WHO operational handbook on tuberculosis

Module 3: Diagnosis

Rapid diagnostics for tuberculosis detection

Third edition

Web Annex A. Information sheets

World Health Organization

WHO operational handbook on tuberculosis

Module 3: Diagnosis

Rapid diagnostics for tuberculosis detection

Third edition

Web Annex A. Information sheets

World Health Organization

WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition. Web Annex A. Information sheets

ISBN 978-92-4-009048-4 (electronic version)

Suggested citation. Web Annex A. Information sheets. In: WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; 2024. Licence: CC BY-NC-SA 3.0 IGO.

Cataloguing-in-Publication (CIP) data. CIP data are available at https://iris.who.int/.

Sales, rights and licensing. To purchase WHO publications, see https://www.who.int/publications/book-orders. To submit requests for commercial use and queries on rights and licensing, see https://www.who.int/copyright.

This publication forms part of the document entitled *WHO operational handbook on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition*. It is being made publicly available for transparency purposes and information.

Design by Inis Communication

Contents

WA1 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 WA1.1 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. **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. **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:

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

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 Amplification 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 RealTi*m*e MTB amplification reagent (90 days from the date of manufacture / 60 days from date of shipment), Abbott RealTi*m*e 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 Realti*m*e 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 Realti*m*e 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 RealTi*m*e 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, RealTi*m*e MTB assay DNA eluant may be used for reflex testing with the RealTi*m*e MTB RIF/INH assay on the m2000 system, to save sample processing and inactivation time. The RealTi*m*e 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 RealTi*me* 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.

  It may be necessary to emphasize training for Realti*me* 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 WA1

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.org/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).

WA2 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. WA2.1 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 WA2

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).

WA3 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 5800/6800/8800 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 5800/6800/8800 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 5800, 6800 or 8800 system (Instrument Gateway and software supplied)

Not supplied but required:

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

- cobas MTB Positive Control Kit
- cobas MTB-RIF/INH Positive Control Kit
- cobas 5800/6800/8800 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 smaller cobas 5800 can produce results for up to 144 samples in a six hour shift. 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 5800/6800/8800 system dimensions and weight**: The cobas 5800 has a footprint of 134 x 79 cm, 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 5800/6800/8800 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 5800/6800/8800 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 5800/6800/8800 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 5800/6800/8800 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 5800/6800/8800 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 WA3

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://elabdoc-prod.roche.com/eLD/api/downloads/0fb803ec-cf25-e711-00ab-00215a9b3428?countryIsoCode=be).

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).

WA4 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 is 15 cfu/mL for the FluoroType MTB test and 20 cfu/mL for the FluoroType MTBDR assay.
- 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 WA4.1, and the equipment, supplies and reagents required are given in Table WA4.2.

Table WA4.1 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 WA4.2 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 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) 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 WA4

1 Unitaid. Tuberculosis – diagnostics technology landscape, 5th edition. Geneva: World Health Organization; 2017 (https://unitaid.org/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).

WA5 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 WA5.1.

Table WA5.1 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 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.

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

Table WA5.2 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 WA5

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).

WA6 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

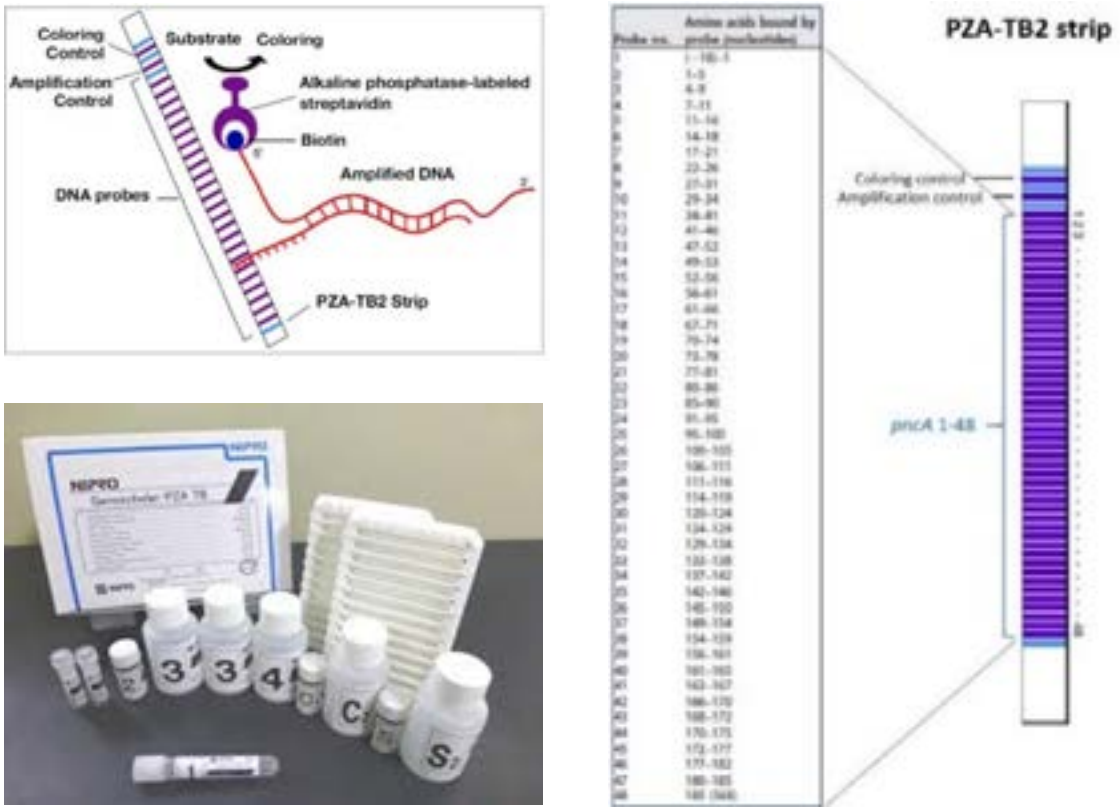
In people with bacteriologically confirmed TB, high complexity reverse hybridization-based nucleic acid amplification tests (NAATs) may be used on culture isolates for detection of PZA resistance (rather than culture-based phenotypic drug susceptibility testing [DST]).
(Conditional recommendation; very 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. WA6.1. Nipro Genoscholar PZA-TB II test procedure and interpretation at-a-glance



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

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 WA6.1 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 WA6

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).

WA7 Information sheet: GenoScreen Deeplex Myc-TB test

Short description

GenoScreen has a kit based on next-generation sequencing (NGS) for the simultaneous identification of mycobacterial species, genotyping and prediction of drug resistance of *Mycobacterium tuberculosis* complex (MTBC) strains; the kit (Deeplex® Myc-TB) can be used directly on clinical samples *(1)*. The assay relies on deep sequencing of a single 24-plex amplicon mix, and it targets 18 main MTBC gene regions associated with resistance to first-line and second-line anti-TB drugs (rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, amikacin, kanamycin, capreomycin, streptomycin, ethionamide, bedaquiline, clofazimine and linezolid). The *hsp65* gene is the target for mycobacterial species identification, whereas the spoligotyping target (CRISPR/direct repeat [DR] locus) and phylogenetic single nucleotide polymorphisms (SNPs) in drug-resistance-associated targets are used for MTBC strain genotyping.

