# WHO operational handbook on tuberculosis

## Module 3: Diagnosis

### Rapid diagnostics for tuberculosis detection

2021 update

**World Health Organization**

# WHO

# operational handbook on tuberculosis

## Module 3: Diagnosis

## Rapid diagnostics for tuberculosis detection

2021 update

World Health Organization

# Contents

# Acknowledgements

# Abbreviations and acronyms

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

| | |
|---|---|
| **PCR** | polymerase chain reaction |
| **PLHIV** | people living with HIV/AIDS |
| **QA** | quality assurance |
| **QC** | quality control |
| **RRDR** | RIF-resistance-determining region |
| **RR-TB** | rifampicin-resistant tuberculosis |
| **SARS-CoV-2** | severe acute respiratory syndrome coronavirus 2 |
| **SL-LPA** | line-probe assay for second-line drugs |
| **SOP** | standard operating procedure |
| **SRL** | supranational reference laboratory |
| **TB** | tuberculosis |
| **$T_m$** | melting temperature |
| **TWG** | technical working group |
| **USA** | United States of America |
| **WHO** | World Health Organization |
| **WRD** | WHO-recommended rapid diagnostic test |
| **XDR-TB** | extensively drug-resistant tuberculosis |

# Abbreviations of TB agents

| | |
|---|---|
| **AMK** | amikacin |
| **BDQ** | bedaquiline |
| **CFZ** | 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 |

WHO operational handbook on tuberculosis:
rapid diagnostics for tuberculosis detection

# 1. Introduction

## 1.1 Background

Globally, tuberculosis (TB) continues to be a significant public health problem, with an estimated 10 million people developing TB in 2019 and 7.1 million reported to have been diagnosed and notified *(1)*. The gap between the numbers estimated and notified is large and has worsened during the coronavirus disease (COVID-19) pandemic *(2)*. Drug-resistant TB (DR-TB) is another area of concern, particularly multidrug- or rifampicin-resistant TB (MDR/RR-TB), which is TB disease caused by *Mycobacterium tuberculosis* complex bacteria (MTBC) with resistance to rifampicin (RIF) or isoniazid (INH), or both. The relative gap was even larger for MDR/RR-TB in 2019, with an estimated 0.5 million new cases of MDR/RR-TB, of which only 206 030 were detected and notified *(1)*. In addition, an estimated 1.1 million people had TB disease caused by MTBC with resistance to INH and susceptibility to RIF (referred to as Hr-TB), which is largely undetected.

The effective management of TB relies on the rapid diagnosis of TB, rapid detection of drug resistance and prompt initiation of an effective treatment regimen. Thus, there is a need for access to fast and accurate detection tests and rapid and accurate drug-susceptibility testing (DST) for all 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 treatment is started. However, the initiation of treatment should not be delayed waiting 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 *(3)* – calls for the early diagnosis of TB and universal access to DST. WHO defines universal access to DST as rapid DST for at least RIF among all patients with bacteriologically confirmed TB, and further DST for at least fluoroquinolones (FQs) and second-line injectable agents among all TB patients with RIF resistance *(4)*. To meet the End TB Strategy targets, molecular WHO-recommended rapid diagnostic tests (mWRDs) should be made available to all individuals with signs or symptoms of TB, all bacteriologically confirmed TB patients should receive DST for at least RIF (in 2018, only about 61% of such patients were tested for RIF resistance), and all patients with RR-TB should receive DST for at least FQs.[1] Recent WHO guidelines stress the importance of DST before treatment, especially for the medicines for which mWRDs are available (e.g. FQs, INH and RIF) but should not delay the start of treatment *(5)*.

---

[1]  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.

Furthermore, as described in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (6)*, all national TB programmes (NTPs) should prioritize the development of a network of TB laboratories that use modern methods of diagnosis (e.g. molecular methods and liquid culture), have efficient referral systems, use electronic data and diagnostics connectivity, use standard operating procedures (SOPs) and appropriate quality assurance (QA) processes, adhere to biosafety principles for all testing and have sufficient human resources. These priorities should be comprehensively addressed in national strategic plans and should be adequately funded.

Over recent decades, considerable effort has gone into building the laboratory, clinical and programmatic capacity to prevent, detect and treat TB and DR-TB. Many tools and guidance documents have been developed, including guidelines for the detection and treatment of MDR/RR-TB and Hr-TB; rapid tests to detect resistance to RIF, INH, FQs, ethionamide (ETO) and amikacin (AMK); model diagnostic testing algorithms; and guidance for scaling up laboratory capacities to combat DR-TB. Based on current treatment recommendations, countries embarking on interventions to detect and treat DR-TB should, in addition, establish laboratory capacity to perform phenotypic DST for drugs that are recommended for use in MDR-TB regimens *(7)* and for which there are reliable DST methods (e.g. bedaquiline [BDQ], linezolid [LZD], clofazimine [CFZ] and delamanid [DLM]). The addition of a new molecular test for pyrazinamide [PZA] testing in the latest guidelines should facilitate testing for this drug. Also, countries should expand their capacity to monitor the culture conversion of patients being treated for DR-TB.

An increasing number of novel tools serve similar purposes; hence, WHO has introduced a class-based recommendation approach. Instead of evaluating and approving individual products, WHO will recommend a *class* that represents a group of products with similar characteristics and performances. This approach is expected to increase competitiveness in price, quality and services. The change was introduced in December 2020, with a Guideline Development Group (GDG) recommending three new classes of tools.

The classes are defined by the type of technology (e.g. automated or reverse hybridization nucleic acid amplification tests [NAATs]), the complexity of the test for implementation (e.g. low, moderate or high – considering the requirements of infrastructure, equipment and technical skills of laboratory staff) and the target conditions (e.g. diagnosis of TB, and detection of resistance to first-line or second-line drugs). The level of complexity is only one of the elements that should guide implementation. Other important elements include diagnostic accuracy, the epidemiological and geographical setting, operational aspects (e.g. turnaround times, throughput, existing infrastructure and specimen referral networks), economic aspects and qualitative aspects on acceptability, equity, and end-user values and preferences.

Linked to this change to class-based recommendations is the joint announcement by the Global TB Programme at WHO (WHO/GTB) and the WHO Prequalification (PQ) Unit on the pathways to a WHO endorsement for new in vitro diagnostics for TB *(8)*. All products will continue to be reviewed by WHO/GTB initially, to determine whether the product warrants a new class:

- if a new class is warranted, the product will be assessed as a "first in class" through a GDG process; and
- if the product is deemed to fall under an existing class with the same recommendations, the dossier will be forwarded to the WHO PQ Unit for assessment, to ensure the quality of the

product and manufacturing processes, and to ensure that the performance of the new test is similar to that of existing products in the class before listing.

## 1.2 About this guide

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

- describes the WHO-recommended tests for detecting TB and DR-TB and the most recent WHO policy guidance for their use, and the processes and steps needed for implementing a diagnostic test for routine use within the TB diagnostic network (Section 2);
- describes the steps that need to be taken to implement a new diagnostic tool (Section 3); and
- outlines TB diagnostic model algorithms that incorporate the most recent WHO recommendations for detecting and treating TB and DR-TB *(9)*, and considerations for the implementation of a new algorithm (Section 4).

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 5 (Suggested reading); rather, the guide provides references and links to original resources.

The most up-to-date WHO policy guidance on TB diagnostics and laboratory strengthening can be found on the WHO/GTB website.[2] Guidance on the implementation of diagnostic testing is also available on the website of the Global Laboratory Initiative (GLI) of the Stop TB Partnership.[3]

## 1.3 Target audience

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

---

[2] See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

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

WHO operational handbook on tuberculosis:
rapid diagnostics for tuberculosis detection

# 2. Diagnostic tests with WHO recommendations

This section provides brief descriptions of WHO-reviewed technologies for the detection of TB and DR-TB, summarizes WHO recommendations for such technologies and refers to WHO policy statements for each test and the WHO *Consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection 2021 update (5)*[4] for a thorough discussion of the technologies and recommendations.

The latest guidelines have added three new classes of NAAT technologies, as shown in Table 2.1.

**Table 2.1. New classes of technologies recommended and associated products evaluated**

| Technology class | Products included in evaluation |
| --- | --- |
| Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid | Abbott RealTi*me* MTB and Abbott RealTi*me* MTB RIF/INH (Abbott) |
| | BD MAX MDR-TB (Becton Dickinson) |
| | cobas MTB and cobas MTB-RIF/INH (Roche) |
| | FluoroType MTBDR and FluoroType MTB (Bruker/Hain Lifescience) |
| Low complexity automated NAATs for detection of resistance to isoniazid and second-line anti-TB agents | Xpert MTB/XDR (Cepheid) |
| High complexity reverse hybridization-based NAATs for detection of resistance to pyrazinamide | Genoscholar PZA-TB II (Nipro) |

NAAT: nucleic acid amplification test; TB: tuberculosis.

The change from product-specific recommendations (e.g. Xpert MTB/RIF or Truenat MTB) to class-based recommendations (e.g. low complexity automated NAATs) is new. Hence, we are in a transitionary period where the previous product-based recommendations have not yet been integrated into the new classes. Integration of previously recommended products into the new classes needs to take into account the product-specific recommendations that, although similar, do differ, particularly for subgroups. The aim is that future updates will consolidate all products into classes.

---

[4]    See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

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

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

The initial tests for the diagnosis of TB are broadly grouped as WHO-approved rapid diagnostic tests (WRDs); these are defined as diagnostic tests that employ molecular or biomarker-based techniques for the diagnosis of TB *(10)*.[5] The newer, rapid and sensitive molecular tests recommended for the initial detection of MTBC and drug resistance are designated as mWRDs; they include Xpert MTB/RIF Ultra and Xpert MTB/RIF (Cepheid, Sunnyvale, United States of America [USA]); Truenat MTB, MTB Plus and MTB-RIF Dx tests (Molbio Diagnostics, Goa, India); and loop-mediated isothermal amplification (TB-LAMP; Eiken Chemical, Tokyo, Japan).

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

In addition, the biomarker-based lateral flow lipoarabinomannan assay (LF-LAM) test (Alere Determine TB LAM Ag, USA) is recommended to assist in diagnosing TB in selected groups of HIV-infected presumptive-TB patients. A positive LF-LAM result is considered as bacteriological confirmation of TB in these patients *(11)*,[6] and this test is also included as a WRD.

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.

The follow-on tests include line-probe assays (LPAs) for detection of resistance to RIF and INH (GenoType MTBDRplus, Bruker/Hain Lifescience, Nehren, Germany; NTM+MDRTB Detection Kit, NIPRO Corporation, Osaka, Japan) and to FQs and second-line injectables agents (GenoType MTBDRsl, Bruker/Hain Lifescience, Nehren, Germany). The two new classes added as follow-on tests include the low complexity automated NAATs for the detection of INH, FQs, ETO and AMK resistance (first in class: Xpert MTB/XDR [Cepheid, Sunnyvale, USA]) and the high complexity reverse hybridization NAAT for the detection of PZA resistance (first in class: Genoscholar PZA-TB II [NIPRO Corporation, Osaka, Japan]).

---

[5]   See https://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf;jsessionid=FD522CF3B90C25716F 96288BFDEA6C75?sequence=1

[6]   See https://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf?sequence=1

## 2.1 Conventional diagnostic tests for the diagnosis of TB

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

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

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

### 2.2.1 Xpert MTB/RIF assay

The Xpert MTB/RIF assay is a cartridge-based automated test that uses 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):*[7]



Source: Reproduced with permission of Cepheid, ©2021. All rights reserved.

- In adults with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used as an initial diagnostic test for TB and detection of RIF resistance rather than smear microscopy, culture and phenotypic DST.
- In children with signs and symptoms of pulmonary TB, Xpert MTB/RIF should be used in sputum, gastric aspirate, nasopharyngeal aspirate or stool specimens as the initial diagnostic test for TB and detection of RIF resistance detection, rather than smear microscopy or culture and phenotypic DST.
- In adults and children with signs and symptoms of TB meningitis, Xpert MTB/RIF should be used in cerebrospinal fluid (CSF) as an initial diagnostic test for TB meningitis rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert MTB/RIF may be used in lymph node aspirate, lymph node biopsy, pleural fluid, peritoneal fluid, pericardial

---

[7]    See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

fluid, synovial fluid or urine specimens as the initial diagnostic test for the corresponding form of extrapulmonary TB rather than smear microscopy or culture.

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

## 2.2.2 Xpert MTB/RIF Ultra assay

The Xpert MTB/RIF Ultra assay (hereafter called Xpert Ultra) uses the same GeneXpert platform as the Xpert MTB/RIF test, and was developed to improve the sensitivity and reliability of detection of MTBC and RIF resistance. To address sensitivity, Xpert Ultra uses two multicopy amplification targets (IS6110 and IS1081) and a larger PCR chamber; thus, Xpert Ultra has a lower LoD than Xpert MTB/RIF (16 colony forming units [cfu]/mL and 131 cfu/mL, respectively). At very low bacterial loads, Xpert Ultra can give a "trace" result, which is not based on amplification of the *rpoB* target and therefore does not give results for RIF susceptibility or resistance. An additional improvement in the Xpert Ultra is that the analysis is based on melting temperature ($T_m$), which allows for better differentiation of resistance-conferring mutations. Planning the transition to the Xpert

Ultra requires special consideration and a GLI document is available to assist in this process *(12)*.[8] WHO recommends using the Xpert Ultra test in the following situations *(5)*:[9]

- In adults with signs and symptoms of pulmonary TB without a prior history of TB or with a remote history of TB treatment (>5 years since end of treatment), Xpert Ultra should be used as the initial diagnostic test for TB and for detection of RIF resistance rather than smear microscopy or culture and phenotypic DST.
- In adults with signs and symptoms of pulmonary TB and a prior history of TB with an end of treatment within the past 5 years, Xpert Ultra may be used as the initial diagnostic test for TB and for detection of RIF resistance rather than smear microscopy or culture and phenotypic DST.
- In children with signs and symptoms of pulmonary TB, Xpert Ultra should be used as the initial diagnostic test for TB rather than smear microscopy or culture in sputum or nasopharyngeal aspirates.

---

[8] See http://stoptb.org/wg/gli/assets/documents/GLI_ultra.pdf

[9] See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

WHO operational handbook on tuberculosis:
rapid diagnostics for tuberculosis detection

- In adults and children with signs and symptoms of TB meningitis, Xpert Ultra should be used in CSF as an initial diagnostic test for TB meningitis rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra may be used in lymph node aspirate and lymph node biopsy as the initial diagnostic test for the detection of lymph node TB, rather than smear microscopy or culture.
- In adults and children with signs and symptoms of extrapulmonary TB, Xpert Ultra should be used for detection of RIF resistance rather than culture and phenotypic DST.
- In adults with signs and symptoms of pulmonary TB who have an Xpert Ultra trace positive result on the initial test, repeated testing with Ultra may *not* be used.
- In children with signs and symptoms of pulmonary TB in settings with a pretest probability of less than 5% and an Xpert Ultra negative result on the initial test, repeated testing with Xpert Ultra in sputum or nasopharyngeal aspirate specimens may *not* be used.
- In children with signs and symptoms of pulmonary TB in settings with a pretest probability of 5% or more and an Xpert Ultra negative result on the first initial test, a repeat of one Xpert Ultra test (for a total of two tests) in sputum and nasopharyngeal aspirate specimens may be used.

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

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

The assays are designed to be operated in peripheral

laboratories with minimal infrastructure and minimally trained technicians, although micropipetting skills are required. A practical guide is available to assist implementers considering these tests *(13)*.[10]

If a positive result is obtained with the MTB or MTB Plus assay, an aliquot of extracted DNA is run on the Truenat MTB-RIF Dx assay to detect mutations associated with RIF resistance. WHO recommends using Truenat MTB, MTB Plus and MTB-RIF Dx tests in the following situations *(5)*:[11]

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

---

[10] See http://stoptb.org/assets/documents/resources/publications/sd/Truenat_Implementation_Guide.pdf

[11] See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

Notes:

- These recommendations apply to the use of the test with sputum specimens from people living with HIV/AIDS (PLHIV), based on extrapolation of the data on test performance with smear-negative sputum specimens; and
- These recommendations apply to the use of the test with sputum specimens from children, based on extrapolation of the data from adults, although the test is expected to be less sensitive in children.

## 2.2.4 Moderate complexity automated NAATs

The moderate complexity automated NAATs class of tests includes rapid and accurate tests for the detection of pulmonary TB from respiratory samples. Overall pooled sensitivity for TB detection was 93.0% (95% confidence interval [CI]: 90.9–94.7%) and specificity 97.7% (95% CI: 95.6–98.8%) (Tables 3.1–3.4 in Section 3). Moderate complexity automated NAATs are also able to simultaneously detect resistance to both RIF and INH, and are less complex to perform than phenotypic DST and LPAs. After the sample preparation step, the tests are largely automated. Overall pooled sensitivity for detection of RIF resistance was 96.7% (95% CI: 93.1–98.4%) and specificity was 98.9% (95% CI: 97.5–99.5%). Fig. 2.1 illustrates the procedures for each test.

**Fig. 2.1. Summary of testing procedures for the newly endorsed moderate complexity automated NAATs** *(14)*

| | Abbott RealTime MTB and MTB RIF/INH | BD MAX MDR-TB | Bruker-Hain FluoroType MTBDR | Roche cobas MTB and MTB-RIF/INH |
|---|---|---|---|---|
| **Maximum # of samples per run** | N = 94 | N = 24 | N = 94 | N = 94 |
| **Specimen reception to results out, measured for 24 samples** | **7 hrs** | **4.6 hrs** | **4.5 hrs** | **5.5 hrs** |
| **Inactivation** | 3:1 IR ↓ Invert several times ↓ Vortex 20/30 sec | 2:1 BD Max STR ↓ Shake 10 times ↓ Pre-incubate 5 min ↓ Shake 10 times | 2:1 Liquefaction ↓ Vortex | 2:1 Cobas MIS ↓ Vortex 30/60 sec |
| **Incubation** | 1 h ↓ Vortex 20/30 sec at 20 min | 25 min | 30 min | 1 h |
| **DNA extraction, amplification & detection** | DNA extraction 4 h 22 min m2000 sp ↓ Seal plate and transfer to instrument ↓ Amplification and detection 2 h 30 min | 3 h 41 min run time to results | DNA extraction 2 h GXT 96 ↓ Seal plate and transfer to instrument ↓ Amplification and detection 1 h 41 min | Sonicate 1 min per sample ↓ Centrifuge 60 sec ↓ 2 h 30 min run time to results |

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

Overall pooled sensitivity for detection of INH resistance was 86.4% (95% CI: 82.8–89.3%) and specificity was 99.2% (95% CI: 98.1–99.7%). These assays offer high-throughput testing and are suitable for high workload settings, so could potentially be used in areas with a large population density or high TB prevalence. However, this class of tests is primarily for laboratory settings, and will require a reliable and rapid system for sample referral and result reporting. Moderate complexity automated NAATs may already be used programmatically for other diseases (e.g. severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2], HIV, and hepatitis B and C) which could potentially facilitate implementation of TB testing on shared platforms.

An information sheet summarizing the individual technologies in this class is at Appendix 2. A detailed comparison of the different products is also available *(14)*.[12]

WHO has made the following recommendation *(5)*:[13]

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

Notes:

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

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

## 2.3.1 TB-LAMP assay

The TB-LAMP assay is designed to detect MTBC directly from sputum specimens. This is a manual assay that provides results in less than 1 hour, does not require sophisticated instrumentation and can be used at the peripheral health centre level, given biosafety requirements similar to those for sputum-smear microscopy. TB-LAMP does not detect resistance to anti-TB drugs. For the detection of TB in adults with signs and symptoms consistent with pulmonary TB, TB-LAMP has demonstrated a sensitivity of 0.78% (95% credible interval [CrI]: 0.71–0.83%) and a specificity of 0.98% (95% CrI: 0.96–0.99%) as compared with a microbiological reference standard.
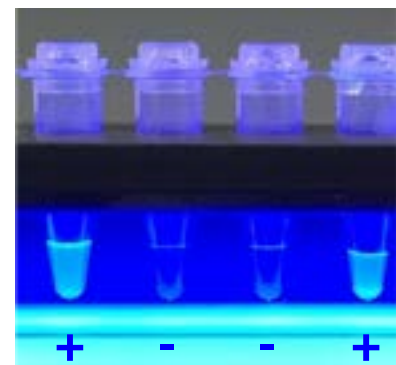


Source: Reproduced with permission of Eiken Chemical CO., LTD., © 2021. All rights reserved.

---

[12]  See https://www.finddx.org/wp-content/uploads/2019/08/FIND_cDST_WHO_Supplement.xlsx

[13]  See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

WHO has made the following recommendations *(5)*:[14]

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

Notes:

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

## 2.3.2 Urine LF-LAM

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

urine does not provide any information on drug resistance. A document is available addressing practical considerations for the implementation of the LF-LAM *(15)*.[15]
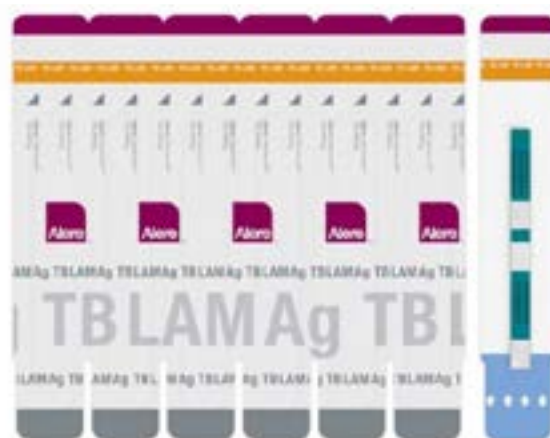
WHO recommends using urine LF-LAM in the following situations *(5)*:

- In inpatient settings, WHO recommends using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:
  - with signs and symptoms of TB (pulmonary or extrapulmonary);
  - with advanced HIV disease;
  - who are seriously ill; or
  - who have a CD4 cell count of less than 200 cells/mm$^3$, irrespective of signs and symptoms of TB.
- In outpatient settings, WHO suggests using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children who:
  - have signs and symptoms of TB (pulmonary or extrapulmonary);
  - are seriously ill; or
  - have a CD4 cell count of less than 100 cells/mm$^3$, irrespective of signs and symptoms of TB.

---

[14] See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

[15] See http://stoptb.org/wg/gli/assets/documents/practical-implementation-lf-lam.pdf

- In outpatient settings, WHO recommends against using LF-LAM to assist in the diagnosis of active TB in HIV-positive adults, adolescents and children:
  - who have not been assessed for TB symptoms; or
  - without TB symptoms and with an unknown CD4 cell count or with a CD4 cell count greater than 100 cells/mm$^3$.

Notes:

- For their initial diagnostic test, all patients with signs and symptoms of pulmonary TB who are capable of producing sputum should have at least one sputum specimen submitted for an mWRD assay. This includes children and adolescents living with HIV who are able to provide a sputum sample. LF-LAM results (test time <15 minutes) are likely to be available before mWRD results; hence, treatment decisions should be based on the LF-LAM result while awaiting the results of other diagnostic tests.
- LF-LAM 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.

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

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

The "first in class" product for low complexity automated NAATs for detection of resistance to INH and second-line anti-TB drugs is the Xpert MTB/XDR Assay (Cepheid, Sunnyvale, USA). This test uses a cartridge designed for the GeneXpert instrument to detect resistance to INH, FQs, ETO and second-line injectable drugs (AMK, kanamycin and capreomycin). However, unlike Xpert MTB/RIF and Xpert MTB/RIF Ultra, which are performed on a GeneXpert instrument that can detect 6 colours, the new test requires a 10-colour GeneXpert instrument. There is an option to combine the 6 and 10-colour systems through a common computer. A bioequivalence study of Xpert MTB/RIF and Xpert MTB/RIF Ultra on the 6- and 10-colour system will be reviewed by WHO later in 2021 and guidance will then be provided.

The low complexity automated NAAT is intended for use as a follow-on test in specimens determined to be MTBC-positive; it offers the chance to improve access to rapid DST in intermediate and even peripheral laboratories. The Xpert MTB/XDR test provides results in less than 90 minutes, leading to faster time to results than the current standard of care (i.e. LPAs or culture-based phenotypic DST). This NAAT requires the same infrastructure and training of technicians as the other Xpert tests.

The overall pooled sensitivity for detection of INH resistance was 94% (95% CI: 89–97%) and specificity was 98% (95% CI: 95–99%) (Table 3.3). Overall pooled sensitivity for detection of FQ resistance was 93% (95% CI: 88–96%) and specificity was 98% (95% CI: 94–99%) (Table 3.4). Thus, Xpert MTB/XDR could be used as a reflex test to complement existing technologies that only test for RIF resistance, allowing the rapid and accurate detection of resistance to INH and FQ in cases of RIF-susceptible TB, and of resistance to FQ, INH, ETO and AMK in cases of RR-TB. The package insert includes the use of the test on culture isolates; performance based

on the FIND study – see Web Annex 4.15 in *(5)*[16] – confirms this potential use case. However, the primary purpose of this test is to achieve rapid and early detection of resistance, and recommendations are for use directly on clinical specimens. Appendix 2 provides an information sheet summarizing this test.

WHO recommends the use of low complexity automated NAATs for detection of resistance to INH and second-line anti-TB drugs in the following situations *(5)*:

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

Notes:

- These recommendations are based on the evidence of diagnostic accuracy in sputum of adults with bacteriologically confirmed pulmonary TB, with or without RIF resistance.
- The recommendations are extrapolated for adolescents and children based on the generalization of data from adults.
- The recommendations apply to PLHIV (studies included a varying proportion of such people). Data stratified by HIV status were not available.
- The recommendations are extrapolated to people with extrapulmonary TB and testing of non-sputum samples was considered appropriate, which affects the certainty. The panel did not evaluate test accuracy in non-sputum samples directly, including in children. However, extrapolation was considered appropriate given that WHO recommendations exist for similar technologies for use on non-sputum samples (e.g. Xpert MTB/RIF and Xpert Ultra).

## 2.4.2 LPAs

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



---
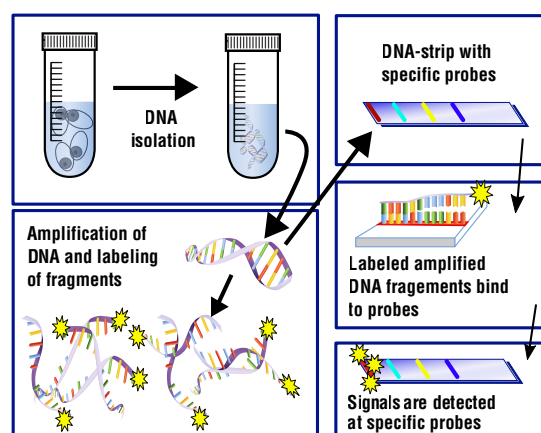
### First-line LPAs

First-line LPAs (FL-LPAs) such as GenoType MTBDR*plus* and NTM+MDRTB Detection Kit allow the detection of resistance to RIF and INH. WHO recommends using FL-LPAs in the following situations *(5)*:[17]

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

Notes:

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

### Second-line LPAs

Second-line LPAs (SL-LPAs) such as the GenoType MTBDR*sl* test allow the detection of resistance to FQs and AMK. WHO recommends using SL-LPAs in the following situations *(5)*:

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

Notes:

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

## 2.4.3 High complexity reverse hybridization NAAT

The "first in class" product for this class is the GenoScholar PZA-TB (Nipro, Osaka, Japan) for the detection of resistance to PZA. The GenoScholar PZA-TB test is based on the same principle as the FL-LPA and SL-LPA but requires the use of a large number of hybridization probes to cover the full *pncA* gene (>700 base pairs [bp]). Reading the hybridization results on the crowded strips with a total of 48 probes requires careful attention to avoid errors. However, it provides faster results than phenotypic DST and is based on molecular detection. The overall pooled sensitivity for the detection of PZA resistance was 81.2% (95% CI: 75.4–85.8%) and the pooled specificity was 97.8% (95% CI: 96.5–98.6%) *(5)*.[18] The hybridization can be performed

---

[17]  See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

[18]  See https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf

on the TwinCubator instruments (Hain Lifescience, Germany) that are used for LPAs *(16)*. An information sheet summarizing high complexity reverse hybridization NAATs is at Appendix 2.

WHO recommends the use of high complexity reverse hybridization NAATs in the following situations *(5)*:

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

Note:

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

# 2.5 Tests WHO recommends against using

Based on reviews of available data, WHO has recommended *against* using tests that do not provide reliable information for diagnosing TB. In 2011, WHO recommended that commercial serologic tests should not be used for the diagnosis of pulmonary and extrapulmonary TB because the commercial serodiagnostic tests available at that time provided inconsistent and imprecise findings; there was no evidence that using those commercial serological assays improved patient outcomes; and the tests generated high proportions of false positive and false negative results, which may have an adverse impact on the health of patients *(17)*.[19]

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, NAATs (e.g. Xpert MTB/RIF, Xpert Ultra or Truenat) are not recommended for use in monitoring the response to treatment. Also, in outpatient settings, WHO recommends against using LF-LAM to assist in the diagnosis of active TB in:

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

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

---

[19]  See https://www.who.int/publications/i/item/9789241502054

# 2.6 Phenotypic and genotypic DST

Treatment of TB has undergone significant changes over recent years, with new drugs and regimens recommended; hence, the definitions for DR-TB have been revised accordingly. The updated pre-XDR-TB definition is "TB caused by *M. tuberculosis* strains that fulfil the definition of MDR/RR-TB and are also resistant to any FQ". The updated XDR-TB definition is "TB caused by *M. tuberculosis* strains that fulfil the definition of MDR/RR-TB and that are also resistant to any FQ and at least one additional Group A drug (i.e. BDQ or LZD)" *(18)*.[20] These changes have important implications for Member States, particularly for scaling up the detection of resistance to FQ and BDQ. In addition, there is an increasing demand for DST for other new and repurposed drugs. In 2018, WHO updated the critical concentrations (CCs) used in phenotypic DST to include the new and repurposed drugs for existing methods *(19)*.[21] A WHO technical manual for DST is also available *(20)*.[22]

The new definition of XDR-TB requires DST results for FQs, BDQ and LZD; thus, testing for resistance to BDQ and LZD has become a priority, particularly testing for BDQ resistance. Culture-based phenotypic DST for BDQ and LZD can be performed using either the mycobacterial growth indicator tube (MGIT) or Middlebrook 7H11 media. The BDQ pure drug substance for use in phenotypic DST is provided free through the US National Institutes of Health (NIH) HIV Reagent Program *(21)*;[23] however, courier costs need to be covered. An information note that explains the request process is also available *(22)*.[24] Delamanid powder can be ordered from the manufacturer, Otsuka, through the website of the American Type Culture Collection (ATCC) (delamanid: NR-51636) *(23)*.[25] Other pure drug substances for laboratory DST and associated catalogues are available through the Global Drug Facility *(24)*.[26]

In addition, CCs for the rifamycins and INH were reviewed, and findings published in 2021 *(25)*.[27] The INH CCs have remained the same, whereas the RIF CCs in MGIT and 7H10 have been revised downwards from 1.0 mg/L to 0.5 mg/L. These revisions are expected to reduce discordance between genotypic and phenotypic DST results, and laboratories are advised to implement these changes immediately. For MGIT, this can be achieved by reconstituting the lyophilized RIF from the BD SIRE kit in 8 mL, instead of 4 mL, of sterile distilled or deionized water.

