

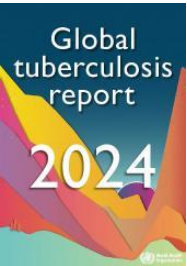
# FIND

## Closing the gap in drug-resistant TB diagnosis: the critical role of sequencing

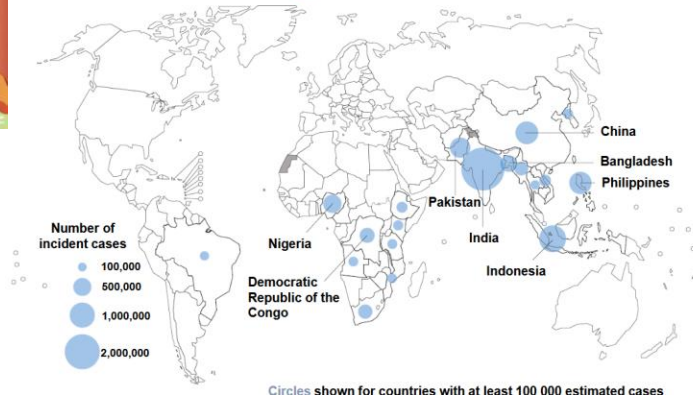
◆ Anita Suresh  
Head of Genomics & Sequencing Unit, FIND



# CRITICAL NEED FOR BETTER AND FASTER DIAGNOSTICS FOR DR-TB



**8 countries, 67% of global cases in 2023**  
**87% in 30 high TB burden countries**



## Updated treatment regimens for DR-TB

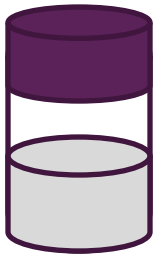
- **DS TB** – **HRZE** for 6 months
- **Hr TB** – **REZLv** for 6 months
- **RR/MDR TB** – **BPaLM/BPaMZ** for 6 months
- **Pre-XDR TB** – **BPaL** for 6-9 months

- TB is treatable and curable - yet, it remains the **leading cause of death** from an infectious disease
- **10.8 million** people fell ill with TB; **1.25 million** people died of TB
- **400,000** people developed drug-resistant TB (DR-TB) in 2023
- Universal access to comprehensive resistance testing not yet achieved
  - **<50%** DR-TB patients get diagnosed
- Early detection and optimal treatment critical
  - Get people on the right treatment from the start
- New drugs and shorter regimens now available
  - Need to **preserve the efficacy of these drug regimens** through timely and comprehensive drug susceptibility testing

# tNGS VS WGS: THE TB USE CASE

## tNGS for drug resistance detection

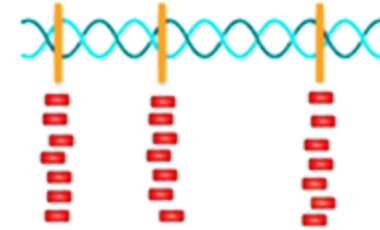
Sputum Sample



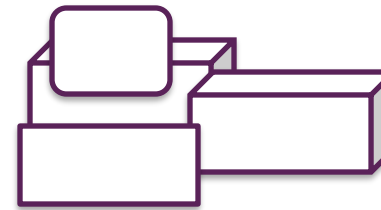
Extract DNA

Human cells  
Bacterial cells  
MTB cells

PCR Enrichment  
& Lib Prep



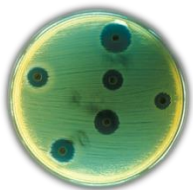
Sequence



Interpret specific variants from amplicons to predict phenotypic resistance

## WGS for surveillance

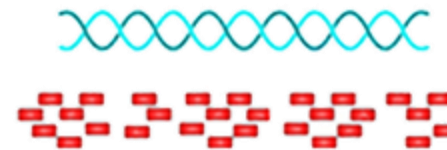
TB Culture



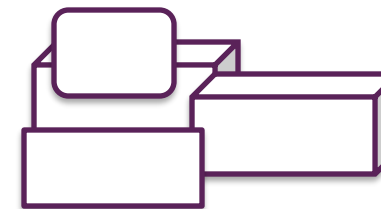
Extract DNA

MTB cells

Fragment & Lib Prep



Sequence

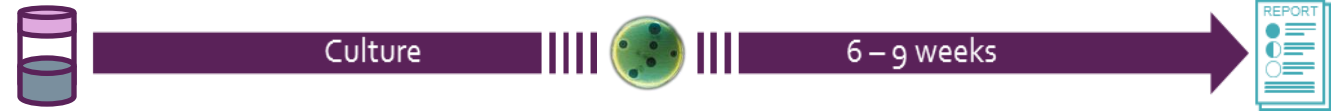


Collate ALL variants across genome for surveillance, research and transmission mapping

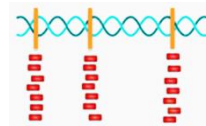
# TARGETED NGS ENABLES FAST & COMPREHENSIVE DR-TB TESTING



**Phenotypic DST**  
(current standard of care)



**Whole-genome sequencing**



**Targeted sequencing**



## Why Targeted NGS for TB AMR?

- Strong genotype → phenotype correlations in TB
- High-throughput, scalable, lower biosafety requirements
- Faster results to inform clinical decision-making
- Adaptable to new genes and mutations
- Multi-disease platform

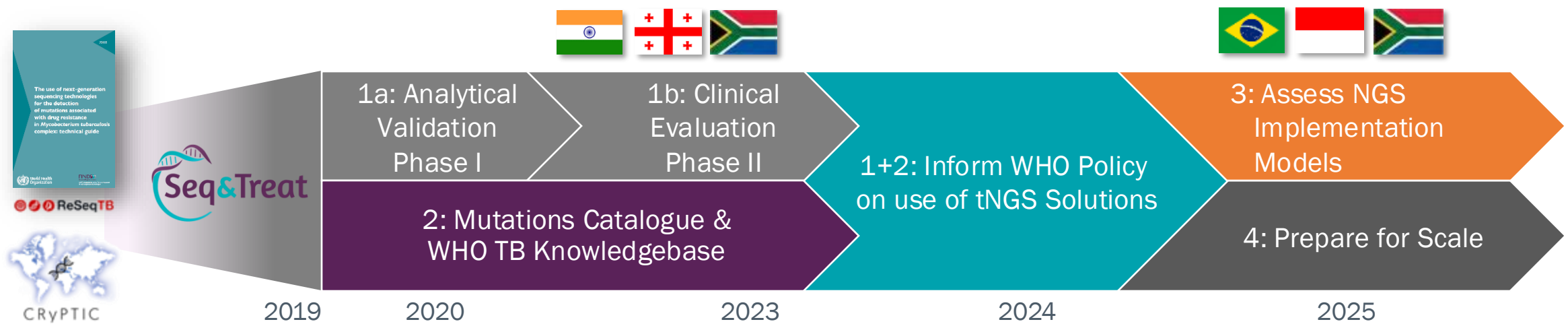


## A PARADIGM SHIFT

Targeted NGS-based **diagnosis** of **DR-TB** direct from clinical samples

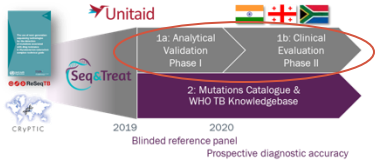
# Seq&Treat RAPID, COMPREHENSIVE tNGS SOLUTIONS FOR DR-TB DIAGNOSIS

