WHO operational handbook on tuberculosis

Module 3: Diagnosis

Rapid diagnostics for tuberculosis detection

World Health Organization

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Abbreviations

AIDS	acquired immunodeficiency syndrome
AFB	acid-fast bacilli
ART	antiretroviral therapy
CRI	colorimetric redox indicator
CSF	cerebrospinal fluid
DR-TB	drug-resistant tuberculosis
DST	drug-susceptibility testing
EPTB	extrapulmonary TB
EQA	external quality assessment
ERPD	Expert Review Panel for Diagnostics of the Global Fund
FIND	Foundation for Innovative New Diagnostics
FL-LPA	line probe assay for first-line drugs
GLI	Global Laboratory Initiative
Global Fund	Global Fund to Fight AIDS, Tuberculosis and Malaria
HIV	human immunodeficiency virus
HR	human resources
Hr-TB	isoniazid-resistant, rifampicin-susceptible TB
LAMP	loop-mediated isothermal amplification
LF-LAM	lateral flow lipoarabinomannan assay
LOD	limit of detection
LPA	line-probe assay
MDR-TB	multidrug-resistant tuberculosis
MIC	minimal inhibitory concentration
MGIT™	mycobacterial growth indicator tube
MoH	ministry of health
MTBC	*Mycobacterium tuberculosis* complex
mWRD	molecular WHO-recommended rapid diagnostic
NGS	next-generation sequencing
NTP	national TB programme
NTRL	national TB reference laboratory
PCR	polymerase chain reaction
PLHIV	people living with HIV/AIDS
PT	proficiency testing
QA	quality assurance

QC	quality control
RRDR	RIF-resistance-determining region
RR-TB	rifampicin-resistant TB
SL-LPA	line probe assay for second-line drugs
SOP	standard operating procedure
SRL	supranational reference laboratory
TB	tuberculosis
Tm	melting temperature
TWG	technical working group
USA	United States of America
WHO	World Health Organization
WRD	WHO-recommended rapid diagnostic

Abbreviations of TB agents

AMK	amikacin
BDQ	bedaquiline
CLF	clofazimine
DLM	delamanid
EMB	ethambutol
ETO	ethionamide
FQ	fluoroquinolone
HREZ	isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z)
INH	isoniazid
LFX	levofloxacin
LZD	linezolid
MFX	moxifloxacin
PZA	pyrazinamide
REZ	rifampicin (R), ethambutol (E) and pyrazinamide (Z)
RIF	rifampicin

1 Introduction

1.1 Background

Globally, tuberculosis (TB) remains an important public health problem, with about 10 million people developing TB in 2018 *(1)*. A major threat to being able to treat and prevent TB is the spread of drug-resistant TB (DR-TB), in particular, multidrug- or rifampicin-resistant TB (MDR/RR-TB), which is TB disease caused by *Mycobacterium tuberculosis* complex (MTBC) bacteria with resistance to rifampicin (RIF) and to isoniazid (INH). In 2018, there were about half a million new cases of RIF-resistant TB (RR-TB), of which 78% had multidrug-resistant TB (MDR-TB). In addition, an estimated 830 000 people had TB disease caused by MTBC with resistance to INH and susceptibility to RIF, referred to as Hr-TB.

The effective management of TB relies on the rapid diagnosis of TB, rapid detection of drug resistance and rapid initiation of an effective treatment regimen. This requires access to rapid and accurate detection tests, as well as rapid and accurate drug-susceptibility testing (DST) for all TB patients. Ideally, to guide the selection of an effective regimen, all TB patients should have DST for all anti-TB drugs that might be included in their treatment regimen before, or at the start of, therapy. However, the initiation of treatment should not be delayed to wait for DST results; also, efforts to build laboratory capacity (especially DST) should not slow the detection and enrolment of DR-TB patients 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) calls for the early diagnosis of TB and universal DST. To meet the End TB Strategy targets, WHO-recommended molecular rapid TB diagnostics (WRDs) should be made available to all individuals with signs or symptoms of TB, all bacteriologically confirmed TB patients should receive DST at least for RIF (in 2018, only about 51% of such patients were tested for RIF resistance), and all patients with RR-TB should receive DST at least for fluoroquinolones (FQs).[1] Updated WHO guidelines stress the importance of DST before treatment, especially for the medicines for which WHO-recommended rapid molecular tests are available (e.g. FQs, INH and RIF).[2]

Furthermore, as described in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (2)*, 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 the past few 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

[1] Note: The original End TB strategy called for the testing of all RR-TB patients 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, which makes rapid DST for amikacin unnecessary.

[2] *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection.*

DR-TB. Based on current treatment recommendations *(3)*, countries embarking on interventions to detect and treat DR-TB should establish laboratory capacity to perform genotypic and phenotypic DST for RIF, INH and FQs (e.g. levofloxacin [LFX] and moxifloxacin [MFX]), and to perform phenotypic DST for drugs that are recommended for use in MDR-TB regimens *(4)* and for which there are reliable DST methods (e.g. bedaquiline [BDQ], linezolid [LZD], clofazimine [CFZ], pyrazinamide [PZA] and delamanid [DLM]). Countries should also expand capacity to monitor the culture conversion of patients being treated for DR-TB.

1.2 About this guide

This guide was developed to assist with the translation of WHO policies on tuberculosis (TB) diagnostic testing into practical guidance on the implementation of WHO-recommended tests and algorithms for TB testing.

Section 2 of the guide describes the WHO-recommended tests for detecting TB and drug-resistant TB (DR-TB), as well as the most recent WHO policy guidance for their use. It also describes the processes and steps needed for implementing a diagnostic test for routine use within the TB diagnostic network. Section 3 describes TB diagnostic algorithms that incorporate the most recent WHO recommendations for detecting and treating TB and DR-TB *(3)*. It also outlines considerations for the implementation of a new algorithm.

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 Section 4 (Suggested reading); therefore, 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 on the WHO Global TB Programme website.[3] Guidance on the implementation of diagnostic testing is also available on the website of the Global Laboratory Initiative (GLI) of the Stop TB Partnership.[4]

1.3 Target audience

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

[3] See http://www.who.int/tb/areas-of-work/laboratory/diagnostics/.

[4] See http://www.stoptb.org/wg/gli/gat.asp.

2 WHO-recommended diagnostic tests

2.1 Conventional diagnostic tests

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. Sputum-smear microscopy is a relatively insensitive test, with a limit of detection (LOD) of 5000–10000 bacilli per millilitre of sputum; also, it cannot distinguish drug-susceptible strains from drug-resistant strains. WHO recommends that TB programmes transition to replacing microscopy as the initial diagnostic test with molecular WRDs that allow detection of 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, infrastructure requirements (biosafety level 3 [BSL-3] or TB containment laboratory) and 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 patient's response to treatment.

The conventional method for detecting resistance to anti-TB drugs relies on culture-based phenotypic DST using liquid or solid media. However, phenotypic testing is time-consuming (taking from weeks to months to generate results), primarily because of the slow growth rate of MTBC. This is often too late to inform therapy, stop the acquisition or spread of additional resistance, or prevent mortality. Another issue is that culture-based phenotypic DST requires sophisticated laboratory infrastructure, qualified staff and strict quality control. Also, reliable phenotypic DST methods are not available for some first-line and second-line anti-TB drugs, and for certain drugs (e.g. pyrazinamide), it is technically difficult to generate reliable DST results *(5)*. The potential harms of incorrect DST results mean that the results of phenotypic DST for cycloserine/terizidone, ethambutol (EMB), imipenem-cilastatin/meropenem, ETO/prothionamide and *p*-aminosalicylic acid (PZA) are not recommended for use in clinical decision-making.

Despite the disadvantages, culture-based phenotypic DST remains essential for those drugs for which there are no reliable molecular tests at present, but for which there are accurate and reproducible phenotypic methods (e.g. BDQ). In addition, phenotypic DST may be needed even for drugs for which there are reliable and accurate molecular tests, if there is a need to investigate discordant results, or to perform further testing in the case of unexpected molecular test results (either resistance or susceptibility).

2.2 WHO-approved rapid tests for diagnosis of TB and DR-TB

In recent years, rapid and sensitive molecular tests have become available to replace or complement existing conventional tests for detecting MTBC and drug resistance. These tests include Xpert® MTB/RIF and Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, United States of America [USA]); loop-mediated

isothermal amplification (TB-LAMP; Eiken Chemical, Tokyo, Japan); Truenat™ MTB, MTB Plus and MTB-RIF Dx tests (Molbio Diagnostics, Goa, India) and line-probe assays (LPAs; GenoType® MTBDR*plus* and GenoType® MTBDR*sl*, HAIN Lifescience, Nehren, Germany; NTM+MDRTB Detection Kit, NIPRO Corporation, Osaka, Japan). 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 HIV-infected presumed TB patients; a positive LF-LAM result is considered as bacteriological confirmation of TB in these patients *(6)*. WHO has reviewed and approved 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.

2.2.1 Xpert MTB/RIF assay

The Xpert MTB/RIF assay is a cartridge-based automated test that uses real-time 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:[5]

- 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 rifampicin-resistance detection rather than smear microscopy/culture and phenotypic drug-susceptibility testing;
- 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 rifampicin-resistance detection rather than smear microscopy/culture and phenotypic drug-susceptibility testing;
- 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/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/culture;
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF should be used for rifampicin-resistance detection rather than culture and phenotypic drug-susceptibility testing;
- 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 pre-test 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 pre-test probability 5% or more and an Xpert MTB/RIF negative result on the initial test, repeated testing with Xpert MTB/RIF (for total of two tests) in sputum, gastric fluid, nasopharyngeal aspirate and stool specimens may be used;

[5] *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection.*

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 reaction chamber; thus, Xpert Ultra has a lower LOD than Xpert MTB/RIF (16 colony forming units [cfu]/mL and 131 cfu/mL, respectively). Furthermore, the use of analysis based on melting temperature instead of real-time PCR analysis allows Xpert Ultra to better differentiate silent from resistance-conferring mutations, and minimizes false results on RIF resistance, especially in samples with a low bacterial load. WHO recommends using the Xpert Ultra test in the following situations:[6]

- 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 rifampicin-resistance detection rather than smear microscopy/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 last five years, Xpert Ultra may be used as the initial diagnostic test for TB and for rifampicin-resistance detection rather than smear microscopy/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/culture in sputum or nasopharyngeal aspirates;
- In adults and children with signs and symptoms of TB meningitis, Xpert Ultra should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for TB meningitis rather than smear microscopy/culture;
- In adults and children with signs and symptoms of extra-pulmonary TB an Xpert Ultra may be used in lymph node aspirate and lymph node biopsy as the initial diagnostic test for the detection of lymph nodes TB, rather than smear microscopy/culture;
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra should be used for rifampicin-resistance detection rather than culture and phenotypic drug susceptibility testing;
- 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 pretest probability below 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 pretest probability 5% or more and an Xpert Ultra negative result on the first initial test, repeated 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 real-time micro PCR for the semiquantitative detection of MTBC directly from sputum specimens, and can report results in less than an hour. The assays use automated, battery-operated devices to extract, amplify and detect specific genomic DNA loci.

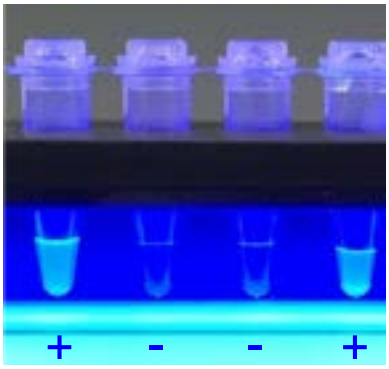
[6] *WHO consolidated guidelines on tuberculosis. Module 3: diagnosis - rapid diagnostics for tuberculosis detection.*

The assays are designed to be operated in peripheral laboratories with minimal infrastructure and minimally trained technicians.

If the MTB or MTB Plus assay result is positive, 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/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 rifampicin resistance rather than culture and phenotypic DST:
  - These recommendations apply to the use of the test with sputum specimens from people living with HIV (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 TB-LAMP assay



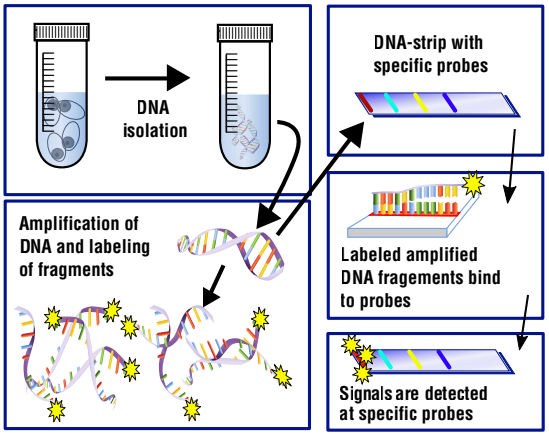
The TB-LAMP assay is designed to detect MTBC directly from sputum specimens. This is a manual assay that requires less than 1 hour to perform, does not require sophisticated instrumentation and can be used at the peripheral health centre level, given biosafety requirements similar to sputum smear microscopy. TB-LAMP does *not* detect resistance to anti-TB drugs. The following recommendations have been done by WHO *(8)*:

- 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:
  - 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.

2.2.5 LPAs

LPAs are a family of DNA strip-based tests that detect mutations associated with drug resistance:

- 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 (FL-LPAs) such as GenoType MTBDR*plus* and NTM+MDRTB Detection Kit allow the detection of resistance to RIF, INH and ETO. WHO recommends using FL-LPAs in the following situations *(9)*:

- For persons with a sputum smear-positive specimen or a cultured isolate of MTBC, commercial molecular LPAs may be used as the initial test instead of phenotypic culture-based DST to detect resistance to RIF and INH:
  - 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 DST for INH may still be used to evaluate patients when the LPA result does not detect INH resistance. This is particularly important for populations with a high pre-test probability of resistance to INH.
  - FL-LPAs are not recommended for the direct testing of sputum smear-negative specimens for the detection of *Mycobacterium tuberculosis* complex (MTBC).

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 *(10)*:

- For patients with confirmed MDR/RR-TB, SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to FQs and AMK
  - These recommendations apply 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 patients with negative SL-LPA results, particularly in populations with a high pre-test probability for resistance to FQs or AMK.
  - SL-LPA tests are also useful for detecting FQ resistance before starting therapy for Hr-TB.

2.2.6 Urine LF-LAM assay

The urine LF-LAM assay is an immunocapture assay based on the detection of the mycobacterial LAM antigen in urine, and 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 patient's survival. The Alere Determine TB LAM Ag is currently the only commercially available urine LAM test endorsed by WHO. The detection of mycobacterial LAM antigen in urine does not provide any information on drug resistance. WHO recommends using urine LF-LAM in the following situations *(11)*:

- 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 or who are seriously ill, or with 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 without TB symptoms and with an unknown CD4 cell count, or with a CD4 cell count greater than 100 cells/mm^3;

- For their initial diagnostic test, all patients with signs and symptoms of pulmonary TB who are capable of producing sputum should have at least one sputum specimen submitted for a molecular WRD assay. This also includes children and adolescents living with HIV who are able to provide a sputum sample. LF-LAM results (test time <15 minutes) are likely to be available before molecular WRD test results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.
- LF-LAM should be used as an add-on to clinical judgement in combination with other tests. It should *not* be used as a replacement or triage test.

2.3 Additional WHO-reviewed genotypic and phenotypic methods

WHO has evaluated a number of tests, and more are on the horizon but have not yet been the subject of formal recommendations. These tests are described here for completeness but are not included in the model diagnostic algorithms.

WHO has recommended selected non-commercial liquid culture systems for detecting MTBC and RIF resistance, with conditions and as an interim solution, pending the development of genotypic or automated liquid culture and DST capacity *(12)*. These methods include microscopic observation of drug susceptibility (MODS), nitrate reductase assay (NRA) and colorimetric redox indicator (CRI). They are suitable for use at central-level laboratories or reference laboratories, and require highly trained personnel. However, their use is not intended to replace conventional culture and DST. Their implementation should be phased in, and should include validation against standard methods. Scaling up the use of CRI, MODS and NRA, and decentralizing their use to lower-level laboratories are *not* recommended.

In October 2019, WHO released a report *(13)* of a technical expert consultation on the accuracy of centralized assays for the detection of MTBC and resistance to RIF and INH. The expert group evaluated four centralized, high-throughput TB assays: Abbott RealTime MTB and MTB RIF/INH assays, Roche cobas® MTB and MTB-RIF/INH assays, Hain FluoroType® MTBDR assay, and BD MAX™ MDR-TB assay. In a study using a well-defined strain panel to determine the analytical sensitivity for MTBC and resistance to RIF and INH, each of the four assays displayed similar performance to WHO-endorsed assays for the detection of TB (Cepheid Xpert MTB/RIF) and RIF and INH resistance (Hain MTBDR*plus*). The technical expert consultation concluded that additional studies were required to validate the performance of the assays with clinical specimens under routine testing conditions before WHO consider them for approval.

- In March 2020, the Expert Review Panel for Diagnostics (ERPD) of the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund) approved the Abbott RealTime MTB and MTB RIF/INH assays, and the BD MAX MDR-TB assay for the detection of TB and resistance to RIF and INH. ERPD approval allows countries to use funding from the Global Fund to procure products for a time-limited period, with a possibility of renewal (for the Abbott and BD assays, until March 2021). ERPD approval of TB diagnostics is intended as an interim approval mechanism on the pathway to potential WHO endorsement.
- Implementation considerations for the centralized assay platforms should be based on where countries would place the tests in the diagnostic algorithm for TB and other diseases, as well as in-country laboratory capacity. To ensure the rapid turnaround of samples referred to testing sites, countries should ensure that an efficient and reliable sample transportation system is available. To bring cost-efficiency to testing services, consideration of integrating TB testing into existing platforms should be prioritized in locations where integrated testing is feasible *(14)*.

An LPA, the Genoscholar® PZA-TB II assay (Nipro, Osaka, Japan), is the only commercially available assay for the detection of mutations within the *pncA* gene (including the promoter region) that are likely to lead to PZA resistance. WHO plans to review this assay.

DNA sequencing using next-generation sequencing (NGS) methods can also rapidly detect mutations associated with drug resistance for many anti-TB drugs *(15)*. NGS-based DST has the potential to reduce the need for phenotypic DST for patient-care decisions and drug-resistance surveys. NGS-based DST may be particularly useful for drugs for which phenotypic testing is unreliable, or settings that do not have the capacity for performing phenotypic DST reliably. The current NGS systems has limitations; in particular, the required computational expertise and resources. Thus, implementation of NGS-based DST is likely to be focused, at least initially, on capacity-building at the national TB reference laboratory and, perhaps, at well-performing regional TB reference laboratories. Amplification-based targeted NGS assays for detecting DR-TB directly from sputum specimens are in the pipeline. The Next Gen-RDST assay (Translational Genomics Research Institute, Phoenix, Arizona, USA) can detect mutations associated with resistance to at least seven drugs, and the Deeplex®-MycTB assay (GenoScreen, Lille, France) can detect mutations in gene regions associated with resistance to at least 13 drugs. These assays have not yet been reviewed or approved by WHO.

2.4 Tests not recommended

Based on reviews of available data, WHO has recommended against using tests that do not provide reliable information useful 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 then-available commercial serodiagnostic tests provided inconsistent and imprecise findings; there was no evidence that using the commercial serological assays available at that time improved patient outcomes; and the tests generated high proportions of false-positive and false-negative results, which may have an adverse impact on the health of patients *(16)*. Similarly, WHO recommends that, in low- and middle-income countries, interferon-gamma-release assays 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.

WHO recommendations are specific for intended uses and, sometimes, even an approved test is not recommended to be used for a specific purpose. For example, nucleic acid amplification tests (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 assessing TB symptoms;
- patients without TB symptoms and unknown CD4 cell count, or without TB symptoms and CD4 cell count greater than or equal to 200 cells/mm^3; and
- patients without TB symptoms and with a CD4 cell count of 100–200 cells/mm^3.

WHO also recommends against using the LF-LAM test for the diagnosis of TB in those who are HIV-negative and do not fall into the specific groups for whom testing is recommended. Countries are encouraged to read the WHO policy statements carefully.

2.5 Implementing a new diagnostic test

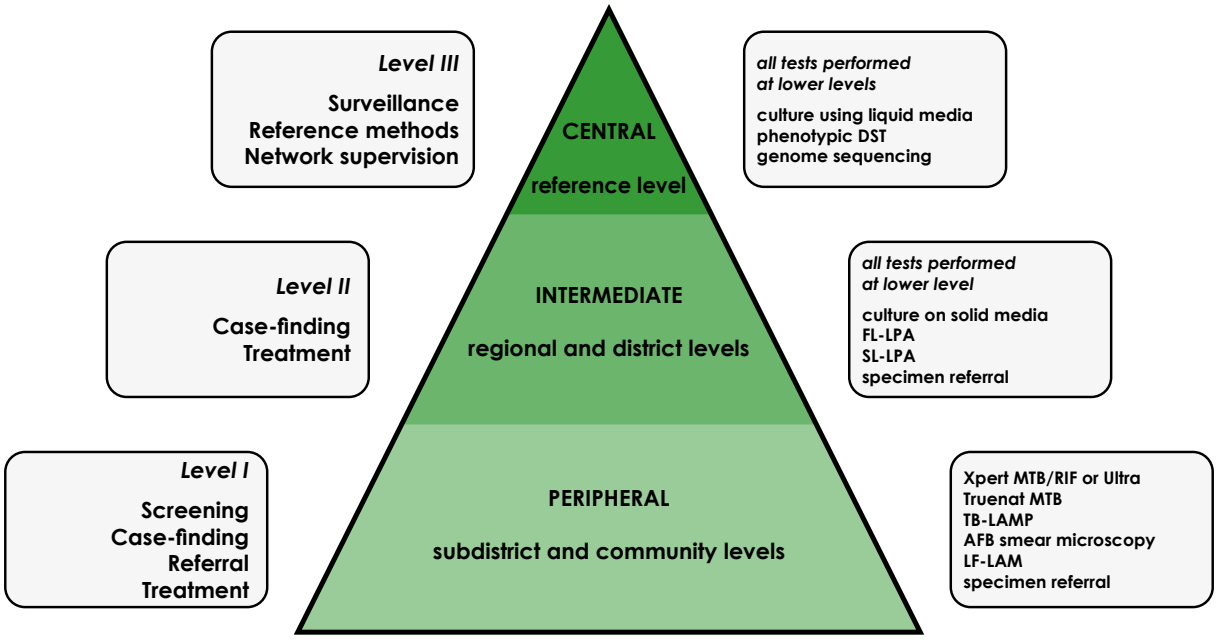
2.5.1 Placement of diagnostic tests in the tiered structure of the TB laboratory network

In many resource-limited or high burden settings, TB laboratory networks have a pyramidal structure, as shown in Fig. 2.1. 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 various activities and diagnostic methods being performed in the laboratories.