The latest CCs for all drugs are listed in Table 2.2 *(22)*.[28]

---

[20] See https://www.who.int/publications/i/item/meeting-report-of-the-who-expert-consultation-on-the-definition-of-extensively-drug-resistant-tuberculosis

[21] See https://www.who.int/publications/i/item/WHO_CDS_TB_2018.5

[22] See https://www.who.int/publications/i/item/9789241514842

[23] See https://www.hivreagentprogram.org/

[24] See http://stoptb.org/assets/documents/resources/publications/sd/BDQ_DEL_access.pdf

[25] See https://www.atcc.org

[26] See http://www.stoptb.org/gdf/drugsupply/product_catalog.asp

[27] See https://www.who.int/publications/i/item/technical-report-on-critical-concentrations-for-drugsusceptibility-testing-of-isoniazid-and-therifamycins-%28rifampicin-rifabutin-and-rifapentine%29

[28] See http://stoptb.org/assets/documents/resources/publications/sd/BDQ_DEL_access.pdf

## Table 2.2. CCs and clinical breakpoints for medicines recommended for the treatment of TB

| Drug groups | Drug | LJ | 7H10 | 7H11 | MGIT[a] |
|---|---|---|---|---|---|
| **First-line agents** *(25)*[29]<br>Isoniazid and rifamycins | Isoniazid | 0.2 | 0.2 | 0.2 | 0.1 |
| | Rifampicin[b] | 40 | 0.5 | 1.0 | 0.5 |
| | Rifabutin[c] | – | – | – | – |
| | Rifapentine[d] | – | – | – | – |
| **First-line agents** *(26)*[30]<br>Ethambutol and pyrazinamide | Ethambutol[e] | 2.0 | 5.0 | 7.5 | 5.0 |
| | Pyrazinamide | – | – | – | 100.0 |
| **Agents for the treatment of rifampicin-resistant and multidrug-resistant TB** *(27)*[31] | | | | | |
| **Group A** | Fluoroquinolones:[f] | | | | |
| | Levofloxacin (CC)[g] | 2.0 | 1.0 | – | 1.0 |
| | Moxifloxacin (CC)[g] | 1.0 | 0.5 | 0.5 | 0.25 |
| | Moxifloxacin (CB)[h] | – | 2.0 | – | 1.0 |
| | Bedaquiline[i] | – | – | 0.25 | 1.0 |
| | Linezolid | – | 1.0 | 1.0 | 1.0 |
| **Group B** | Clofazimine[i] | – | – | – | 1.0 |
| | Cycloserine/<br>terizidone[j] | – | – | – | – |
| **Group C** | Ethambutol | 2.0 | 5.0 | 7.5 | 5.0 |
| | Delamanid[i] | – | – | 0.016 | 0.06 |
| | Pyrazinamide | – | – | – | 100.0 |
| | Imipenem-cilastatin/<br>meropenem | – | – | – | – |
| | Amikacin | 30.0 | 2.0 | – | 1.0 |
| | Streptomycin | 4.0 | 2.0 | 2.0 | 1.0 |
| | Ethionamide | 40.0 | 5.0 | 10.0 | 5.0 |
| | Prothionamide | 40.0 | – | – | 2.5 |
| | *p*-aminosalicylic acid | – | – | – | – |

CB: clinical breakpoint; CC: critical concentration; DST: drug-susceptibility testing; LJ: Löwenstein–Jensen; MGIT: mycobacterial growth indicator tube; RFB: rifabutin; RIF: rifampicin; RPT: rifapentine; TB: tuberculosis; WHO: World Health Organization.

Note: All concentrations are in mg/L and apply to the proportion method, with 1% as the critical proportion. Unless otherwise stated, they are CCs rather than CBs. Red font indicates updated CC for RIF in 2021.

[a] MGIT is proposed as the reference method for performing DST for second-line anti-TB medicines.

---

[29] See https://www.who.int/publications/i/item/technical-report-on-critical-concentrations-for-drugsusceptibility-testing-of-isoniazid-and-therifamycins-%28rifampicin-rifabutin-and-rifapentine%29

[30] See http://www.stoptb.org/wg/gli/assets/documents/Updated%20critical%20concentration%20table_1st%20and%202nd%20line%20drugs.pdf

[31] See http://apps.who.int/iris/bitstream/10665/260470/1/WHO-CDS-TB-2018.5-eng.pdf

<sup>b</sup> Additional data are needed to clarify whether the RIF CC for LJ is set correctly. The RIF CC for 7H11 was based on limited data and might be too high given that the RIF CC for 7H10 had to be lowered to 0.5 mg/L.

<sup>c</sup> No CCs were adopted because RFB is not currently recommended for TB treatment by WHO, but the validity of the current CCs of 0.5 mg/L for 7H10, 7H11 and MGIT set by the Clinical and Laboratory Standards Institute could not be confirmed by WHO *(27)*. The conservative approach would be to use genotypic DST and, where applicable, phenotypic DST for RIF as surrogates for RFB DST.

<sup>d</sup> Genotypic DST and, where applicable, phenotypic DST for RIF should serve as surrogates for RPT DST.

<sup>e</sup> Ethambutol 5 mg/L in MGIT is not equivalent to other methods. Ethambutol testing in 7H11 is not equivalent to 7H10. There is insufficient evidence to recommend a change in concentration for any method.

<sup>f</sup> Testing of ofloxacin is not recommended because it is no longer used to treat drug-resistant TB and laboratories should transition to testing the specific fluoroquinolones used in treatment regimens. During this transition, testing of ofloxacin at the CCs (i.e. 4.0 mg/L on LJ, 2.0 mg/L on 7H10, 2.0 mg/L on 7H11 and 2.0 mg/L in MGIT) may be performed instead of testing at the CCs for levofloxacin and moxifloxacin, but not for the CBs for moxifloxacin.

<sup>g</sup> Levofloxacin and moxifloxacin interim CCs for LJ have been established despite very limited data.

<sup>h</sup> CBs for 7H10 and MGIT apply to high-dose moxifloxacin (i.e. 800 mg daily).

<sup>i</sup> Interim CCs have been established.

<sup>j</sup> Cycloserine CC on LJ has been withdrawn due to limited evidence.

Phenotypic DST remains the reference standard for most anti-TB compounds; however, this test is slow and it requires specialized infrastructure and highly skilled staff. New and rapid next-generation technologies are needed at the peripheral level to expedite appropriate therapy and impact patients' outcomes. WHO has developed a target product profile, planned for release in the second half of 2021, to guide research and development to address this need.[32]

DNA sequencing using next-generation sequencing (NGS) is a promising method for rapid detection of mutations associated with drug resistance for many anti-TB drugs *(28)*.[33] NGS-based DST could reduce the need for phenotypic DST for patient-care decisions and DR-TB surveys, and it may be particularly useful for drugs for which phenotypic DST is unreliable or settings that do not have the capacity to perform phenotypic DST. The current NGS systems have limitations, especially 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 central level (i.e. national TB reference laboratory [NTRL] and well-performing regional TB reference laboratories).

Amplification-based targeted NGS assays for detecting DR-TB directly from sputum specimens have been developed. The Deeplex-MycTB assay *(29)*[34] (GenoScreen, Lille, France) is commercially available (CE-IVD and RUO versions available). The assay allows prediction of resistance to 15 anti-TB drugs, with a turnaround time of less than 48 hours. Similarly, the Translational Genomics Research Institute (Phoenix, Arizona, USA) is currently developing a next-generation rapid DST assay that can detect mutations associated with resistance to at least seven drugs *(30)*.[35] Also, through ABL it has commercialized an assay that allows detection of resistance to RIF and INH (i.e. DeepChek-TB RpoB/InhA Genotyping Assay) *(31)*.[36] Furthermore, a new product, the NanoTB Drug Resistance Assay (Oxford Nanopore Technologies, Oxford, United Kingdom of Great Britain and Northern Ireland), can detect resistance to 11 drugs and has the

---

[32] The document will be titled *WHO target product profiles for next-generation drug susceptibility tests at peripheral level*.

[33] See https://apps.who.int/iris/bitstream/handle/10665/274443/WHO-CDS-TB-2018.19-eng.pdf

[34] See https://www.genoscreen.fr/en/genoscreen-services/products/deeplex

[35] See https://www.tgen.org/news/2019/march/18/tgen-tb-test-to-help-eliminate-disease-worldwide/

[36] See https://www.ablsa.com/laboratory-applications/deepchek-hbv-rt-genotyping-dr-assay-2-2/

advantage of working on small devices such as the MinION, a handheld compact device *(32)*.[37] These assays have not yet been reviewed or approved by WHO for clinical use.

One important issue identified when using these technologies is the lack of a standardized and robust single reference source for interpretation of mutations. To address this need, WHO has developed guidance *(33)*[38] and has released a catalogue of mutations in MTBC and their association with resistance. This is the largest collated database of MTBC isolates (>38 000) with matched phenotypic DST and whole genome sequences. The methods used to generate the catalogue and findings are presented in the report that accompanies the full catalogue, which is now available.[39] Future regular updates are planned. The catalogue is also expected to support the development of other rapid molecular DST methods.

---

[37]  See https://nanoporetech.com/resource-centre/rapid-diagnosis-drug-resistant-tuberculosis-using-nanopore-sequencing

[38]  See https://www.who.int/publications/i/item/WHO-CDS-TB-2018.19

[39]  Catalogue of mutations in *Mycobacterium tuberculosis* complex and associated drug resistance.

WHO operational handbook on tuberculosis:
rapid diagnostics for tuberculosis detection

# 3. Implementing a new diagnostic test

## 3.1 Placement of diagnostic tests in the tiered laboratory network

Diagnostic tests that are implemented should:

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

The decision on where to place a specific test is an important one that can lead to success or failure in achieving these desired outcomes. Also, a diagnostic test should not be seen in isolation from the broader ecosystem of tests (TB and non-TB) used to deliver results for clinical management.

In many resource-limited or high-burden settings, TB laboratory networks have a pyramidal structure, as shown in Fig. 3.1. This structure has the largest number of laboratories at the peripheral level (Level I); a moderate number of intermediate laboratories (Level II), usually located in mid-sized population centres and health facilities; and a single (or more than one in large countries) central laboratory (Level III) at the provincial, state or national level. Each level or tier has specific requirements for infrastructure and biosafety, defined by the activities and diagnostic methods being performed in the laboratories.

### 3.1.1 Peripheral level

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

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

These mWRDs offer the advantage of higher sensitivity for TB detection and detection of RIF resistance. The Truenat MTB can run on battery for periods of time and is thus useful where the electricity supply is unstable; also, it can operate at higher temperatures (up to 40 °C) *(34)*.[40]

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

### 3.1.2 Intermediate level

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

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

### 3.1.3 Central level

At the central level (Level III), testing requiring highly advanced skills, infrastructure and biosafety precautions is offered. An important expectation at this level is to provide testing to resolve discord results, troubleshooting, training support to other levels, QA and monitoring, and surveillance.

---

[40] See http://stoptb.org/assets/documents/resources/wd/Practical%20Considerations%20for%20Implementation%20of%20Truenat.pdf

- FL-LPA, SL-LPA and NGS are all applicable at this level. The moderate complexity automated NAATs with high throughput could also be considered at this level. In addition, establishing capacity for sequencing is becoming increasingly important. The new class of high complexity reverse hybridization NAAT for PZA is suited to this level. It is the first rapid molecular test for resistance to this important drug and can make use of existing LPA infrastructure.
- Culture and phenotypic DST using solid or liquid media should be available at this level. At a minimum, phenotypic DST for the new and repurposed drugs should be available.

**Fig. 3.1. Organization of a TB diagnostic network**

**Level III**
Surveillance
Reference methods
Network supervision

**CENTRAL**
reference level

all tests performed at lower levels and culture using liquid media, phenotypic DST, genome sequencing

**Level II**
Case-finding
treatment

**INTERMEDIATE**
regional and district levels

all tests performed at lower levels
culture on solid media
FL-LPA, SL-LPA, moderate complexity automated NAAT, high complexity reverse hybridisation NAAT, genome sequencing

**Level I**
Screening
Case-finding
Referrals
Treatment

**PERIPHERAL**
subdistrict and community levels

Xpert MTB/RIF, Xpert Ultra, Truenat MTB, TB-LAMP, AFB smear microscopy, LF-LAM, low complexity automated NAAT, specimen referral

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

## 3.1.4 Structure of network and testing packages

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

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

Although the levels described are useful conceptually, in practice they may overlap considerably. Careful logistical planning by mapping the current network of health facilities, population densities, the testing burden across the different facilities, transport infrastructure and the available laboratory network will aid placement. As an example, primary TB testing of presumptive patients will be relevant across all health facilities where individuals are screened for TB. In contrast, patients with RR-TB may be managed at selected sites, and placement of testing for FQ and BDQ resistance may only be needed in selected laboratories that serve those sites.

The *Framework of indicators and targets for laboratory strengthening under the End TB Strategy* can serve as a guide for implementing and monitoring improvements to TB testing and TB diagnostic networks *(6)*.[41]

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

- resources available for implementation;
- infrastructural requirements;
- biosafety requirements;
- specimen types and collection procedures;
- projected testing volumes;
- requirement for rapid diagnosis of severely ill patients;
- minimum number of tests needed to maintain expertise and optimal use of instruments;
- current and planned testing algorithms;
- trained human resources (HR) capacity;
- links to other laboratories for further testing;
- specimen referral and result reporting systems; and
- possibility of integration with testing, specimen referral and reporting systems for other diseases.

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

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

The *GLI guide to TB specimen referral systems and integrated networks (35)*[42] and the *GLI specimen referral toolkit (36)*[43] are useful sources of information for designing, implementing and monitoring systems for referring specimens and reporting results.

---

[41] See https://www.who.int/publications/i/item/9789241511438

[42] See http://www.stoptb.org/wg/gli/assets/documents/GLI_Guide_specimens_web_ready.pdf

[43] See http://www.stoptb.org/wg/gli/srt.asp

## 3.2 Pretest probability and test accuracy considerations

The predictive values of a test vary depending on the prevalence of TB in the patient population being tested. Table 3.1 provides examples of population-level projections of the results of testing with the various mWRDs in settings with different TB prevalence, based on pooled sensitivity and specificity estimates that were extracted from the WHO diagnostic policy statements for each test. Tables 3.2–3.4 provide those same parameters for detection of resistance to RIF, INH and FQs, respectively. The sensitivity of the test may be lower when used for active case finding in a population screening context because such patients would be less sick and have a lower bacillary burden. In choosing a test to implement, countries will need to consider the possible trade-offs between higher or lower sensitivity and higher or lower specificity, based on the prevalence of TB in their country. False negative results may lead to missed opportunities to treat TB. False positive results may lead to the overtreatment of 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.

Usually, a decision to undertake a diagnostic work-up of an individual for TB begins with assessing symptoms and signs of TB disease. However, many individuals with culture-positive TB may not have symptoms or may consider the symptoms too insignificant to report, leading to missed diagnostic opportunities. To improve TB case detection and identify individuals suitable for TB preventive treatment, WHO has updated the TB screening guidelines.[44] Several modalities are recommended for screening: symptom screening, chest X-ray and mWRDs. For screening PLHIV, recommended screening modalities include the classical four-symptom screen, chest X-ray, and an mWRD or positive C-reactive protein test (>5 mg/L).

Chest X-ray as a screening or triage tool can identify individuals to be tested with an initial molecular test and can thus reduce the number of individuals tested and the associated costs but the CXR cost would need to be lower than the test costs *(37, 38)*.[45] This approach is likely to improve the pretest probability for TB and therefore should improve the predictive value of the molecular test and reduce false positive results, especially in populations with a low prevalence of TB. For example, the addition of chest X-ray as a screening tool to an algorithm in which all individuals with an abnormal chest X-ray receive mWRD was calculated to increase the positive predictive value of the mWRD from 56.8% to 78.5% and the prevalent cases detected from 69% to 80%, compared with testing with an mWRD, irrespective of symptoms, in a population with a TB prevalence of 1% *(39)*.[46]

---

[44] See https://www.who.int/publications/i/item/9789240022676

[45] See https://www.who.int/publications/i/item/9789241548601 and https://www.who.int/publications/i/item/9789241511506

[46] See https://www.who.int/publications/i/item/9789240022676

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

| Intervention | Test accuracy (%) | Studies (participants) | Certainty of evidence | 2.5% prevalence | 10% prevalence | 30% prevalence |
|---|---|---|---|---|---|---|
| **Xpert MTB/RIF** | Se: 0.85 (95% CrI: 0.82–0.88) | 70 (10 409) | High | TP: 21 / FN: 4 | TP: 85 / FN: 15 | TP: 255 / FN: 45 |
| | Sp: 0.98 (95% CrI: 0.97–0.98) | 70 (26 828) | High | TN: 965 / FP: 10 | TN: 891 / FP: 9 | TN: 693 / FP: 7 |
| **Xpert MTB/RIF Ultra** | Se: 0.90 (95% CrI: 0.84–0.94) | 6 (960) | High | TP: 22 / FN: 3 | TP: 90 / FN: 10 | TP: 269 / FN: 31 |
| | Sp: 0.96 (95% CrI: 0.93–0.98) | 6 (1694) | High | TN: 932 / FP: 43 | TN: 860 / FP: 40 | TN: 669 / FP: 31 |
| **MC-aNAAT** | Se: 0.93 (95% CI: 0.91–0.95) | 29 (4767) | Moderate | TP: 23 / FN: 2 | TP: 93 / FN: 7 | TP: 279 / FN: 21 |
| | Sp: 0.98 (95% CI: 0.96–0.99) | 29 (9085) | High | TN: 953 / FP: 22 | TN: 879 / FP: 21 | TN: 684 / FP: 16 |
| **Truenat MTB** | Se: 0.73 (95% CI: 0.68–0.78) | 1 (258) | Moderate | TP: 18 / FN: 7 | TP: 73 / FN: 27 | TP: 220 / FN: 80 |
| | Sp: 0.98 (95% CI: 0.97–0.99) | 1 (1122) | High | TN: 957 / FP: 18 | TN: 884 / FP: 16 | TN: 687 / FP: 13 |
| **Truenat MTB Plus** | Se: 0.80 (95% CI: 0.75–0.84) | 1 (261) | Moderate | TP: 20 / FN: 5 | TP: 80 / FN: 20 | TP: 239 / FN: 61 |
| | Sp: 0.96 (95% CI: 0.95–0.97) | 1 (1087) | High | TN: 940 / FP: 25 | TN: 868 / FP: 32 | TN: 675 / FP: 25 |
| **TB-LAMP** | Se: 0.78 (95% CrI: 0.71–0.83) | 7 (1810) | Very low | TP: 20 / FN: 5 | TP: 78 / FN: 22 | TP: 234 / FN: 66 |
| | Sp: 0.98 (95% CrI: 0.96–0.99) | 7 (1810) | Very low | TN: 955 / FP: 20 | TN: 882 / FP: 18 | TN: 686 / FP: 14 |

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

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

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

| Intervention | Test accuracy | Studies (participants) | Certainty of evidence | 2% prevalence | 10% prevalence | 15% prevalence |
|---|---|---|---|---|---|---|
| **Xpert MTB/RIF** | Se: 0.96 (95% CI: 0.94–0.97) | 48 (1775) | High | TP: 19 / FN: 1 | TP: 96 / FN: 4 | TP: 144 / FN: 6 |
| | Sp: 0.98 (95% CI: 0.98–0.99) | 48 (6245) | High | TN: 960 / FP: 20 | TN: 882 / FP: 18 | TN: 833 / FP: 17 |
| **Xpert MTB/RIF Ultra** | Se: 0.94 (95% CI: 0.87–0.97) | 5 (240) | High | TP: 19 / FN: 1 | TP: 94 / FN: 6 | TP: 141 / FN: 9 |
| | Sp: 0.99 (95% CI: 0.98–1.00) | 5 (690) | High | TN: 970 / FP: 10 | TN: 891 / FP: 9 | TN: 842 / FP: 8 |
| **Truenat MTB-RIF Dx** | Se: 0.84 (95% CI: 0.72–0.92) | 1 (51) | Very Low | TP: 17 / FN: 3 | TP: 84 / FN: 16 | TP: 126 / FN: 24 |
| | Sp: 0.97 (95% CI: 0.95–0.99) | 1 (258) | Moderate | TN: 951 / FP: 29 | TN: 873 / FP: 27 | TN: 825 / FP: 25 |
| **MC-aNAAT** | Se: 0.97 (95% CI: 0.93–0.98) | 18 (702) | Moderate | TP: 19 / FN: 1 | TP: 97 / FN: 3 | TP: 146 / FN: 4 |
| | Sp: 0.99 (95% CI: 0.97–0.99) | 18 (2172) | High | TN: 970 / FP: 10 | TN: 891 / FP: 9 | TN: 842 / FP: 8 |
| **FL-LPA by direct testing of SS+ samples** | Se: 0.96 (95% CI: 0.95–0.97) | 48 (2876) | Moderate | TP: 19 / FN: 1 | TP: 96 / FN: 4 | TP: 144 / FN: 6 |
| | Sp: 0.98 (95% CI: 0.97–0.99) | 48 (7684) | Moderate | TN: 960 / FP: 20 | TN: 882 / FP: 18 | TN: 833 / FP: 17 |

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

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

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

| Intervention | Test accuracy | Studies (participants) | Certainty of evidence | 2% prevalence | 10% prevalence | 15% prevalence |
|---|---|---|---|---|---|---|
| **MC-aNAAT** | Se: 0.86 (95% CI: 0.83–0.89) | 18 (854) | Moderate | TP: 17 / FN: 3 | TP: 86 / FN: 14 | TP: 129 / FN: 21 |
| | Sp: 0.99 (95% CI: 0.98–1.00) | 18 (1904) | High | TN: 970 / FP: 10 | TN: 891 / FP: 9 | TN: 842 / FP: 8 |
| **LC-aNAAT** | Se: 0.94 (95% CI: 0.89–0.97) | 3 (994) | Moderate | TP: 19 / FN: 1 | TP: 94 / FN: 6 | TP: 141 / FN: 9 |
| | Sp: 0.98 (95% CI: 0.95–0.99) | 3 (611) | Moderate | TN: 960 / FP: 20 | TN: 882 / FP: 18 | TN: 833 / FP: 17 |
| **FL-LPA by direct testing of SS+ samples** | Se: 0.89 (95% CI: 0.86–0.92) | 46 (3576) | Moderate | TP: 18 / FN: 2 | TP: 89 / FN: 11 | TP: 134 / FN: 16 |
| | Sp: 0.98 (95% CI: 0.97–0.99) | 46 (6896) | Moderate | TN: 960 / FP: 20 | TN: 882 / FP: 18 | TN: 833 / FP: 17 |

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

[a] The isoniazid resistance detection by MC-aNAAT occurs only in cases where TB is detected. That is why suggested prevalence, reflecting INH resistance in newly detected TB patients, also applies to this technology class.

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

| Intervention | Test accuracy | Studies (participants) | Certainty of evidence | 1% prevalence | 5% prevalence | 10% prevalence |
|---|---|---|---|---|---|---|
| **LC-aNAAT** | Se: 0.93 (95% CI: 0.88–0.96) | 3 (384) | High | TP: 9 / FN: 1 | TP: 47 / FN: 3 | TP: 93 / FN: 7 |
| | Sp: 0.98 (95% CI: 0.94–0.99) | 3 (953) | Moderate | TN: 973 / FP: 17 | TN: 934 / FP: 16 | TN: 885 / FP: 15 |
| **SL-LPA by direct testing of SS+ samples** | Se: 0.86 (95% CI: 0.75–0.93) | 9 (519) | Moderate | TP: 9 / FN: 1 | TP: 43 / FN: 7 | TP: 86 / FN: 14 |
| | Sp: 0.99 (95% CI: 0.97–0.99) | 9 (1252) | High | TN: 980 / FP: 10 | TN: 937 / FP: 13 | TN: 887 / FP: 13 |

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

# 3.3 Epidemiologic considerations

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

- TB cases resistant to RIF, INH and FQs;
- PLHIV;
- TB that is extrapulmonary;
- TB among children;
- those with severe illness requiring rapid diagnosis; and
- hospitalized versus ambulatory patients.

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

# 3.4 Multi-disease platform considerations

Health needs are diverse, and programmes are expected to provide a range of diagnostics to assist health workers in managing patients. The diagnosis of TB often begins with symptom screening, which is not specific to TB, given that cough and fever overlap with COVID-19 and other respiratory infections. Additionally, TB patients may be coinfected with HIV, particularly in sub-Saharan Africa, and services for both diseases are usually provided at the same levels of care. The relative diagnostic volumes are also quite heterogeneous and can be low when considered by disease and by day at peripheral health centres, justifying the need for multi-disease testing using the same equipment.

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

Multi-disease testing has the advantage of shared financial costs for equipment purchasing and maintenance, as well as human resources. Efficiencies could also be achieved because such testing could result in optimal use of equipment and batch sizes. However, if not planned well, such testing could have the opposite effect. Equitable access and shared prioritization of testing are important to ensure that a group of patients with a particular disease are not disadvantaged. The overall laboratory budgets should ensure fair distribution based on burden of disease and need.

Multi-disease testing is mainly useful where the numbers of tests by individual programmes are small within a particular setting. In contrast, scenarios where there are large TB and HIV

testing needs and infrastructure is installed to meet the demand, multi-disease testing will be less relevant. Nonetheless, the burden of disease and testing volumes change over time; hence, the use of equipment should be monitored and programmes may need to adapt.

## 3.5 Steps and processes for implementing a new diagnostic test

**Box 3.1 Key steps in implementing a new diagnostic test**

➜ Establish a technical working group to lead the process

➜ Define the intended use of the new test, and update diagnostic algorithms

➜ Develop a realistic costed implementation plan and budget for ongoing costs

➜ Procure and install equipment in safe, functional testing sites

➜ Ensure a reliable supply of quality-assured reagents and consumables

➜ Develop SOPs and clinical protocols

➜ Implement a comprehensive QA programme

➜ Implement training, mentoring and competency assessment programmes

➜ Monitor and evaluate the implementation and impact of the new test

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

The key steps in implementing a new test are listed in Box 3.1. Critical early steps include defining the intended use of the new test, developing a costed implementation plan, building the infrastructure (instruments and facilities) and developing the human resources needed for the new test. The following sections organize the key steps into 10 main areas:

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

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

## Area 1 – Policies, budgeting 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 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;
- specimen transport systems logisticians for centralized or regional testing (TB and non-TB);
- community representatives; and
- clinical staff.

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

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

The TWG members should familiarize themselves with the contents of the relevant WHO policies, guidance and reports, as well as any available implementation guides from WHO, GLI, FIND and implementing partners. Particular attention should be paid to WHO policies and recommendations on using the test to aid in the diagnosis of TB or detection of drug resistance, the test's limitations and interpretation of test results.

## Step 1.3 – Define immediate and future purposes of the test

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

## Step 1.4 – Update national diagnostic algorithm and guidelines

The TWG should lead a review of existing national diagnostic algorithms, taking into consideration the needs of TB patients, clinical needs, country epidemiology, existing testing algorithms, sample referral systems and other operational considerations, and make recommendations to the MoH and NTP. Section 4 provides details on model algorithms for the use of WHO-recommended tests in detail.

The TWG should also lead a review of guidelines for the use of the new diagnostic test results in patient-care decisions. Clinical guidelines should provide clear guidance to clinicians, nurses and health care professionals on the intended use of the new diagnostic test; outline target 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. Of particular relevance is the specimen referral system. A checklist for evaluating a specimen referral system can be found in the relevant GLI publication *(35)*.[47]

For the prospective testing site, detailed assessments of the laboratory's readiness with respect to physical facilities, staffing and infrastructure will be needed. Because laboratory-acquired TB infection is a well-recognized risk for laboratory workers, undertaking a risk assessment for conducting the new test in the prospective site is critical, to ensure that the required biosafety requirements are in place before the new test is implemented *(40)*.[48]

## Step 1.6 – Develop a costed operational plan for phased implementation

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

---

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

[48]   See https://www.who.int/publications/i/item/9789241504638

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 issues

> **2.1 Determine importation requirements**
>
> **2.2 Conduct country validation and verification studies 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 validation and verification studies as required

Validation includes conducting large-scale evaluation studies to measure performance of the test if there is any possibility that country-specific factors (e.g. prevalence of different mutations or microorganism strains) may cause performance to deviate substantially from the manufacturer's results or other evaluation studies. Validation is also required before commencing testing of clinical specimens in cases where laboratories perform non-standard or modified methods, use tests outside their intended scope (e.g. specimens for which the test has not been validated) or use methods developed in-house. These studies, in addition to testing a well-characterized panel of known positive and negative samples, may include prospectively testing the current gold standard and the new test in parallel on clinical specimens *(41).*[49]

Verification includes small-scale method evaluation studies in cases where commercial tests are used according to the manufacturer's intended use. This usually involves testing a well-characterized panel of known positive and negative samples (in a blinded fashion) in line with requirements for national or international accreditation schemes *(41)*.

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 such large-scale studies. Instead, implementing laboratories should conduct small-scale verification *(42)*[50] studies to demonstrate that the laboratory can achieve the same performance characteristics that were obtained during

---

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

[50]  See https://www.iso.org/standard/56115.html

the validation studies when using the test as described in those 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 need 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. Sufficient time 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 in the implementation process is selecting appropriate instruments that fit the needs of the clinical or microbiological laboratory and can be used to perform the new diagnostic test. The most suitable instrument for a country will depend on the intended use of the diagnostic test. In general, it is important to choose an instrument that is broadly available and has good manufacturer supply distribution and support.

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 *(43)*.[51] In settings where TB diagnostic services are standalone and there is a high workload for TB testing, dedicated instruments may be preferred.

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

### Step 3.2 – Instrument verification and maintenance

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

---

[51]   See https://www.who.int/publications/i/item/WHO-HTM-TB-2017.06

Many tests rely on precision instruments that require regular preventive maintenance, and ad hoc servicing and maintenance. The end-user should perform regular preventive maintenance, to ensure good performance of the instrument. Suppliers or authorized service providers should perform on-request maintenance, as necessary. Countries should take advantage of any available extended warranties or service contracts to ensure continued functioning of the instruments.

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

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

A functional testing site requires testing instruments to be properly positioned in a clean, secure and suitable location. Most instruments will require an uninterrupted supply of power, and appropriate working and storage conditions (e.g. humidity and temperature controlled). A safe environment requires WHO biosafety recommendations for conducting the diagnostic test to be followed in appropriate containment facilities with adequate ventilation; it also requires appropriate personal protective equipment to be used, and biologic waste to be disposed of safely and in accordance with regulations. Failure to provide a functional and safe work environment can affect the quality and reliability of testing.

## 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; and streamlining of importation and in-country distribution procedures to ensure sufficient shelf life of reagents and consumables, once they reach testing sites;
- careful monitoring of consumption rates, tracking of reagent-specific shelf lives and forecasting to avoid expirations or stock-outs;
- careful planning to ensure that sites have received training and that equipment has been installed ahead of shipment of reagents;

- ongoing monitoring of all procurement and supply chain steps, to ensure that delays are minimized and that sites receive correct reagents as per the planned schedule; and
- regular reassessment of purchasing and distribution strategies, to ensure that they are responsive to needs and the current situation.

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

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

New-lot testing, also known as lot-to-lot verification, should be performed on new batches of reagents or test kits. Such testing usually involves testing a sample of the new materials and comparing the results to an existing lot of materials with known performance. Preferably, new-lot testing of commercially available test kits is performed at the central (e.g. NTRL) or regional level, thereby ensuring that kits with test failures are not distributed. At the testing site, new-lot testing is needed for reagents prepared at that site; it may also be needed to monitor conditions during transport and storage of test kits within the country. For quality control (QC), 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 QC) and external quality assessment (see Area 7);
- recording and reporting of results (see Area 8); and
- waste management.
- A well-defined, comprehensive set of SOPs that addresses all aspects of the laboratory testing processes – from sample collection to reporting of results – will be essential; in part, because errors at any step can have a significant impact on the quality of testing. Some SOPs will rely on the manufacturer's protocols included with commercial kits whereas others will need to be developed. SOPs must be made readily available for staff and must be updated regularly.

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

A comprehensive plan to implement a new diagnostic test must address all relevant parts of the diagnostic cascade, not just what happens in the laboratory. In addition to laboratory-related SOPs, clear clinical protocols and guidance will be needed for selecting patients to be tested, ordering tests, interpreting test results, reporting and making patient-care decisions. Before the introduction of a new diagnostic test or any changes in an existing test, all clinical staff involved in diagnosis and management of patients must be informed about the planned changes, and relevant training must be conducted. Information must also be shared with clinical staff at all referral sites through staff training opportunities and through use of standardized educational materials developed by the NTP.

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

## Area 6 – Digital data

**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 consider software and hardware requirements, to take advantage of digital data. "Diagnostics connectivity" refers to the ability to connect diagnostic test devices that produce results in a digital format, in such a way as to transmit data reliably to a variety of users *(44)*.[52] Key features of the systems are the ability to monitor performance remotely, conduct QA and manage inventory. With remote monitoring, designated individuals can use any internet-enabled computer to access the software, providing an overview of the facilities, devices and commodities in the network. Software can track consumption and inventory to avoid stock-outs and expiring supplies. It can also identify commodity lots or specific instruments with poor performance or abnormal error rates for QA purposes, and provide a pre-emptive service to avoid instrument failure. This approach is a highly cost-effective way to ensure that a diagnostic device network functions properly; it is also useful for reporting and connecting with treatment sites.

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

- clinicians and patients, which allows for faster patient follow-up;
- laboratory information management systems or electronic registers, reducing staff time and the chance of transcription errors, and greatly facilitating monitoring and evaluation processes; and

---

[52] See http://www.stoptb.org/WG/gli/assets/documents/gli_connectivity_guide.pdf

- the NTP, to assist with surveillance of disease trends or resistance patterns and rates, and to enhance the capacity of the NTP to generate the data needed for performance indicators of the End TB Strategy.

