Stop TB Partnership

March 2021

AUTOMATED RAPID NUCLEIC ACID AMPLIFICATION TESTS (NAATs) FOR DETECTION OF TB AND RESISTANCE TO RIFAMPICIN AND ISONIAZID STOP TB INFORMATION NOTE

On 17 February 2021 the WHO Global TB Programme published a Rapid Communication on the key findings of a December 2020 Guideline Development Group that reviewed several classes of nucleic acid amplification tests (NAATs) for detection of TB and drug-resistant TB. The Rapid Communication was released in advance of updated WHO guidelines expected later in 2021, in order to allow for rapid transition and planning at country level.

One of the three classes of technologies reviewed was **automated NAATs for detection of TB and resistance to rifampicin and isoniazid** and categorized as having "moderate complexity", considering the requirements of infrastructure, equipment and technical skills. These NAATs are run on high-throughput instruments that automate the DNA extraction, PCR amplification and detection steps. The instruments also have a menu of other tests available, allowing for opportunities to multiplex.

Products included in the WHO evaluation:

- Abbott RealTime MTB and MTB RIF/INH Resistance assays, using the RealTime System
- BD MAX MDR-TB assay, using the BD MAX System
- Hain Lifescience FluoroType MTBDR VER 2.0 assay, using the GenoXtract 96 and FluoroCyclerXT Systems
- Roche cobas MTB and MTB-RIF/INH assays, using the cobas6800/8800 Systems

The WHO Guideline Development Group found the use of these NAATs to be **highly accurate** when using respiratory samples from people with signs and symptoms of pulmonary TB, comparing to a reference standard of culture and phenotypic DST: overall pooled sensitivity (95% CI) for TB detection was 93.0% (90.9 to 94.7) and specificity was 97.7% (95.6 to 98.8); overall pooled sensitivity (95% CI) for rifampicin resistance detection was 96.7% (93.1 to 98.4) and specificity was 98.9% (97.5 to 99.5); overall pooled sensitivity (95% CI) for isoniazid resistance detection was 86.4% (82.8 to 89.3) and specificity was 99.2% (98.1 to 99.7).

The Rapid Communication concludes that available **evidence supports the use of these moderate complexity automated NAATs for the detection of TB and resistance to rifampicin and isoniazid.** These products meet the criteria of the Global Fund Quality Assurance policy and are **now eligible for procurement using Global Fund funds**.

ERPD approval for TB diagnostics: what is it?

These 4 moderate complexity automated NAATs evaluated by the WHO Global TB Proramme have been eligible for procurement using Global Fund (GF) funds since 2020, as they had been earlier approved by the GF's Expert Review Panel for Diagnostics (ERPD). The ERPD is a group of independent experts who review the potential risks and benefits associated with the use of diagnostic products and make recommendations to the GF and Unitaid on their use. The WHO Regulation and Prequalification Department hosts the ERPD. ERPD approval of TB diagnostics is intended as an interim approval mechanism on the pathway to potential WHO endorsement, either as a WHO Global TB Programme recommendation or by WHO prequalification (WHO prequalification assessments for TB diagnostics are expected to start in 2021).

ERPD approval allows countries to use GF funding to procure products for a time-limited period, with possibility for renewal. The current list of TB diagnostics approved by the ERPD can be found on the GF eligible products list. ERPD approval of products is categorized as either Risk Category 1 or 2. While products in both Categories meet established ERPD standards around manufacturing site quality and risk management systems and have adequate evidence of analytical performance, Risk Category 2 products have limited clinical performance data in the settings of intended use and/or limited stability data to assign shelf-life.

Product descriptions and operational considerations

Abbott RealTi*m*e MTB and MTB RIF/INH Resistance assays, using the *m*2000 RealTi*m*e System

The Abbott *m*2000 RealTi*m*e System comprises two instruments:

- The *m*2000*sp* instrument automatically extracts DNA from the specimen and prepares the PCR plate. The dimensions of the instrument and accompanying cabinet are 145 x 79.4 x 217.5 cm, with a weight of 314.4 kg.
- The *m*2000*rt* instrument automates the PCR amplification and detection of DNA. The instrument can be placed on a benchtop and has dimensions of 34 x 49 x 45 cm, with a weight of 34.1 kg.

