global laboratory initiative
advancing TB diagnosis

World Health Organization

Manual for selection of molecular WHO-recommended rapid diagnostic tests for detection of tuberculosis and drug-resistant tuberculosis

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Contents

About this manual

This manual provides practical guidance for the selection of molecular World Health Organization (WHO)-recommended rapid diagnostic test(s) for tuberculosis (TB) and drug-resistant TB, which countries can implement to meet the goals of their national strategic plan for TB.

The manual is designed to be suitable for use in any country; however, countries may need to modify or customize the approach described in the guide to meet the local context of their health system.

Target audience

This manual is intended to inform those interested in implementation of molecular WHO-recommended rapid diagnostic tests to detect TB and drug-resistant TB. The target audience includes ministry of health officials, national TB programme managers, national TB reference laboratory staff, donors, implementing partners, and international agencies and organizations.

About the Global Laboratory Initiative

The Global Laboratory Initiative (GLI) is a network of international partners dedicated to accelerating and expanding access to quality-assured TB laboratory services; GLI has been a working group of the United Nations (UN) Stop TB Partnership since 2007. Coordinated by its core group with support from its Secretariat at the WHO Global TB Programme, GLI's mission is to serve as a collaborative platform for the development and uptake of practical guidance and tools for building and sustaining high-quality TB diagnostic networks. The GLI core group has representation from key constituencies including national and supranational reference laboratories, programmes from countries with a high TB and multidrug-resistant TB burden, technical partners, donors and civil society. More information about GLI can be found on its website[1] or through its Secretariat.[2]

[1] www.stoptb.org/wg/gli.

[2] gli_secretariat@who.int.

Acknowledgements

The development of this manual was led by Thomas Shinnick (independent consultant) under the coordination of the UN Stop TB Partnership GLI Working Group and its Secretariat within the WHO Global TB Programme. Lead authors of the manual included Thomas Shinnick and GLI core group member Patricia Hall (United States Centers for Disease Control and Prevention [US CDC], Atlanta, GA, United States of America). Technical input and critical reviews were provided by GLI core group members Elisa Tagliani (San Raffaele Scientific Institute, Milan, Italy), Christopher Gilpin (International Organization for Migration, Geneva, Switzerland), Sarabjit Singh Chadha (Foundation for Innovative New Diagnostics [FIND], New Delhi, India) and Sarder Tanzir Hossain (United States Agency for International Development [USAID] Infectious Disease Detection and Surveillance Project, Dhaka, Bangladesh). Special thanks to Erin Rottinghaus Romano (US CDC) and Heidi Albert (FIND, Cape Town, South Africa) for technical review and valuable feedback on the content of the manual.

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Abbreviations and acronyms

AIDS	acquired immunodeficiency syndrome
AMK	amikacin
DR-TB	drug-resistant TB
DST	drug-susceptibility testing
ETO	ethionamide
FQ	fluoroquinolone
GDF	Global Drug Facility
GLI	Global Laboratory Initiative
Global Fund	Global Fund to Fight AIDS, Tuberculosis and Malaria
HIV	human immunodeficiency virus
Hr-TB	isoniazid-resistant, rifampicin-susceptible TB
INH	isoniazid
MDR-TB	multidrug-resistant TB
MTBC	*Mycobacterium tuberculosis* complex bacteria (e.g. *M. tuberculosis* or *M. bovis* bacteria)
mWRD	molecular WHO-recommended rapid diagnostic test (for TB)
RIF	rifampicin
RR-TB	rifampicin-resistant TB
TB	tuberculosis
WHO	World Health Organization
XDR-TB	extensively drug-resistant TB; that is, MDR/RR-TB that is also resistant to a fluoroquinolone and one other Group A drug (bedaquiline or linezolid)

Background

To meet the targets of the World Health Organization (WHO) End TB Strategy *(1)*, WHO recommends that :

- people with signs or symptoms of tuberculosis (TB) receive a molecular WHO-recommended rapid diagnostic (mWRD) test to detect TB;
- people with bacteriologically confirmed TB receive a rapid molecular test to detect resistance to at least the first-line drug rifampicin (RIF); and
- people with RIF-resistant TB (RR-TB) receive a rapid molecular test to detect resistance to at least fluoroquinolones (FQs; for example, evofloxacin and moxifloxacin).[3]

More recently, WHO guidelines have stressed the importance of drug-susceptibility testing (DST) before treatment. This emphasizes the need for countries to implement rapid molecular DST for the medicines for which mWRDs are available, such as RIF, isoniazid (INH) and FQs.

There are a growing number of mWRDs to aid in the diagnosis of TB and drug-resistant TB (DR-TB). Each of the mWRDs has good sensitivity and specificity for the detection of *Mycobacterium tuberculosis* complex mycobacteria (MTBC). Most, but not all, mWRDs used to detect MTBC are also capable of detecting DR-TB (see **Table 1**). All mWRDs that detect DR-TB also test for resistance to RIF, and some mWRDs test for resistance to both RIF and INH, facilitating detection of mono-resistance to each of these key first-line anti-TB medicines, as well as combined resistance (i.e. multidrug-resistant TB [MDR-TB]). Lastly, mWRDs are also available to test all those with bacteriologically confirmed TB for resistance to RIF, INH, FQs, amikacin (AMK), pyrazinamide (PZA) and ethionamide (ETO).

Importantly, the transition to rapid molecular testing does not eliminate the need for culture and phenotypic DST. Those tests are still needed for conducting DST for drugs for which an mWRD is not available, conducting DST to guide drug dosing determinations, monitoring the response to TB treatment and investigating discordant results from diagnostic testing or DST. In particular, phenotypic DST is needed for testing the new and repurposed Group A drugs used to treat RR-TB and MDR-TB, and for detecting extensively drug-resistant TB (XDR-TB) *(2)*. Thus, the national TB diagnostic network will need to provide both molecular and phenotypic DST services; it will also need to have effective referral linkages between sites conducting phenotypic DST and sites conducting mWRDs.

[3] 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 drug-susceptibility testing for amikacin unnecessary.

This manual describes a process that is designed to assist countries to identify which mWRDs may be suitable for addressing the diagnostic needs in their specific setting. The stepwise process includes considerations of national policies and goals, epidemiology of TB and DR-TB, diagnostic network structure and capacity, facility and infrastructure requirements, and implementation considerations. These factors may lead to the adoption of at least two mWRDs for use in a country, to ensure that testing needs for all clients are met.

Successful implementation of the mWRD selected will require strong government commitment, support from donors and implementing partners, and allocation of sufficient financial and human resources for the implementation process. The required resources include those for annual operating costs, performance monitoring and continuous quality improvement of the mWRDs, and revised testing network.

Molecular WHO-recommended rapid diagnostic tests for selection

The mWRDs discussed in this manual are listed in **Table 1**. They include initial diagnostic tests for diagnosis of TB (without or with detection of drug resistance) and follow-on diagnostic tests for detection of drug resistance.

WHO recommendations and policy guidance for each of these tests are available in the *WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update (3)*. Implementation guidance is available in the *WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update (4)* and, for some mWRDs, in Global Laboratory Initiative (GLI) implementation manuals and information sheets.[4]

Table 1. Molecular WHO-recommended rapid diagnostic tests to detect TB and DR-TB

Test	Manufacturer	Description	Type[a] of approval	Resistance detected
Initial diagnostic tests for diagnosis of TB without detection of drug resistance				
Loopamp™ MTBC detection kit	Eiken Chemical, Tokyo, Japan	Manual or automated NAAT	Individual	None
FluoroType® MTB	Bruker/Hain Lifescience, Nehren, Germany	Automated NAAT	MC-aNAAT	None
Initial diagnostic tests for diagnosis of TB with detection of drug resistance				
Xpert® MTB/RIF	Cepheid, Sunnyvale, CA, USA	Automated NAAT	Individual	RIF
Xpert MTB/RIF Ultra	Cepheid, Sunnyvale, CA, USA	Automated NAAT	Individual	RIF
Truenat® MTB or MTB Plus for TB detection, reflexing[b] to Truenat MTB-RIF-Dx for DR-TB detection	Molbio Diagnostics, Goa, India	Automated NAAT	Individual	RIF
RealTi*me* MTB for TB detection, reflexing[b] to RealTi*me* MTB RIF/INH for DR-TB detection	Abbott Molecular, Des Plaines, IL, USA	Automated NAAT	MC-aNAAT	RIF, INH[c]
BD MAX™ MDR-TB	Becton Dickinson, Sparks, MD, USA	Automated NAAT	MC-aNAAT	RIF, INH[c]
FluoroType MTBDR	Bruker/Hain Lifescience, Nehren, Germany	Automated NAAT	MC-aNAAT	RIF, INH[c]

[4] Individual implementation manuals are available for some of the mWRDs on the GLI website: https://www.stoptb.org/wg/gli/gat.asp.