- At the *peripheral level* (level I), peripheral laboratories offer a range of basic diagnostic tests, such as acid-fast bacilli (AFB) smear microscopy, Xpert MTB, TB-LAMP, Truenat MTB or LF-LAM. Further testing is usually accomplished by referring specimens to a higher level testing facility.
- At the *intermediate level* (level II), advanced technologies requiring greater infrastructure, expertise or biosafety precautions are offered, such as culture on liquid or solid media, or FL-LPA or SL-LPA (or both) using sputum specimens. Again, samples requiring additional testing may be referred to a higher level laboratory.
- At the *central level* (level III), testing requiring advanced skills, infrastructure and biosafety precautions are offered, such as culture using solid and liquid media, phenotypic DST using solid or liquid media, or FL-LPA or SL-LPA using isolates and NGS.

Fig. 2.1. Organization of a TB diagnostic network



The structure of the network and testing packages available at each level should be tailored to meet the needs of the community and the local epidemiology of TB; that is, targets should be demand based rather than population based. For example, when considering placement of a diagnostic test:

- more or higher volume laboratories may be needed in high- than in low-prevalence settings; and
- optimal access to quality testing may be achieved 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 optimal strategy will probably vary by geography and epidemiologic situation.

The organization and structure of the TB diagnostic network can be informed by optimal scenario planning combined with detailed mapping of the network. 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 *(2)*.

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

- available resources for implementation;
- infrastructure requirements;
- biosafety requirements;
- specimen requirements and collection procedures;
- projected testing volumes;
- 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 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 under-served settings.

The *GLI guide for TB specimen referral systems (17)* and the *GLI specimen referral toolkit (18)* are good sources of information for designing, implementing and monitoring systems for referring specimens and reporting results.

2.5.2 Test accuracy considerations for selecting a diagnostic test

The predictive values of a test vary, depending on the prevalence of TB in the patient population being tested. Table 2.1 provides examples of population-level projections of the results of testing with the various molecular WRDs in settings with different prevalence of TB, based on pooled sensitivity and specificity estimates that were extracted from the WHO policy statements for each test. 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 over-treatment of patients without 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.

Table 2.1. Performance characteristics of molecular tests for the detection of TB

Performance of molecular WRDs for the detection of MTBC in adults with signs and symptoms being evaluated for pulmonary TB compared with a microbiological reference standard				
Test	**Test accuracy**	**2.5% prevalence**	**10% prevalence**	**30% prevalence**
Xpert MTB/RIF	Sensitivity: 0.85 (95% CrI: 0.82–0.88)	TP: 21 / FN: 4	TP: 85 / FN: 15	TP: 255 / FN: 45
	Specificity: 0.99 (95% CrI: 0.97–0.98)	TN: 965 / FP: 10	TN: 891 / FP: 9	TN: 693 / FP: 7
Xpert Ultra	Sensitivity: 0.90 (95% CrI: 0.84–0.94)	TP: 22 / FN: 3	TP: 90 / FN: 10	TP: 269 / FN: 31
	Specificity: 0.96 (95% CrI: 0.93–0.98)	TN: 932 / FP: 43	TN: 860 / FP: 40	TN: 669 / FP: 31
Truenat MTB[a]	Sensitivity: 0.73 (95% CI: 0.68–0.78)	TP: 18 / FN: 7	TP: 73 / FN: 27	TP: 220 / FN: 80
	Specificity: 0.98 (95% CI: 0.97- 0.99)	TN: 957 / FP: 18	TN: 884 / FP: 16	TN: 687 / FP: 13
Truenat MTB Plus	Sensitivity: 0.80 (95% CI: 0.75–0.84)	TP: 20 / FN: 5	TP: 80 / FN: 20	TP: 239 / FN: 61
	Specificity: 0.96 (95%CI: 0.95–0.97)	TN: 940 / FP: 25	TN: 868 / FP: 32	TN: 675 / FP: 25
TB-LAMP	Sensitivity: 0.78 (95%CrI: 0.71–0.83)	TP: 20 / FN: 5	TP: 78 / FN: 22	TP: 234 / FN: 66
	Specificity: 0.98 (95%CrI: 0.96–0.99)	TN: 955 / FP: 20	TN: 882 / FP: 18	TN: 686 / FP: 14

CI: confidence interval; CrI: credible interval; FN: false negative; FP: false positive; MTBC: *Mycobacterium tuberculosis* complex; TB: tuberculosis; TN: true negative; TP: true positive; WRD: WHO-recommended rapid diagnostic.

[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.

The predictive values in Table 2.1 assume that the indicated test is the initial diagnostic test for TB. Countries may consider using chest X-ray to triage who should be tested with a molecular test, to reduce the number of individuals tested and the associated costs *(19, 20)*. This approach should improve the pre-test probability for TB and should thus improve the predictive value of the molecular test and reduce false-positive test results, especially in populations with a low prevalence of TB. For example, the addition of chest X-ray as a triage test to an algorithm in which all patients with signs and symptoms of TB receive an Xpert MTB/RIF test was calculated to increase the positive predictive value of the Xpert MTB/RIF test from 69% to 82%, in a population with a prevalence of 1% *(20)*.

Epidemiologic considerations for selecting a diagnostic test

In selecting a diagnostic test to implement, it is important to consider the characteristics (i.e. risk groups) of the population being served. These characteristics should be derived from population-based studies, if available, including the proportion of TB cases resistant to RIF, INH and FQs; the proportion among people who are HIV-positive; the proportion that is extrapulmonary; and the proportion among children. 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.

2.5.3 Steps and processes for implementing a new diagnostic test

Box 2.1 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, Global Laboratory Initiative (GLI), 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 2.1. 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 HR needed for the new test. The following sections organize the key steps into 10 main areas:

- Area 1 – Policies and planning
- Area 2 – Regulatory issues
- Area 3 – Equipment
- Area 4 – Supply chain

- Area 5 – Procedures
- Area 6 – Digital data
- Area 7 – Quality assurance
- Area 8 – Recording and reporting
- Area 9 – Training and competency assessment
- Area 10 – Monitoring and evaluation

This section discusses the steps in each of these areas.

Area 1 – Policies and planning

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

1.2 Review WHO policies and available technical and implementation guides

1.3 Define immediate and future purposes of the test

1.4 Update national diagnostic algorithm and guidelines

1.5 Perform a situational analysis, including biosafety

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. The TWG's establishment 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 information technology (IT) experts; and
- specimen transport systems for centralized or regional testing (TB and non-TB).

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 roles and responsibilities of members of the implementation team, and 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, in light of the intended use of the new diagnostic test, country epidemiology, existing testing algorithms, sample referral systems and other operational considerations, and make recommendations to the MoH and NTP. Section 3 describes 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 patient 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; 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.

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, at least, the minimum biosafety requirements are in place before the new test is implemented *(21)*.

The situational analysis will also need to assess the relevant portions of the TB diagnostic network. Of particular relevance is the specimen referral system. A checklist for evaluating a specimen referral system can be found in the relevant GLI publication *(17)*.

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); need for specialized, skilled and well-trained staff; need for expert technical assistance; maintenance of confidentiality of patient information; and establishment of a QA system.

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

Area 2 – Regulatory

2.1 Determine importation requirements

2.2 Conduct country verification study as required

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 service providers of equipment and consumables, to determine importation and registration requirements, and to initiate country verifications, if required.

Step 2.2 – Conduct country verification study as required

Validation[7] studies are large-scale studies that are done to establish the performance characteristics (e.g. sensitivity, specificity, accuracy, positive and negative predictive values, robustness, reliability and reproducibility), and to test limitations and results-acceptance criteria for a test. Validation studies are an essential part of the WHO review process and 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 these large-scale studies. Instead, implementing laboratories should conduct small-scale verification *(22)* 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 the validation studies, and that the method is suitable for its intended use in the population of patients being tested. Countries must make their own determination on the needs for verification, based on national guidelines and accreditation requirements.

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. An appropriate time period must be allowed to submit the application and provide any required supplementary evidence.

Area 3 – Equipment

3.1 Select, procure, install and set up equipment

3.2 Instrument verification and maintenance

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 of the implementation process is selecting appropriate instruments to fit the needs of the clinical or microbiological laboratory, and 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 broadly available, and has good local supply distribution and support.

[7] The terms validation and verification are used almost interchangeably, which may generate confusion. During implementation, the focus should be on small-scale studies to verify the test's performance in the intended testing laboratory.

To bring cost-efficiency to testing services, a priority should be to consider the integration of TB testing on existing platforms, in locations where integrated testing is feasible *(14)*. 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, most instruments will require expert set up, with the manufacturer's engineers performing the installation. Potential setup complexities include power supply and backup options, electrical 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.

Step 3.2 – Instrument verification and maintenance

All instruments must be documented to be "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. 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 determine which sites will conduct diagnostic testing, based on factors such as TB epidemiology, geographic considerations, testing workload, availability of qualified staff, efficiency of referral networks and patient access to services. Each testing site should be evaluated for readiness using a standardized checklist before the site starts to test clinical specimens. 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 (i.e. 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.

Area 4 – Supply chain

4.1 Review forecasting, ordering and distribution procedures

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;

- 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; and
- On-going monitoring of all procurement and supply chain steps, to ensure that delays are minimized and that sites receive correct reagents as per planned schedule.

Purchasing and distribution strategies should be reassessed at regular intervals, 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 standard operating procedures for handling the reagents and chemicals used to ensure both quality and safety.

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 test kits with test failures are not distributed. At the testing site, new-lot testing is needed for reagents prepared at that site, and may also be needed to monitor conditions during transport and storage of test kits in-country. For quality control, WHO recommends using positive and negative controls when testing new batches of reagents.

Area 5 – Procedures

5.1 Develop SOPs

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 patients 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 quality controls) and external quality assessment (see Area 7); and
- recording and reporting of results (see Area 8).

A well-defined, comprehensive set of SOPs that addresses all aspects of the laboratory testing processes – from sample collection to results reporting – 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; others will need to be developed. SOPs must be made readily available for staff and 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 patients to be tested, ordering tests, interpreting test results and making patient care decisions. All clinical staff involved in diagnosis and management of patients must be sensitized on updated procedures before a new diagnostic test is used. Clinical staff from referral sites must also be sensitized, using staff trainings in combination with standardized printed materials developed by the NTP.

The rate of ordering of the new test must be monitored, to ensure that clinical staff at all sites that should be offering the test do actually order the test. Clinical staff at sites with a low testing rate may need additional training and sensitization.

Area 6 – Digital data

6.1 Develop the use of digital data and diagnostics connectivity

6.2 Develop procedures for data backup, security and confidentiality

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 take into account software and hardware requirements, to take advantage of digital data.

Diagnostics connectivity *(23)* 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. 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 a pre-emptive service to avoid instrument failure. This approach can provide a highly cost-effective way to ensure that a diagnostic device network functions properly.

Data can also be transmitted automatically to:

- clinicians and patients, which allows for faster patient follow-up;
- laboratory information management systems or electronic registers, which reduces staff time and the chance of transcription errors, and greatly facilitates monitoring and evaluation processes; and
- the NTP, to assist with surveillance of disease trends or resistance patterns, and to enhance the capacity of the NTP to generate the data needed for several of the 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. There also must be policies and procedures to ensure the security of laboratory data and confidentiality of patient data, in line with national and international regulations.

Area 7 – Quality assurance, control and assessment

7.1 Implement a comprehensive QA programme

7.2 Establish and monitor quality controls (QCs)

7.3 Develop an external quality assessment (EQA) programme

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 competence assessment (Area 9);
- instrument verification and maintenance (Area 3);
- method validation or verification (Area 2);
- lot-to-lot testing (Area 4);
- internal QC;
- EQA; and
- quality indicator monitoring and continuous quality improvement.

A comprehensive discussion of the essential elements of a QA system can be found in the *GLI practical guide to TB laboratory strengthening (24)*. 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.

Step 7.3 – Develop an EQA programme

An EQA programme includes quality and performance indicator monitoring, proficiency testing, re-checking or making inter-laboratory comparisons, regular on-site supportive supervision and timely feedback, corrective actions and follow-up. On-site supervision should be prioritized to poorly performing sites identified through proficiency testing, monthly monitoring of performance indicators or site assessments. Failure to enroll in a comprehensive EQA program 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 between testing sites. The purpose of such testing is to identify sites with serious testing deficiencies, help with targeting 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 re-testing of samples at a higher level laboratory. Many TB laboratories are familiar with this approach, because blinded rechecking 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, and to enable monitoring of trends and 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 relevant GLI guide *(24)*, quality indicators specific to the new diagnostic should be adapted from international guidelines or developed from scratch. 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 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.

Area 8 – Recording and reporting

8.1 Review and revise request for examination and reporting forms

8.2 Review and revise laboratory and clinical registers

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

Depending on the current format of the country's requisition (i.e. specimen examination request) form, it may be necessary to make revisions to accommodate a new diagnostic test. Countries should determine whether an update of the examination forms is needed, taking into account 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 patient in order 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, fields for such data are already on request forms, but the fields are completed incompletely or inconsistently. Refresher training to clinical and laboratory staff should be conducted, to ensure that forms are filled out correctly and completely.

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 those results. An easy-to-read format is important because there is likely to be a wide range of expertise among the clinicians interpreting test results.

Step 8.2 – Review and revise laboratory and clinical registers

Current laboratory and clinical registers that are based on the WHO reporting framework *(6)* 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 use the approach consistently across all testing and clinical sites.

Area 9 – Training and competency assessment

9.1 Develop and implement a training curriculum and strategy

9.2 Assess and document the competence of staff

Step 9.1 – Develop and implement a training curriculum and strategy

Training and competency assessment are critical for generating quality assured test results. 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 laboratory staff to ensure that all clinicians involved in the screening and care of TB patients:

- 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 9.2 – 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 an 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 the 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.

Area 10 – Monitoring and evaluation

10.1. Monitor implementation of the diagnostic test

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. For each objective of a test, indicators to assess its impact should be developed. For each indicator, programmes should define its purpose, target, data elements and data sources; how it 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 the outcome and identify opportunities for interventions to increase impact. As part of demonstrating a test's impact, and to assist with planning and policy-making, programmes should consider evaluating the cost-effectiveness and end-user perspective of a test 1 year after implementation, and regularly thereafter over the next 3–5 years. The end-user perspective should include acceptability and feasibility aspects of the principal user groups; that is, health workers (e.g. clinicians, nurses and community health workers), laboratory technicians and patients.

3 Diagnostic algorithms

Effective and efficient TB diagnostic algorithms are key components of a diagnostic cascade designed to ensure that patients with TB are diagnosed accurately and rapidly, and are promptly placed on appropriate therapy. In turn, that therapy should reduce morbidity and mortality, 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 algorithms, which emphasize the use of WHO-recommended rapid diagnostics (WRDs), are illustrative and countries must adapt them to their local situation.

As mentioned in Section 2.5.2, when selecting a diagnostic algorithm to implement, it is important to consider the characteristics of the population being served. Thus, the four model algorithms are as follows:

- **Algorithm 1** relies on molecular WRDs as the initial diagnostic tests, and is appropriate for all settings, although the choice of which molecular WRD to use may differ in a setting with high MDR/RR-TB prevalence (e.g. a test that detects MTBC and RIF resistance may be needed) or with high HIV prevalence (e.g. a more sensitive test may be needed).
- **Algorithm 2** incorporates the most recent WHO recommendations for the use of the LF-LAM assay as an aid in the diagnosis of TB in PLHIV, and may be most relevant to settings with a high HIV prevalence. However, Algorithm 2 is applicable to any patient living with HIV who meets the testing criteria, regardless of the underlying prevalence of HIV in that setting.
- **Algorithm 3** and **Algorithm 4** are for follow-up testing to detect drug resistance other than RIF resistance:
  - Algorithm 3 is used when the purpose is to detect resistance to second-line drugs in patients with RIF resistance;
  - Algorithm 4 is used when the purpose is to detect resistance to INH in patients at risk of Hr-TB and with RIF susceptibility.

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

Each algorithm is accompanied by explanatory notes and is followed by a decision pathway that provides a detailed description of the various decisions in the algorithm.

3.1 Model algorithms

3.1.1 Algorithm 1 – molecular WRD 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. In this algorithm, molecular WRDs are used as the initial diagnostic test to detect TB and RIF resistance (i.e. this algorithm meets the goals of the End TB Strategy for the use of molecular WRDs and universal DST). This algorithm is designed to be used with each of the WHO-approved rapid molecular tests for detection of MTBC (Xpert MTB/RIF, Xpert MTBRIF Ultra, Truenat MTB, Truenat MTB Plus and TB-LAMP), although it may require minor modification based on which molecular WRD is used and in which population. For example, although this algorithm is appropriate for both low and high burden MDR-TB settings, in a setting with a high MDR-TB burden, it would be preferable to use a molecular WRD that detects MTBC and RIF resistance simultaneously (e.g. Xpert MTB/RIF), rather than one that detects only MTBC (e.g. TB-LAMP).

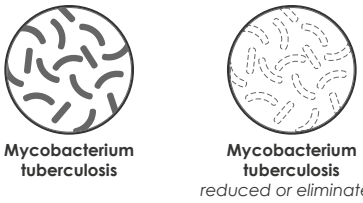
This algorithm is feasible when the molecular WRD testing can be conducted on site or can be accessed through a reliable referral system with short turnaround times.

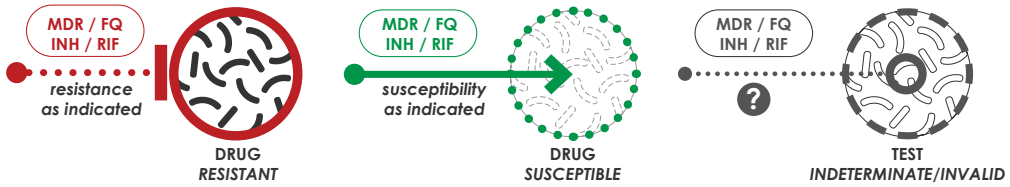
Graphic legend

Document organization

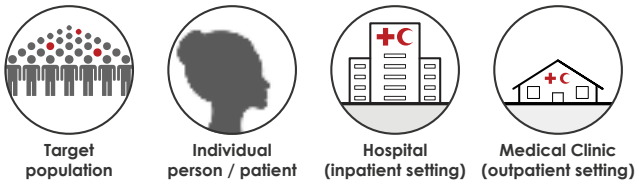
Test results

Resistance & susceptibility

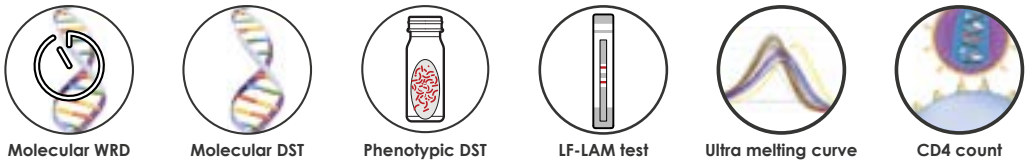




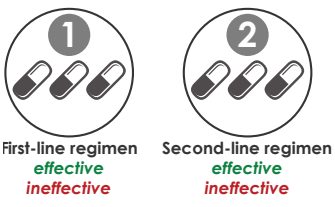
People & Places



Tests & Analytical Protocols

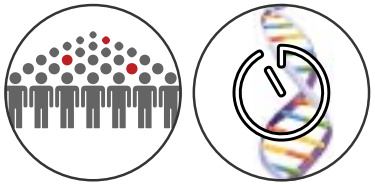


Treatments



Algorithm 1

1 *Molecular WRD as the initial diagnostic test for TB*



Persons to be evaluated for TB[1]

Collect one specimen[2] and perform mWRD[3]

A — MTB *not* detected

Re-evaluate the patient clinically and conduct additional testing to confirm or exclude TB in accordance with national guidelines[8]

Consider repeat mWRD testing in children[9]

Use clinical judgement for treatment decisions

B — MTB detected (not trace)[4] RIF resistance *not* detected[5]

Treat with first-line regimen in accordance with national guidelines[10]

Consider DST for INH if risk of INH mono- or poly-resistance is high[11]

C — MTB detected (not trace)[4] RIF resistance detected

Evaluate the patient for **MDR-TB risk factors**[12]

- **HIGH**
  - Treat with **MDR-TB regimen** in accordance with national guidelines[14]
  - **Follow Algorithm** 3 for further testing and assessment
- **LOW**
  - **Repeat mWRD** test with a fresh specimen[13]
  - **Follow *this* algorithm** to interpret results

D — MTB detected (not trace)[4] RIF indeterminate[6]

- **Ultra**
  - evaluate **Ultra melting curve**[6]
    - **RIF resistance detected**
      - Follow C
    - **RIF resistance *not* detected**
      - **Repeat mWRD**[13]
- **Other mWRDs**
  - **Repeat mWRD** test[13]
  - **Follow *this* algorithm** to interpret results
  - If both tests give indeterminate results, treat with **first-line regimen**[9]
  - Promptly conduct additional investigations to **assess resistance to RIF**[14]
  - Review treatment based on **DST** result

E — MTB detected trace[7] RIF indeterminate

Evaluate person for pulmonary or extrapulmonary TB, HIV, age, and prior TB treatment

- **PLHIV[14] or children** being evaluated for pulmonary TB and persons being evaluated for extrapulmonary TB and **adults** being evaluated for pulmonary TB who are not at risk for HIV and who do not have a history of prior TB or TB treatment within the past 5 years
  - Treat with **first-line regimen**[10]
  - Conduct additional investigations to **assess resistance to rifampicin**[16]
  - Review treatment based on **DST** result
- **Adults** being evaluated for pulmonary TB who are not at risk for HIV and who have a history of prior TB or whose TB treatment ended less than 5 years ago
  - Recent TB treatment may generate a **false-positive** result [17]
  - **Re-evaluate** patient clinically[8]
  - Conduct **additional testing for TB and RIF resistance** in accordance with national guidelines[16]
  - Use **clinical judgement** for treatment decisions

F — No result, error or invalid test

Use **clinical judgement** for treatment decisions

Conduct **additional testing to confirm or exclude TB** in accordance with national guidelines[8]

Consider repeat mWRD test[13]

AIDS: acquired immunodeficiency syndrome; CSF: cerebrospinal fluid; DNA: deoxyribonucleic acid; DST: drug-susceptibility testing; FL-LPA: line probe assay for first-line drugs; HIV: human immunodeficiency virus; INH: isoniazid; LAMP: loop-mediated isothermal amplification; MDR-TB: multidrug-resistant tuberculosis; MTB: *Mycobacterium tuberculosis*; mWRD: molecular WHO-recommended rapid diagnostic; PLHIV: people living with HIV/AIDS; RIF: rifampicin; RR-TB: rifampicin-resistant tuberculosis; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic.

