FIND

Seq&Treat: Genomics for drug-resistant TB diagnosis

2 July 2025 | IPSN EPI-WIN webinar series

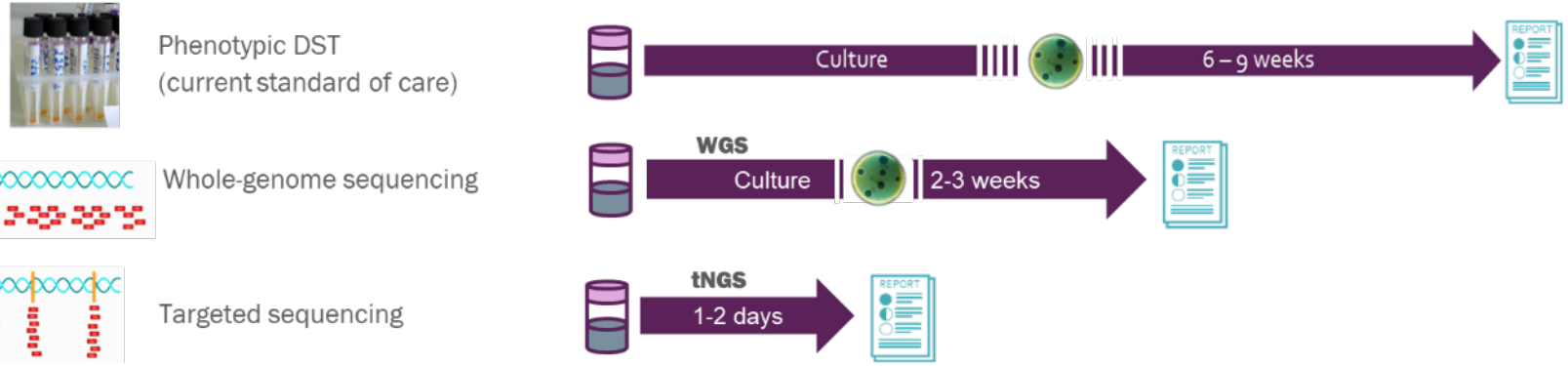
Anita Suresh
Head of Genomics & Sequencing Unit, FIND

CRITICAL NEED FOR BETTER AND FASTER DIAGNOSTICS FOR DR-TB

- Early detection and optimal treatment critical for tackling TB
- New drugs and shorter regimens now available
  - Get people on the right treatment from the start
  - Preserve the efficacy of new drug regimens so more people can avail of the treatment for as long as possible

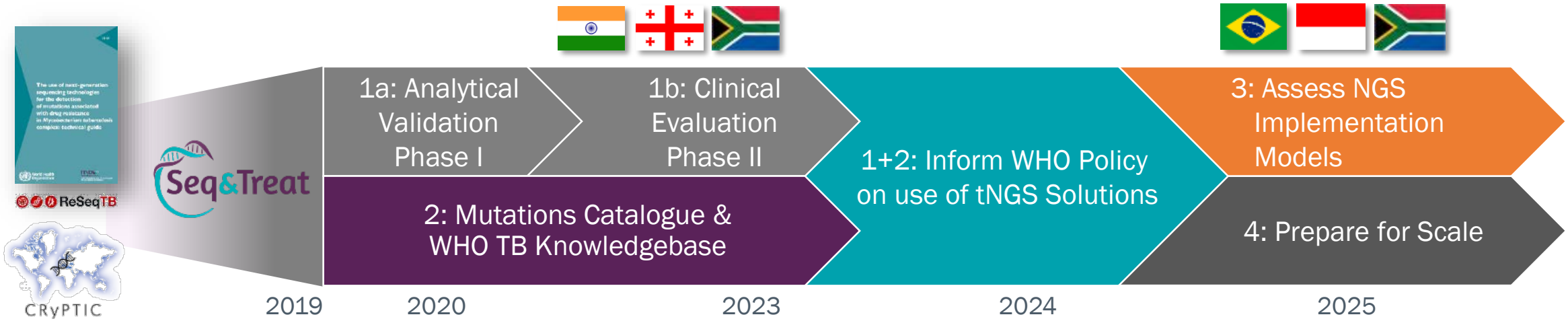
Targeted NGS-based diagnosis of DR-TB direct from clinical samples presents a paradigm shift

- Strong genotype → phenotype correlations in TB
- Adaptable to new genes and mutations
- Scalable, lower biosafety requirements; multi-disease platform
- Faster results to inform clinical decision-making



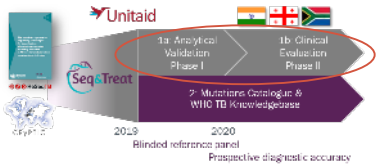
Seq&Treat RAPID, COMPREHENSIVE tNGS SOLUTIONS FOR DR-TB DIAGNOSIS

