQ and other medicines, and undertake culture in parallel 3A.
4. If the targeted NGS test result is indeterminate, the targeted NGS test can be repeated with a fresh sample in cases where the bacterial load is expected to give a definitive result (smear positive of high/medium grade on mWRD) and treatment decisions are based on clinical assessments, the epidemiologic situation and the results of phenotypic DST. Use the results of the targeted NGS test to modify treatment if appropriate, and select the drugs requiring phenotypic DST when the culture is positive 3B. Note: although the results from sequencing produce information on multiple drugs simultaneously, Table 4.3.1 takes a single-drug approach for interpreting targeted NGS test results, for simplicity, although the information on the resistance or susceptibility of each of the medicines should be taken into consideration when designing a treatment regimen.
    a. If the targeted NGS test detects one or more mutations associated with resistance to BDQ, 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%):
        - If the risk of BDQ resistance is low (e.g. no prior BDQ exposure, the prevalence of resistance to BDQ is less than 5% in the population, or there is no history of contact with a person known to have BDQ-resistant TB) and the targeted NGS test does not detect mutations associated with resistance to FQs and LZD, continue the BDQ-containing regimen while awaiting the results of phenotypic DST.
        - If the risk of BDQ resistance is low and the targeted NGS test does detect other mutations associated with resistance to FQs, stop moxifloxacin and continue with BPaL. If resistance to LZD is detected, change to another regimen based on the targeted NGS test result and follow-up phenotypic DST results.
        - If the risk of BDQ resistance is high (e.g. prior BDQ exposure, the prevalence of resistance to BDQ is more than 5% in the population, or there is history of contact with a person known to have BDQ-resistant TB), change to an individualized regimen based on the targeted NGS test results and follow-up phenotypic DST results.
    b. If the targeted NGS test does not detect mutations associated with resistance to BDQ, clinical decisions should consider the risk of BDQ resistance and the results of the targeted NGS test for other medicines, particularly for FQs and LZD. People should be closely

monitored, and additional DST performed on any culture isolated at month 2 or later during treatment:

- If the risk of BDQ resistance is low, continue the BDQ-containing regimen. The decision to perform phenotypic DST for BDQ for this group will be context dependent, taking into account the prevalence of resistance, the number of samples to be tested and the expected number missed by targeted NGS. Ideally, where resources are available, phenotypic DST would be done for all samples. Modify the regimen as appropriate if resistance to other medicines in the regimen is detected.
- If the targeted NGS test detects other mutations associated with FQ resistance but not LZD, treat with BPaL (i.e. discontinue the use of FQ).
- If the targeted NGS-based test detects other mutations associated with LZD resistance, change to a BDQ-containing individualized regimen based on the targeted NGS test results and follow-up phenotypic DST results.
- If the risk of BDQ resistance is high, continue the BDQ-containing regimen while awaiting the results of phenotypic DST for BDQ. Modify as appropriate if resistance to other medicines in the regimen is detected (as described in Step 5). Closely monitor the person and conduct DST on those who remain culture positive at month 2 or later and those who are not responding clinically.

Table 4.3.1. Treatment modifications and follow-on DST for MDR/RR-TB based on results from targeted NGS

Initial regimen	Drug	Targeted NGS result	Is phenotypic DST to specific drug required?[a]	Suggested treatment modification
BPaLM	BDQ	Resistant		If TB is susceptible to FQs and LZD, continue BPaLM pending phenotypic DST results and clinical assessment If TB is resistant to FQs, stop moxifloxacin and continue with BPaL. If TB is resistant to LZD, change to another regimen based on targeted NGS and phenotypic DST results Change to an individualized regimen based on targeted NGS and follow-up phenotypic DST results
		Low risk of BDQ resistance[b]	Yes	
		High risk of BDQ resistance[c]	Yes	
BPaLM	BDQ	Susceptible		
		Low risk of BDQ resistance[b]	No. Defer (2 months or later culture)	Continue BPaLM
		High risk of BDQ resistance[c]	Yes or deferred	Continue BPaLM, monitor the person closely, conduct DST on those who remain culture positive at month 2 or later and those who are not responding clinically
	FQ	Resistant	No	Drop M, continue BPaL
		Susceptible	Yes/no[d]	Continue BPaLM
	LZD	Resistant	No	Change to a 9-month regimen using ETO if only LZD resistant; otherwise, change to an individualized regimen based on targeted NGS and phenotypic DST results
		Susceptible	Yes/no[d]	Continue BPaLM

Initial regimen	Drug	Targeted NGS result	Is phenotypic DST to specific drug required?[a]	Suggested treatment modification
9-month	FQ, BDQ, LZD or CFZ	Resistant (any of the 4 drugs)	BDQ/CFZ: yes Others: no	If only LZD resistant, replace with ETO; otherwise, change to an individualized regimen based on targeted NGS and phenotypic DST results
		Susceptible (all 4 drugs)	Yes/no[d]	No treatment modification; continue the 9-month regimen
	Other drugs[e] PZA EMB	Resistant	No	Discontinue the specific drug. Where both drugs are resistant, change to an individualized regimen
		Susceptible	No[f]	No treatment modification; continue with the specific drug
Individualized 18 month	Do not use drugs with resistance detected by targeted NGS. The exception is BDQ, where in low-risk individuals the drug may be used pending the results of phenotypic DST. Where results indicate susceptibility to FQ, BDQ, LZD, CFZ and PZA, these drugs may be used but phenotypic DST should be performed to definitively exclude resistance to these drugs. In addition, phenotypic DST to DLM, Pa and CS should be performed.			

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; DST: drug susceptibility testing; EMB: ethambutol; ETO: ethionamide; FQ: fluoroquinolone; LZD: linezolid; M: moxifloxacin; NGS: next-generation sequencing; Pa: pretonamid; PZA: pyrazinamide; TB: tuberculosis.

[a] Where a targeted NGS result is indeterminate, phenotypic DST should be performed.

[b] Low risk: no prior BDQ exposure, the prevalence of resistance to BDQ is <5% in the population, or there is no history of contact with a person with TB with a known resistance to BDQ.