[1] Persons to be evaluated for TB include adults and children with signs or symptoms suggestive of TB, or with a chest X-ray with abnormalities suggestive of TB. This algorithm may also be followed for the diagnosis of extrapulmonary TB using CSF, lymph node and other tissue specimens.

[2] Programs may consider collecting two specimens upfront. The first specimen should be promptly tested using the molecular WRD test. The second specimen may be used for the additional testing described in this algorithm. For persons 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. molecular WRD, culture, DST or histology).

[3] Molecular WRD tests appropriate for this algorithm include Xpert MTB/RIF, Xpert Ultra, Truenat MTB, Truenat MTB Plus and TB-LAMP.

[4] "MTB detected (not trace)" includes MTB detected as high, moderate, low or very low. These categories apply to the original Xpert MTB/RIF and Xpert Ultra tests. Results of the Truenat MTB and MTB Plus tests and the TB-LAMP test also fall into the category of "MTB detected (not trace)".

[5] Determination of RIF resistance occurs simultaneously in the Xpert MTB/RIF and Xpert Ultra 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, see Fig. 3.1) and in the TB-LAMP test, which requires a fresh specimen to be collected and a molecular or phenotypic DST to be conducted.

[6] The interpretation and follow-up testing for "MTB detected rifampicin indeterminate" results for the Xpert Ultra test differs from the interpretation of results for other mWRDs. MTB detected RIF indeterminate results obtained with the Xpert Ultra test (especially those with high and medium semi-quantitative 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 FL-LPA will be needed to confirm or exclude RIF resistance. Indeterminate results for the other molecular WRDs are usually related to very low numbers of bacilli in the sample.

[7] "MTB detected trace" applies only to the Xpert Ultra test.

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

[9] In children with signs and symptoms of pulmonary TB in settings with a pre-test 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 in sputum or nasopharyngeal aspirate (for a total of two tests). In addition Xpert MTB/RIF can be used in gastric fluid and stool. Data were not 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 molecular WRD test should be repeated at the same testing site with a fresh specimen, with the result of the repeat test interpreted as shown in this algorithm. The result of the second test is the result that should be used for clinical decisions.

[10] Patients should be initiated on a first-line regimen according to national guidelines, unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen.

[11] A sample may be sent for molecular or phenotypic DST for INH if there is a high prevalence of INH resistance not associated with RIF resistance (i.e. INH mono- or poly-resistance) in this setting.

[12] Patients at high risk for MDR-TB include previously treated patients, including those who had been lost to follow-up, relapsed or failed a treatment regimen; non-converters (smear positive at end of intensive phase); MDR-TB contacts; and any other MDR-TB risk groups identified in the country.

[13] The mWRD test 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.

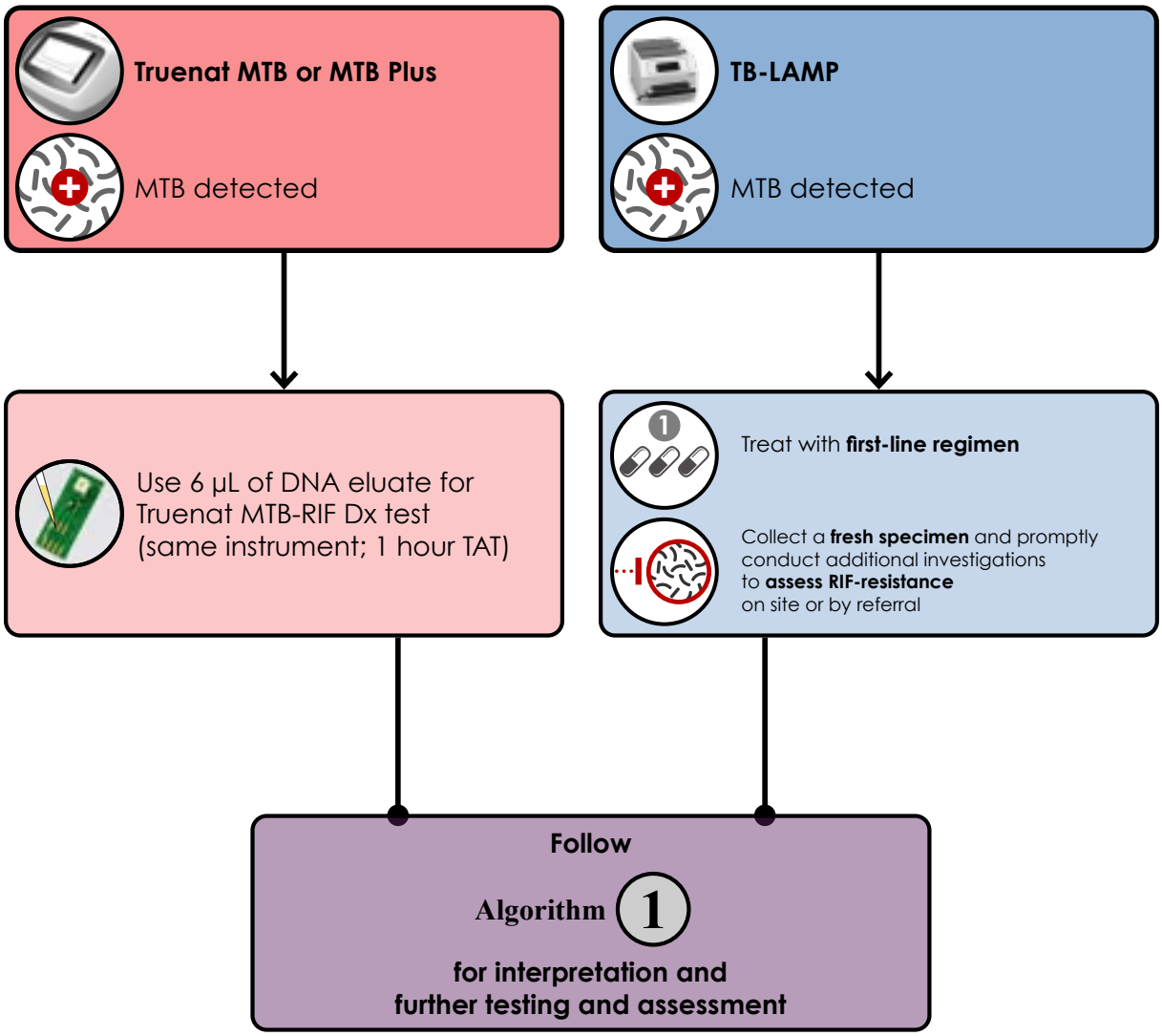
[14] PLHIV include those who are HIV positive or whose HIV status is unknown, but who present with strong clinical evidence of HIV infection, in settings where there is a high prevalence of HIV or among members of a risk group for HIV. For all those with unknown HIV status, HIV testing should be performed according to national guidelines.

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

[16] Phenotypic (culture and DST) and molecular (e.g. LPAs, DNA sequencing and high-throughput centralized assay) methods are available for evaluating drug resistance. Rapid molecular methods are preferred.

[17] In patients with a prior history of TB within the past 5 years, or whose TB treatment was completed less than 5 years ago, Xpert Ultra trace results (and occasionally Xpert MTB/RIF "MTB detected low or very low") may be positive, not because of active TB but because of the presence of non-viable bacilli. Repeat Xpert MTB/RIF or Ultra testing is not recommended, but may be used as an initial attempt to assess RIF resistance. Culture and DST may be of benefit for detecting TB and drug resistance. Clinical decisions must be made on all available information and clinical judgment.

Fig. 3.1. Two-step testing for RIF resistance for Truenat and TB-LAMP



DNA: deoxyribonucleic acid; LAMP: loop-mediated isothermal amplification; MTB: *Mycobacterium tuberculosis*; RIF: rifampicin; TAT: turn-around-time; TB: tuberculosis.

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

Tests

Molecular WRDs appropriate for this algorithm include the Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, Truenat MTB Plus and TB-LAMP tests.

- "Xpert MTB test" designates either the original 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 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.
- With respect to detection of RIF resistance, the Xpert MTB tests detect MTB and RIF resistance simultaneously. A sample with a positive result in the Truenat MTB or MTB Plus test is further tested with Truenat MTB-RIF Dx test, using the same DNA sample that was isolated for the initial Truenat test. A positive TB-LAMP test requires a fresh specimen to be collected, and molecular or phenotypic DST to be conducted, to detect RIF resistance.

General considerations

WHO recommends the use of a molecular WRD (Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, Truenat MTB Plus, Truenat MTB-RIF Dx or TB-LAMP) 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 patients who are on treatment or have been previously treated, if the patient is being evaluated for possible RR-TB (e.g. non-converters at the end of the intensive phase of treatment) or for a new or continuing episode of TB (e.g. relapse cases or previously treated patients, including those who had been lost to follow-up). TB programmes should transition to replacing microscopy as the initial diagnostic test with molecular WRDs that allow for the detection of MTBC.

This algorithm is designed to be used with each of the WHO-approved rapid molecular tests for the detection of MTBC, although the algorithm may require minor modification based on which molecular WRD is used and in which population.

- The Xpert MTB/RIF and Xpert Ultra tests are recommended for use with adults and children being evaluated for pulmonary TB; these tests simultaneously detect MTBC and RIF resistance.
  - The Xpert MTB test is also 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.
  - Minor variations for use of the Xpert MTB 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 pre-test probability of 5% or more and an Xpert MTB negative result on the initial test, repeated testing with Xpert MTB 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 tests are recommended for use in testing adults and children with signs and symptoms of pulmonary TB. These tests only detect MTBC. A second test (Truenat MTB-RIF Dx test) on DNA isolated for the Truenat MTB or MTB Plus test is conducted to assess RIF

resistance (this test uses the same instrument and takes about 1 hour; see Fig. 3.1). Considerations for these tests 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 smear-negative patients 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, and 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).

The Xpert Ultra test has two significant differences compared with the other molecular WRDs that have been incorporated into Algorithm 1: first, Xpert Ultra has an additional semi-quantitative result of "MTB detected trace"; and second, the evaluation of a result of "MTB detected, RIF-resistance indeterminate" differs between Xpert Ultra and other molecular WRDs. These differences are incorporated in Algorithm 1.

- The Xpert Ultra test has a higher sensitivity than the other molecular WRDs for the detection of MTBC. However, the higher sensitivity of Xpert Ultra is accompanied by a slight loss of specificity (i.e. an increase in the number of patients incorrectly identified as having active TB). This is because the Xpert Ultra assay can detect very small numbers of non-viable or non-replicating bacilli, particularly in patients with a history of TB treatment (i.e. completed within the past 5 years). Such non-viable bacteria may also be detected by the other molecular WRDs, albeit less frequently. Because the increased sensitivity and loss of specificity are primarily related to the Xpert Ultra "trace" call, the algorithm and decision pathway includes criteria for interpreting the "MTB detected trace" results, to balance the potential harms of overtreating patients with a false-positive result with the potential benefits of increased numbers of correctly diagnosed TB patients and decreased mortality associated with TB.
- The Xpert Ultra test uses a melting temperature (Tm)-based analysis to detect RIF resistance, instead of the real-time PCR used by the other molecular WRDs. This change enables the Xpert Ultra test to better differentiate between silent and resistance-conferring mutations, and improve the accuracy of determining RIF resistance. The Xpert Ultra assay software reports a result as "RIF indeterminate" when one or more of the *rpoB* probes do not produce any measurable Tm peak *(25)*. This may happen when there are large deletions or multiple mutations in the RIF resistance-determining region (RRDR). Detailed analysis of the melting curves generated by the Xpert Ultra test can identify such mutations, and thus can detect RIF resistance without requiring the additional testing that is needed for RIF indeterminate results obtained with other molecular WRDs.

Molecular WRDs 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 molecular WRD 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 patient routinely, instead of only collecting a second specimen when additional testing is needed. If so, the first specimen should be tested promptly using the molecular WRD test. The second specimen

may be used for the additional testing described in the algorithm (e.g. repeat molecular WRD testing), or for smear microscopy or culture as a baseline for treatment monitoring.

- If more than one specimen cannot 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 using the molecular WRD test. If additional TB testing is warranted, an option is to consider using any portions of the sample remaining after the molecular WRD for other tests (e.g. culture, histology, LPA and DST). Clinical decisions should be made based on clinical judgement and the results of available laboratory tests.

With respect to the detection of MTBC, the Xpert MTB/RIF, Xpert Ultra and Truenat MTB tests report "MTB not detected", "MTB detected (high, medium, low or very low)", "no result", "error" or "invalid". The Xpert Ultra test has an additional semi-quantitative category of "trace". The TB-LAMP test only reports "MTB detected" or "MTB not detected".

- Each of the semi-quantitative categories of MTB detected, including "trace", is considered as bacteriological confirmation of TB.
- In HIV-negative, symptomatic adult patients with a recent history of TB treatment (i.e. completed less than 5 years ago), Xpert Ultra "trace" results (and occasionally other molecular WRD "MTB 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 judgment.

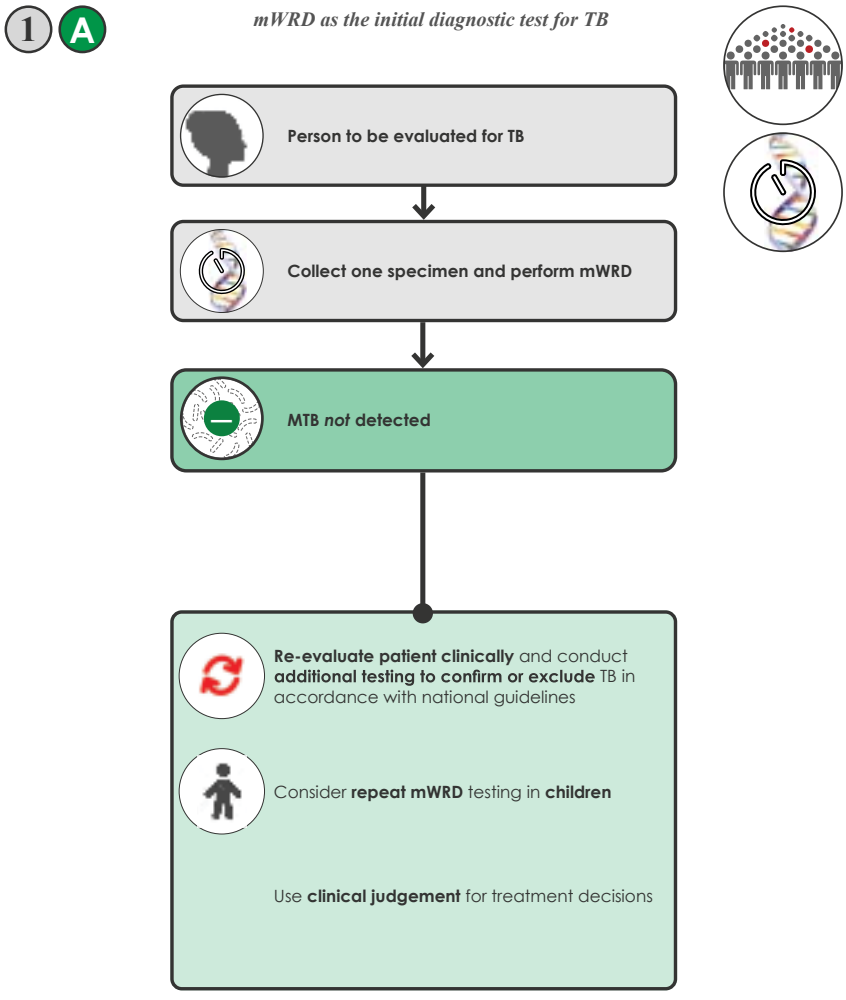
With respect to the detection of RIF resistance, the Xpert MTB/RIF, Xpert Ultra and Truenat MTB-RIF Dx tests report the results as "RIF resistance detected", "not detected" or "indeterminate". These assays infer RIF resistance by the absence of binding the amplicons to wild-type sequences. The use of Tm-based analysis, instead of real-time PCR analysis, in the Xpert Ultra and Truenat MTB/RIF tests improves the reliability of the detection of resistance-conferring mutations.

The use of a molecular WRD to detect RIF resistance does not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.

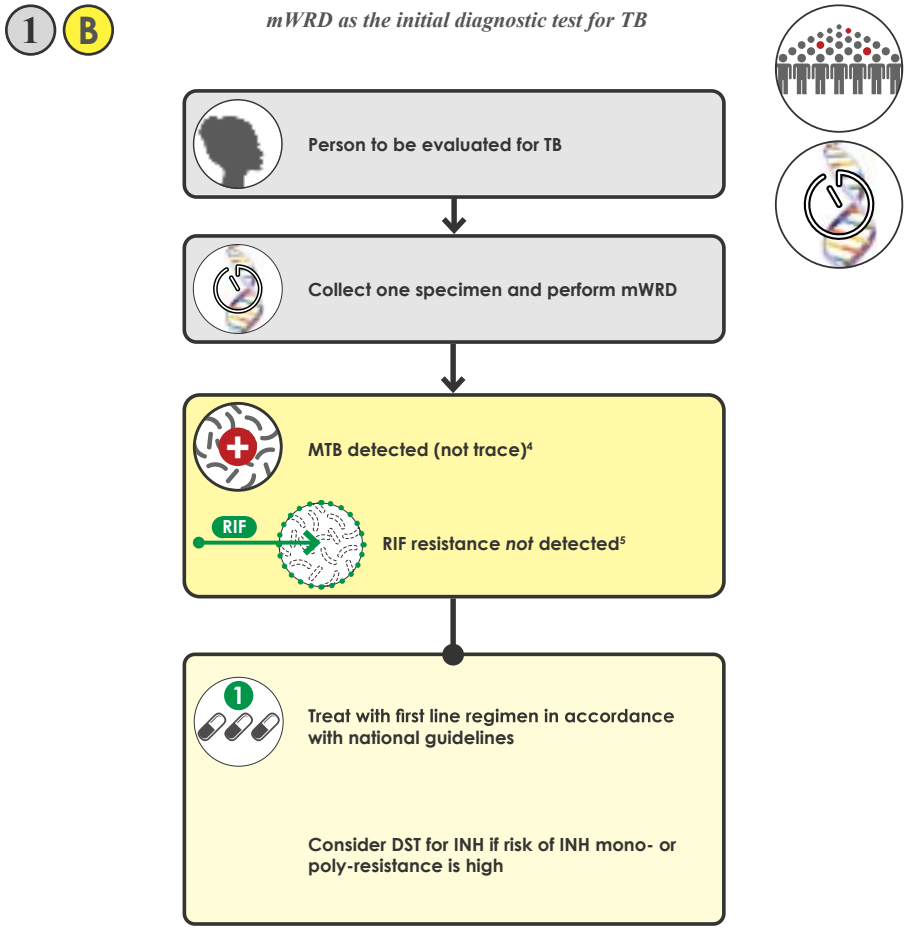
Decision pathway

1. Collect a good-quality specimen and transport it to the testing laboratory. Conduct the molecular WRD. 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 WRD, see section 2.2 above or individual WHO policy statements.)

2. If the molecular WRD test result is "MTB not detected" (1)(A), re-evaluate the patient and conduct additional testing in accordance with national guidelines.
    a. Further investigations for TB may include chest X-ray, additional clinical assessments, additional molecular WRD testing or culture, and, finally, clinical response following treatment with broad-spectrum antimicrobial agents (FQs should not be used).
        i. In children with signs and symptoms of pulmonary TB in settings with a pre-test probability exceeding 5% (but not in settings with pre-test probability below 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).
        ii. The performance of the other molecular WRDs in repeat testing is not known.
    b. Consider the possibility of clinically defined TB (i.e. TB without bacteriological confirmation). Use clinical judgement for treatment decisions.