System testing capacity: The *m*2000 RealTi*m*e System can run up to 96 tests for *Mycobacterium tuberculosis* complex (MTBC) in a batch using the MTB assay. Purified nucleic acid from up to 22 MTB-positive patient samples can then be reflexed for detection of resistance to rifampicin and isoniazid, using the MTB RIF/INH Resistance assay. The MTB RIF/INH Resistance assay can also be used as a stand-alone test, starting from nucleic acid extraction.

Time to detection: For batches of up to 96 tests, MTBC is detected within 8.25 hours, with RIF/INH testing requiring a further 3 hours, resulting in complete results within 11.25 hours. For batches of up to 24 tests, MTBC is detected within 6 hours, with RIF/INH testing requiring a further 2.5 hours, resulting in complete results within 10.5 hours. For detection of resistance to isoniazid, the assay can discriminate and report high (*katG*) and low (*inhA*) isoniazid resistance.

Both scenarios are possible to accomplish during an 8-hour working shift, as completion of testing using the *m*2000*rt* instrument is automatic and does not require presence of a technician.

Specimen types: Inactivated sputum, bronchoalveolar lavage, NALC sediments

Multiplexing possibilities:

Many high TB burden countries are already using the *m*2000 system, including to test for HIV-1 viral load and early infant diagnosis, as well as for other diseases. Concurrent multiplexing (i.e., running tests for TB and HIV on the same instrument at the same time) is not a possibility, but a laboratory may arrange serial multiplexing of a batch of TB tests and a batch of HIV tests within laboratory working shifts.

The wide menu of available tests with GF ERPD, WHO PQ, US FDA and/or CE marking is listed below.

Assay	MTB and MTB INH/RIF Resistance	HIV-1 Qualitative	HIV-1	HBV	HCV Viral Load	HCV Geno-typing II	High Risk HPV	CT/NG	CT	CMV	EBV	SARS-CoV-2
Approval	GF ERPD, CE	WHO PQ, CE	WHO PQ, US FDA, CE	US FDA, CE	WHO PQ, US FDA, CE	US FDA, CE	WHO PQ, CE	US FDA, CE	CE	US FDA, CE	CE	WHO EUL, US FDA EUA, CE

Pricing: Global pricing is not yet available; contact the manufacturer for prices. Discussions are underway with Stop TB Partnership's Global Drug Facility (GDF) for inclusion in the GDF Catalog.

Manufacturer links: Abbott *m*2000 System, Abbott MTB assay, Abbott MTB RIF/INH Resistance assay

BD MAX™ MDR-TB assay, using the BD MAX™ System

The BD MAX system is an integrated instrument that automates the DNA extraction, PCR amplification and detection steps. The BD MAX MDR-TB assay simultaneously detects MTBC and resistance to rifampicin and isoniazid, without the need for reflexing. The only manual steps are for sample preparation and loading, and are similar to those for Xpert MTB/RIF. The instrument can be placed on a benchtop and has dimensions of 94 x 75.4 x 72.4 cm, with a weight of 113.4 kg.

System testing capacity: The BD MAX system can test up to 24 patient samples at once.

Time to detection: Test results for MTBC detection and resistance to rifampicin and isoniazid are provided within 4 hours. For detection of resistance to isoniazid, the assay can discriminate and report high (*katG*) and low (*inhA*) isoniazid resistance. Two batches, equaling up to 48 samples, can be tested within an 8-hour working shift.

Specimen types: Inactivated sputum, NALC sediments

Multiplexing possibilities:

BD MAX offers a broad syndromic menu focused on infectious disease and women's health. Due to its specific assay technology, BD MAX MDR-TB is run by itself in the instrument whereas the other BD MAX assays can be mixed in a run for concurrent multiplexing.