Test	Manufacturer	Description	Type[a] of approval	Resistance detected
cobas® MTB for TB detection, reflexing[b] to cobas MTB RIF/INH for DR-TB detection	Roche Molecular Diagnostics, Pleasanton, CA, USA	Automated NAAT	MC-aNAAT	RIF, INH[c]
Follow-on diagnostic tests for detection of drug resistance				
Xpert MTB/XDR	Cepheid, Sunnyvale, USA	Automated NAAT	LC-aNAAT	INH, FQ, ETO, AMK
GenoType MTBDR*plus*	Bruker/Hain Lifescience, Nehren, Germany	Manual reverse hybridization assay	FL-LPA	RIF, INH, ETO
Genoscholar™ NTM + MDRTB Detection Kit	NIPRO Corporation, Osaka, Japan	Manual reverse hybridization assay	FL-LPA	RIF, INH[b]
GenoType MTBDR*sl*	Bruker/Hain Lifescience, Nehren, Germany	Manual reverse hybridization assay	Individual	FQ, AMK
Genoscholar PZA-TB	NIPRO Corporation, Osaka, Japan	Manual reverse hybridization assay	HC-rNAAT	PZA

AMK: amikacin; DR-TB: drug-resistant TB; ETO: ethionamide; FL-LPA: first-line line-probe assay; FQ: fluoroquinolone; HC-rNAAT: high complexity reverse hybridization nucleic acid amplification test; INH: isoniazid; LC-aNAAT: low complexity automated nucleic acid amplification test (for isoniazid and second-line drugs); MC-aNAAT: moderate complexity automated nucleic acid amplification test; MTBC: *Mycobacterium tuberculosis* complex bacteria; PTO: prothionamide; PZA: pyrazinamide; RIF: rifampicin; TB: tuberculosis; USA: United States of America.

[a] Only WHO-recommended tests are listed. WHO approval is based on the review of evidence for an individual test or class of tests; the classes include MC-aNAAT, LC-aNAAT, FL-LPAs and HC-rNAAT.

[b] These assays are designed as a two-step process with two separate amplification reactions. The first step is detection of MTBC; the second step is detection of drug resistance.

[c] This assay detects mutations in the *inh* promoter region that confer resistance to INH and ETO/PTO; however, the performance for the test for detecting resistance to ETO/PTO resistance has not yet been reviewed.

In the past, WHO issued policies and recommendations for individual tests based on reviews – undertaken by guideline development groups (GDGs) – of test diagnostic accuracy, feasibility, balance of benefits and harms, cost considerations and acceptability. However, in 2021, WHO developed a class-based scheme for mWRD test classification *(3)*. The classes were defined by the type of test technology, the complexity of test implementation and the target conditions for use. Diagnostic accuracy of the individual members of the class were combined and reviewed, then used to establish class-wide recommendations and design mWRD-inclusive algorithms *(4)*.

Part A. Identifying mWRDs to meet a country's diagnostic needs

This section of the manual describes a stepwise process designed to assist countries to identify one or more mWRDs that are suitable for addressing the diagnostic testing needs for their specific setting.

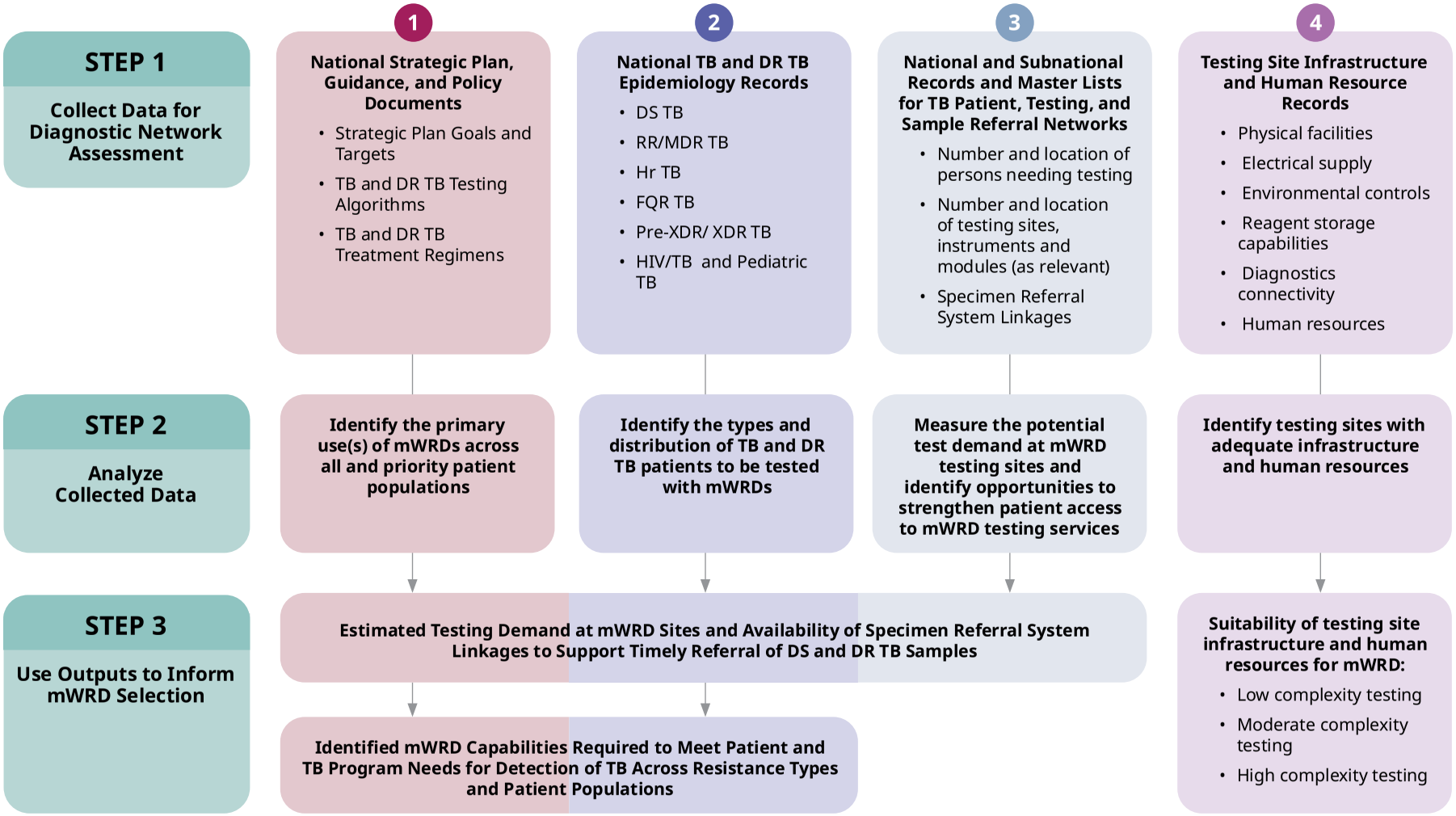
- **Part A1** describes the collection and analysis of information needed for selecting one or more mWRDs. It outlines the main factors that influence the identification of the testing instruments and mWRDs that best address a country's diagnostic testing needs.
- **Part A2** describes how the information collected in Part A1 can be used to identify mWRDs that are suitable to meet the diagnostic needs of the country and ensure patient access to diagnostic services.
- **Part 3** describes factors to consider when determining which suitable mWRDs could be implemented; such factors include issues related to registration and importation of instruments and commodities (including customs clearance), supply chain requirements, service and maintenance requirements, and availability of in-country support from the manufacturer, distributors and authorized local service providers.

A1 Preselection data and analyses

The suitability of an mWRD to meet a country's diagnostic testing or DST needs is determined by:

- national policies, goals and targets, which inform testing priorities;
- subnational epidemiology of TB and DR-TB in the country, which informs diagnostic service needs; and
- the existing TB diagnostic network structure and components, which are reviewed to identify successes and gaps in current testing services and may be used to inform strategies and opportunities for providing and improving diagnostic services.