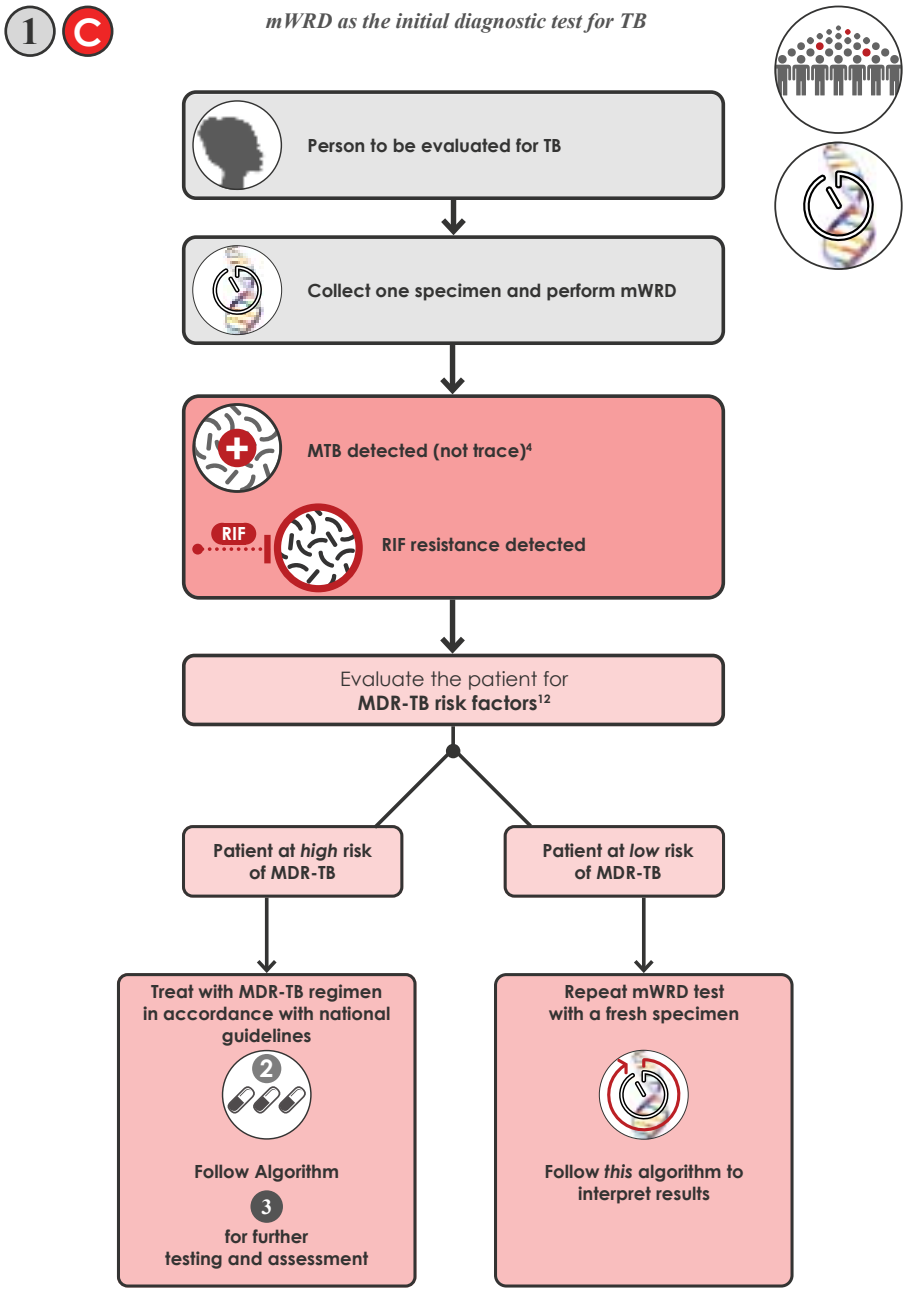
3. If the molecular WRD test result is "MTB detected, RIF resistance not detected" (1)(B):
   a. Initiate the patient on an appropriate regimen using first-line TB drugs in accordance with national guidelines.
   b. Request additional DST in the following cases:
      i. Molecular or phenotypic DST for INH is indicated particularly:
         1. if the patient has been treated with INH or is a contact of a known Hr-TB patient; or
         2. if there is high prevalence of INH resistance that is not associated with RIF resistance (i.e. INH mono-resistance or poly-resistance, not MDR-TB) in this setting. (See Algorithm 4 for follow-up testing.)
      i. Molecular or phenotypic DST for resistance to RIF may be requested if the patient is considered to be at risk of having RR-TB despite the initial molecular WRD result. False RIF-susceptible Xpert MTB results are rare but have been observed in 1–5% of TB cases tested in various epidemiologic settings. In contrast, phenotypic DST for RIF, especially using liquid culture, is associated with a higher proportion of false-susceptible results *(26)*.
   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 testing, because of technical issues related to producing an appropriate inoculum and loss to contamination.
      iii. A rapid molecular test is preferred. Currently, FL-LPA is the only WHO-approved rapid molecular test for INH resistance. FL-LPA can identify *inhA* and *katG* mutations, which can guide clinicians on the composition of INH-resistant TB regimen (see Algorithm 3). Guidance



on interpreting LPA results may be found in the relevant GLI guide *(27)*. DNA sequencing has proven useful in many cases but WHO has not yet evaluated it.

iv. Culture-based phenotypic DST for INH and RIF requires 3–8 weeks to produce a result. Phenotypic DST may be useful for evaluating patients with a negative FL-LPA result, particularly in populations with a high pre-test probability for resistance to INH.

4. If the molecular WRD test result is "MTB detected, RIF resistance detected" 1C, an MDR-TB risk assessment is needed. Patients at high risk for MDR-TB include previously treated patients, such as those who had been lost to follow-up, relapsed or failed a treatment regimen; non-converters (e.g. smear positive at end of intensive phase of treatment for drug-susceptible TB); contacts of MDR-TB patients; and any other MDR-TB risk groups identified in the country. In high MDR-TB burden countries, every TB patient is considered to be at high risk of having MDR-TB.

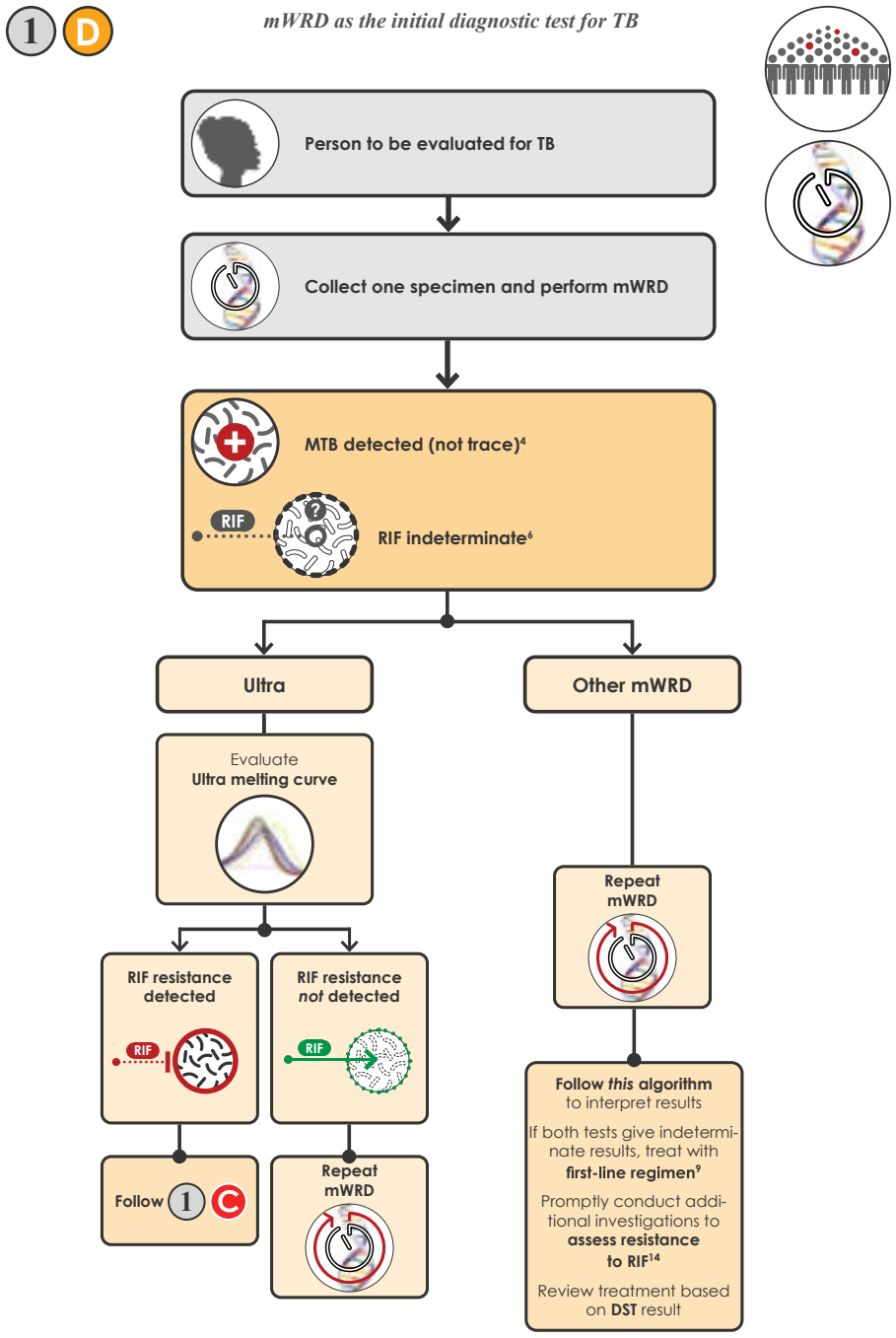


a. If the patient is at high risk of having MDR-TB and the RIF-resistant test result is definitive, initiate the patient on a regimen for RR-TB or MDR-TB in accordance with national guidelines *(3, 4)*. Follow Algorithm 3 for additional testing.
b. If the patient is at low risk of having MDR-TB, repeat the molecular WRD test with a second sample. If FL-LPA is available at the site and the sputum specimen is smear positive, FL-LPA can be used for confirming the RIF-resistant result.
    i. If the second test also indicates RIF resistance, initiate an MDR-TB regimen in accordance with national guidelines and WHO recommendations *(26, 27)*, and follow Algorithm 3 for additional testing.
    ii. If the molecular WRD result for the second sample is "MTB 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, false-positive RIF-resistant results due to laboratory or clerical errors may occur. It is assumed that the repeat test will be performed with more caution, that the result of the second test is correct, and that the result of the first test may have been due to a laboratory or clerical error.
c. For all patients with RR-TB or MDR-TB, conduct additional investigations to assess resistance to the drugs being used in the treatment regimen. 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 are preferred.
    i. For MDR-TB regimens that rely on the use of FQs, submit a sample for molecular testing for FQ resistance (see Algorithm 3).
    ii. Ideally, a specimen from each patient 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 to 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.

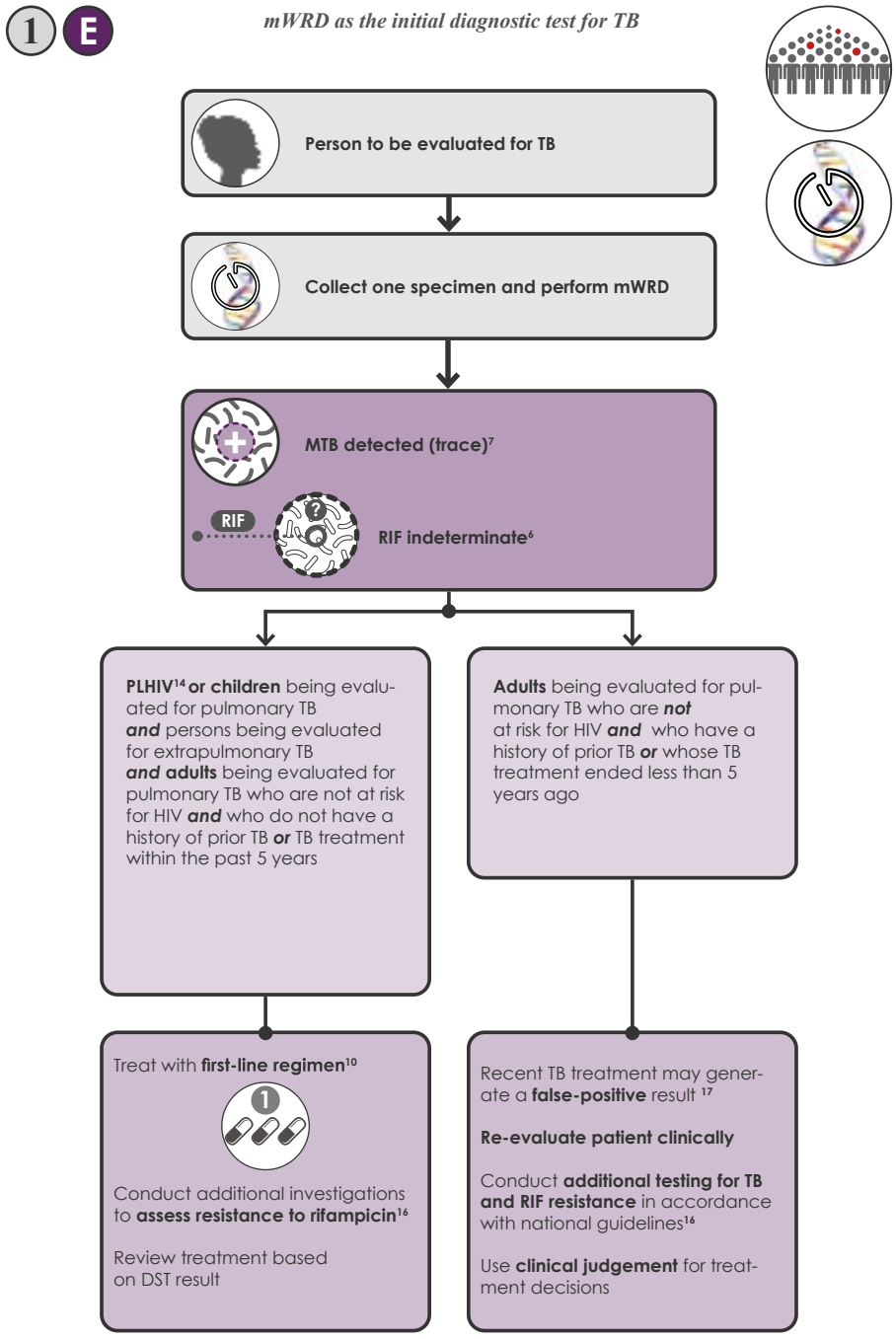
5. If the molecular WRD gives a result of "MTB detected, RIF indeterminate" (1)(D), the interpretation and follow-up testing for Xpert Ultra differs from that for other molecular WRD tests (e.g. Xpert MTB/RIF or Truenat MTB-RIF Dx test). With any of the molecular WRD tests, the initial result of "MTB detected" should be considered as bacteriological confirmation of TB. The patient should be initiated on an appropriate regimen using first-line TB drugs in accordance with national guidelines, unless the patient is at high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. In most settings, a history of prior TB treatment is not sufficient to indicate that the patient is at high risk of having MDR-TB for the purpose of making treatment decisions.
    a. For most molecular WRD assays, an "MTB detected, RIF resistance indeterminate" result is generally caused by a paucibacillary TB load in the sample; in such cases, retesting a fresh specimen at the same testing site is useful.
        i. If the result of the second molecular WRD test is "MTB detected, RIF resistance not detected", follow Step 3. If the result is "MTB detected, RIF-resistance detected", follow Step 4.
        ii. In some cases, testing a second sample, which might also contain very few bacteria, may generate a result of "MTB detected, RIF indeterminate" or "MTB not detected". In these situations, additional investigations such as culture and phenotypic DST may be needed to confirm or exclude resistance to RIF, because the indeterminate result provides no information on resistance.
    b. "MTB 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.
        i. The Ultra melt curves from "MTB 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.

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 Step 4.
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 5a for additional testing.
3. If the semiquantitative result is high or medium, FL-LPA or DNA sequencing may be useful.

c. Culture and DST or FL-LPA may be performed for follow-up testing to confirm or exclude RIF resistance.



6. If the Xpert Ultra test result is "MTB detected trace" (1)(E), additional evaluations 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 evaluation 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, in settings where there is a high prevalence of HIV or among members of a risk group 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 molecular



WRD test result is highest immediately after completing treatment, and slowly declines with time *(7)*. 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 patients 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 care workers must endeavour to obtain a reliable history of TB treatment, recognizing that some patients may not communicate their treatment history because of stigma or concern over legal status for migrants.

b. For 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 MTB 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 patient on an appropriate regimen using first-line TB drugs in accordance with national guidelines, unless the patient is at very high risk of having MDR-TB. Initiate such patients on an MDR-TB regimen.

iii. Undertake additional investigations (e.g. culture and phenotypic 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.

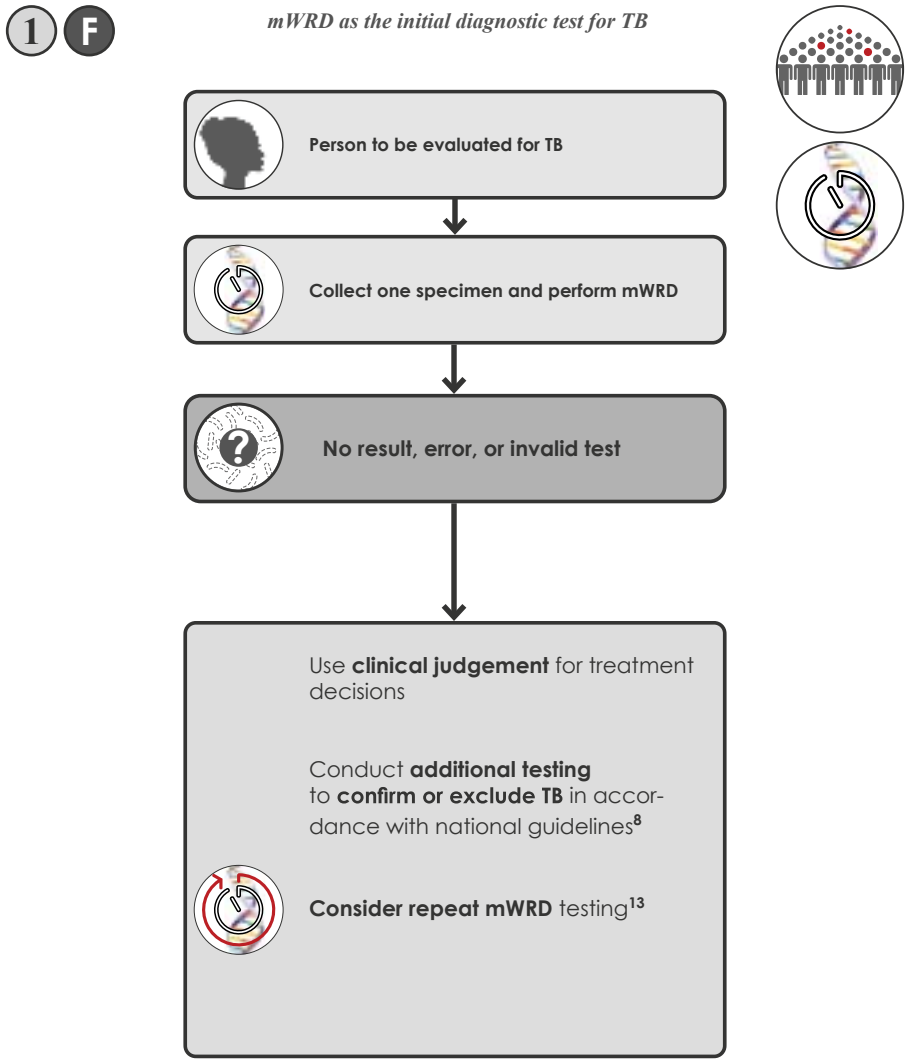
ii. Clinically re-evaluate the patient and conduct additional testing in accordance with national guidelines. Consider the possibility of TB caused by reactivation, relapse or reinfection.

iii. In initiating treatment, consider the clinical presentation and context of the patient. Make clinical decisions based on all available information and clinical judgment.

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 Guideline Development Group recommended against repeat Xpert Ultra testing for individuals with an initial Xpert Ultra trace result for the detection of MTBC.
2. Culture and DST may be of benefit to detect TB and drug resistance. The trace result provides no information on RIF resistance.

7. If the Xpert MTB test does not give a result (1)(F), or gives a result of error or invalid, repeat the Xpert MTB test at the same testing site with a second specimen. If FL-LPA is available at the site and the second specimen is smear positive, FL-LPA can be used for the repeat testing (although repeat Xpert MTB testing is preferred because it has a lower LOD than the FL-LPA).



Interpretation of discordant results

This algorithm relies on testing of a sample with the molecular WRD test to detect MTBC and assess susceptibility to RIF. 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. Molecular WRD result "MTB detected other than trace", culture negative (see Point 5 for trace).
    a. The molecular WRD result and clinical judgement should have been used to guide the treatment decision, pending additional testing.
    b. The molecular WRD 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 patient 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 patient 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. Molecular WRD result "MTB not detected", culture positive.
    a. Treatment decision should be based on the culture result. If the patient started treatment based on clinical judgement, continue treatment. Record the patient 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, WRD tests have a pooled sensitivity of 83% to 90% for detecting pulmonary TB compared to culture *(28)*. 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 patient for TB, conducting additional testing using WRD test; culturing additional samples, and evaluating the possibility of laboratory or clerical error. If the patient was initiated on anti-TB therapy based on clinical judgement, evaluate the response to therapy.
3. Molecular WRD result "MTB detected, RIF resistance detected"; RIF susceptible by phenotypic DST.
    a. Use the molecular WRD result to guide treatment decisions pending additional testing.
    b. Certain mutations are known to generate this discordant result, particularly in the BACTEC™ mycobacterial growth indicator tube (MGIT™) system (i.e. a false-susceptible phenotypic result). Patients infected with strains carrying these mutations often fail treatment with RIF-based first-line regimens *(26)*.
    c. In some low MDR-TB prevalence settings, silent mutations have been observed that generate a false-resistant WRD result, but these are very rare.
    d. False RIF-resistant results have been observed with the Xpert MTB/RIF G4 cartridge when the MTB detected result was "very low". Follow-up action may include WRD testing of the culture.
    e. Follow-up actions may include DNA sequencing, FL-LPA, phenotypic DST using solid media and evaluation of the possibility of laboratory or clerical error.
4. Molecular WRD result "MTB 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 molecular WRD results are rare, but have been observed in 1–5% of 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".

c. Follow-up actions may include DNA sequencing, repeating the phenotypic DST and evaluating the possibility of laboratory or clerical error.

5. Xpert Ultra "MTB detected trace", culture negative.

The interpretation of this result must consider patient characteristics, specimen type and whether the person had been previously treated for TB:

- Cultures may be negative for several reasons, including the patient 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 very small numbers of bacilli in a sample that generates an "MTB 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 "MTB 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, "MTB 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 "MTB detected trace" result is considered as bacteriological confirmation of TB (i.e. true positive results) and such patients 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 "MTB 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 have been 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 patients placed on anti-TB therapy may include re-evaluating the patient 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 "MTB 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 such patients 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.