The assay is performed using a single ready-to-use polymerase chain reaction (PCR) amplification mix included in the Deeplex Myc-TB kit, followed by sequencing on the Nextera® XT or DNA Flex library preparation kits on the iSeq 100, MiniSeq, MiSeq or NextSeq sequencing platforms (Illumina®). The assay includes an automated analysis pipeline of the sequencing data in a secure web application, with integrated databases for results interpretation and different reporting formats.

WHO recommendations for use

Assessment details

The Deeplex Myc-TB was assessed for diagnosis of drug resistance to the following drugs: rifampicin, isoniazid, pyrazinamide, ethambutol, levofloxacin, moxifloxacin, amikacin, streptomycin. bedaquiline, clofazimine and linezolid.

Recommendations

In people with bacteriologically confirmed **pulmonary TB disease**, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol rather than culture-based phenotypic drug susceptibility testing.
(Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide] or low [rifampicin, fluoroquinolones and ethambutol])

- Priority should be assigned to those at higher risk of resistance to first-line treatment medications, including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or experience treatment failure;
  - have previously had TB treatment;
  - are in contact with a person known to have resistance to TB drugs; or
  - reside in settings or belong to subgroups where there is a high probability of resistance to either rifampicin, isoniazid or fluoroquinolones (used in new shorter regimens), or where there is a high prevalence of *M. tuberculosis* strains harbouring mutations not detected by other rapid molecular tests.

In people with bacteriologically confirmed **rifampicin-resistant pulmonary TB disease**, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin rather than culture-based phenotypic drug susceptibility testing.
(Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate [ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin] or very low [amikacin])

- Priority should be given to those at a higher risk of resistance to medications used for the treatment of rifampicin-resistant TB (RR-TB), including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or have experienced treatment failure;
  - have previously had TB treatment, including with the new and repurposed drugs;
  - are in contact with a person known to have resistance to TB drugs, including the new and repurposed drugs; or
  - have pre-extensively drug-resistant TB (pre-XDR-TB) with resistance to fluoroquinolones.

The Deeplex Myc-TB product met the class-based performance criteria for rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, bedaquiline, linezolid, clofazimine, amikacin and streptomycin.

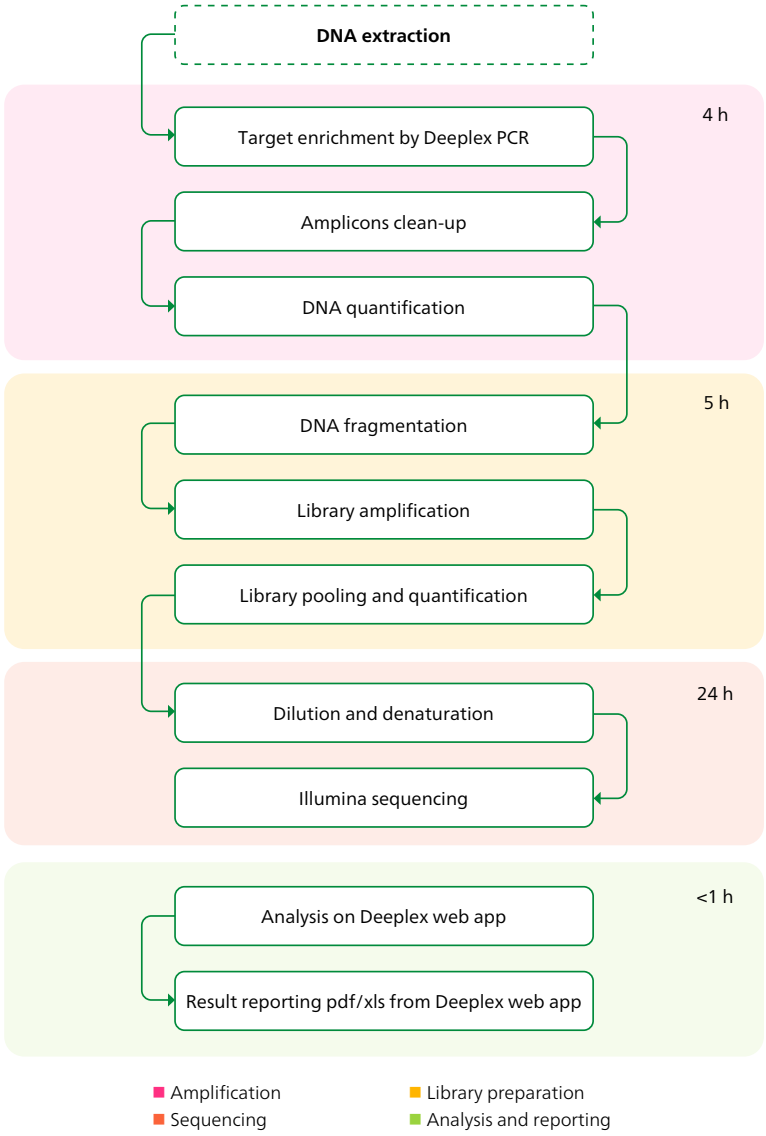
Key performance conclusions

- Pooled sensitivity and specificity data for the class as presented in the *WHO consolidated guidelines on tuberculosis, third edition (2)*.
- Detection of heteroresistance down to 3% subpopulations (reported by the company).
- Detection of DNA loads down to 100 genomes *(1)*.
- More than 100 non-tuberculous mycobacterial species identified, with 93.5% concordance with *rpoB* and 16S rDNA reference sequencing data, as evaluated on 292 isolates from 69 different species or species complexes *(1)*.

Test procedure at a glance

The Deeplex Myc-TB 48-test kit includes a master mix ready for multiplexed amplification (Table WA7.1), a positive (bacille Calmette-Guérin [BCG]) and internal DNA control (non-mycobacterial) and an activation code to access the Deeplex web app. The assay is applied on genomic DNA extracted from inactivated clinical samples (e.g. sputum) or positive mycobacterial culture (Fig. WA7.1). After single multiplex PCR and purification of amplicons, DNA libraries are prepared and sequenced on Illumina platforms (Table WA7.2). The sequencing data are then uploaded to the web app for automated analysis and interpretation. Results from extracted DNA are obtained in less than 48 hours.

Fig. WA7.1. Summary of Deeplex Myc-TB workflow



DNA: deoxyribonucleic acid; PCR: polymerase chain reaction.