Generate evidence and boost in-country capacity to support the global adoption of end-to-end tNGS solutions for comprehensive diagnosis of DR-TB



https://www.finddx.org/what-we-do/projects/seqtreat/

ANALYTICAL AND CLINICAL EVALUATION OF tNGS FOR DR-TB DETECTION



GenoScreen

illumina

Oxford NANOPORE Technologies

Tbilisi
Georgia

Mumbai
India

Johannesburg
South Africa

- **Phase 1:** Internal analytical validation of tNGS solutions demonstrated reproducibility, and SNP calling accuracy
  - When a SNP is present, it is called accurately
- **Phase 2:** Multicenter, cross-sectional, diagnostic accuracy study in three countries
  - **Trial population:** Confirmed pulmonary TB patients at risk/proven to have DR-TB
  - **Study size:** 750 participants
  - **Composite Reference:** Phenotypic DST & WGS
  - **Comparators:** Xpert MTB/RIF+Hain LPA
  - **Objective:** Assessment of diagnostic accuracy for resistance to 13 anti-TB drugs: RIF INH MOX LEV PZA AMK KAN CAP BDQ LZD CLF STR EMB

GENOSCREEN AND ONT TNGS WORKFLOWS

GenoScreen illumina

Oxford NANOPORE Technologies

Instrument	Promega Maxwell	Thermocycler Qubit Fluorometer	Thermocycler	Qubit Fluorometer	Illumina MiSeq Sequencer	Deeplex cloud-based analysis
Genoscreen	1. 200uL of sediment 2. 2x Lysis Buffer 3. Maxwell® FFPE Plus DNA Kit 4. Eluted in 50uL molecular water	1. Quantify DNA 2. One reaction Multiplex PCR 3. Bead-based clean-up 4. Quantify PCR product	1. Illumina Nextera XT library preparation 2. Bead-based clean-up	1. Quantify library product 2. Pool libraries 3. 45 samples + 3 controls	1. Denature and dilute library pool 2. Load onto sequencer 3. Run sequencer	1. Load data to Deeplex web application 2. Run pipeline 3. Control validation 4. View results
Sputum sample	DNA extraction	Mtb target amplification	Library preparation	Pool samples	Sequence	Bioinformatics
ONT	1. 700uL of sediment 2. Bead beating 3. Maxwell RSC PureFood Pathogen Kit 4. Eluted in 50uL elution buffer	1. One reaction Multiplex PCR	1. ONT rapid barcode library preparation	1. Pool libraries 2. 22 samples + 2 controls 3. Bead-based clean-up 4. Quantify pool 5. Add adapter	1. Prime flowcell 2. Load MinION flowcell 3. Run sequencer	1. Load data to server 2. Run pipeline 3. Control validation 4. View results
Instrument	Promega Maxwell FastPRep-24 5G homogenizer	Thermocycler	Thermocycler	Qubit Fluorometer	ONT MinION Sequencer	ONT local instance of software analysis

Report

CLINICAL RESULTS PUBLISHED!

Evaluating culture-free targeted next-generation sequencing for diagnosing drug-resistant tuberculosis: a multicentre clinical study of two end-to-end commercial workflows

Rebecca E Colman, Marva Seifert, Andres De la Rossa, Sophia B Georghiou, Christine Hoogland, Swapna Uplekar, Sacha Laurent, Camilla Rodrigues, Priti Kambli, Nestani Tukvadze, Nino Maghradze, Shaheed V Omar, Lavania Joseph, Anita Suresh, Timothy C Rodwell

Summary

Background Drug-resistant tuberculosis remains a major obstacle in ending the global tuberculosis epidemic. Deployment of molecular tools for comprehensive drug resistance profiling is imperative for successful detection and characterisation of tuberculosis drug resistance. We aimed to assess the diagnostic accuracy of a new class of molecular diagnostics for drug-resistant tuberculosis.

Lancet Infect Dis 2024
Published Online
October 29, 2024
https://doi.org/10.1016/S1473-3099(24)00586-3

This study represents the first **large-scale multicentre clinical evaluation** of two commercial end-to-end tNGS workflows for diagnosing drug-resistant tuberculosis in diverse settings.

By implementing GenoScreen and ONT workflows, the study assessed **diagnostic accuracy and failure rates** across various drug compounds.

The research demonstrates the **potential of tNGS to replace conventional methods** by providing **accurate and comprehensive drug resistance** profiles directly from tuberculosis clinical specimens.