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



# SEQ&TREAT OUTPUT 1- EVIDENCE GENERATION FOR TNGS SOLUTIONS

## ANALYTICAL AND CLINICAL EVALUATION OF TNGS FOR DR-TB DETECTION



- **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



# TARGETED NGS END-TO-END SOLUTIONS EVALUATED

## GENOSCREEN AND ONT TNGS WORKFLOWS



Instrument	Thermocycler					
	Promega Maxwell	Qubit Fluorometer	Thermocycler	Qubit Fluorometer	Illumina MiSeq Sequencer	Deeplex cloud-based analysis
<b>Genoscreen</b>	<ol style="list-style-type: none"> <li>200uL of sediment</li> <li>2x Lysis Buffer</li> <li>Maxwell® FFPE Plus DNA Kit</li> <li>Eluted in 50uL molecular water</li> </ol>	<ol style="list-style-type: none"> <li>Quantify DNA</li> <li>One reaction Multiplex PCR</li> <li>Bead-based clean-up</li> <li>Quantify PCR product</li> </ol>	<ol style="list-style-type: none"> <li>Illumina Nextera XT library preparation</li> <li>Bead-based clean-up</li> </ol>	<ol style="list-style-type: none"> <li>Quantify library product</li> <li>Pool libraries</li> <li>45 samples + 3 controls</li> </ol>	<ol style="list-style-type: none"> <li>Denature and dilute library pool</li> <li>Load onto sequencer</li> <li>Run sequencer</li> </ol>	<ol style="list-style-type: none"> <li>Load data to Deeplex web application</li> <li>Run pipeline</li> <li>Control validation</li> <li>View results</li> </ol>
<b>ONT</b>	<ol style="list-style-type: none"> <li>700uL of sediment</li> <li>Bead beating</li> <li>Maxwell RSC PureFood Pathogen Kit</li> <li>Eluted in 50uL elution buffer</li> </ol>	<ol style="list-style-type: none"> <li>One reaction Multiplex PCR</li> </ol>	<ol style="list-style-type: none"> <li>ONT rapid barcode library preparation</li> </ol>	<ol style="list-style-type: none"> <li>Pool libraries</li> <li>22 samples + 2 controls</li> <li>Bead-based clean-up</li> <li>Quantify pool</li> <li>Add adapter</li> </ol>	<ol style="list-style-type: none"> <li>Prime flowcell</li> <li>Load MinION flowcell</li> <li>Run sequencer</li> </ol>	<ol style="list-style-type: none"> <li>Load data to server</li> <li>Run pipeline</li> <li>Control validation</li> <li>View results</li> </ol>
<b>Instrument</b>	Promega Maxwell FastPrep-24 5G homogenizer	Thermocycler	Thermocycler	Qubit Fluorometer	ONT MinION Sequencer	ONT local instance of software analysis



Sputum sample



DNA extraction



Mtb target amplification



Library preparation



Pool samples



Sequence



Bioinformatics



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.

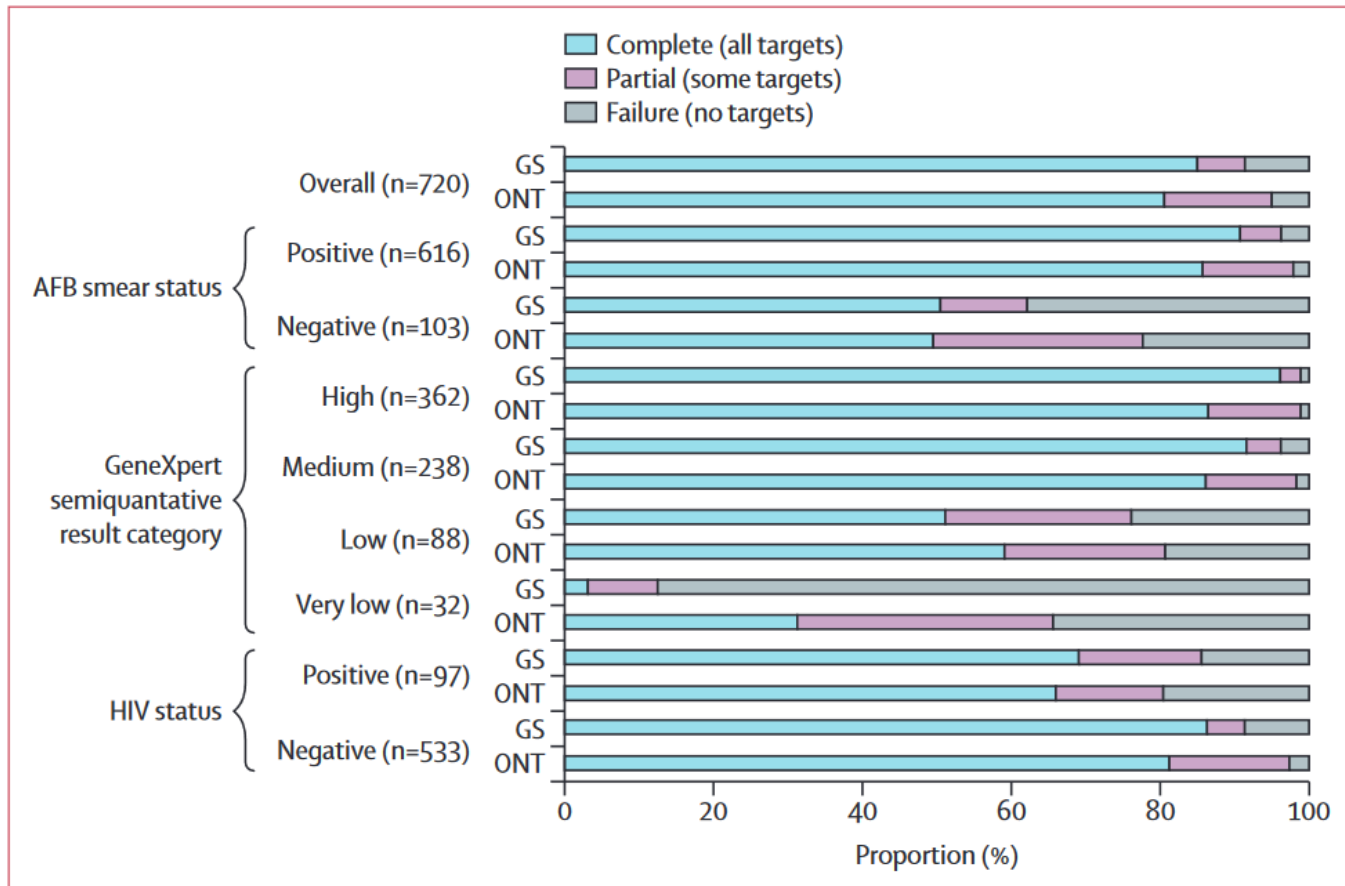
**Lancet Infect Dis 2024**  
 Published Online  
 October 29, 2024  
[https://doi.org/10.1016/S1473-3099\(24\)00586-3](https://doi.org/10.1016/S1473-3099(24)00586-3)

