Workshop

Joint SEAR-WPR workshop to plan the accelerated implementation of new WHO TB policies

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Hanoi, Viet Nam

WHO Policy Updates on Targeted Next Generation Sequencing End-to-End Solutions for the Detection of Drug-Resistant Tuberculosis

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Presentation Outline

- Next Generation Sequencing
- WHO Recommendations on use of tNGS for surveillance
- WHO Recent and Forthcoming Policy on use of tNGS for clinical management
- Next Steps and Resources

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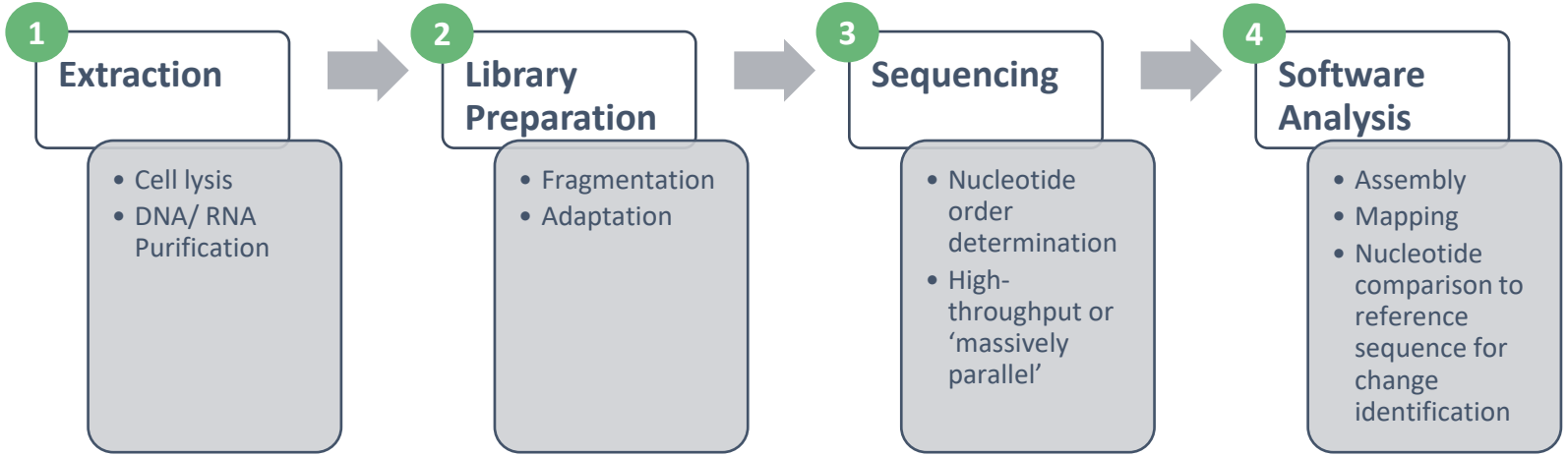
Next Generation Sequencing

Overview

Next Generation Sequencing (NGS) Overview

Sequencing *is a laboratory procedure that determines the order of nucleotides in the genome (RNA or DNA) of an organism.*

NGS is a high-throughput sequencing method used to determine the nucleotide sequence of a genome in a single biochemical reaction.

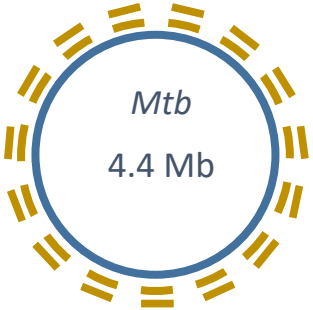


Whole Genome & Targeted NGS

Whole Genome Sequencing (WGS)

- Investigates the entire genome
- Most comprehensive approach
- Less depth (fewer reads at each point of the genome)
- Requires TB culture leading to a longer turnaround time
- More data allows for more complete comparison of strains useful for surveillance