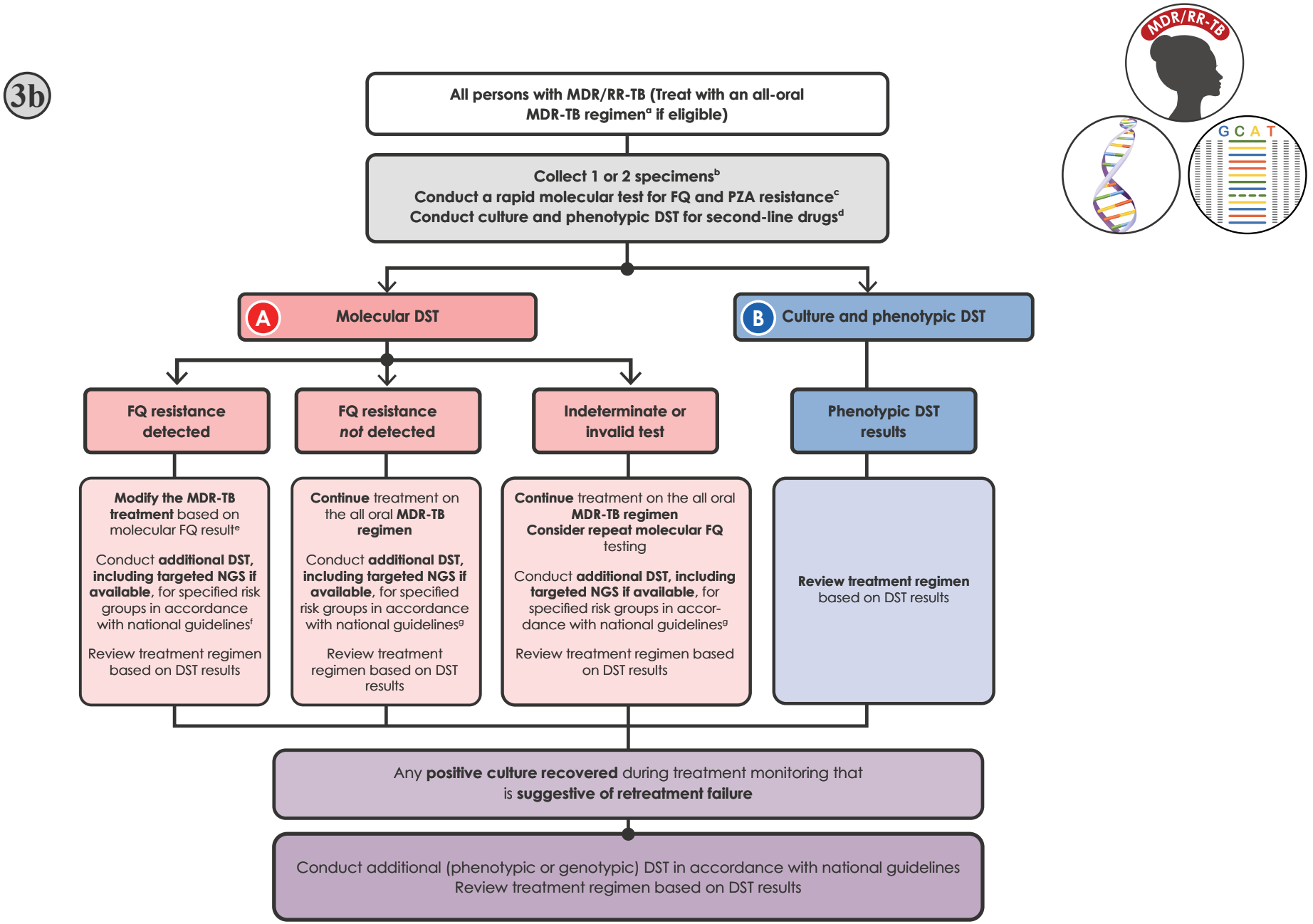
[c] High risk: prior BDQ exposure, the prevalence of resistance to BDQ is >5% in the population, or there is history of contact with a person with TB with a known resistance to BDQ.

[d] In individuals not at high risk for resistance (e.g. no prior drug exposure, the prevalence of resistance to the specific drug is <5% in the population, or there is no history of contact with a person with TB with known drug resistance) phenotypic testing is advised but is not an absolute requirement. This is particularly true where access to phenotypic DST is limited, in which case, higher risk groups should be prioritized.

[e] Where possible, these drugs should only be reported for people requiring 9-month or individualized regimens. If there is no option to selectively report drugs, it should be clear that results relevant to the current regimen should be primarily considered and the additional results should be used only when needed.

[f] Phenotypic DST may not be more accurate than targeted NGS for these drugs. The targeted NGS results can be used for clinical management.

Fig. 4.6. Algorithm 3b: DST for people with MDR/RR-TB (limited or no targeted NGS capacity)



AMK: amikacin; 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; DR-TB: drug-resistant TB; DST: drug susceptibility testing; EMB: ethambutol; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; MDR-TB: multidrug-resistant TB; MDR/RR-TB: multidrug- or rifampicin-resistant TB; mWRD: molecular WHO-recommended rapid diagnostic test; NGS: next-generation sequencing; Pa: pretonamid; PZA: pyrazinamide; RIF: rifampicin; SL-LPA: line probe assay for second-line drugs; SRL: supranational reference laboratory; STR: streptomycin; TB: tuberculosis; WHO: World Health Organization.

[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 (BPaL or BPaLM) 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. Rapid 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, 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 2022 update of the WHO consolidated guidelines on the treatment of DR-TB *(9)*. BPaL may be used for people with FQ-resistant MDR-TB *(9)*. See Table 4.3.1.

[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. 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. 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.