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.

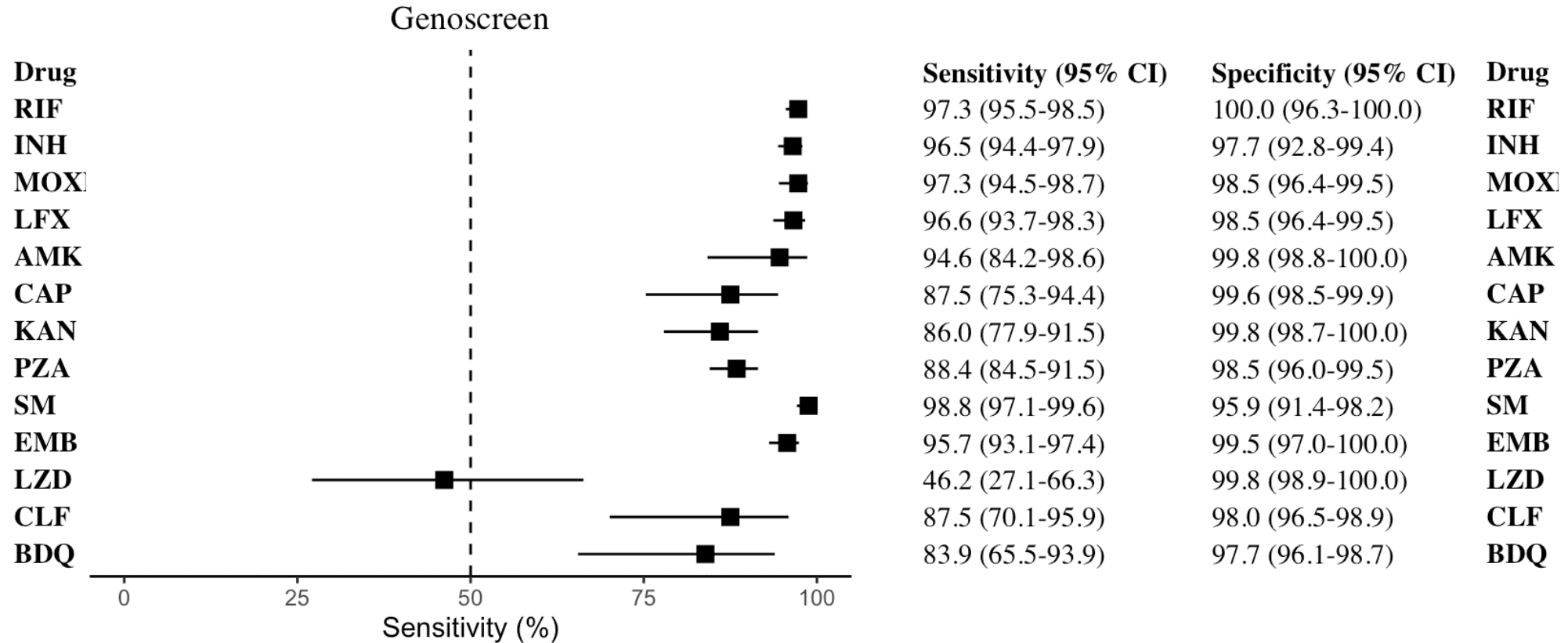
# RESULTS SUMMARY



- 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

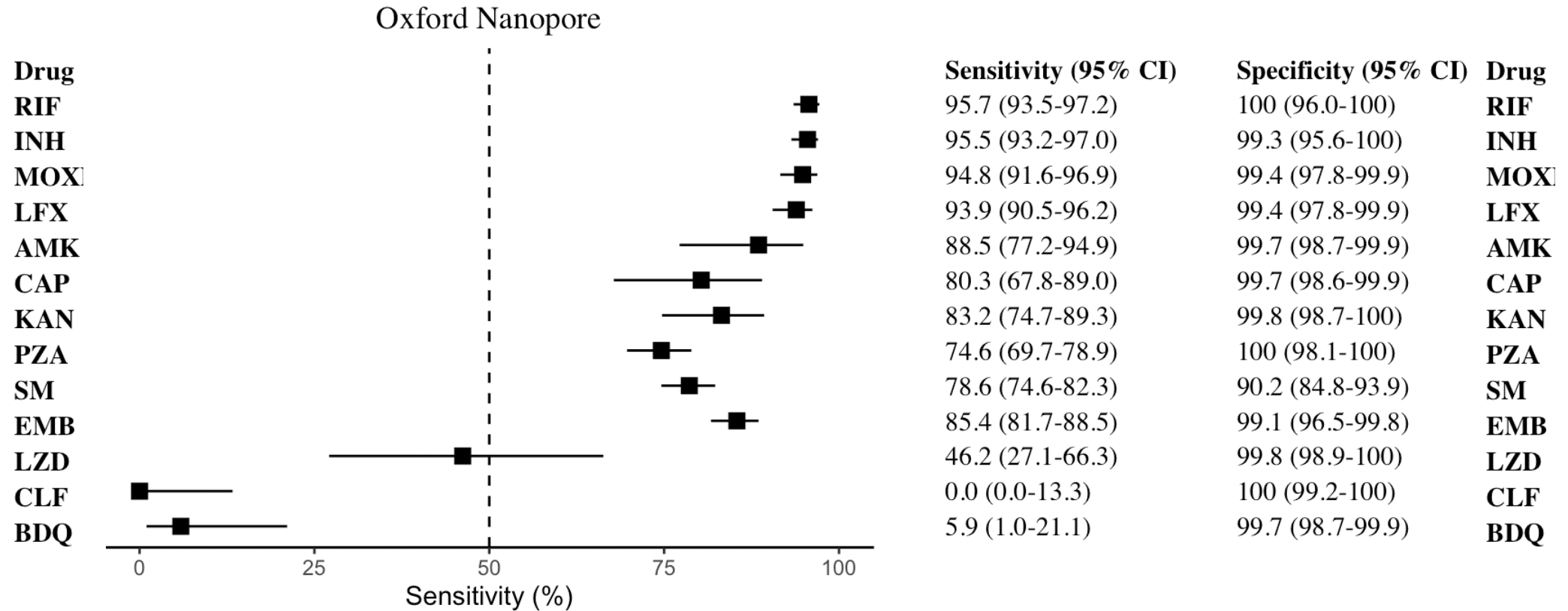
Figure 2: Sequencing success by bacterial load proxies (AFB smear status, study GeneXpert semiquantitative result category, and HIV status)