Table WA7.1. Deeplex Myc-TB mycobacterial targets

Gene region	Target	Gene region	Target
hsp65	Species ID	*gyrA, gyrB*	Fluoroquinolones
CRISPR/DR	Spoligotyping	*rrs*	Amikacin
PhyloSNPs	Genotyping	*eis, rrs*	Kanamycin
		tlyA,[a] *rrs*	Capreomycin
rpoB	Rifampicin	*gidB,*[a] *rrs, rpsL*[a]	Streptomycin
ahpC, fabg1, katG, inhA	Isoniazid	*ethA,*[a] *inhA, fabG1*	Ethionamide
pncA[a]	Pyrazinamide	*rv0678*[a]	Bedaquiline, clofazimine
embB	Ethambutol	*rrl, rplC*	Linezolid

[a] Full genes.

Table WA7.2. Deeplex Myc-TB specifications for the Illumina platforms

Platform	Kit	Run time (2x150 bp)	Number of samples
iSeq 100	i1 Reagent 1.2 Gb	19 hours	13 + 3 controls
MiniSeq	Mid output (2.1 Gb) High output (6.6 Gb)	17 hours 24 hours	21 + 3 controls 66/69 + 3 controls
MiSeq	Nano kit (300 Mb) Micro kit (1.2 Gb) Full kit (4.5 Gb)	17 hours 19 hours 24 hours	1 + 3 controls 13 + 3 controls 45 + 3 controls
NextSeq	Mid output (32.5 Gb) High output (100 Gb)	26 hours 29 hours	372 + 3 controls Not applicable[a]

[a] Sequence output per sample is expected to exceed maximal limits, using available sets of 384 indices maximum.

Gb: gigabases; Mb: megabases.

Equipment, supplies and reagents required

Table WA7.3. Equipment, supplies and reagents required for Deeplex Myc-TB

Supplied	Not supplied but required
Reagents	
Deeplex Myc-TB amplification master mix	Ultra-pure PCR-grade water
Deeplex Myc-TB external positive control (BCG)	Beckman Coulter Agencourt AMPure XP® or Macherey-Nagel NucleoMag® NGS clean-up and size select magnetic beads
Deeplex Myc-TB internal amplification control (non-mycobacterial DNA)	1.0 N or 10 N NaOH, molecular grade
	NaClO
	Illumina index kits
	Tween20, molecular grade
	Tris-HCl 10 mM, pH 8.5, molecular grade
	Illumina PhiX control
	Illumina sequencing kit
	Illumina library preparation kit (Nextera XT DNA or DNA Flex)
	Fluorometer assay reagents
	Ethanol 100%, molecular grade
	PCR-grade 1M Tris-HCl, pH 7.8, molecular grade
Consumables	
	Personal protective equipment
	0.2 ml 96-well PCR plates for PCR amplification or PCR microtubes or strips
	Adhesive PCR plate films
	0.5 and 1.5 mL low binding microtubes
	Filter tips PCR clean
Equipment	
	Single channel and multi-channel pipettes (p10, p100, p200 and single channel p1000)
	Centrifuges for 1.5 mL microtubes

Supplied	Not supplied but required
Equipment	
	Vortex mixers
	Heat block for 1.5 mL microtubes
	Illumina sequencer (iSeq 100, MiniSeq, MiSeq, NextSeq)
	PCR amplification systems, or 96-well PCR amplification systems if 96-well plates are used
	Invitrogen DynaMag™-2 Magnet, or DynaMag-96 side skirted magnet if 96-well plates are used
	Fluorometer
	Computer
	Internet connection
Software	
Deeplex web application activation code	

BCG: bacille Calmette-Guérin; DNA: deoxyribonucleic acid; N: normal; NaClO: sodium hypochlorite; NaOH: sodium hydroxide; PCR: polymerase chain reaction.

Operational considerations

Sample types: DNA extracted from *N*-acetyl-l-cysteine–sodium hydroxide (NALC)-decontaminated, heat- or ethanol-inactivated clinical samples, and from heat-inactivated cultures.

Storage and handling: Deeplex Myc-TB test kit components should be stored at –20 °C. If properly handled, the kit can be stored for up to 1 year. It is recommended to aliquot the components and to avoid repeated freeze–thaw cycles. DNA extraction, library preparation,

quantification and sequencing components should be stored as per the manufacturer's instructions; they typically require storage at –25 °C to –15 °C, 2 °C to 8 °C, or 15 °C to 30 °C.

Testing capacity: There are 48 tests per kit. The maximum number of tests and run times for each Illumina platform are given in Table WA7.2.

Time to detection: Deeplex Myc-TB PCR takes about 4 hours for master mix preparation, PCR amplification, clean-up and quantification of PCR products. The turnaround time – including multiplex PCR, library preparation, sequencing and analysis – is about 48 hours, and it depends on the multiplexing and sequencing platform.

Result reporting: Results are automatically generated via the Deeplex web app in less than 1 hour. Once the FASTQ files have been uploaded and analysed, integrated reference databases, including the WHO catalogue *(3)*, are interrogated to identify mutations associated with mycobacterial species, MTBC lineages and sublineages and spoligotypes, resistance and susceptibility to anti-TB drugs. Mutations that are not in the databases are classified as uncharacterized. Spoligotypes are identified based on the profile of spacers at the MTBC CRISPR/DR locus. The Deeplex web app generates automatically detailed reports in different formats (e.g. PDF and Microsoft Excel®), including one summary format using plain language.

The kit and workflow and the means through which the product is executed are under active development and update.

Shelf life: The shelf life is 12 months.

Implementation considerations

Area 1 – Policies, budgeting and planning (Section 3.5.1)

The assay may be placed in centralized reference settings. It will not replace the WHO-recommended rapid diagnostic tests (WRDs) as the initial test for diagnosis of TB, but could be used for prioritized patient populations requiring comprehensive drug susceptibility testing (DST) (including group A agents for the treatment of RR-TB and multidrug-resistant TB), faster than phenotypic DST.

The Illumina systems on which Deeplex Myc-TB tests are run are capable of multidisease testing, which may be considered because of the potential cost savings across programmes for communicable and noncommunicable diseases.

The WHO implementation manual– The use of next-generation sequencing for the surveillance of drug-resistant tuberculosis: an implementation manual (*4*) – provides practical guidance for national TB programmes and laboratories to plan and implement NGS-based approaches for the characterization of MTBC bacteria to detect mutations associated with drug resistance.

Area 2 – Regulatory issues (Section 3.5.2)

The kit is CE marked for in vitro diagnostic use (CE-IVD).

Area 3 – Equipment (Section 3.5.3)

Illumina instruments have high infrastructure and working requirements, and should be placed at laboratories that can accommodate molecular workflow (e.g. with separate and dedicated preparation, amplification and sequencing spaces). Resource capacities (e.g. electrical supply and network connection), and service and maintenance agreements to ensure optimal system functionality should be considered. 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 4 – Supply chain (Section 3.5.4)

Procurement and delivery of any third-party equipment, consumables and reagents not supplied but required for the workflow should be ensured.

Illumina and GenoScreen established a partnership to enable global access to a package combining Illumina sequencing products and the GenoScreen Deeplex Myc-TB assay.