[g] 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 3b – testing for FQ resistance

1. Promptly initiate the person on an MDR-TB regimen in accordance with national guidelines. The most recent WHO recommendation is to use an all-oral 6-month regimen composed of BDQ, Pa, LZD and MFX (BPaLM) *(9)*. The use of an all-oral BDQ-containing treatment regimen of 9–12 months is now limited to those not eligible for the BPaLM or BPaL regimens (e.g. aged below 14 years, or pregnant or breastfeeding) *(9).*
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, collect two specimens and conduct the molecular and phenotypic testing in parallel. Transport sputum specimens or isolates to the appropriate testing laboratory, if necessary.
3. Conduct the low complexity automated NAAT or SL-LPA to detect mutations associated with FQ resistance. Note that targeted NGS tests can also detect mutations associated with resistance to FQ. If a targeted NGS test is available, follow Algorithm 3a for interpretation of results and follow-up actions.
4. If the low complexity automated NAAT or SL-LPA detects one or more mutations associated with resistance to FQs and:
   a. the individual is on a BPaLM regimen, discontinue MFX and continue with BPaL treatment while awaiting the results of the phenotypic DST;
   b. the individual is on the 9-month all-oral regimen, change to an individualized longer regimen, designed using the WHO priority grouping of medicines *(9)*:
      - the first-in-class low complexity automated NAAT (Xpert MTB-XDR) provides results for INH, FQs, ETO and AMK, and can be used to inform individualized regimen selection;
      - collect a specimen and submit 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
      - perform DST for MFX at the clinical breakpoint to determine the potential use of high-dose (800 mg) MFX for treatment *(9)* (Web Annex C).
5. If the low complexity automated NAAT or SL-LPA is negative for mutations associated with resistance to FQs:
   a. Continue people on the all-oral BDQ-containing MDR-TB regimen (6- or 9-month), while awaiting the results of the phenotypic DST (Step 6).
   b. In settings with high underlying prevalence of resistance to FQs or for people considered at high risk of resistance, refer a specimen for culture and phenotypic DST for FQs, because the sensitivity of the low complexity automated NAAT 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. The phenotypic DST should also include testing at the clinical breakpoint to inform individualized drug selection. Modify the regimen as necessary, based on the phenotypic DST results.
6. Perform culture and phenotypic DST for each of the drugs included in the treatment regimen for which there are accurate and reproducible methods. For the preferred regimens, reliable phenotypic DST methods 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 (high complexity reverse hybridization NAAT) but is currently limited to use on culture isolates.

   a. If the isolate is susceptible to all drugs, continue the person on the preferred MDR-TB regimen.
   b. If resistance to a drug is detected, refer to Table 4.3.1 to guide treatment modification. Given that results for phenotypic DST are slow, reassess the person's response 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, ensure that treatment monitoring includes the collection of samples for culturing as described in the WHO consolidated guidelines *(9).* Any positive culture suggestive of treatment failure should undergo phenotypic DST. Modify the regimen as necessary, based on the phenotypic DST 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 and, in particular, to limit the risk of prolonging a failing regimen.

4.4 Algorithm 4 – Follow-on testing for individuals with RIF-susceptible TB at risk of resistance to other drugs

Algorithm 4 is a follow-on algorithm, the purpose of which is to detect resistance in individuals with RIF-susceptible TB at risk of having DR-TB and individuals with Hr-TB. 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 known person with 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 may 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).

Tests

- The moderate complexity automated NAAT class of initial tests for TB detection that simultaneously detects resistance to RIF and INH is recommended, and should improve rapid

identification of Hr-TB. People from Algorithm 1, where this class of test was used and Hr-TB was identified, would enter this algorithm for further investigation and management. This test could also be used for detection of INH resistance for those with only RIF results.

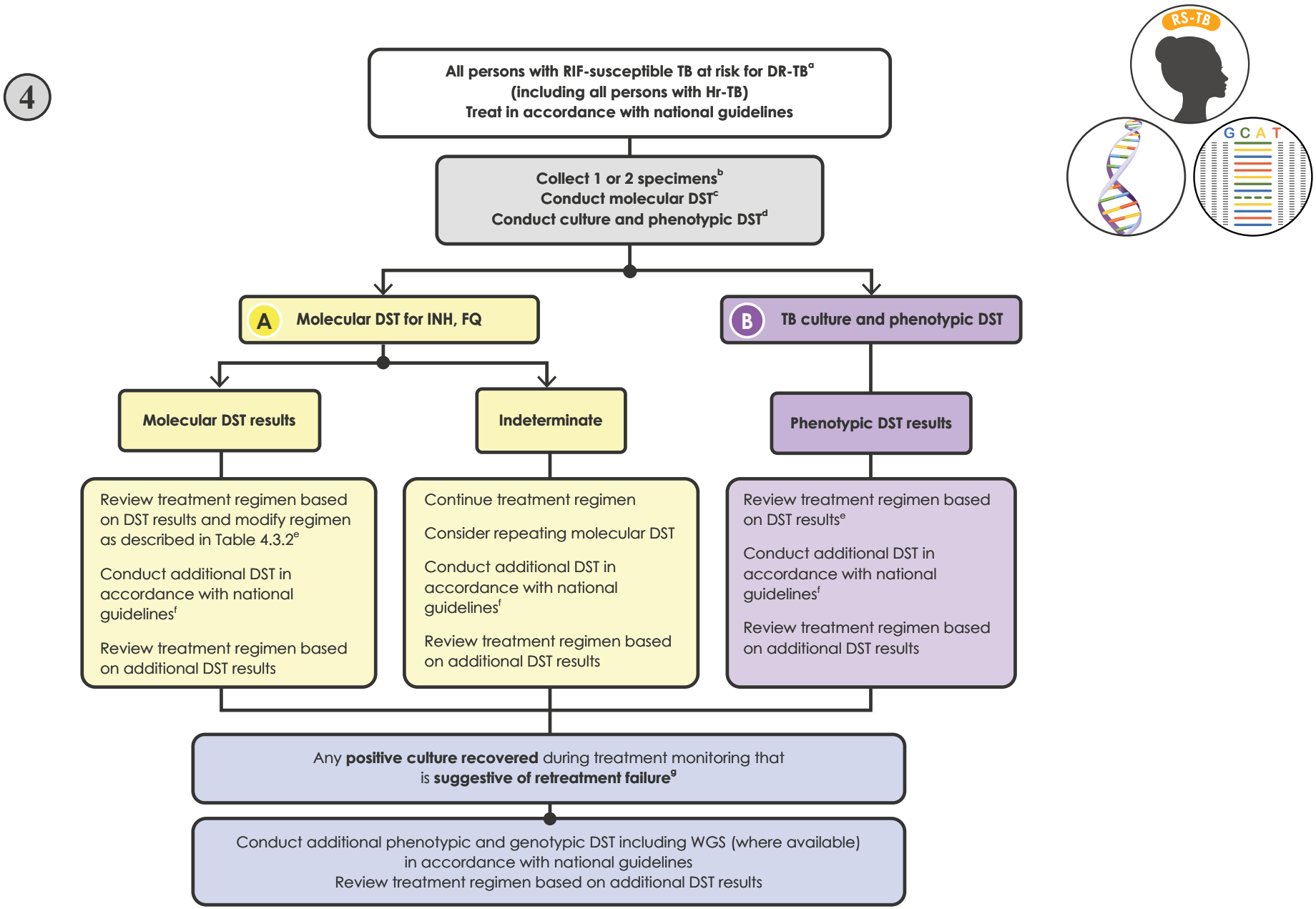
- The low complexity automated NAAT (Xpert MTB-XDR) is recommended as a follow-on test that simultaneously detects resistance to INH and FQ, is suited to the peripheral level and complements existing WRDs that detect only RIF resistance.
- Targeted NGS tests, such as the Deeplex® Myc-TB test (GenoScreen), which is recommended for detecting resistance to RIF, INH, PZA, EMB, FQ, BDQ, LZD, CFZ, AMK and STR; and the AmPORE TB test (Oxford Nanopore Technologies), which is recommended for detecting resistance to RIF, INH, FQ, LZD, AMK and STR.
  - The use of targeted NGS tests to detect resistance does not eliminate the need for conventional phenotypic DST, which will be necessary for determining resistance to anti-TB agents not assessed by the targeted NGS test (or suboptimal by that test) and for monitoring the emergence of additional drug resistance.
  - Targeted NGS tests are suitable for use at the central laboratory or NTRL level. They may also be used at the regional level if the appropriate infrastructure, human resources and QA systems are available. Implementation of targeted NGS testing depends on the availability of a reliable specimen transport system and an efficient mechanism for reporting results.
- The FL-LPA and SL-LPA are useful alternatives where these tests are already available and can detect INH and FQ resistance, respectively.
- The use of molecular tests to detect INH resistance does not eliminate the need for conventional culture-based phenotypic DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.
- Detection of FQ resistance, along with PZA resistance, is important for people with Hr-TB, to ensure that an effective treatment is offered and to prevent amplification of resistance to RIF or FQ.
- Phenotypic DST to PZA is desirable if a quality-assured reliable phenotypic DST for PZA has been established in the country. An alternative is the new high complexity reverse hybridization NAAT, which is recommended for use on culture isolates for PZA resistance detection. Sequencing of the *pncA* gene is another option, if available.