RESULTS SUMMARY

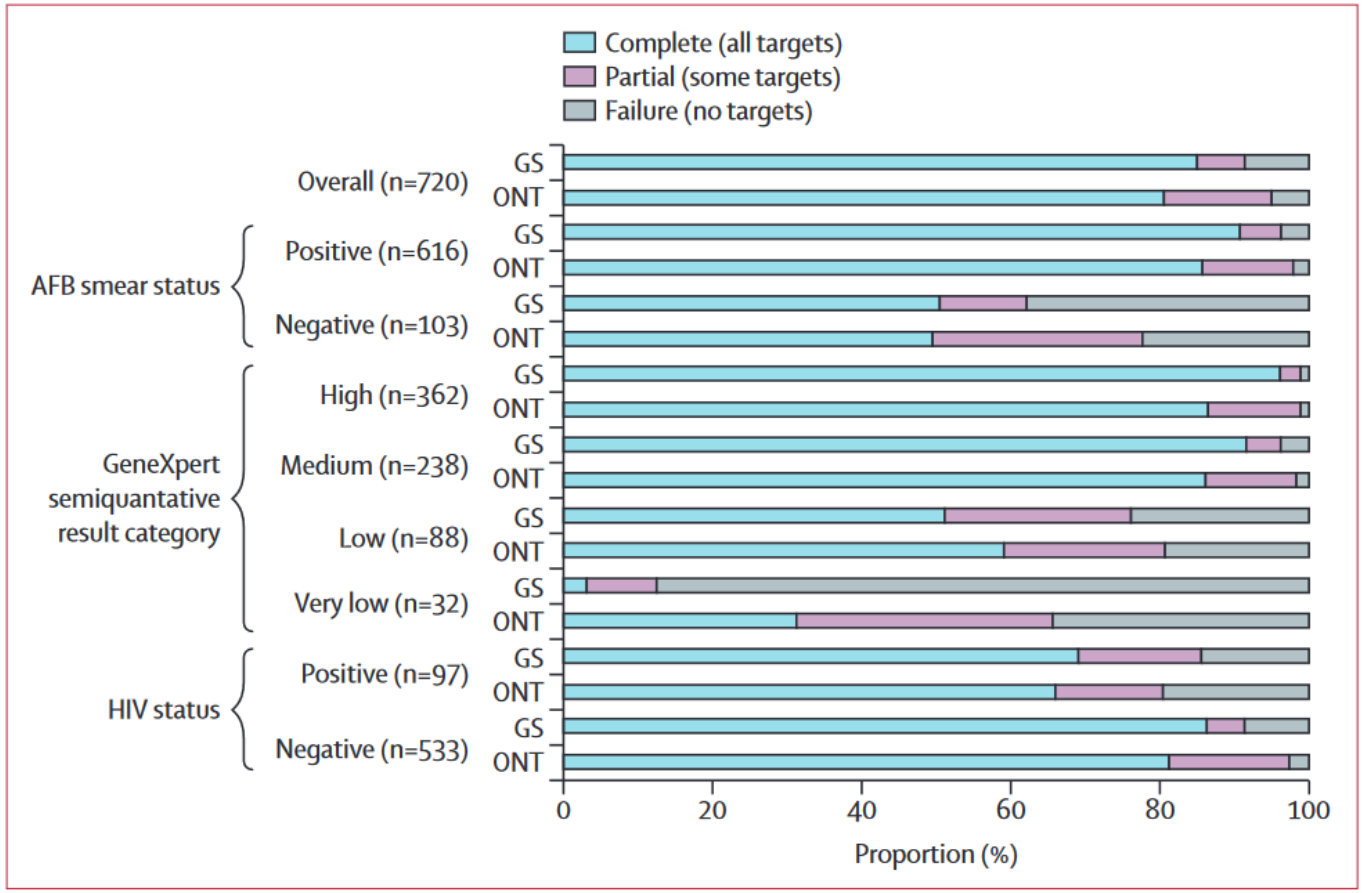
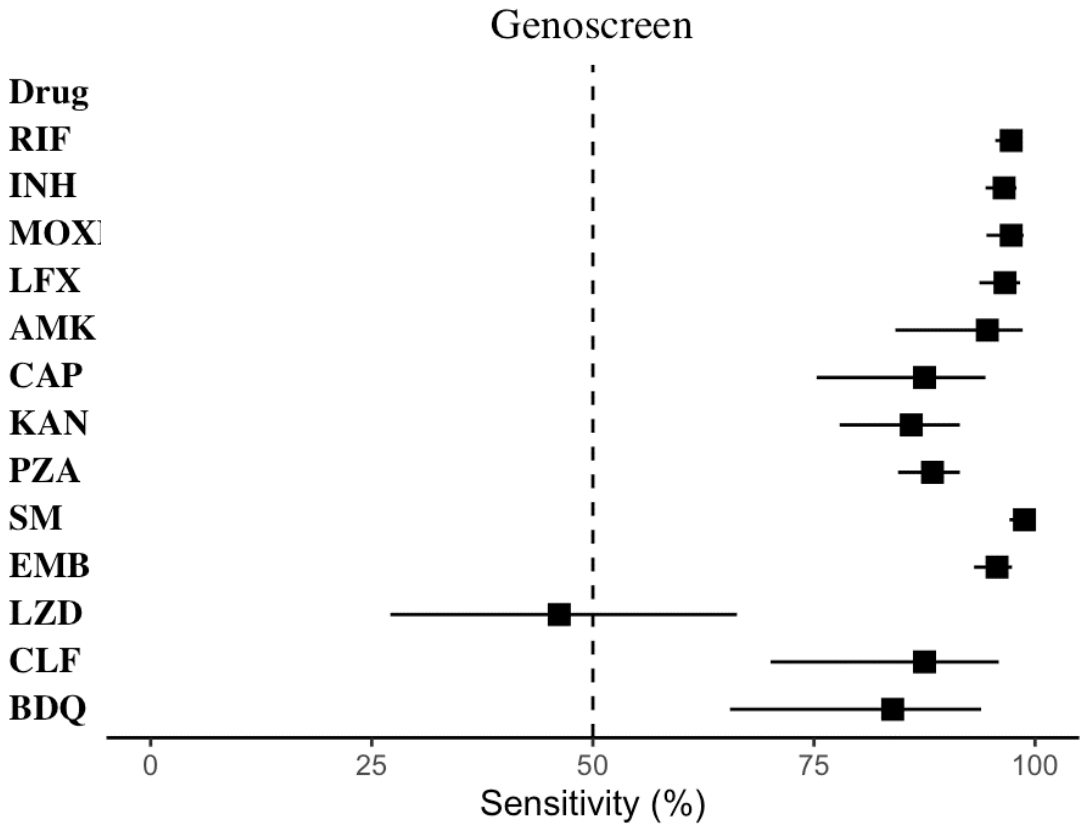