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

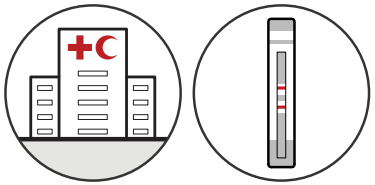
Algorithm 2 is the preferred algorithm for testing to support the diagnosis of TB in PLHIV. It is appropriate for use in settings with a high burden of HIV and for use with individual patients living with HIV who meet the testing criteria, regardless of the overall HIV burden. The algorithm emphasizes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

Currently available urinary LF-LAM assays 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 urinary 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 *(11)*. 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, and 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 burden settings. The choice of which molecular test to use may be different in a low or high MDR-TB burden setting. For example, in a setting with a high burden of MDR-TB, it would be preferable to use a molecular WRD that detects MTBC and RIF resistance simultaneously (e.g. Xpert MTB/RIF), rather than a molecular WRD that uses a two-step process to detect RIF resistance.

Algorithms 2a and 2b

2a *LF-LAM testing to aid in the diagnosis of TB among PLHIV in inpatient settings*



All hospitalized patients, including adults, adolescents, and children living with HIV[1]

Assess patient for TB signs and symptoms, being seriously ill[2], having AHD[3] and CD4 count

A Positive for TB signs and symptoms

Collect a urine sample & perform urine LF-LAM[4]
Collect a sample & perform mWRD test

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 (+)

Initiate TB treatment based on mWRD

mWRD (−)

Clinical Management

TB is not ruled out[8]

Conduct additional evaluations for TB[5]

B No TB signs or symptoms *and* AHD+ or seriously ill or CD4 < 200

Collect a urine sample & perform urine LF-LAM

LF-LAM (−)

evaluate CD4 Count

CD4 <200

Clinical Management

TB is not ruled out[8]

Conduct additional evaluations for TB[5]

CD4 >200

Apply AHD package of care

LF-LAM (+)

Initiate TB treatment
Collect specimen & perform mWRD test

mWRD (+)

Adjust treatment based on WRD results if needed

mWRD (−)

Continue TB treatment

Perform workup to exclude DR-TB[7]

C No TB signs or symptoms and CD4 > 200 or unknown

Clinical management

AHD: advanced HIV disease; AIDS: acquired immunodeficiency syndrome; DR-TB: drug-resistant tuberculosis; DST: drug-susceptibility testing; HIV: human immunodeficiency virus; LF-LAM: lateral flow lipoarabinomannan assay; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic; PLHIV: people living with HIV/AIDS; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic.

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

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

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

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

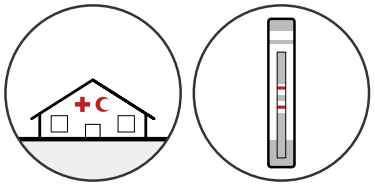
[5] Patients should be initiated on a first-line regimen according to national guidelines, unless they are at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen.

[6] Negative LF-LAM results do not rule out TB in symptomatic persons. The mWRD test result should be evaluated when it becomes available for treatment decisions. See Algorithm 1 for interpretation of molecular WRD results.

[7] 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-RIF Dx tests) are preferred.

[8] Negative Xpert and LF-LAM results do not rule out TB in symptomatic persons. Conduct additional clinical evaluations for TB. Further investigations for TB may include chest X-ray, additional clinical assessments, clinical response following treatment with broad-spectrum antimicrobial agents, and additional WRD testing or culture. Consider initiating treatment for bacterial infections using antibiotics with broad-spectrum antibacterial activity (FQs should not be used) and for *Pneumocystis* pneumonia. The clinical response should be evaluated after 3–5 days of treatment.

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 who are ART naive
2. HIV patients returning for care following a treatment interruption
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] and low CD4 count

A Positive for TB signs and symptoms *and/or* seriously ill

Collect a urine sample & perform urine LF-LAM[4]
Collect a sample & perform mWRD test

- 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 (+) → Initiate TB treatment based on mWRD results
  - mWRD (−) → Clinical Management. TB is not ruled out[8]. Conduct additional evaluations for TB[6]

B No TB signs or symptoms *and not* seriously ill

CD4 assessment

- CD4 <100 *or* Stage 3 or 4 → perform urine LF-LAM
  - LF-LAM (+) → Initiate TB treatment[5]. Conduct mWRD test and perform workup to exclude DR-TB[7]
  - LF-LAM (−) → Apply AHD package of care
- CD4 100 - 200 → Do Not Perform LF-LAM → Apply AHD package of care
- CD4 >200 *or* unknown → Do Not Perform LF-LAM → Clinical management

C Without assessing symptoms → Do Not Perform LF-LAM → Clinical management

AHD: advanced HIV disease; ART: antiretroviral therapy; DR-TB: drug-resistant tuberculosis; DNA: deoxyribonucleic acid; DST: drug-susceptibility testing; HIV: human immunodeficiency virus; LF-LAM: lateral flow lipoarabinomannan assay; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic; PLHIV: people living with HIV/AIDS; RIF: rifampicin TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic.

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

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

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

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

[5] Patients should be initiated on a first-line regimen according to national guidelines, unless the patient is at very high risk of having MDR-TB. Such patients should be initiated on an MDR-TB regimen. Treatment regimens should be modified as needed based on the results of the mWRD testing.

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

[7] 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-RIF Dx tests) are preferred. Results of the WRD test should be interpreted as shown in this figure.

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

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

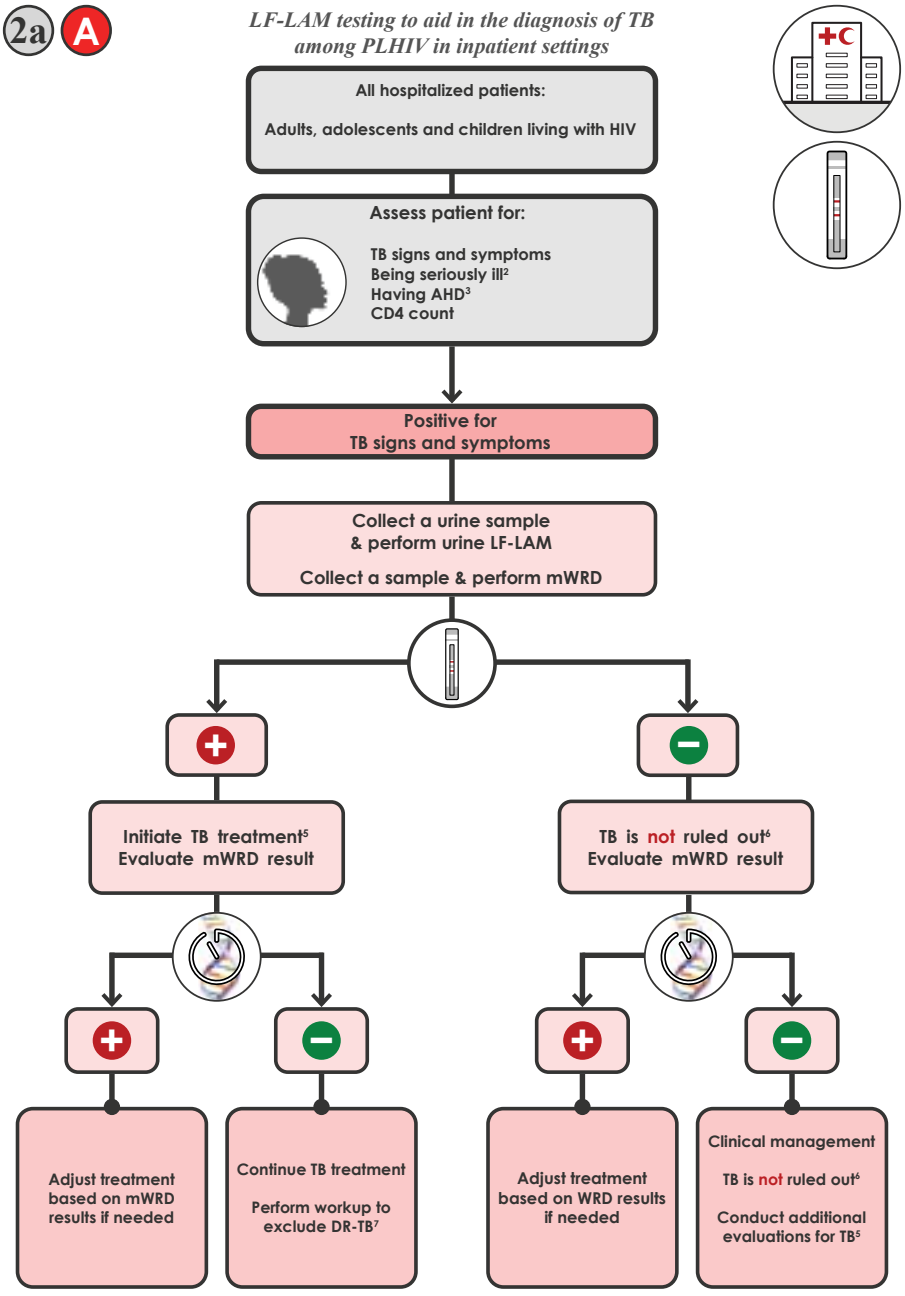
- The LF-LAM assay is a point-of-care test that may be implemented outside the laboratory (e.g. at the bedside in clinics that see critically ill PLHIV), for rapid diagnosis of TB and treatment initiation.
- The algorithms may be used for individuals being evaluated for pulmonary or extrapulmonary TB.
- The algorithms are appropriate for all individuals living with HIV 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 burden settings. The choice of which molecular test to use may be different in a low or high burden MDR-TB setting.
- The algorithms emphasize the use of the urinary LF-LAM test as the add-on diagnostic test to molecular WRD 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
  - in outpatient settings, for HIV-positive adults, adolescents and children who are seriously ill or PLHIV with a CD4 cell count of less than 100 cells/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 persons, because of suboptimal sensitivity and specificity in HIV-negative persons.
- All patients with signs and symptoms of pulmonary TB who are capable of producing sputum should have at least one specimen submitted for molecular WRD 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 patient 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 assay.
   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 patients who have advanced HIV disease (AHD), are seriously ill or have CD4 counts of less than 200/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, in settings where there is a high prevalence of HIV or among members of a risk group 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 antiretroviral therapy (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, who are positive for signs and symptoms of TB 2a A:
    a. Collect a urine specimen and conduct the LF-LAM assay **and** collect a specimen and conduct molecular WRD testing. If the molecular WRD test is available on site, perform the molecular WRD testing in parallel to the LF-LAM testing.
        i. For individuals being evaluated for pulmonary TB, the following samples may be used for the molecular WRD test: induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage or aspirate, nasopharyngeal aspirate and stool samples.

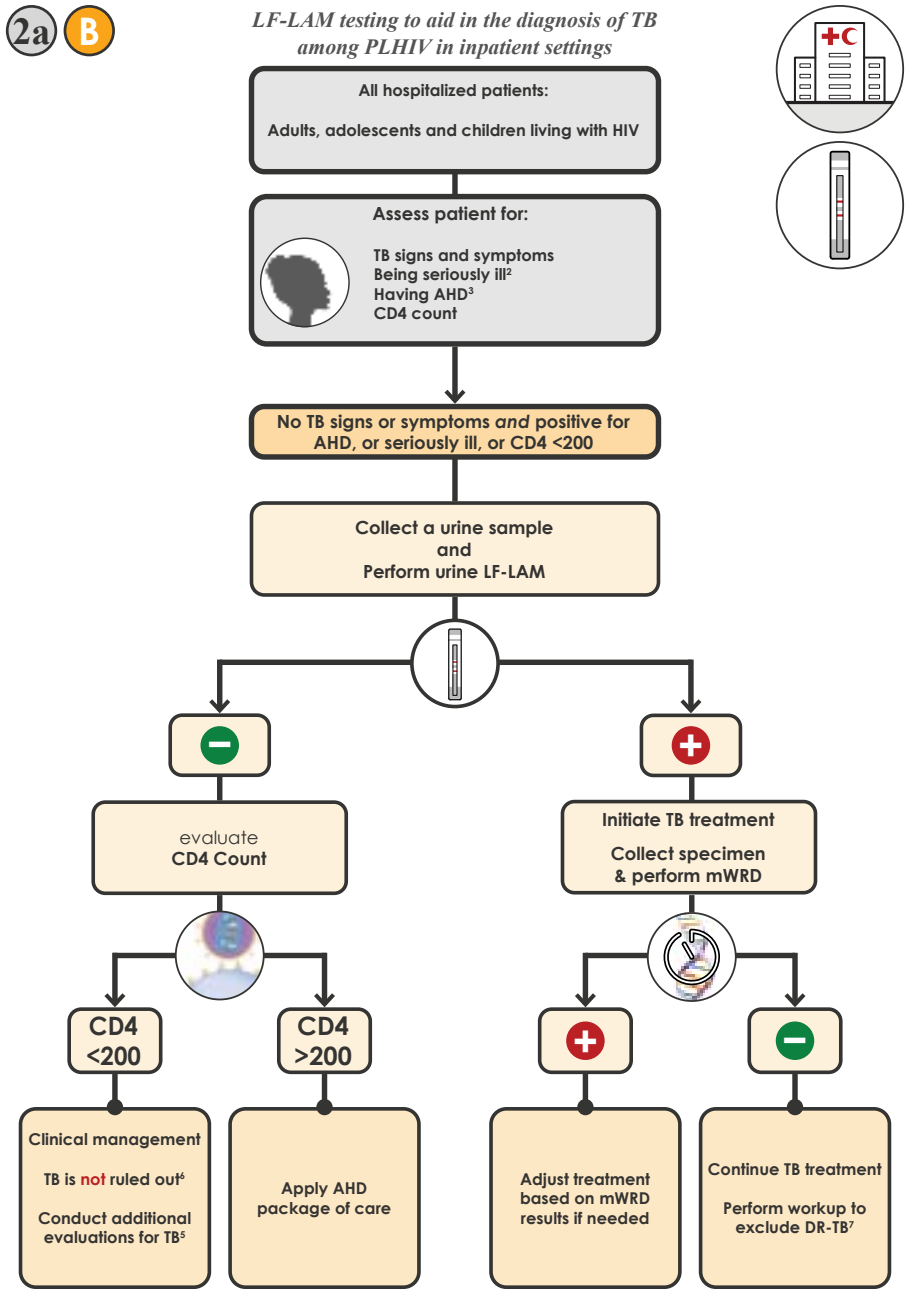
2a A



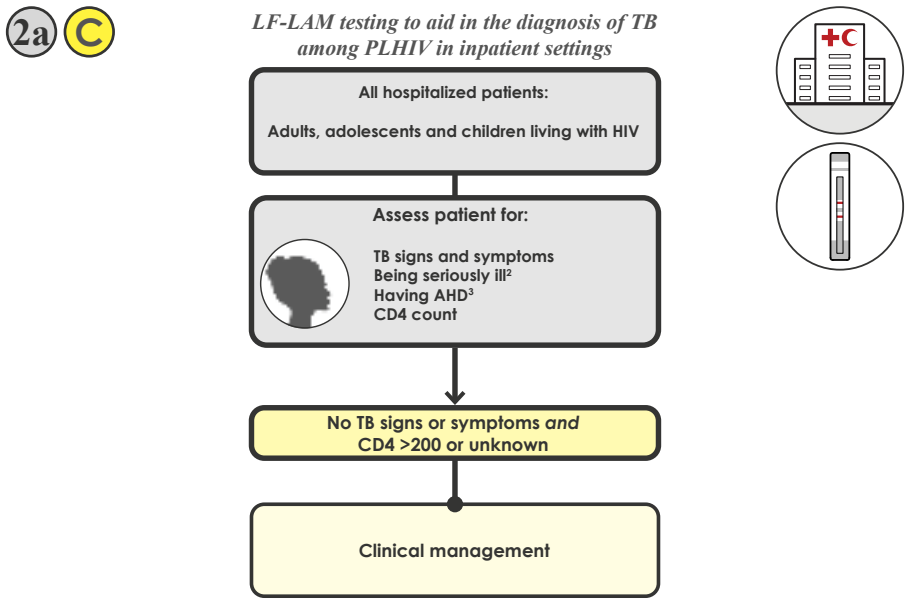
ii. For individuals being evaluated for extrapulmonary TB, the Xpert MTB test is recommended for use with CSF (preferred sample for TB meningitis), lymph node aspirates and lymph node biopsies. In addition, Xpert MTB/RIF is recommended for pleural fluid, peritoneal fluid, pericardial fluid, synovial 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.

b. The LF-LAM result (test time <15 minutes) is likely to be available before the molecular WRD test result, and should be interpreted in the context of clinical judgment, chest X-ray findings (if available) and any available bacteriological results.
c. All patients meeting the testing requirements who have a positive LF-LAM result should be initiated on TB treatment immediately, while awaiting results of the molecular WRD test. Follow Algorithm 1 for the interpretation of molecular WRD results, and modify therapy as needed. Additional studies to assess drug resistance may be needed. Phenotypic (culture and DST) and molecular (e.g. LPAs, DNA sequencing and high-throughput assays) methods are also available to evaluate drug resistance. Rapid molecular methods are preferred.
d. TB is not ruled out if the LF-LAM test result is negative. Evaluate the results of the molecular WRD test, and follow Algorithm 1 for interpretation of results and follow-up testing.
e. Treat all patients with a molecular WRD test result of "MTB detected" for TB (see Algorithm 1), regardless of LF-LAM result.
f. TB is not ruled out if both the LF-LAM result and molecular WRD test results are negative (or if no molecular WRD test is performed). Re-evaluate the patient 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 patient 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 patient 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 patients 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 assay.
   b. If the LF-LAM is negative and the CD4 is <200 cells/mm^3, re-evaluate the patient and conduct additional testing in accordance with national guidelines (see Step 2f).
   c. If the LF-LAM is negative and the CD4 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 judgment. Collect a specimen and conduct a WRD test to assess the possibility of rifampicin resistance.
      i. If the molecular WRD result is "MTB detected", follow Algorithm 1 for interpretation, testing and treatment recommendations.
      ii. If the molecular WRD result is "MTB not detected", treat the patient for TB and conduct additional laboratory testing (e.g. culture and DST) to assess drug resistance.



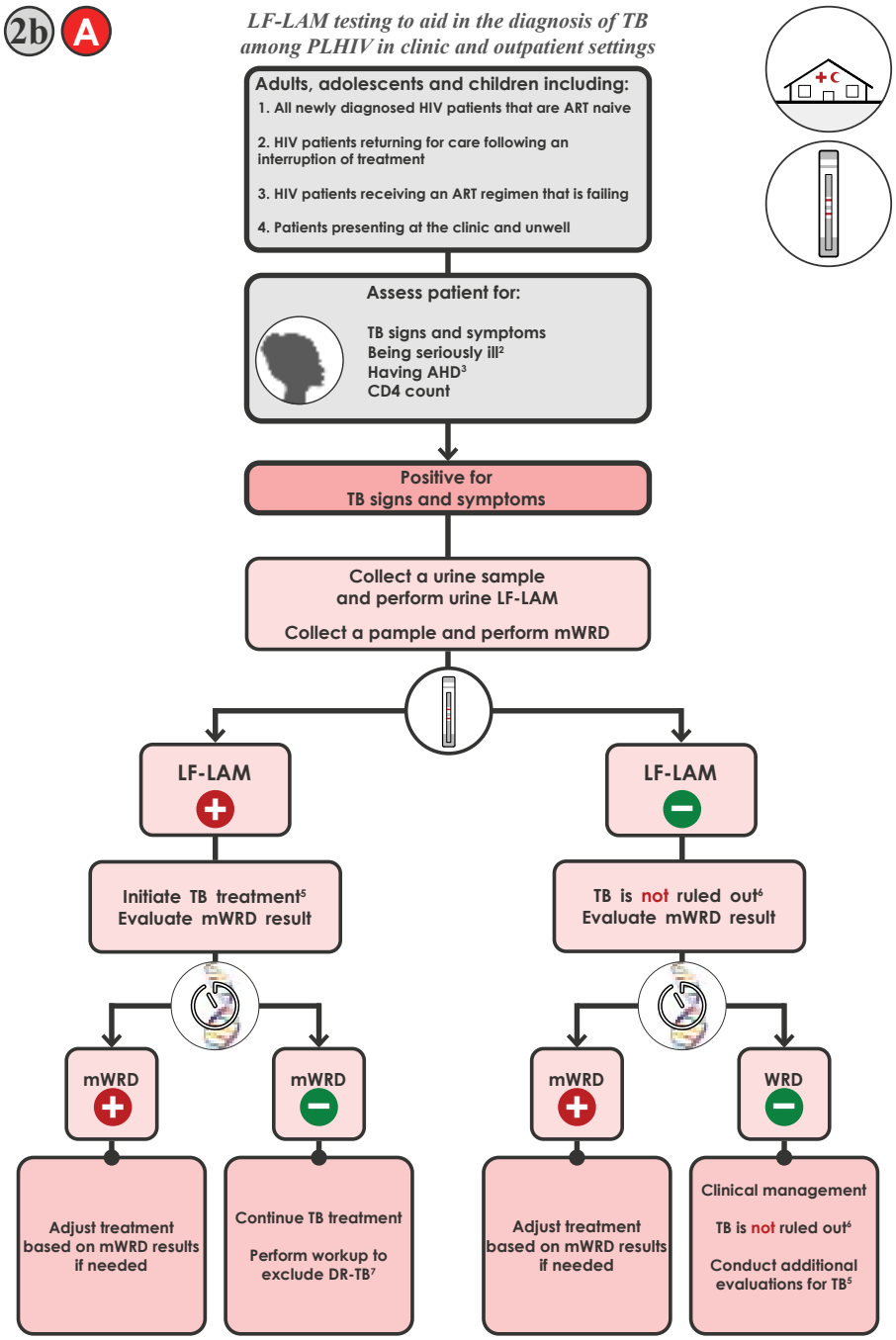
4. For hospitalized PLHIV without signs or symptoms of TB and whose CD4 is 200 cells/mm^3 or above (or is unknown) 2a C, do not conduct an LF-LAM test.



