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

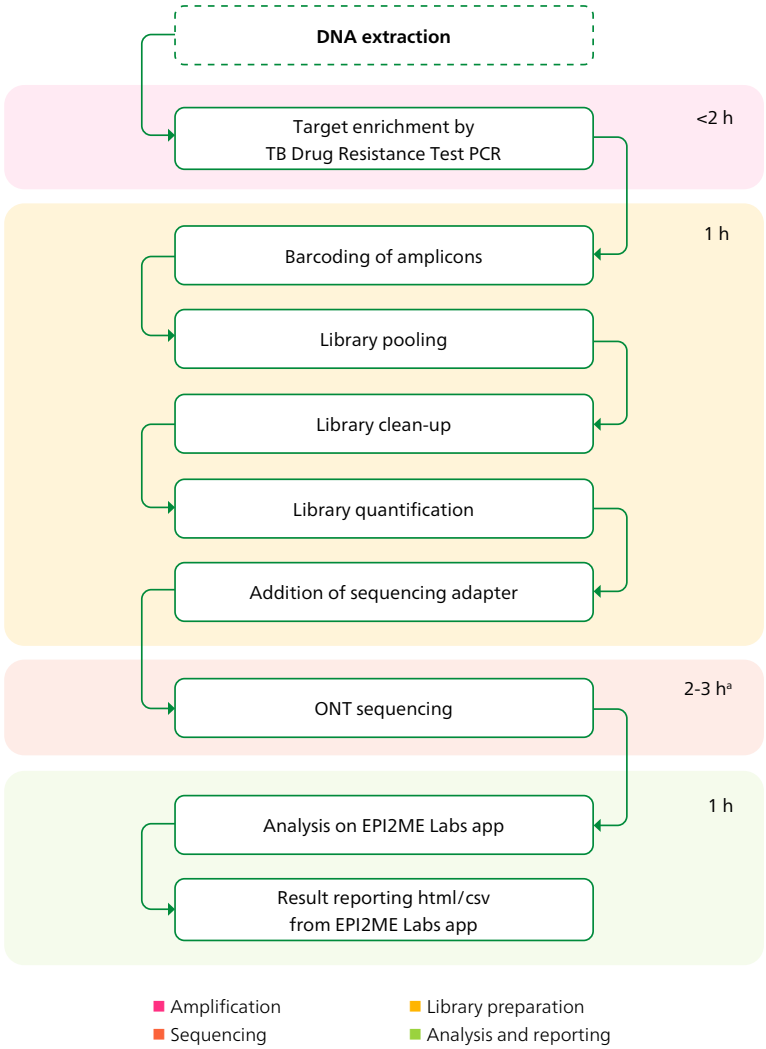
- Pooled sensitivity and specificity data for the class as presented in the *WHO consolidated guidelines on tuberculosis, third edition (1)*.
- Fast turnaround time for 22 sample runs.
- Detection of heteroresistance down to 10% subpopulations (reported by the company).

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 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. 1). After single multiplex PCR, DNA libraries are prepared and sequenced on MinION (Table 2). Sequencing data are automatically analysed on the device. Results are obtained in less than 5 hours using extracted DNA.

Fig. 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 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 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 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

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).