Figure 2: **Sequencing success by bacterial load proxies (AFB smear status, study GeneXpert semiquantitative result category, and HIV status)**

- Both tNGS solutions provide accurate & reproducible results from direct clinical samples
  - >95% of clinical samples produced TB sequence data on tNGS
- High rate of tNGS sequence data generation
  - Sequence failure is associated with lower bacterial loads using Xpert semi-quantitative proxy
    - Genoscreen: >91% of sediment samples produced DR information; 1.2% drug target failure
    - ONT: >94% of sediment samples produced DR information, 5% drug target failure

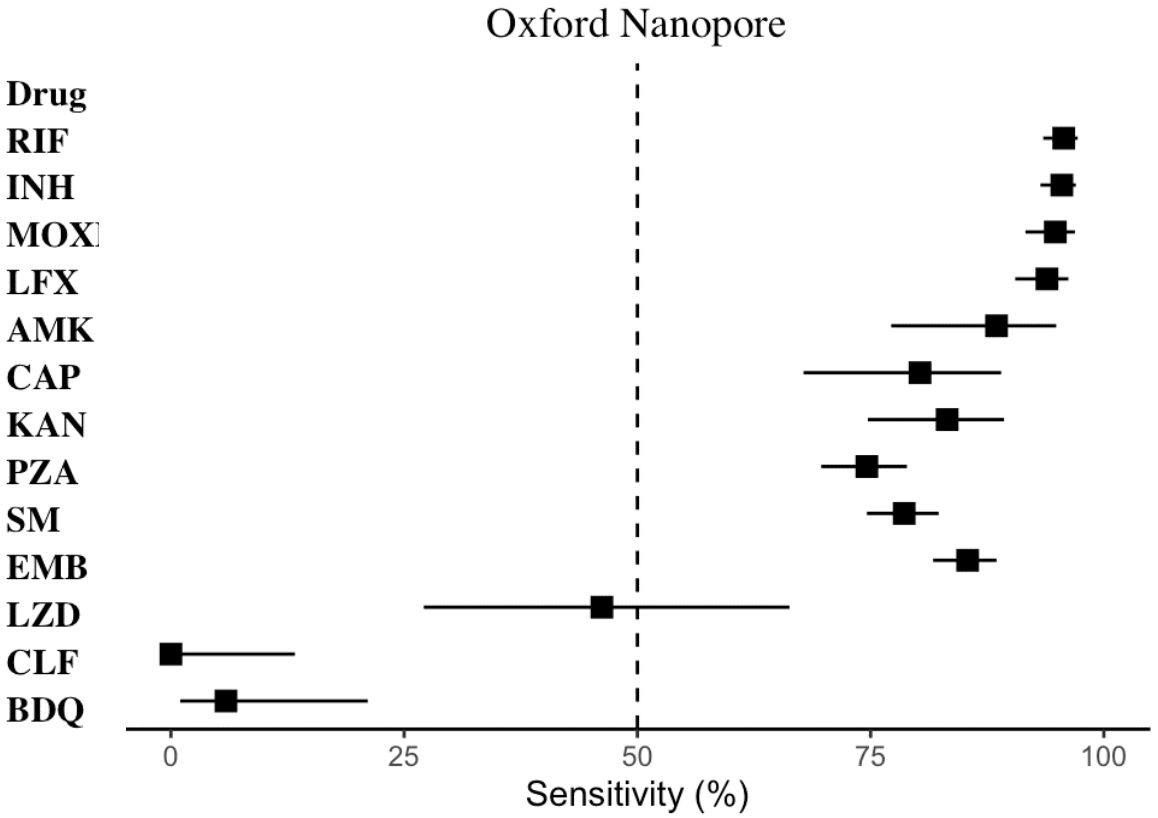
GENOSCREEN ACCURACY



Drug	Sensitivity (95% CI)	Specificity (95% CI)	Drug
RIF	97.3 (95.5-98.5)	100.0 (96.3-100.0)	RIF
INH	96.5 (94.4-97.9)	97.7 (92.8-99.4)	INH
MOXI	97.3 (94.5-98.7)	98.5 (96.4-99.5)	MOXI
LFX	96.6 (93.7-98.3)	98.5 (96.4-99.5)	LFX
AMK	94.6 (84.2-98.6)	99.8 (98.8-100.0)	AMK
CAP	87.5 (75.3-94.4)	99.6 (98.5-99.9)	CAP
KAN	86.0 (77.9-91.5)	99.8 (98.7-100.0)	KAN
PZA	88.4 (84.5-91.5)	98.5 (96.0-99.5)	PZA
SM	98.8 (97.1-99.6)	95.9 (91.4-98.2)	SM
