

Programmatic implementation of targeted next-generation sequencing (tNGS) for DR-TB management in South Africa

From pilot validation to routine national integration of an end-to-end Deeplex tNGS solution

Dr Shaheed V Omar
Centre Head
Centre for Tuberculosis, NICD/NHLS
National TB Reference Laboratory
WHO Supranational Reference Laboratory
Johannesburg, South Africa

Why tNGS? tNGS WORKSTREAM BACKGROUND

- WHO-recommended platform for comprehensive detection of drug-resistance–associated mutations
- Mutation-level resistance profiling across multiple drug classes
- Seq&Treat Multi-country Study demonstrated clinical and operational feasibility for DR-TB management
- The South African implementation transitions tNGS from pilot validation to routine national integration

THE SOUTH AFRICAN CONTEXT

- Among the first LMICs implementing routine tNGS for DR-TB
- Integrated within the national DR-TB reflex testing algorithm
- ~7,000 DR-TB patients annually eligible for reflex resistance testing
- BDQ resistance (~6.5%) increases the need for rapid, comprehensive profiling

OBJECTIVE: establish programmatic tNGS capacity for all RR/DR-TB patients



Use of targeted next-generation sequencing
to detect drug-resistant tuberculosis

Rapid communication, July 2023

Comprehensive DST Matrix

Simultaneous, 13-drug
susceptibility profiling
covering core first-line
(INH, RIF), FQ, SLID, and
critical newer agents
(BDQ, LZD, CFZ).



Addressing Bedaquiline Resistance
Monday 29 April, 09:00 – 10:00

Introduction:
South Africa ranks amongst the countries with the highest burden of TB, drug-resistant TB, and TB/HIV co-infection. In September 2023, the National TB Control and Management Programme (NCTM) successfully launched the new BDRx, a treatment regimen for drug-resistant TB (DR-TB) in First Line. The new BDRx, a DR-TB treatment regimen requires the patient to complete the medication over a shorter period (18 months) of time compared to previous DR-TB treatment regimens implemented in the National TB Programme.

The introduction of new and improved TB drugs has helped significantly improve the prognosis of cured DR-TB patients. However, evidence is emerging of bedaquiline resistance. This leads to treatment failure and new drug-resistant TB. While most new and improved drugs have proven to have a low resistance profile, we have noted that one of our drugs called bedaquiline has shown increase in resistance. Resistance to bedaquiline is worrying because of inadequate adherence to TB treatment, particularly in some of our high-risk sites like the Cape Town. The National TB Programme has established a rigorous monitoring system, and we have started to implement a plan to manage bedaquiline resistance among DR-TB patients.

Plan to Address Bedaquiline Resistance aligned to the TB Recovery Plan Pillars

Pillar I: Communicate & Advocate	Pillar II: Find & Link	Pillar III: Treat & Refer	Pillar IV: Monitor & Prepare	Pillar V: Monitor & Assess
Enable adherence of DR-TB patients Active health management and patient engagement in sites and education for medication Regular monitoring of adherence and report routine testing of BDQ resistance Responsible management of DR-TB patients Disseminate data among healthcare workers in South Africa and globally	Enable diagnosis of DR-TB patients Urgent introduction of targeted next-generation sequencing (tNGS) to detect bedaquiline resistance in DR-TB patients Strengthen TB testing (Tb-Hist) in the National TB Programme Strengthen TB testing (Tb-Hist) in the National TB Programme Strengthen TB testing (Tb-Hist) in the National TB Programme	Strengthen treatment for DR-TB patients Urgent introduction of targeted next-generation sequencing (tNGS) to detect bedaquiline resistance in DR-TB patients Strengthen TB testing (Tb-Hist) in the National TB Programme Strengthen TB testing (Tb-Hist) in the National TB Programme	Strengthen monitoring of DR-TB patients Urgent introduction of targeted next-generation sequencing (tNGS) to detect bedaquiline resistance in DR-TB patients Strengthen TB testing (Tb-Hist) in the National TB Programme Strengthen TB testing (Tb-Hist) in the National TB Programme	Strengthen assessment of DR-TB patients Urgent introduction of targeted next-generation sequencing (tNGS) to detect bedaquiline resistance in DR-TB patients Strengthen TB testing (Tb-Hist) in the National TB Programme Strengthen TB testing (Tb-Hist) in the National TB Programme