4.4.1 General considerations

- WHO guidelines stress the importance of DST before treatment, especially for medicines for which mWRDs are available.
- People with TB that is RIF susceptible, INH susceptible or unknown should be started on a first-line regimen for drug-susceptible TB *(55)*.
- 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 *(56)*. The prevalence in some settings can exceed 25%. 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%) *(56)*.

- 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 low complexity automated NAATs for follow-up detection of INH resistance can be valuable tools owing to their ease of use and the possibility of implementing these tests in the lower levels of the health system.
- The recommended Hr-TB treatment regimen is RIF, EMB, PZA and LFX for 6 months *(9, 57)*.
- 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, where this is not possible, the results should make 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 *(53)* and technical manual (Web Annex C). Member States should ensure 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/meropenem; hence, results should not be used for clinical decision-making.
- Initiation of treatment should not be delayed while waiting for the results of DST.

Fig. 4.7. New Algorithm 4: Follow-on testing for people with RIF-susceptible TB at risk of resistance to other drugs



AMK: amikacin; BDQ: bedaquiline; CFZ: clofazimine; CS: cycloserine; DLM: delamanid; DR-TB: drug-resistant TB; DST: drug susceptibility testing; EMB: ethambutol; FL-LPA: line probe assay for first-line drugs; FQ: fluoroquinolone; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; INH: isoniazid; LZD: linezolid; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant TB; MDR/RR-TB: multidrug- or rifampicin-resistant TB; mWRD: molecular WHO-recommended rapid diagnostic test; NGS: next-generation sequencing; Pa: pretomanid; PZA: pyrazinamide; RIF: rifampicin; RR-TB: rifampicin-resistant TB; SL-LPA: line probe assay for second-line drugs; SRL: supranational reference laboratory; STR: streptomycin; TB: tuberculosis; WHO: World Health Organization.

[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 *(9, 55)*. 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 *(9)* and drug-susceptible TB *(55)*.

[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 4 – Follow-on testing for individuals with RIF-susceptible TB at risk of resistance to other drugs

1. Promptly initiate the person on a regimen for the treatment of RIF-susceptible TB in accordance with national guidelines *(55)*. Individuals with Hr-TB should be started on an Hr-TB regimen *(9, 57)*.
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, collect two specimens and conduct the molecular and phenotypic testing in parallel. Transport sputum specimens or isolates to the appropriate testing laboratory, if necessary.
3. Conduct molecular testing, and perform culture in parallel.
   a. If targeted NGS tests are used:
      - Modify the treatment if appropriate, and perform phenotypic DST when the culture is positive based on Table 4.3.2. Note: the results from sequencing produce information on multiple drugs simultaneously; however, for simplicity, Table 4.3.2 takes a single-drug approach for interpreting targeted NGS test results, although all results should be taken into consideration when designing a treatment regimen.

Table 4.3.2 Treatment modifications and follow-on DST for Hr-TB based on results from targeted NGS

Drug	Targeted NGS result	Is DST to 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 3a
	Susceptible	No	Continue with the first-line regimen, including RIF if no other resistance is detected, otherwise use an individualized regimen
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; EMB: ethambutol; FQ: fluoroquinolone; HPMZ: isoniazid (H), rifapentine (P), moxifloxacin (M) and pyrazinamide (Z); Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; HRZE: isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E); INH: isoniazid; MDR/RR-TB: multidrug- or rifampicin-resistant TB; NGS: next-generation sequencing; PZA: pyrazinamide; RH: rifampicin (R) and isoniazid (H); RIF: rifampicin; TB: tuberculosis.