# GENOSCREEN ACCURACY



- 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**

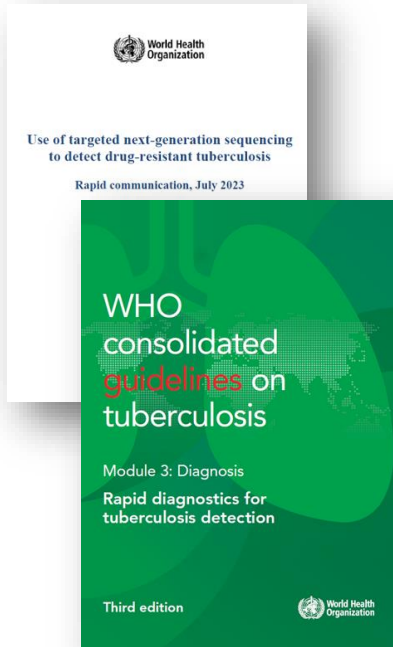
# ONT ACCURACY



- 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.

- Priority should be assigned to those at higher risk of resistance to first-line treatment medications
- Conditional due to lack of data on health benefits, the variable certainty of evidence on diagnostic accuracy, and the fact that accuracy is suboptimal for certain drugs
- 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.

# MOVING FROM POLICY TO IMPLEMENTATION OF tNGS

## User perspectives, economic and qualitative findings on tNGS

### Acceptability

- ✓ Comprehensive
- ✓ Convenient
- ✓ Rapid and Adaptable

### Challenges

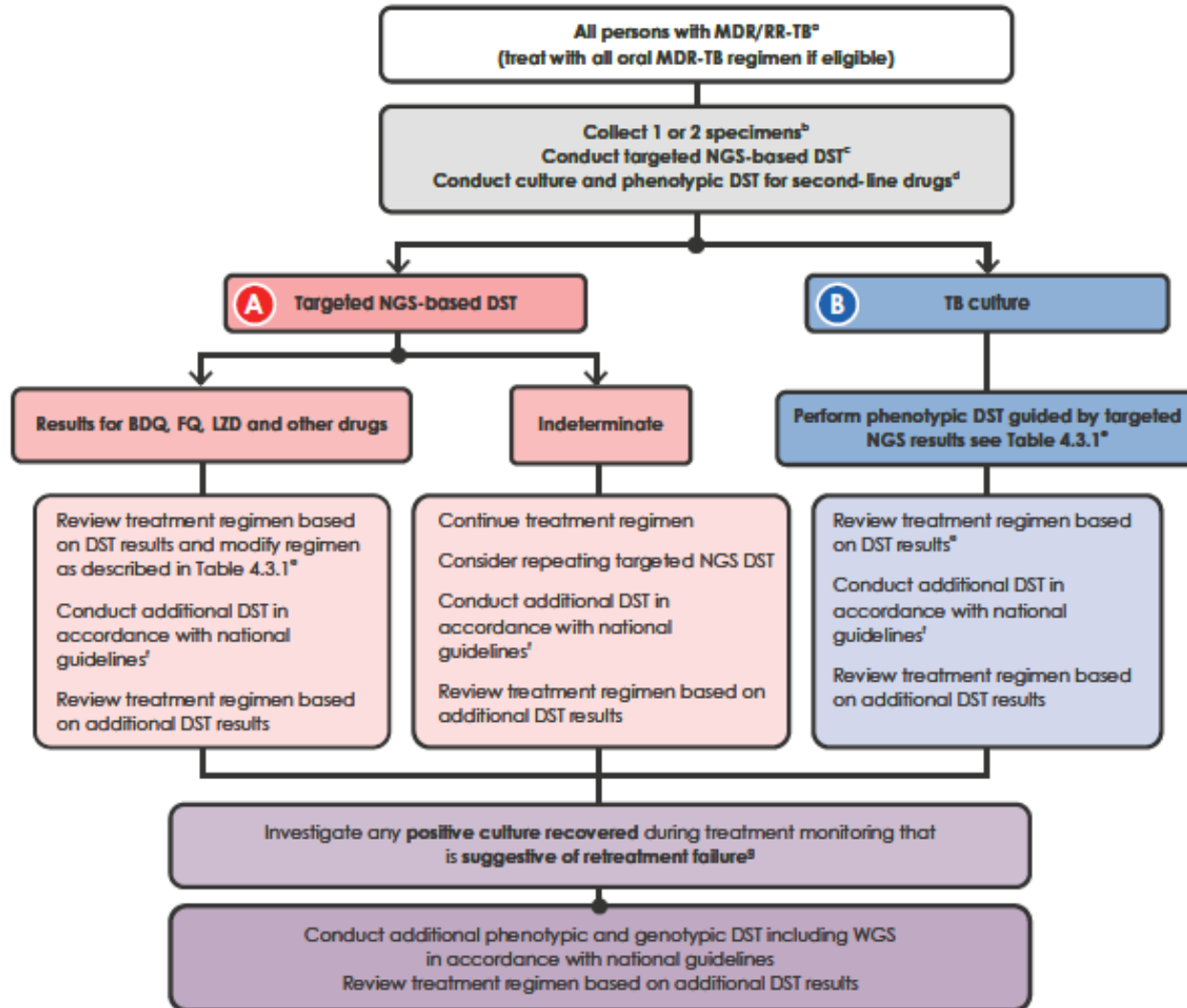
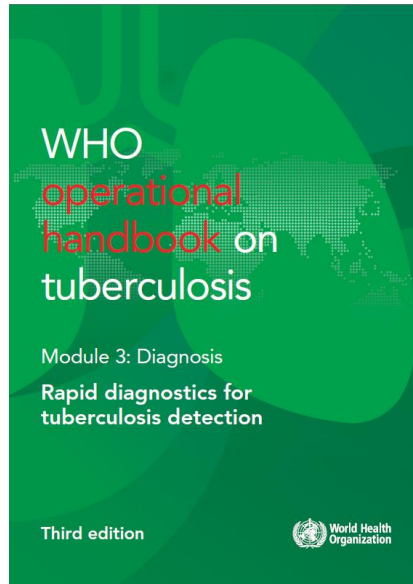
- Set-up, high technical complexity
- Specialized infrastructure and expertise
- Procurement and supply chain
- Data management and storage
- Routine update of the mutations catalogue