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

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

## Area 7 – Quality assurance, control and assessment

> **7.1 Implement a comprehensive QA programme**
>
> **7.2 Establish and monitor QCs**
>
> **7.3 Develop an external quality assessment 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 competency assessment (Area 9);
- instrument verification and maintenance (Area 3);
- method validation or verification (Area 2);
- lot-to-lot testing (Area 4);
- internal QC;
- external quality assessment (EQA); and
- quality indicator monitoring and continuous quality improvement.

A comprehensive discussion of the essential elements of a QA system can be found in the *GLI practical guide to TB laboratory strengthening (41)*.[53] 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; the aim is to detect 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

---

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

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 comparisons between laboratories, regular on-site supportive supervision and timely feedback, corrective actions and follow-up. On-site supervision should be prioritized at poorly performing sites identified through proficiency testing, monthly monitoring of performance indicators or site assessments. Failure to enrol in a comprehensive EQA programme is a missed opportunity to identify and correct problems that affect the quality of testing.

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

## Proficiency testing

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

## Re-checking of samples

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

## On-site supervisory visits

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

*Step 7.4 – Monitor and analyse quality indicators*

Routine monitoring of quality indicators, also known as performance indicators, is a critical element of assuring the quality of any diagnostic test. In addition to the general laboratory quality indicators recommended in the relevant GLI guide *(41)*,[54] 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 format of the country's current test requisition form (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, considering the cost and time required for such a revision. If a system is not already in place, countries should establish a numbering system to identify repeat samples from the same patient, to monitor the proportion and performance of repeat tests.

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

---

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

The forms used for reporting test results must balance the need to convey the test information while also conveying the information that is essential to allow a clinician to interpret the results and act promptly on them. An easy-to-read format is important because there is likely to be a wide range of expertise among the clinicians interpreting test results.

### Step 8.2 – Review and revise laboratory and clinical registers

Current laboratory and clinical registers that are based on the WHO reporting framework *(11)*[55] may need to be modified to record the results of the diagnostic test being implemented. Forms for laboratory records may also need to be modified. Countries should implement a standardized approach for recording test results in laboratory and clinical registers, and should use the approach consistently across all testing and clinical sites. Countries with electronic laboratory information management systems may need to include news tests in the software package.

## Area 9 – Human resource 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, and should be offered for the different levels of personnel (e.g. managers, senior technologists, technicians and laboratory assistants). Implementing a diagnostic test requires training beyond the steps required to carry out the test, and the manufacturer-supplied on-site training following installation is often too short to cover QA activities. The testing site manager must ensure that test users are trained in the operation and maintenance of the test instrument, correct performance of the test and associated QA activities.

Clinician training or sensitization must be done in parallel with training of laboratory staff, to ensure that all clinicians involved in the screening and care of 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 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.

---

[55]  See https://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf?sequence=1

# 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. Indicators to assess the impact of test objectives should be developed. For each such indicator, programmes should define the purpose, target, data elements and data sources as well as how the indicator is to be calculated, process indicators and corresponding data elements that contribute to the main indicator.

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

As part of demonstrating a test's impact, and to assist with planning and policy-making, programmes should also consider evaluating the cost–effectiveness and end-user perspective of a test 1 year after its implementation, followed by regular evaluation 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.

# 4. Model 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 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 WRDs, are illustrative and countries must adapt them to their local situation.

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 mWRDs as the initial diagnostic tests and is appropriate for all settings, although the choice of which mWRD to use may differ in a setting with high MDR/RR-TB prevalence (e.g. a test that detects MTBC and RIF with or without INH resistance may be needed), with high HIV prevalence (e.g. a more sensitive test may be needed) or with high Hr-TB prevalence (where a test that detects MTBC, RIF and INH resistance simultaneously will be needed).
- **Algorithm 2** incorporates the most recent WHO recommendations for the use of the LF-LAM as an aid in the diagnosis of TB in PLHIV and is most relevant to settings with a high HIV prevalence. However, Algorithm 2 is applicable to any PLHIV who meet the testing criteria, regardless of the underlying prevalence of HIV in that setting.
- **Algorithm 3** and **Algorithm 4** are for follow-up testing, after TB is diagnosed, to detect additional drug resistance:
  - Algorithm 3 is used when the purpose is to detect resistance to second-line drugs in patients with RIF resistance; and
  - Algorithm 4 is used when the purpose is to detect resistance to INH in patients at risk of Hr-TB and with RIF susceptibility, or when Hr-TB (RIF-susceptible and INH-resistant TB) is detected and follow-on testing is needed (e.g. FQ resistance detection).

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 settings 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. In general, the algorithms do not show a specific test; rather, they provide a flow of the expected outcomes of the test that are common across the algorithms (e.g. TB detected) and the follow-up action required.

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

The diagnostic pathway begins with a person being screened positive for TB. WHO has released updated recommendations on TB screening, and readers are encouraged to consult the latest guidance (39). However, presumptive TB patients may not always present with symptoms that match the latest screening guideline recommendations but still have an increased probability for TB disease requiring diagnostic testing. The modalities for screening, beyond the four-symptom screen, now include chest X-ray, an mWRD used as a screening tool or C-reactive protein in PLHIV. The addition of mWRD for screening of selected at-risk populations and settings goes beyond its primary use as an initial line diagnostic tool and the different uses should not be confused. However, priority should be given to ensuring universal access to mWRDs as a diagnostic test for TB and DR-TB before extending its use to screening. Furthermore, the use of mWRDs as a screening test would have large financial and operational implications, and should be carefully considered.

People who are referred for diagnostic evaluation after a positive screen for TB using modalities other than an mWRD should go through a clinical evaluation, including other relevant investigations if available (e.g. chest X-ray) and follow Algorithm 1 to reach a bacteriologically confirmed diagnosis. Among those who are referred for diagnostic evaluation after a positive screen for TB using an mWRD,[56] i.e. used in a screening rather than a diagnostic context, the pretest probability is an important consideration in addition to the clinical picture when deciding to repeat the mWRD or proceed with treatment. Due to the high specificity even when used as a screening test (99%), a positive test is likely to be a true positive when the pretest probability is high.

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

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

---

[56] See https://www.who.int/publications/i/item/9789240022676

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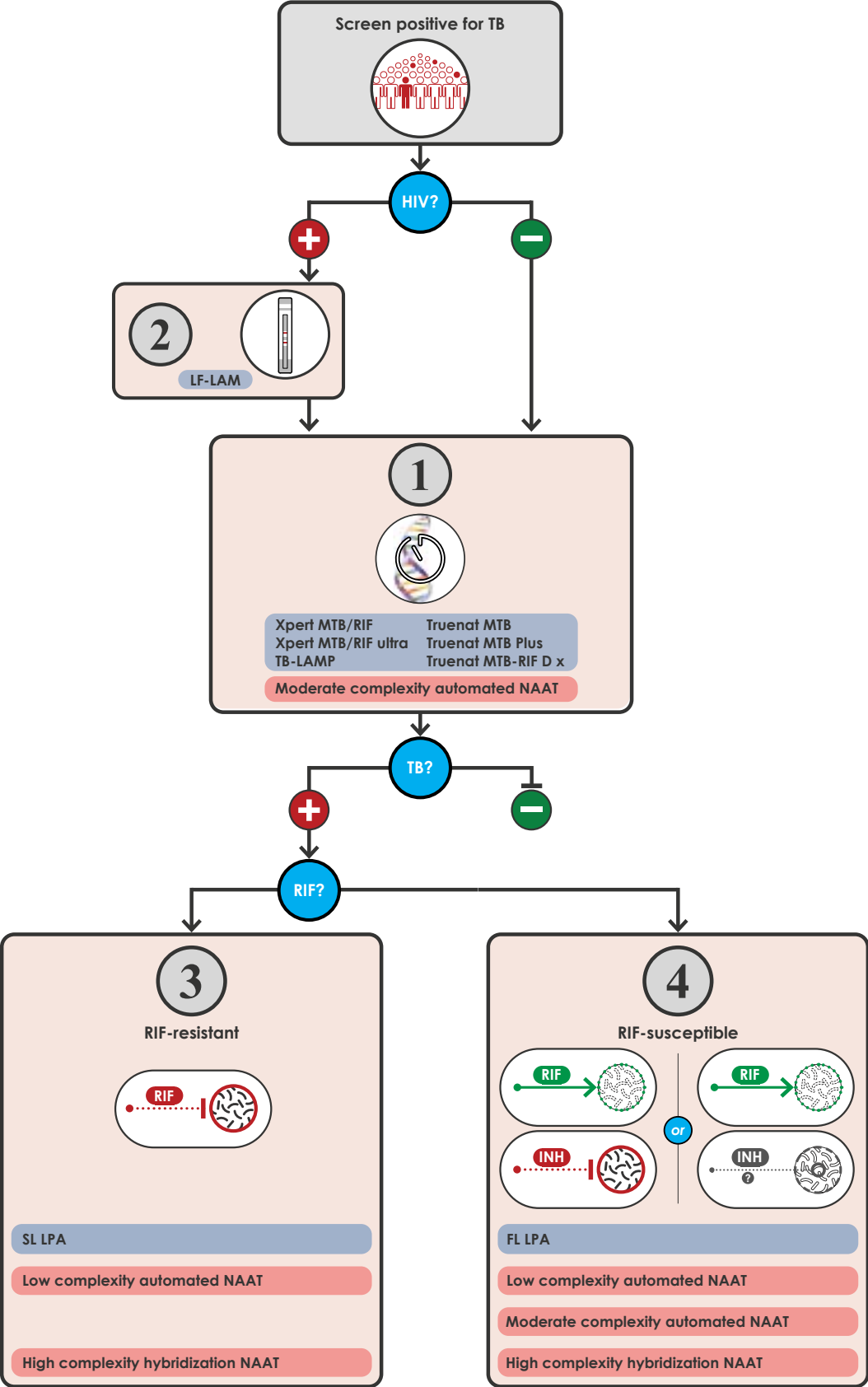


FL-LPA: line-probe assay for first-line drugs; LF-LAM: lateral flow lipoarabinomannan assay; NAAT: nucleic acid amplification test; RIF: rifampicin; SL-LPA: line-probe assay for second-line drugs; TB: tuberculosis.

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

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

The test options for Algorithm 1 vary in complexity. The Xpert MTB/RIF, Xpert Ultra, Truenat MTB and Truenat MTB Plus all require basic pipetting skills and are easy to decentralize but have limited throughput with the commonly used instruments. In contrast, although some of the moderate complexity automated NAATs have minimal hands-on time, they have large infrastructure requirements; also, most of these tests provide higher throughput and are suited to established laboratories with reliable sample referral networks, and they detect resistance to INH in addition to RIF. In practice, test needs and associated choices are likely to vary, depending on the setting within a country or province. Consideration should be given towards hybrid models using a combination of tests from different manufacturers; this has the added advantage of providing a safety mechanism in the event of an expected problem with a supplier.

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

Algorithms 3 and 4 follow on from Algorithm 1, with the split based on the RIF result. Algorithm 3 is for those with confirmed RR-TB and is aimed at providing additional rapid DST. The options are the FL-LPA and SL-LPA, and the recently recommended low complexity automated NAATs with both test groups providing similar DST results. The major difference is the complexity of the test procedures. The low complexity automated NAATs are easier to perform, can be used irrespective of smear grade and may be better suited for decentralization. Rapid early diagnosis of FQ resistance among MDR/RR-TB patients is important, and the low complexity automated NAATs would be the preferred option to scale up and increase access. A new molecular test for PZA is recommended and it belongs to the class of high complexity reverse hybridization NAATs. Currently, the recommendation is for use on isolates only, with testing at higher levels of the laboratory network for selected patients. Here again an interlinked laboratory network offering a range of tests may best cater for patient needs.

Algorithm 4 is a follow-on algorithm for those with RIF-susceptible TB, and is aimed at detecting INH resistance (where INH testing was not included in Algorithm 1) or detecting FQ resistance when Hr-TB has been identified by moderate complexity automated NAATs in Algorithm 1. Currently, INH detection is not widely implemented at initial diagnosis, but this will change with the adoption of moderate complexity automated NAATs as initial tests that also detect

INH resistance. The adoption of the low complexity automated NAAT for follow-on testing of RIF-susceptible TB could also increase the detection of INH resistance. The estimated incidence of Hr-TB is greater than that of RR-TB, and both require modification of the treatment regimen. Hr-TB patients would need an FQ added to the therapy. For patients diagnosed through a moderate complexity automated NAAT that detects both RIF and INH resistance in Algorithm 1, there are two options for FQ resistance detection: low complexity automated NAAT, which is less complex and can be decentralized; or SL-LPA which, although more complex, may already be available in the same laboratory as the moderate complexity automated NAAT. In scenarios where diagnostic tests for only RIF resistance are available (e.g. Xpert MTB/RIF and Truenat MTB), follow-on testing in settings or subpopulations with high Hr-TB burden using the low complexity automated NAAT will be appropriate, being simpler and cheaper than performing both the FL-LPA and SL-LPA.

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

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

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

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

**(1)**

**Person screened positive for TB[1]**

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

**(A) MTBC *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) MTBC detected (not trace)[4] RIF resistance *not* detected[5]**

**INH susceptible or not known**

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]

**INH Resistant[18]**

Treat with Hr-TB regimen

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

Follow Algorithm **(4)**

**(C) MTBC 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) MTBC 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) MTBC 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

**(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]

**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

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

1   People screened positive for TB include adults and children with signs or symptoms suggestive of TB, with a chest X-ray showing abnormalities suggestive of TB, a positive mWRD used as a screening tool or positive C-reactive protein test (>5 mg/L) in PLHIV. A person with a positive mWRD used as a screening tool and a low pretest probability should be clinically assessed and, if deemed a presumptive TB patient, should have a repeat mWRD performed and follow Algorithm 1. If the pretest probability is high and the clinical picture is consistent with TB disease, then this test could be considered diagnostic and the patient should be managed based on the result of the test and, if relevant, should continue on to Algorithm 3 or 4. This algorithm may also be followed for the diagnosis of extrapulmonary TB using CSF, lymph node and other tissue specimens. However, mWRDs that are recommended for use in the diagnosis of extrapulmonary TB investigations are currently limited to Xpert MTB/RIF and Xpert Ultra.

2   Programmes may consider collecting two specimens upfront. The first specimen should be promptly tested using the mWRD. The second specimen may be used for the additional testing described in this algorithm. For individuals being evaluated for pulmonary TB, sputum is the preferred specimen. Tissue biopsy samples are difficult or impossible to obtain repeatedly; therefore, they should be tested with as many methods as possible (e.g. mWRD, culture, DST or histology).

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

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

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

6   The interpretation and follow-up testing for "MTBC detected rifampicin indeterminate" results for the Xpert Ultra test differs from the interpretation of results for other mWRDs. MTBC detected that RIF indeterminate results obtained with the Xpert Ultra test (especially those with high and medium semiquantitative results) may be due to large deletions or multiple mutations that confer RIF resistance. Analysis of the Ultra melt curves can detect such resistance-conferring mutations. In some cases, culture and DST, sequencing or alternative mWRD will be needed to confirm or exclude RIF resistance. Indeterminate results for the other mWRDs are usually related to very low numbers of bacilli in the sample.

7   "MTBC detected trace" applies only to the Xpert Ultra test.

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

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

10  Patients should be initiated on a first-line regimen according to national guidelines, unless the patient is at very high risk of having MDR-TB. Such patients should be further investigated and initiated on an MDR-TB regimen. In situations where INH results are available (e.g. MC-aNAAT) and INH resistance has not been detected, the probability of having MDR-TB would be lower.

11  A sample may be sent for molecular or phenotypic DST for INH if there is a high prevalence of INH resistance not associated with RIF resistance (i.e. INH mono- or poly-resistance) in this setting. Where a result for INH resistance is "not detected" (e.g. MC-aNAAT), and the pretest probability for Hr-TB is high, phenotypic DST for INH should be performed because 6–14% of resistance can be missed by molecular assays.

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

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

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

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

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

[17] In patients with a prior history of TB within the past 5 years or whose TB treatment was completed less than 5 years ago, Xpert Ultra trace results (and occasionally Xpert MTB/RIF "MTBC detected low or very low") may be positive, not because of active TB but because of the presence of non-viable bacilli. Clinical decisions must be made based on all available information and clinical judgement.

[18] Patients diagnosed using an MC-aNAAT and whose result is RIF resistance not detected and INH resistance detected should be treated for Hr-TB with RIF/EMB/PZA (REZ) and levofloxacin. For practical purposes, HREZ fixed-dose combination tablets may be used instead of REZ. Consider including high-dose INH in the Hr-TB regimen if low-level resistance is detected (*inhA* mutation only). Follow Algorithm 4.

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

*Tests*

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

- "Xpert MTB test" designates either the Xpert MTB/RIF test or the Xpert MTB/RIF Ultra (hereafter referred to as "Xpert Ultra") test. The individual tests are named when describing test-specific features. The Xpert Ultra test has an additional semiquantitative category called "trace", caused by small amounts of bacterial DNA.
- The Truenat MTB and MTB Plus assays use the same results categories as the Xpert MTB/RIF assay, and the decision pathway for the Truenat tests is the same as that for the Xpert MTB/RIF test.
- Moderate complexity automated NAATs, recommended by WHO in 2021, include a number of tests for detection of MTBC as well as RIF and INH resistance. Currently, tests from four manufacturers have been reviewed and recommended, but only for use on respiratory samples:
  - Abbott RealTi*me* MTB and Abbott RealTi*me* MTB RIF/INH (Abbott);
  - FluoroType MTBDR and FluoroType MTB (Bruker/Hain Lifescience);
  - BD MAX MDR-TB (Becton Dickinson); and
  - cobas MTB and cobas MTB-RIF/INH (Roche).
- Simultaneous detection of MTBC and RIF resistance applies to the Xpert MTB/RIF, Xpert Ultra and moderate complexity automated NAAT tests. The Truenat MTB or MTB Plus test requires subsequent testing with Truenat MTB-RIF Dx. Some moderate complexity automated NAATs (Abbott RealTi*me* MTB and cobas MTB) have the option of simultaneous or manual reflex testing for resistance to RIF or INH (or both). For the two-step procedures, the same DNA sample that was isolated for the initial test is used.
- The TB-LAMP test only detects MTBC and it requires a fresh specimen to be collected for subsequent testing with an alternative molecular test or phenotypic DST to detect RIF resistance.

*General considerations*

WHO recommends the use of an mWRD (Xpert MTB/RIF, Xpert MTB/RIF Ultra, Truenat MTB, Truenat MTB Plus, Truenat MTB-RIF Dx, TB-LAMP or moderate complexity automated NAAT) as the initial diagnostic test, rather than microscopy or culture, for all individuals with signs and symptoms of TB. This includes all newly presenting symptomatic individuals; it may also include patients who are on treatment or have been previously treated, if the patient is being evaluated for possible RR-TB or Hr-TB (e.g. non-converters at the end of the intensive phase of treatment despite treatment adherence) or for a new or continuing episode of TB (e.g. relapse cases or 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 mWRDs that show a higher sensitivity for the diagnosis of TB as well as simultaneous detection of resistance to RIF (and, for moderate complexity automated NAATs, INH as well).

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

- The Xpert MTB/RIF and Xpert Ultra tests have specific recommendations for extrapulmonary TB and children being evaluated.
  - These tests are recommended for use with CSF (preferred sample for TB meningitis), lymph node aspirates and lymph node biopsies. In addition, the Xpert MTB/RIF test is recommended for pleural fluid, peritoneal fluid, pericardial fluid, synovial fluid or urine, as an initial diagnostic test for the corresponding extrapulmonary TB disease. Blood may also be used as a specimen for HIV-positive adults and children with signs and symptoms of disseminated TB. Sample-specific optimization steps may be needed to achieve optimal results, especially for paucibacillary samples.
  - Minor variations for use of these tests with children with signs and symptoms of pulmonary TB include the following:
    - Xpert MTB/RIF can be used as the initial diagnostic test for pulmonary TB with sputum, gastric aspirate, nasopharyngeal aspirate or stool samples, whereas Xpert Ultra is recommended for use with sputum and nasopharyngeal aspirate specimens; and
    - in settings with a pretest probability of 5% or more and an Xpert MTB/RIF or Ultra negative result on the initial test, repeated testing with Xpert MTB/RIF or Ultra with the same or different specimen types (for a total of two tests) may be used; otherwise, repeat testing is not recommended.
- The Truenat MTB and MTB Plus test considerations include the following:
  - There is uncertainty about use of this test in PLHIV, because insufficient data were available on the performance of these tests in PLHIV. The indirect data on test performance in 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; it would be suitable for use in settings that have a low prevalence of HIV and MDR-TB. Considerations for the use of this test include the following:
  - In populations with a high burden of MDR-TB, TB-LAMP should not replace the use of rapid molecular tests that detect RIF resistance (e.g. Xpert MTB/RIF), because TB-LAMP does not provide any information on RIF resistance.
  - In populations with a high prevalence of HIV, TB-LAMP should not replace the use of rapid molecular tests that have a higher sensitivity for detection of TB (e.g. Xpert Ultra).
- Considerations for the moderate complexity automated NAATs include the following:
  - The recommendations apply to PLHIV. Performance on smear-negative samples was reviewed but was only available for TB detection, not for RIF and INH resistance. Data stratified by HIV status were not available.
  - The recommendations apply to adolescents and children based on the generalization of data from adults. An increased rate of false negative or indeterminate results may be found with paucibacillary TB disease in children.

– Extrapolation to use in people with extrapulmonary TB and testing on non-sputum samples was not considered because data on diagnostic accuracy of technologies in the class for non-sputum samples were limited.

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

Algorithm 1 describes the collection of one initial specimen to be used for mWRD testing and the collection of additional specimens as needed. For operational issues, programmes may consider collecting two specimens (e.g. spot and morning sputum samples, or two spot specimens) from each patient routinely, instead of only collecting a second specimen when additional testing is needed. If two specimens are collected, the first should be tested promptly using the mWRD. The second specimen may be used for the additional testing described in the algorithm (e.g. repeat mWRD testing for failed tests or follow-on resistance testing, or for smear microscopy or culture as a baseline for treatment monitoring).

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

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

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

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

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

## *Decision pathway*

1. Collect a good-quality specimen and transport it to the testing laboratory. Conduct the mWRD. For individuals being evaluated for pulmonary TB, the following specimens may be used: induced or expectorated sputum (preferred), bronchoalveolar lavage, gastric lavage or aspirates, nasopharyngeal aspirates and stool samples. (For information on which specimens may be used with which mWRD, see Section 2.2 above or individual WHO policy statements.)

2. If the mWRD result is "MTB not detected" (1)(A) re-evaluate the patient and conduct additional testing in accordance with national guidelines.

    a. Further investigations for TB may include chest X-ray, additional clinical assessments, additional mWRD testing or culture and clinical response following treatment with broad-spectrum antimicrobial agents (FQs should *not* be used).

    b. In children with signs and symptoms of pulmonary TB in settings with a high pretest probability (>5%) and a negative Xpert MTB result on the first initial test, repeat the Xpert MTB test for a total of two tests. The tests may use the same specimen types or different specimen types (e.g. one sputum specimen and one nasopharyngeal aspirate sample).

    c. The performance of the other mWRDs in repeat testing is not known.

    d. Consider the possibility of clinically defined TB (i.e. TB without bacteriological confirmation). Use clinical judgement for treatment decisions.

*Molecular WRD as the initial diagnostic test for TB*

**Person screened positive for TB[1]**

↓

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

↓

**MTB *not* detected**

**Re-evaluate 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
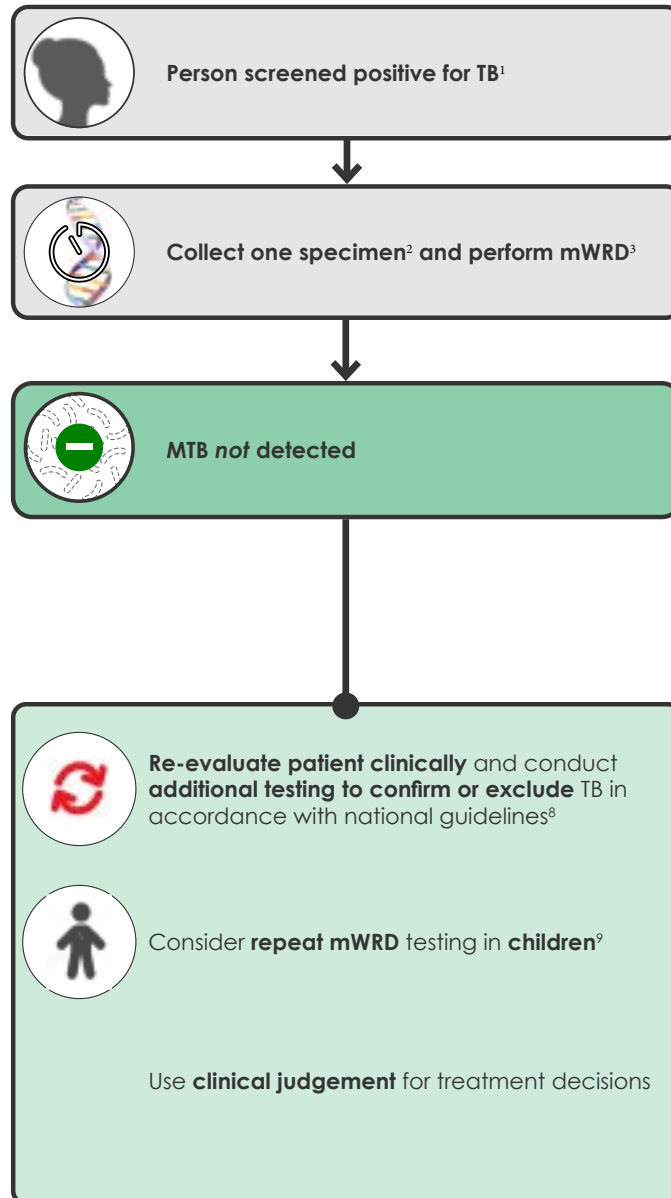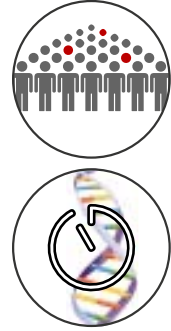
For footnotes please see page 53

3. If the mWRD result is "MTBC detected, RIF resistance not detected" and "INH resistance not detected" or INH results are unknown (1)(B):

a. Initiate the 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 (see Algorithm 4 for follow-up testing):
      - if the patient has been treated with INH or is a contact of a known Hr-TB patient; or
      - if there is high prevalence of INH resistance that is not associated with RIF resistance (i.e. Hr-TB or poly-resistance, not MDR-TB) in this setting.

      If the INH resistance is "not detected" by the moderate complexity automated NAAT and the patient has a high risk of Hr-TB, phenotypic DST for INH should be performed because 6–14% of INH resistance can be missed by current molecular tests.

   ii. Molecular or phenotypic DST for RIF resistance may be requested if the patient is at risk of having RR-TB despite the initial mWRD result showing "susceptibility". Sometimes, these anomalous results may be due to sample labelling errors and a repeat test may resolve the issue. False RIF-susceptible Xpert MTB results can occur but are uncommon (1–5% of RIF-resistant TB cases tested) and the level of such results depends on the epidemiologic settings. In contrast, phenotypic DST for RIF, especially using liquid culture, is associated with a higher proportion of false-susceptible results *(45)*. The updated critical concentration for RIF which should be used will reduce, but not eliminate, this issue. Sequencing should be performed when available, and should cover not only the RIF-resistance-determining region (RRDR) but regions outside as well (e.g. codons 170 and 491).

c. If additional molecular or phenotypic testing is performed:

   i. The molecular and phenotypic testing may be performed in different laboratories. Perform these tests in parallel – do not wait for the results of one test before initiating another test.

   ii. The molecular and phenotypic DST may be performed using the specimen (direct DST) or using bacteria recovered by culture (indirect DST). Direct DST is preferred for molecular testing, whereas indirect DST may be preferred for phenotypic DST, because of technical issues related to producing an appropriate inoculum and loss to contamination.

   iii. A rapid molecular test is preferred using WHO-recommended tests. Mutation interpretation can also be found in the WHO catalogue of mutations.[57] DNA sequencing has proven useful in many cases but WHO has not yet evaluated it for clinical use.

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

---

[57] See https://www.who.int/publications/i/item/9789240028173

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

Person screened positive for TB[1]

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

MTB detected (not trace)[4]

**RIF** RIF resistance *not* detected[5]

**1** INH susceptible or not known
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]

**Hr-TB** INH Resistant[18]
Treat with Hr-TB regimen
Consider including high-dose isoniazid in the Hr-TB regimen if low-level resistance detected
Follow Algorithm **4**

For footnotes please see page 52

4. If the mWRD test result is "MTBC detected, RIF resistance not detected and INH resistance detected" (currently only applicable to the moderate complexity automated NAATs):

   a. Initiate the patient on an appropriate Hr-TB regimen in accordance with national guidelines. The WHO recommendation for patients with Hr-TB is treatment with RIF, ethambutol (EMB), PZA and levofloxacin (LFX) for a duration of 6 months *(7)*.

   b. Follow Algorithm 4 for Hr-TB:

      i. Additional DST for RIF may be required in settings where RIF-resistant mutations outside the RRDR are common. Decision on the choice between phenotypic testing or sequencing will depend on the type of mutation expected. In places where the *rpoB* I491F mutation is common, sequencing is preferred because phenotypic DST, even with the lower CC, will still miss many resistant infections; in other cases (e.g V170F) phenotypic testing is appropriate *(25)*.

5. If the mWRD result is "MTBC detected, RIF resistance detected" irrespective of the INH result **1** **C**, an MDR-TB risk assessment is needed. Patients at high risk for MDR-TB include previously treated patients (e.g. 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 groups at risk for MDR-TB identified in the country. In high MDR-TB burden countries, every TB patient is considered to be at high risk of having MDR-TB.