Area 5 – Procedures (Section 3.5.5)

Given the complexity of the targeted NGS workflow, a comprehensive set of standard operating procedures (SOPs) must be developed, covering sample collection, storage and referral; sample processing and DNA extraction; DNA library preparation and sequencing; and NGS data analysis and interpretation. A panel of local or international experts that includes laboratory and clinical staff should cooperate in developing a standard, targeted NGS reporting system that will support clinical decisions.

The product performance depends on the efficiency of DNA isolation and purification methods used.

Resistance is reported when a documented resistance-conferring mutation is detected in targets of interest. Where mutations are not detected, this suggests strain sensitivity but does not exclude the possibility of resistance. Low-frequency variants below the limit of detection may affect the quality of results and their interpretation. The interpretation provided is based on the current understanding of genotype–phenotype relationships.

Area 6 – Digital data (Section 3.5.6)

The assay includes an easy-to-use web application for uploading and analysing raw sequencing data rapidly interpretating the results. The app is hosted on a secure cloud digital platform and is accessed via a code provided with the kit. Opportunities for integration of e-systems may be explored.

Area 7 – Quality assurance, control and assessment (Section 3.5.7)

Quality assurance (QA) systems and activities for the Deeplex Myc-TB assay mimic those of the moderate complexity automated nucleic acid amplification tests (NAATs). The assay results should be monitored carefully based on expected outcomes to promptly detect false positive and false negative trends, and laboratories should regularly participate in external QA programmes. Potential contamination of the molecular workflow means that each run on the

Illumina instrument must include positive and negative controls to ensure run validity, and laboratory spaces should be tested for contamination at least monthly. Control interpretation guidance is included in the manufacturer's instructions for use and should be included in user training and competency assessments.

New method validation for the Deeplex Myc-TB test should include well-characterized MTBC positive and negative samples, and precision and accuracy measurements for drugs targeting all drug-resistance loci. Samples should be well-characterized strains with and without known resistance-associated mutations.

Area 8 – Recording and reporting (Section 3.5.8)

The Deeplex Myc-TB web app generates automatic reports that include sample information, the date, analysis mode, quality summary, experiment set, control results and all mutation details as derived by the software. The report can be exported in different formats, and users should follow national requirements for results reporting. Revision of laboratory registers and reporting forms may be needed.

Source: Reproduced with permission of GenoScreen, © 2024. All rights reserved.

Area 9 – Human resource training and competency assessment (Section 3.5.9)

Laboratory, clinical and programme staff should be extensively trained on testing principles, methods and the appropriate review and interpretation of results, for at least 1 week. Laboratory technicians should be fully trained on all steps and should be able to troubleshoot where necessary. Competency assessments should be performed after training and periodically thereafter.

The manufacturer offers training programmes for users.

References for WA7

1 Jouet A, Gaudin C, Badalato N, Allix-Beguec C, Duthoy S, Ferre A et al. Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs. Eur Respir J. 2021;57. doi: https://doi.org/10.1183/13993003.02338-2020.

2 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; 2024 (https://iris.who.int/handle/10665/376221).

3 Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, second edition. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/9789240082410).

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

WA8 Information sheet: AmPORE TB Oxford Nanopore Diagnostics test

Short description

Oxford Nanopore Diagnostics (OND) AmPORE TB® is a test based on targeted next-generation sequencing (NGS). It can simultaneously identify mycobacterial species and detect *Mycobacterium tuberculosis* complex (MTBC) genetic variants associated with antimicrobial resistance in DNA extracted from sputum samples. The assay relies on sequencing of a single 27-plex amplicon mix: 24 drug-resistance targets, a genotyping target, a non-tuberculous mycobacteria (NTM) identification target (*hsp65*) and an internal control.

The 24 drug-resistance targets are MTBC genic regions that are associated with resistance to first-line and second-line anti-tuberculosis (anti-TB) drugs (rifampicin, isoniazid, pyrazinamide, ethambutol, moxifloxacin, levofloxacin, amikacin, kanamycin, capreomycin, streptomycin, ethionamide, bedaquiline, clofazimine, linezolid, delamanid and pretomanid). The *hsp65* gene is the target for mycobacterial species identification, whereas the spoligotyping target (CRISPR/direct repeat [DR] locus) is used for MTBC strain genotyping.

The assay is performed using the Rapid Barcoding Kit 96 (SQK-RBK110.96), TB Drug Resistance Test Kit (OND-CUST-KIT) and Flow Cells (FLO-MIN106D) on MinION.

The sequencing control software and AmPORE TB workflow analysis pipeline on the host computer process reads for results interpretation and different reporting formats.

WHO recommendations for use

Assessment details

AmPORE TB was assessed for diagnosis of drug resistance to the following drugs: rifampicin, isoniazid, pyrazinamide, ethambutol, moxifloxacin, levofloxacin, amikacin, streptomycin, bedaquiline, linezolid and clofazimine).

Recommendations

In people with bacteriologically confirmed **pulmonary TB disease**, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol rather than culture-based phenotypic drug susceptibility testing.

(Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide] or low [rifampicin, fluoroquinolones and ethambutol])

- Priority should be assigned to those at higher risk of resistance to first-line treatment medications, including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or experience treatment failure;
  - have previously had TB treatment;
  - are in contact with a person known to have resistance to TB drugs; or
  - reside in settings or belong to subgroups where there is a high probability of resistance to either rifampicin, isoniazid or fluoroquinolones (used in new shorter regimens), or where there is a high prevalence of *M. tuberculosis* strains harbouring mutations not detected by other rapid molecular tests.

In people with bacteriologically confirmed **rifampicin-resistant pulmonary TB disease**, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin rather than culture-based phenotypic drug susceptibility testing.
(Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate [ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin] or very low [amikacin])

- Priority should be given to those at a higher risk of resistance to medications used for the treatment of rifampicin-resistant TB (RR-TB), including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or have experienced treatment failure;
  - have previously had TB treatment, including with the new and repurposed drugs;
  - are in contact with a person known to have resistance to TB drugs, including the new and repurposed drugs; or
  - have pre-extensively drug-resistant TB (pre-XDR-TB) with resistance to fluoroquinolones.

The AmPORE TB product met the class-based performance criteria for rifampicin, isoniazid, fluoroquinolones, linezolid, amikacin and streptomycin.