Fig. 1. Preselection data and analyses



DR-TB: drug-resistant TB; DS-TB: drug-susceptible TB; FQR-TB: fluoroquinolone-resistant TB; HIV: human immunodeficiency virus; HIV/TB: HIV-associated TB; Hr-TB: isoniazid-resistant, rifampicin-susceptible TB; MDR/RR-TB: multidrug-resistant TB or rifampicin-resistant TB; mWRD: molecular WHO-recommended rapid diagnostic; TB: tuberculosis; WHO: World Health Organization; XDR-TB: extensively drug-resistant TB.

The first step in the process for evaluating the suitability of the various mWRDs for use in the different geographical and epidemiological settings in a country is to collect and analyse information needed to inform key decisions. Relevant information includes the country's national policies and strategic plans, epidemiology of drug-susceptible TB and DR-TB, intended uses of the tests, anticipated testing volumes, coverage and accessibility (**Fig. 1**).

A1.1 National strategic plan, guidance and policies

The national strategic plan or national TB laboratory strategic plan should define which mWRD capabilities are needed to inform the mWRD targets.

A1.2 National TB and DR-TB epidemiology

Estimating future testing demand (i.e. number of samples for each test type) requires information about the epidemiology of TB and DR-TB in the various settings in the country, to understand the types and distribution of tests required to meet patient needs. Important information includes the number of estimated, tested and notified cases of TB, paediatric TB, TB/HIV and DR-TB, and the proportion of cases with resistance to key anti-TB medicines (i.e. RIF, INH and FQs). Where possible, the epidemiological data should be stratified by district, region or other subnational geographical area. Much of this information should be available in annual and quarterly reports or from prevalence and drug-resistance surveys.

Key questions to consider:

- What are the objectives of national testing algorithms?
- What is the intended primary use of national testing with mWRDs (e.g. detection of TB, detection of RR-TB or detection of TB and resistance to RIF and INH), and what will the testing be used for as capacity grows?
- Which groups are priority populations for testing with mWRDs?
- Which anti-TB medicines are used in current treatment regimens for which molecular or phenotypic DST is needed?

A1.3 National and subnational TB patient population, testing and sample referral networks

The capacities and capabilities of the TB diagnostic network will be important for determining which mWRDs could be implemented in which settings and at which tiers of the network. Key aspects include the structure of the existing network and its relationship to the clinical service delivery network, such as the diagnostic services and facilities available in each tier of the network (centralized, decentralized and mixed-model services); molecular testing platforms currently in use at different levels of the laboratory network; linkages between laboratories and health care facilities (public sector and private sector); and processes and pathways for referring specimens from health care facilities to centralized or decentralized mWRD testing facilities.

The diagnostic network structure and referral linkages should be assessed, to understand how diagnostic services are organized in a country, identify gaps in access to diagnostic testing services, and identify opportunities to optimize coverage and turnaround times of diagnostic services for all clients. The assessment may be conducted by compiling country-specific, national health facility and testing site data, including health facility presumptive and confirmed TB patient volumes; linkages to on-site or referral-based testing sites; testing site instruments, tests and testing capacity; and testing turnaround

times for clients or health facilities. These data are then analysed to identify gaps in testing coverage by geography or patient population, and gaps in test service turnaround time that limit patient access to diagnostic testing services. Identified gaps that could be closed by addressing minor or localized system challenges should be prioritized for resolution, with activities monitored for their impact on gaps or for further action. However, if significant gaps in testing services are identified that cannot be completely addressed through activities that strengthen existing or new networks, or if new testing needs are identified that cannot be met by the existing network, a full diagnostic network optimization exercise may be conducted (see **Annex 3**).

Potential sources of data for the assessment:

- Population distribution – www.worldpop.org
- Distribution of TB – prevalence, DR-TB surveys or reports of notified cases
- Number and locations of health facilities – master facility list, public databases or survey
- Number and locations of testing sites – master facility list or survey
- Capabilities and capacities of individual sites – annual or quarterly reports
- Specimen referral linkages – survey or network map

Box 1. Key definitions for mWRD site and instrument assessment

Capacity: The number of tests that can be completed by a defined site, instrument or testing unit (i.e. module) in a defined period.

Example: One module of a Cepheid GeneXpert IV instrument in Country X runs three tests in a workday that includes ≤8 hours for testing. Thus, the instrument's capacity, given the availability of four testing modules, is 12 tests per day (3 tests per module × 4 modules = 12), although minimum, average and maximum capacities may differ.

Example: One module of a Cepheid GeneXpert IV instrument in Country X can run a minimum of one test, an average of two tests and a maximum of four tests in the same 8-hour workday.

Given the differences between these examples, it is important to define and specify the type of capacity that is being calculated and used in network analyses.

Utilization: The percentage of maximum site, instrument or testing unit (i.e. module) capacity that is used for testing in a defined period.

Example: 24 BD MAX tests are run in an 8-hour workday, for which the BD MAX has a maximum capacity of 48 tests. The BD MAX instrument therefore has a utilization rate of 50% (24/48). Note that "optimal" utilization of testing site and instrument capacity is often less than 100%, to ensure that sufficient surge capacity is available in the event of increased workload or testing equipment service needs.

Coverage: The percentage of patients that needed an mWRD and received mWRD testing.

Example: Of 200 patients presenting with presumptive TB at health facility X, 150 received mWRD diagnostic and RIF resistance testing, representing a coverage of 75% (150/200). Note that coverage may be calculated at the site level or the above-site (regional or national) level. Similarly, coverage may be calculated for all eligible patients or patient subpopulations (e.g. paediatric patients and patients living with HIV).

A1.4 Testing site infrastructure and human resources

A1.4.1 Anticipated workload

The epidemiology of TB in the local area should provide an estimation of test-specific volumes by assessing the number of people that need an mWRD test, as well as the types of diagnostic and drug-susceptibility tests needed for each anti-TB medicine. The diagnostic network analysis that maps people needing testing alongside existing or needed mWRD sites should enable estimation of the anticipated workload at each mWRD site. Using these data, the WHO TB Diagnostics Capacity Calculator *(5)*[5] may be modified to calculate the number of mWRDs needed at each individual mWRD testing site. Also, current workload information for existing sites should be available from quarterly or annual reports, and can be combined with network analysis data to confirm or refine estimates of the anticipated workload. Anticipated workload is important for the following reasons:

- The workload must be supported by sufficient mWRD test and instrument capacity. Although some instruments can process a maximum of 10 samples per day, others can process 50–100 or even more samples per day (see **Annex 2**). Both overuse and underuse of instrument capacity should be avoided.
- Staffing at the testing site should support completion of the required number of tests per day at each testing site. Necessary modifications to staffing plans can be determined by considering the current number of staff, workhours per day, standard and shifted start and end times, workdays per week and "real-world" throughput of the mWRD platform. For example, this might be the theoretical throughput adjusted for the actual number of test cycles possible in a workday that is inclusive of sample preparation and result reporting time, as well as any instrument downtime for cleaning and maintenance.
- The anticipated workload should also consider the possibility of multidisease testing. For instruments that are shared with other disease testing (e.g. HIV or COVID-19), the total number of samples to be tested per day should be considered when determining the efficient use of the instrument. There are potential cost advantages to sharing an instrument used for multidisease testing (e.g. sharing of purchase and maintenance costs) provided sufficient capacity for each programme's testing demand is ensured. Also, having laboratory staff testing samples for multiple diseases may improve testing efficiencies and staff proficiencies, and facilitate scheduling and shift creation.

A1.4.2 Resource availability

The selection of an mWRD will be influenced by the availability of a testing facility that has the infrastructure needed to support its use. Important considerations for mWRD testing site infrastructure include:

- reliability of the electricity supply;
- availability of temperature and humidity control;
- manufacturer recommendations for sample, equipment and reagent storage;
- access to the required waste disposal supplies and methods;
- biosafety requirements;
- space and security requirements for physical facilities;

[5] See Microsoft Excel spreadsheet at http://www.who.int/entity/tb/publications/calculations_of_lab_capacity.xls?ua=1.

- number and technical skill of laboratory staff (e.g. precision pipetting and computer skills);
- availability of internet; and
- diagnostic connectivity for health care provider and programme reporting.
- Guidance for assessing the suitability of testing sites is available in the implementation guides for the various mWRDs available on the Stop TB Partnership website *(6)*.

In addition to the categories above, this preselection phase should also include reviewing relevant WHO policies and guidance *(3, 4)*, to become familiar with the recommended uses and operational characteristics of the various mWRDs.