*Molecular WRD as the initial diagnostic test for TB*
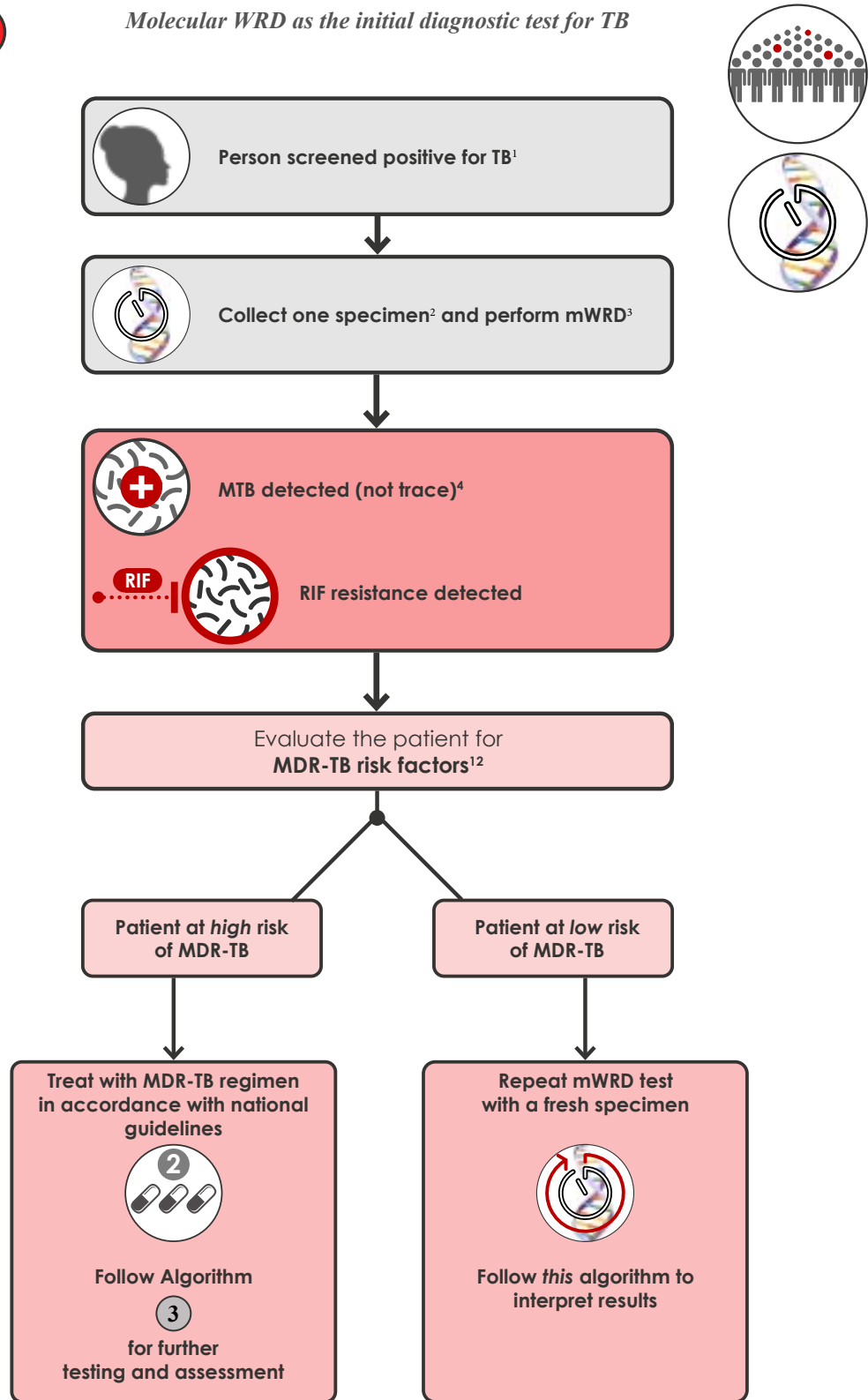
For footnotes please see page 52

a. If the patient is at high risk of having MDR-TB and the test result is RIF resistant, initiate the patient on a regimen for MDR/RR-TB in accordance with national guidelines. Follow Algorithm 3 for additional testing.

b. If the patient is not at high risk of having MDR-TB, repeat using an mWRD with a second sample. To aid interpretation, the initial instrument output for the result can be reviewed when available. Probe binding delay and samples having a low bacillary load, which can be inferred from the semiquantitative readings (e.g. low and very low), have been associated with increased false resistance in some settings *(46–48)*.

   i. If the second test also indicates RIF resistance, initiate an MDR/RR-TB regimen in accordance with national guidelines and WHO recommendations, and follow Algorithm 3 for additional testing.

   ii. If the mWRD result for the second sample is "MTBC detected, RIF resistance not detected", initiate treatment with a first-line regimen in accordance with national guidelines. In most situations, false positive RIF-resistant results due to technical performance of the assay are rare, but such results may occur because of laboratory or clerical errors. It is assumed that the repeat test will be performed with more caution, that the result of the second test is correct, and that the result of the first test may have been due to a laboratory or clerical error. Mixed infections in high-burden settings could also explain such discordance and patients should be closely followed up repeat tested if response is poor on first line treatment. If an INH resistance or susceptibility result is available, interpret and follow-up as described in Algorithm 4.

   iii. In the event that the mWRD result for the second sample is "MTBC detected, RIF resistance is inconclusive", the patient will require further investigation. A possible mixed infection may explain such a scenario. History of prior treatment and TB contact history should be reassessed. The decision to manage the patient as Hr-TB or MDR/RR-TB will need to be based on further investigation that includes phenotypic DST to RIF and INH, and, where available, DNA sequencing. A third mWRD should be performed to decide on the initial therapy; the patient should be closely followed up while awaiting the final definitive results and the appropriate algorithm should be followed.

   iv. In the event that a moderate complexity automated NAAT was performed and INH results are also available, this could be useful to provide certainty. INH resistance is associated with RIF resistance and the finding of INH resistance should prompt further investigation to exclude RIF resistance.

c. For all patients with RR-TB or MDR-TB, conduct additional investigations to assess resistance to the drugs being used in the treatment regimen. Rapid detection of FQ resistance is essential in determining the regimen to be selected. The recent addition of a low complexity automated NAAT for detection of FQ resistance provides a rapid and accurate peripheral level solution that can be performed directly on specimens. Phenotypic (culture and DST) and molecular (e.g. SL-LPA, and DNA sequencing) methods are available to evaluate drug resistance beyond RIF and INH. Rapid molecular methods are preferred. However, for resistance detection to the new and repurposed drugs, phenotypic DST is the only currently available option. Thus, two separate specimens may be required.

   i. MDR/RR-TB regimens rely on the use of 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 or even months to provide results).

iii. Any positive culture recovered during treatment monitoring that is suggestive of treatment failure should undergo DST for the drugs used in the treatment regimen.

6. If the mWRD gives a result of "MTBC detected, RIF indeterminate" ①Ⓓ, the patient will require further investigation. The interpretation and follow-up testing for Xpert Ultra differs from that for other mWRDs. With any of the mWRDs, the initial result of "MTBC detected" should be considered as bacteriological confirmation of TB. The 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 (in which case, the patient should be initiated on an MDR-TB regimen). In most settings, for the purpose of making treatment decisions, a history of prior TB treatment is not sufficient to indicate that the patient is at high risk of having MDR-TB.

a. For most mWRDs, an "MTBC detected, RIF resistance indeterminate" result is generally caused by a paucibacillary TB load in the sample; in such cases, retesting a fresh specimen is recommended.

i. If the result of the second mWRD is "MTBC detected, RIF resistance not detected", follow Steps 3.13.3. If the result is "MTBC detected, RIF-resistance detected", follow Steps 6.1 and 6.2.

ii. In some cases, testing a second sample, which might also contain very few bacteria, may generate a result of "MTBC detected, RIF indeterminate" or "MTB not detected". In these situations, additional investigations such as culture and phenotypic DST or molecular testing of the isolate or sequencing may be needed to confirm or exclude resistance to RIF, because the indeterminate result provides no information on resistance.

*Molecular WRD as the initial diagnostic test for TB*

**Person screened positive for TB**[1]

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

**MTB detected (not trace)**[4]

RIF **RIF indeterminate**[6]

**Ultra**

Evaluate
**Ultra melting curve**

**Other mWRD**

RIF resistance
detected

RIF resistance
*not* detected

RIF

RIF

**Repeat
mWRD**

Follow **1** **C**

**Repeat
mWRD**

**Follow *this* algorithm**
to interpret results

If both tests give indetermi-
nate results, treat with
**first-line regimen**[9]

Promptly conduct addi-
tional investigations to
**assess resistance
to RIF**[14]

Review treatment based
on **DST** result

For footnotes please see page 52

b. "MTBC detected (non-trace), RIF indeterminate" results obtained with the Xpert Ultra test (especially those with high or medium semiquantitative results) may be due to the presence of large deletions or multiple mutations in the RRDR or mutations that pose a challenge with mutation analysis software *(49)*.

    i. The Ultra melt curves from "MTBC detected (non-trace), RIF indeterminate" samples should be reviewed (preferably by an advanced Xpert user or supervisor), including a review of the amplification of the probes and melt curve profile *(49)*.

        1. Melt curves that suggest the presence of a large deletion or multiple mutations in the RRDR should be interpreted as "RIF resistance detected". In such cases, follow Steps 6.1 and 6.2.

        2. If the melt curve is not consistent with the presence of a large deletion or multiple mutations in the RRDR, the result is interpreted as "indeterminate". In such cases, follow Step 7.1 for additional testing.

        3. If the semiquantitative result is high or medium, FL-LPA or DNA sequencing may be useful.

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

7. If the Xpert Ultra test result is "MTBC detected trace" **(1)(E)**, additional considerations are needed. However, WHO suggests *not* repeating Xpert Ultra testing in adults who have an initial Xpert Ultra trace result to confirm the result.

a. Review the clinical characteristics to determine the person's age, HIV-infection status and history of TB treatment, and determine whether the samples are pulmonary or extrapulmonary.

    i. PLHIV include individuals who are HIV-positive or whose HIV status is unknown but who present with strong clinical evidence of HIV infection, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all those with unknown HIV status, perform HIV testing in accordance with national guidelines.

    ii. Children are defined as those aged under 15 years.

    iii. Individuals with a history of recent TB treatment include those who successfully completed a course of therapy within the past 5 years. The likelihood of a false positive mWRD test result is highest immediately after completing treatment, and slowly declines with time *(50, 51)*. 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 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, in the case of migrants, concern over legal status.

*Molecular WRD as the initial diagnostic test for TB*

**Person screened positive for TB[1]**

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

**MTB detected (trace)[7]**

RIF

**RIF indeterminate[6]**

---

**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

**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

---

Treat with **first-line regimen[10]**

1

Conduct additional investigations to **assess resistance to rifampicin[16]**

Review treatment based on DST result

Recent TB treatment may generate a **false-positive** result [17]

**Re-evaluate patient clinically**

Conduct **additional testing for TB and RIF resistance** in accordance with national guidelines[16]

Use **clinical judgement** for treatment decisions

For footnotes please see page 52

b. For certain populations – PLHIV and children who are being evaluated for pulmonary TB; for individuals being evaluated for extrapulmonary TB using CSF, lymph nodes and tissue specimens; and for adults being evaluated for pulmonary TB, who are not at risk for HIV and who do not have a history of prior TB treatment within the past 5 years:

   i. Consider the MTBC detected trace result obtained with the first specimen as bacteriological confirmation of TB (i.e. a true positive result) and use for clinical decisions.

   ii. Initiate the patient 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 (in which case, initiate the patient on an MDR-TB regimen).

   iii. Undertake additional investigations (e.g. culture and DST) to confirm or exclude resistance to RIF.

c. For adults being evaluated for pulmonary TB, who are not at risk of HIV and have a history of TB treatment in the past 5 years:

   i. For adults with a history of recent TB treatment or unknown treatment history, consider the possibility of the Xpert Ultra trace result being a false positive result because of the presence of non-viable bacilli.

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

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

   iv. Further investigations for TB may include chest X-ray, additional clinical assessments and clinical response following treatment with broad-spectrum antimicrobial agents (FQs should not be used).

      1. Repeat Xpert Ultra testing is of uncertain benefit. A recent WHO GDG recommended against repeat Xpert Ultra testing for individuals with an initial Xpert Ultra trace result for the detection of MTBC.

      2. Culture and phenotypic DST may be of benefit to detect TB and drug resistance. The trace result provides no information on RIF resistance.

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

*Molecular WRD as the initial diagnostic test for TB*

**Person screened positive for TB**[1]

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

**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** testing[13]
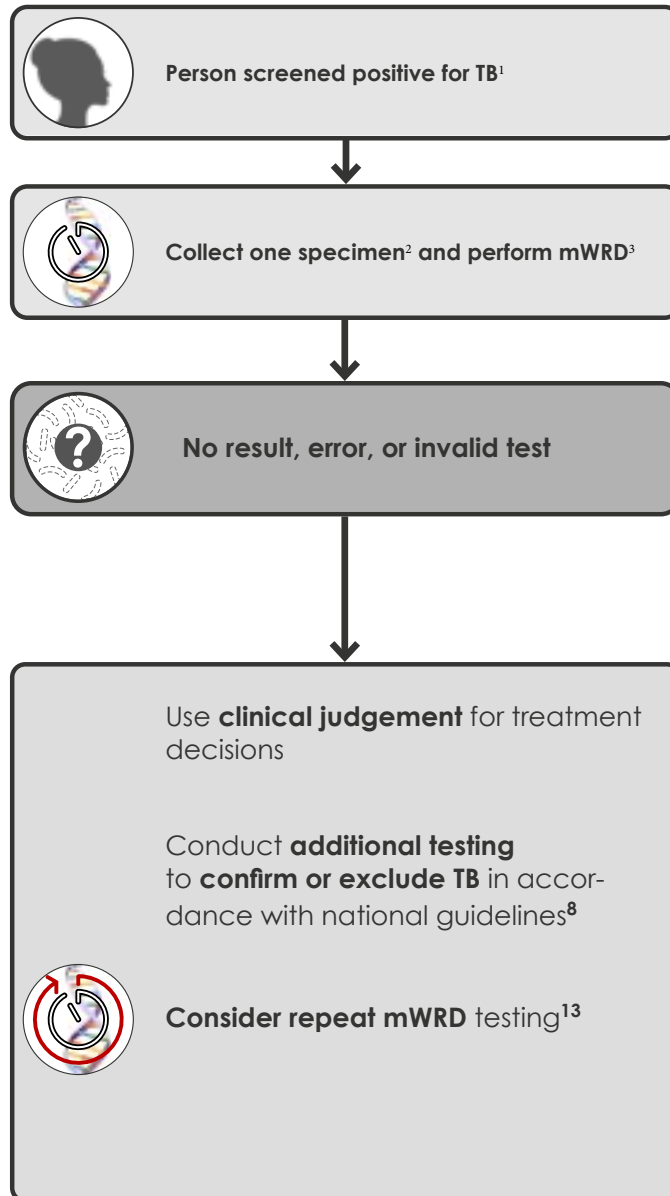
For footnotes please see page 52

*Interpretation of discordant results*

This algorithm relies on testing of a sample with an mWRD to detect MTBC and assess susceptibility to RIF. Discordance in resistance to INH is described in Algorithm 4. On occasion, follow-up testing is recommended to ensure that clinical decisions are well informed. However, discordant results may occur, usually when comparing culture-based results with molecular results. Each discordant result will need to be investigated on a case-by-case basis. General considerations are outlined below.

1. mWRD result "MTBC detected other than trace", culture negative (see Point 5 for trace):
   a. The mWRD result and clinical judgement should be used to guide the treatment decision, pending additional testing.
   b. The mWRD result should be considered as bacteriological confirmation of TB, if the sample was collected from a person who was not recently receiving treatment with anti-TB drugs. Cultures from individuals with pulmonary TB may be negative for several reasons, including that the 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. mWRD 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, WRDs have a pooled sensitivity of 83–90% for detecting pulmonary TB compared with culture *(52)*. 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 mWRDs, 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. mWRD result "MTBC detected, RIF resistance detected"; RIF-susceptible by phenotypic DST:
   a. Use the mWRD result to guide treatment decisions pending additional testing.
   b. Borderline resistant mutations are known to generate this discordant result, particularly in the BACTEC 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 *(45)*.

c. In some settings with a low prevalence of MDR-TB, silent mutations have been observed that generate a false-resistant mWRD result, but these are rare.

d. A review of the probe melting temperatures when available *(53)* or banding pattern on the FL-LPA can aid in determining of inferring the specific mutation (e.g. borderline resistant or silent).

e. False RIF-resistant results have been observed with the Xpert MTB/RIF G4 cartridge when the MTBC detected result was "very low" and associated with probe binding delay *(46)*. Follow-up action may include mWRD testing of the culture.

f. Follow-up actions may include DNA sequencing, confirmatory testing on other mWRD testing platform, phenotypic DST using solid media and evaluation of the possibility of laboratory or clerical error.

4. mWRD result "MTBC detected, RIF resistance not detected"; RIF resistant by phenotypic DST:

a. The treatment regimen should be modified based on the results of the phenotypic DST.

b. False RIF-susceptible mWRD results are rare but have been observed in 1–5% of RIF-resistant TB cases tested with the Xpert MTB/RIF test in various epidemiologic settings. Mutations in the region of the *rpoB* gene sampled by the Xpert MTB tests have been shown to account for 95–99% of RIF resistance. The remainder of RIF resistance arises from mutations outside the sampled region, which produce an Xpert MTB result of "RIF resistance not detected". In settings with a prevalent clone that harbours a mutation outside the RRDR, for example Eswatini *(54)*, this may be more common; however, this has not been identified as a major concern in other settings *(55)*. Surveillance to monitor emergence of such clones over time should be considered.

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

5. Xpert Ultra "MTBC detected trace", culture negative:

The interpretation of this result must consider 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 small numbers of bacilli in a sample that generates an "MTBC detected trace" result may be due to active TB disease, laboratory cross-contamination, recent exposure to (or infection with) tubercle bacilli (incipient TB), and current or past treatment for TB.

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

a. For PLHIV and children who are being evaluated for pulmonary TB, or when extrapulmonary specimens (CSF, lymph nodes and tissue specimens) are tested, the benefits of the increased sensitivity for the detection of MTBC (i.e. true positives) outweighs the potential harm of decreased specificity (i.e. false positives).

i. The "MTBC detected trace" result is considered as bacteriological confirmation of TB (i.e. true positive results) and such 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 "MTBC detected trace" results should be considered as bacteriological confirmation of TB (i.e. true positive results), any clinical decision (e.g. to treat for TB) should be made based on all available laboratory, clinical and radiological information, and clinical judgement.

      2. Consider the possibility that the culture result was a false negative result, if the samples were collected from a person who was not receiving treatment with anti-TB drugs, because of the paucibacillary nature of the sample. Follow-up actions for 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 "MTBC detected trace" result was a false positive result because of the presence of non-viable bacilli. A culture-negative result is consistent with this possibility.

      2. If these 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.

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

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

The currently available LF-LAMs have sufficient sensitivity and specificity to aid in the diagnosis of TB among individuals coinfected with HIV, but have suboptimal sensitivity and specificity in those who are HIV-negative. Hence, this algorithm emphasizes the use of the LF-LAM test as a diagnostic test in all PLHIV with signs and symptoms of TB, as well as in other specific scenarios (described below) for the diagnosis of TB among PLHIV *(56)*. The ease of use of the LF-LAM test makes it suitable for implementation outside of the laboratory – for example, in clinics (especially in those that see critically ill PLHIV) – for rapid diagnosis of TB and treatment initiation in urgent cases of suspected TB among PLHIV. Algorithm 2a is used for PLHIV being evaluated for TB (pulmonary or extrapulmonary) in an inpatient setting. The updated WHO screening guidelines now recommend adult and adolescent inpatients with HIV in medical wards where the TB prevalence is more than 10% should be tested systematically for TB disease with an mWRD, as described in Algorithm 1 *(7)*. This is in addition to the recommendations on the use of LF-LAM among inpatient PLHIV *(56)*. Algorithm 2b is used for PLHIV being evaluated for TB (pulmonary or extrapulmonary) in an outpatient setting or clinic.

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

## Fig. 4.3. Algorithm 2a: LF-LAM to aid in the diagnosis of TB among PLHIV in inpatient settings
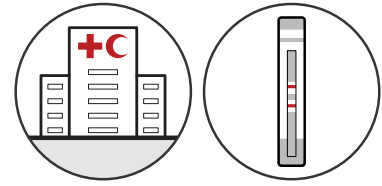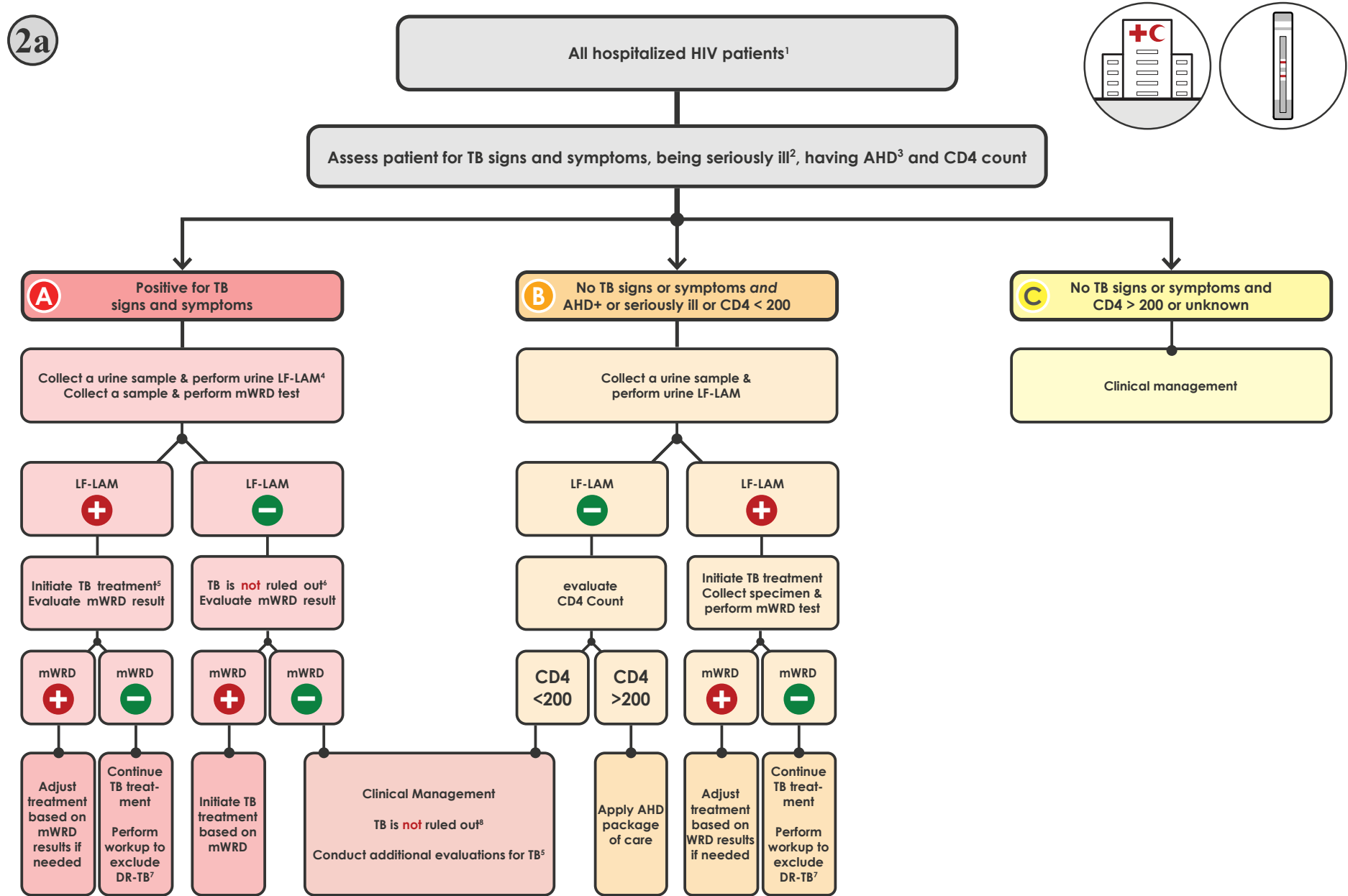
AHD: advanced HIV disease; AIDS: acquired immunodeficiency syndrome; DNA: deoxyribonucleic acid; DR-TB: drug-resistant tuberculosis; DST: drug-susceptibility testing; FQ: fluoroquinolone; HIV: human immunodeficiency virus; LF-LAM: lateral flow lipoarabinomannan assay; LPA: line-probe assay; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant tuberculosis; mWRD: molecular WHO-recommended rapid diagnostic test; PLHIV: people living with HIV/AIDS; TB: tuberculosis; WHO: World Health Organization; WRD: WHO-recommended rapid diagnostic test.

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

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

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

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

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

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

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

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

**Fig. 4.4. Algorithm 2b: LF_LAM testing to aid in the diagnosis of TB among PLHIV in clinic and outpatient settings**

**2b**

**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**

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

**C** — **Without assessing symptoms**

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

**CD4 assessment**

**LF-LAM ➕**

**LF-LAM ➖**

**CD4 <100 *or* Stage 3 or 4**

**CD4 100 - 200**

**CD4 >200 *or* unknown**

**Do Not Perform LF-LAM**

**Initiate TB treatment[5]
Evaluate mWRD Result**

**TB is not ruled out[6]
Evaluate mWRD Result**

**perform urine LF-LAM**

**Do Not Perform LF-LAM**

**Do Not Perform LF-LAM**

**mWRD ➕**

**mWRD ➖**

**mWRD ➕**

**mWRD ➖**

**LF-LAM ➕**

**LF-LAM ➖**

**Adjust treatment based on mWRD results if needed**

**Continue TB treatment

Perform workup to exclude DR-TB[7]**

**Initiate TB treatment based on mWRD results**

**Clinical Management

TB is not ruled out[8]

Conduct additional evaluations for TB[6]**

**Initiate TB treatment[5]

Conduct mWRD test and perform workup to exclude DR-TB[7]**

**Apply AHD package of care**

**Clinical management**

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

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

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

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

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

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

[6] LF-LAM negative results do not rule out TB in symptomatic persons. The result of the mWRD 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-Truenat MTB Plus or MC-aNAATs) are preferred.

[8] The mWRD negative and LF-LAM negative results do not rule out TB in symptomatic persons. Conduct additional clinical evaluations for TB. Further investigations for TB may include chest X-ray, additional clinical assessments, 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.

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

*General considerations for Algorithm 2a and Algorithm 2b*

- The LF-LAM is a point-of-care test that may be implemented outside the laboratory (e.g. at the bedside in clinics that see critically ill PLHIV) for rapid diagnosis of TB and treatment initiation.
- The algorithms may be used for PHLIV being evaluated for pulmonary or extrapulmonary TB.
- The algorithms are appropriate for all PLHIV who meet the testing requirements, regardless of the overall prevalence of HIV in the setting.
- The algorithms are appropriate for both low and high MDR-TB or Hr-TB burden settings. The choice of which mWRD to use may be different in a low- or high-burden MDR-TB setting.
- The algorithms emphasize the use of the LF-LAM test in addition to an mWRD in all PLHIV with signs and symptoms of TB, and in:
  - inpatient settings, for HIV-positive adults, adolescents and children with advanced HIV disease or who are seriously ill, or PLHIV with a CD4 cell count of less than 200 cells/mm$^3$, irrespective of signs and symptoms of TB; and
  - 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 mWRD testing. This also includes children and adolescents living with HIV who are able to provide a sputum sample (see Algorithm 1).

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

1. Evaluate the hospitalized 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.

   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, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all those with unknown HIV status, perform HIV testing in accordance with national guidelines. For all

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

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

d. For adults, adolescents and children aged more than 5 years, AHD is defined as CD4 cell count <200 cells/mm3 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 **and** collect a specimen and conduct mWRD testing. If the mWRD is available on site, perform the mWRD testing in parallel to the LF-LAM testing.

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

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

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



**All hospitalized HIV patients[1]**

**Assess patient for:**

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

**Positive for
TB signs and symptoms**

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

**Collect a sample & perform mWRD test**

**+**

**−**

**Initiate TB treatment[5]
Evaluate mWRD result**

**TB is not ruled out[6]
Evaluate mWRD result**

**+**

**−**

**+**

**−**

**Adjust treatment
based on mWRD
results if needed**

**Initiate TB treatment**

**Perform workup to
exclude DR-TB[7]**

**Adjust treatment
based on WRD results
if needed**

**Clinical management**

**TB is not ruled out[6]**

**Conduct additional
evaluations for TB[5]**
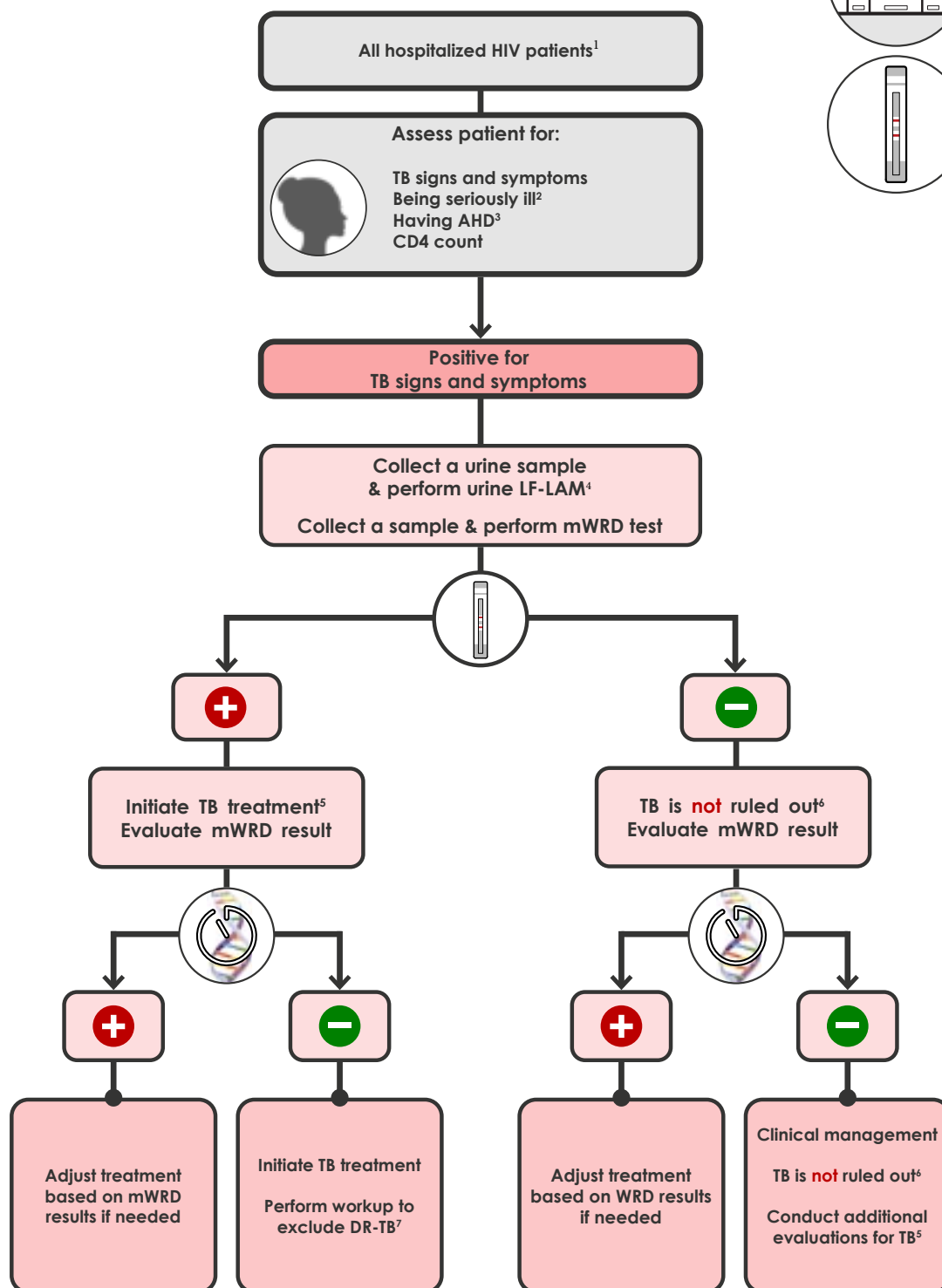
For footnotes please see page 75

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

c.  All patients meeting the testing requirements who have a positive LF-LAM result should be initiated on TB treatment immediately, while awaiting results of the mWRD. Follow Algorithm 1 for the selection and interpretation of mWRD results, which include resistance detection, and modify therapy as needed.

d.  TB is not ruled out if the LF-LAM test result is negative. Evaluate the results of the mWRD, and follow Algorithm 1 for interpretation of results and follow-up testing.

e.  Treat all patients with an mWRD result of "MTBC detected" for TB (see Algorithm 1), regardless of LF-LAM result.

f.  TB is not ruled out if both the LF-LAM result and mWRD results are negative (or if no mWRD is performed). Re-evaluate the 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/mm³ **(2a) (B)**:

   a.  Collect a urine specimen and conduct the LF-LAM.

   b.  If the LF-LAM is negative and the CD4 count is <200 cells/mm³, 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 count is >200 cells/mm³, apply an AHD package of care.

   d.  If the LF-LAM is positive, initiate TB treatment based on this result and clinical judgement. Collect a specimen and conduct an mWRD to assess the possibility of rifampicin resistance.

      i.  If the mWRD result is "MTBC detected", follow Algorithm 1 for interpretation, testing and treatment recommendations.

      ii.  If the mWRD result is "MTBC not detected", continue treating the patient for TB and conduct additional laboratory testing (e.g. culture and phenotypic DST) to assess drug resistance.

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

All hospitalized HIV patients[1]

**Assess patient for:**

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

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

Collect a urine sample &
perform urine LF-LAM

**(−)**

evaluate
**CD4 Count**

**CD4 <200**

Clinical management

TB is **not** ruled out[6]

Conduct additional
evaluations for TB[5]

**CD4 >200**

Apply AHD
package of care

**(+)**

**Initiate TB treatment**

**Collect specimen
& perform mWRD**

**(+)**

Adjust treatment
based on mWRD
results if needed

**(−)**

Continue TB treatment

Perform workup to
exclude DR-TB[7]

For footnotes please see page 75

4. For hospitalized PLHIV without signs or symptoms of TB and whose CD4 is 200 cells/mm³ or above (or is unknown) **2a** **C**, do not conduct an LF-LAM test. The latest WHO TB screening guidelines, however, recommend that all hospitalized PLHIV irrespective of signs and symptoms should be routinely tested using an mWRD for TB.

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



All hospitalized HIV patients[1]

Assess patient for:

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

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

Clinical management
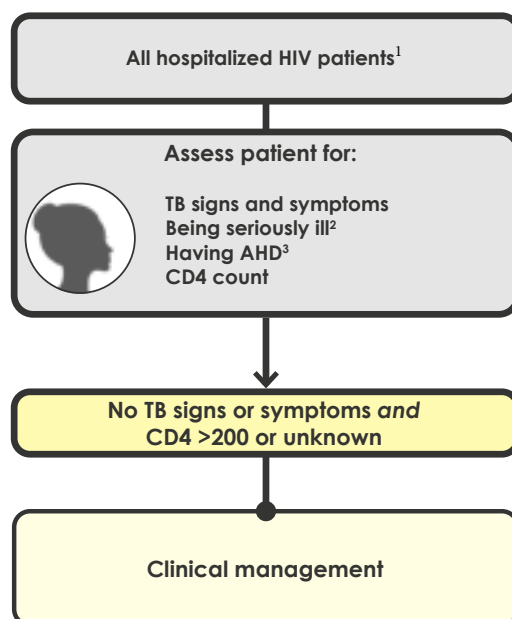
For footnotes please see page 75

## 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:

   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, reside in settings where there is a high prevalence of HIV or are members of a group at risk for HIV. For all those with unknown HIV status, perform HIV testing in accordance with national guidelines.