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

b. If a WHO recommended follow-on molecular test other than targeted NGS tests is used:
   - For a person with RR-TB by DST, detected by either a molecular (e.g. Xpert MTB/RIF, Xpert Ultra or Truenat) or phenotypic DST, but no results are available for INH and the person is at high risk for Hr-TB, start at Step 1 below.
   - For a person who had an initial TB test that included RIF and INH results (e.g. a moderate complexity automated NAAT was used) in Algorithm 1, go to Step 4 below.

   1. Collect a good-quality specimen and transport it to the testing laboratory for molecular or phenotypic testing for INH resistance:
      a. Testing could follow a two-step process: detection of INH resistance followed by detection of FQ resistance. The two-step process is applicable when a moderate complexity automated NAAT or FL-LPA is used for Hr-TB detection, followed by the low complexity automated NAAT or SL-LPA for FQ resistance detection. A single step option is now available using the first-in-class low complexity automated NAAT, which detects both INH and FQ resistance simultaneously.
      b. 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 in Section 3). Phenotypic DST will be relevant when the person is at high risk for Hr-TB. If both molecular and phenotypic tests are performed, initiate the tests in parallel; do not wait for the results of one test before initiating the other test.
      c. 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.
   2. If INH resistance is not detected, continue treatment with a first-line regimen in accordance with national guidelines:
      a. Conduct additional DST in accordance with national guidelines.
      b. Consider 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. Using the low complexity automated NAAT will provide simultaneous detection of resistance to INH and FQ. If FQ resistance is not detected, follow Steps 3b and 5. If FQ resistance is detected, follow Steps 3d(ii) and 5. If the FQ result is unknown or unsuccessful, follow Step 3c.
      b. Initiate treatment with an Hr-TB regimen *(9)*:
         i. There is no clear evidence showing that adding INH at the usual doses adds benefits or harms to people. For the convenience of 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. According to emerging evidence, 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 isoniazid in the regimen, even when used at the higher dose, declines as MICs increase further.

c. Refer a specimen from a person with laboratory-confirmed Hr-TB for molecular (e.g. low complexity automated NAAT 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, so go to Step 3d.
   i. Rapid molecular testing for FQ resistance is preferred. When used for direct testing of sputum specimens, the low complexity automated NAAT and SL-LPA detects 93% and 86% of people with FQ resistance, respectively (Table 3.4 in Section 3):
      - Low complexity automated NAATs 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 *(49)*. 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 a higher indeterminate rate will occur when testing smear-negative specimens.
      - Despite good specificity and sensitivity of low complexity automated NAATs and SL-LPA for the detection of FQ resistance, phenotypic DST is required to exclude resistance to individual FQs completely. 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. Review FQ resistance results:
   i. If FQ resistance is not detected, continue treatment with the LFX-containing Hr-TB regimen.
   ii. If FQ resistance is detected:
      - Discontinue use of LFX and change to a 6-month regimen of (INH)/RIF/EMB/PZA; that is, 6(H)REZ, where the "(H)" indicates that the INH is optional) or an individualized Hr-TB regimen.
      - Refer a specimen for PZA DST if reliable PZA DST is available in the country. Options include the high complexity reverse hybridization NAAT, phenotypic DST in the MGIT system and *pncA* sequencing. For more details, see 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, continue therapy 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, repeat the low or moderate complexity automated NAAT or FL-LPA with a fresh specimen. Consider 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 collecting 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.

4.4.2 Discordant results

Interpretation of discordant results

This algorithm follows from Algorithm 1 with an mWRD that detected MTBC and was RIF susceptible. In the scenario where the moderate complexity automated NAAT was used, the INH result would already be available. In this algorithm, the follow-up testing could have a second RIF result when the FL-LPA is used as a follow-on, or it could have a second INH result when the moderate complexity automated NAAT is followed by the low complexity automated NAAT. Sometimes, results may be discordant. Each discordant result will need to be investigated on a case-by-case basis. General considerations 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. 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 data are too sparse to assess how frequently 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 be helpful 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 moderate complexity automated NAAT result is "MTBC detected, RIF resistance not detected, INH resistance detected" but the result is "INH susceptible" by low complexity automated NAAT:
   a. This result is expected to be rare because both assays interrogate the same region of the *katG* and *inhA* genes.
   b. The existence of heteroresistant populations (i.e. mixtures of susceptible and resistant bacteria) is a more likely reason, especially in high-burden settings where the force of infection is high. A review of the low complexity automated NAAT probe melting temperatures *(52)* 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. Reassess the risk for Hr-TB, and if a high risk for Hr-TB or administration errors (e.g. mislabelling) are not the reason, treatment decisions should cover the worst-case scenario and be based on the moderate complexity automated NAAT result.
4. Where the moderate complexity automated NAAT result is "MTBC detected, RIF resistance not detected, INH resistance not detected" and the sample is INH resistant by low complexity automated NAAT:
   a. Treatment decisions should be based on the low complexity automated NAAT 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 low complexity automated NAAT 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 low complexity automated NAAT 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. Targeted NGS detects resistance and other molecular tests show susceptibility or vice versa:
   a. Check 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 taken as the final result.
   b. Check 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. Check 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.

4.5 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. 4.8 to Fig. 4.10. Three scenarios are provided, with two simulated pathways in each. The scenarios are based on three epidemiologic 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.