Key performance conclusions

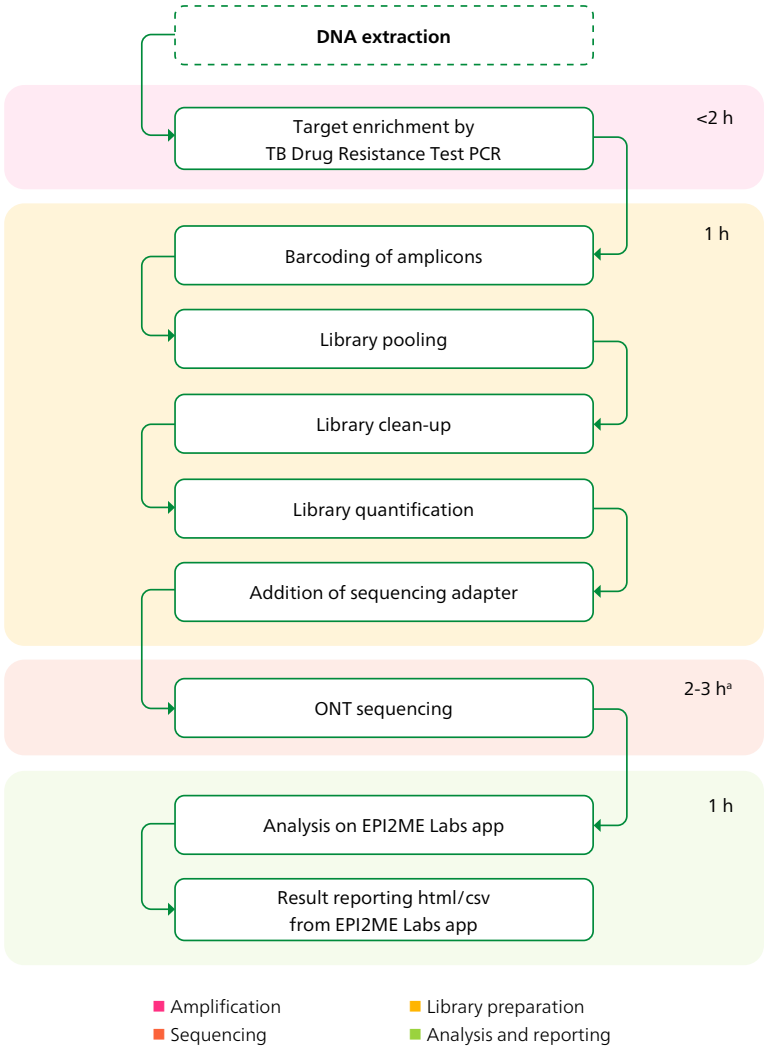
- Pooled sensitivity and specificity data for the class as presented in the *WHO consolidated guidelines on tuberculosis, third edition (1)*.
- Fast turnaround time for 22 sample runs.
- Detection of heteroresistance down to 10% subpopulations (reported by the company).

Test procedure at a glance

The AmPORE TB kit includes reagents for creating a polymerase chain reaction (PCR) master mix for multiplexed amplification of targets (Table WA8.1) of 22 individual samples, a positive

control and a no template control. The assay is applied on genomic DNA extracted from decontaminated sputum samples (Fig. WA8.1). After single multiplex PCR, DNA libraries are prepared and sequenced on MinION (Table WA8.2). Sequencing data are automatically analysed on the device. Results are obtained in less than 5 hours using extracted DNA.

Fig. WA8.1. Summary of AmPORE TB Drug Resistance Test workflow



DNA: deoxyribonucleic acid; ONT: Oxford Nanopore Technologies; PCR: polymerase chain reaction; TB: tuberculosis.

[a] Recommended minimum required threshold of 20× median reads coverage. Longer runs provide higher coverage.

Table WA8.1. AmPORE TB Drug Resistance Test mycobacterial targets

Gene region	Target	Gene region	Target
hsp65	NTM identification	*eis, rrs*	Kanamycin
CRISPR/DR	Genotyping	*tlyA, rrs*	Capreomycin
rpoB	Rifampicin	*gidB, rrs, rpsL*	Streptomycin
fabG1, katG, inhA	Isoniazid	*ethA, inhA, fabG1*	Ethionamide
pncA	Pyrazinamide	*rv0678*	Bedaquiline, clofazimine
embA, embB	Ethambutol	*atpE*	Bedaquiline
gyrA, gyrB	Fluoroquinolones	*rrl, rplC*	Linezolid
rrs, eis	Amikacin	*ddn, fgd1, fbiA, fbiB, fbiC*	Delamanid

DR: direct repeat; NTM: non-tuberculous mycobacteria.

Table WA8.2. AmPORE TB Drug Resistance Test specifications

Platform	Kit	Run time	Number of samples
MinION	OND Tuberculosis Drug Resistance Test Kit and OND Flow Cell	2 hours	22 + 2 controls per flow cell[a]

OND: Oxford Nanopore Diagnostics.

[a] Flow cells can be washed and reused according to manufacturer's instructions.

Equipment, supplies and reagents required

Table WA8.3. Equipment, supplies and reagents required for AmPORE TB

Supplied	Not supplied but required
Reagents	
Rapid Barcoding Kit 96 (SQK-RBK110.96) and AmPORE TB Test Kit (OND-CUST-KIT) **Kits contents:** Rapid barcode plate AMPure XP beads Sequencing buffer II Rapid adapter F Elution buffer Loading beads II Loading solution Flush tether Flush buffer Primers Internal control Positive control	Ultra-pure PCR-grade water Platinum II Taq HS DNA polymerase 5X platinum II PCR buffer Platinum GC enhancer dNTP mix (10 mM)
	Ethanol 100%, molecular grade
	Fluorometer assay reagents[a]
Consumables	
FLO-MIN106D	Personal protective equipment
	DNA sample-to-surface binding reducing tubes (1.5 mL tubes)
	96-well PCR plate, semi-skirted, straight edges
	Adhesive 96-well PCR plate films
	Fluorometer assay tubes[a]
	Filter tips PCR clean
Equipment	
	Centrifuge for 1.5 tubes
	Centrifuge for 96-well plate
	Thermal cycler suitable for 96-well PCR plates
	Invitrogen DynaMag-2 Magnet
	Fluorometer[a]

Supplied	Not supplied but required
Equipment	
	Single channel and multi-channel pipettes (p10, p100, p200 and single channel p1000)
	Vortex mixer
	Sample mixer (optional)
Software Software provided as standard on MinION	

DNA: deoxyribonucleic acid; PCR: polymerase chain reaction.

[a] Optional.

Operational considerations

Sample types: DNA extracted from decontaminated sputum specimens.

Storage and handling: AmPORE TB kit components should be stored at –20 °C to –30 °C. DNA extraction, library preparation, quantification and sequencing components should be stored as per the manufacturer's instructions: they typically require storage at –25 °C to –15 °C, 2 °C to 8 °C, or 15 °C to 30 °C.

Testing capacity: The kit provides reagents for up to 96 tests (96 barcodes to be supplied). There are 22 test samples per run and two additional controls.

Time to detection: AmPORE TB takes about 2 hours for master mix preparation and PCR amplification. The turnaround time – including multiplex PCR, library preparation, sequencing and analysis – is about 5 hours.

Result reporting: Results are generated on the host computer in less than 1 hour after sequencing completion. Raw data are analyzed to provide assay validity, MTBC lineages and resistance to anti-TB drugs, based on the mutations associated with resistance included in the WHO mutation catalogue *(2)*. Mutations that remain unclassified are not reported. The

test detects single nucleotide polymorphisms (SNPs), multinucleotide polymorphisms, small insertions and deletions, and whole gene deletions. Spoligotypes are identified based on the profile of spacers at the MTBC CRISPR/DR locus. AmPORE TB generates reports in various formats.