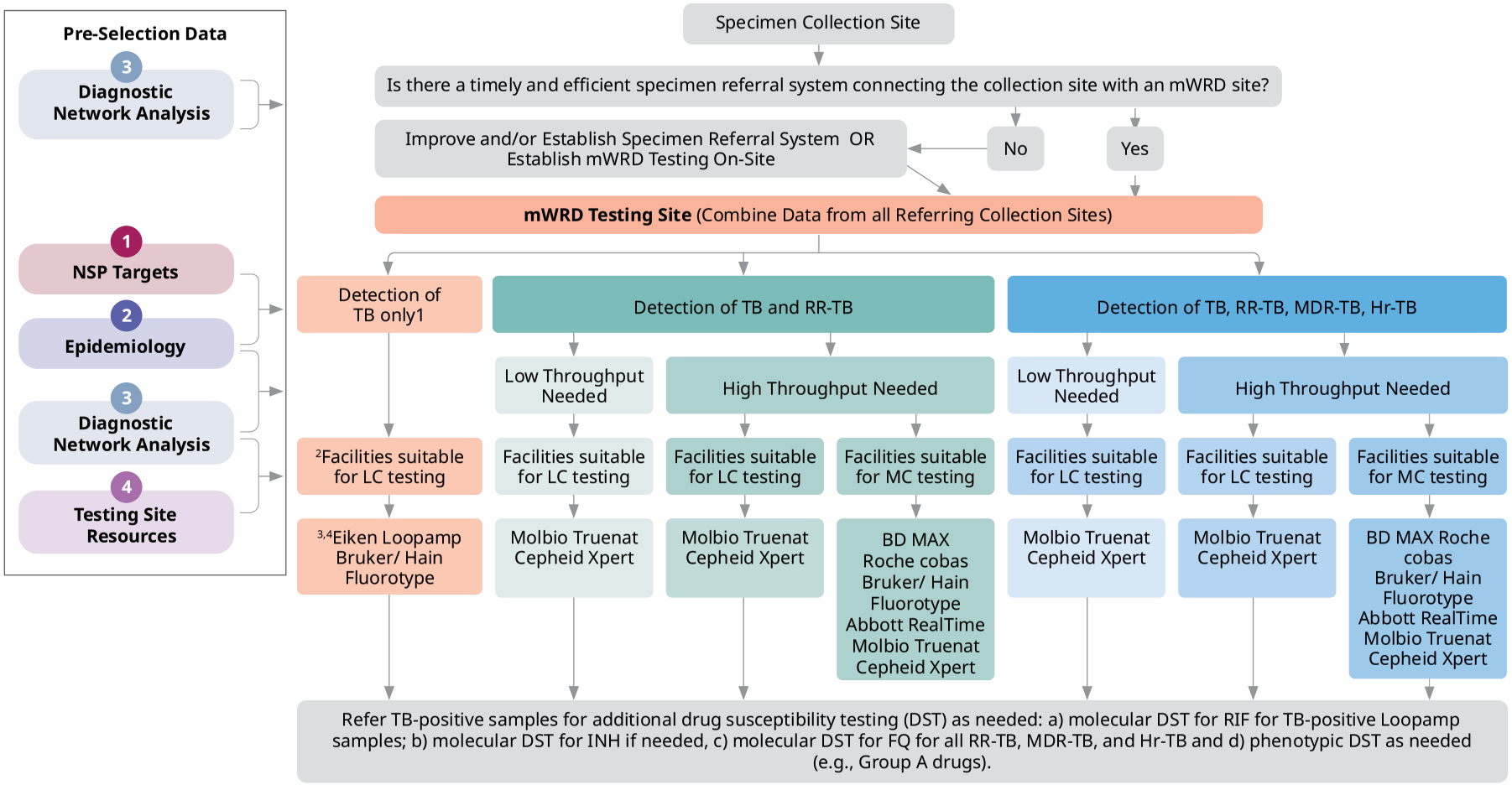
A2 Decision pathway for identifying mWRDs for implementation

Collated and analysed preselection data (see **Part A1**) will provide information on where persons seek care (e.g. specimen collection sites), linkages to testing (e.g. on-site testing or specimen referral) and testing site demands and capabilities (e.g. estimated workload). The decision pathway (**Fig. 2**) uses this information to identify which mWRDs could be used at each individual mWRD testing site to address the molecular diagnostic needs of the patient population being served. The results of the site analyses can be combined to identify the type and potential number of mWRDs needed to meet the molecular diagnostic needs of an epidemiological or geographical setting, and ultimately of the country and the patients being served.

Although the focus is on the individual mWRD testing site, the decision pathway begins with a consideration of the specimen collection sites that are linked or are expected to be linked in the future to an mWRD testing site. This is important for determining the anticipated workload for a testing site, and for patient access to mWRD testing. It may also identify gaps in coverage.

For specimen collection sites that are not currently linked to an mWRD testing site, access to mWRD testing can be provided by either implementing such testing at that specimen collection site or by establishing a linkage (e.g. specimen referral system) to an existing or planned mWRD testing site. The decision pathway can be used to determine which mWRDs would be suitable for implementation at a new mWRD testing site. The decision pathway can also be used to reevaluate an mWRD testing site when the linkage of an additional specimen collection site (or sites) significantly alters the anticipated workload.

Fig. 2. Decision pathway



DR-TB: drug-resistant TB; DST: drug-susceptibility testing; FQ: fluoroquinolone; Hr-TB: INH-resistant, RIF-susceptible TB; INH: isoniazid; LC: low complexity; MC: moderate complexity; MC-aNAAT: moderate complexity automated nucleic acid amplification test; MDR-TB: multidrug-resistant TB; MTBC: *Mycobacterium tuberculosis* complex bacteria; mWRD: molecular WHO-recommended rapid diagnostic; NSP: national strategic plan for TB; RIF: rifampicin; RR-TB: RIF-resistant TB; SRS: specimen referral system; TB: tuberculosis; WHO: World Health Organization.

[a] Any of the tests for detection of TB and DR-TB could also be used in a situation where only the detection of TB is needed. If so, follow the pathway for "Detection of TB and RR-TB".

[b] Complexity of testing relates to the requirements of infrastructure, equipment and the technical skills of laboratory staff.

[c] The Eiken Loopamp MTBC assay and the Bruker/Hain FluoroType MTB assay are designed as tests for the detection of MTBC only. Although the Roche cobas MTB, RealTi*me* MTB and Truenat MTB tests could be used for the detection of MTBC only, they are designed for use as initial assays in a two-test procedure that includes resistance tests. If used for detection of MTBC only, the decision pathway for "Detection of TB and RR-TB" can be used to evaluate their suitability. However, programmes are strongly encouraged to use these tests to detect MTBC and drug resistance, to meet the goal of universal testing for rifampicin resistance.

[d] Tests listed are described in **Table 1** and compared in **Annex 2**. The mWRD abbreviations are defined as follows: Eiken Loopamp (Loopamp MTBC Detection Kit); Bruker/Hain FluoroType (FluoroType MTB); Molbio Truenat (Truenat MTB, MTB Plus and MTB-RIF-Dx); Cepheid Xpert (Xpert MTB/RIF or Xpert MTB/RIF Ultra); BD MAX (BD MAX MDR-TB); Roche cobas (cobas MTB and cobas MTB RIF/INH); and Abbott RealTi*me* (RealTi*me* MTB and RealTi*me* MTB RIF/INH).

A2.1 Steps for identifying suitable mWRDs for use as the initial diagnostic test at a testing site

The steps for identifying suitable mWRDs are outlined below.

Step 1

Identify specimen collection sites with acceptable turnaround times

The diagnostic network analysis in **Part A1** should identify the specimen collection sites (and hence the patients) that are linked to an mWRD site by a specimen referral system[6] that supports an overall, acceptable turnaround time (≤48 hours) from specimen collection to result reporting.

a. The projected test demand from all linked collection sites should be combined to inform the anticipated workload of the mWRD testing site.

b. The network analysis may identify sites that are not currently linked to an mWRD testing site but could be linked, and where a specimen referral system could be established or strengthened to achieve a turnaround time of 48 hours or less. The projected test from any newly linked collection sites should be added to the anticipated workload of the newly linked mWRD testing site.

c. For some specimen collection sites, it may be necessary to implement molecular testing at the collection site to provide access to timely molecular results for all those needing testing. The decision tree can be used to determine which tests would be suitable to establish at such a standalone site.