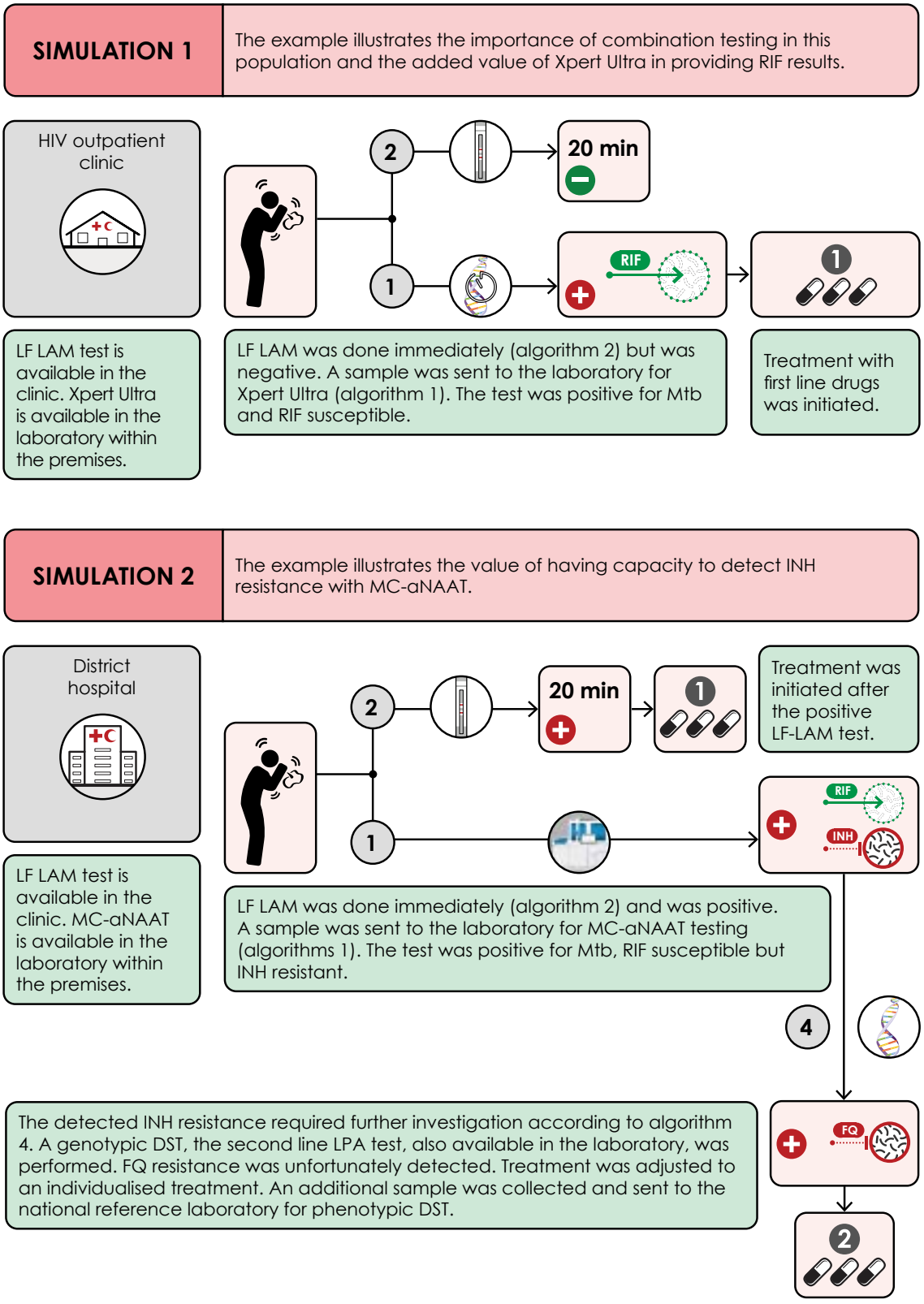
4.5.1 Implementing a new diagnostic algorithm

Modifications to diagnostic algorithms must be put in place only after a formal evaluation, review and approval by officials within the MoH, 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 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, PLHIV and people with extrapulmonary TB, and the proportion of TB among children.

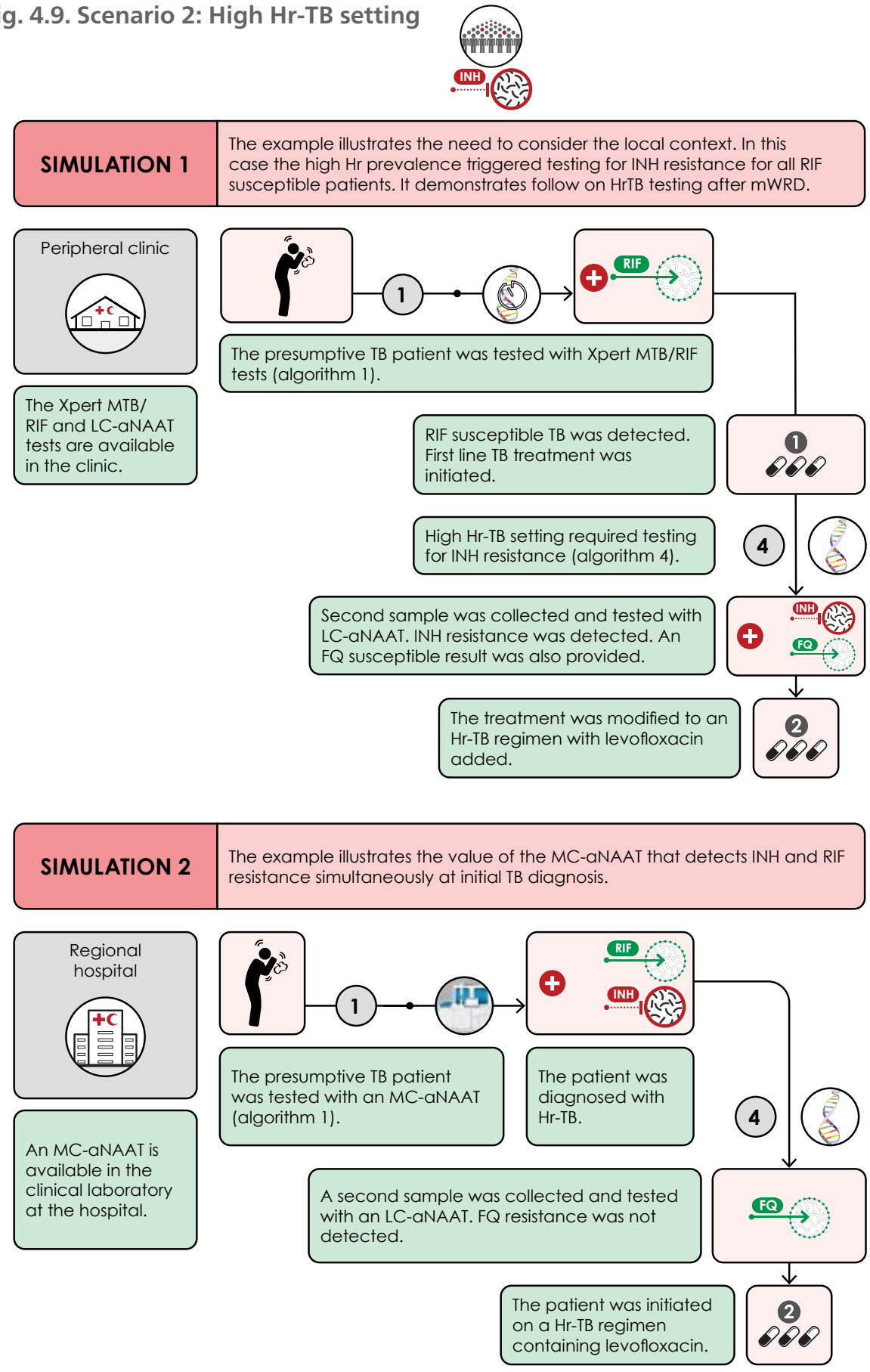
Algorithms should be designed to use existing laboratory services and networks, so that specimens can be referred to the appropriate level for tests that are not available at peripheral-level laboratories. Such referrals are particularly important when evaluating individuals for DR-TB or HIV-associated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