EMB	95.7 (93.1-97.4)	99.5 (97.0-100.0)	EMB
LZD	46.2 (27.1-66.3)	99.8 (98.9-100.0)	LZD
CLF	87.5 (70.1-95.9)	98.0 (96.5-98.9)	CLF
BDQ	83.9 (65.5-93.9)	97.7 (96.1-98.7)	BDQ

- Genoscreen: >95% sensitivity for RIF, INH, FQ, AMK, SM, and EMB; >83% for PZA, BDQ, CLF, CAP, and KAN
- GS performance on BDQ and CLF was above TPP minimum [84% sens, 98% spec]
- Performance on LZD is reduced [GS:46% sens, 99% spec
- Indicates performance will improve as knowledge of mutations associated with resistance is expanded

ONT ACCURACY

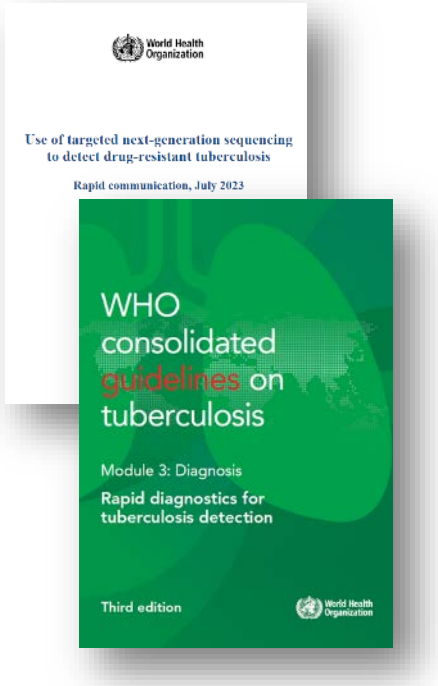


Drug	Sensitivity (95% CI)	Specificity (95% CI)	Drug
RIF	95.7 (93.5-97.2)	100 (96.0-100)	RIF
INH	95.5 (93.2-97.0)	99.3 (95.6-100)	INH
MOXI	94.8 (91.6-96.9)	99.4 (97.8-99.9)	MOXI
LFX	93.9 (90.5-96.2)	99.4 (97.8-99.9)	LFX
AMK	88.5 (77.2-94.9)	99.7 (98.7-99.9)	AMK
CAP	80.3 (67.8-89.0)	99.7 (98.6-99.9)	CAP
KAN	83.2 (74.7-89.3)	99.8 (98.7-100)	KAN
PZA	74.6 (69.7-78.9)	100 (98.1-100)	PZA
SM	78.6 (74.6-82.3)	90.2 (84.8-93.9)	SM
EMB	85.4 (81.7-88.5)	99.1 (96.5-99.8)	EMB
LZD	46.2 (27.1-66.3)	99.8 (98.9-100)	LZD
CLF	0.0 (0.0-13.3)	100 (99.2-100)	CLF
BDQ	5.9 (1.0-21.1)	99.7 (98.7-99.9)	BDQ