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

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

2. For PLHIV being evaluated for TB who are positive for signs and symptoms, or who are seriously ill regardless of TB symptoms (2b) (A):

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

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

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

**2b** **A**

*LF-LAM testing to aid in the diagnosis of TB among PLHIV in clinic and outpatient settings*
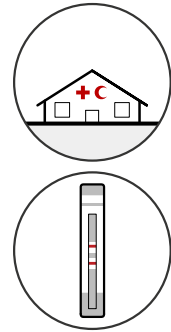
**Adults, adolescents and children including:**

1. All newly diagnosed HIV patients that are ART naive

2. HIV patients returning for care following an interruption of treatment

3. HIV patients receiving an ART regimen that is failing

4. Patients presenting at the clinic and unwell

**Assess patient for:**

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

**Positive for
TB signs and symptoms**

**Collect a urine sample
and perform urine LF-LAM**

**Collect a pample and perform mWRD**

**LF-LAM ➕**

**Initiate TB treatment[5]
Evaluate mWRD result**

**mWRD ➕**

**Adjust treatment
based on mWRD results
if needed**

**mWRD ➖**

**Continue TB treatment**

**Perform workup to
exclude DR-TB[7]**

**LF-LAM ➖**

**TB is not ruled out[6]
Evaluate mWRD result**

**mWRD ➕**

**Adjust treatment
based on mWRD results
if needed**

**WRD ➖**

**Clinical management**

**TB is not ruled out[6]**

**Conduct additional
evaluations for TB[5]**

For footnotes please see page 77

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

c. All patients meeting the testing requirements who have a positive LF-LAM result should be initiated on TB treatment immediately, while awaiting results of the mWRD. Follow Algorithm 1 for selection and interpretation of mWRD results which include resistance detection, and modify therapy as needed.

d. TB is not ruled out if the LF-LAM test result is negative. Evaluate the results of the mWRD, and follow Algorithm 1 for result interpretation and follow-up testing.

e. Treat all patients with an mWRD result of "MTBC detected" for TB (see Algorithm 1), regardless of LF-LAM result.

f. TB is not ruled out if both the LF-LAM and mWRD results are negative (or if no mWRD is performed). Re-evaluate the 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 PLHIV being evaluated for TB who do not have signs or symptoms of TB, or who are not seriously ill, determine their CD4 count and whether they have AHD (2b) (B).

  a. If the CD4 is <100 cells/mm$^3$ or the patient presents with a WHO stage 3 or 4 event, collect a urine specimen and perform an LF-LAM.

    i. If the LF-LAM test is positive, initiate TB treatment immediately. Conduct additional studies to assess drug resistance. Rapid molecular methods are preferred (see Algorithm 1) and include drug-resistance detection.

    ii. If the LF-LAM test is negative, apply an AHD package of care.

  b. If CD4 is 100–200 cells/mm$^3$, DO NOT perform an LF-LAM; apply an AHD package of care.

  c. If the CD4 is >200 cells/mm$^3$ or unknown, DO NOT perform an LF-LAM; clinically manage the patient.

**2b** **B**

*LF-LAM testing to aid in the diagnosis of TB among PLHIV in clinic and outpatient settings*
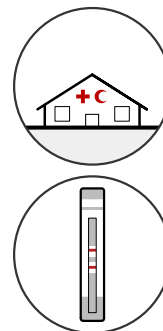
**Adults, adolescents and children including:**

1. All newly diagnosed HIV patients that are ART naive

2. HIV patients returning for care following an interruption of treatment

3. HIV patients receiving an ART regimen that is failing

4. Patients presenting at the clinic and unwell

**Assess patient for:**

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

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

**CD4 assessment**

| **CD4 <100** *or* **Stage 3 or 4** | **CD4** **100-200** | **CD4** **unknown *or*** **>200** |
|---|---|---|

**LF-LAM** ✚

**LF-LAM** ⊖

**Continue TB treatment**

**Perform workup to exclude DR-TB[7]**

**Apply AHD package of care**

**Clinical management**
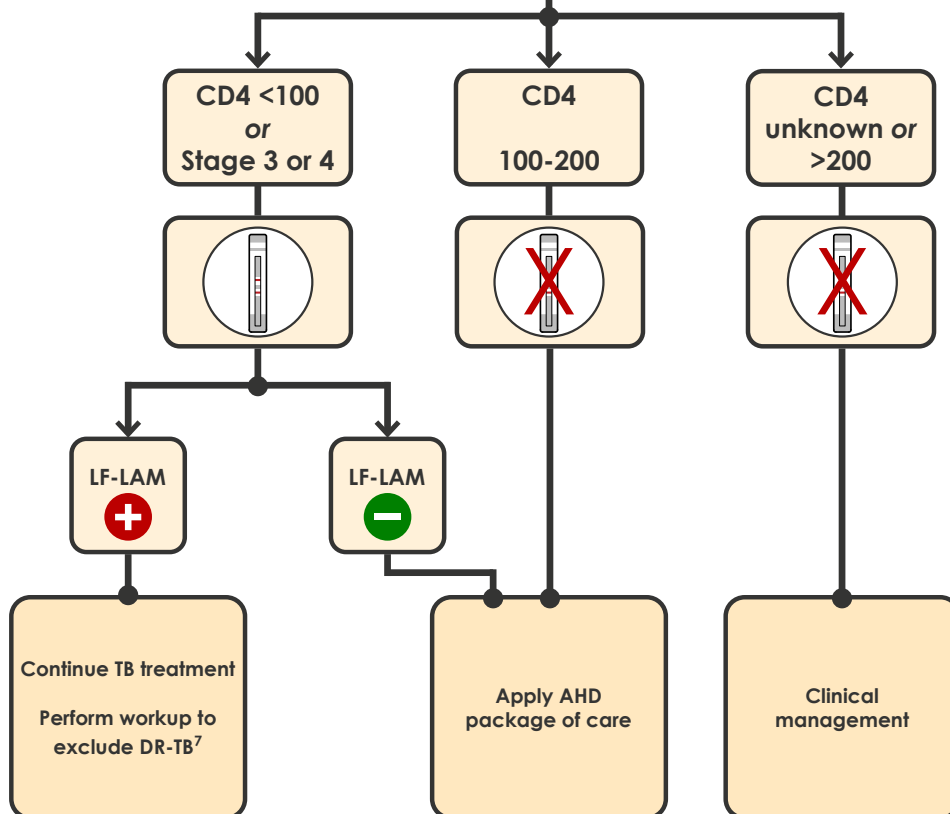
For footnotes please see page 77

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

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

**2b** **C**

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

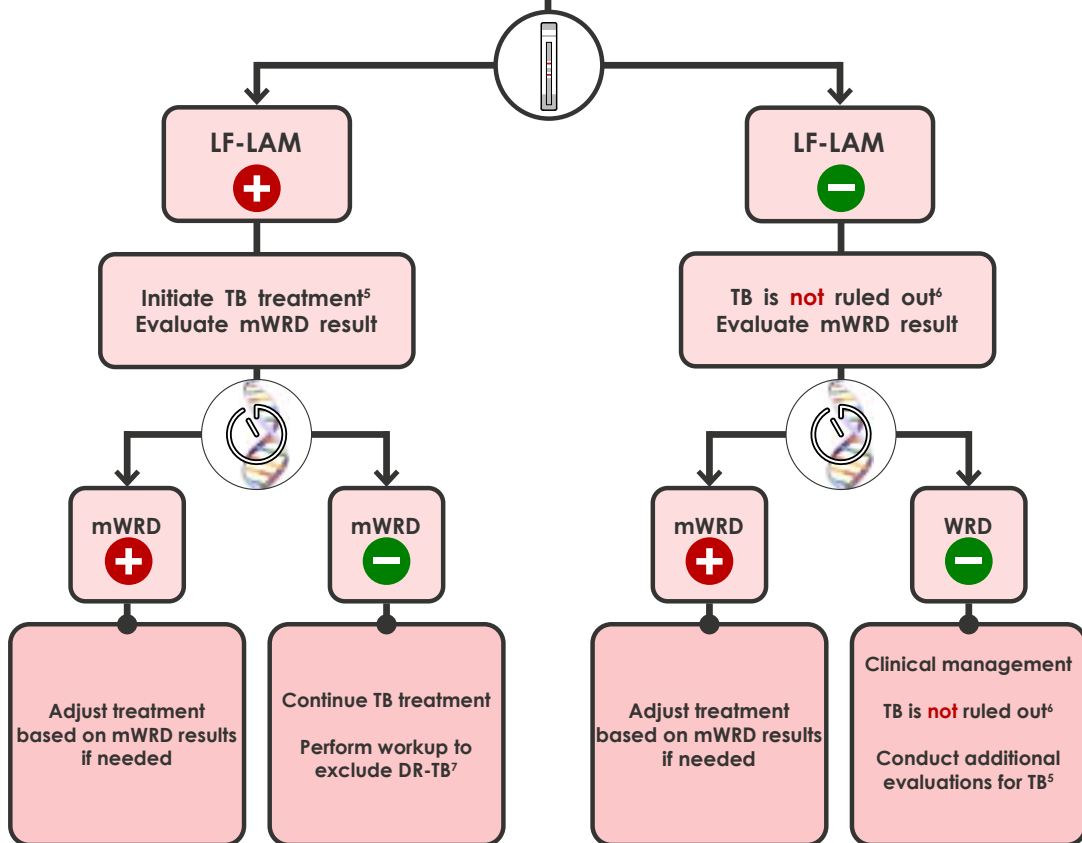**Adults, adolescents and children including:**

1. All newly diagnosed HIV patients that are ART naive

2. HIV patients returning for care following an interruption of treatment

3. HIV patients receiving an ART regimen that is failing

4. Patients presenting at the clinic and unwell

**Assess patient for:**

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

**Without assessing symptoms**

**Do not perform urine LF-FAM**

**Clinical management**
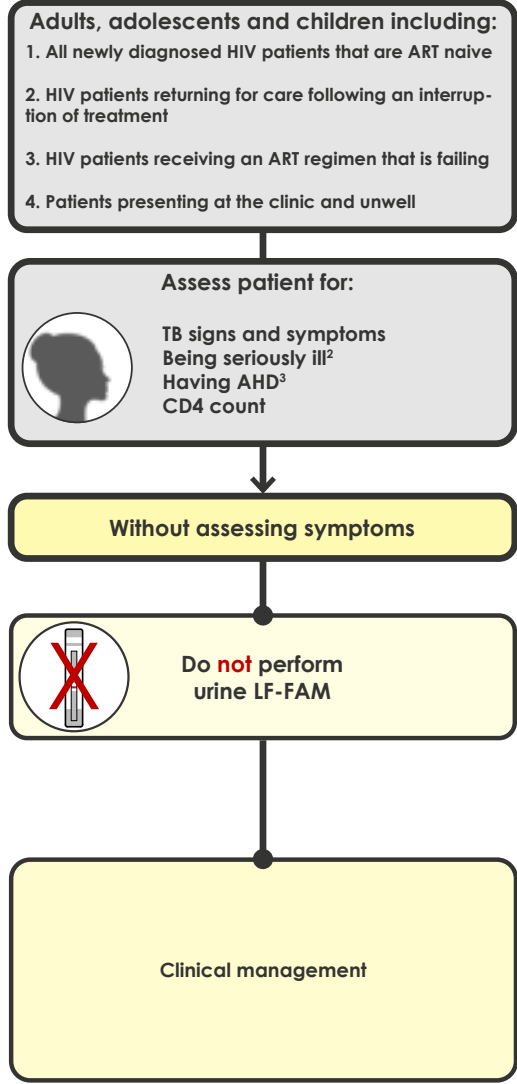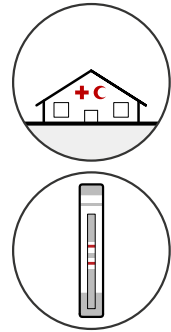
For footnotes please see page 77

## 4.3 Algorithm 3 – DST for second-line drugs for RR-TB or MDR-TB patients

Algorithm 3 is for further evaluation of patients with RR-TB or MDR-TB. In its most recent recommendations *(57)*, WHO stresses the importance of DST before starting the preferred shorter all-oral BDQ-containing MDR-TB regimen, especially for medicines for which mWRDs 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 (CFZ) 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.

# Fig. 4.5. DST for second-line drugs for RR- 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 and PZA 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 molecular FQ 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 molecular FQ** 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 failture[8]**

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

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

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

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

[3] WHO recommends getting the rapid DST results for FQs before the start of treatment, although this testing should not delay the start of treatment. Currently, LC-aNAAT and SL-LPA are the WHO-approved rapid molecular tests for detecting FQ resistance.

[4] Phenotypic DST should be conducted for each of the drugs included in the treatment regimen for which there are accurate and reproducible methods. Reliable phenotypic DST methods when performed in a quality-assured laboratory are available for BDQ, FQ, CFZ, INH, PZA, DLM and LZD. A new molecular class of tests, the reverse hybridization high complexity NAAT, is available for PZA resistance detection on culture isolates. The initiation of treatment should not be delayed while awaiting the results of the phenotypic DST.

[5] For more details regarding individualized regimens, see the *WHO consolidated guidelines on drug-resistant tuberculosis treatment (58)*.

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

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

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

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

*Tests*

- A new test, the low complexity automated NAAT, is recommended and is an alternative to SL-LPA:
  - It can be used in PLHIV, children and people with extrapulmonary TB.
  - The first in class test, the Xpert MTB-XDR test, provides results in under 2 hours, requires minimal hands-on time, can be used at the peripheral level and provides results simultaneously for FQ, INH, ETO and AMK. This test requires a 10-colour GeneXpert instrument unlike the current Xpert MTB/RIF and Xpert Ultra test that use the 6-colour GeneXpert instruments. The new instruments can be linked to existing 6-colour GeneXpert systems through a common computer.
  - The use of low complexity automated NAAT to detect FQ resistance does not eliminate the need for conventional culture-based phenotypic DST, which will be necessary for determining resistance to other anti-TB agents and monitoring the emergence of additional drug resistance.
- The SL-LPA is still recommended and used where available. The following should, however, be noted:
  - 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, human resources and QA systems are available. Implementation of SL-LPA testing depends on the availability of a reliable specimen transport system and an efficient mechanism for reporting results.
  - The use of SL-LPA to detect FQ resistance does not eliminate the need for conventional culture-based phenotypic DST, which will be necessary for determining resistance to other anti-TB agents and monitoring the emergence of additional drug resistance.

*General considerations*

- A shorter all-oral BDQ-containing treatment regimen of 9–12 months in duration is the preferred option for eligible MDR/RR-TB patients *(7)*.
  - The preferred regimen contains BDQ, LFX or moxifloxacin (MFX), ETO, EMB, INH (high-dose), PZA and CFZ (4–6 months of BDQ-LFX-ETO-EMB-PZA-high dose INH-CFZ / 5 months of LFX -CFZ-PZA-EMB).
  - Individualized all-oral longer regimens, designed using the WHO priority grouping of medicines, may still be used for MDR/RR-TB patients who do not meet the eligibility criteria for an all-oral shorter BDQ-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 low complexity automated NAAT or 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 *(59)*.

- WHO guidelines stress the importance of DST before treatment, especially for medicines for which mWRDs are available.
    - WHO-approved rapid molecular tests are available for RIF, INH and FQs. Genetic tests, including targeted NGS, are being developed for some of the other drugs (e.g. BDQ and LZD) included in MDR-TB regimens.
    - WHO recommends a new molecular test for PZA resistance detection belonging to the class high complexity reverse hybridization NAAT. Its use is limited to culture isolates. Alternatively, *pncA* sequencing should be performed when available. In a quality-assured laboratory, with careful attention to inoculum preparation, a susceptible phenotypic DST result using MGIT960 for PZA can be used to guide the inclusion of PZA in a DR-TB treatment regimen *(20)*.
    - Reliable phenotypic DST methods are available for RIF, INH, FQs, BDQ, CFZ, LZD, AMK and DLM. Testing algorithms that rely on culture and phenotypic DST are described in the relevant WHO policy framework *(60)* and technical manual *(20)*. Member States should ensure there is capacity for DST for drugs used for treatment and for which reliable testing is available.
    - No reliable phenotypic DST methods are available for EMB, ETO/prothionamide, cycloserine/terizidone or imipenem-cilastatin/meropenem; hence, results should not be used for clinical decision-making.
    - If phenotypic DST to second-line drugs is not available in-country, specimens or isolates may be shipped to an external laboratory for testing (e.g. a WHO supranational reference laboratory [SRL]). Material transfer agreements and import/export permits may be needed.
    - Currently, availability of phenotypic DST for BDQ and LZD is limited in many settings, and resistance levels are likely to be low. There is, however, increasing evidence that BDQ resistance even in unexposed patients 1.4–3.4% *(61)*. BDQ is a core drug for DR-TB treatment and included in the revised XDR-TB definition. Thus, building testing capacity to this drug and other drugs used in treatment (e.g. LZD, CFZ and DLM) is essential. If resistance is suspected during treatment and DST is not available, the strains should be referred to an SRL for further testing.
    - Do not delay initiation of treatment while waiting for the results of DST.

*Decision pathway for Algorithm 3*

1.  Promptly initiate the patient on an MDR-TB regimen, in accordance with national guidelines. The most recent WHO recommendations is for the use of a shorter all-oral BDQ-containing treatment regimen of 9–12 months in duration *(57, 58).*

2.  If molecular and phenotypic testing are performed in the same laboratory, collecting one specimen may be sufficient. If testing is performed in two laboratories, collect two specimens and conduct the molecular and phenotypic testing in parallel. Transport sputum specimens or isolates to the appropriate testing laboratory, if necessary.

3.  Conduct the low complexity automated NAAT or SL-LPA to detect mutations associated with FQ resistance.

4.  If the low complexity automated NAAT or 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.  The first in class low complexity automated NAAT (Xpert MTB-XDR) provides results for INH, ETO and AMK and can be used to inform individualized regimen selection. Collect a specimen and submit for phenotypic DST to the WHO Group A, B and C drugs (e.g. DST for BDQ, DLM and LZD), if phenotypic DST is not already being done as described in Step 6. Testing moxifloxacin at the clinical breakpoint should be performed to determine the potential use of high-dose (800 mg) moxifloxacin for treatment *(19).*

**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 and PZA resistance[3]**
**Conduct culture and phenotypic DST for second-line drugs[4]**

**Molecular DST**

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

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

For footnotes please see page 92

5. If the low complexity automated NAAT or SL-LPA is negative for mutations associated with resistance to FQs:

   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 the low complexity automated NAAT and SL-LPA to detect mutations associated with FQ resistance is about 93% and 86%, respectively. The phenotypic DST should include testing for resistance to the FQs used in the country. The phenotypic DST should also include testing moxifloxacin at the clinical breakpoint to inform individualized drug selection. Modify the regimen as necessary, based on the phenotypic DST results.

6. Perform culture and phenotypic DST for each of the drugs included in the treatment regimen for which there are accurate and reproducible methods ③ Ⓑ. For the preferred regimens, reliable phenotypic DST methods when performed in a quality-assured laboratory are available for BDQ, FQs, CFZ, PZA and INH. A WHO-approved molecular test for PZA resistance detection is available (high complexity reverse hybridization NAAT) but currently is limited to use on culture isolates.

   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. As results for phenotypic DST are slow, reassess patient response when these results are available. The decision to change from a shorter to the longer MDR-TB regimen should consider the phenotypic DST result and clinical response. Monthly monitoring is important, and the patient should be closely followed up.

**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 and PZA resistance[3]
Conduct culture and phenotypic DST for second-line drugs[4]

**Phenotypic DST**

**Phenotypic 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

For footnotes please see page 92

7. For all patients, ensure that treatment monitoring includes the collection of samples for culturing as described in the WHO consolidated guidelines *(58).* Any positive culture suggestive of treatment failure should undergo phenotypic DST. Modify the regimen as necessary, based on the phenotypic DST results.

   a. WHO recommends that all 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.

# 4.4 Algorithm 4 – mWRD as the initial or follow-on test to detect Hr-TB

Algorithm 4 is for either detection of Hr-TB or follow-on testing for those with Hr-TB already identified. Detection of Hr-TB would be applicable in patients or settings with a high risk for Hr-TB and with RIF-susceptible TB (e.g. those with a molecular WRD result of "MTB detected, RIF resistance not detected") and isoniazid unknown. All patients with RIF-susceptible TB and INH susceptible/unknown 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.

**Tests**

- The new moderate complexity automated NAAT class of initial tests for TB detection that simultaneously detects resistance to RIF and INH is recommended and should improve rapid identification of Hr-TB. Patients from Algorithm 1, where this class of test was used and Hr-TB was identified, would enter this algorithm for further investigation and management. This test could also be used for detection of isoniazid resistance for those with only rifampicin results.
- The new first in class low complexity automated NAAT class (Xpert MTB-XDR) is recommended as a follow-on test that simultaneously detects resistance to INH and FQ, is suited to the peripheral level and complements existing WRDs that detect only RIF resistance.
- The FL-LPA and SL-LPA are useful alternatives where these tests are already available and can detect INH and FQ resistance, respectively.
- The use of molecular tests to detect INH resistance does not eliminate the need for conventional culture-based phenotypic DST, which will be necessary to determine resistance to other anti-TB agents and to monitor the emergence of additional drug resistance.
- Detection of FQ resistance, along with PZA resistance, is important for patients with Hr-TB to ensure that an effective treatment is offered and prevent amplification of resistance to RIF or FQ.

- Phenotypic DST to PZA is desirable if a quality-assured reliable DST for PZA has been established in the country. An alternative is the new high complexity reverse hybridization NAAT which is recommended for use on culture isolates for PZA resistance detection. Sequencing of the *pncA* if available is another option.

**General considerations**

- Hr-TB prevalence is 7.4% (95% CI: 6.5–8.4%) in new cases and 11.4% (95% CI: 9.4–13.4%) in previously treated patients *(62)*. The prevalence in some settings can exceed 25%. Contacts of a known Hr-TB patient are also at increased risk. The prevalence of any INH resistance is particularly high in some parts of the WHO European Region and Western Pacific Region.
- Hr-TB is currently largely undetected but clinically relevant. 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% versus 2%), relapse (10% versus 5%) and acquiring additional drug resistance (8% versus 1%) *(63)*.
- 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.
- The recommended Hr-TB treatment regimen is :RIF, EMB, PZA and LFX for 6 months *(63)*.

## Fig. 4.6. Molecular WRD as the initial test to detect Hr-TB in patients with RIF-susceptible TB

**4**

**Entry point 1: All patients with MTB detected, rifampicin resistance not detected[1].**
Treat with first line regimen in accordance with national guidelines

**Entry point 2: Patient with MTB detected, rifampicin resistance not detected and isoniazid resistance detected**

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

**A** — INH resistance *not* detected

**B** — 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**. If LC-aNAAT used no need for additional specimen, FQ result available[4]

**FQ resistance detected**

**FQ resistance *not* detected**

**Indeterminate result, no result, error, or invalid test**

**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

**Initiate individulized Hr-TB treatment**[6]

Conduct **molecular or phenotypic 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

**Continue Hr-TB treatment**

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

Conduct **molecular or phenotypic DST for PZA**

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

**Continue Hr-TB treatment**

**Consider repeating molecular FQ testing** 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

INH

?

INH

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

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

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

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

[4]  For each patient with Hr-TB, a specimen should be referred for molecular DST for FQs. The LC-aNAAT can simultaneously detect INH and FQ resistance. The alternative for FQ resistance detection is the SL-LPA. PZA resistance detection should also be performed where this is available and quality assured.

[5]  Despite good sensitivity of LC-aNAAT (93%) and SL-LPA (86%) for detecting FQ resistance, culture and phenotypic DST may be needed for patients with a high pretest probability for FQ resistance (e.g. setting with a high underlying prevalence of resistance to FQs or patient risk factors) when the resistance is not detected by the molecular test.

[6]  Patients with FQ-resistant Hr-TB may be treated with a 6-month regimen of (H)REZ or an individualized Hr-TB regimen.

[7]  For all Hr-TB patients with concurrent resistance to FQ, phenotypic or molecular DST (e.g. HC-rNAAT) for PZA is desirable if a reliable DST for PZA has been established in the country. When resistance to PZA is confirmed, appropriate treatment regimens may have to be designed individually, especially if resistance to both FQ and PZA are detected.
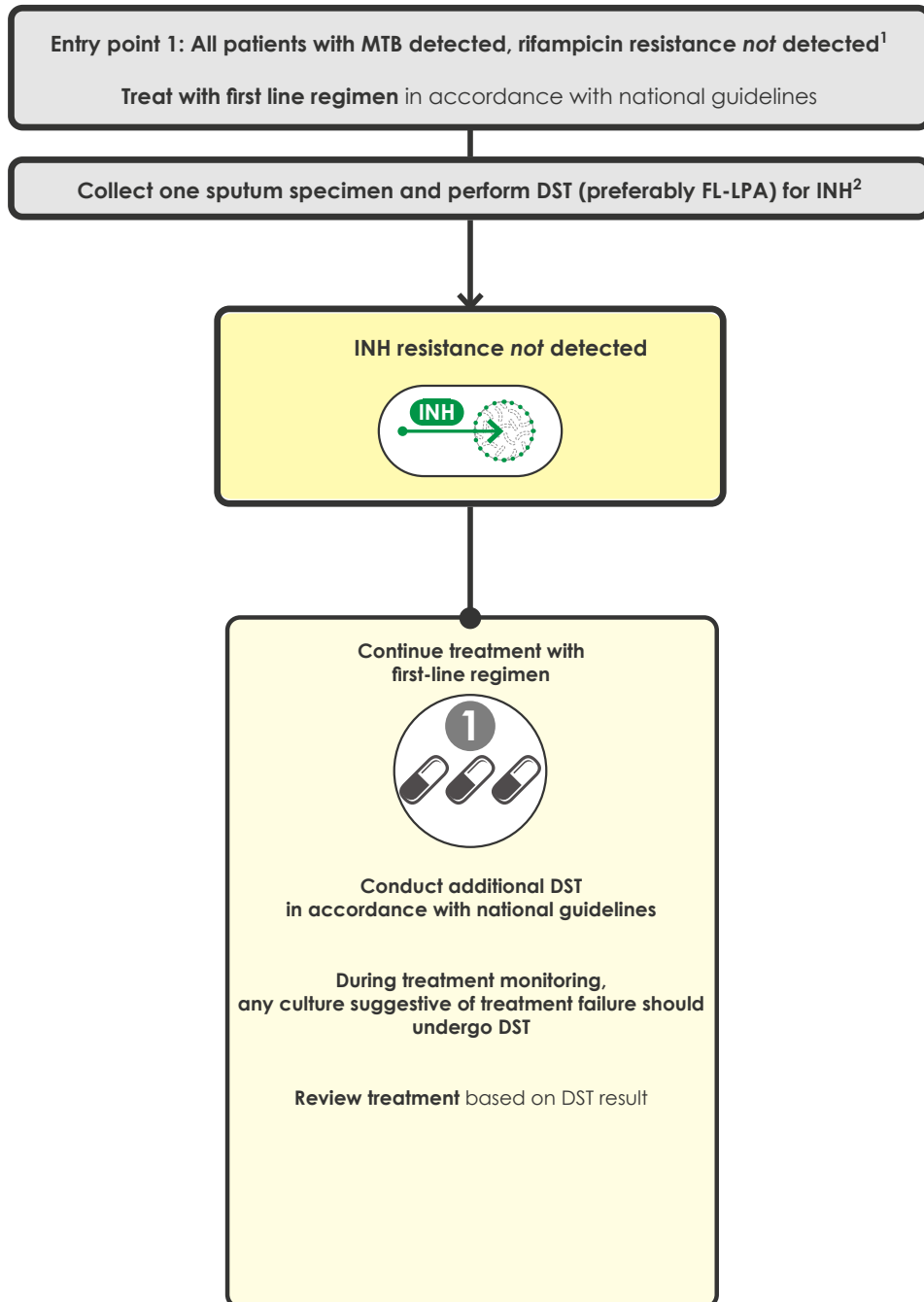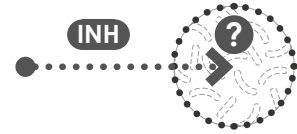
## 4.4.1 Decision pathway for Algorithm 4

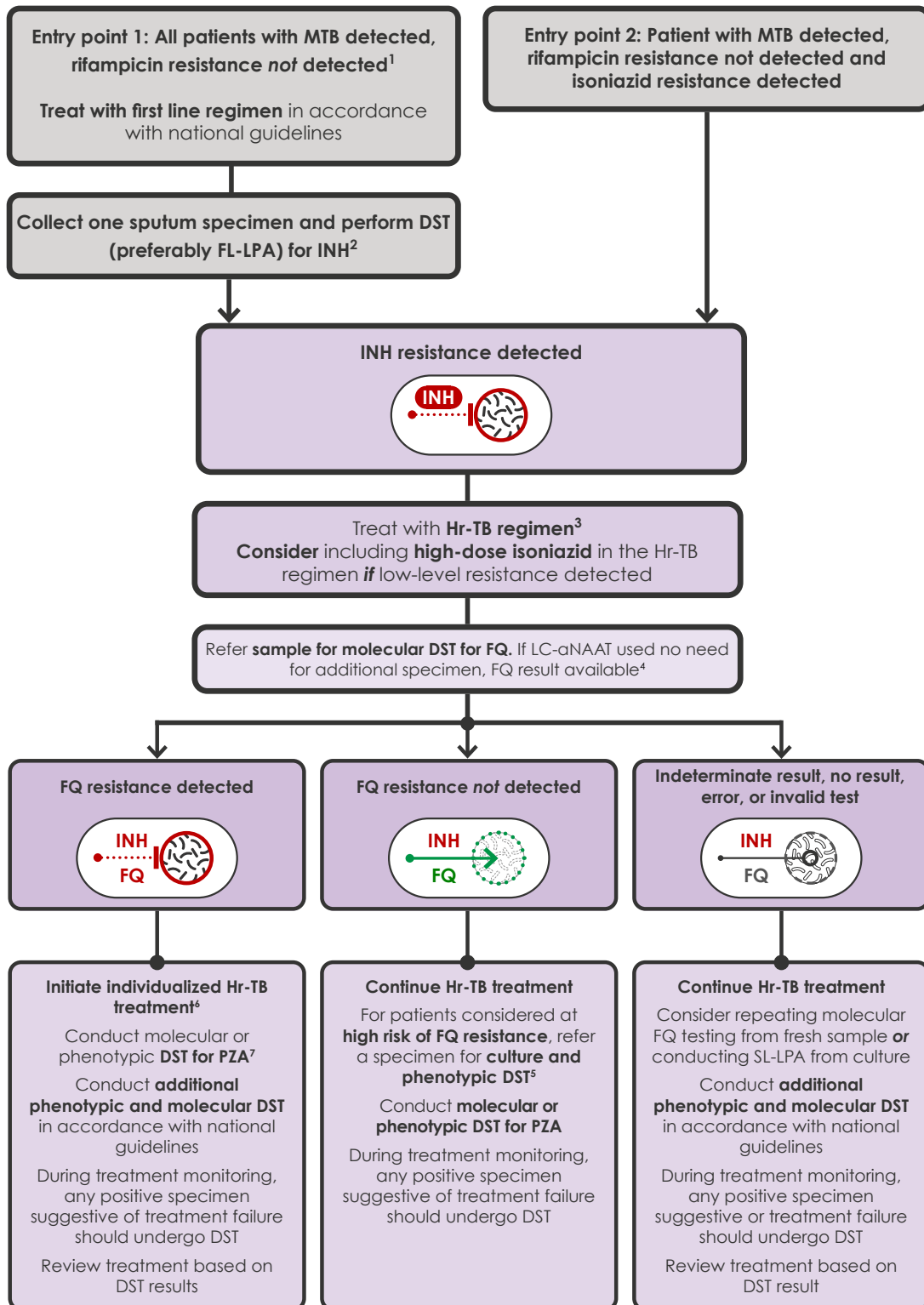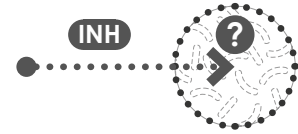There are two starting points for this algorithm:

- For a patient who has RIF-susceptible TB by DST, detected by either a molecular (e.g. Xpert MTB/RIF, Xpert Ultra or Truenat) or phenotypic test, but no results are available for INH and the patient is at a high risk for Hr-TB, start at Step 1.
- For a patient who has had an initial TB test that included RIF and INH results (e.g. a moderate complexity automated NAAT was used) in Algorithm 1, skip to Step 4.