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

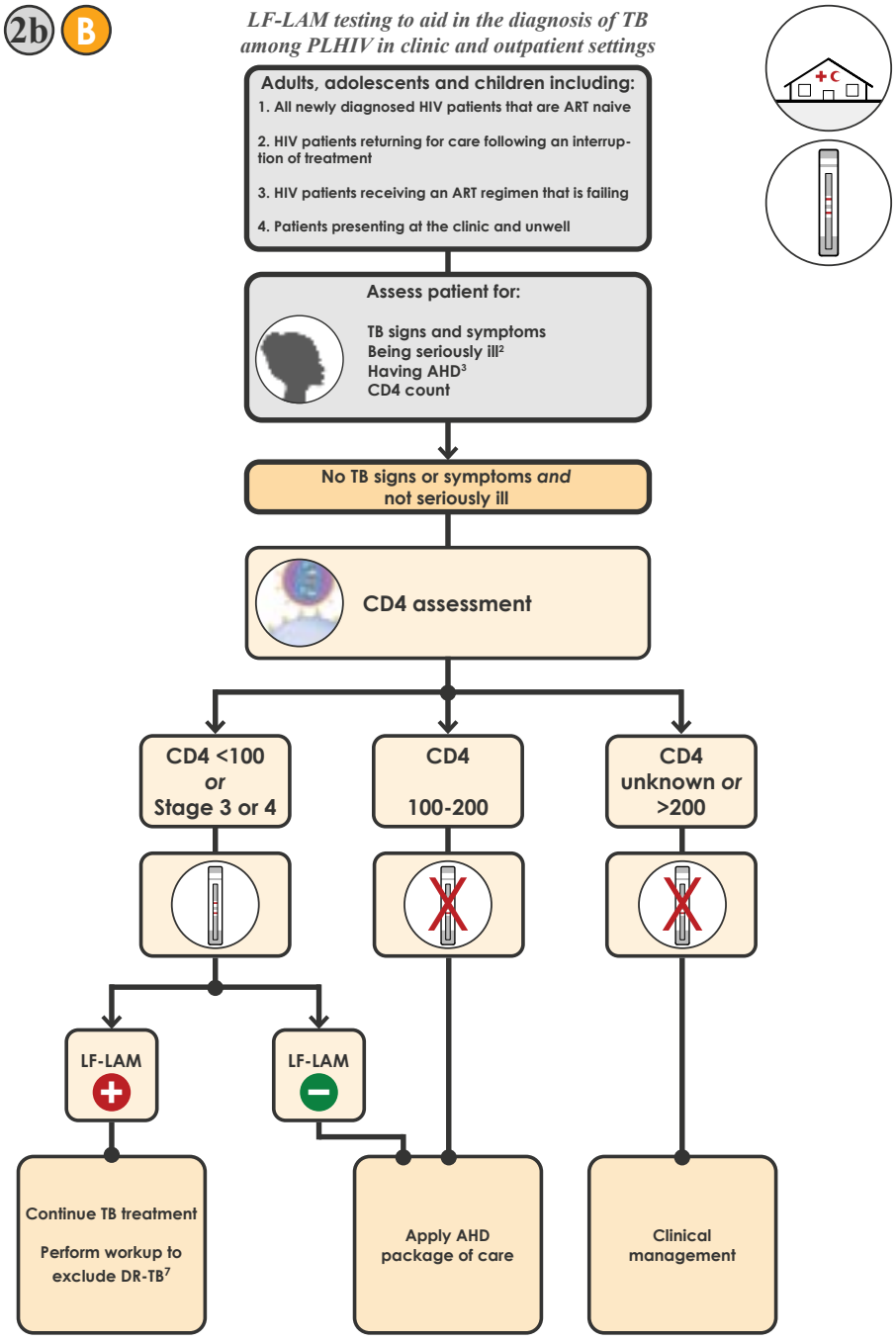
1. Evaluate the patient for TB, determine HIV status, and assess the presence of AHD and danger signs for being seriously ill. In PLHIV who are not seriously ill, also consider measuring CD4 cell counts, to assess eligibility for testing with the LF-LAM assay:
   a. Individuals to be evaluated for TB include HIV-positive adults, adolescents and children, including: all newly diagnosed HIV patients who are ART naive, HIV patients returning for care following an interruption of treatment, HIV patients receiving an ART regimen that is failing, and patients 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, in settings where there is a high prevalence of HIV or among members of a risk group 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 assay **and** collect a specimen and conduct molecular WRD testing. If the molecular WRD test is available on site, do the molecular WRD testing in parallel to the LF-LAM testing.
        i. For individuals being evaluated for pulmonary TB, the following samples may be used for the molecular WRD test: induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage or aspirate, nasopharyngeal aspirate and 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.



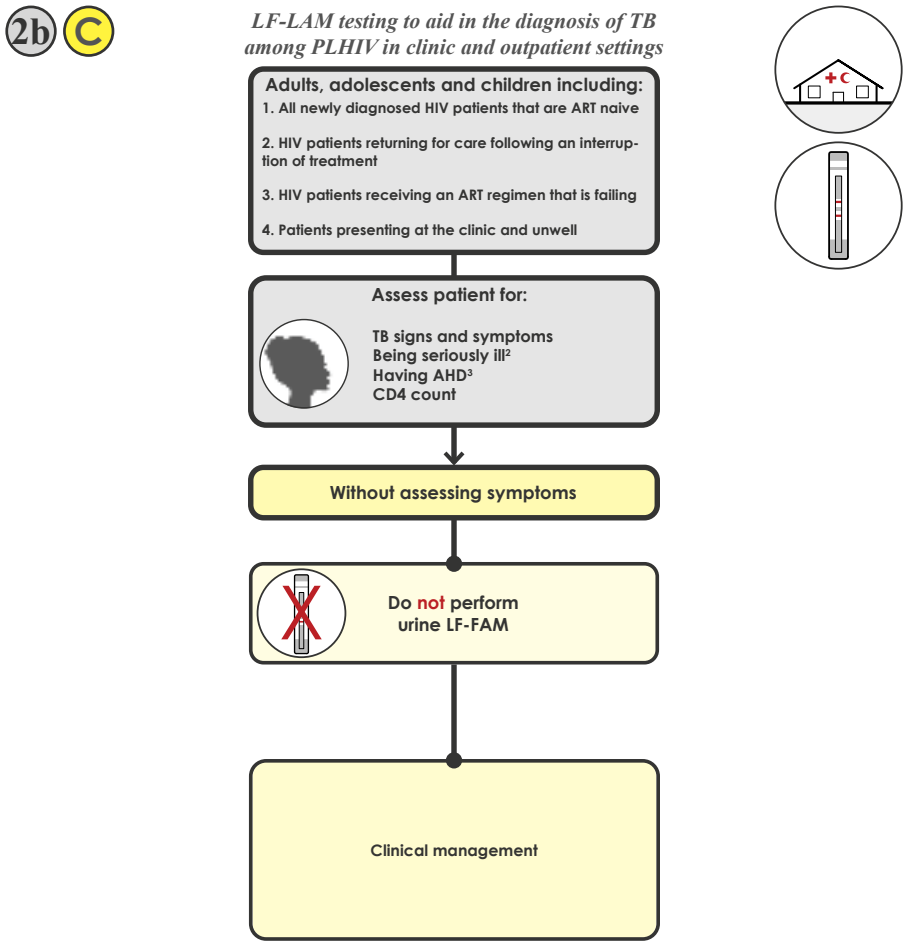
b. The LF-LAM result (test time <15 minutes) is likely to be available before the molecular WRD test result, and it should be interpreted in the context of clinical judgment, chest X-ray findings (if available) and any available bacteriological results.
c. All patients meeting the testing requirements who have a positive LF-LAM result should be initiated on TB treatment immediately, while awaiting results of the molecular WRD test. Follow Algorithm 1 for interpretation of molecular WRD results, and modify therapy as needed. Additional studies to assess drug resistance may be needed. Phenotypic (culture and DST) and molecular (e.g. LPAs, DNA sequencing and high-throughput assays) methods are also available to evaluate drug resistance. Rapid molecular methods are preferred.
d. TB is not ruled out if the LF-LAM test result is negative. Evaluate the results of the molecular WRD test, and follow Algorithm 1 for result interpretation and follow-up testing.
e. Treat all patients with a molecular WRD test result of "MTB detected" for TB (see Algorithm 1), regardless of LF-LAM result.
f. TB is not ruled out if both the LF-LAM and molecular WRD test results are negative (or if no molecular WRD test is performed). Re-evaluate the patient 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 patient 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 patient has TB and a bacterial infection, i.e. clinical improvement may not rule out TB.
        2. If there is high clinical suspicion of TB in the patient (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 patients 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 the patient has AHD 2b B.
   a. If the CD4 is <100 cells/mm^3, or the patient presents with a WHO stage 3 or 4 event, collect a urine specimen and perform an LF-LAM assay.
      i. If the LF-LAM test is positive, initiate TB treatment immediately. Conduct additional studies to assess drug resistance. Rapid molecular methods (e.g. Xpert MTB or Truenat MTB-RIF Dx tests) are preferred (see Algorithm 1). Phenotypic (culture and DST) and molecular (e.g. LPAs, DNA sequencing and high-throughput assays) methods are also available to evaluate drug resistance.
      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 assay; apply an AHD package of care.
   c. If the CD4 is >200 cells/mm^3 or unknown, DO NOT perform an LF-LAM assay; clinically manage the patient.



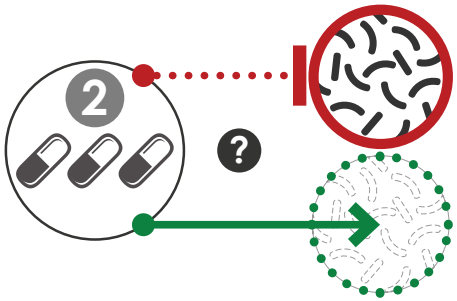
Considerations when using the LF-LAM test are as follows:

- 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 assay does not eliminate the need for other diagnostic tests for TB, such as Xpert MTB, other molecular WRD or culture tests. 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 molecular WRD, or bacteriological culture and DST.
- Published studies reveal that the LF-LAM test may give a different result than a molecular WRD test or culture (e.g. LF-LAM positive, molecular WRD result "MTB 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.



Algorithm 3

3 *DST for second-line drugs for RR-TB or MDR-TB patients*



All patients with RR-TB or MDR-TB
(Treat with the all-oral MDR-TB regimen[1])

Collect one or two specimens[2]
Conduct a rapid molecular test for FQ resistance[3]
Conduct culture and phenotypic DST for second-line drugs[4]

A **Molecular DST**

B **Phenotypic DST**

FQ resistance detected	**FQ resistance *not* detected**	**Indeterminate or invalid test**	**Phenotypic DST results**
Initiate individualised MDR-TB treatment based on SL-LPA result[5] Conduct **additional DST** in accordance with national guidelines[6] Review treatment regimen based on DST results	**Continue** treatment on the **shorter MDR-TB regimen** Conduct **additional DST** in accordance with national guidelines[7] Review treatment regimen based on DST results	**Continue** treatment on the **shorter MDR-TB regimen** **Consider repeat SL-LPA** testing Conduct **additional DST** in accordance with national guidelines[7] Review treatment regimen based on DST results	**Review treatment regimen** based on DST results Conduct **additional DST** in accordance with national guidelines

Any **positive culture recovered** during treatment monitoring that is **suggestive of treatment failure[8]**

Conduct additional (phenotypic) DST in accordance with national guidelines
Review treatment regimen based on DST results

Algorithm 3 is for further evaluation of patients with RR-TB or MDR-TB. In its most recent recommendations *(4)*, WHO stresses the importance of DST before starting the preferred shorter all-oral bedaquiline-containing MDR-TB regimen, especially for medicines for which molecular WRDs are available. These medicines currently include RIF, INH and FQs. In addition, WHO stresses the need to scale up laboratory DST capacity for medicines for which there are accurate and reproducible phenotypic methods, including BDQ, LZD, clofazimine (CLF) and DLM. As in any potentially life-saving situation, treatment for DR-TB should not be withheld from a patient because of a lack of complete DST results.

BDQ: bedaquiline; CLF: clofazimine; DLM: delamanid; DST: drug-susceptibility testing; FQ: fluoroquinolone; INH: isoniazid; LZD: linezolid; MDR-TB: multidrug-resistant tuberculosis; MDR/RR-TB: multidrug- or rifampicin-resistant tuberculosis; PZA: pyrazinamide; RR-TB: rifampicin-resistant tuberculosis; SL-LPA: line probe assay for second-line drugs; TB: tuberculosis; WHO: World Health Organization.

[1] Patients should be promptly initiated on an MDR-TB regimen in accordance with national guidelines and WHO recommendations. A shorter all-oral bedaquiline-containing treatment regimen of 9–12 months duration is the preferred option for eligible MDR/RR-TB patients.

[2] If molecular and phenotypic testing are performed in the same laboratory, one specimen may be sufficient. If testing is performed in two laboratories, two specimens should be collected, and the molecular and phenotypic testing conducted in parallel.

[3] WHO recommends getting the rapid DST results for FQs before the start of treatment, although this testing should not delay the start of treatment. Currently, SL-LPA is the only WHO-approved rapid molecular test for detecting FQ resistance. Diagnostic accuracy is similar when SL-LPA is performed directly on sputum or from cultured isolates. In this group of patients, SL-LPA can be used on sputum smear-positive or smear-negative specimens, although a higher indeterminate rate will occur when testing smear-negative specimens.

[4] Phenotypic DST should be conducted for each of the drugs included in the treatment regimen for which there are accurate and reproducible methods. Reliable phenotypic DST methods are available for BDQ, FQ, CLF, INH, PZA, DLM and LZD. The initiation of treatment should not be delayed while awaiting the results of the phenotypic DST.

[5] For more details regarding individualized regimens, see the WHO *Consolidated guidelines on drug-resistant tuberculosis treatment*, 2019 *(3)*.

[6] For FQ-resistant MDR/RR-TB, a specimen should be collected and submitted for phenotypic DST to the WHO Group A and B drugs (e.g. DST for DLM and LZD), if not already being done as described in note 4.

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

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

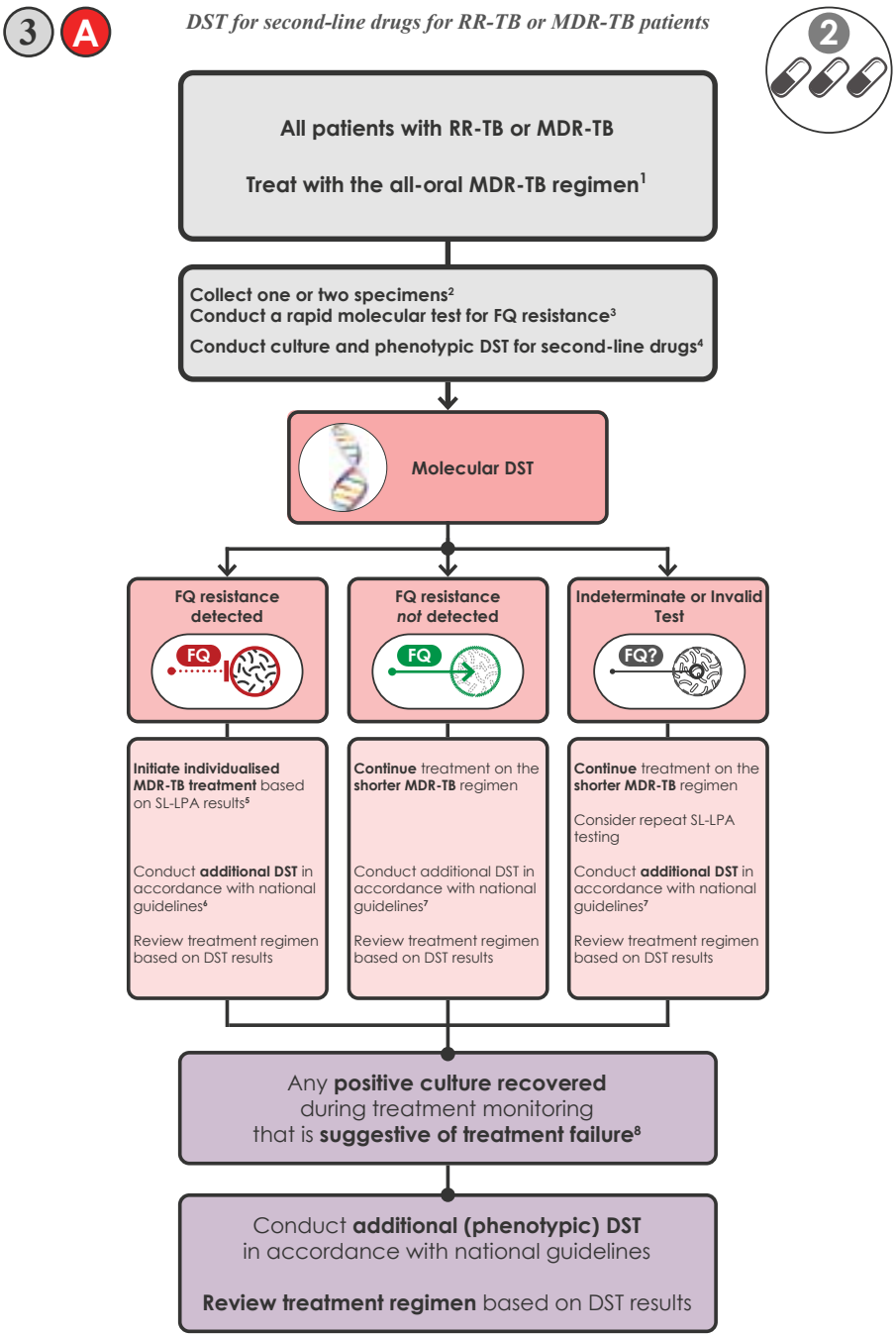
Decision pathway for Algorithm 3 – DST for second-line drugs for RR-TB or MDR-TB patients

General considerations

- A shorter all-oral bedaquiline-containing treatment regimen of 9–12 months' duration is the preferred option for eligible MDR/RR-TB patients *(4)*.
  - The preferred regimen contains bedaquiline, levofloxacin or moxifloxacin, ethionamide, ethambutol, isoniazid (high-dose), pyrazinamide and clofazimine (4–6 months of BDQ-LFX/MFX-ETO-EMB-PZA-INH-CFZ / 5 months of LFX/MFX-CFZ-PZA-EMB).
  - Individualized all-oral longer regimens, designed using the WHO priority grouping of medicines, may be still used for MDR/RR-TB patients who do not meet the eligibility criteria for an all-oral shorter bedaquiline-containing regimen.
  - Injectable medicines (e.g. AMK) should be phased out as a matter of priority in all treatment regimens and replaced by BDQ. If AMK is still used in the country, WHO recommends that, for patients being considered for the shorter AMK-containing MDR-TB regimen, FQ and AMK susceptibility (e.g. no mutations detected by SL-LPA) should be demonstrated before initiating the shorter AMK-containing MDR-TB regimen (see Algorithm 3 of the GLI model diagnostics algorithms for recommended testing *(29)*).
- WHO guidelines stress the importance of DST before treatment, especially for medicines for which molecular WRDs are available.
  - WHO-approved rapid molecular tests are available for RIF, INH and FQs. Genetic tests, including whole genome sequencing, are being developed for some of the other dugs included in MDR-TB regimens.
  - Reliable phenotypic DST methods are available for RIF, INH, FQs, PZA, BDQ, CFZ, LZD, AMK and DLM. Testing algorithms that rely on culture and phenotypic DST are described in the relevant WHO policy framework *(30)* and technical manual*(5) (5)*.
  - No reliable phenotypic DST methods are available for EMB, ethionamide/prothionamide, cycloserine/terizidone, imipenem-cilastatin/meropenem and PZA; 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]).
  - Currently, availability of DST for BDQ and LZD is limited in many settings, and resistance levels are likely to be very low. Therefore, at present, performing DST for BDQ and LZD is not essential before using these medicines; however, NTPs are strongly advised to start building capacity for drug resistance surveillance of these medicines. If resistance is suspected during treatment, and DST is not available, the strains should be conserved and referred to an SRL for further testing.
  - Do not delay initiation of treatment while waiting for the results of DST.
- Currently, SL-LPA is the only WHO-approved rapid molecular test for detecting FQ resistance.
  - 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 national reference laboratory level. It may also be used at the regional level if the appropriate infrastructure, HR 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 culture-based DST, which will be necessary for determining resistance to other anti-TB agents and monitoring the emergence of additional drug resistance.