- ONT: >94% sensitivity for RIF, INH, and FQ; >80% for INJ, EMB; >75% for PZA, and SM
- Performance on BDQ & CLF was poor [6% sens, 99.8% spec]
- Performance on LZD is reduced [ONT:50% sens, 100% spec]
- Sequence data generated for targets → performance will improve with expanded ONT mutation list

TNGS GUIDELINE RELEASE

FROM EVIDENCE TO POLICY ON NGS FOR PATIENT CARE



- Rapid Communications issued by WHO Jul 2023 ahead of guidelines
- Final guidelines issued March 2024: **class-based recommendations on use of tNGS for DR-TB diagnosis**

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])*

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])

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

Where a product has not yet met the requirements for a specific drug (i.e., the drug is not listed), further improvements to the product are needed, and a review of the evidence is necessary before clinical use.

WHO consolidated guidelines on tuberculosis: rapid diagnostics for tuberculosis detection. 3rd ed. https://www.who.int/publications/i/item/9789240089488

WRD: WHO-recommended rapid diagnostics for TB and MDR-TB detection

MOVING FROM POLICY TO IMPLEMENTATION OF tNGS

User perspectives, economic and qualitative findings on tNGS

Acceptability
- ✓ Comprehensive
- ✓ Convenient
- ✓ Rapid and Adaptable

Challenges
- – Set-up, high technical complexity
- – Specialized infrastructure and expertise
- – Procurement and supply chain
- – Data management and storage
- – Routine update of the mutations catalogue

Values, preferences, and equity considerations
- Implications for access based on placement
- Affordability and cost-effectiveness

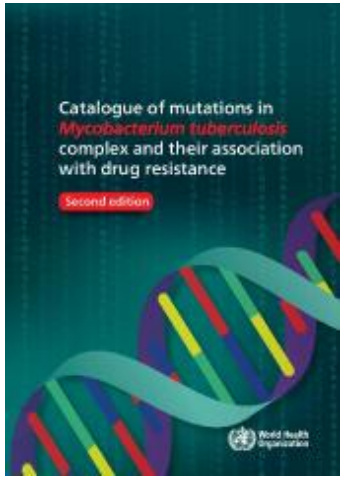
- **tNGS was cost-effective depending on context, costs varied by**
  - ▶ prevalence of resistance
  - ▶ current standard of care
  - ▶ potential impact of loss to follow-up
  - ▶ implementation approach
- **tNGS was acceptable and implementable** in routine programmatic conditions **despite inherent complexity**
- Optimal **implementation models and algorithms** will be **context dependent,** need further investigation

WHO consolidated guidelines on tuberculosis: rapid diagnostics for tuberculosis detection. Third edition. https://www.who.int/publications/i/item/9789240089488

TRANSLATING MUTATIONS TO RESISTANCE INFORMATION

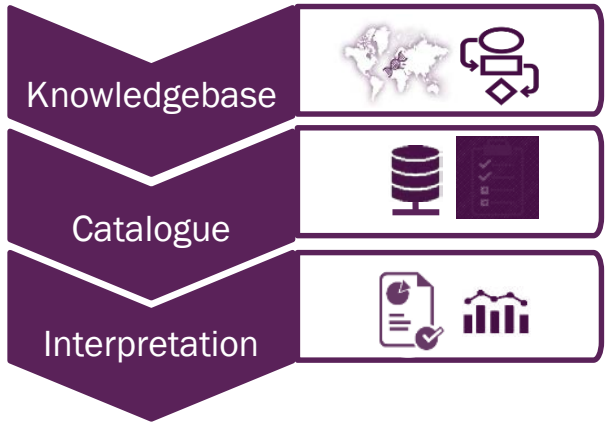
- The WHO now endorses **tNGS** for direct-from-sample resistance profiling across 10+ drugs
- But, tNGS identifies **mutations**, not **resistance** — clinical interpretation is essential to translate mutations into actionable treatment decisions
- **Not all mutations confer resistance** — some are benign, some uncertain, others indicate high or low-level resistance
- **Accurate interpretation is central to effective DR-TB care** — but it requires standardized tools and practical guidance