### 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

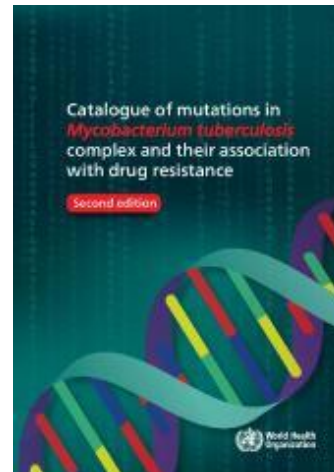
# WHERE DOES tNGS FIT IN THE DR-TB TESTING ALGORITHM?



## 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!

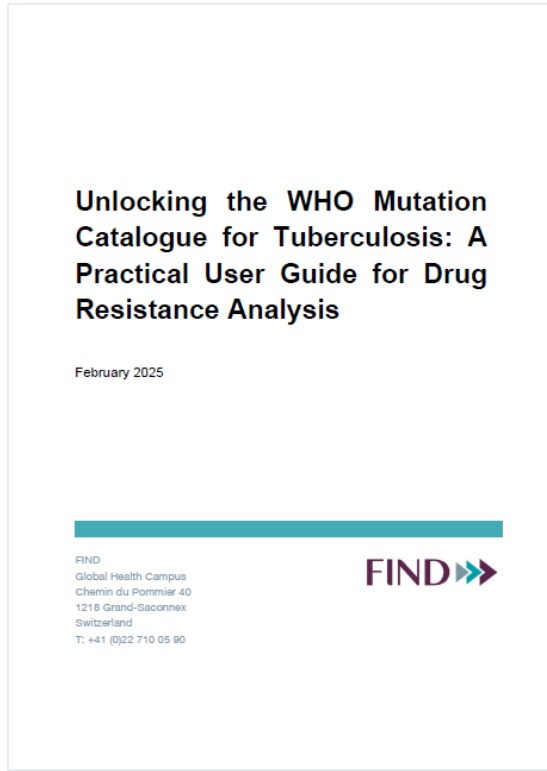


# BASIS FOR PREDICTING RESISTANCE FROM SEQUENCE MUTATIONS CATALOGUE AND WHO TB KNOWLEDGEBASE



- ◆ 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

# MAKING THE MUTATION CATALOGUE MORE ACCESSIBLE



What Makes the Catalogue Powerful – Also Makes it Challenging to Use

## Key Benefits of the Catalogue User Guide

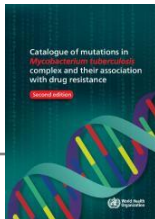
- **Simplified Mutation Tables**
  - Filterable layout with only final grading
- **Mutation Grading Explained**
  - Groups and Clinical Actions
- **Additional Grading Rules Decoded**
  - Includes examples and common use cases

# 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 the gene regions were covered in the assay for amplification, only changes needed are in bioinformatics and reporting
- In case of drugs with low to no coverage in catalogue 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



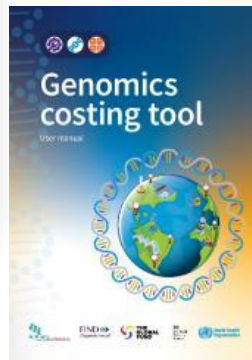
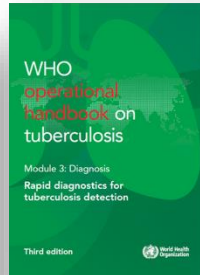
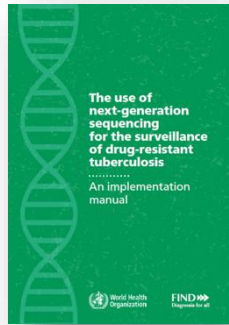
Seq&Treat Phase II performance of **WGS** compared to **phenotypic DST** reference based on 1<sup>st</sup> and 2<sup>nd</sup> editions of WHO mutations catalogue



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

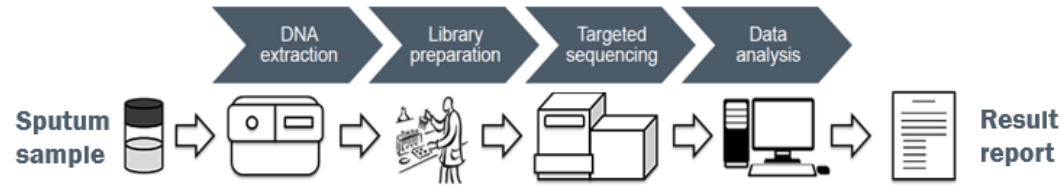
# SEQ&TREAT OUTPUTS 3 AND 4

## PRACTICAL CONSIDERATIONS FOR NGS IMPLEMENTATION AND SCALE



- ◆ **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
- ◆ Manufacturer engagement and **market shaping** to improve access
- ◆ **Genomics costing tool** for country planning and forecasting