Targeted NGS

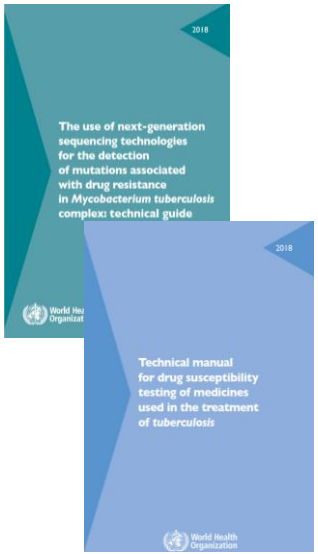


- Targets specific areas of the genome known to harbor mutations associated with DR-TB
- Adaptable to new genes through addition of new primers
- In-depth (more reads at each point of the genome)
- Faster turnaround time using primary clinical specimens (sputa) useful for clinical care
- Less data reduces amount of storage and analytics capacity needed

WHO Recommendations for NGS

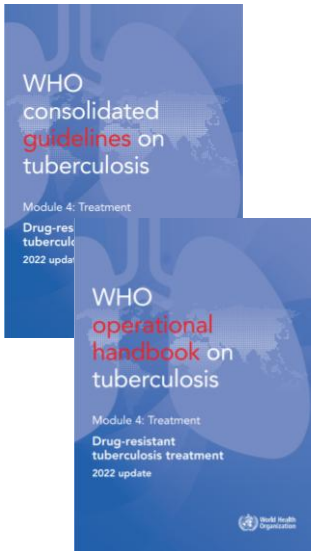
WHO Product Timeline Related to NGS

2018



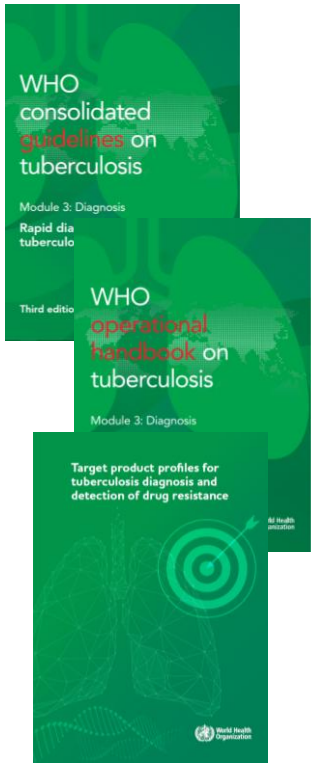
2021

2022



2023

2024



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WHO Guidelines on the Use of Targeted NGS (tNGS) to Diagnose Drug-Resistant Tuberculosis

Recommendations on Use of targeted NGS

Contents

New TB Diagnostic Class: Targeted Next Generation Sequencing

Uses massively parallel sequencing to detect resistance to TB drugs, starting from a processed clinical sample and ending with an end-user report that relates detected *Mycobacterium tuberculosis* mutations to the presence (or absence) of drug resistance, based on the interpretation of a standard catalogue of mutations.

The products and drugs for which eligible data met the class-based performance criteria are listed below:

Deeplex® Myc-TB (Genoscreen, France): rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, bedaquiline, linezolid, clofazimine, amikacin and streptomycin

AmPORE-TB® (Oxford Nanopore Diagnostics, United Kingdom): rifampicin, isoniazid, fluoroquinolones, linezolid, amikacin and streptomycin

TBseq® (Hangzhou ShengTing Medical Technology Co., China): ethambutol

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tNGS Found to be Accurate for DR-TB Detection

Among people with bacteriologically confirmed pulmonary TB

Accurate for all drugs assessed.

Pooled sensitivity of ≥ 95% for rifampicin, isoniazid, moxifloxacin and ethambutol, 94% for levofloxacin and 88% for pyrazinamide.

Specificity was ≥ 96% for all drugs.

Among people with bacteriologically confirmed rifampicin-resistant pulmonary TB (Table)

Accurate for isoniazid, levofloxacin, moxifloxacin, pyrazinamide and ethambutol (pooled sensitivity ≥ 95%; yellow in table).

Acceptable for bedaquiline (68%), linezolid (69%), clofazimine (70%), amikacin (87%) and pyrazinamide (90%) (orange in table).

Specificity was ≥ 95% for all drugs except streptomycin (75%).