The wide menu of available tests with GF ERPD approval, US FDA clearance and/or CE marking is listed below. Partner assays developed by other manufacturers using Open Systems Reagents can also be run on BD MAX.

Assay	MDR-TB	Cdiff	StaphSR	MRSA XT	CPO	Enteric Bacterial	Enteric Parasite	Enteric Viral	GBS	CT/GC/TV	Vaginal Panel	SARS-CoV-2
Approval	GF ERPD, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA, CE	US FDA EUA, CE

Pricing: Global pricing is not yet available; contact the manufacturer for prices. Discussions are underway with Stop TB Partnership's Global Drug Facility (GDF) for inclusion in the GDF Catalog.

Manufacturer links: BD MAX System

Hain Lifescience FluoroType® MTBDR VER 2.0 assay, using the GenoXtract® 96 and FluoroCycler®XT Systems

The FluoroType MTBDR assay simultaneously detects MTBC and resistance to rifampicin and isoniazid, without the need for reflexing. DNA extraction can be performed manually using FluoroLyse or fully automated with the GenoXtract 96 instrument. Using this instrument, a completely automated processing is possible: after DNA isolation and PCR set-up, the plate can be directly placed into the FluoroCyclerXT instrument for fully automated amplification and detection. The GenoXtract 96 instrument can be placed on a benchtop and has dimensions of 112.3 x 77.4 x 82.5 cm, with a weight of approximately 140 kg. The FluoroCyclerXT can be placed on a benchtop and has dimensions of 43 x 57 x 73 cm, with a weight of approximately 65 kg.

The FluoroSoftware evaluates and interprets the results, with a high number of resistance-mediating mutations specified in the results report. Silent mutations within the rpoB gene can be identified. In addition, rare or so far unknown mutations in the target genes are also shown. Furthermore, it is possible to enter information on novel mutations into the machine-learning software in order to ensure their specification in the future.

System testing capacity: The FluoroCyclerXT can run up to 96 patient samples at once.

Time to detection: Test results for MTBC detection and resistance to rifampicin and isoniazid are provided within 2.5 hours. Three batches, equaling up to 288 samples, can be tested within an 8-hour working shift.

Specimen types: Sputum specimens or cultures

Multiplexing possibilities: The full menu of FluoroType assays is available here.

Pricing: Global pricing is not yet available; contact the manufacturer for prices. Discussions are underway with Stop TB Partnership's Global Drug Facility (GDF) for inclusion in the GDF Catalog.

Manufacturer links: FluoroType MTBDR VER 2.0 assay, GenoXtract 96, FluroCyclerXT

cobas® MTB and cobas® MTB-RIF/INH assays, using the cobas 6800/8800 Systems

The **cobas®** 6800 System and **cobas®** 8800 System are high volume molecular diagnostic systems that automate DNA extraction, PCR amplification and detection steps. The systems are designed to seamlessly integrate testing across disciplines and are compatible with the **cobas® prime** Pre-analytical System. The resulting Molecular Work Area brings efficiencies to multiple programmes simultaneously.

System testing capability: The cobas 6800 System can run up to 864 results from an eight-hour shift, and up to 1,440 results in 24-hours. The cobas 8800 System can run up to 1,824 results from an 8 hour shift. Workflow demands and assay mix influence the capacity.

Time to detection: The cobas 6800/8800 Systems can generate 96 results in about 3 hours with subsequent results released approximately every 90 minutes thereafter.

cobas 6800 System

Specimen types: The tests are intended for use on either acid-fast bacilli (AFB) smear-positive or smear-negative, raw sputum, and digested and decontaminated (N-acetyl-L-cysteine/ NaOH treated) sputum and bronchoalveolar lavage (BAL) samples.