Fig. 4.8. Scenario 1: High TB/HIV setting



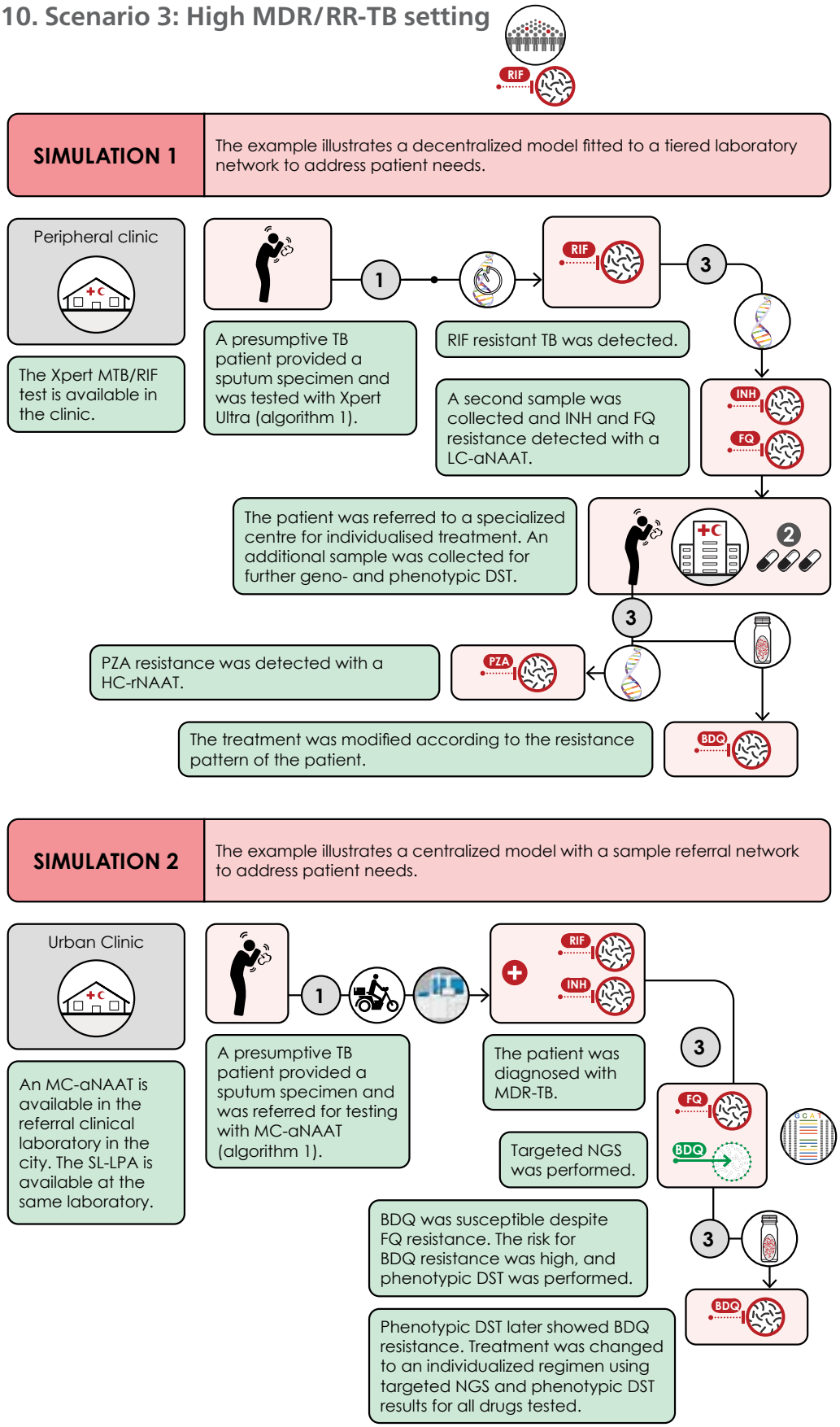
DST: drug susceptibility testing; FQ: fluoroquinolone; HIV: human immunodeficiency virus; INH: isoniazid; LF-LAM: lateral flow lipoarabinomannan assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; Mtb: *Mycobacterium tuberculosis*; NTRL: national TB reference laboratory; RIF: rifampicin; SL-LPA: second-line line probe assay.

Fig. 4.9. Scenario 2: High Hr-TB setting



FQ: fluoroquinolone; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; INH: isoniazid; LC-aNAAT: low complexity automated nucleic acid amplification test; LFX: levofloxacin; MC-aNAAT: moderate complexity automated nucleic acid amplification test; mWRD: molecular World Health Organization–recommended rapid diagnostic test; RIF: rifampicin; TB: tuberculosis.

