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.

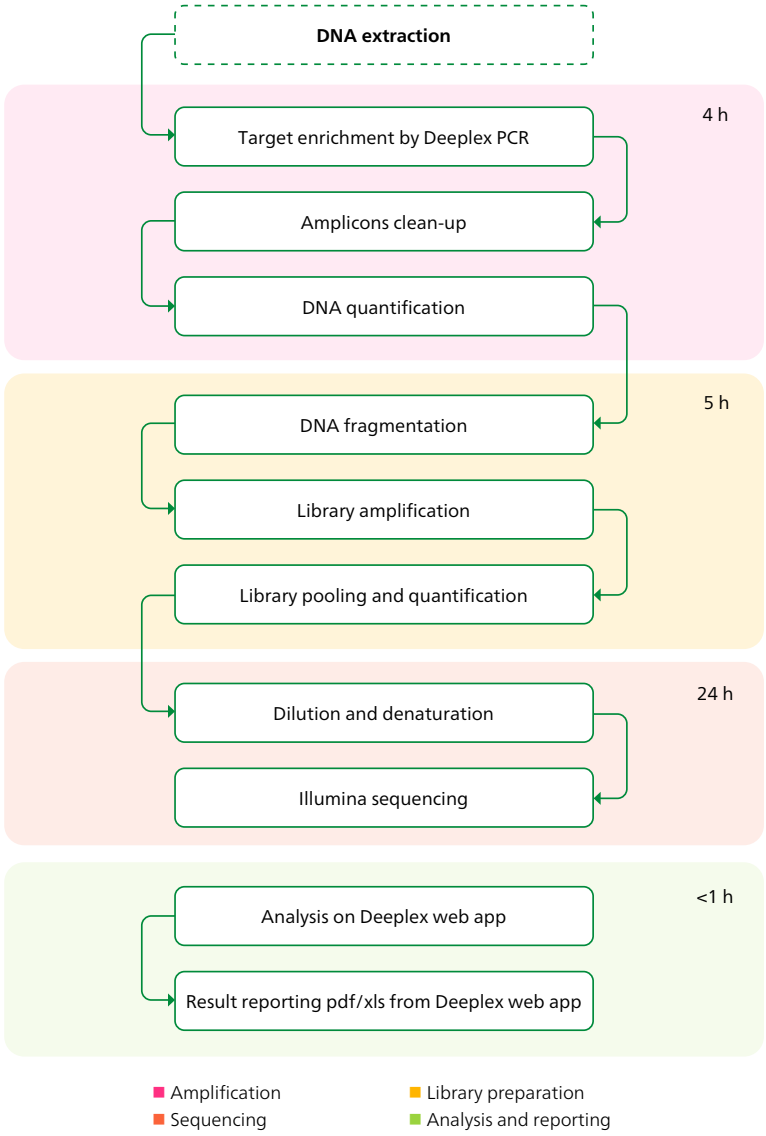
Key performance conclusions

- 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 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. 1). After single multiplex PCR and purification of amplicons, DNA libraries are prepared and sequenced on Illumina platforms (Table 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. 1. Summary of Deeplex Myc-TB workflow



DNA: deoxyribonucleic acid; PCR: polymerase chain reaction.

Table 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 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)	17 hours	21 + 3 controls
	High output (6.6 Gb)	24 hours	66/69 + 3 controls
MiSeq	Nano kit (300 Mb)	17 hours	1 + 3 controls
	Micro kit (1.2 Gb)	19 hours	13 + 3 controls
	Full kit (4.5 Gb)	24 hours	45 + 3 controls
NextSeq	Mid output (32.5 Gb)	26 hours	372 + 3 controls
	High output (100 Gb)	29 hours	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 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.

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

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 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,

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

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).