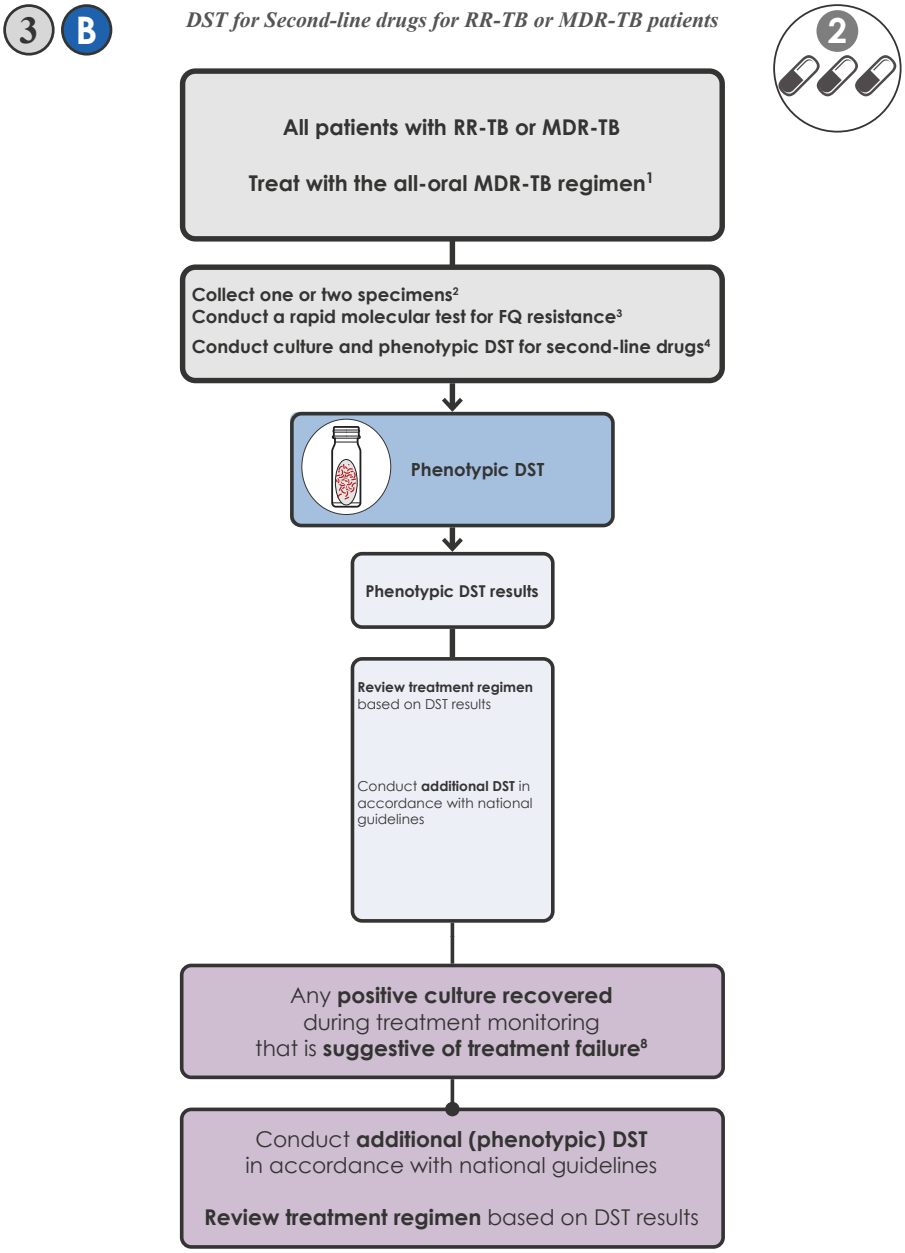
Decision pathway for Algorithm 3

1. Promptly initiate the patient on an MDR-TB regimen, in accordance with national guidelines and the most recent WHO recommendations for the use of a shorter all-oral BDQ-containing treatment regimen of 9–12 months' duration *(3, 4)*.
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 SL-LPA to detect mutations associated with FQ resistance.
4. If SL-LPA detects one or more mutations associated with resistance to FQs (3)(A):



a. Place MDR/RR-TB patients with resistance to FQs on an individualized longer regimen, designed using the WHO priority grouping of medicines recommended in 2018.
b. Collect a specimen and submit for phenotypic DST to the WHO Group A, B and C drugs (e.g. DST for DLM and LZD), if DST is not already being done as described in Step 6. Molecular tests may also be performed (e.g. FL-LPA for ETO).

5. If SL-LPA is negative for mutations associated with resistance to FQs (3)(A):
   a. Continue patients on the all-oral BDQ-containing shorter MDR-TB regimen, while awaiting the results of the phenotypic DST (Step 6).
   b. In settings with high underlying prevalence of resistance to FQs, or for patients considered at high risk of resistance, refer a specimen for culture and phenotypic DST for FQs, because the sensitivity of SL-LPA to detect mutations associated with FQ resistance is about 86%. The phenotypic DST should include testing for resistance to the FQs used in the country. 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 (3)(B). For the preferred regimens, reliable DST methods are available for BDQ, FQs, CFZ, PZA and INH.



a. If the isolate is susceptible to all drugs, continue the patient on the preferred MDR-TB regimen.
b. If resistance is detected to any drug, place the patient on an individualized longer MDR-TB regimen, designed using the WHO priority grouping of medicines recommended in 2018.

7. For all patients, ensure that treatment monitoring includes the collection of samples for culturing, as described in the WHO consolidated guidelines *(3)*. Any positive culture suggestive of treatment failure should undergo phenotypic DST. Modify the regimen as necessary, based on the DST results.
   a. WHO recommends that all patients 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.

Considerations for using SL-LPA

SL-LPAs are WHO-approved DNA strip-based tests that determine the drug-resistance profile of MTBC through the pattern of binding amplicons (DNA amplification products) to probes targeting the most common mutations to key second-line drugs (FQs and AMK), and probes targeting the corresponding wild-type DNA sequence *(9, 10)*. Mutations are **detected** by the binding to probes targeting the most commonly occurring mutations (MUT probes) or **inferred** by the lack of hybridization (i.e. binding) of the amplicons to the corresponding wild-type probes.

Depending on the specific genomic region interrogated by the SL-LPA probe, WHO either recommends or suggests as optimal one or more follow-up diagnostic actions, depending on the specific drug considered, to better guide the treatment regimen. If using sequencing to identify the specific mutation, guidance on interpreting individual mutations can be found in the WHO/FIND *Technical guide on the use of next-generation sequencing technology (31)*. For example, if mutations associated with MFX MIC above the critical concentration but below the clinical breakpoint (i.e. mutations associated with a low-level increase in MIC) are detected, high-dose MFX (up to 800 mg daily to adults) is likely to be effective. If mutations associated with MFX MIC increases above the clinical breakpoint (i.e. mutations associated with a high-level increase in MIC) are detected, the drug is unlikely to be an effective medicine *(32)*.

When used to test sputum specimens directly from patients with RR-TB or MDR-TB, SL-LPAs will detect 86% of patients with FQ resistance, and rarely give a positive result for patients without resistance, as described in the 2016 WHO policy guidance *(10)*. Thus, WHO recommends that treatment decisions should be made on the basis of the SL-LPA results with the following considerations:

- Despite good specificity and sensitivity of SL-LPA for detecting FQ resistance (pooled sensitivity of 86% and specificity of 99%) compared with phenotypic DST, culture and phenotypic DST may be required in some situations, to exclude resistance to the individual drugs in the FQ class completely, as well as to other second-line drugs. In particular, phenotypic DST may be needed in settings with a high pre-test probability for resistance to FQs, to exclude resistance when the SL-LPA does not detect mutations associated with resistance.
- SL-LPA detects resistance-conferring mutations that are highly correlated with phenotypic resistance to ofloxacin, gatifloxacin, LFX and MFX.
  - If mutations associated with a low-level increase in MIC are **detected** (e.g. MUT1, MUT2 or MUT3A probes developed in *gyrA* region, or MUT1 or MUT2 probes developed in *gyrB* region), WHO recommends phenotypic DST for MFX to exclude resistance at the clinical breakpoint.
  - If mutations associated with a low-level increase in MIC are **inferred** by the absence of amplicons binding to wild-type probes in the *gyrA* or *gyrB* regions (i.e. wild-type probes not developed), WHO recommends phenotypic DST for MFX to exclude resistance at the clinical breakpoint. Optional follow-up actions include sequencing of *gyrA* or *gyrB* to identify the specific mutation

or silent mutations (associated with false resistance), and phenotypic DST for MFX (or LFX, or both) at the critical concentration.

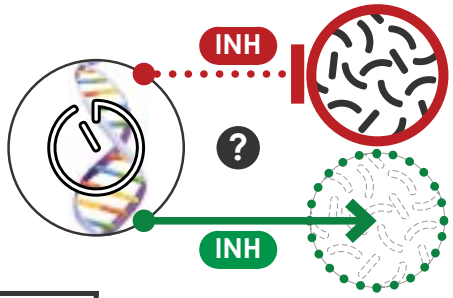
- For recording and reporting purposes SL-LPA results "resistance detected" and "resistance inferred" should be considered as resistant.

3.1.4 Algorithm 4 – molecular WRD as the initial test to detect Hr-TB in patients with RIF-susceptible TB

Algorithm 4 is for further evaluation of patients with RIF-susceptible TB (e.g. those with a molecular WRD result of "MTB detected, RIF resistance not detected") to determine whether the patient has Hr-TB. All patients with RIF-susceptible TB should be started on an appropriate first-line regimen, in accordance with national guidelines, while awaiting the results of follow-up testing. 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 patients with Hr-TB and placing them on effective treatment regimens. Compared with patients with drug-susceptible TB, patients with Hr-TB who are treated with the recommended regimen for drug-susceptible TB have a much higher risk of treatment failure (11% vs 2%), relapse (10% vs 5%) and acquiring additional drug resistance (8% vs 1%). This testing algorithm incorporates the testing requirements to ensure that the recommended Hr-TB treatment regimen (RIF, EMB, PZA and LFX for 6 months) *(33)* will be effective.


Algorithm 4

4 *Molecular WRD as the initial test to detect Hr-TB in patients with RIF-susceptible TB*



All patients with MTB detected, rifampicin resistance not detected[1]
(Treat with first-line regimen in accordance with national guidelines)

Collect one sputum specimen and perform DST (preferably molecular DST) for INH[2]

A **INH resistance *not* detected**

Continue treatment with **first-line regimen**

Conduct **additional DST** in accordance with national guidelines

During treatment monitoring, any positive culture suggestive of treatment failure should undergo DST

Review treatment based on DST result

B ***Low-level* INH resistance detected**

C ***High-level* INH resistance detected**

Treat with **Hr-TB regimen[3]**

Consider including **high-dose isoniazid** in the Hr-TB regimen ***if*** low-level resistance detected

Refer a **sample for molecular DST for FQ[4]**

FQ resistance detected

Initiate individualized Hr-TB treatment[6]

Conduct **DST for PZA[7]**

Conduct **additional phenotypic and molecular DST** in accordance with national guidelines

During treatment monitoring, any positive specimen suggestive of treatment failure should undergo DST

Review treatment based on DST results

FQ resistance *not* detected

Continue Hr-TB treatment

For **patients considered at high risk of FQ resistance**, refer a **specimen for culture and phenotypic DST[5]**

During treatment monitoring, any positive specimen suggestive of treatment failure should undergo DST

Indeterminate result, no result, error, or invalid test

Continue Hr-TB treatment

Consider repeating SL-LPA from fresh sample **or** conducting SL-LPA from culture

Conduct **additional phenotypic and molecular DST** in accordance with national guidelines

During treatment monitoring, any positive specimen suggestive of treatment failure should undergo DST

Review treatment based on DST result

DST: drug-susceptibility testing; EMB: ethambutol; FL-LPA: line-probe assay for first-line drugs; FQ: fluoroquinolone; HREZ: isoniazid (H), rifampicin (R), ethambutol (E) and pyrazinamide (Z); Hr-TB: isoniazid-resistant, rifampicin-susceptible tuberculosis; INH: isoniazid; LFX: levofloxacin; MTB: *Mycobacterium tuberculosis*; PZA: pyrazinamide; REZ: rifampicin (R), ethambutol (E) and pyrazinamide (Z); RIF: rifampicin; SL-LPA: line-probe assay for second-line drugs; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnosis.

[1] All patients with MTB detected, RIF resistance not detected should be initiated on a first-line regimen according to national guidelines.

[2] Patients at high risk for Hr-TB should be given priority for molecular testing for INH resistance. Patients at high risk of Hr-TB include previously treated patients such as those who had been lost to follow-up, relapsed and failed a treatment regimen; Hr-TB contacts; and any other Hr-TB risk groups identified in the country (e.g. from populations with a high prevalence of Hr-TB). Molecular DST (e.g. FL-LPA) is preferred.

[3] Patients should be initiated on an Hr-TB regimen in accordance with national guidelines. The preferred regimen is 6 month regimen of RIF-EMB-PZA-LFX (6 REZ-LFX) after confirmation of INH resistance, so long as RIF resistance has been reliably excluded. INH may be included in the regimen to enable the use of HREZ fixed-dose combination tablet. The use of high doses of INH (up to 15 mg/kg) may be useful for patients whose isolate displays low-level resistance to INH (e.g. isolate with mutations in the inhA promoter region only).

[4] For each patient with Hr-TB, a specimen should be referred for molecular DST for FQs. Currently, SL-LPA is the only WHO-approved rapid molecular test for detecting FQ resistance.

[5] Despite good sensitivity (>85%) of SL-LPA for detecting FQ resistance, culture and phenotypic DST may be needed for patients with a high pre-test probability for FQ resistance (e.g. setting with a high underlying prevalence of resistance to FQs or patient risk factors) to exclude resistance when the SL-LPA does not detect mutations associated with resistance.

[6] Patients with FQ-resistant Hr-TB may be treated with a 6-month regimen of (INH)-RIF-EMB-PZA or an individualised Hr-TB regimen.

[7] For all Hr-TB patients with concurrent resistance to FQ, phenotypic or molecular DST for PZA is desirable if a reliable DST for PZA has been established in the country. When resistance to PZA is confirmed, appropriate treatment regimens may have to be designed individually, especially if resistance to both FQ and PZA are detected.

Decision pathway for Algorithm 4 – molecular WRD as the initial test to detect Hr-TB in patients with RIF-susceptible TB

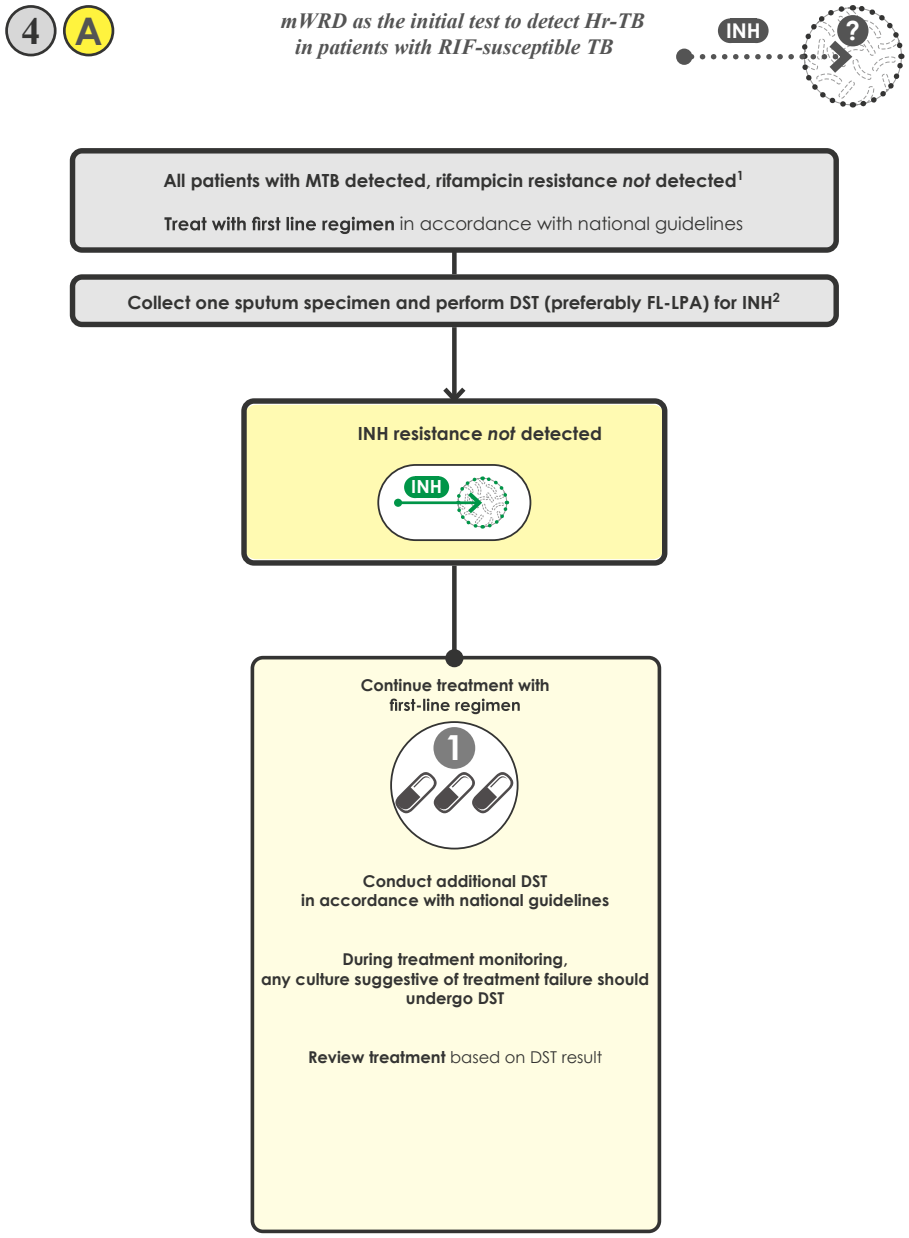
General considerations

- The starting point for this algorithm is a patient who has been shown to have RIF-susceptible TB by DST, either molecular (e.g. Xpert MTB/RIF, Xpert Ultra or Truenat) or phenotypic. It is essential that resistance to RIF is ruled out to avoid acquiring LFX resistance during Hr-TB treatment.
- Ideally, submit a specimen from each patient with RIF-susceptible TB for molecular (preferred) or phenotypic DST for INH.
  - Give priority to patients at high risk for Hr-TB for molecular testing for INH resistance.
    - Risk factors for Hr-TB include being a contact of a known Hr-TB patient and a high prevalence of Hr-TB in the setting.
    - About three-quarters of Hr-TB will be found in individuals who have not been previously treated with INH, and contact information will not be available for many patients.
  - Patients at low risk of having Hr-TB may be tested for INH resistance, or the patient may be treated with a full-course of the standard regimen for drug-susceptible TB.
    - If treatment is conducted in the absence of DST (i.e. empiric first-line therapy), any positive culture recovered during treatment monitoring that suggests treatment failure should undergo DST for INH and RIF.
- For patients with Hr-TB, assess resistance to LFX. If LFX resistance is detected in an Hr-TB patient, remove LFX from the regimen and treat the patient for 6 months with RIF, EMB and PZA.
- For Hr-TB patients with concurrent resistance to FQ, phenotypic or molecular DST to PZA is desirable if a reliable DST for PZA has been established in the country.
- The use of FL-LPA to detect INH resistance does not eliminate the need for conventional culture-based DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance. Also, conventional culture-based DST for isoniazid may still be used for further evaluation of patients when the LPA result does not detect isoniazid resistance (FL-LPA sensitivity is ~85% for detecting INH resistance), particularly for populations with a high pre-test probability of resistance to isoniazid.

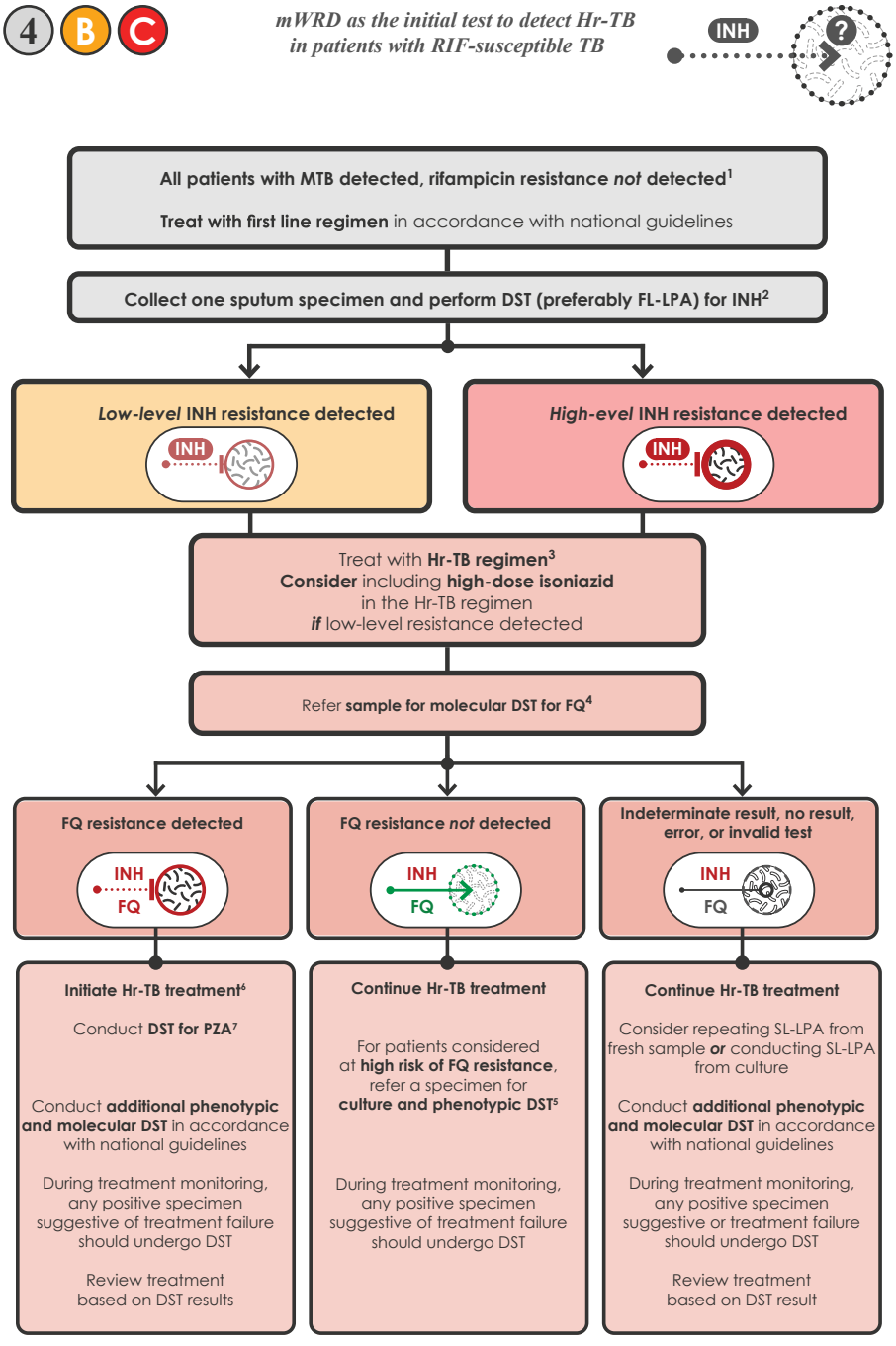
Decision pathway for Algorithm 4

1. Collect a good-quality specimen and transport it to the testing laboratory for molecular or phenotypic testing for INH resistance.
   a. The molecular and phenotypic testing may be performed in different laboratories. If both tests are performed, initiate the tests in parallel; do not wait for the results of one test before initiating the other test.
   b. The molecular and phenotypic DST may be performed using the specimen (direct DST) or using bacteria recovered by culture (indirect DST). Direct phenotypic DST has a much shorter turnaround time, but indirect phenotypic DST may be preferred because of technical issues.
   c. A rapid molecular test is preferred. Currently, FL-LPA is the only WHO-approved rapid molecular test for INH resistance. DNA sequencing has proven useful in many cases but WHO has not yet evaluated this.
   d. Culture-based phenotypic DST for INH requires 3–8 weeks to produce a result. Phenotypic DST may be useful for evaluating patients with a negative FL-LPA result, particularly in populations with a high pre-test probability for resistance to INH.
2. Interpret the FL-LPA results as described in *Line probe assays for drug-resistant tuberculosis detection: interpretation and reporting guide for laboratory staff and clinicians (27)*.