# EXPECTED IMPROVEMENTS IN END-TO-END tNGS WORKFLOWS FOR TB

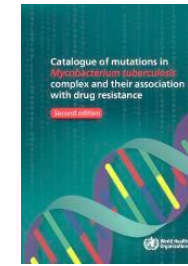


## ONT sensitivity vs. reference

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

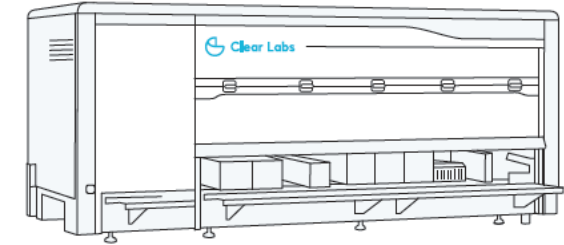
Now

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



Future

- ✓ Newer versions of tNGS assays with new gene targets based on updates to Catalogue and/or growing knowledge of novel targets and resistance mutations
- ✓ Generative AI for 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

### NIH RePORT RePORTER

<b>A novel cartridge-based sequencing solution for decentralized M. tuberculosis resistance detection</b>					
1	R01AI177637-01	METCALFE, JOHN ZAPATA CHAKRAVORTY, SOUMITESH	UNIVERSITY OF CALIFORNIA, SAN FRANCISCO	2023	NIAID
<b>Handheld and population-based sequencing for rapid detection of new and repurposed drug resistance in M. tuberculosis</b>					
5	R01AI153213-03	METCALFE, JOHN ZAPATA ENGELTHALER, DAVID M.	UNIVERSITY OF CALIFORNIA, SAN FRANCISCO	2024	NIAID
<b>Automated Sequencing for Culture-free Diagnosis of Drug Resistant TB</b>					
5	R01AI176481-02	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



...and the FIND sequencing team



- WHO GTB
- Ospedale San Raffaele Milan
- Mutation Catalogue Experts
- Brazil NTP, MoH
- Indonesia BGSi, NTP, MoH
- South Africa NTP, NICD
- UCSD
- DataArt
- Several Technical Consultants



[www.finddx.org/sequencing](http://www.finddx.org/sequencing)

[Anita.suresh@finddx.org](mailto:Anita.suresh@finddx.org)

...

FIND 



BACKUP

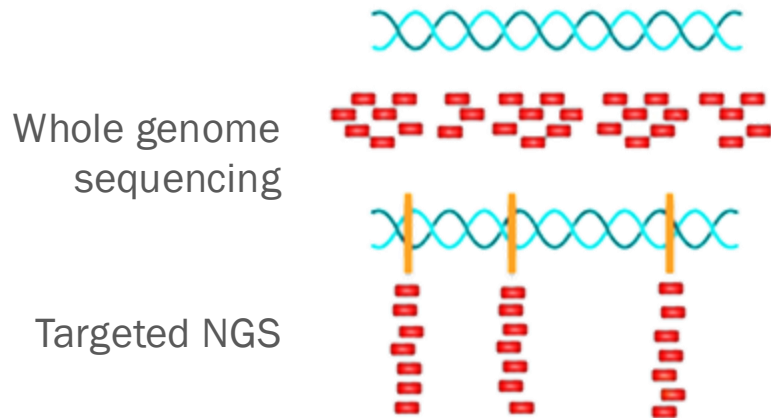
# SEQUENCING CHEAT SHEET

**Genomics:** study of an organism's entire genome = all its genetic material, DNA + RNA

**Sequencing:** process of determining the precise order of nucleotides (A, C, G, and T) in a sample of DNA or RNA

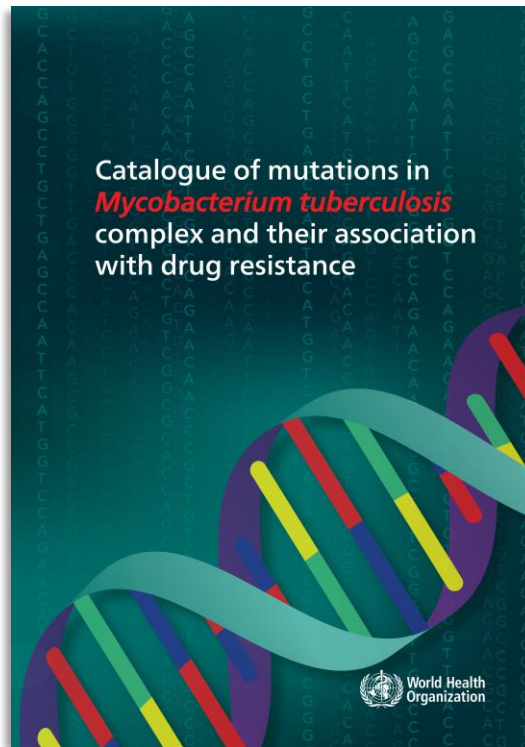
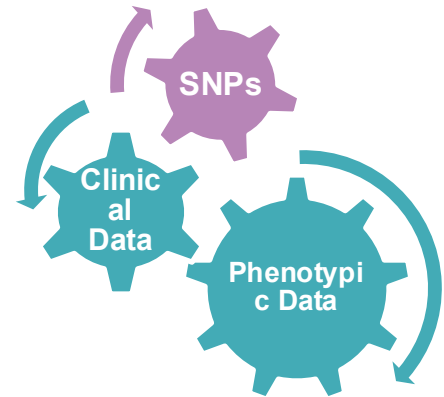
**Next-generation sequencing (NGS):** massively parallel sequencing of large amounts of DNA or RNA, can identify variants or mutations across 1000s of target regions in 1 test