The kit, the workflow and the means through which the workflow is executed are under active development and update.

Shelf life: No information on shelf life is available.

Implementation considerations

Area 1 – Policies, budgeting and planning (Section 3.5.1)

The assay may be placed in centralized reference settings. It will not replace the WHO-recommended rapid diagnostic tests (WRDs) as the initial test for diagnosis of TB, but could be used for prioritized patient populations requiring comprehensive drug susceptibility testing (DST) (including group A agents for the treatment of RR-TB and multidrug-resistant TB), faster than phenotypic DST.

The OND devices on which the TB Drug Resistance Test is run are capable of multidisease testing, which may be considered because of the potential cost savings across programmes for communicable and noncommunicable diseases.

The WHO implementation manual provides practical guidance for national TB programmes and laboratories to plan and implement NGS-based approaches for the characterization of MTBC bacteria to detect mutations associated with drug resistance *(3)*.

Area 2 – Regulatory issues (Section 3.5.2)

The test is currently for research use only.

Area 3 – Equipment (Section 3.5.3)

OND instruments have moderate to high infrastructure requirements; hence, they should be placed in laboratories that can accommodate molecular workflow (e.g. with separate and dedicated preparation, amplification and sequencing spaces). Service and maintenance agreements to ensure optimal system functionality should be considered. 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 4 – Supply chain (Section 3.5.4)

Procurement and delivery of any third-party equipment, consumables and reagents not supplied but required for the workflow should be ensured.

Oxford Nanopore Technologies plc entered a strategic partnership with bioMérieux SA in April 2023. One pillar of this partnership is for bioMérieux to distribute and support the TB Drug Resistance Test.

Area 5 – Procedures (Section 3.5.5)

Given the complexity of the targeted NGS workflow, a comprehensive set of standard operating procedures (SOPs) must be developed, covering sample collection, storage and referral; sample processing and DNA extraction; DNA library preparation and sequencing; and interpretation of results. A panel of local or international experts that includes laboratory and clinical staff should cooperate in developing a standard targeted NGS reporting system that will support clinical decisions.

The product performance depends on the efficiency of the DNA isolation and purification methods used.

Resistance is reported when a documented resistance-conferring mutation is detected in targets of interest. Where mutations are not detected, this suggests strain sensitivity but does not exclude the possibility of resistance. Low-frequency variants below the limit of detection may affect the quality of results and their interpretation. The interpretation provided is based on the current understanding of genotype–phenotype relationships.

Area 6 – Digital data (Section 3.5.6)

The TB Drug Resistance Test produces data in FASTQ format, and those data are analysed at the end of the sequencing run by the provided workflow. A user account is required for access, authentication and authorization. The analysis produces easy-to-read reports and summaries. Other outputs of the analysis are available for troubleshooting or storage to meet local regulatory requirements. No internet connection is required after the installation of bioinformatics tools and resources. No data are uploaded to a cloud platform. Opportunities for integration of e-systems may be explored.

Area 7 – Quality assurance, control and assessment (Section 3.5.7)

Quality assurance (QA) systems and activities for the TB Drug Resistance Test mimic those of the moderate complexity automated nucleic acid amplification tests (NAATs). The assay results should be correlated with other available clinical information, and laboratories should regularly participate in external QA programmes. Because of potential contamination of the molecular workflow, each run on the OND instrument must include supplied internal controls, positive controls and no-template controls, to ensure sample and run validity; also, laboratory spaces should be tested for contamination monthly. Control interpretation guidance is included in the manufacturer's instructions for use and such guidance should be included in user training and competency assessments. This also applies for the quality control steps defined for recalibrating and reusing the flow cells.

A new method validation for the TB Drug Resistance Test should include well-characterized MTBC positive and negative samples, and precision and accuracy measurements for drugs targeting all drug-resistance loci. Samples should be well-characterized strains with and without known resistance-associated mutations.

Area 8 – Recording and reporting (Section 3.5.8)

The TB Drug Resistance Test generates automatic reports that include sample information, the date, analysis mode, quality summary, experiment set and all mutation details as derived by the software. The report can be exported in different formats, and users should follow national requirements for results reporting. Revision of laboratory registers and reporting forms may be needed.

Area 9 – Human resource training and competency assessment (Section 3.5.9)

Laboratory, clinical and programme staff should be extensively trained in testing principles, methods and the appropriate review and interpretation of results, for as long as is necessary to gain competence. Laboratory technicians should be fully trained on all steps and should be able to troubleshoot where necessary. Competency assessments should be performed after training and periodically thereafter.

References for WA8

1 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; 2024 (https://iris.who.int/handle/10665/376221).

2 Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, second edition. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/9789240082410).

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

WA9 Information sheet: Hangzhou ShengTing Medical Technology Co. TBSeq test

Short description

Hangzhou ShengTing Medical Technology Co. has a kit based on targeted next-generation sequencing (NGS) for the simultaneous identification of mycobacterial species and the prediction of drug resistance of *Mycobacterium tuberculosis* complex (MTBC) strains. The kit, TBseq®, is directly applicable to clinical specimens such as sputum, bronchoalveolar lavage fluid, pleural effusion or mycobacteria-positive culture. It relies on deep sequencing of a primer multiplex amplification mix, and targets 21 main MTBC genes associated with resistance to first-line and second-line anti-tuberculosis (anti-TB) drugs (rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, amikacin, kanamycin, capreomycin, streptomycin, para-aminosalicylic acid, cycloserine, ethionamide/prothionamide, bedaquiline, clofazimine and linezolid). Mycobacterial species identification is performed by targeting the *16S* and *hsp65* gene regions.

The assay is performed using the Universal Gene Sequencing Kit (ShengTing) to generate libraries that are sequenced on either a MinION or a GridION platform (Oxford Nanopore Technologies [ONT]). It includes automated analysis software (Nano TNGS) for sequencing data processing and a secure web application (TBseq web app) with integrated databases for result interpretation.

WHO recommendations for use

Assessment details

TBseq was assessed for diagnosis of drug resistance to the following drugs: rifampicin, isoniazid, pyrazinamide, ethambutol, moxifloxacin, levofloxacin amikacin, streptomycin bedaquiline, clofazimine and linezolid.

Recommendations

In people with bacteriologically confirmed **pulmonary TB disease**, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to rifampicin, isoniazid, fluoroquinolones, pyrazinamide and ethambutol rather than culture-based phenotypic drug susceptibility testing.

(Conditional recommendation, certainty of evidence moderate [isoniazid and pyrazinamide] or low [rifampicin, fluoroquinolones and ethambutol])

- Priority should be assigned to those at higher risk of resistance to first-line treatment medications, including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or who experience treatment failure;
  - have previously had TB treatment;
  - are in contact with a person known to have resistance to TB drugs; or
  - reside in settings or belong to subgroups where there is a high probability of resistance to either rifampicin, isoniazid or fluoroquinolones (used in new shorter regimens), or where there is a high prevalence of *M. tuberculosis* strains harbouring mutations not detected by other rapid molecular tests.