3. If INH resistance is *not* detected (4)(A), continue treatment with a first-line regimen in accordance with national guidelines:
   a. Conduct additional DST in accordance with national guidelines.
   b. Additional molecular or phenotypic DST for resistance to INH may be requested if the patient is considered to be at risk of having Hr-TB, despite the FL-LPA result. False INH-susceptible FL-LPA results have been observed in about 10% of INH-resistant TB cases tested in various epidemiologic settings. Compared with phenotypic DSTs, FL-LPAs have a pooled sensitivity of about 90% and a pooled specificity of about 99% for detecting INH resistance *(9)*.



4. If INH resistance *is* detected 4 B and C:
    a. Initiate treatment with an Hr-TB regimen *(33)*:
        i. There is no clear evidence showing that adding INH at the usual doses adds benefits or harms to patients. For patient convenience and 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, patients infected with strains with only *inhA* promoter mutations, and corresponding modest MIC increases, may benefit from high-dose INH therapy *(34)*. 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.



b. Refer a specimen from each patient with laboratory-confirmed Hr-TB for molecular (e.g. SL-LPA) or phenotypic DST for LFX.
    i. Rapid molecular testing for FQ resistance is preferred. Currently, SL-LPA is the only WHO-approved rapid molecular test for FQ resistance.
        1. The diagnostic accuracy of SL-LPA is similar when it is performed directly on sputum or from 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.
        2. SL-LPA is suitable for use at the central or national reference laboratory level; it may also be used at the regional level if the appropriate infrastructure and HR are available. Implementation of SL-LPA testing must ensure the availability of a reliable specimen transport system and an efficient mechanism for reporting results.
        3. When used for direct testing of sputum specimens from patients with MDR/RR-TB, SL-LPA detects 86% of patients with FQ resistance *(10)*.
        4. Despite good specificity and sensitivity of SL-LPA for the detection of FQ resistance, culture and phenotypic DST are required to exclude resistance to individual FQs completely. In particular, phenotypic DST may be needed in settings with a high pre-test probability for resistance to LFX, to exclude resistance when the SL-LPA does not detect mutations associated with resistance.
    ii. If LFX resistance is *not* detected, continue treatment with the LFX-containing Hr-TB regimen.
    iii. If LFX resistance *is* detected:
        1. Discontinue use of LFX and change to a 6-month regimen of (INH)/RIF/EMB/PZA (i.e. 6(H) REZ, where the "(H)" indicates that the INH is optional) or an individualized Hr-TB regimen.
        2. Refer a specimen for PZA DST if reliable PZA DST is available in the country. Options include phenotypic DST in the MGIT system and *pncA* sequencing (Sanger or NGS). For more details, see the *WHO technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis (5)*.
            a. If PZA resistance is *not* detected, or if PZA DST is not available, continue therapy with the regimen designed based on the SL-LPA results.
            b. If PZA resistance *is* detected, appropriate treatment regimens may have to be designed individually, especially if resistances to both FQ and PZA are detected.

5. If the INH result is uninterpretable or invalid, repeat the FL-LPA with a fresh specimen. Consider conducting culture and molecular or phenotypic DST for INH on the isolate, if the patient is considered to be at risk of having Hr-TB.

6. For all patients, 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 patients on first-line regimens, and for RIF, FQs and PZA (if available) for patients on Hr-TB regimens. The treatment regimen should be modified as necessary, based on the results of the DST.

Interpretation of discordant results

This algorithm relies on testing of a sample with a WRD test to detect MTBC and assess susceptibility to RIF. and follow-up testing with FL-LPAs, primarily to detect resistance to INH, although it also provides information on the detection of MTB and resistance to RIF. 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. Molecular WRD (e.g. Xpert Ultra) result "MTB detected, FL-LPA MTB not detected or uninterpretable on follow-up testing".
    a. Molecular WRDs have a lower LOD than the FL-LPA; thus, FL-LPA may fail to detect TB in WRD-positive samples that contain few bacilli. For example, it is estimated that about 80% of specimens detected by Xpert MTB/RIF MTB will generate an interpretable FL-LPA result.

b. The molecular WRD 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. Molecular WRD result "MTB detected, RIF resistance not detected"; RIF resistant by FL-LPA.
   a. Treatment decisions should be based on the FL-LPA result.
   b. This result is expected to be rare because both assays interrogate the same region of *rpoB*. There have been reports of molecular WRD RIF susceptible and FL-LPA RIF resistant discordances, but few data are available to assess how frequently this occurs.
   c. FL-LPA is more sensitive for identifying RIF resistance than molecular WRD in hetero-resistant populations (mixtures of susceptible and resistant bacteria), when resistance is detected by hybridization to a MUT probe that could lead to this discordance.
   d. Follow-up actions may include DNA sequencing, conducting phenotypic DST and evaluating the possibility of laboratory or clerical error.

Considerations for the use of FL-LPA to detect resistance to INH

FL-LPAs are a family of WHO-approved DNA strip-based tests that determine the drug-resistance profile of MTB through the pattern of binding amplicons (DNA amplification products) to probes targeting the most common mutations to first-line drugs (INH and RIF), and to probes targeting the corresponding wild-type DNA sequence *(9, 10)*. Mutations are **detected** by the binding to probes targeting the most commonly occurring mutations (MUT probes), or **inferred** by the lack of binding of the amplicons to the corresponding wild-type probes.

Depending on the specific genomic region interrogated by the FL-LPA probe, one or more follow-up diagnostic actions are either recommended or suggested as an option, to better guide the treatment regimen based on the detection of specific mutations *(27)*. If sequencing is used to identify the specific mutation, guidance on the interpretation of individual *inh*A or *katG* mutations can be found in the relevant WHO technical guide *(31)*. Phenotypic DST or determination of MICs can also provide important information (e.g. low- or high-level) about INH resistance.

- If resistance is i**nferred** by the absence of binding of the amplicons to wild-type probes in the *katG* region (i.e. one or more wild-type probes not developed), sequencing of the *katG* gene is suggested as an option to identify the specific mutation.
- If mutations associated with a low-level increase in MIC are **detected**, sequencing of *inhA* coding region and *katG* gene is suggested as an option to confirm the mutation.
- If mutations associated with a low-level increase in MIC are **inferred** by the absence of binding of the amplicons to wild-type probes in the *inhA* promoter region (and no mutations in the *katG* target region are detected), it is recommended to repeat the testing to confirm the result. Optional follow-up diagnostic actions include sequencing of the *inhA* promoter to identify the specific mutation, or performing phenotypic DST for INH, or determining the INH MIC.
- For recording and reporting purposes FL-LPA results "resistance detected" and "resistance inferred" should be considered as resistant.

3.2 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 and for what purposes the tests are recommended by WHO;
- 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 (risk groups) of the population 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.

Because the risk factors for TB and DR-TB vary widely among countries, it is essential to carefully assess risks at the country and local levels. Algorithms for testing patients suspected of having DR-TB depend on the local epidemiology of TB, local treatment policies, existing laboratory capacity, mechanisms for specimen referral and transport, and human and financial resources.

4 Suggested reading

4.1 WHO policy guidance on TB diagnostics and laboratory strengthening

Systematic screening for active tuberculosis: principles and recommendations. WHO/HTM/TB/2013.04. Geneva: World Health Organization. 2013. https://www.who.int/tb/tbscreening/en/

Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. WHO/HTM/TB/2013.16. Geneva: World Health Organization. 2013. http://www.who.int/tb/publications/xpert-mtb-rif-assay-diagnosis-policy-update/en/

The End TB Strategy – global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization. 2014. http://www.who.int/tb/strategy/End_TB_Strategy.pdf

Policy framework for implementing new tuberculosis diagnostics. WHO/HTM/TB/2015.11. Geneva: World Health Organization. 2015. http://www.who.int/tb/publications/implementing_TB_diagnostics/en/

Chest radiography in tuberculosis detection. Summary of current WHO recommendations and guidance on programmatic approaches. Geneva: World Health Organization. 2016. https://www.who.int/tb/publications/chest-radiography/en/

Framework of indicators and targets for laboratory strengthening under the End TB Strategy. WHO/HTM/TB/2016.18. Geneva: World Health Organization. 2016. http://www.who.int/tb/publications/labindicators/en/

Frequently asked questions about the implementation of the new WHO recommendation on the use of the shorter MDR-TB regimen under programmatic conditions, Version: 20 December 2016. Geneva: World Health Organization. 2016. http://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/FAQshorter_MDR_regimen.pdf

The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin. Policy update. WHO/HTM/TB/2016.12. Geneva: World Health Organization. 2016. http://www.who.int/tb/publications/molecular-test-resistance

The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance. WHO/HTM/TB/2016.07. Geneva: World Health Organization. 2016. http://www.who.int/tb/publications/lpa-mdr-diagnostics

Considerations for adoption and use of multi-disease testing devices in integrated laboratory networks. WHO/HTM/TB/2017.05. Geneva: World Health Organization; 2017. https://www.who.int/tb/publications/2017/considerations_multidisease_testing_devices_2017/en/

Planning for country transition to Xpert® MTB/RIF Ultra cartridges. Geneva: Global Laboratory Initiative. 2017. http://www.stoptb.org/wg/gli/assets/documents/GLI_ultra.pdf

WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF [WHO/HTM/TB/2017.04]. Geneva: World Health Organization. 2017. https://www.who.int/tb/publications/2017/XpertUltra/en/

Line probe assays for drug-resistant tuberculosis detection: Interpretation and reporting guide for laboratory staff and clinicians. Geneva: Global Laboratory Initiative. 2018. http://www.stoptb.org/wg/gli/assets/documents/LPA_test_web_ready.pdf

Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. WHO/CDS/TB/2018.24. Geneva: World Health Organization. 2018. https://www.who.int/tb/publications/2018/WHO_technical_drug_susceptibility_testing/en/

WHO treatment guidelines for isoniazid-resistant tuberculosis: supplement to the WHO treatment guidelines for drug-resistant tuberculosis. WHO/CDS/TB/2018.7. Geneva: World Health Organization. 2018. http://www.who.int/tb/publications/2018/WHO_guidelines_isoniazid_resistant_TB/en/

Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Policy update 2019. WHO/CDS/TB/2019.16. Geneva: World Health Organization. 2019. https://www.who.int/tb/publications/2019/LAMPolicyUpdate2019/en/

Rapid communication: key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB). WHO/CDS/TB/2019.26. Geneva: World Health Organization. 2019. https://www.who.int/tb/publications/2018/rapid_communications_MDR/en/

WHO consolidated guidelines on drug-resistant tuberculosis treatment. WHO/CDS/TB/2019.7. Geneva: World Health Organization. 2019. https://www.who.int/tb/publications/2019/consolidated-guidelines-drug-resistant-TB-treatment/en/

Frequently asked questions on the WHO rapid communication 2019: key changes to the treatment of drug-resistant TB. Geneva: World Health Organization. 2020. https://www.who.int/tb/areas-of-work/drug-resistant-tb/faqs-updated-final-version.pdf?ua=1

Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB in adults and children. Policy update. Geneva: World Health Organization; 2020.

4.2 Guidance on implementation of diagnostic testing

GLI model TB diagnostic algorithms. Geneva: Global Laboratory initiative. 2017. http://www.stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf

GLI practical guide to TB laboratory strengthening. Geneva: Global Laboratory initiative. 2017. http://www.stoptb.org/wg/gli/gat.asp

GLI specimen referral toolkit. Geneva: Global Laboratory Initiative. 2017. http://www.stoptb.org/wg/gli/srt.asp

4.3 Training packages

Training package on culture on solid and liquid medium. Global Laboratory Initiative. 2012. http://www.stoptb.org/wg/gli/assets/documents/Training%20Package%20Culture_October%202012.zip

Training package on DST by phenotypic and molecular method. Global Laboratory Initiative. 2012. http://www.stoptb.org/wg/gli/assets/documents/Training%20Package%20DST_October%202012.zip

Training package on line probe assays (LPAs). Global Laboratory Initiative. 2012. http://www.stoptb.org/wg/gli/assets/documents/Training%20Package%20LPA_%20October%202012.zip

Training package on Xpert MTB/RIF. Global Laboratory Initiative. 2014. http://www.stoptb.org/wg/gli/TrainingPackage_XPERT_MTB_RIF.asp

Training package: programme modules for diagnostic network strengthening. Global Laboratory Initiative. 2018. http://www.stoptb.org/wg/gli/TrainingPackage_Programme.asp

References

1 Global tuberculosis report 2019. Geneva: World Health Organization; 2019 (https://www.who.int/tb/publications/global_report/en/, accessed 8 June 2020).

2 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/tb/publications/labindicators/en/, accessed 28 May 2020).

3 Consolidated guidelines on drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2019 (https://www.who.int/tb/publications/2019/consolidated-guidelines-drug-resistant-TB-treatment/en/, accessed 28 May 2020).

4 Rapid communication: key changes to treatment of multidrug- and rifampicin-resistant tuberculosis (MDR/RR-TB) (WHO/CDS/TB/2019.26). Geneva: World Health Organization; 2018 (https://www.who.int/tb/publications/2018/rapid_communications_MDR/en/, accessed 28 May 2020).

5 Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis (WHO/CDS/TB/2018.24). Geneva: World Health Organization; 2018.

6 Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014) [WHO/HTM/TB/2013.2]. Geneva, Switzerland: World Health Organization; 2013 (https://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf?sequence=1, accessed 20 March 2020).

7 Molecular assays intended as initial tests for the diagnosis of pulmonary and extrapulmonary TB and rifampicin resistance in adults and children: rapid communication. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/bitstream/handle/10665/330395/9789240000339-eng.pdf, accessed 5 June 2020).

8 The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis. Policy guidance. Geneva: World Health Organization; 2016 (https://www.who.int/tb/publications/lamp-diagnosis-molecular/en/, accessed 28 May 2020).

9 The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin. Policy update. Geneva: World Health Organization; 2016 (https://www.who.int/publications/guidelines/tuberculosis/en/, accessed 28 May 2020).

10 The use of molecular line probe assays for the detection of resistance to second-line anti-tuberculosis drugs: policy guidance [WHO/HTM/TB/2016.07]. Geneva: World Health Organization; 2016 (https://apps.who.int/iris/bitstream/handle/10665/246131/9789241510561-eng.pdf?sequence=1, accessed 20 March 2020).

11 Lateral flow urine lipoarabinomannan assay (LF-LAM) for the diagnosis of active tuberculosis in people living with HIV. Policy update. Geneva: World Health Organization; 2019 (https://www.who.int/tb/publications/2019/diagnose_tb_hiv/en/, accessed 5 June 2020).

12 Non-commercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis. Policy statement. WHO/HTM/TB/2011.9. , 2011. Geneva: World Health Organization; 2011 (http://www.who.int/tb/publications/2011/mdr_tb_diagnostics_9789241501620/en/, accessed 8 June 2020).

13 WHO meeting report of a technical expert consultation: accuracy of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid (WHO/CDS/TB/2019.14). Geneva: World Health Organization; 2019 (https://www.who.int/tb/publications/2019/EvaluationOfAssaysforTBDetection/en/, accessed 28 May 2020).

14 Considerations for adoption and use of multi-disease testing devices in integrated laboratory network (WHO/HTM/TB/2017.05). Geneva: World Health Organization; 2017 (https://www.who.int/tb/publications/2017/considerations_multidisease_testing_devices_2017/en/, accessed 8 June 2020).

15 World Health Organization, Foundation for Innovative New Diagnostics. 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/bitstream/handle/10665/274443/WHO-CDS-TB-2018.19-eng.pdf, accessed 10 April 2020).

16 Commercial serodiagnostic tests for diagnosis of tuberculosis: policy statement (WHO/HTM/TB/2011.5). Geneva: World Health Organization; 2011 (https://www.who.int/tb/publications/tb-serodiagnostic-policy/en/ accessed 8 June 2020).

17 GLI guide to TB specimen referral systems and integrated networks. Geneva: Global Laboratory Initiative; 2017 (http://www.stoptb.org/wg/gli/gat.asp, accessed 8 June 2020).

18 GLI specimen referral toolkit. Geneva: Global Laboratory Initiative; 2017 (http://www.stoptb.org/wg/gli/srt.asp accessed 8 June 2020).

19 Chest radiography in tuberculosis detection. Summary of current WHO recommendations and guidance on programmatic approaches Geneva: World Health Organization; 2016 (https://www.who.int/tb/publications/chest-radiography/en/ accessed 6 June 2020).

20 Systematic screening for active tuberculosis: principles and recommendations (WHO/HTM/TB/2013.04). Geneva: World Health Organization; 2013 (https://www.who.int/tb/tbscreening/en/ accessed 8 June 2020).

21 Tuberculosis laboratory biosafety manual (WHO/HTM/TB/2012.11). Geneva: World Health Organization; 2012 (https://www.who.int/tb/publications/2012/tb_biosafety/en/, accessed 28 May 2020).

22 ISO 15189: 2012 Medical laboratories – requirements for quality and competence. Geneva: International Organization for Standardization; 2017 (https://www.iso.org/standard/56115.html, accessed 8 June 2020).

23 GLI quick guide to TB diagnostics connectivity solutions Geneva: Global Laboratory Initiative; 2016 (http://www.stoptb.org/wg/gli/assets/documents/gli_connectivity_guide.pdf accessed 8 June 2020).

24 GLI practical guide to TB laboratory strengthening. Geneva: Global Laboratory Initiative; 2017 (http://stoptb.org/wg/gli/gat.asp, accessed 8 June 2020).

25 Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J et al. The New Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. mBio. 2017;8(4):e00812–17 (https://pubmed.ncbi.nlm.nih.gov/28851844, accessed 28 May 2020).

26 Van Deun A, Aung KJM, Bola V, Lebeke R, Hossain MA, de Rijk WB et al. Rifampin drug resistance tests for tuberculosis: challenging the gold standard. J Clin Microbiol. 2013;51(8):2633–40 (https://pubmed.ncbi.nlm.nih.gov/23761144, accessed 28 May 2020).

27 Line probe assays for drug-resistant tuberculosis detection: interpretation and reporting guide for laboratory staff and clinicians. Geneva: Global Laboratory Initiative; 2018 (http://www.stoptb.org/wg/gli/assets/documents/LPA_test_web_ready.pdf, accessed 26 May 2020).

28 Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014;2014(1):CD009593 (https://pubmed.ncbi.nlm.nih.gov/24448973, accessed 28 May 2020).

29 GLI model diagnostic algorithms. Geneva: Global Laboratory Initiative; 2017 (http://www.stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf, accessed 8 June 2020).

30 Implementing tuberculosis diagnostics: a policy framework (WHO/HTM/TB/2015.11). Geneva, World Health Organization. 2015 (https://www.who.int/tb/publications/implementing_TB_diagnostics/en/, accessed 8 June 202).

31 World Health Organization, Foundation for Innovative New Diagnostics. Technical guide on next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex (WHO/CDS/TB/2018.19). Geneva: World Health Organization; 2018 (https://www.who.int/tb/publications/2018/WHO_technical_guide_nextgen_sequencing/en/, accessed 10 April 2020).

32 Technical report on critical concentrations for TB drug susceptibility testing of medicines used in the treatment of drug-resistant TB. Geneva: World Health Organization; 2018 (https://www.who.int/tb/publications/2018/WHO_technical_report_concentrations_TB_drug_susceptibility/en/, accessed 20 March 2020).

33 WHO treatment guidelines for isoniazid-resistant tuberculosis. Supplement to the WHO treatment guidelines for drug-resistant tuberculosis (WHO/CDS/TB/2018.7). Geneva World Health Organization 2018 (https://apps.who.int/iris/bitstream/handle/10665/260494/9789241550079-eng.pdf, accessed 20 March 2020).

34 Dooley KE, Miyahara S, von Groote-Bidlingmaier F, Sun X, Hafner R, Rosenkranz SL et al. Early bactericidal activity of different isoniazid doses for drug resistant TB (INHindsight): a randomized open-label clinical trial. Am J Respir Crit Care Med. 2020;201(11):1416–24.

Annex 1. Budgetary considerations for implementing a new diagnostic test

Successful implementation of the plan will require financial and human resource commitments from MOH/NTP, with possible support of implementing partners. Consider integrating TB testing on existing multi-disease platforms in locations where integrated testing is feasible, as a way to share costs across disease programmes. A budget should be developed to address activities in collaboration with key partners. 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, HR
Equipment
• Costs of assessing site readiness – travel, HR • Costs of upgrading laboratory facilities and infrastructure (e.g. electricity, air conditioning, etc.) to ensure a safe and functional testing site • Costs to adhere to biosafety precautions, and biological and chemical waste disposal requirements • Select, procure and install equipment – Purchase (or lease) of instrument and needed ancillary equipment – Delivery and importation costs – Installation by manufacturer or authorized service provider (e.g. per diems, 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
- Cost of maintaining centralized stores and costs of distribution
- Material cost per test, including but not limited to test reagents, consumables, sample collection items, printing paper, etc. Additional equipment costs that include additional equipment requirements (printer, computer, 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 patients to be tested, ordering tests, interpreting test results and making patient care decisions

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

Quality assurance, control and assessment

- Preparation and regular review of all testing and QA documents (SOPs, checklists, etc.) 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 – travel, HR, 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, reporting, etc.), 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 trainings and sensitization meetings
- Printing and distribution of updated training manuals and sensitization materials
- Costs associated with facility and classroom-based training, including 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; QC: quality control; SOP: standard operating procedure; TWG: technical working group.

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