Step 2

Identify the categories of tests needed

For established or new mWRD sites that provide the diagnostic testing at the specimen collection sites, national policies and goals for testing using mWRDs, along with the epidemiology of TB and DR-TB in the population to be tested, should identify the category of test needed (i.e. detection of TB, or detection of resistance to RIF or any other anti-TB medicines). For example, in a setting with a high prevalence of MDR/RR-TB and a national algorithm indicating that all patients with presumptive TB should receive DST for RIF, an mWRD that detects TB and assesses RIF resistance (and possibly INH resistance) would be preferred over one that only detects TB. Also, mWRD sites that serve different geographical and epidemiological settings in the country may require different classes of mWRDs or different mWRDs within a class, to provide tailored services to their local clients.

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[6] Specimen referral systems include a policy and governance framework, standard operating procedures (SOPs), mechanisms and equipment to move specimens safely and to report results promptly, logistics, trained personnel, data management, financing, and monitoring and evaluation. See the *GLI Guide to TB specimen referral systems and integrated networks*, which can be found on the Stop TB Partnership website *(7)*.

Step 3

Identify the instrument capacity needed at each testing site

The estimation of the anticipated workload at each testing site should identify whether low- or high-throughput systems will be needed to adequately complete the volume of testing and ensure result reporting within 48 hours of specimen collection.

a. mWRD sites that anticipate high volumes of samples may best meet testing demand using an instrument with a high throughput (e.g. cobas 6800 or 8800) or by using multiple instruments with a low throughput (e.g. multiple Truelab Quatro microPCRs).

b. Some mWRD instruments are available in modular formats to accommodate a wide range of tests per day. For example, Xpert MTB/RIF Ultra tests can be conducted on GeneXpert instruments with 1, 2, 4, 8, 16 or more modules, and more than one instrument can be connected to run on a single computer.

Step 4

Identify infrastructure improvements that may be needed at testing sites

The availability of suitable testing facilities may influence the choice of test or may indicate what infrastructure improvements will be needed to implement a specific test. For example:

a. In a setting with an unreliable electrical supply, an mWRD that uses a battery-operated instrument may be preferable to making significant investments in infrastructure upgrades (e.g. installation of solar power, generators or uninterruptible power supply [UPS]).

b. The choice of mWRD may be limited by the number of rooms available for testing, the potential for establishment of molecular testing unidirectional workflow across multiple rooms, and the presence of environmental conditions (e.g. temperature, humidity and dust):

    i. number of rooms required for mWRD testing is 1–3 rooms;

    ii. mWRD testing instruments vary in size from under 0.5 m wide to over 4 m wide and from under 10 kg to over 2400 kg in weight;

    iii. the maximum operating temperature of mWRD testing instruments ranges from 28 °C to 40 °C; and

    iv. mWRD reagent storage conditions vary from –20 °C to 30 °C.

c. If a molecular testing platform is currently in use by another disease programme (e.g. HIV or COVID-19) at a testing facility in the TB diagnostic network, or accessible by that network, implementing the corresponding mWRD for TB would be logical. Platforms that use random access approaches (e.g. GeneXpert) or that allow different types of tests to be performed in the same batch (e.g. cobas 6800/8800 and BD MAX) would be preferable, to ensure that the needs of all patients and programmes can be met. Careful planning will be needed to ensure cross-disease equitable access to testing instrumentation and resources, predefined prioritization of sample or test types, cross-programme implementation and optimization of the multidisease testing strategy, and use of non-duplicative data management and reporting solutions.

d. mWRD sites may be able to accommodate low, moderate or high complexity mWRDs, as indicated by their WHO class assignment. Complexity of the testing is based on each test's requirements for infrastructure, equipment and laboratory staff technical skill:

   i. *Low complexity* – Facilities suitable for low complexity testing are typically found in the lower tiers of TB laboratory network (e.g. peripheral and district laboratories) and have few or no special laboratory infrastructure requirements (e.g. reliable supply of electricity or low dust environment) and laboratory staff with basic technical skills (e.g. basic pipetting, and precision not critical). Instruments required for the mWRD used in such facilities are relatively easy to install, operate and maintain. The mWRDs suitable for such facilities include Xpert MTB/RIF, Xpert MTB/RIF Ultra and Xpert MTB/XDR; Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF-Dx; and Loopamp MTBC tests. Low complexity testing can be performed in facilities suitable for moderate or high complexity testing.

   ii. *Moderate complexity* – Facilities suitable for moderate complexity tests are typically found in the intermediate or central tiers of the TB laboratory network (e.g. regional, intermediate or national laboratories), have specific laboratory infrastructure requirements (e.g. multiple rooms), and have qualified laboratory staff with specific computer and moderate complexity testing skills, such as the ability to successfully complete multi-step procedures with precision. Required instruments for moderate complexity mWRDs have more extensive installation, operational and maintenance requirements. The mWRDs suitable for such facilities include RealTime MTB and RealTime MTB RIF/INH; BD MAX MDR-TB; FluoroType MTB and FluoroType MTBDR; and cobas MTB and cobas MTB RIF/INH tests. Moderate complexity testing can also be performed in facilities suitable for high complexity testing, but not in low complexity settings.

   iii. *High complexity* – Facilities suitable for high complexity tests are typically found in the intermediate or central tiers of the TB laboratory network, and their requirements are similar to those of moderate complexity laboratories. The key difference is that a higher level of technical skills is required to perform the assay and interpret results. The mWRDs suitable for such facilities include the follow-on Genoscholar PZA-TB line-probe assay for the detection of PZA resistance. Low and moderate complexity testing can be performed in facilities suitable for high complexity testing.

Step 5

Consider the need for follow-up testing

The diagnostic pathway does not always stop with mWRD testing; it can include necessary follow-up testing, such as testing for resistance to additional anti-TB medicines. Thus, the following are important:

a. Consider whether multiple mWRDs can be used in combination to meet testing demand. For example, in certain circumstances, tests listed as "initial diagnostic tests for diagnosis of TB with drug-resistance detection" can also be used as follow-on tests to detect drug resistance. The BD MAX MDR-TB, for example, can be used as a follow-on test to detect resistance to INH and RIF for TB-positive samples identified by the Loopamp MTBC detection test.

b. Consider WHO and national recommendations for comprehensive diagnostic and DST services. For example, WHO recommends that all persons with MDR/RR-TB or INH-resistant,

RIF-susceptible TB (Hr-TB) receive a drug-susceptibility test for FQ resistance. This can be achieved by having an mWRD for FQ resistance at the mWRD site or by referring a sample to a second mWRD site that offers an mWRD for FQ resistance. The decision process described here can be applied to choosing follow-on tests to detect resistance to additional drugs. It may also be necessary to refer samples for phenotypic DST, particularly for the new and repurposed drugs (e.g. bedaquiline, delamanid and linezolid) for which an mWRD is not available, or for drugs for which clinical dosing may benefit from determination of critical concentrations (e.g. moxifloxacin). Lastly, DST should be available for all drugs included in a treatment regimen for which there is a reliable DST method *(8)*.

A2.2 Steps for identifying suitable mWRDs for use as follow-on tests at a testing site

A similar process is employed for selecting a suitable mWRD to use as a follow-on test to detect drug resistance. A critical step is to determine which drug resistances to assess. This should be guided by national policies and testing algorithms, along with the epidemiology of DR-TB in the population to be tested. Patients with resistance detected at initial testing (e.g. RIF) can be referred to specific treatment centres and testing at laboratories linked to these sites; alternatively, provision of on-site testing at the treatment site needs to be considered. A low complexity follow-on test (Xpert MTB/XDR) is available to assess resistance to INH, FQ, AMK and ETO. Follow-on tests of increased complexity are available that assess resistances to RIF and INH (GenoType MTBDR*plus* and Genoscholar NTM + MDRTB Detection Kit) and to FQ and AMK (GenoType MTBDR*sl*). A high complexity follow-on test (Genoscholar PZA-TB) is available to assess resistance to PZA. Each of these tests can be configured to be used in a low- or high-throughput setting.