1. Collect a good-quality specimen and transport it to the testing laboratory for molecular or phenotypic testing for INH resistance.

   a. Testing could follow a two-step process: detection of INH resistance followed by detection of FQ resistance. The two-step process is applicable when a moderate complexity automated NAAT or FL-LPA is used for Hr-TB detection and followed by the low complexity automated NAAT or SL-LPA for FQ resistance detection. A single step option is now available using the first in class low complexity automated NAAT which detects both INH and FQ resistance simultaneously.

   b. Phenotypic testing may be required for INH resistance determination as the sensitivity, depending on the test used, may miss ~15% of resistant samples (Table 3.3 in Section 3). Phenotypic testing will be relevant when the patient is at high risk for Hr-TB. If both molecular and phenotypic tests are performed, initiate the tests in parallel; do not wait for the results of one test before initiating the other test.

   c. Culture-based phenotypic DST for INH requires 3–8 weeks to produce a result. Phenotypic DST may be useful for evaluating patients with a susceptible mWRD INH result, particularly in populations with a high pretest probability for resistance to INH.

2. If INH resistance is *not* detected ④Ⓐ, 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 mWRD result.

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

INH ?

**Entry point 1: 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 FL-LPA) for INH[2]**

**INH resistance *not* detected**

INH

**Continue treatment with
first-line regimen**

**1**

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

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

**Review treatment** based on DST result

3. If INH resistance *is* detected (4) (B):

   a. Using the low complexity automated NAAT will provide simultaneous detection of resistance to INH and FQ. If FQ resistance is not detected follow Steps 3b and 5. If FQ resistance is detected follow Steps 3c iii and 5. If the FQ result is unknown or unsuccessful follow Step 3c.

   b. Initiate treatment with an Hr-TB regimen *(63)*:

      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 minimal inhibitory concentration (MIC) increases, may benefit from high-dose INH therapy. Thus, additional INH up to a maximum dose of 15 mg/kg per day may be considered for use with the Hr-TB regimen for such isolates. The added value of isoniazid in the regimen, even when used at the higher dose, declines as MICs increase further.

   c. Refer a specimen from a patient with laboratory-confirmed Hr-TB for molecular (e.g. low complexity automated NAAT or SL-LPA) or phenotypic DST for FQ and PZA. (Note that if the Xpert MTB/XDR test was used in Step 1, the FQ result will already be available, so skip to Step 3d.)

      i. Rapid molecular testing for FQ resistance is preferred. When used for direct testing of sputum specimens from patients, the low complexity automated NAAT and SL-LPA detects 93% and 86% of patients with FQ resistance, respectively (Table 3.4 in Section 3).

         1. Low complexity automated NAATs provide rapid results and are suitable for use at the peripheral level. The first in class test, Xpert MTB/XDR, reports low-level FQ resistance when the mutations *gyrA* A90V, *gyrA* S91P and *gyrA* D94A are detected from the probe melting temperature *(64)*. Phenotypic DST at the clinical breakpoint for moxifloxacin should be performed to confirm the potential value of high-dose moxifloxacin treatment for such patients.

         2. 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.

         3. Despite good specificity and sensitivity of low complexity automated NAATs and 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 pretest probability for resistance to FQ, to exclude resistance when the SL-LPA does not detect mutations associated with resistance.

INH

**Entry point 1: All patients with MTB detected, rifampicin resistance *not* detected[1]**

**Treat with first line regimen** in accordance with national guidelines

**Entry point 2: Patient with MTB detected, rifampicin resistance not detected and isoniazid resistance detected**

**Collect one sputum specimen and perform DST (preferably FL-LPA) for INH[2]**

**INH resistance detected**

INH

Treat with **Hr-TB regimen[3]**
**Consider** including **high-dose isoniazid** in the Hr-TB regimen *if* low-level resistance detected

Refer **sample for molecular DST for FQ.** If LC-aNAAT used no need for additional specimen, FQ result available[4]

**FQ resistance detected**

INH
FQ

**FQ resistance *not* detected**

INH
FQ

**Indeterminate result, no result, error, or invalid test**

INH
FQ

**Initiate individualized Hr-TB treatment[6]**

Conduct molecular or phenotypic **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

**Continue Hr-TB treatment**

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

Conduct **molecular or phenotypic DST for PZA**

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

**Continue Hr-TB treatment**

Consider repeating molecular FQ testing 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 or treatment failure should undergo DST

Review treatment based on DST result

For footnotes please see page 102

d. Review FQ resistance results.
   i. If FQ resistance is *not* detected, continue treatment with the LFX-containing Hr-TB regimen.
   ii. If FQ resistance *is* detected:
      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 the high complexity reverse hybridization NAAT, phenotypic DST in the MGIT system and *pncA* sequencing. For more details, see the WHO *Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis (20)*.
         a. If PZA resistance is *not* detected, or if PZA DST is not available, continue therapy with the regimen designed based on the previous DST results.
         b. If PZA resistance *is* detected, individualized treatment regimens may have to be designed, especially if resistance to both FQ and PZA is detected.

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

5. 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 follows from Algorithm 1 with an mWRD that detected MTBC and was RIF-susceptible. In the scenario where the moderate complexity automated NAAT was used, the INH result would already be available. In this algorithm the follow-up testing could have a second RIF result when the FL-LPA is used as a follow-on or a second INH result when the moderate complexity automated NAAT is followed by the low complexity automated NAAT. Sometimes, results may be discordant. Each discordant result will need to be investigated on a case-by-case basis. General considerations are outlined below.

1. mWRD (e.g. Xpert Ultra) result "MTBC detected", follow-on FL-LPA "MTB not detected" or "uninterpretable".[58]
   a. mWRDs approved for detection of TB have a lower LoD than the FL-LPA; thus, FL-LPA may fail to detect TB in mWRD-positive samples that contain few bacilli. For example, it is estimated that about 80% of specimens with "MTBC detected" by Xpert MTB/RIF will generate an interpretable FL-LPA result.
   b. The initial mWRD result should be used to guide treatment decisions, pending additional testing.

---

[58] The FL-LPA is not classified as an mWRD because it is not an initial test for TB detection but rather a follow-on test.

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. Initial mWRD result "MTBC detected, RIF resistance not detected"; RIF resistant by FL-LPA.

   a. Treatment decisions should be based on the FL-LPA result (i.e. treat based on the worst-case scenario).

   b. This result is expected to be rare because both assays interrogate the same region of the *rpoB* gene. There have been reports of mWRD RIF-susceptible and FL-LPA RIF-resistant discordances, but little data are available to assess how frequently this occurs.

   c. FL-LPA is more sensitive for identifying RIF resistance than most mWRDs in hetero-resistant populations (mixtures of susceptible and resistant bacteria). The test includes hybridization probes specific to both the common mutated and the wild-type sequences in the bacterial genome. If the Xpert Ultra was used, a review of the probe melting temperature curves may be helpful to identify hetero-resistant populations (e.g. a double peak).

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

3. Moderate complexity automated NAAT result "MTBC detected, RIF resistance not detected, INH resistance detected"; but "INH susceptible" by low complexity automated NAAT.

   a. This result is expected to be rare because both assays interrogate the same region of the *katG* and *inhA* genes.

   b. The existence of hetero-resistant populations (i.e. mixtures of susceptible and resistant bacteria) is a more likely reason, especially in high-burden settings where the force of infection is high. A review of the low complexity automated NAAT probe melting temperatures *(53)* may identify such a possibility (e.g. dual peak).

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

   d. Reassess risk for Hr-TB, and if high risk for Hr-TB or admin errors (e.g. mislabelling) are not the reason, treatment decisions should cover the worst-case scenario and be based on the moderate complexity automated NAAT result.

4. Moderate complexity automated NAAT result "MTBC detected, RIF resistance not detected, INH resistance not detected"; INH resistant by low complexity automated NAAT.

   a. Treatment decisions should be based on the low complexity automated NAAT result (i.e. treat based on the worst-case scenario).

   b. This result is expected to be rare because both assays interrogate the same region of the *katG* and *inhA* genes. However, the low complexity automated NAAT is more sensitive for INH detection as it includes additional gene targets (fabG1 and oxyR-ahpC intergenic regions).

   c. The existence of hetero-resistant populations (i.e. mixtures of susceptible and resistant bacteria) is another possible reason, especially in high-burden settings where the force of infection is high. A review of the low complexity automated NAAT probe melting temperatures may identify such a possibility (e.g. dual peak).

   d. Follow-up actions may include DNA sequencing, conducting phenotypic DST and evaluating the possibility of laboratory or clerical error.
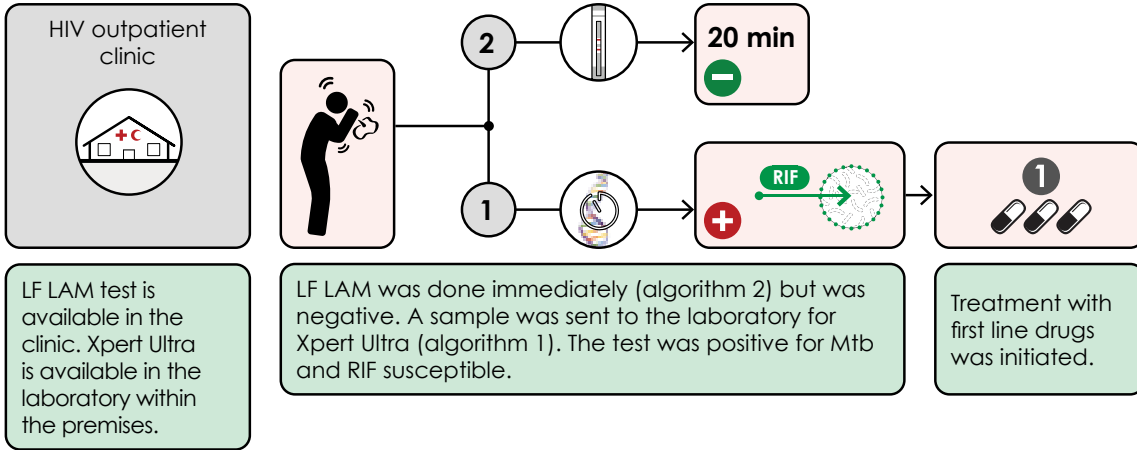
## 4.5 Illustrative algorithm combinations

To aid understanding of how the different algorithms interlink to provide a final diagnosis for a patient, illustrative scenarios are presented in Fig. 4.7 to Fig. 4.9. Three scenarios are provided, with two simulated pathways in each. The scenarios are based on three epidemiological settings: high TB/HIV, high Hr-TB and high MDR/RR-TB. These examples are for illustrative purposes only – they do not represent a specific recommendation. There could be many alternative combinations that could achieve the same outcome; the choice to use one test instead of another would depend on factors such as availability, ease of use, in-country product support and costs.

## Fig. 4.7. Scenario 1: High TB/HIV setting
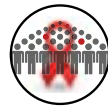


**SIMULATION 1** — The example illustrates the importance of combination testing in this population and the added value of Xpert Ultra in providing RIF results.

**HIV outpatient clinic**

LF LAM test is available in the clinic. Xpert Ultra is available in the laboratory within the premises.

LF LAM was done immediately (algorithm 2) but was negative. A sample was sent to the laboratory for Xpert Ultra (algorithm 1). The test was positive for Mtb and RIF susceptible.

Treatment with first line drugs was initiated.

**SIMULATION 2** — The example illustrates the value of having capacity to detect INH resistance with MC-aNAAT.

**District hospital**

LF LAM test is available in the clinic. MC-aNAAT is available in the laboratory within the premises.

Treatment was initiated after the positive LF-LAM test.

LF LAM was done immediately (algorithm 2) and was positive. A sample was sent to the laboratory for MC-aNAAT testing (algorithms 1). The test was positive for Mtb, RIF susceptible but INH resistant.

The detected INH resistance required further investigation according to algorithm 4. A genotypic DST, the second line LPA test, also available in the laboratory, was performed. FQ resistance was unfortunately detected. Treatment was adjusted to an individualised treatment. An additional sample was collected and sent to the national reference laboratory for phenotypic DST.

DST: drug-susceptibility testing; INH: isoniazid; LF-LAM: lateral flow lipoarabinomannan assay; MC-aNAAT: moderate complexity automated NAAT; MTBC: *Mycobacterium tuberculosis* complex bacteria; NAAT: nucleic acid amplification test; RIF: rifampicin; SL-LPA: line-probe assay for second-line drugs; TB: tuberculosis. Model algorithm number shown in shaded circles.

WHO operational handbook on tuberculosis: rapid diagnostics for tuberculosis detection

# Fig. 4.8. Scenario 2: High Hr-TB setting



**SIMULATION 1**

The example illustrates the need to consider the local context. In this case the high Hr prevalence triggered testing for INH resistance for all RIF susceptible patients. It demonstrates follow on HrTB testing after mWRD.

**Peripheral clinic**

The Xpert MTB/RIF and LC-aNAAT tests are available in the clinic.

The presumptive TB patient was tested with Xpert MTB/RIF tests (algorithm 1).

RIF susceptible TB was detected. First line TB treatment was initiated.

High Hr-TB setting required testing for INH resistance (algorithm 4).

Second sample was collected and tested with LC-aNAAT. INH resistance was detected. An FQ susceptible result was also provided.

The treatment was modified to an Hr-TB regimen with levofloxacin added.

**SIMULATION 2**

The example illustrates the value of the MC-aNAAT that detects INH and RIF resistance simultaneously at initial TB diagnosis.
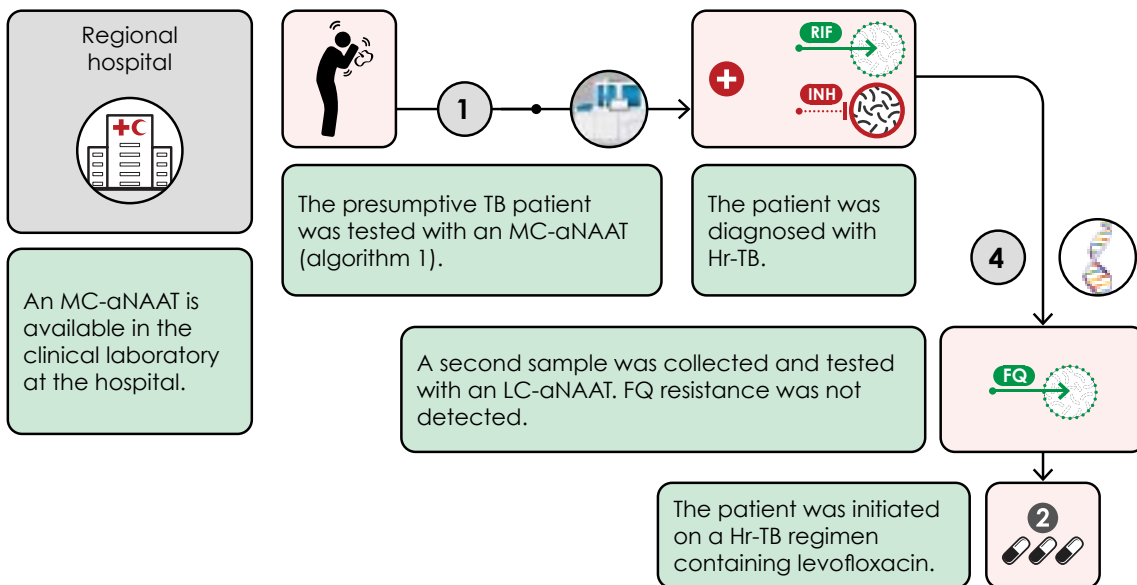
**Regional hospital**

An MC-aNAAT is available in the clinical laboratory at the hospital.

The presumptive TB patient was tested with an MC-aNAAT (algorithm 1).

The patient was diagnosed with Hr-TB.

A second sample was collected and tested with an LC-aNAAT. FQ resistance was not detected.

The patient was initiated on a Hr-TB regimen containing levofloxacin.

Hr-TB: isoniazid-resistant, rifampicin-susceptible tuberculosis; INH: isoniazid; LC-aNAAT: low complexity automated NAAT; MC-aNAAT: moderate complexity automated NAAT; MTBC: *Mycobacterium tuberculosis* complex bacteria; NAAT: nucleic acid amplification test; TB: tuberculosis. Model algorithm number shown in shaded circles.

# Fig. 4.9. Scenario 3: High MDR/RR-TB setting



**SIMULATION 1** — The example illustrates a decentralized model fitted to a tiered laboratory network to address patient needs.

Peripheral clinic

The Xpert MTB/RIF test is available in the clinic.

1 — A presumptive TB patient provided a sputum specimen and was tested with Xpert Ultra (algorithm 1).

RIF resistant TB was detected.

A second sample was collected and INH and FQ resistance detected with a LC-aNAAT.

The patient was referred to a specialized centre for individualised treatment. An additional sample was collected for further geno- and phenotypic DST.

PZA resistance was detected with a HC-rNAAT.

The treatment was modified according to the resistance pattern of the patient.

**SIMULATION 2** — The example illustrates a centralized model with a sample referral network to address patient needs.

Urban Clinic

An MC-aNAAT is available in the referral clinical laboratory in the city. The SL-LPA is available at the same laboratory.

1 — A presumptive TB patient provided a sputum specimen and was referred for testing with MC-aNAAT (algorithm 1).

The patient was diagnosed with MDR-TB.

SL-LPA was performed.

PZA resistance was detected with a HC-rNAAT.

Phenotypic DST was performed and treatment was modified according to the resistance pattern of the patient.

HC-rNAAT: high complexity reverse hybridization NAAT; LC-aNAAT: low complexity automated NAAT; LFX: levofloxacin; MC-aNAAT: moderate complexity automated NAAT; MTBC: *Mycobacterium tuberculosis* complex bacteria; NAAT: nucleic acid amplification test; PZA: pyrazinamide; RIF: rifampicin; SL-LPA: line-probe assay for second-line drugs; TB: tuberculosis. Model algorithm number shown in shaded circles.

## 4.5.1 Implementing a new diagnostic algorithm

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

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

The risk factors for TB and DR-TB vary widely among countries; hence, 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.

WHO operational handbook on tuberculosis:
rapid diagnostics for tuberculosis detection

# 5. Suggested reading

## WHO policy guidance on TB diagnostics and laboratory strengthening

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 (https://apps.who.int/iris/bitstream/handle/10665/112472/9789241506335_eng.pdf;jsessionid=C6DD5DFBE6B8B0264DE8E02946EBF290?sequence=1).

WHO End TB Strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2015 (https://www.who.int/publications/i/item/WHO-HTM-TB-2015.19).

Implementing tuberculosis diagnostics: a policy framework (WHO/HTM/TB/2015.11). Geneva: World Health Organization; 2015 (https://www.who.int/publications/i/item/9789241508612).

Chest radiography in tuberculosis detection: summary of current WHO recommendations and guidance on programmatic approaches. Geneva: World Health Organization; 2016 (https://www.who.int/publications/i/item/9789241511506).

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

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 (https://www.who.int/tb/areas-of-work/drug-resistant-tb/treatment/FAQshorter_MDR_regimen.pdf?ua=1).

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 (https://www.who.int/publications/i/item/9789241511261).

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://www.who.int/publications/i/item/9789241510561).

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

Planning for country transition to Xpert MTB/RIF Ultra cartridges. Geneva: Global Laboratory Initiative; 2017 (http://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/publications/i/item/WHO-HTM-TB-2017.04).

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/publications/i/item/9789241514842).

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).

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/2019/WHO_RapidCommunicationMDRTB2019.pdf?ua=1).

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/publications/i/item/9789241550604).

WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020. (https://www.who.int/publications/i/item/9789240007048)

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).

# 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://stoptb.org/wg/gli/gat.asp).

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

# Training packages

Training package on culture in solid and liquid media. Geneva: Global Laboratory Initiative; 2012 (http://www.stoptb.org/wg/gli/documents.asp?xpand=2).

Training package on DST by phenotypic and molecular method. Geneva: Global Laboratory Initiative; 2012 (http://www.stoptb.org/wg/gli/documents.asp?xpand=2).

Training package on line probe assays (LPAs). Geneva: Global Laboratory Initiative; 2012 (http://www.stoptb.org/wg/gli/documents.asp?xpand=2).

Training package on Xpert MTB/RIF. Geneva: Global Laboratory Initiative; 2014 (http://www.stoptb.org/wg/gli/documents.asp?xpand=2).

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

# 6. References

1   Global tuberculosis report 2020. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240013131).

2   Impact of the COVID-19 pandemic on TB detection and mortality in 2020. Geneva: World Health Organization; 2021 (https://www.who.int/publications/m/item/impact-of-the-covid-19-pandemic-on-tb-detection-and-mortality-in-2020).

3   WHO End TB Strategy: global strategy and targets for tuberculosis prevention, care and control after 2015. Geneva: World Health Organization; 2015 (https://www.who.int/publications/i/item/WHO-HTM-TB-2015.19).

4   Report of the 16th meeting of the Strategic and Technical Advisory Group for Tuberculosis (WHO/HTM/TB/2016.10). Geneva: World Health Organization; 2016 (https://www.who.int/tb/advisory_bodies/stag_tb_report_2016.pdf?ua=1).

5   WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

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

7   WHO consolidated guidelines on tuberculosis Module 4: treatment – drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240007048).

8   Public announcement to TB in vitro diagnostics manufacturers. Geneva: World Health Organization; 2021 (https://www.who.int/publications/m/item/public-announcement-to-tb-in-vitro-diagnostics-manufacturers ).

9   Consolidated guidelines on drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240007048).

10  Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014) (WHO/HTM/TB/2013.2). Geneva: World Health Organization; 2013 (https://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf;jsessionid=FD522CF3B90C25716F96288BFDEA6C75?sequence=1).

11  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).

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

13  Practical guide to implementation of Truenat tests for the detection of TB and rifampicin resistance (Version 2). Geneva: Stop TB Partnership, USAID and GLI; 2021 (http://stoptb.org/assets/documents/resources/publications/sd/Truenat_Implementation_Guide.pdf).

14  FIND cDST WHO supplement. Geneva: World Health Organization; 2019 (https://www.finddx.org/wp-content/uploads/2019/08/FIND_cDST_WHO_Supplement.xlsx).

15  Practical implementation of lateral flow urine lipoarabinomannan assay (LF-LAM) for detection of active tuberculosis in people living with HIV. Geneva: Global Laboratory Initiative; 2021 (http://stoptb.org/wg/gli/assets/documents/practical-implementation-lf-lam.pdf).

16  Sengstake S, Rigouts L. A multicenter evaluation of the Genoscholar PZA-TB II line probe assay to detect pyrazinamide resistance in *Mycobacterium tuberculosis* isolates: study report. Institute of Tropical Medicine, Antwerp. 2020.

17  Commercial serodiagnostic tests for diagnosis of tuberculosis: policy statement (WHO/HTM/TB/2011.5). Geneva: World Health Organization; 2011 (https://www.who.int/publications/i/item/9789241502054).

18  Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/meeting-report-of-the-who-expert-consultation-on-the-definition-of-extensively-drug-resistant-tuberculosis).

19  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/publications/i/item/WHO_CDS_TB_2018.5).

20  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/publications/i/item/9789241514842).

21  HIV Reagent Program [website]. Bethesda, MA: NIH HIV Reagent Program; 2021 (https://www.hivreagentprogram.org/).

22  Access to pure drug substances for DST: bedaquiline and delamanid StopTB information note. Geneva: Stop TB Partnership; 2021 (http://stoptb.org/assets/documents/resources/publications/sd/BDQ_DEL_access.pdf).

23  American Type Culture Collection [website]. Manassas, VA: ATCC; 2021 (https://www.atcc.org).

24  GDF product catalog. Geneva: Stop TB Partnership; 2021 (http://www.stoptb.org/gdf/drugsupply/product_catalog.asp).

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

26  Updated interim critical concentrations for first-line and second-line DST (as of May 2012) Geneva: World Health Organization; 2012 (http://www.stoptb.org/wg/gli/assets/documents/Updated%20critical%20concentration%20table_1st%20and%202nd%20line%20drugs.pdf).

27  Technical report on critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis (WHO/CDS/TB/2018.5). Geneva: World Health Organization; 2018 (http://apps.who.int/iris/bitstream/10665/260470/1/WHO-CDS-TB-2018.5-eng.pdf).

28  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).

29  Genoscreen. Deeplex Myc-TB – A new *Mycobacterium tuberculosis* drug resistance prediction assay [website]. (https://www.genoscreen.fr/en/genoscreen-services/products/deeplex).

30  Tgen. Tgen and ABL pursue global rollout of advanced TB test [website]. (https://www.tgen.org/news/2019/march/18/tgen-tb-test-to-help-eliminate-disease-worldwide/).

31  Advanced Biological Laboratories. Deepchek-TB Rpob/Inha genotyping assay [website]. (https://www.ablsa.com/laboratory-applications/deepchek-hbv-rt-genotyping-dr-assay-2-2/).

32  Oxford Nanopore Technologies. Rapid diagnosis of drug resistant tuberculosis using nanopore sequencing [website]. (https://nanoporetech.com/resource-centre/rapid-diagnosis-drug-resistant-tuberculosis-using-nanopore-sequencing).

33  Technical guide on next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex. Geneva: World Health Organization; 2018 (https://www.who.int/publications/i/item/WHO-CDS-TB-2018.19).

34  Practical considerations for implementation of Truenat. Geneva: Stop TB Partnership and USAID; 2020 (http://stoptb.org/assets/documents/resources/wd/Practical%20Considerations%20for%20Implementation%20of%20Truenat.pdf).

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

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

37  Chest radiography in tuberculosis detection: summary of current WHO recommendations and guidance on programmatic approaches. Geneva: World Health Organization; 2016 (https://www.who.int/publications/i/item/9789241511506).

38  Systematic screening for active tuberculosis: principles and recommendations (WHO/HTM/TB.2013.04). Geneva: World Health Organization; 2013 (https://www.who.int/publications/i/item/9789241548601).

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

40  Tuberculosis laboratory biosafety manual (WHO/HTM/TB/2012.11). Geneva: World Health Organization; 2012 (https://www.who.int/publications/i/item/9789241504638).

41  GLI practical guide to TB laboratory strengthening. Geneva: Global Laboratory Initiative; 2017 (http://stoptb.org/wg/gli/gat.asp).

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

43  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/publications/i/item/WHO-HTM-TB-2017.06).

44  GLI quick guide to TB diagnostics connectivity solution. Geneva: Global Laboratory Initiative; 2016 (http://www.stoptb.org/WG/gli/assets/documents/gli_connectivity_guide.pdf).

45  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).

46  Berhanu RH, Schnippel K, Kularatne R, Firnhaber C, Jacobson KR, Horsburgh CR et al. Discordant rifampicin susceptibility results are associated with Xpert MTB/RIF probe B and probe binding delay. Int J Tuberc Lung Dis. 2019;23(3):358–62.

47  Ngabonziza JCS, Decroo T, Migambi P, Habimana YM, Van Deun A, Meehan CJ et al. Prevalence and drivers of false-positive rifampicin-resistant Xpert MTB/RIF results: a prospective observational study in Rwanda. Lancet Microbe. 2020;1(2):e74-e83 (https://doi.org/10.1016/S2666-247(20)30007-0).

48  Beylis N, Ghebrekristos Y, Nicol M. Management of false-positive rifampicin resistant Xpert MTB/RIF. Lancet Microbe. 2020;1(6):e238 (https://doi.org/10.1016/S2666-5247(20)30123-3).

49  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).

50  Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B et al. Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis. 2018;18(1):76–84 (https://doi.org/10.1016/S1473-3099(17)30691-6).

51  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).

52  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).

53  Cao Y, Parmar H, Gaur RL, Lieu D, Raghunath S, Via N et al. Xpert MTB/XDR: a 10-color reflex assay suitable for point-of-care settings to detect isoniazid, fluoroquinolone, and second-line-injectable-drug resistance directly from *Mycobacterium tuberculosis*-positive sputum. Journal of clinical microbiology. 2021;59(3):e02314–20.

54  Sanchez-Padilla E, Merker M, Beckert P, Jochims F, Dlamini T, Kahn P et al. Detection of drug-resistant tuberculosis by Xpert MTB/RIF in Swaziland. N Engl J Med. 2015;372(12):1181–2.

55  Ismail NA, McCarthy K, Conradie F, Stevens W, Ndjeka N. Multidrug-resistant tuberculosis outbreak in South Africa. Lancet Infect Dis. 2019;19(2):134–5 (https://doi.org/10.1016/S1473-3099(18)30715-1).

56  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/publications/i/item/9789241550604).

57  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/2019/WHO_RapidCommunicationMDRTB2019.pdf?ua=1).

58  WHO consolidated guidelines on tuberculosis. Module 4: treatment - drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240007048).

59  GLI model diagnostic algorithms. Geneva: Global Laboratory Initiative; 2017 (http://www.stoptb.org/wg/gli/assets/documents/GLI_algorithms.pdf).

60  Implementing tuberculosis diagnostics: a policy framework (WHO/HTM/TB/2015.11). Geneva, World Health Organization. 2015 (https://www.who.int/publications/i/item/9789241508612).

61  Ismail NA, Aono A, Borroni E, Cirillo DM, Desmaretz C, Hasan R et al. A multimethod, multicountry evaluation of breakpoints for bedaquiline resistance determination. Antimicrob Agents Chemother. 2020;64(9):e00479–20 (http://aac.asm.org/content/64/9/e00479-20.abstract).

62  Dean AS, Zignol M, Cabibbe AM, Falzon D, Glaziou P, Cirillo DM et al. Prevalence and genetic profiles of isoniazid resistance in tuberculosis patients: A multicountry analysis of cross-sectional data. PLoS Med. 2020;17(1):e1003008 (https://doi.org/10.1371/journal.pmed.1003008).

63  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).

64  Cao Y, Parmar H, Gaur RL, Lieu D, Raghunath S, Via N et al. Xpert MTB/XDR: A 10-color reflex assay suitable for point of care settings to detect isoniazid-, fluoroquinolone-, and second-line-injectable-drug resistance directly from *Mycobacterium tuberculosis*-positive sputum. bioRxiv. 2020:2020.09.08.288787 (http://biorxiv.org/content/early/2020/09/09/2020.09.08.288787.abstract).