In people with bacteriologically confirmed **rifampicin-resistant pulmonary TB disease**, targeted next-generation sequencing technologies may be used on respiratory samples to diagnose resistance to isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, ethambutol, amikacin and streptomycin rather than culture-based phenotypic drug susceptibility testing.
(Conditional recommendation, certainty of evidence high [isoniazid, fluoroquinolones and pyrazinamide], moderate [ethambutol], low [bedaquiline, linezolid, clofazimine and streptomycin] or very low [amikacin])

- Priority should be given to those at a higher risk of resistance to medications used for the treatment of rifampicin-resistant TB (RR-TB), including individuals who:
  - continue to be smear or culture positive after 2 months or more of treatment, or have experienced treatment failure;
  - have previously had TB treatment, including with the new and repurposed drugs;
  - are in contact with a person known to have resistance to TB drugs, including the new and repurposed drugs; or
  - have pre-extensively drug-resistant TB (pre-XDR-TB) with resistance to fluoroquinolones.

The TBseq product met the class-based performance criteria for ethambutol.

Key performance conclusions

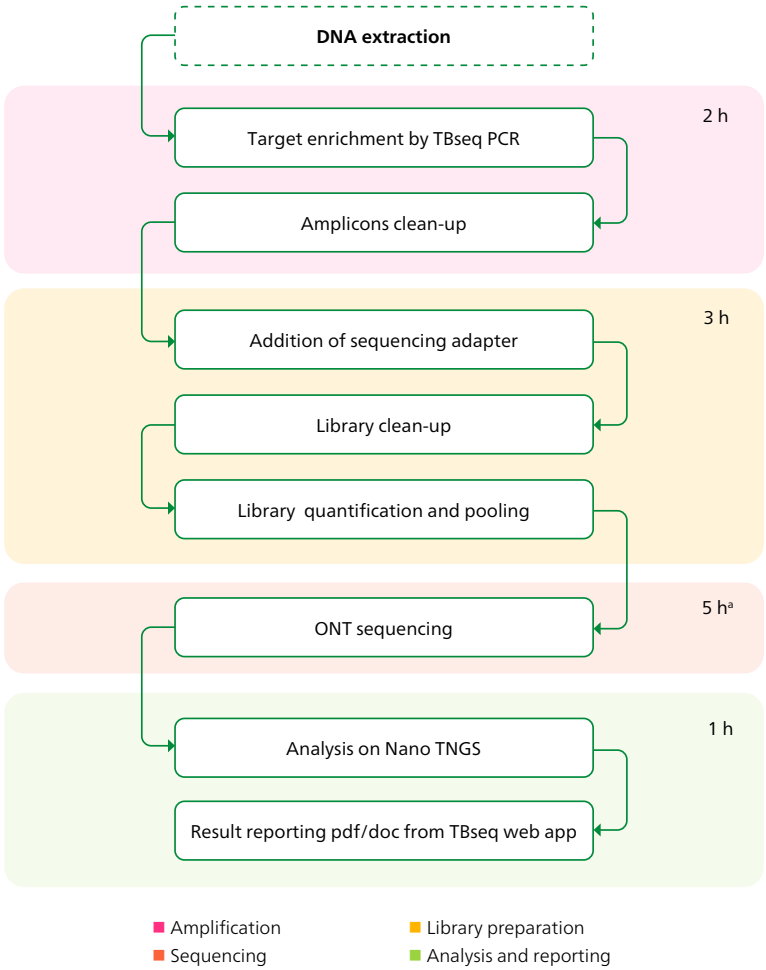
- Pooled sensitivity and specificity data for the class as presented in the *WHO consolidated guidelines on tuberculosis, third edition (1)*.
- Detection of heteroresistance down to 10% subpopulations (reported by the company).
- Detection of DNA loads down to 200 colony forming units (cfu)/mL (reported by the company).

Test procedure at a glance

The TBseq kit includes a master mix ready for multiplex amplification (Table WA9.1), and positive and negative controls. The assay is applied to genomic DNA extracted from inactivated clinical samples (e.g. sputum) or mycobacteria-positive culture (Fig. WA9.1). After single multiplex polymerase chain reaction (PCR), barcoding and purification of the amplicons, DNA libraries are prepared and sequenced on ONT platforms (Table WA9.2). The sequencing data are then

uploaded to an analysis program (Nano TNGS V1.0) for automated analysis. The turnaround time is about 12 hours. Flow cells can be washed and reused up to three times.

Fig. WA9.1. TBseq workflow



DNA: deoxyribonucleic acid; ONT: Oxford Nanopore Technologies; PCR: polymerase chain reaction.

[a] Recommended minimum required threshold of 500x median reads coverage. Longer runs provide higher coverage.

Table WA9.1. TBseq mycobacterial targets

Gene region	Target	Gene region	Target
16s, hsp65	Species ID	*eis, rrs*	Kanamycin
rpoB	Rifampicin	*tlyA,*[a] *rrs*	Capreomycin
ahpC, katG, inhA	Isoniazid	*folC, thyA*[a]	Para-aminosalicylic acid
pncA[a]	Pyrazinamide	*ethA,*[a] *ahpC, inhA*	Ethionamide/prothionamide
embB, embA	Ethambutol	*rv0678,*[a] *atpE*[a]	Bedaquiline, clofazimine
rrs, rpsL,[a] *gibB*[a]	Streptomycin	*rplC*[a]	Linezolid
gyrA, gyrB	Fluoroquinolones	*alr*[a]	Cycloserine
rrs	Amikacin		

[a] Full genes.

Table WA9.2. TBseq specifications for the ONT platforms

Platform	Kit	Run time	Number of samples
MinION/GridION	Flow cell R9.4.1	~5 hours	22 + 2 controls

ONT: Oxford Nanopore Technologies.

Equipment, supplies and reagents required

Table WA9.3. Equipment, supplies and reagents required for TBSeq

Supplied	Not supplied but required
Reagents	
TBseq targeted PCR primer mix (A)	Ultra-pure PCR-grade water
TBseq PCR mix (A)	Universal Gene Sequencing Kit (ShengTing)
TBseq lysozyme (A)	Fluorometer assay reagents
TBseq lysis enzymes (A)	Ethanol molecular grade
TBseq positive control (A)	Ligation Sequencing Kit V14 (Oxford Nanopore, SQK-LSK114)
TBseq negative control (A)	
TBseq magnetic beads solution (B)	
TBseq elution solution (B)	
TBseq proteinase K (B)	

Supplied	Not supplied but required
TBseq conditioning fluid (B)	
Consumables	
	Personal protective equipment
	0.2 mL 96-well plates or PCR microtubes or strips
	1.5 mL microtubes PCR grade
	Filter tips PCR clean
	96-well deep-well PCR plates
	Tip combs
Equipment	
	Single- and multi-channel pipettes (10 µL, 100 µL, 200 µL) and 1000 µL single-channel pipette
	Fluorometer
	Microcentrifuge
	Vortex mixer
	MinION or GridION sequencing device (Oxford Nanopore)
	Thermal cycler suitable for 96-well PCR plates
	Magnetic stand (e.g. Thermo Fisher™,12321D)
	Computer with the following minimal requirements for MinION or GridION: • Operating system: Windows 10 or Linux Ubuntu 20.04 and 18.04 • Memory/RAM: 16 GB RAM or higher • CPU: Intel i7, i9, Xeon, or better, with at least 4 cores/threads or Ryzen 5, 7, or better, with at least 4 cores or 8 threads • GPU: NVIDIA GPU RTX 2060 SUPER or better, with at least 8 GB of GPU memory • Storage: 1 TB internal SSD or highe • Ports: USB3.0
Software	
Nano TNGS V1.0	
TBseq web app	

CPU: central processing unit; GPU: graphics processing unit; PCR: polymerase chain reaction; SSD: solid-state drive.