Cost-effective depending on setting and context

Acceptable and implementable, despite inherent complexity

Table 2.3.6. The accuracy and certainty of evidence of targeted NGS for the detection of resistance to anti-TB drugs among bacteriologically confirmed rifampicin-resistant pulmonary TB

Drug	Reference standard	Accuracy % (95% CI)	Studies (persons)	Certainty in evidence
Isoniazid	Phenotypic DST	Se: 96.5 (93.8–99.2)	12 (1440)	High
	Phenotypic DST	Sp: 95.8 (91.8–99.8)	12 (517)	High
Levofloxacin	Phenotypic DST	Se: 95.8 (90.4–100)	6 (654)	Moderate
	Phenotypic DST	Sp: 96.0 (93.1–98.9)	7 (913)	High
Moxifloxacin	Phenotypic DST	Se: 96.5 (93.6–99.5)	6 (652)	High
	Phenotypic DST	Sp: 95.2 (91.0–99.4)	8 (921)	High
Pyrazinamide	Phenotypic DST+WGS	Se: 90.0 (86.8–93.2)	3 (346)	High
	Phenotypic DST+WGS	Sp: 98.6 (96.8–100)	3 (269)	High
Bedaquiline	Phenotypic DST	Se: 67.9 (42.6–93.2)	3 (31)	Low
	Phenotypic DST	Sp: 97.0 (94.3–99.7)	4 (519)	High
Linezolid	Phenotypic DST	Se: 68.9 (38.7–99.1)	4 (31)	Low
	Phenotypic DST	Sp: 99.8 (99.6–100)	6 (1093)	High
Clofazimine	Phenotypic DST	Se: 70.4 (34.6–100)	4 (36)	Low
	Phenotypic DST	Sp: 96.3 (93.2–99.3)	6 (789)	High
Amikacin	Phenotypic DST	Se: 87.4 (74.5–100)	5 (115)	Very low
	Phenotypic DST	Sp: 99.0 (98.4–99.6)	8 (1003)	Moderate
Ethambutol	Phenotypic DST+WGS	Se: 96.7 (95.0–98.4)	4 (431)	Moderate
	Phenotypic DST+WGS	Sp: 98.4 (96.1–100)	4 (123)	Moderate
Streptomycin	Phenotypic DST	Se: 98.1 (96.1–100)	5 (493)	High
	Phenotypic DST	Sp: 75.0 (59.5–90.5)	5 (250)	Low

CI: confidence interval; DST: drug susceptibility testing; NGS: next-generation sequencing; Se: sensitivity; Sp: specificity; TB: tuberculosis; WGS: whole genome sequencing

2023 Recommendations on use of targeted NGS

Recommendations

1. 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], low [rifampicin, fluoroquinolones and ethambutol])*

Remarks

- 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 or more months 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 fluoroquinolone (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.
- This recommendation is conditional because of the lack of data on health benefits, the variable certainty of evidence on diagnostic accuracy, and the fact that accuracy is suboptimal for certain drugs. In addition, because this is a new technology that has not yet been widely implemented, there is still limited and variable evidence on costs, cost–effectiveness and feasibility of implementation.

2. In people with bacteriologically confirmed rifampicin-resistant pulmonary TB disease, targeted NGS 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], very low [amikacin])

Remarks

- Priority should be given to those at a higher risk of resistance to medications used for the treatment of 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-XDR-TB with resistance to fluoroquinolones.
- As above, this recommendation is conditional because of the lack of data on health benefits, the variable certainty of evidence on diagnostic accuracy, the fact that accuracy is suboptimal for certain drugs, and limited and variable evidence on costs, cost–effectiveness and feasibility of implementation.

Implementation Considerations for tNGS

- Targeted NGS solutions **do not replace existing rapid tests that are more accessible and easier to perform** for detecting resistance to **RIF, INH and FQ**.
  - However, if targeted NGS can be performed rapidly, it can be considered as an alternative initial option for prioritized populations.
- Priority should be given to samples with a **high bacillary load** as determined by initial bacteriological tests (e.g., semi-quantitative high/medium or smear-positive grading).
  - Where the bacillary load is low (e.g. semi-quantitative low/very low/trace or smear-negative grading) indeterminate results are likely to be higher and culture-based DST is likely still required
- Since sensitivity for BDQ, LZD and CFZ resistance is **suboptimal**, consideration of the **pretest probability** is important in interpreting the targeted NGS results for these drugs
  - Resistant result may be used to guide therapy
  - Sensitive result should be followed up with culture-based DST if risk of resistance is high