WHO operational handbook on tuberculosis:
rapid diagnostics for tuberculosis detection

# Annex 1: Budgetary considerations for implementing a new diagnostic test

Successful implementation of the plan will require financial and human resource commitments from the ministry of health (MoH) or national tuberculosis (TB) programme (NTP), with possible support from implementing partners. Consider integrating TB testing on existing multi-disease platforms in locations where integrated testing is feasible, to share costs across disease programmes. A budget should be developed to address activities in collaboration with key partners, using the considerations outlined below. Technical assistance may be needed.

| Budgetary considerations |
| --- |
| **Policies and planning** |

- Workshop for stakeholder engagement and planning
- Costs of TWG meetings
- Technical workshop for guideline and algorithm update
- Situational analysis costs (HR, travel and report writing)
- Printing and distribution costs for revised guidelines and algorithms
- Development of a costed operational plan
- External technical assistance costs, if needed

| **Regulatory** |
| --- |

- Regulatory submission costs, if applicable
- Local travel costs to regulatory authority
- Importation processes and costs
- Verification study, if required (samples, reagents and HR)

## Equipment

- Costs of assessing site readiness (travel and HR)
- Costs of upgrading laboratory facilities and infrastructure (e.g. electricity and air conditioning) to ensure a safe and functional testing site
- Costs to adhere to biosafety precautions, and biological and chemical waste disposal requirements
- Costs of selecting, procuring and installing equipment:
  - purchase (or lease) of instruments and any necessary ancillary equipment
  - delivery and importation costs
  - installation by manufacturer or authorized service provider (e.g. per diems and travel)
  - training
  - instrument verification
  - extended warranty or service contract
- Costs of routine preventive maintenance
- Costs of annual maintenance or calibration

## Supplies

- Workshop for stakeholders involved in procurement, to strengthen the supply chain
- Costs of maintaining centralized stores and costs of distribution
- Material cost per test (e.g. test reagents, consumables, sample collection items and printing paper), and additional equipment costs such as requirements for additional equipment (e.g. printer, computer and printer cartridges), shipping and courier costs
- Costs of new-lot testing

## Procedures

- Workshop and HR for the development of SOPs
- Printing and dissemination of revised SOPs
- Development, printing and dissemination of revised clinical protocols and guidance for selecting patients to be tested, ordering tests, interpreting test results and making decisions on patient care

## Digital data

- Purchase and implementation of a laboratory information management system, if applicable
- Purchase and installation of a diagnostics connectivity solution, if applicable
- HR and training
- Costs of data transmission (e.g. high-speed internet service)
- Costs associated with providing and maintaining a remote monitoring system in-country

## Quality assurance, control and assessment

- Preparation and regular review of all testing and QA documents (e.g. SOPs and checklists) based on national requirements
- Cost of conducting quality controls (e.g. testing known positives or negatives)
- Costs of HR for routinely collecting and analysing quality indicators
- Costs of conducting on-site visits (e.g. travel, HR, and preparation of checklists and reports)
- Costs associated with hosting an on-site visit and preparation of documents
- Costs associated with providing PT panels and overseeing PT, reporting results and corrective actions, and costs associated with testing PT panels at each site
- Costs associated with retesting samples at a higher level laboratory (e.g. shipment of samples, testing and reporting), if applicable

## Recording and reporting

- Workshop and HR to update recording and reporting forms, registers, etc.
- Preparation, printing and distribution of standardized forms (e.g. test request and results reporting) and logbooks

## Training and competency assessment

- Workshop and HR to update training packages for laboratory and clinical staff
- Training-of-trainers workshop, participant and instructor travel, on-site training and sensitization meetings
- Printing and distribution of updated training manuals and sensitization materials
- Costs associated with facility and classroom-based training (e.g. travel, accommodation, printing materials, venue hire and catering)
- Costs associated with annual competency testing of staff

## Monitoring and evaluation

- Meetings to update monitoring and evaluation system, and regular meetings to review impact of transition and re-planning
- Monitoring and evaluation of refresher training
- Operational research study to measure clinical impact

## Annual ongoing costs

- Consumables and reagents for diagnostic testing
- Costs associated with repeat testing and proficiency testing
- Specimen referral and results reporting
- HR
- Equipment calibration and servicing
- Diagnostics connectivity
- QA

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

# Annex 2: Information sheets on the newly recommended products

# A2.1 Information sheet: Practical considerations for implementation of the Abbott RealTi*me* MTB and Abbott RealTi*me* MTB RIF/INH tests

*Abbott Molecular* diagnostic solution for tuberculosis (TB) has two nucleic acid amplification tests (NAATs), one for detection of *Mycobacterium tuberculosis* complex (MTBC) (RealTi*me* MTB test) and one for detection of resistance to both rifampicin (RIF) and isoniazid (INH) (RealTi*me* MTB RIF/INH) *(1)*. TB detection is based on the IS6110 genetic element and the *pab* gene targets. The RIF and INH resistance test uses eight dye-labelled probes to detect mutations in the RIF-resistance determining region (RRDR) of the *rpo*B gene for RIF resistance and four probes to detect INH resistance, with two probes each for the *kat*G and *inh*A genes. The test is performed on the m2000 platform, m2000*sp* instrument used for automated DNA extraction and the m2000*rt* for performing real-time polymerase chain reaction (PCR). The World Health Organization (WHO) includes these tests within the class of moderate complexity automated NAATs, and the recommendations below apply to these tests *(2)*.

## WHO recommendations for use

In people with signs and symptoms of pulmonary TB, moderate complexity automated NAATs may be used on respiratory samples for detection of pulmonary TB, RIF resistance and INH resistance, rather than culture and phenotypic drug susceptibility testing (DST). *(Conditional recommendation; moderate certainty of evidence for diagnostic accuracy)*

There are several subgroups to be considered for this recommendation:

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

## Key performance conclusions

- Both RealTi*me* MTB and RealTi*me* MTB RIF/INH assays perform well for the diagnosis of TB and drug-resistant TB (DR-TB) compared with culture and phenotypic DST.
- Limit of detection reported by the company: TB detection = 17 colony forming units (CFU)/mL, RIF/INH detection = 60 CFU/mL *(3–5)*.
- The pooled sensitivity and specificity data for the class are presented in the Web Appendix of the WHO consolidated guidelines *(6)*.

## Test procedures at-a-glance

The automated RealTi*me* MTB assay targets DNA insertion element IS*6110* and the *pab* genes from eight members of the MTBC (*M. tuberculosis*, *M. africanum*, *M. bovis*, *M. bovis BCG*, *M. microti*, *M. caprae*, *M. pinnipedii* and *M. canetti*), using real-time PCR technology to detect the presence of MTBC. For RealTi*me* MTB RIF/INH testing, raw or processed sputum specimens may be inactivated and prepared, or DNA eluates from RealTi*me* MTB-positive specimens may be used directly for testing. The RealTi*me* MTB RIF/INH assay uses real-time PCR-based fluorescent probe technology to detect RIF resistance (*rpo*B gene), and high-level (*kat*G gene) and low-level (*inh*A promoter region) INH resistance. Use of both assays together allows for detection of MTBC with or without rifampicin-resistant TB (RR-TB), isoniazid-resistant, rifampicin-susceptible TB (Hr-TB) or multidrug-resistant TB (MDR-TB) within 10.5 hours.

**Table A2.1. Characteristics of Abbott RealTi*me* MTB and Abbott RealTi*me* MTB RIF/INH platforms**

| Step | RealTi*me* MTB | RealTi*me* MTB RIF/INH |
|---|---|---|
| **Sample inactivation** | Decontamination or liquefaction of sputum (optional) and inactivation with inactivation reagent (1 hour) | **Sputum**: Same as RealTi*me* MTB.<br>**RealTime MTB DNA**: Not required. |
| **Sample preparation** | Automated (m2000*sp* instrument) or manual sample preparation using the required Abbott mSample Preparation System$_{DNA}$ kit, wherein cells are lysed and magnetic microparticles capture and purify MTBC DNA (4.5 hours) | **Sputum**: Same as RealTi*me* MTB.<br>**RealTime MTB DNA**: Not required. |
| **Reagent preparation and reaction plate assembly** | Automated (m2000*sp* instrument) or manual addition of prepared sample and test reagents to a 96-well plate for real-time PCR. | |
| **DNA amplification and target detection** | Amplification reagents are automatically (m2000*sp* instrument) or manually added to the prepared sample and loaded onto the m2000*rt* instrument for RT-PCR, fluorescence detection and automated reporting (2 hours) | |

DNA: deoxyribonucleic acid; IR: inactivation reagent; MTBC: Mycobacterium tuberculosis complex; PCR: polymerase chain reaction.

## Equipment, supplies and reagents required

*Supplied:*

**RealTi*me* MTB Reagents**: RealTi*me* MTB Amplification Reagent Kit
(one reagent pack and an internal control) and the RealTi*me* MTB
Control Kit (includes positive and negative internal controls).

**RealTi*me* MTB RIF/INH Reagents**: RealTi*me* MTB RIF/INH Amplifi-
cation Reagent Kit (includes three reagent packs, an internal control,
a positive control and a negative control) and the RealTi*me* MTB RIF/
INH Control Kit (includes external positive and negative controls).

**Other reagents**: Abbott mSample Preparation System$_{DNA}$ Kit
(192 sample preparations/kit) and inactivation reagent (IR).

**m2000 system**: m2000*sp* instrument (optional for automated,
medium throughput sample processing, 145.0 × 79.4 × 217.5 cm,
weight 314.4 kg), m24*sp* instrument (optional for automated,
low throughput sample processing, 88.1 × 75.9 × 69.6 cm, weight
84 kg) and m2000*rt* instrument (required for RT-PCR amplification,
fluorescence detection and result reporting, 34 × 49 × 45 cm, weight
34.1 kg).

*Not supplied but required*

- Class II Biological Safety Cabinet, 2000 x g plate centrifuge and vortex
- Calibrated precision pipettes, aerosol barrier pipette tips, sample racks,
  reaction vessels, tubes and microcentrifuge tubes
- 95–100% ethanol, 10 M NaOH, isopropanol, Tween20 and molecular water
- Abbott 96-well optical reaction plates and adhesive plate covers with applicator
- Abbott 96-deep-well plates and plate holder(s)
- Personal protective equipment (e.g. chemical-resistant gloves, laboratory coat and
  eye protection)

*Operational considerations*

- **Testing capacity**: RealTi*me* MTB assay (96 samples per kit, including two assay controls),
  RealTi*me* MTB RIF/INH assay (24 samples per kit, including two assay controls).
- **Service and maintenance**: Abbott m2000*sp* (daily maintenance), Abbott m2000*rt* (weekly
  and monthly maintenance) and Abbott m2000 system (annual, manufacturer-provided
  with contract).
- **Storage conditions**: RealTi*me* MTB amplification and control reagents (–15 °C to –25 °C
  storage; 2–8 °C after opening), partially used packs must be capped and kept upright,
  protected from light and used within 14 days. Abbott RealTi*me* MTB RIF/INH Resistance
  Amplification Reagent Kit (–15 °C to –25 °C storage and after opening), partially used packs
  must be used within 90 days. Abbott m2000 instrumentation (15–28 °C, relative humidity
  30–80%).

- **Connectivity**: The instruments can be connected, and data exported, to the laboratory information management service. The m2000 system does not have a USB port enabled for connectivity, so data must be exported manually using a CD-ROM.
- **Shelf life**: Abbott RealT*ime* MTB amplification reagent (90 days from the date of manufacture / 60 days from date of shipment), Abbott RealT*ime* MTB RIF/INH reagents (90 days from date of manufacture / 30 days from date of shipment).
- **Unit price**: Global prices are not yet available through the Stop TB Partnership Global Drug Facility, although discussions are underway. Abbott is currently offering prices based on order size. Reagent rental agreements may be considered with committed test volumes.

## *Implementation considerations*

In addition to the general guidance provided in **Section 3.5** of the main text, consider the following test-specific implementation considerations:

- **Area 1 – Policies and planning**: Given Abbott m2000 infrastructure requirements and the moderate complexity of the Realt*ime* tests, countries may prioritize instrument placement at a national or central reference laboratory for testing single or multiple diseases *(7)*. Alternatively, countries may have adequate infrastructure available and sufficient sample volume to consider deployment at regional referral laboratories.

  The Abbott m2000 system that runs both Realt*ime* TB tests is capable of multi-disease testing, which may be considered for holistic patient testing and potential cost savings across disease programmes *(8)*. Abbott RealT*ime* assays are available for HIV: HIV-1 viral load (VL) and HIV-1 quantitative; for hepatitis C virus (HCV): HCV VL and HCV guideline test (GT); for hepatitis B virus (HBV): HBV VL; for cytomegalovirus (CMV); for Epstein-Barr virus (EBV); for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CT/NGF); for high-risk human papillomavirus (HPV); and for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although the Abbott m2000*sp* system requires batching per assay protocol, assays for HIV-1 and HCV, as well as those for CMV and EBV, can be run on the same plate.
- **Area 3 – Equipment**: Abbott m2000 instruments have high infrastructure requirements and should be placed at laboratories that can accommodate molecular workflow, including separate and dedicated preparation and amplification spaces (one for sample preparation and one for amplification via real-time PCR). If automated sample preparation is desired, the m2000*sp* instrument must be procured and maintained through regular service in the sample preparation area.
- **Area 5 – Procedures**: If desired, RealT*ime* MTB assay DNA eluant may be used for reflex testing with the RealT*ime* MTB RIF/INH assay on the m2000 system, to save sample processing and inactivation time. The RealT*ime* MTB RIF/INH assay uses mutations in the *rpo*B gene, *kat*G gene and *inh*A promoter region of MTBC DNA to detect resistance to RIF and INH. Based on level of detection of wild-type probes (*rpo*B, *kat*G or *inh*A promoter) and mutant probes (*kat*G or *inh*A promoter), resistance may be reported as negative (R-), detected (R-det), indeterminate (indet) or undetected (below limit of detection). Additionally, detection of specific INH resistance-associated mutations can result in low-level (INH low R) and high-level (INH high R) resistance results. A resistance negative result suggests a lack of detection of targeted resistance-associated mutations, which may correlate with strain sensitivity, but may not include all potentially resistance-conferring mutations.

- **Area 6 – Digital data**: Opportunities for integration of diagnostic connectivity solutions and e-systems may be explored to meet targets established in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (9)*.
- **Area 7 – Quality assurance**: Quality assurance systems and activities for the Abbott assays mimic those of other moderate complexity automated NAATs. New method validation for the RealT*ime* MTB RIF/INH test should include precision and accuracy measurement for drugs targeting all drug-resistance loci, using well-characterized strains with and without known resistance-associated mutations. Because of potential contamination of the molecular workflow, each run on the m2000*rt* instrument must include positive and negative controls to ensure run validity, and laboratory spaces should be tested for contamination at least monthly, using the procedure outlined in the manufacturer's instructions for use.
- **Area 9 – Training and competency assessment**: Training on the Abbott m2000 system was reported by the Foundation for Innovative Diagnostics (FIND) as being more complex than on other centralized TB testing platforms, requiring theoretical and practical training for at least 5 days *(10)*.

It may be necessary to emphasize training for Realt*ime* assay reagent management and handling, because these reagents are stored under different conditions, and the DNA extraction and PCR reagents require reconstitution and must be manually inserted into the correct positions in the m2000*sp* instrument. These steps within the procedure can be time consuming and must be done accurately for successful testing runs.

## References for A2.1

1    Wang MG, Xue M, Wu SQ, Zhang MM, Wang Y, Liu Q et al. Abbott RealTime MTB and MTB RIF/INH assays for the diagnosis of tuberculosis and rifampicin/isoniazid resistance. Infect Genet Evol. 2019;71:54–9 (https://pubmed.ncbi.nlm.nih.gov/30902741/).

2    Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB: rapid communication. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/update-on-the-use-of-nucleic-acid-amplification-tests-to-detect-tb-and-drug-resistant-tb-rapid-communication).

3    Unitaid. Tuberculosis – diagnostics technology landscape, 5th edition. Geneva: World Health Organization; 2017 (https://unitaid.eu/assets/2017-Unitaid-TB-Diagnostics-Technology-Landscape.pdf).

4    Abbott RealTime RIF/INH [website]. Chicago: Abbott; 2019 (https://www.molecular.abbott/int/en/products/infectious-disease/realtime-mtb-rif-inh-resistance).

5    Abbott RealTime MTB [website]. Chicago: Abbott; 2019 (https://www.molecular.abbott/int/en/products/infectious-disease/realtime-mtb).

6    WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

7    Evaluation of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid: WHO Technical Expert Consultation Report. Geneva: World Health Organization; 2019 (https://www.who.int/publications/i/item/WHO-CDS-TB-2019.14).

8    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/publications/i/item/WHO-HTM-TB-2017.06).

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

10  FIND cDST WHO supplement. Geneva: World Health Organization; 2019 (https://www.finddx.org/wp-content/uploads/2019/08/FIND_cDST_WHO_Supplement.xlsx).

# A2.2 Information sheet: Practical considerations for implementation of the BD MAX MDR-TB test

Becton Dickinson (BD) has a multiplexed real-time polymerase chain reaction (PCR) nucleic acid amplification tests (NAAT) (BD MAX MDR-TB) for the detection of *Mycobacterium tuberculosis* complex (MTBC) and resistance to both rifampicin (RIF) and isoniazid (INH) in tuberculosis (TB). For MTBC detection, this test targets the multicopy genomic elements IS6110 and IS1081, as well as a single copy genomic target. For detection of RIF resistance, the test targets the RIF-resistance determining region (RRDR) codons 507–533 of the *rpo*B gene; for detection of resistance to INH it targets both the *inh*A promoter region and the 315 codon of the *kat*G gene. The test is performed on the BD Max platform, in which the DNA is automatically extracted and real-time PCR is performed. The World Health Organization (WHO) includes these tests within the class of moderate complexity automated NAATs, and the recommendations below apply to them *(1, 2)*.

## WHO recommendations for use

In people with signs and symptoms of pulmonary TB, moderate complexity automated NAATs may be used on respiratory samples for detection of pulmonary TB, RIF resistance and INH resistance, rather than culture and phenotypic drug-susceptibility testing (DST). *(Conditional recommendation; moderate certainty of evidence for diagnostic accuracy)*

There are several subgroups to be considered for this recommendation:

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

## Key performance conclusions

- The BD MAX MDR-TB test performs well for the diagnosis of TB and DR-TB compared with culture and phenotypic DST.
- Limit of detection reported by the company: TB detection = 0.5 colony forming units (CFU)/mL, RIF/INH detection = 6 CFU/mL.
- The pooled sensitivity and specificity data for the class are presented in the Web Appendix of the WHO consolidated guidelines *(3)*.

## Test procedure at-a-glance

1. **Sample treatment**: Raw or concentrated sputum is manually mixed 2:1 with BD MAX STR (sample treatment reagent), shaken and incubated twice, then transferred to a BD MAX MDR-TB sample tube.

2. **Sample preparation and real-time PCR**: The BD MAX MDR-TB sample tube is manually transferred to the BD MAX instrument, which then automates all sample preparation processes (cell lysis, DNA extraction, magnetic bead-based DNA concentration, heat and elution buffer-based DNA purification) and real-time PCR (reagent rehydration and DNA amplification).

3. **Detection and reporting**: Automated by the BD MAX instrument, specific fluorescent probes are used to detect MTBC DNA (IS6110, IS1081 and a single gene target, *dev*R), RIF resistance (RRDR codons 507–533) and INH resistance (*inh*A promoter and *kat*G codon 315). Results are automatically determined and digitally reported by the instrument within 4 hours.

4. **Result interpretation**: BD MAX automatically reports detection status (detected/ not detected) for MTBC, RIF resistance and INH resistance. RIF and INH may be reported as "unreportable" (i.e. fluorescence not measurable) and INH may have results disaggregated by *kat*G and *inh*A promoter region targets (mutation detected/ not detected). Also reported are MTBC detection with very low mycobacterial loads without resistance results (MTB low ), lack of sample processing control detection (MTB unresolved), system failure (indeterminate) and incomplete runs (incomplete) *(4)*.

# Equipment, supplies and reagents

## Fig. A2.1. BD MAX System and BD MAX PCR Cartridges

**MDR-TB assay:** 24 tests, master mixes, reagent strips, extraction tubes, sample tube, transfer pipettes, septum caps

**BD MAX system:** A fully-integrated, automated instrument that performs nucleic acid extraction and real-time PCR providing results for up to 24 samples per run on a benchtop with a computer

**Required, provided separately by BD:** BD MAX STR (sample treatment reagent), BD MAX PCR Cartridges (for RT-PCR)

**Not provided by BD:** Timer (required), personal protective equipment (lab coat, eye protection, powderless gloves), universal power source (UPS; recommmended), waste containers, external controls

## Operational considerations

- **Testing capacity**: 48 tests (24 in <4 hours) per 8-hour workday *(4)*.
- **BD MAX system dimensions and weight**: The BD MAX system includes a benchtop instrument (94.0 × 75.4 × 72.4 cm, weight of 113.4 kg).
- **Sputum storage and testing conditions**: For sputum decontamination N-acetyl-L-cysteine/ sodium hydroxide (NALC/NaOH) should be used. Sample processing may be conducted on unprocessed sputum transported at 2–35 °C within 3 days of collection and subsequently stored at 2–8 °C for an additional 7 days. BD MAX STR-treated samples can be stored at 2–8 °C for up to 72 hours and may be retested within this same time period.
- **Storage temperature**: BD MAX MDR-TB reagents (2–28 °C) and BD MAX instrument (18– 28 °C with 20–80% relative humidity).
- **Shelf life**: At least 9 months.
- **Unit prices for low- and middle-income countries**: Global prices are not yet available from the Stop TB Partnership Global Drug Facility, but may become available in the future.

## Implementation considerations

In addition to the general guidance provided in **Section 3.5**, consider the following test-specific implementation considerations:

- **Area 1 – Policies and planning**: BD MAX MDR-TB integration into national algorithms and network placement should consider that (1) the test requires moderate complexity molecular infrastructure, (2) efficient specimen transport may be required to ensure quality sputum specimens are received under appropriate temperature within timelines for BD MAX MDR-TB testing, and (3) any multi-disease testing needs or plans to optimize resource use. Note that the BD MAX system also has assays available to detect *Clostridium difficile*, enteric bacterial and parasitic pathogens, Group B *Streptococcus*, methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus*, and bacterial vaginosis as well as Chlamydia, gonorrhoea and trichinosis.

- **Area 3 – Equipment**: The BD MAX system, including the BD MAX instrument and all accompanying reagents, are required for BD MAX MDR-TB testing. Budget planning for test implementation should therefore consider any existing BD MAX systems, available infrastructure and other resource capacities and service and maintenance agreements to ensure optimal system functionality.

- **Area 6 – Digital data**: Opportunities for diagnostic connectivity solution and e-systems integration may be explored to meet targets established in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (5)*.

- **Area 7 – Quality assurance**: Quality assurance systems and activities for BD MAX MDR-TB mimic those of other moderate complexity automated NAATs. New method verifications should be conducted using a panel of well-characterized *M. tuberculosis* strains such that sensitivity and resistance for each TB medicine included in the assay (RIF and INH) is represented to demonstrate that the laboratory can achieve expected performance characteristics. Due to potential contamination of the molecular workflow, each run on the instrument must include positive and negative controls to ensure run validity and laboratory spaces should be tested for contamination at least monthly.

- **Area 8 – Recording and reporting**: Given the potential for decentralized testing sites to report an expanded set of drug sensitivity results for the first time, countries should consider any additional communication routes or reporting procedures that may be required. In addition, diagnostic connectivity solutions may be used to automate reporting.

- **Area 9 – Training and competency assessment**: As with other molecular drug-susceptibility tests, laboratory staff and clinicians should be trained on appropriate review and interpretation of resistance results for all included medicines.

# References for A2.2

1   Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB: rapid communication. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/update-on-the-use-of-nucleic-acid-amplification-tests-to-detect-tb-and-drug-resistant-tb-rapid-communication).

2   WHO consolidated guidelines on tuberculosis Module 4: treatment – drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240007048).

3   WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

4   BD MAX MDR-TB instructions for use. Franklin Lakes, NJ: Becton Dickinson; 2020 (https://www.bd.com/resource.aspx?IDX=35759).

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

# A2.3 Information sheet: Practical considerations for implementation of the Roche cobas MTB and cobas MTB-RIF/INH assays

Roche Molecular Systems, Inc. (RMS, Roche) has two nucleic acid amplification tests (NAATs), the cobas MTB and cobas MTB-RIF/INH tests, to detect *Mycobacterium tuberculosis* complex (MTBC) and drug resistance (rifampicin [RIF] and isoniazid [INH]), respectively *(1, 2)* in tuberculosis (TB). The MTB assay detects both 16S rRNA and *esx* genes as target genes for MTBC detection. The rifampicin resistance determining region (RRDR) is targeted for detection of RIF resistance, whereas the *inh*A promoter region and the *kat*G gene are targeted for detection of INH resistance. The tests are run on the cobas 6800/8800 Systems, which automatically extract DNA for real-time polymerase chain reaction (PCR). Also, these tests have been assessed in various studies *(3–6)*. The World Health Organization (WHO) includes these tests within the class of moderate complexity automated NAATs, and the recommendations below apply to them *(7)*.

## Key recommendations for use

In people with signs and symptoms of pulmonary TB, moderate complexity automated NAATs may be used on respiratory samples for detection of pulmonary TB, RIF resistance and INH resistance, rather than culture and phenotypic drug susceptibility testing (DST). *(Conditional recommendation; moderate certainty of evidence for diagnostic accuracy)*

There are several subgroups to be considered for this recommendation:

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

## WHO performance conclusions

- Both cobas MTB and cobas MTB-RIF/INH assays perform well for the diagnosis of TB and drug-resistant TB (DR-TB) compared with culture and phenotypic DST.
- **Limit of detection** reported by the company: TB detection = 7.6 colony forming units (CFU)/mL (sputum or bronchoalveolar lavage [BAL] sediments), 8.8 CFU/mL (raw sputum). RIF detection = 94 CFU/mL (sputum or BAL sediments), 182 CFU/mL (raw sputum); INH detection = 12.6 CFU/mL (sputum or BAL sediments) and 27.5 CFU/mL (raw sputum).
- The pooled sensitivity and specificity data for the class are presented in the Web Appendix of the WHO consolidated guidelines *(8)*.

## Test procedures at-a-glance

After manual liquefaction and mycobacterial inactivation using both chemical and physical methods (i.e. lysis reagent and sonication, respectively), inactivated samples are tested on the cobas 6800/8800 instruments, which automatically extract DNA and perform real-time PCR with fluorescence detection *(1, 9)*. For MTBC detection, the MTB assay targets both 16S rRNA and five *esx* genes, whereas the MTB-RIF/INH assay detects resistance to RIF and INH by targeting the RRDR of the *rpo*B gene and both the *inh*A promoter region and the *kat*G gene *(1)*. Results are generated for the first 96 samples within 3 hours, with further results released in subsequent 90-minute intervals.

## Equipment, supplies and reagents required

### *Supplied:*

- cobas MTB 384 test cassette (proteinase, amplification reagents, internal controls and elution buffer)
- cobas MTB-RIF/INH 72 test cassette (proteinase, amplification reagents, internal controls and elution buffer)
- cobas 6800 or 8800 system (Instrument Gateway and software supplied)

### *Not supplied but required:*

- cobas MTB Positive Control Kit
- cobas MTB-RIF/INH Positive Control Kit
- cobas 6800/8800 Buffer Negative Control Kit
- cobas omni MGP, SPEC DIL, LYS and WASH reagents
- cobas microbial inactivation solution
- cobas omni processing plate and amplification plate
- Pipettors and sterile, filtered pipette tips
- Class II biosafety cabinet
- Personal protective equipment (e.g. chemical-resistant gloves, laboratory coat and eye protection)
- Centrifuge and vortex
- Tube sonicator TS 5
- 5 mL polypropylene screw cap tubes with round bases

- Thermostable barcode labels

## Operational considerations

- **Testing capacity**: Each cobas MTB kit includes sufficient reagents for 40 runs. The cobas 6800/8800 instruments can process 96 tests including controls per run. The cobas 6800 System can run a maximum of 384 tests per 8-hour workday, whereas the cobas 8800 System can run a maximum of 1056 tests per 8-hour workday *(1)*.
- **cobas 6800/8800 system dimensions and weight**: The cobas 6800 is 292 x 216 x 129 cm and weighs 1624 kg, inclusive of its server. The cobas 8800 is 429 x 216 high x 129 cm and weighs 2405 kg, inclusive of its server.
- **Time to detection**: The cobas MTB produces 96 results within 3.5 hours, with subsequent results released about 90 minutes thereafter; the cobas MTB-RIF/INH produces results in an additional 3.5 hours.
- **Sputum storage and testing conditions**: Testing may be conducted on unprocessed sputum stored or transported at 2–35 °C for no more than 3 days, followed by no more than 7 days at 2–8 °C. Sputum sediment specimens may be stored at 2–8 °C for no more than 7 days. When not loaded on a cobas instrument, the MTB kit and associated reagents must be stored at 2–8 °C (once loaded, reagents are automatically temperature controlled, with expiration monitored). Once opened, the cobas MTB kit must be used within 90 days while stored on the system and the associated cobas omni reagents must be used within 30 days.
- **Service and maintenance**: The cobas 6800/8800 Systems require weekly maintenance and cleaning *(9)*. If users observe droplets on the instrument deck, the local Roche Service organization should be contacted immediately for support.
- **Unit price**: Global pricing is available through Roche's Global Access Program across several disease areas, including those for TB. Although not yet available, discussions are underway with Stop TB Partnership's Global Drug Facility (GDF) for inclusion in the GDF catalogue.

## Implementation considerations

In addition to general guidance provided in **Section 3.5**, consider the following test-specific implementation considerations:

- **Area 1 – Policies and planning**: Given cobas 6800/8800 Systems infrastructure requirements and the moderate complexity of cobas tests, countries may prioritize instrument placement at a national reference laboratory, which may be used centrally for single or multiple disease testing *(10)*. Alternatively, countries may have adequate infrastructure available and sufficient sample volume to consider deployment at regional referral laboratories.

  The Roche cobas 6800/8800 Systems on which both cobas tests are run are capable of multi-disease and biochemical testing, which may be considered for holistic patient testing and potential cost savings across disease programmes. Immunoassays based on cobas are used for detection of markers associated with anaemia, bone health, diabetes, tumours, fertility, thyroid and cardiac function, growth hormones, sepsis and arthritis; the assays are also available for certain infectious diseases, including HIV, hepatitis virus (type A-C), cytomegalovirus (CMV), herpes simplex virus (HSV), syphilis, rubella, *Trichomonas vaginalis* and/or *Mycoplasma genitalium* (TV/MG), methicillin-resistant *Staphylococcus aureus* (MRSA), West Nile virus (WNV) and coronavirus disease (COVID-19). The cobas omni Utility Channel

may also be considered for implementation by users interested in running third-party molecular tests or laboratory-developed tests.

- **Area 3 – Equipment**: Roche cobas 6800/8800 Systems have high infrastructure requirements and should be placed at laboratories that can accommodate molecular workflow, including separate and dedicated preparation and amplification spaces (one for sample preparation and one for amplification via real-time PCR). If automated sample preparation is desired, the cobas instrument must be procured and maintained with regular service.

- **Area 6 – Digital data**: Opportunities for integration of diagnostic connectivity solutions and e-systems may be explored to meet targets established in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (11)*.

- **Area 7 – Quality assurance**: Quality assurance systems and activities for the cobas assays mimic those of other moderate complexity automated NAATs. New method validation for the cobas MTB test should include well-characterized MTBC positive and negative samples to determine assay accuracy and precision as compared with manufacturer-reported performance characteristics. New method validation for the cobas MTB-RIF/INH assay should include precision and accuracy measurement for both drugs targeting all drug-resistance loci, using well-characterized strains with and without known resistance-associated mutations. Because of potential contamination of the molecular workflow, each run on the instrument must include positive and negative controls to ensure run validity, and laboratory spaces should be tested for contamination at least monthly.

- **Area 9 – Training and competency assessment**: Given that DNA extraction, amplification and fluorescence detection and reporting are automated by the cobas 6800/8800 systems, laboratory technicians should be fully trained on all automated steps to appropriately troubleshoot systems and conduct maintenance activities across instrumentation. As with other molecular drug sensitivity tests, laboratory staff and clinicians should be trained on appropriate review and interpretation of resistance results for all included medicines.

## References for A2.3

1   Roche Diagnostics. Cobas MTB and cobas MDR-TB instructions for use documents.

2   Roche Diagnostics. cobas MTB-RIF/INH [website]. 2021 (https://diagnostics.roche.com/global/en/products/params/cobas-mtb-rif-inh.html).

3   Nadarajan D, Hillemann D, Kamara R, Foray L, Conteh OS, Merker M et al. Evaluation of the Roche cobas MTB and MTB-RIF/INH assays in samples from Germany and Sierra Leone. J Clin Microbiol. 2021;59(5)(https://pubmed.ncbi.nlm.nih.gov/33658264/).

4   Scott L, David A, Govender L, Furrer J, Rakgokong M, Waja Z et al. Performance of the Roche cobas MTB assay for the molecular diagnosis of pulmonary tuberculosis in a high HIV burden setting. J Mol Diagn. 2020;22(10):1225–37 (https://pubmed.ncbi.nlm.nih.gov/32745613/).

5   Melanson SE, Lindeman NI, Jarolim P. Selecting automation for the clinical chemistry laboratory. Arch Pathol Lab Med. 2007;131(7):1063–9 (https://pubmed.ncbi.nlm.nih.gov/17616992/).

6   Characteristics of four molecular assays designed for the screening of Mycobacterium tuberculosis disease, including the detection of RIF and INH susceptibility. 2020 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7527868/table/tbl1/?report=objectonly).

7   Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB: rapid communication. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/update-on-the-use-of-nucleic-acid-amplification-tests-to-detect-tb-and-drug-resistant-tb-rapid-communication).

8   WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

9   cobas 6800/8800 systems processing transfer head: potential for leakage – diagnostics. Rotkreuz, Switzerland: Roche Diagnostics; 2019 (https://www.who.int/diagnostics_laboratory/procurement/190618_roche_molecular_system_sbn_rmd_2019_001.pdf).

10  Evaluation of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid: WHO Technical Expert Consultation Report. Geneva: World Health Organization; 2019 (https://www.who.int/publications/i/item/WHO-CDS-TB-2019.14).

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

# A2.4 Information sheet: Practical considerations for implementation of the Bruker-Hain Lifesciences FluoroType MTB and FluoroType MTBDR

Bruker-Hain Diagnostics has two real-time nucleic acid amplification tests (NAATs), the FluoroType MTB to detect *Mycobacterium tuberculosis* complex (MTBC) and the FluoroType MTBDR, to detect MTBC, and resistance to rifampicin (RIF) and isoniazid (INH) in tuberculosis (TB). The MTB test (VER 1.0) targets the *IS6110* DNA insertion element for MTBC detection, while the MTBDR test (VER 2.0) targets the *rpo*B gene for detection of MTBC and RIF resistance, and the *inh*A promoter and *kat*G gene for detection of INH resistance. For DNA extraction, both manual (FluoroLyse) and automated (GenoXtract) options are available. The instruments used for amplification and detection are the Bruker-Hain Diagnostics FluoroCycler 12 and FluoroCycler XT for MTB and MTBDR assays. The World Health Organization (WHO) includes these tests within the class of moderate complexity automated NAATs, and the recommendations below apply to them *(4)*.