Multiplexing possibilities: The systems are compatible with more than 30 molecular assays, including HIV-1, HBV, HCV, HPV, MTB and MTB-RIF/INH which are included in Roche's Global Access Program. The SARS-CoV-2 and SARS-CoV-2 + Influenza A/B respiratory assays are available as standard assays that can be multiplexed. Additional flexibility is achieved utilizing the **cobas omni** Utility Channel for both third-party tests and laboratory developed tests (LDT's).

Pricing: Access pricing is available through Roche's Global Access Program for specific disease areas including TB. Discussions are underway with Stop TB Partnership's Global Drug Facility (GDF) for inclusion in the GDF Catalog.

Manufacturer links: cobas MTB test, cobas MTB-RIF/INH test, cobas 6800 System, cobas 8800 System, cobas 6800/8800 System menu, Molecular Work Area, Global Access Program

Implementations considerations from the 2019 WHO Technical Expert Group Consultation that reviewed the moderate-complexity automated NAATs for detection of TB and resistance to RIF and INH

"Implementation considerations for the centralized assay platforms should be based on where countries would place the tests in the diagnostic algorithm for TB and other diseases, as well as in-country laboratory capacity. For example, countries may consider placement of a centralized assay platform at a national reference laboratory only, which may be used for single or multiple disease testing on one platform. Alternatively, countries may have adequate infrastructure available and sufficient sample volume to consider deployment at regional referral laboratories. Consideration of the overall testing volume, for TB and other diseases for which tests are run on the platform, should be made, and the efficiencies of different run sizes determined. To ensure rapid turnaround time of samples referred to testing sites, countries should ensure that an efficient and reliable sample transportation system is available. To bring cost efficiency to testing services, consideration of integration of TB testing on existing platforms should be prioritized in locations where integrated testing is feasible. In other settings where TB diagnostic services are stand-alone and there is a high workload for TB testing, dedicated instruments may be preferred."

See also WHO Considerations for adoption and use of multidisease testing devices in integrated laboratory networks

WHO statements on the importance of testing for isoniazid resistance

From the 2018 WHO treatment guidelines for isoniazid-resistant tuberculosis:

"The overall aim of TB treatment is to achieve cure without relapse in all patients, interrupting *M. tuberculosis* transmission and preventing the acquisition (or amplification) of additional drug resistance. Globally, Hr-TB (rifampicin-susceptible, isoniazid-resistant TB) is more prevalent than MDR-TB. Efforts need to be made by all countries to move towards universal testing of both isoniazid and rifampicin at the start of TB treatment and to ensure the careful selection of patients eligible for the (H)RZE-Lfx regimen."

From the Frequently asked questions on the 2018 WHO treatment guidelines for isoniazid-resistant tuberculosis:

"How can the national TB programme expand its capacity to detect Hr-TB?

There is as yet no diagnostic platform approved for the detection of Hr-TB which matches the rapidity and convenience of Xpert MTB/RIF for rifampicin resistance. First line LPA can diagnose isoniazid resistance, complete with genotyping detail of clinical relevance, but requires substantial infrastructure typically available in a provincial or central level facility. The cost of the equipment to perform the test ranges from about USD8,000 to USD40,000, depending on local needs and if results are read automatically. Dedicated rooms in the laboratory would also be necessary. In countries eligible for preferential concessional pricing (138 eligible countries as of March 2018), the cost of a single LPA test strip is USD9.30. However, considering additional laboratory reagents and consumables required to perform the test, the total cost of performing an LPA test is approximately USD20-25.

Liquid culture (or MGIT) could also detect Hr-TB at the level of a reference laboratory; this option has the disadvantage of an obligatory processing delay of at least 10 days. Testing on solid media is also an option but may take several months to obtain results and is therefore of limited use for baseline testing and monitoring non-response. Nonetheless, a phenotypic or molecular test result confirming Hr-TB is of equal value for clinical purposes once the method used is reliable and quality assured. Increased capacity to undertake phenotypic testing on liquid and solid media also requires substantial investment in infrastructure."