A3 Considerations for suitable mWRD implementation

The decision process described above should help to identify which mWRDs are suitable to address the diagnostic needs of the country. The decision regarding which of the suitable mWRDs to implement in the various epidemiological and geographical settings will involve country-specific considerations such as:

- existing molecular testing platforms and capacity for TB and other diseases;
- financial aspects (e.g. available budget, cost of instruments and commodities, availability of Global Drug Facility [GDF] pricing, implementation costs and annual operating costs);
- instrument capabilities, service and maintenance requirements and the availability of authorized local service providers, extended warranties and service agreements;
- sample referral network and its ability to deliver timely results in different areas;
- procurement issues (e.g. supply chain, shelf life, storage conditions, importation formalities, customs regulations and in-country distributors);
  - shelf lives range from 9 months to 24 months;
  - storage conditions for reagents range from –15 °C to –25 °C, to 2 °C to 8 °C and 2 °C to 30 °C;
- need for upgrading facilities to meet infrastructure requirements and operating conditions;
- availability of enough staff with the appropriate skills; and
- availability of in-country technical support and assistance.

Questions to consider when comparing suitable mWRDs are listed in **Annex 1**.

Finally, the decision process focuses on individual mWRD testing sites. The results of the analyses of individual sites in an epidemiological or geographical setting should be combined to obtain an overview of the mWRDs appropriate for use in the setting. When considering the setting as a whole, issues related to procurement, equipment maintenance and quality assurance (for example) may influence which mWRDs will be feasible to implement and maintain over the long term. A mixture of different options that fit the needs of each localized setting may be needed, to provide the best overall solution. Programmes will also need to take into account plans for future expansion of mWRD testing in the country.

Part B. Suggested reading and resources

B1 WHO guidelines and policies[7]

- *WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update (3).*
- *WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detention, 2021 update (4).*
  - The narrative summarizes the individual mWRDs, their performance characteristics and recommended uses.
  - Information sheets in Annex 2 provide overviews of the Abbott RealTi*m*e MTB and MTB RIF/INH tests, Becton Dickinson BD MAX MDR-TB test, Roche cobas MTB and cobas MTB-RIF/INH tests, Bruker/Hain Lifescience FluoroType MTB and FluoroType MTBDR, Cepheid Xpert MTB/XDR and Nipro Genoscholar PZA-TB II tests.

B2 GLI implementation manuals[8]

- *Implementing a quality assurance system for Xpert MTB/RIF testing*.
- *GLI planning for country transition to Xpert MTB/RIF Ultra cartridges.*
- *Practical guide to implementation of Truenat tests.*
- *GLI guide for the interpretation and reporting of line probe assays*.

B3 GLI information sheets[9]

- Practical considerations for implementation of the Abbott RealTime MTB and Abbott RealTime MTB RIF/INH tests.
- *Practical considerations for implementation of the BD MAX MDR-TB test*.
- *Practical considerations for implementation of the Roche cobas MTB and cobas MTB-RIF/INH assays*.
- *Practical considerations for implementation of the Bruker/Hain Lifescience FluoroType MTB and FluoroType MTBDR*.

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

[8] See the Stop TB Partnership website *(7)*.

[9] See the Stop TB Partnership website *(7)*.

- *Practical considerations for implementation of the Cepheid Xpert MTB/XDR test.*
- *Practical considerations for implementation of the Nipro Genoscholar PZA-TB II assay*.
- *Practical considerations for implementation of the loop-mediated isothermal amplification (TB-LAMP) test*.

B4 Stop TB Partnership information notes and GDF publications

- *Xpert® MTB/RIF and Ultra: technical information note (9)*.
- *Automated rapid nucleic acid amplification tests (NAATs) for detection of TB and resistance to rifampicin and isoniazid: Stop TB information note (10)*.
- *Practical considerations for implementation of Truenat (11)*.
- *Diagnostics, medical devices & other health products catalog (12)* – Describes test specifications, ordering information, shelf lives and storage conditions for tests eligible for purchase using funds from the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund).

B5 Global Fund to Fight AIDS, Tuberculosis and Malaria

List of TB diagnostic test kits and equipment classified according to the Global Fund quality assurance policy (13) – Describes prices of equipment, consumables and warranties available through the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund).

B6 Diagnostic network analysis and optimization

- *Bringing data analytics to the design of optimized diagnostic networks in low- and middle-income countries: process, terms and definitions.* Nichols et al. (2020) *(14)*.
- *Designing an optimized diagnostic network to improve access to TB diagnosis and treatment in Lesotho.* Albert et al. (2020) *(15)*.
- *Laboratory mapping program (LabMaP) – what we do.* African Society for Laboratory Medicine (2022) *(16)*.

B7 Specimen referral systems

- *Guide to TB specimen referral systems and integrated networks* – available from the Stop TB Partnership website *(6)*.

References

1. The End TB Strategy. Geneva: World Health Organization; 2015 (https://www.who.int/publications/i/item/WHO-HTM-TB-2015.19, accessed January 2022).

2. Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27–29 October 2020. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/338776, accessed January 2022).

3. WHO consolidated guidelines on tuberculosis Module 3: Diagnosis rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/342331, accessed January 2022).

4. WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/342369, accessed January 2022).

5. Framework of indicators and targets for laboratory strengthening under the End TB Strategy. Geneva: World Health Organization; 2016 (https://apps.who.int/iris/handle/10665/250307, accessed January 2022).

6. Guidance and tools [website]. Stop TB Partnership; (https://stoptb.org/wg/gli/gat.asp, accessed January 2022).

7. GLI specimen referral toolkit. Stop TB Partnership; 2020 (https://stoptb.org/wg/gli/srt.asp, accessed January 2022).

8. Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis. Geneva: World Health Organization; 2018 (https://apps.who.int/iris/handle/10665/275469, accessed January 2022).

9. Xpert® MTB/RIF and Ultra. Stop TB Partnership; 2019 (https://stoptb.org/assets/documents/gdf/drugsupply/Xpert_info_note.pdf, accessed January 2022).

10. Automated rapid nucleic acid amplification tests (NAATs) for detection of TB and resistance to rifampicin and isoniazid – Stop TB information note. Geneva: Stop TB Partnership; 2021 (https://stoptb.org/assets/documents/resources/publications/sd/RIH_INH_NAATs.pdf, accessed January 2022).

11. Practical considerations for implementation of Truenat. Geneva: Stop TB Partnership;(https://stoptb.org/assets/documents/resources/wd/Practical%20Considerations%20for%20Implementation%20of%20Truenat.pdf, accessed January 2022).

12. Diagnostics, medical devices & other health products catalog. Geneva: Stop TB Partnership; 2021 (https://stoptb.org/assets/documents/gdf/drugsupply/GDFDiagnosticsCatalog.pdf, accessed January 2022).

13. List of TB diagnostic test kits and equipments classified according to the Global Fund quality assurance policy. Geneva: The Global Fund; 2020 (https://www.theglobalfund.org/media/9461/psm_productsdiagnosticstb_list_en.pdf, accessed January 2022).

14. Nichols K, Girdwood SJ, Inglis A, Ondoa P, Sy KTL, Benade M et al. Bringing data analytics to the design of optimized diagnostic networks in low- and middle-income countries: process, terms and definitions. Diagnostics (Basel). 2020;11(1) (https://pubmed.ncbi.nlm.nih.gov/33374315/, accessed January 2022).

15. Albert H, Purcell R, Wang YY, Kao K, Mareka M, Katz Z et al. Designing an optimized diagnostic network to improve access to TB diagnosis and treatment in Lesotho. PLoS One. 2020;15(6):e0233620 (https://pubmed.ncbi.nlm.nih.gov/32492022/, accessed January 2022).

16. Laboratory mapping program (LabMaP). What we do. [website]. African Society for Laboratory Medicine; (https://aslm.org/what-we-do/labmap/, accessed January 2022).

Annex 1. Questions to consider when comparing suitable mWRDs

The questions in this annex are designed to help countries to decide which of the "suitable" molecular World Health Organization (WHO)-recommended rapid diagnostic tests (mWRDs) for tuberculosis (TB) identified by the decision pathway meet the diagnostic testing needs of respective mWRD sites selected for test introduction by the TB diagnostic network analysis. The included questions are not exhaustive; instead, they are designed to highlight the different characteristics of mWRDs that have the same level of complexity and similar throughputs. Annex 2 contains tables that compare mWRD test specifications, for reference.

The questions given here were identified by consensus among members of the Global Laboratory Initiative (GLI) Core Group. Country-specific factors will affect the priority of the questions and the relative importance of the answers in determining which mWRD best meets the needs of a particular epidemiological and geographical setting.

mWRD site testing demand

- Is the test needed for initial detection of TB alone, or for initial or follow-on detection of resistance to TB specific medicines (if so, which medicines?) at the selected mWRD site or sites?
- Which level of mWRD test class complexity and operational requirements can be accommodated by existing site infrastructure and human resources; also, if upgrades are needed, are they feasible?
- What level of throughput (i.e. tests per day) is needed to meet the anticipated demand for testing at the mWRD site?