## WHO recommendations for use

In people with signs and symptoms of pulmonary TB, moderate complexity automated NAATs may be used on respiratory samples for detection of pulmonary TB, RIF resistance and INH resistance, rather than culture and phenotypic drug susceptibility testing (DST). *(Conditional recommendation; moderate certainty of evidence for diagnostic accuracy)*

There are several subgroups to be considered for this recommendation:

- The recommendation is based on evidence of diagnostic accuracy in respiratory samples of adults with signs and symptoms of pulmonary TB.
- This recommendation applies to people living with HIV (PHLIV) (studies included a varying proportion of such people). Performance on smear-negative samples was reviewed but was only available for TB detection and not for RIF and INH resistance. Data stratified by HIV status were not available.
- This recommendation applies to adolescents and children based on the generalization of data from adults. Indeterminate results are more likely to be found with paucibacillary TB disease in children.

- Extrapolation for use in people with extrapulmonary TB and testing on non-sputum samples was not considered because data on diagnostic accuracy of technologies in the class for non-sputum samples were limited.

## Key performance conclusions *(5, 6)*

- Both FluoroType MTB and FluoroType MTBDR assays perform well for the diagnosis of TB, RIF and INH detection compared with culture and phenotypic DST.
- The limit of detection reported by the company for TB detection with FluoroLyse: FluoroType MTB (Version 1.0) = 15 colony forming units (CFU)/mL, FluoroType MTBDR (Version 2) = 9 CFU/mL, while for RIF/INH resistance detection = 14 CFU/mL).
- The pooled sensitivity and specificity data for the class are presented in a Web Appendix of the WHO consolidated guidelines *(4)*.

## Test procedure at-a-glance

The FluoroType MTB and MTBDR assays accommodate manual (FluoroLyse kit) or automated DNA extraction (GenoXtract (12) or GenoXtract 96/fleXT instruments with corresponding extraction kits), followed by polymerase chain reaction (PCR) on the FluoroCycler 12 (MTB VER 1.0) or FluoroCycler XT (MTB VER 2.0) instruments. The MTB (VER 1.0) assay uses high resolution melt analysis to detect and automatically report fluorescence detection associated with probes specific for the MTBC insertion element IS*6110*. The MTBDR assay LiquidArray technology combines sensitive amplification with high resolution melt curve analysis lights-on/lights-off chemistry to simultaneously detect MTBC and resistance to RIF and INH; it targets the *rpo*B gene associated with RIF resistance, and the *kat*G gene and the *inh*A promoter associated with INH resistance. The assay differentiates between high-level and low-level INH resistance, and the FluoroSoftware automatically reports specific mutations identified for each gene target.

Possible results and their causative explanations are given in Table A2.2, and the equipment, supplies and reagents required are given in Table A2.3.

**Table A2.2. Interpretation of the FluoroType MTB and FluoroType MTBDR tests**

| Result | Explanation |
|---|---|
| **FluoroType MTB** | |
| **MTBC DNA detected** | There is at least one valid MTBC peak |
| **No MTBC DNA detected** | There is no valid MTBC peak |
| **MTB complex peak in threshold zone** | MTBC peak is in the threshold zone |
| **Invalid** | No mutation was detected in the katG and inhA loci |
| **MTBC DNA detected** | The melting curve is invalid |
| **FluoroType MTBDR** | |
| **No MTBC DNA detected** | The *rpo*B locus was not detected |

| Result | Explanation |
|---|---|
| **RIF: sensitive** | No mutation or a silent mutation detected in the *rpo*B locus |
| **RIF: resistant** | The *rpo*B locus carries a resistance-mediating mutation |
| **RIF: indeterminate** | The *rpo*B mutation status could not be determined |
| **INH: sensitive** | No mutation was detected in the *kat*G and *inh*A loci |
| **INH: resistant** | The *inh*A and/or the *kat*G loci are mutated |
| **INH: indeterminate** | The *kat*G and/or *inh*A mutations could not be determined |
| **Invalid** | The fluorescence signature is invalid |

DNA: deoxyribonucleic acid; INH: isoniazid; MTB: *Mycobacterium tuberculosis*; MTBC: *Mycobacterium tuberculosis* complex; RIF: rifampicin.

## Table A2.3. Equipment, supplies and reagents required

| Supplied with kit(s) | Not supplied but required (test specific) | Not supplied but required (general) |
|---|---|---|
| **Amplification reagents** | FluoroLyse extraction kit (manual) or<br><br>GenoXtract or GenoXtract 96/fleXT with corresponding extraction kits (automated) | Personal protective equipment: chemical-resistant gloves, laboratory coat, eye protection |
| **Internal and external controls** | FluoroCycler 12 instrument (18.5 × 24 × 24 cm, weight 5 kg)<br><br>FluoroCycler XT instrument (43 × 57 × 73 cm, weight 65 kg) | Pipettors – calibrated (10–1000 µL) |
| **Barcode label for FluoroSoftware** | Multiply-µStripPro or FrameStar 96-well PCR plate | Sterile, filtered, DNAse-free pipette tips |
| **Lot label** | 4s3 Semi-automatic sheet heat sealer | Sterile, DNAase-free PCR tubes |
| | Clear Weld Seal Mark II | Tabletop centrifuge (96-well PCR plates,1.5 mL and 2.0 mL tubes) |
| | Inactivation Set (for automated extraction for MTB VER 2.0 and MTBDR VER 2.0) | Vortex |
| | | Class II biosafety cabinet |

DNA: deoxyribonucleic acid; PCR: polymerase chain reaction; RNA: ribonucleic acid.

## Operational considerations

- **Sample types**: Decontaminated sputum specimens (FluoroType MTB), and decontaminated sputum specimens and culture isolates (FluoroType MTBDR).
- **Storage and handling**: Both amplification reagents and controls (internal and positive MTB) should be stored at −20 °C to −18 °C, refrozen immediately after use and freeze/thawed no more than four times.
- **Testing capacity**: 12 samples (including assay controls) per FluoroCycler 12 run with potential to for simultaneous connection of multiple units to increase testing capacity. 96 samples (including assay controls) per FluoroCycler XT run.
- **Time to detection**: Within 3 hours for both FluoroType assays.
- **Result reporting**: Results are automated via FluoroSoftware, including high-level and low-level INH resistance reporting. Mutations that are rare or are associated with unknown resistance profiles in the target genes are also shown. Additionally, users can share run files or DNA extracts with verified new resistance conferring mutations with the Hain Technical Support Team. These data can be used for machine learning that may improve resistance prediction as new mechanisms of resistance are reported. Note: The *rpo*B H526C (*Escherichia coli* nomenclature, equivalent to the H445C *M. tuberculosis* nomenclature), is not detected by the FluoroType MTBDR.
- Connectivity: Both FluoroCycler instruments can be connected, and data exported, to laboratory information management service.
- **Shelf life**: As reported on each kit box when stored as directed.
- **Unit price**: A global price is not yet available through the Stop TB Partnership Global Drug Facility, although discussions are underway.

## Implementation considerations

In addition to general guidance provided in **Section 3.5**, consider the following test-specific implementation considerations:

- **Area 1 – Policies and planning**: Because of the FluoroCycler and FluoroType tests' high complexity infrastructure and molecular workflow requirements, the FluoroType MTB and MTBDR assays are best suited for centralized reference laboratories *(7)*.

  The FluoroCycler instruments on which FluoroType tests are run is capable of multi-disease testing, which may be considered for holistic patient testing and potential cost savings across disease programmes. The following FluoroType assays are available: BK virus, cytomegalovirus, Epstein–Barr virus, varicella-zoster virus, herpes simplex virus, parvovirus B19, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (CT/NGF), methicillin-resistant *Staphylococcus aureus* (MRSA), Bordetella and Borrelia, sexually transmitted infections (7-plex), severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) and different human genetics.

- **Area 3 – Equipment**: Programmes should review existing, local equipment and Bruker-Hain Diagnostics testing protocols, as available, to inform selection and procurement of manual (FluoroLyse) or automated, instrument-based (GenoXtract) extraction resources, noting that the FluoroType tests have not yet been demonstrated as being compatible with existing Hain GenoLyse extraction kits. Similarly, FluoroType MTB and FluoroType MTBDR tests are only compatible with the FluoroCycler instruments and may not be run on any other

existing Hain equipment, including the GT Blot system. In addition to obtaining the required instrumentation, testing volumes should be calculated before procurement to maximize resources (human, budgetary and testing) and ensure availability of sufficient testing supplies and reagents to meet clinical demand.

- **Area 6 – Digital data**: Opportunities for FluoroCycler 12 and FluoroCycler XT integration of diagnostic connectivity solutions and e-systems exists and may be explored to meet targets established in the *Framework of indicators and targets for laboratory strengthening under the End TB Strategy (8)*.

- **Area 7 – Quality assurance**: Quality assurance systems and activities for the FluoroType assays mimic those of other moderate complexity automated NAATs. Internal and external control reagents are provided and must be included with each run samples to ensure results are accurate and not affected by contamination, amplification inhibition or amplification failure. Control interpretation guidance is included in the manufacturer's instructions for use and should be included in tester trainings and competency assessments. In addition, laboratory spaces should be tested for contamination at least monthly.

- **Area 8 – Recording and reporting**: The FluoroCycler FluoroSoftware generates automatic reports that include the date, run name and all sample information, as well as the respective fluorescence signatures and the interpretations as derived by the software. Users should follow national requirements for results reporting.

- **Area 9 – Training and competency assessment**: As with other molecular drug sensitivity tests, laboratory staff and clinicians should be trained on testing principles, methods and the appropriate review and interpretation of results, particularly those related to high and low isoniazid resistance.

## References for A2.4

1   Unitaid. Tuberculosis – diagnostics technology landscape, 5th edition. Geneva: World Health Organization; 2017 (https://unitaid.eu/assets/2017-Unitaid-TB-Diagnostics-Technology-Landscape.pdf).

2   Bruker-Hain Diagnostics. FluoroType MTBDR VER 2.0 – your test system for true MDR-TB testing [website]. (https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/fluorotype-mtbdr.html).

3   Bruker-Hain Diagnostics. FluoroType MTB – the direct detection test for innovative labs [website]. (https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/fluorotype-mtb.html).

4   WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

5   de Vos M, Derendinger B, Dolby T, Simpson J, van Helden PD, Rice JE et al. Diagnostic accuracy and utility of FluoroType MTBDR, a new molecular assay for multidrug-resistant tuberculosis. J Clin Microbiol. 2018;56(9)(https://pubmed.ncbi.nlm.nih.gov/29976588/#:~:text=FluoroType%20identified%2098%25%2C%2097%25,testing%20for%20rifampin%20and%20isoniazid.).

6   Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB: rapid communication. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/update-on-the-use-of-nucleic-acid-amplification-tests-to-detect-tb-and-drug-resistant-tb-rapid-communication).

7   Evaluation of centralized assays for TB detection and detection of resistance to rifampicin and isoniazid: WHO Technical Expert Consultation Report. Geneva: World Health Organization; 2019 (https://www.who.int/publications/i/item/WHO-CDS-TB-2019.14).

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

# A2.5 Information sheet: Practical considerations for implementation of the Cepheid Xpert MTB/XDR test

The Xpert MTB/XDR detects *Mycobacterium tuberculosis* complex (MTBC) DNA and genomic mutations associated with resistance to isoniazid (INH), fluoroquinolones (FQs), ethionamide (ETH) and second-line injectable drugs (amikacin [AMK], kanamycin and capreomycin) in a single cartridge. Xpert MTB/XDR tests are run on Cepheid's GeneXpert instruments, using 10-colour modules that differ from the 6-colour modules traditionally used for Xpert MTB/RIF and Xpert MTB/RIF Ultra (Xpert Ultra) testing. Xpert MTB/XDR is intended for use as a reflex test in tuberculosis (TB) specimens (unprocessed sputum or concentrated sputum sediments) determined to be MTBC-positive *(1)*. These tests have been assessed in various studies *(2–4)*. The World Health Organization (WHO) includes this test within the class of low complexity automated nucleic acid amplification tests (NAATs), and the recommendations below apply to this test *(6, 7)*.

## WHO recommendations for use

WHO recommends the use of low complexity automated NAATs in the following situations *(8)*:

- In people with bacteriologically confirmed pulmonary TB, low complexity automated NAATs may be used on sputum for initial detection of resistance to INH and FQs, rather than culture-based phenotypic drug-susceptibility testing (DST).
  *(Conditional recommendation; moderate certainty of evidence for diagnostic accuracy)*
- In people with bacteriologically confirmed pulmonary TB and resistance to RIF, low complexity automated NAATs may be used on sputum for initial detection of resistance to ETH, rather than DNA sequencing of the *inh*A promoter
  *(Conditional recommendation; very low certainty of evidence for diagnostic accuracy)*
- In people with bacteriologically confirmed pulmonary TB and resistance to RIF, low complexity automated NAATs may be used on sputum for initial detection of resistance to amikacin, rather than culture-based phenotypic DST.
  *(Conditional recommendation; low certainty of evidence for diagnostic accuracy)*

There are several subgroups to be considered for these recommendations:

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

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

## Key performance conclusions

- Xpert MTB/XDR assay performs well as a follow-on test for patients with bacteriologically confirmed TB for the detection of resistance to INH, FQ, ETH and AMK.
- Limit of detection as reported by the manufacturer for TB detection is 136 colony forming units (CFU)/mL for unprocessed sputum and 86 CFU/mL for sputum sediment.
- The pooled sensitivity and specificity data for the class are presented in the Web Appendix of the WHO consolidated guidelines *(8)*.

## Test procedure at-a-glance

The sample processing procedure and cartridge handling are the same as for Xpert MTB/RIF and Ultra tests. However, the Xpert MTB/XDR test runs on a GeneXpert platform that supports multiplexing via 10-colour technology, instead of the 6-colour technology used with Xpert MTB/RIF and Ultra tests.

The test targets the genes, codon regions and nucleotide sequences given in Table A2.4.

**Table A2.4. Gene targets, codon regions and nucleotide sequences that determine presence of variants associated with drug resistance in the Xpert MTB/XDR test**

| Drug | Gene target | Codon regions | Nucleotide |
|---|---|---|---|
| **Isoniazid** | *inh*A promoter | Not applicable | −1 to −32 intergenic region |
| | *kat*G | 311–319 | 939–957 |
| | *fabG1* | 199–210 | 597–630 |
| | *oxyR-ahpC* intergenic region | Not applicable | −5 to −50 intergenic region (or −47 to −92)[a] |
| **Ethionamide** | *inh*A promoter | Not applicable | −1 to −32 intergenic region |
| **Fluoroquinolones** | *gyrA* | 87–95 | 261–285 |
| | *gyrB* | 531–544 (or 493–505)[a] | 1596–1632 |

| Drug | Gene target | Codon regions | Nucleotide |
|---|---|---|---|
| **Amikacin, kanamycin, capreomycin** | *rrs* | Not applicable | 1396–1417 |
| **Amikacin, kanamycin** | *eis* promoter | Not applicable | −6 to −42 intergenic region |

[a] Codon numbering system according to Camus et al. (2002) *(9)*, as reported in Cepheid, Clinical evaluation of the Xpert MTBXDR assay, Report R244C2 Xpert MTB/XDR Rev 1.0.

# Equipment, supplies and reagents

**10-colour GeneXpert module(s):** Available for GeneXpert instrument models GX I to GX XVI (**Fig. A2.2**) may be procured as new modules, new systems (i.e. instrument, computer or laptop, or barcode scanner) or as satelites (instrument only) to connect to existing GeneXpert systems. There is also the possibility to convert an existing GeneXpert system with 6 colours modules to 10 colour modules, but hybrid 6 and 10-colours instruments are not supported by Cepheid for now.

**Table A2.5. Equipment, supplies and reagents required**

| Supplied with kit(s) | Not supplied but required |
|---|---|
| **Xpert MTB/XDR cartridges (10 per kit)** | Timer |
| **Sample reagent (10 × 8 mL bottles)** | Universal power source (UPS) |
| **Disposable transfer pipettes (12 per kit)** | Personal protective equipment for low complexity molecular testing |
| **CD (containing assay definition file instructions for use)** | |

# Operational considerations

- **Testing capacity**: Maximum of five 90-minute tests per module in an 8-hour workday *(10)*.
- **GeneXpert instrument service and maintenance**: Various options are offered by the manufacturer.
- **Sputum storage and testing conditions**: Testing may be conducted on raw sputum stored at 2–35 °C for no more than 7 days or sputum sediment stored at 2–8 °C for no more than 7 days. After addition of sample reagent, test within 2.5 hours if sample is stored at up to 35 °C or test within 4 hours if it is kept at 2–8 °C *(10)*.
- **Storage temperature**: Xpert MTB/XDR cartridges and reagents (2–28 °C) and 10-colour GeneXpert instruments (≤30 °C).

- **Unit prices for low- and middle-income countries**: Xpert MTB/XDR cartridges cost US$ 19.80 per test or US$ 198.00 per kit (10 tests) *(8)*. 10-colour GeneXpert modules range from US$ 3860 (single module kit) to US$ 72 350 (new GX XVI system) *(11)*.

## Implementation considerations

In addition to general guidance provided in **Section 3.5**, consider the following test-specific implementation considerations:

- **Area 1 – Policies and planning**: Xpert MTB/XDR integration into national algorithms and network placement should consider that the test may be placed as low as near-point-of-care settings, and that patient specimens may need to undergo further testing such as culture or DST (e.g. to detect resistance to bedaquiline, delamanid or other medicines *(8)*). Because this test may primarily be used as a follow-on test for laboratory-confirmed TB, budget planning for test implementation should consider test volumes as well as strategic procurement of 10-colour modules, and instruments or systems that leverage or complement existing GeneXpert networks.
- **Area 3 – Equipment**: Xpert MTB/XDR cartridges are only compatible with 10-colour GeneXpert modules and will not function on the 6-colour modules traditionally used for Xpert MTB/RIF and Xpert Ultra testing.
- **Area 6 – Digital data**: GeneXpert instruments with 10-colour modules have the same diagnostic connectivity opportunities as instruments with 6-colour modules, allowing for SMS-based and e-based transmission of results and other connectivity application features, such as commodity and quality monitoring. The assay results could be transferred to laboratory information systems (LIS), as with previous TB Xpert tests. The addition of the Xpert MTB/XDR test to your LIS will probably require a collaboration with the LIS provider to implement the new test settings because the result reporting will be different from that of currently used TB Xpert assays.
- **Area 7 – Quality assurance**: Quality assurance systems and activities for Xpert MTB/XDR mimic those for Xpert MTB/RIF *(12)* or Xpert Ultra, with a few differences based on differences in test design. Given inclusion of an expanded set of probes for detection to a wider range of TB medicines, new method verifications should be conducted using a panel of well-characterized *M. tuberculosis* strains, such that sensitivity and resistance for each TB medicine included in the assay (RIF, INH, FQ, ETH and AMK) is represented, to demonstrate that the laboratory can achieve expected accuracy and precision of resistance detection. Once completed, decentralized testing sites do not need to repeat a new method verification with a comprehensive resistance panel unless required by national policy or one or more accrediting body, but should conduct new method verifications, as was done for the introduction of Xpert MTB/RIF or Xpert Ultra tests. Post-market validation and new-lot testing should be conducted according to established TB Xpert protocols.
- **Area 8 – Recording and reporting**: Given the potential for decentralized testing sites to report an expanded set of drug-susceptibility results for the first time, countries should consider whether any additional communication routes or reporting procedures may be required. In addition, diagnostic connectivity solutions may be used to automate reporting. Revisions to laboratory registers and reporting forms may be needed.

- **Area 9 – Training and competency assessment**: Training on new cartridges should be incorporated into current refresher training programmes as well as pre-implementation sensitization on the technology updates. As with other molecular DST, laboratory staff and clinicians should be trained on appropriate review and interpretation of resistance results for all included medicines.

## References for A2.5

1   Cepheid. Package insert portal [website]. 2020 (https://www.cepheid.com/en_US/package-inserts/1615).

2   Cao Y, Parmar H, Gaur RL, Lieu D, Raghunath S, Via N et al. Xpert MTB/XDR: a 10-color reflex assay suitable for point-of-care settings to detect isoniazid, fluoroquinolone, and second-line-injectable-drug resistance directly from Mycobacterium tuberculosis-positive sputum. Journal of clinical microbiology. 2021;59(3):e02314–20.

3   Penn-Nicholson A, Georghiou SB, Ciobanu N, Kazi M, Bhalla M, David A et al. Clinical evaluation of the Xpert MTB/XDR assay for rapid detection of isoniazid, fluoroquinolone, ethionamide and second-line drug resistance: A cross-sectional multicentre diagnostic accuracy study. medRxiv. 2021;(https://www.medrxiv.org/content/10.1101/2021.05.06.21256505v1).

4   Bainomugisa A, Gilpin C, Coulter C, Marais BJ. New Xpert MTB/XDR: added value and future in the field. Eur Respiratory Soc. 2020.

5   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).

6   WHO consolidated guidelines on tuberculosis Module 4: treatment – drug-resistant tuberculosis treatment. Geneva: World Health Organization; 2020 (https://www.who.int/publications/i/item/9789240007048).

7   Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB: rapid communication. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/update-on-the-use-of-nucleic-acid-amplification-tests-to-detect-tb-and-drug-resistant-tb-rapid-communication).

8   WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

9   Camus J-C, Pryor MJ, Médigue C, Cole ST. Re-annotation of the genome sequence of Mycobacterium tuberculosis H37Rv. Microbiol. 2002;148(10):2967–73.

10  Cepheid. Xpert MTB/XDR [website]. 2021 (https://www.cepheid.com/en/tests/Critical-Infectious-Diseases/Xpert-MTB-XDR).

11  Genexpert: negotiated prices [website]. Geneva: Foundation for Innovative New Diagnostics (FIND); 2021 (https://www.finddx.org/pricing/genexpert/).

12  Stop TB Partnership. Practical guide to implementing a quality assurance system for Xpert MTB/RIF testing. 2021 (http://www.stoptb.org/wg/gli/pgiqas.asp).

# A2.6 Information sheet: Practical considerations for implementation of the Nipro Genoscholar PZA-TB II assay

Nipro (Osaka, Japan) developed Genoscholar PZA-TB, a reverse hybridization-based technology for detection of pyrazinamide (PZA) resistance in tuberculosis (TB) *(1, 2)*. Compared with MTBDR*plus* and MTBDR*sl* LPA, the Genoscholar PZA-TB line-probe assay (LPA) does not include specific mutant probes, because resistance mutations are widespread across the entire *pncA* gene with no predominant mutations. Instead, the Genoscholar PZA-TB assay targets a 700 base pair (bp) fragment that covers the entire *pncA* gene and promoter region up to nucleotide −18 of the wild-type H37Rv reference strain that is known to harbour resistance-associated mutations. The first version of the assay contained 47 probes that covered the *pncA* promoter and open reading frame. The second version contained 48 probes. Three of the 48 probes (*pncA* 16, 17 and 35) in the second version represent silent mutations known to be genetic markers not associated with PZA resistance: Gly60Gly (probe 16), Ser65Ser (probe 17) and Thr142Thr (probe 35). The World Health Organization (WHO) includes this test as the first member within the class of high complexity reverse hybridization NAATs, and the recommendations below apply to this test *(3)*.

## WHO recommendations for use

In people with bacteriologically confirmed TB, high complexity reverse hybridization-based nucleic acid amplification tests (NAATs) may be used on culture isolates for detection of PZA resistance (rather than culture-based phenotypic drug susceptibility testing [DST]). *(Conditional recommendation; very low certainty of evidence for diagnostic accuracy)*

No special considerations are required in terms of subgroups (e.g. for children, people living with HIV [PLHIV] and those with extrapulmonary TB), given that the test is recommended for use on culture isolates.

## Key performance conclusions

- The Nipro Genoscholar PZA-TB II test performs well for PZA resistance compared with phenotypic DST.
- The performance data on direct testing was limited and the recommendation currently only includes use on isolates.
- The pooled sensitivity and specificity data for the class are presented in a Web Appendix of the WHO consolidated guidelines *(4)*.

## Fig. A2.3. Nipro Genoscholar PZA-TB II test procedure and interpretation at-a-glance
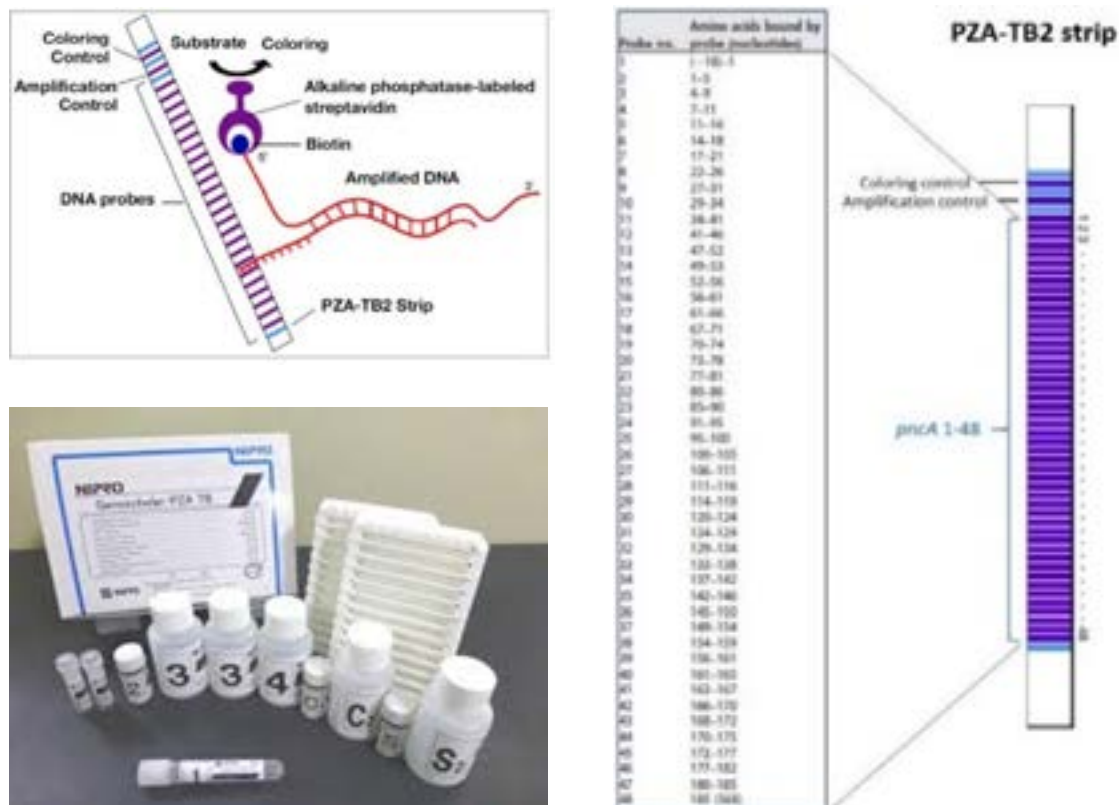
DNA is extracted from cultures, 5–10 μl of extract is amplified by polymerase chain reaction (PCR), denatured and hybridized using the MULTIBLOT NS-4800 system to complementary probes bound to a membrane-based strip. After hybridization, alkaline phosphatase-labelled streptavidin is added to bind any hybrids formed in the previous step and bound to the strip. The enzymatic reaction results in purple bands which are visually interpreted. The absence of wild type probe binding indicates the presence of a mutation.

## Table A2.6. Equipment, supplies and reagents required

| Supplied with kit(s) | Not supplied but required |
| --- | --- |
| **DNA amplification reagents** | Class II biosafety cabinet (for DNA extraction) |
| **DNA detection solutions** | Thermocycler |
| **Strips** | DNA amplification consumables |
| | Pipettors – calibrated (10–1000 μl) |
| | Sterile, filtered, DNAse-free pipette tips |

| Supplied with kit(s) | Not supplied but required |
|---|---|
| | Water bath with a shaking platform (with an inclined hood; temperature adjustable at 62 ±0.5 °C) or MULTIBLOT NS-4800 (Nipro) for the automated hybridization process |
| | Tabletop centrifuge (1.5 mL and 0.2 mL) |
| | Vortex |
| | Tweezers, measuring cylinder, beaker, etc. |
| | Sterile purified water |
| | TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH8.0) |
| | Personal protective equipment for high complexity molecular testing |
| | Infectious medical waste container, autoclave |

DNA: deoxyribonucleic acid; EDTA: ethylenediaminetetraacetic acid.

## Operational considerations

- **Testing capacity**: MULTIBLOTNS-4800 (48 strips per run), TwinCubator (12 strips per run)
- **Storage temperature**: 2–10 °C
- **Shelf life**: 18 months
- **Unit price (consumable only)**: US$ 16 per test and US$ 14 000 per MULTIBLOTNS 4800 instrument

## Implementation considerations

In addition to general guidance provided in **Section 3.5**, consider the following test-specific implementation considerations:

- **Area 1 – Policies and planning**: Genoscholar PZA-TB II integration into national algorithms and placement into networks should consider that the test should be placed at reference laboratories with adequate infrastructure; a well-functioning sample referral system from peripheral laboratories to reference laboratory should be in place; and patients with DR-TB (i.e. confirmed resistance to rifampicin [RIF] or isoniazid [INH]) may be prioritized for testing.
- **Area 3 – Equipment**: High complexity hybridization NAATs require multiple pieces of equipment for molecular processing (see Equipment, supplies and reagents above). High-throughput laboratories should consider procuring the Nipro automated MULTIBLOT NS-4800 instrument, which can increase testing capacity from 12 to 48 samples per run.
- **Laboratory design and infrastructure**: Precautions to reduce the risk of cross-contamination are critical. As a minimum requirement, three separate rooms for the different molecular steps should be established – one for DNA extraction, one for pre-amplification procedures, and one for amplification and post-amplification processes. Critical to attaining satisfactory results are restricted access, attention to the direction of workflow and meticulously followed procedures for cleaning.

- **Area 5** – **Procedures**: Since mutations are only inferred by the absence of probes, the presence of mutations not associated with resistance may lead to reporting of resistance in the absence of resistance-associated mutations (false resistance). This limitation could be overcome by the sequencing of the *pncA* gene, especially if the pretest probability is low (e.g. RIF-susceptible TB case) and interpreting results based on the latest WHO catalogue of mutations.
- **Area 7** – **Quality assurance**: High complexity reverse hybridization NAATs require strict adherence to a number of procedures to minimize the risk of contamination; therefore, the use of appropriate positive and negative controls, the monitoring of results based on expected outcomes to promptly detect false positive and false negative trends, and the regular participation to external quality assurance programmes should all be observed. New method validations should use PZA-sensitive and PZA-resistant, well-characterized isolates of *Mycobacterium tuberculosis*. PZA-resistant isolates should be selected to ensure a range of resistance-associated mutations are represented in the validation panel to ensure precision and accuracy of resistance detection can be achieved in relation to manufacturer-reported performance characteristics across differing resistance profiles.
- **Area 9** – **Training and competency assessment**: Well-trained staff are needed to carry out a complex procedure that involves several manual steps, timed incubations, precise pipetting and care to avoid cross-contamination. In addition, special training and experience is required for reading of banding patterns on the strip and appropriate interpretation of results.

## References for A2.6

1  Sekiguchi J, Nakamura T, Miyoshi-Akiyama T, Kirikae F, Kobayashi I, Augustynowicz-Kopec E et al. Development and evaluation of a line probe assay for rapid identification of pncA mutations in pyrazinamide-resistant Mycobacterium tuberculosis strains. J Clin Microbiol. 2007;45(9):2802–7 (https://pubmed.ncbi.nlm.nih.gov/17596354/).

2  Nipro. Genoscholar PZA-TB II [website]. 2021 (https://www.nipro-group.com/en/products-services/genoscholartm-pza-tb-ii).

3  Update on the use of nucleic acid amplification tests to detect TB and drug-resistant TB: rapid communication. Geneva: World Health Organization; 2021 (https://www.who.int/publications/i/item/update-on-the-use-of-nucleic-acid-amplification-tests-to-detect-tb-and-drug-resistant-tb-rapid-communication).

4  WHO consolidated guidelines on tuberculosis Module 3: diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2021 update (https://apps.who.int/iris/bitstream/handle/10665/342331/9789240029415-eng.pdf).

For further information, please contact:

**World Health Organization**

20, Avenue Appia CH-1211 Geneva 27 Switzerland

Global TB Programme

Web site: www.who.int/tb

World Health
Organization

9789240030589