Fig. 4.10. Scenario 3: High MDR/RR-TB setting



BDQ: bedaquiline; DST: drug susceptibility testing; FQ: fluoroquinolone; HC-rNAAT: high complexity reverse hybridization nucleic acid amplification test; INH: isoniazid; LC-aNAAT: low complexity automated nucleic acid amplification test; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant TB; NGS: next-generation sequencing; PZA: pyrazinamide; RIF: rifampicin; RR-TB: rifampicin-resistant TB; TB: tuberculosis.

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Annex 1. Budgetary considerations for implementing a new diagnostic test

Successful implementation of the plan will require financial and human resource commitments from the ministry of health (MoH) 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)

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

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 manual in *Web Annex C* (WHO *Technical manual for culture-based drug susceptibility testing of medicines used in the treatment of tuberculosis)* 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 *(1)* as well as newly developed critical concentrations for pretonamid 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	Moxifloxacin Hydrochloride 249ug/vial	BD REF: 215404	6 vials of 249 µg each	Please refer to GDF catalogue or contact GDF or manufacturer for more information	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 https://www.beiresources.org	20 mg	Not applicable	Not applicable	RT
Bedaquiline	Bedaquiline fumarate 170 µg (active) Ficoll (inactive). Contents of vial soluble in 2 mL DMSO)	BD REF: 215449	170 µg (2 vials)	Please refer to GDF catalogue or contact GDF or manufacturer for more information	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	5 g	2–8°C

Drug	Description and ingredients	Manufacturer (Catalogue No.)	Quantity	GDF Catalogue No	GDF Quantity	Storage
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]	10 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	TB Alliance	10 or 50 mg	Contact TB Alliance	Contact TB Alliance	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]

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

[b] 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 six months (i.e. lower temperatures should not be used and vials should never be re-frozen).

RT – room temperature

Reference for Annex 2

1. Technical report on critical concentrations for drug susceptibility testing of isoniazid and the rifamycins (rifampicin, rifabutin and rifapentine). Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/9789240017283).

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 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)*, which 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 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 the ones 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 a safe, secure and functional testing site or 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, 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

1. The use of next-generation sequencing for the surveillance of drug-resistant tuberculosis: an implementation manual. Geneva: World Health Organization; 2023. (https://www.who.int/publications/i/item/9789240078079).
2. The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex: technical guide. (WHO/CDS/TB/2018.19). Geneva: World Health Organization; 2018 (https://apps.who.int/iris/handle/10665/274443).
3. Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, second edition. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/9789240082410).

Annex 4. Conflict of interest assessment

The DOI forms submitted by the experts reviewing this handbook and information retrieved from the internet, were examined by WHO staff members Nazir Ismail and Carl-Michael Nathanson to assess whether there were, or might be, actual or perceived conflicts of interest and, if so, whether a management plan was required. This evaluation process, and resultant management plans, were based on the Guidelines for declaration of interests (WHO experts) and the WHO handbook for guideline development (2nd edition).

Both financial and non-financial interests were considered. A "significant" conflict of interest would include:

- "intellectual bias",where an individual may have repeatedly and publicly taken a position on an issue under review, which may affect the individual's objectivity and independence in the global policy development process;
- involvement in research or publication of materials related to issues under review; and
- a financial interest above US$ 5000.

Developers of any assay are never involved in the process of policy development – such involvement is automatically considered a conflict of interest. Upon review no significant conflict of interest were identified. The review findings are summarized in Table A2.1.

Table A2.1. Declarations of interests

Expert	Interest declared	Conclusion
David Branigan	None declared	No conflict of interest
Andrea Cabibbe	None declared	No conflict of interest
Patricia Hall	None declared	No conflict of interest
Rumina Hasan	None declared	No conflict of interest
Brian Kaiser	None declared	No conflict of interest
Shaheed V Omar	Reserarch grant from Janssen Pharmaceuticals to assess prevalence of BDQ resistance using WGS	Conflict of interest not significant
Mark Nicol	None declared	No conflict of interest
Thomas Shinnick	None declared	No conflict of interest
Wayne Van Gemert	None declared	No conflict of interest

Annex 5. Additional resources

WHO policy guidance on TB diagnostics and laboratory strengthening

WHO consolidated guidelines on tuberculosis. Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; In press.WHO End TB Strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2015 (https://www.who.int/publications/i/item/WHO-HTM-TB-2015.19).

Framework of indicators and targets for laboratory strengthening under the End TB Strategy (WHO/HTM/TB/2016.18). Geneva: World Health Organization; 2016 (https://www.who.int/publications/i/item/9789241511438).

Considerations for adoption and use of multi-disease testing devices in integrated laboratory networks (WHO/HTM/TB/2017.06). Geneva: World Health Organization; 2017 (https://www.who.int/publications/i/item/WHO-HTM-TB-2017.06).

WHO policy guidance on TB screening and treatment

WHO consolidated guidelines on tuberculosis. Module 2 – Screening: systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/9789240022676).

WHO consolidated guidelines on tuberculosis. Module 4 – Treatment: drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240063129).

WHO consolidated guidelines on tuberculosis. Module 4 – Treatment: drug-susceptible tuberculosis treatment. Geneva: World Health Organization; 2022 (https://www.who.int/publications/i/item/9789240048126).

Guidance on implementation of diagnostic tests

Practical manual on tuberculosis laboratory strengthening, 2022 update. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/9789240061507).

The use of next-generation sequencing for the surveillance of drug-resistant tuberculosis: an implementation manual. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/9789240078079).

Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, 2nd edition. Geneva: World Health Organization; 2023 (https://iris.who.int/handle/10665/374061).

Manual for selection of molecular WHO recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis. Geneva: Global Laboratory Initiative; 2022 (https://www.stoptb.org/manual-selection-of-molecular-who-recommended-rapid-diagnostic-tests-detection-of-tuberculosis-and-0).

GLI specimen referral toolkit. Geneva: Global Laboratory Initiative; 2017 (https://www.stoptb.org/gli-guidance-and-tools/gli-specimen-referral-toolkit).

GLI practical guide to implementation of Truenat™ Tests for the detection of TB and Rifampicin resistance. Geneva: Global Laboratory Initiative; 2021 (https://www.stoptb.org/gli-guidance-and-tools/practical-guide-to-implementation-of-truenat-tests).

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