<p><b>qPCR</b> qPCR analyses <b>limited</b> variants/mutations at specific locations</p>	<p><b>Targeted NGS</b> Targeted NGS simultaneously analyses <b>100s-1000s</b> of genes</p>
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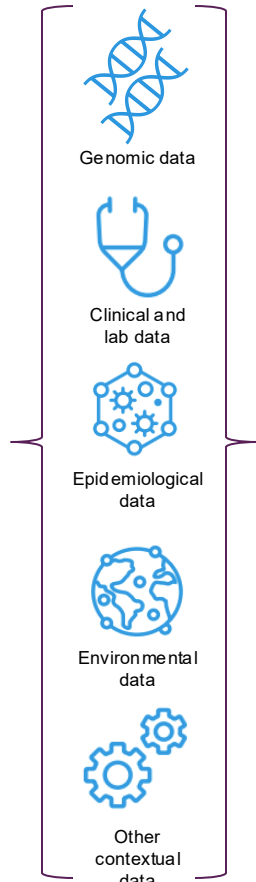
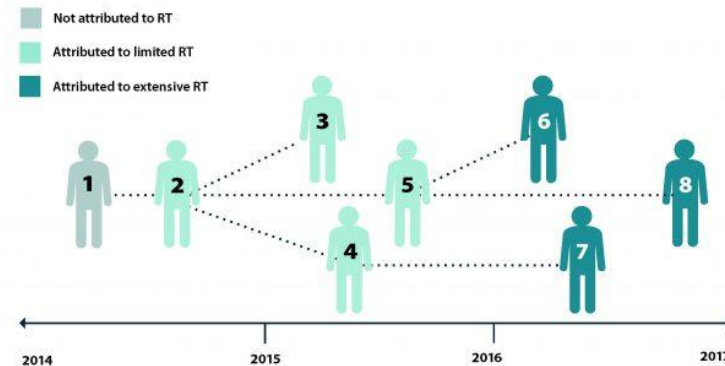
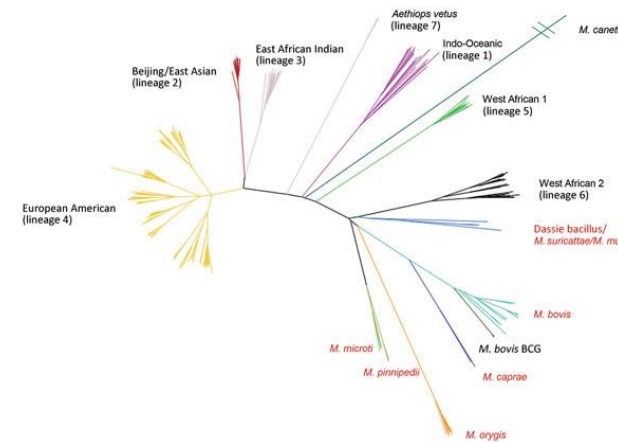


# THE TB USE CASE: WHY WGS?

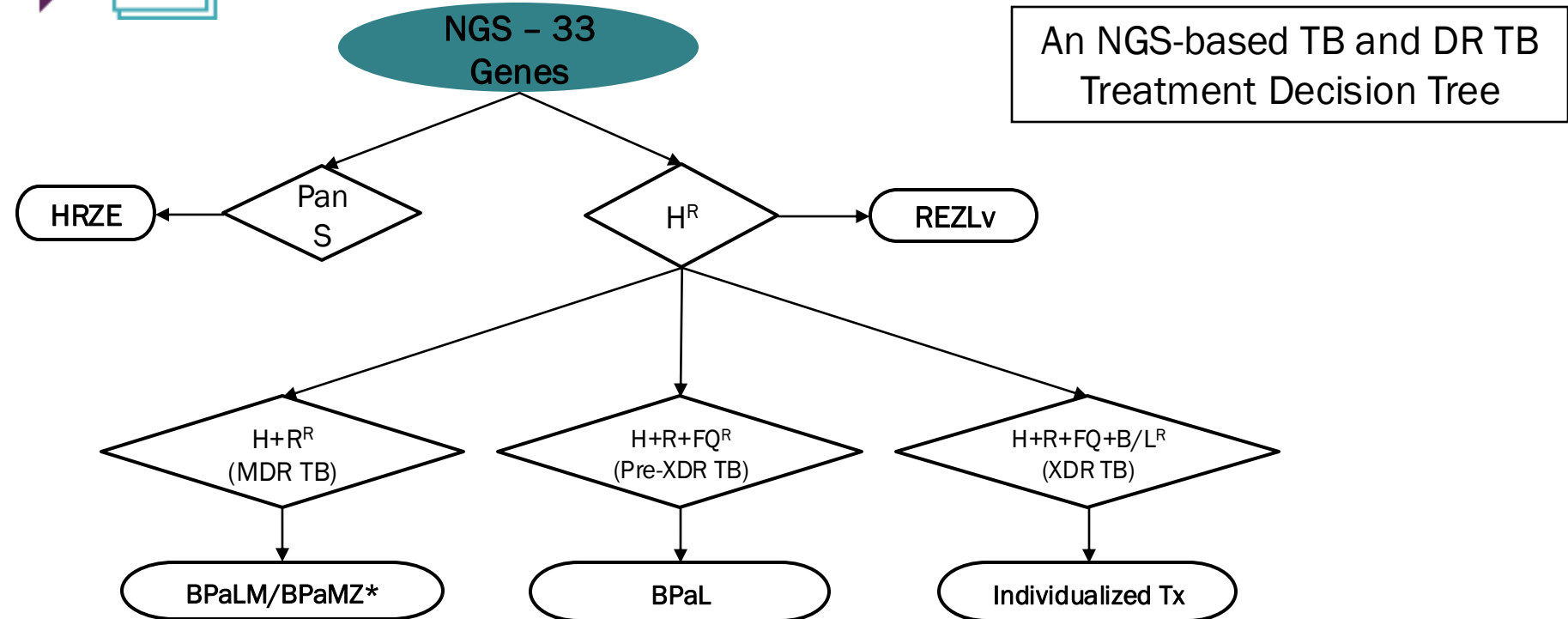
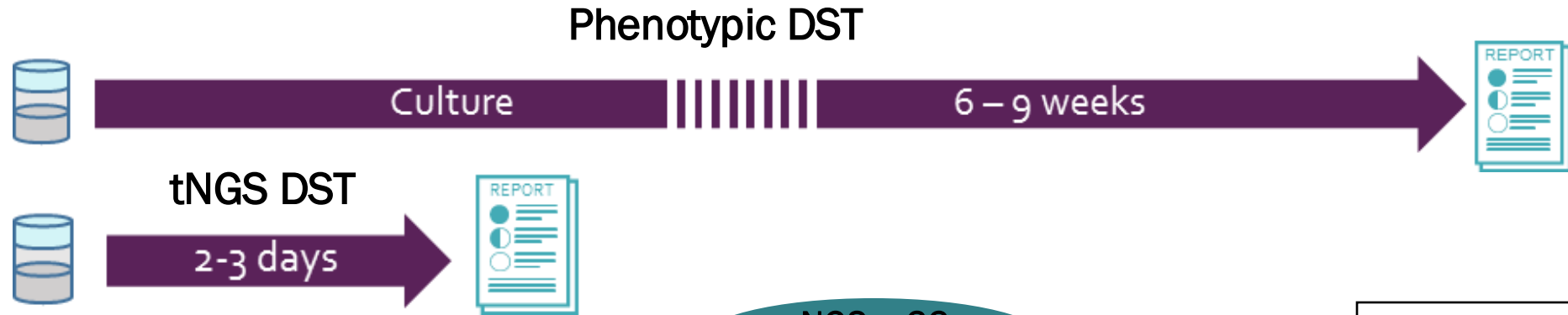
Building knowledge for interpreting variants and understanding the genotypic basis of observed drug resistant phenotypes