→ **Mutation Catalogue!**



BASIS FOR PREDICTING RESISTANCE FROM SEQUENCE

MUTATIONS CATALOGUE AND WHO TB KNOWLEDGEBASE



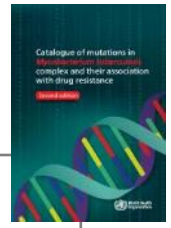
- Standardized data collection and analysis
- **v1** released 2021: 38,000 isolates from 40 countries
- **v2** released 2023: 53,000 isolates (+15,000)
  - Expanded geographic coverage
  - Mutations for BDQ, LZD, DLM, CFZ resistance added
- **v3 to be released ~June 2025**
  - ~82,000 clinical Mtb isolates (+ 29,000)
  - Approx 20,000 isolates w BDQ, LZD and DLM, with WGS & pDST data
  - Includes mutations associated with Pa resistance
- Powered by the **WHO TB Sequencing Knowledgebase** which collates global TB genotypic-phenotypic datasets

https://www.who.int/publications/i/item/9789240028173

IMPROVED PERFORMANCE WITH UPDATED MUTATION LISTS

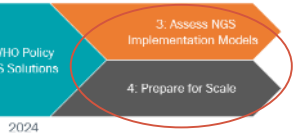
- Where tNGS sensitivity was low
  - Sequence data was generated for targets
  - Thus, performance will improve with added mutation-to-resistance calls
- Additional mutations in WHO mutation catalogue v2
  - >70% sensitivity of BDQ and CLF
  - As gene regions were amplified in the assay, only changes needed are in bioinformatics and reporting
- In case of drugs with low to no coverage in v2 – LZD, Pretomanid
  - Performance will likely improve as knowledge of mutations associated with resistance expands - v3
  - May require assay wet-lab changes to add genes or regions not yet covered

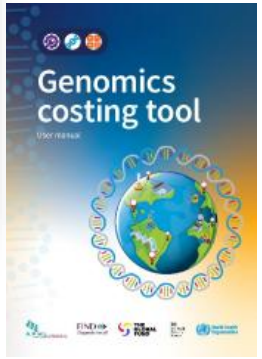
Seq&Treat Phase II performance of WGS compared to phenotypic DST reference based on 1st and 2nd editions of WHO mutations catalogue

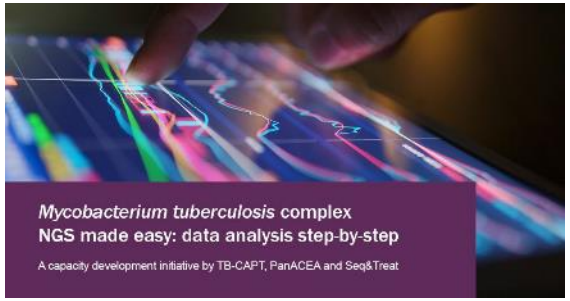


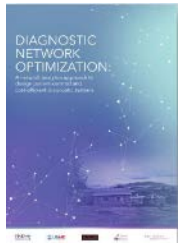
	Catalogue version 1		Catalogue version 2	
	v1 Sensitivity	v1 Specificity	v2 Sensitivity	v2 Specificity
RIF	0.98 (0.96, 0.99)	0.76 (0.69, 0.82)	0.98 (0.96, 0.99)	0.79 (0.73, 0.85)
INH	0.95 (0.93, 0.97)	0.99 (0.97, 1.00)	0.95 (0.93, 0.97)	0.99 (0.97, 1.00)
MOX	0.96 (0.94, 0.98)	0.98 (0.96, 0.99)	0.97 (0.94, 0.98)	0.97 (0.95, 0.98)
LEV	0.96 (0.92, 0.97)	0.98 (0.95, 0.99)	0.96 (0.93, 0.98)	0.97 (0.94, 0.98)
AMK	0.90 (0.79, 0.96)	1.00 (0.99, 1.00)	0.92 (0.81, 0.97)	1.00 (0.99, 1.00)
CAP	0.78 (0.65, 0.88)	0.99 (0.98, 1.00)	0.78 (0.65, 0.88)	0.99 (0.98, 0.99)
KAN	0.79 (0.69, 0.86)	0.96 (0.94, 0.97)	0.79 (0.70, 0.87)	0.96 (0.94, 0.97)
PZA	0.91 (0.88, 0.94)	0.94 (0.91, 0.96)	0.87 (0.83, 0.90)	0.96 (0.93, 0.98)
SM	0.92 (0.90, 0.95)	0.92 (0.88, 0.95)	0.92 (0.89, 0.94)	0.93 (0.88, 0.95)
EMB	0.87 (0.84, 0.90)	0.81 (0.76, 0.85)	0.84 (0.79, 0.87)	0.81 (0.76, 0.85)
LZD	0.39 (0.23, 0.58)	1.00 (0.99, 1.00)	0.52 (0.32, 0.71)	1.00 (0.99, 1.00)
CLF			0.78 (0.61, 0.89)	0.98 (0.96, 0.99)
BDQ			0.74 (0.58, 0.86)	0.98 (0.96, 0.99)