Source: Reproduced with permission of Hangzhou ShengTing Medical Technology Co. , © 2024. All rights reserved.

Operational considerations

Sample types: DNA extracted from inactivated clinical samples, and from heat-inactivated cultures.

Storage and handling: The reagents in Components A and B (Table WA9.3) should be stored and shipped in dark containers at –20 °C and 2–8 °C, respectively. When properly stored, the kit is stable for up to 1 year. The reagents are stable for up to three freeze–thaw cycles. Reagents for DNA extraction, library preparation, library DNA quantification and sequencing should be stored according to the manufacturer's instructions: they typically require storage at –25 °C to –15 °C, 2 °C to 8 °C, or 15 °C to 30 °C.

Testing capacity: There are 24 tests per kit.

Time to detection: TBseq PCR takes about 12 hours to test 24 samples.

Result reporting: Results are automated via Nano TNGS V1.0. Once the FASTQ files are uploaded and have been analysed, integrated reference databases, including the WHO mutation catalogue *(2)*, are queried to identify mutations associated with mycobacterial species, resistance and susceptibility to anti-TB drugs. Mutations that are not in the databases are classified as uncharacterized. The TBseq web app automatically generates detailed reports in different formats (e.g. PDF and Microsoft Word®).

The kit, the workflow and the means with which the workflow is executed are under active development and update.

Shelf life: The shelf life is 12 months.

Implementation considerations

Area 1 – Policies, budgeting and planning (Section 3.5.1)

The assay may be placed in centralized reference settings. It will not replace the WHO-recommended rapid diagnostic tests (WRDs) as the initial test for diagnosis of TB, but could be used for prioritized patient populations requiring comprehensive drug susceptibility testing (DST) (including group A agents for the treatment of RR-TB and multidrug-resistant TB), faster than phenotypic DST.

The ONT systems on which TBseq tests are run are capable of multidisease testing, which may be considered for potential cost savings across programmes for communicable and noncommunicable diseases.

The WHO implementation manual provides practical guidance for national TB programmes and laboratories to plan and implement NGS-based approaches for the characterization of MTBC bacteria to detect mutations associated with drug resistance *(3)*.

Area 2 – Regulatory issues (Section 3.5.2)

The Universal Gene Sequencing Kit has completed the registration process in China (National Medical Products Administration [NMPA]); the registration number is "Zhejiang Device Registration Approval No. 20201178".

Area 3 – Equipment (Section 3.5.3)

ONT instruments have moderate to high infrastructure requirements and should be placed in laboratories that can accommodate molecular workflow (e.g. with separate and dedicated preparation, amplification and sequencing spaces). Computational and other resource capacities (e.g. electrical supply and network connection), and service and maintenance agreements to ensure optimal system functionality should be considered. Testing volumes should be calculated before procurement to maximize resources (human, budgetary and testing) and ensure that sufficient testing supplies and reagents are available to meet clinical demand.

Area 4 – Supply chain (Section 3.5.4)

Procurement and delivery of any third-party equipment, consumables and reagents not supplied but required for the workflow should be ensured.

Area 5 – Procedures (Section 3.5.5)

Given the complexity of the targeted NGS workflow, a comprehensive set of standard operating procedures (SOPs) must be developed, covering sample collection, storage and referral; sample processing and DNA extraction; DNA library preparation and sequencing; and NGS data analysis and interpretation. A panel of local or international experts that includes laboratory and clinical staff should cooperate in developing a standard targeted NGS reporting system that will support clinical decisions.

The product performance depends on the efficiency of the DNA isolation and purification methods used.

Resistance is reported when a documented resistance-conferring mutation is detected in targets of interest. Where mutations are not detected, this suggests strain sensitivity but does not exclude the possibility of resistance. Low-frequency variants below the limit of detection may affect the quality of results and their interpretation. The interpretation provided is based on the current understanding of genotype–phenotype relationships.

Area 6 – Digital data (Section 3.5.6)

The assay includes an easy-to-use web application for uploading and analysing raw sequencing data and rapidly interpreting the results. Opportunities for integration of e-systems may be explored.

Area 7 – Quality assurance, control and assessment (Section 3.5.7)

Quality assurance (QA) systems and activities for the TBseq assay mimic those of the moderate complexity automated nucleic acid amplification tests (NAATs). Assay results should be monitored carefully based on expected outcomes to promptly detect false positive and false negative trends, and laboratories should regularly participate in external QA programmes. Potential contamination of the molecular workflow means that each run on the ONT instrument must include positive and negative controls to ensure run validity, and laboratory spaces should be tested for contamination at least monthly. Control interpretation guidance is included in the manufacturer's instructions for use, and such guidance should be included in user training and competency assessments.

New method validation for the TBseq test should include well-characterized MTBC positive and negative samples, and precision and accuracy measurements for drugs targeting all drug-resistance loci. Samples should be well-characterized strains with and without known resistance-associated mutations.

Area 8 – Recording and reporting (Section 3.5.8)

The TBseq generates automatic reports that include sample information, the date, analysis mode, quality summary, experiment set and all mutation details as derived by the software. The TBseq web app can integrate all the results of a sample, and can generate reports in different formats (e.g. PDF or Word). Reports can be downloaded from the TBseq web app directly. Users should follow national requirements for results reporting. Revision of laboratory registers and reporting forms may be needed.

Area 9 – Human resource training and competency assessment (Section 3.5.9)

Laboratory, clinical and programme staff should receive at least 1 week of in-depth training on test principles, methods, and appropriate review and interpretation of results. Laboratory technicians should be fully trained in all steps and should be able to troubleshoot when necessary. Competency assessments should be conducted after training and periodically thereafter.

References for WA9

1 WHO consolidated guidelines on tuberculosis. Module 3: diagnosis – rapid diagnostics for tuberculosis detection, third edition. Geneva: World Health Organization; 2024 (https://iris.who.int/handle/10665/376221).

2 Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, second edition. Geneva: World Health Organization; 2023 (https://www.who.int/publications/i/item/9789240082410).

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

For further information, please contact:

Global Tuberculosis Programme
World Health Organization
20, Avenue Appia CH-1211 Geneva 27 Switzerland
Web site: www.who.int/tb

9789240090484