New tNGS Solutions for Drug-Resistant TB Detection

Updated

ONT AmPORE-TB Reagent Kit

Data Analysis Completed

ABL Diagnostics SA DeepChek Assay 13-Plex KB Drug Susceptibility Testing Kit

- Advances in tNGS Solutions: One updated resistance interpretation software (ONT AmPORE-TB) and one solution new diagnostic accuracy dataset (ABL Diagnostics SA DeepChek Assay 13-Plex KB)
- 2024 public and targeted call for evidence was done and evidence review completed for 2025 Technical Advisory Group assessment
- Solutions assessed for detection of first-line drug resistance among people with bacteriologically-confirmed and second-line resistance among people with rifampicin-resistant TB

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Performance of the Updated AmPORE-TB for Drug-Resistant TB

	Drug	Reference standard	AmPORE-TB (ONT) (679 patients)		tNGS Within-Class (pooled per drug)	
			Sensitivity[95% CI]	Specificity [95% CI]	Sensitivity [95% CI]	Specificity [95% CI]
Bacteriologically Confirmed TB	RIF	cDST	94.6% [92.3-96.3]	98.3% [93.9-99.8]	93.1% [87.0-99.2]	96.2% [88.6-100]
	INH	pDST	95.3% [93.0-96.9]	97.3% [93.1-99.2]	95.8% [92.8-98.7]	97.0% [95.1-98.9]
	MXF	pDST	95.4% [92.4-97.5]	96.4% [93.9-98.1]	95.6% [92.4-98.7]	96.3% [93.2-99.5]
	LFX	pDST	94.8% [91.6-97.0]	96.1% [93.5-97.9]	94.2% [88.4-99.9]	96.2% [93.4-98.9]
	PZA	cDST	91.1% [87.7-93.9]	98.4% [95.9-99.6]	88.4% [85.2-91.7]	98.5% [97.1-100]
	EMB	cDST	84.7% [81.0-87.9]	98.6% [96.1-99.7]	95.8%[94.-0-97.6]	99.3% [98.2-100]
Rifampicin-Resistant TB	INH	pDST	96.0% [93.8-97.5]	97.7% [87.7-99.9]	96.5% [93.8-99.2]	95.8% [91.8-99.8]
	MXF	pDST	96.7% [94.0-98.4]	95.4% [91.9-97.7]	96.5% [93.6-99.5]	95.2% [91.0-99.4]
	LFX	pDST	97.0% [94.3-97.0]	95.0% [91.4-97.4]	95.8% [90.4-100]	96.0% [93.1-98.9]
	PZA	cDST	92.1% [87.7-94.7]	98.1% [94.6-99.6]	90.0% [86.8-93.2]	98.6% [96.8-100]
	BDQ	pDST	73.5% [55.6-87.1]	97.2% [95.3-98.5]	67.9% [42.6-93.2]	97.0% [94.3-99.7]
	LZD	pDST	77.8% [52.4-93.6]	99.6% [98.6-100]	68.9% [38.7-99.1]	99.8% [99.6-100]
	CFZ	pDST	68.8% [50.0-83.9]	97.4% [95.6-98.6]	70.4% [34.6-100]	96.3% [93.2-99.3]
	AMK	pDST	87.9% [76.7-95.0%]	99.0% [97.6-99.7]	87.4% [74.5-100]	99.0% [98.4-99.6]
	EMB	cDST	85.4% [81.7-88.6]	98.6% [96.1-99.7]	96.7% [95.0-98.4]	98.4% [96.1-100]
	SM	pDST	88.0% [84.5-91.0]	85.1% [77.2-91.1]	98.1% [96.1-100]	75.0% [59.5-90.5]

pDST = phenotypic drug susceptibility testing, cDST = composite phenotypic and WGS drug susceptibility testing

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New tNGS Solutions for Drug-Resistant TB Detection