Mapping TB strain relatedness and resistance profiles to understand trends, and to track and interrupt TB transmission

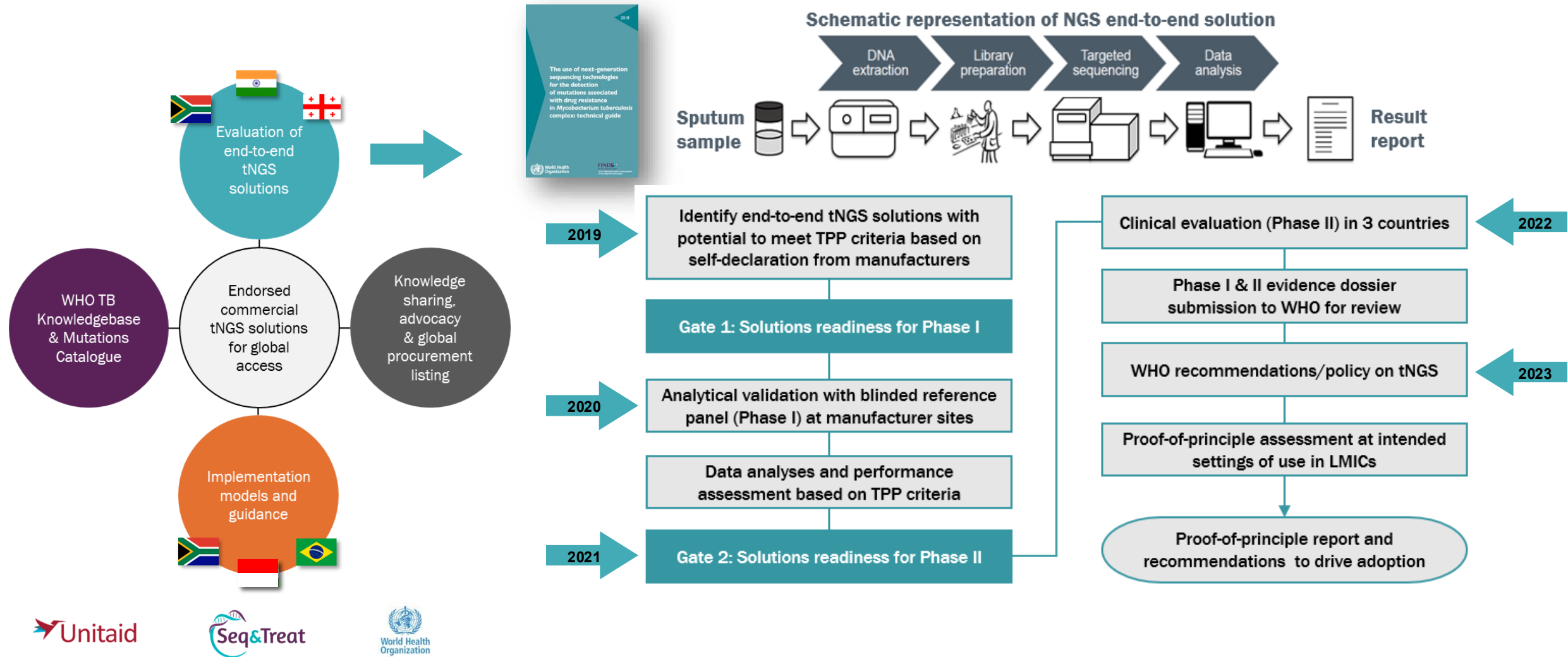


# THE TB USE CASE: WHY tNGS?



# SEQ&TREAT OUTPUT 1

## EVIDENCE GENERATION FOR END-TO-END tNGS SOLUTIONS



# SEQUENCING DELIVERY MODELS



## Centralized

- Established sample referral network
- High-throughput sequencers
- Data management and reporting



## Decentralized

- Geographic distance/spread, e.g. islands
- Low throughput, benchtop sequencing
- Centralized, local or cloud-based analysis



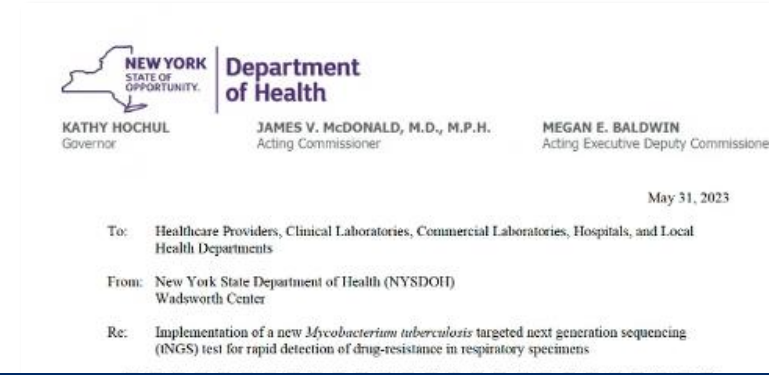
## Service model

- Send out samples or DNA in country or region
- Sequencing service providers
- Core sequencing facility (cross-programme)
- Low cost, high throughput setup

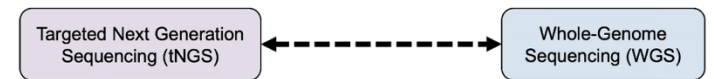
# PROGRAMMATIC IMPLEMENTATION OF NGS FOR DR-TB



- tNGS implementation and operational assessment in **Brazil** with NTP and MoH
- National genomics pilot implementation for DR-TB in **Indonesia** with MoH, BGSi and NTP
  - Support for Unpad-West Java Provincial Health Lab tNGS implementation
- Programmatic implementation pilot in **South Africa** with NTP
- tNGS Implementers Network
  - Learning and knowledge-sharing
- Ongoing tNGS programmatic implementation in other countries – eSwatini, Bangladesh, Kyrgyzstan



## Impact of tNGS as an early detection method for drug-resistant MTBC



Average **15 day** improvement for primary specimens;  
*First time NYS has detected XDR TB directly from primary specimen*

Average **43 day** improvement for contaminated cultures

Stat results available in < 24 hr



The tNGS assay will largely replace pyrosequencing in our current testing algorithm. WGS testing will still be performed on all MTBC culture-positive isolates for more comprehensive drug resistance prediction and for genotyping and surveillance to determine recent transmission.