WHO recommendations for mWRD use and performance *(1)*

- Do the WHO recommendations for test use, target populations (e.g. adults, children, people living with HIV) and specimen types match the needs of the patient population served by the mWRD site? Note: all currently recommended mWRDs are approved for use with sputum and bronchial alveolar lavage samples, but only some mWRDs approved for use with various extrapulmonary samples.
- At the mWRD site's TB prevalence rate, what are the performance parameters of the test (e.g. sensitivity, specificity, positive and negative predictive value, and error rates) in the population served by the mWRD testing site?
- How flexible and adaptable are the test and test platform? Can the test and test platform be used to address current and planned diagnostic targets of the national strategic plan?

Costs

- What commodity-specific costs are associated with the mWRDs (e.g. test, supplies, reagents, instruments and equipment)?
- What annual operating costs are associated with the mWRDs (e.g. consumables, instruments, service and maintenance, human resources and external quality assurance)?
- Is Global Drug Facility (GDF) pricing needed for the mWRDs; if so, is it available?
- What are the costs to support the introduction of each mWRD and how do they differ (e.g. facility upgrades, national and site-level documentation revisions, clinical and laboratory trainings, diagnostic connectivity solutions and external quality assurance)?[10]
- Does the mWRD instrument have the capacity to test for multiple diseases – TB, HIV, COVID-19 or other diseases – if so, can costs for introduction, implementation and maintenance be shared across disease programmes?

Procurement and supply chain

- Does the test have regulatory approval to be used in the country and can customs clearance be achieved? What are the importation requirements for instruments, reagents and supplies?
- Is a cold chain required for distribution of reagents and commodities?
- What is the shelf life of the required reagents and commodities?

Instrumentations, maintenance and service

- Is a molecular testing instrument in use at a current or planned mWRD testing site? If so, does it have capacity available to accommodate the anticipated TB testing workload, either alone or in combination with testing for other diseases?
- What are the service and maintenance requirements for required instruments?
- What are the calibration requirements for necessary ancillary equipment?
- Is support from the manufacturer or authorized local service providers for installation, implementation and equipment maintenance (warranties or service contracts) available in-country?
- Is there in-built or potential diagnostic connectivity, to allow for rapid transfer of results and remote monitoring?

Facility requirements

- What biosafety precautions are needed? Is a biological safety cabinet required and available?
- Is the main electricity supply reliable? Is there a need for alternative energy sources, generators or uninterruptible power supply (UPS)? Can the test be conducted with a battery-powered instrument, if necessary?

[10] For a discussion of budgetary needs for implementation, see the Global Laboratory Initiative Planning and Budgeting Tool for TB and Drug Resistant TB Testing on the Stop TB Partnership website *(2)*.

- Will upgrades to the facility be needed to meet the operating environment requirements (e.g. temperature, humidity and dust free), room and bench space requirements, and security requirements?
- What are the recommended storage conditions for the components of the test, and can the selected mWRD sites accommodate any necessary freezers and refrigerators?

Human resources

- What is the complexity of instrument use and site-level maintenance, and the degree of automation available? Is the level of complexity suitable for testers at the mWRD testing site?
- What is the required hands-on time and the complexity of the hands-on steps? Does the assay require precision pipetting?
- Is detection of *Mycobacterium tuberculosis* complex bacteria (MTBC) and drug resistance accomplished in a one-step process or is it a two-step process that requires separate assays for detection of MTBC and detection of resistance?
- How many staff will be required to handle the anticipated workload?
- Where multidisease testing is conducted, could staff be supported by multiple disease programmes?
- What is the total time required for a test (hands-on time plus instrument run time) and how can it fit into the workflow of the laboratory?

References for Annex 1

1. WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection, 2021 update. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/342331, accessed January 2022).

2. Guidance and tools [website]. Geneva: Stop TB Partnership; 2022 (https://stoptb.org/wg/gli/gat.asp, accessed January 2022).

Annex 2. Test specifications

	Initial molecular diagnostic tests for detection of TB							
	With detection of drug resistance						Without detection of drug resistance	
	Cepheid Xpert® MTB/ RIF and Ultra	**Molbio Truenat® MTB, MTB Plus and MTB-RIF-Dx**	**Abbott RealTime MTB and MTB RIF/ INH**	**BD MAX™ MDR-TB**	**Bruker/Hain FluoroType® MTBDR**	**Roche cobas® MTB and MTB RIF/INH**	**Bruker/Hain FluoroType MTB**	**Eiken Loopamp™ MTBC Detection (TB-LAMP)**
WHO class	N/A[a]	N/A	MC-aNAAT[b]	MC-aNAAT	MC-aNAAT	MC-aNAAT	MC-aNAAT	N/A
Drug resistance detected	RIF	RIF	RIF, INH	RIF, INH	RIF, INH	RIF, INH	None	None
Maximum capacity (maximum no. of tests/ workday)	12 (GX-IV) to 48 (GX-XVI)[c]	9 to 36	94	48	Up to 288	384 to 1056[d]	Up to 288	70
Batch size	4 (GX-IV) to 16 (GX-XVI)	1 (Uno) to 4 (Quatro)	Up to 94	Up to 24	Up to 94	Up to 94	Up to 94	Up to 14
Run time	2 hours (MTB/RIF) 90 minutes (Ultra)	1 hour (detection) plus 1 hour (resistance)	7 hours (detection) plus 3.5 hours (resistance)	4.5 hours	2.5 hours	3.5 hours (detection) plus 3.5 hours (resistance)	2.5 hours	90 minutes
Required instrumentation	6-color GeneXpert	Trueprep sample prep device and Truelab microPCR analyzer	m2000sp and m2000rt microplate centrifuge	BD MAX	GXT96 and Fluorocycler XT	cobas 6800/8800	GXT96 and Fluorocycler XT	HumaLoop T or HumaTurb system

	Initial molecular diagnostic tests for detection of TB							
	With detection of drug resistance						Without detection of drug resistance	
Dimensions	GX-IV 27.94 cm × 30.48 × 29.72 cm, 11.8 kg GX-XVI: 57.8 × 65.6 × 33.7 cm, 21.5 kg	Trueprep device 21.5 × 23.5 × 11.5 cm, 2.5 kg Truelab Uno PCR 24 × 18.5 × 112 cm, 1.5 kg	m2000sp 145 × 79.4 × 217.5 cm, 314.4 kg m2000rt 34 × 49 × 45 cm, 34.1 kg	BD MAX 94 × 75.4 × 72.4 cm, 113.4 kg	GXT96 112.3 × 77.4 × 82.5 cm, 140 kg FluoroCycler XT 43 × 57 × 73 cm, 65 kg	cobas 6800 292 × 216 × 129 cm, 1624 kg cobas 8800 429 × 216 × 129 cm, 2405 kg	GXT96 112.3 × 77.4 × 82.5 cm, 140 kg FluoroCycler XT 43 × 57 × 73 cm, 65 kg	HumaLoop T 25 × 30.6 × 18.2 cm, 9.5 kg
Molecular workflow required	No	No	Yes	No	Yes	No	Yes	No
Operating conditions	<30 °C	15 °C to 40 °C, with 10–80% relative humidity	15 °C to 28 °C, with 30–80% relative humidity	18 °C to 28 °C with 20–80% relative humidity	-	-	-	<30 °C
Reagent storage	2 °C to 28 °C	2 °C to 30 °C	–15 °C to –25 °C	2 °C to 28 °C	–20 °C to –18 °C	2 °C to 8 °C	–20 °C to –18 °C	2 °C to 30 °C
Reagent shelf life	9 months	2 years	12 months (MTB RIF/INH) or 18 months (MTB)	9 months	On request	16 months (MTB RIF/INH) or 18 months (MTB)	On request	14 months
Connectivity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No

INH: isoniazid; MC-aNAAT: moderate complexity automated nucleic acid amplification test; N/A: not applicable; PCR: polymerase chain reaction; RIF: rifampicin; TB: tuberculosis; WHO: World Health Organization.

[a] Not applicable: The test has not been assigned to a class. WHO recommendations were made following a review of the performance of the individual test.

[b] Detailed operational characteristics of the individual moderate complexity (MC-aNAATs) are available *(1)*

[c] Individual GeneXpert instruments can be connected to run on a single computer and thereby increase throughput. Also, the GeneXpert infinity system can process more than 2000 samples per day.