Updated

ONT AmPORE-TB Reagent Kit

Data Analysis Completed

ABL Diagnostics SA DeepChek Assay 13-Plex KB Drug Susceptibility Testing Kit

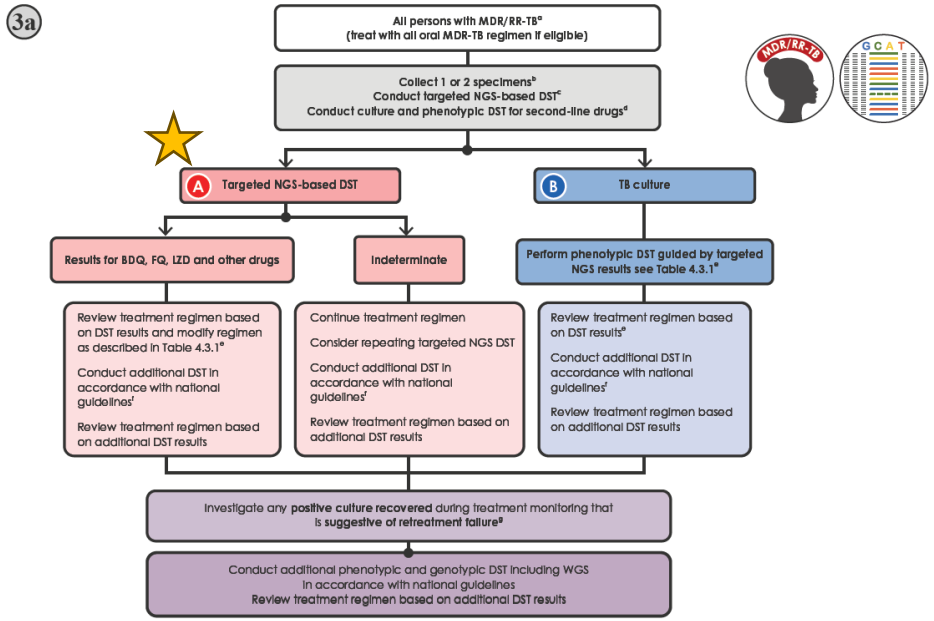
- Performance of the AmPORE-TB solution **is comparable** to that of other WHO-recommended tNGS solutions for the detection of resistance to:
  - ✓ the first-line drugs rifampicin, isoniazid, fluroquinolones, and pyrazinamide among people with bacteriologically confirmed pulmonary TB
  - ✓ isoniazid, fluoroquinolones, bedaquiline, linezolid, clofazimine, pyrazinamide, amikacin, and streptomycin among people with rifampicin-resistant TB

- Performance of the DeepChek Assay 13-Plex KB Drug Susceptibility Testing solution **could not be adequately compared** to that of WHO-recommended tNGS solutions for the detection of drug resistance among people with bacteriologically confirmed pulmonary TB with or without rifampicin resistance.

- WHO recommendations for the use of tNGS **are valid** for the AmPORE-TB (ONT) solution, which now meets class-based performance criteria for detection of resistance to **three additional drugs** (pyrazinamide, bedaquiline, and clofazimine).

Operational Handbook: Use of targeted NGS

Fig. 4.5. New Algorithm 3a: DST for MDR/RR-TB using targeted NGS



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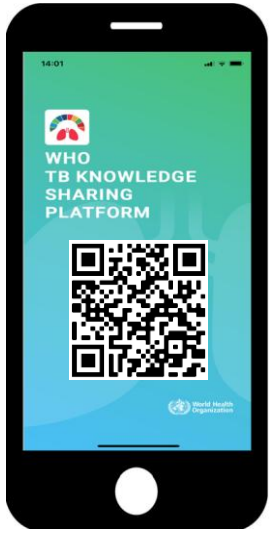
Summary and Next Steps

- ❑ Two new tNGS end-to-end solutions recommended by WHO for detection of first- and second-line drug-resistant TB
- ❑ Forthcoming 2025 Operational Handbook on TB Diagnosis will include new solution policy statements and underlying reports
- ❑ Advances in our understanding of the molecular mechanisms associated with resistance are expected to improve solution performance over time; updates will undergo WHO review and assessment
- ❑ More evidence is needed on the new drugs and the impact of targeted NGS on patient important outcomes
- ❑ 2025 Additional Resources: Mutation Catalogue Version 3, WHO Academy tNGS eCourse, WHO Prequalification process development for the tNGS class



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