PRACTICAL CONSIDERATIONS FOR NGS IMPLEMENTATION AND SCALE







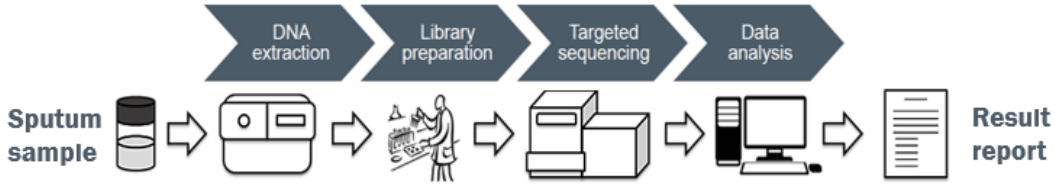


OptiDx.org

Shaheed's talk

- **Assess country readiness** for NGS implementation
- **Technical** and **operational guidance** for uptake of NGS for DR-TB diagnosis and surveillance
- **Training** in partnership with key stakeholders: WHO, National TB Programmes, implementers, advocacy
- TB NGS products included in **global procurement lists** with access pricing - Global Fund, GDF, UNDP
  - Genoscreen-Illumina partnership for kit packages and supply
  - ONT-bioMérieux partnership for supply and distribution
- **Cost-effectiveness** and impact modelling
- **Genomics costing tool** for country planning and forecasting

WHAT'S NEXT FOR NGS SOLUTIONS FOR DR-TB DIAGNOSIS?



Now

- ✓ Updated Mutations Catalogue(s) v3 and more as knowledge of targets and mutations associated with drug resistance grows
- ✓ Re-analysis of performance *in silico* for updated software-analytic pipelines

Future

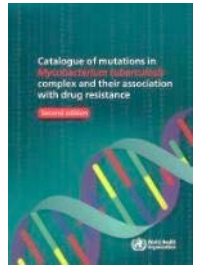
- ✓ Newer versions of tNGS assays with new gene targets based on updated Catalogue and/or growing knowledge of resistance mutations
- ✓ Generative AI and predictive analytics for future mutation catalogue updates

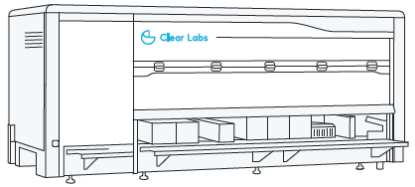
In development

- ✓ Increased level of **automation** at various steps in the workflow
- ✓ Simplified DNA **extraction** and processing
- ✓ Reduced number of steps in library preparation
- ✓ **Combined** sample and library prep
- ✓ **WGS direct** from sputum samples
- ✓ tNGS/WGS from **non-sputum** samples

ONT sensitivity vs. reference

Drug	Original pipeline	Updated pipeline
BDQ	5.9%	69.4%
CLF	0	66.7%





ABL Advanced Biological Laboratories

HaystackAnalytics

nature communications

Article https://doi.org/10.1038/s41467-025-57174-1

Multivariable regression models improve accuracy and sensitive grading of antibiotic resistance mutations in *Mycobacterium tuberculosis*

Sanjana G. Kulkarni[1], Sacha Laurent[2], Paolo Miotto[3], Timothy M. Walker[4,5], Leonid Chindelevitch[6], Carl-Michael Nathanson[7], Nazir Ismail[7,8], Timothy C. Rodwell[2,9] & Maha R. Farhat[1,10]

NIH RePORT RePORTER

A novel cartridge-based sequencing solution for decentralized M. tuberculosis resistance detection
1 R01AI177637-01 METCALFE, JOHN ZAPATA; CHAKRAVORTY, SOUMITESH UNIVERSITY OF CALIFORNIA, SAN FRANCISCO 2023 NIAID

Baseline pRescription According to Direct from Sputum Sequencing and TArgeted drug Concentration Strategy (BRASS TACS)
5R01AI168371-02 TORNHEIM, JEFFREY JOHNS HOPKINS UNIVERSITY 2024 NIAID

Automated Sequencing for Culture-free Diagnosis of Drug Resistant TB
5 R01AI176401-02 RODWELL, TIMOTHY CHARLES; COLMAN, REBECCA ELIZABETH UNIVERSITY OF CALIFORNIA, SAN DIEGO 2024 NIAID

ACKNOWLEDGEMENTS

Our work is made possible by our donors

alongside significant contributions from our private sector partners

our country and technical partners

- WHO GTB
- Ospedale San Raffaele Milan
- Mutation Catalogue Experts
- Brazil NTP, MoH
- Indonesia BGSi, NTP, MoH
- South Africa NTP, NICD
- Hinduja Hospital, Mumbai
- Georgia NTP
- UCSD
- DataArt
- Several Technical Consultants and Academic Partners

...

...and the FIND sequencing team

www.finddx.org/sequencing

Anita.suresh@finddx.org