[d] Maximum throughput for the cobas instruments reflects the number of liquefied, lysed and inactivated samples that can be processed in one day.

	Follow-on mWRDs for detection of drug resistance			
	Cepheid Xpert MTB/XDR	**Nipro Genoscholar™ PZA-TB II**	**Bruker/Hain MTBDR*plus***	**Bruker/Hain MTBDR*sl***
WHO class	LC-aNAAT	HC-rNAAT	FL-LPA	SL-LPA
Drugs tested	INH, FQ, AMK, ETO	PZA	RIF, INH, ETO	FQ, AMK
Sample type	Sputum, BAL	Cultures	Sputum or cultures	Sputum or cultures
Maximum tests per day	16 (GX4) to 62 (GX16)	12 or 48	12 or 48	12 or 48
Batch size	Up to 4 with GX4 or up to 16 with GX16	Up to 12 with TwinCubator or up to 48 with Multi-Blot NS-4800	Up to 12 with TwinCubator or up to 48 with GT-Blot 48	Up to 12 with TwinCubator or up to 48 with GT-Blot 48
Run time	90 minutes	1–2 days plus time required for culture	1–2 days	1–2 days
DNA extraction	Integrated into assay	Manual	Manual or separate instrument	Manual or separate instrument
Testing process	Low complexity automated NAAT	High complexity reverse hybridization	Manual reverse hybridization test	Manual reverse hybridization test
Required instrumentation	GeneXpert (10 color)	Multi-Blot NS-4800 or TwinCubator, Thermocycler	TwinCubator or GT-Blot 48, Thermocycler	TwinCubator or GT-Blot 48, Thermocycler
Molecular workflow required	No	Yes	Yes	Yes
Operating conditions	<30 °C	-	Ambient to 55 °C	Ambient to 55 °C
Reagent storage	2 °C to 28 °C	2 °C to 10 °C	Kit 1: 2 °C to 8 °C Kit 2: –20 °C	Kit 1: 2 °C to 8 °C Kit 2: –20 °C
Reagent shelf life	-	12 months	18 months	18 months
Connectivity	Yes	-	No	No
Multiplexing	Yes	-	No	No

AMK: amikacin; BAL: bronchoalveolar lavage; ETO: ethionamide; FL-LPA: first-line line probe assay; FQ: fluoroquinolone; HC-rNAAT: high complexity reverse hybridization NAAT; INH: isoniazid; LC-aNAAT: low complexity automated NAAT; MC-aNAAT: moderate complexity automated NAAT; mWRD: molecular WHO-recommended rapid diagnostic; N/A: not applicable; NAAT: nucleic acid amplification test; RIF: rifampicin; SL-LPA: second-line line probe assay; WHO: World Health Organization.

Reference for Annex 2

1. FIND cDST. WHO supplement. Geneva: World Health Organization; 2019 (https://www.finddx.org/wp-content/uploads/2019/08/FIND_cDST_WHO_Supplement.xlsx, accessed January 2022).

Annex 3. Diagnostic network optimization

Diagnostic network optimization is a three-step process *(1):*

Step 1: Geographical mapping and baseline scenario model creation.

Step 2: Alternative scenario creation and analysis.

Step 3: Network optimization (i.e. comparison of the scenarios to identify the optimal network design).

The first step relies on information that may already be available in the ministry of health, national TB programme (NTP) or national TB laboratory, or from simple surveys, and it can provide important information. In contrast, a full analysis and network optimization exercise requires considerable human resources and time (3–6 months), and countries may require expert technical assistance. Optimal network configurations may differ between different geographical and epidemiological settings.

Step 1: Geographical mapping and baseline scenario model creation

The first step involves mapping – that is, spatial analysis using geographic information system (GIS) coordinates – of the populations that require testing; the number and locations of health facilities where people seek care; the number, locations, capabilities and capacities of testing sites; and referral linkages.

Baseline scenario

- What is the potential demand for testing?
- Where are specimens collected for testing?
- Where is testing done?
- How do specimens get from collection sites to testing sites?

Guidance on collecting the information needed for a spatial analysis can be found in these resources:

- *Laboratory mapping program (LabMaP) – what we do (2);*
- *Master facility list resource package: guidance for countries wanting to strengthen their master facility list (3);* and
- *How to include laboratories in a master facility list: preliminary guidance (4).*

Also, various databases of geocoded health facilities in sub-Saharan Africa are available *(5, 6)*.

The main purpose of this step is to generate a baseline model of the diagnostic network for use in the network optimization process. However, the inventory of GIS-mapped health facilities and GIS-mapped TB laboratories (including current inventory of diagnostic tests and instruments) should

be useful to the NTP for strategic planning, allocating resources and planning for continuation of TB services in case of service disruptions. For example:

- the inventory of laboratories and current mWRD instruments and test volumes may identify underused and overused instruments, and opportunities to redistribute instruments to improve the efficiency of testing;
- overlaying the maps of specimen collection sites, testing sites and transportation routes might identify potential specimen referral linkages and inform the design of specimen transport routes; and
- comparison of the TB network baseline model with other network models (e.g. for HIV) may identify opportunities to collaborate or cost-share services while providing clients with one-stop-shop testing services.

Step 2: Alternative scenario creation and analysis

The next step is to develop alternative scenarios to the baseline model, in consultation with key stakeholders. These scenarios should reflect decision points such as the following:

- Where can new sites for mWRDs be established to increase detection rates of TB or drug-resistant (DR-TB), or to address national strategic plan goals and priorities for improving TB testing in populations that are underserved or a priority?
- Are there opportunities for linking a cluster of specimen collection sites to generate sufficient test demand to justify establishing a molecular testing site with high throughput? Sites that could form a cluster include those within 40–50 km of an mWRD site (which is a feasible daily driving distance for a courier) or those that can be linked by a specimen referral system that allows the testing laboratory to report results within the recommended turnaround time (≤48 hours from specimen collection).
- How can molecular testing services be provided for difficult-to-reach areas or areas where specimen referral systems with short turnaround times are not currently feasible?
- Are there opportunities for using existing molecular testing platforms (e.g. a Roche cobas 8800 or Abbott m2000 instrument, used for HIV testing) for TB testing?
- How does changing the acceptable turnaround time affect access to molecular testing?

Although the next step is formal – software-driven evaluation of the alternative scenarios – programmes will be able to generate useful insights from a less formal interim analysis. For example, a visual analysis of a map that overlays population distribution and existing mWRD sites may be able to quickly identify areas that would benefit from the establishment of a new mWRD site and thus help programmes to decide where to place new mWRD instruments.

Step 3: Network optimization

The third step, network optimization, relies on specialized software and modelling approaches to evaluate baseline and alternative network configurations using a set of predefined outputs *(7)*. The aim of network optimization is to increase patient access to testing services and optimize the delivery of those services. The predefined outputs assess the impact of various diagnostic placement,

number and use scenarios on the effectiveness, efficiency and adaptability of the diagnostic network. Possible outputs include improved:

- availability of molecular diagnostic testing services for TB – that is, the proportion of specimen collection sites that are linked to an mWRD site;
- accessibility of molecular diagnostic testing for TB – that is, the proportion of the population that live within walking distance[11] of a specimen collection site that has onsite mWRD testing or is linked to an mWRD site by a specimen referral system that enables an overall turnaround time for mWRD testing of 48 hours or less (i.e. from specimen collection to return of results);
- promptness of molecular diagnostic testing for TB – that is, the proportion of mWRD sites that achieve the target turnaround time of 48 hours or less; and
- quality of molecular diagnostic testing for TB – that is, the proportion of mWRD sites that have sufficient trained and competent staff, and that meet GLI key performance indicators (e.g. expected rates of error, failures and specimen rejection) and standards for internal and external quality assurance.

References for Annex 3

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3. Master facility list resource package: guidance for countries wanting to strengthen their master facility list. Geneva: World Health Organization; 2018 (https://apps.who.int/iris/handle/10665/326848, accessed January 2022).

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6. LabMap dashboard [website]. Addis Ababa: African Society for Laboratory Medicine; 2022 (https://aslm.org/labmap-dashboard/, accessed January 2022).

7. https://www.finddx.org/wp-content/uploads/2021/11/Guide-to-Diagnostic-Network-Optimization_15.11.2021.pdf, accessed January 2022.

[11] For accessing primary health care, a walking distance of 5 km is often considered acceptable. Terrain-specific accessibility algorithms are available that consider geographical variability in determining acceptable walking distances.

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