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.

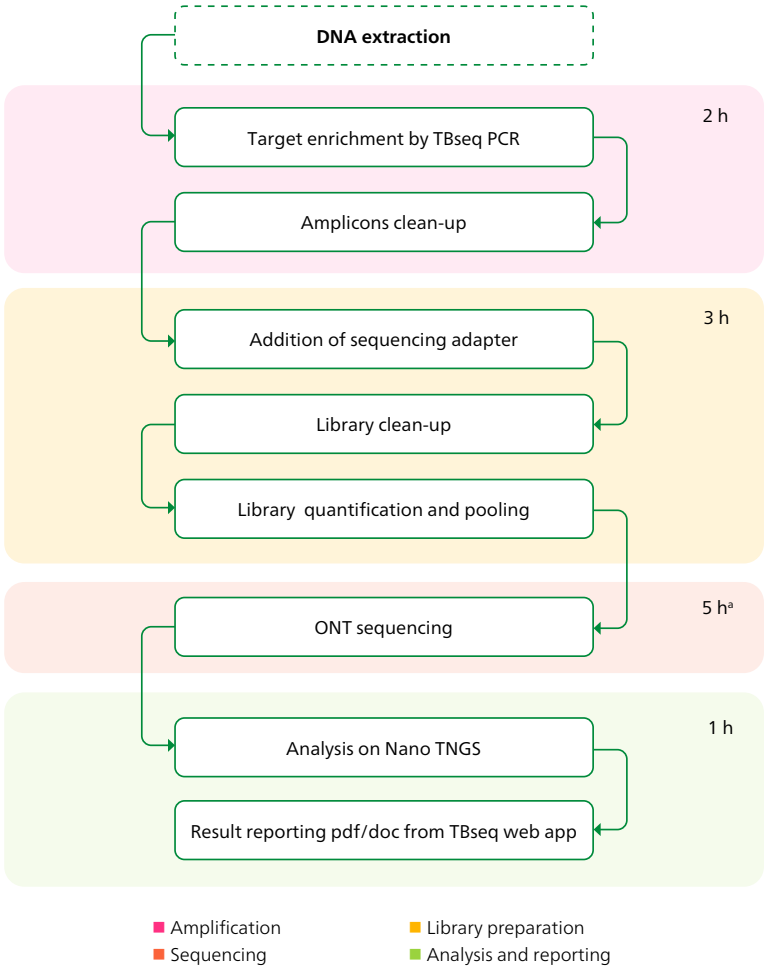
Key performance conclusions

- 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 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. 1). After single multiplex polymerase chain reaction (PCR), barcoding and purification of the amplicons, DNA libraries are prepared and sequenced on ONT platforms (Table 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. 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 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 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 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)	
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

Supplied	Not supplied but required
	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 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

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).