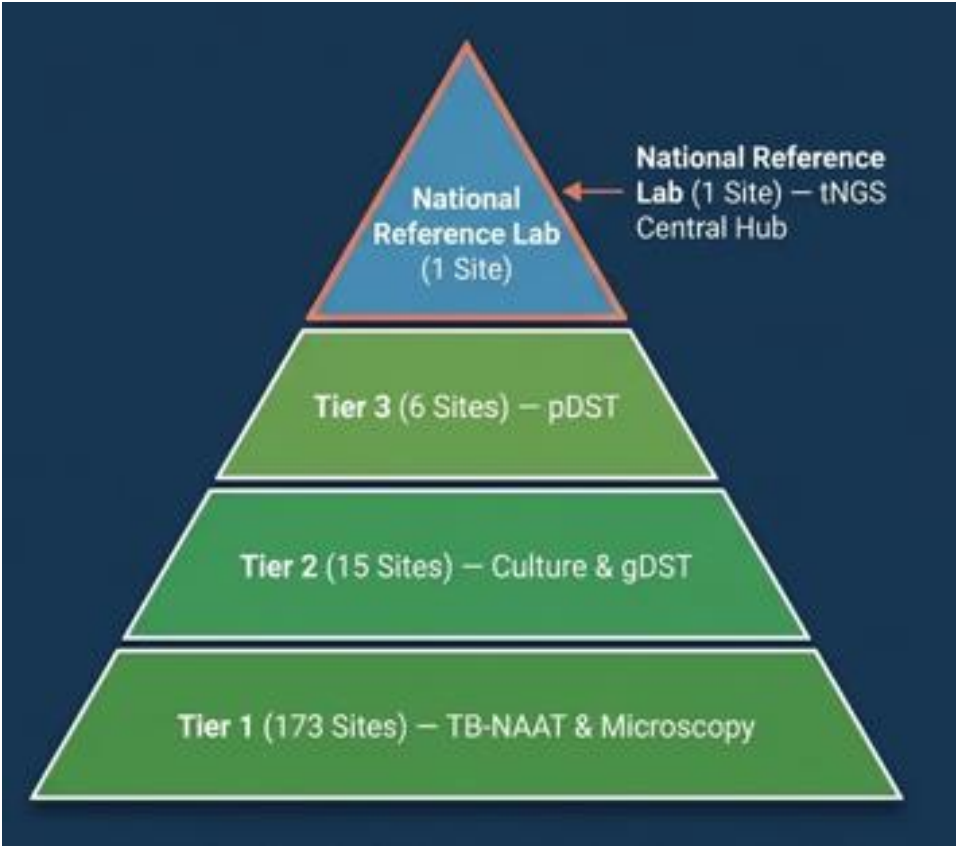
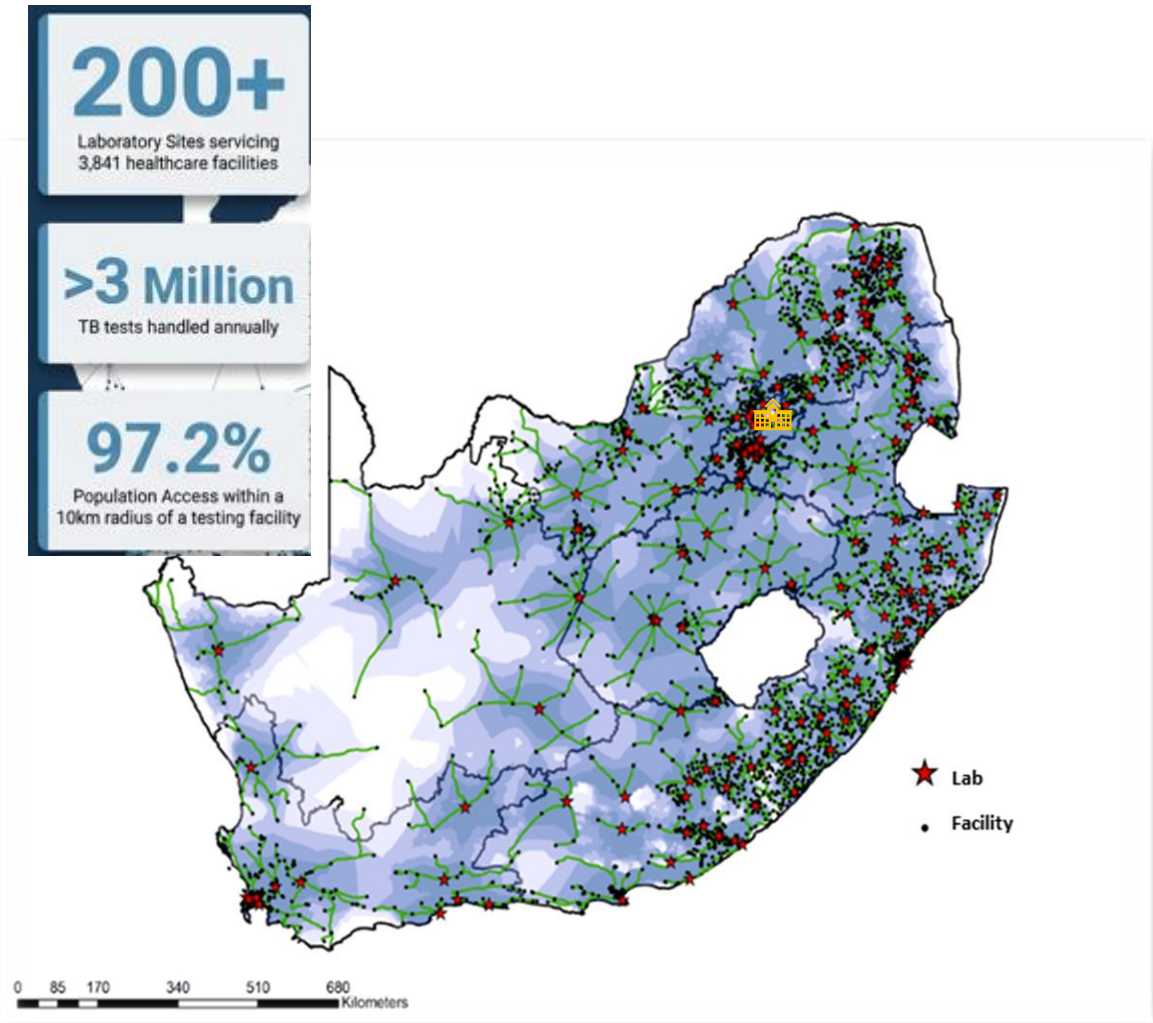
Pillar II Find & Link

People with TB
are linked to care
within one week

Accelerate diagnosis of BDQ resistance

Urgent introduction
of targeted
next generation
sequencing,
starting with most
affected areas

SOUTH AFRICA Diagnostic Network



A PHASED approach from validation to sustainable integration

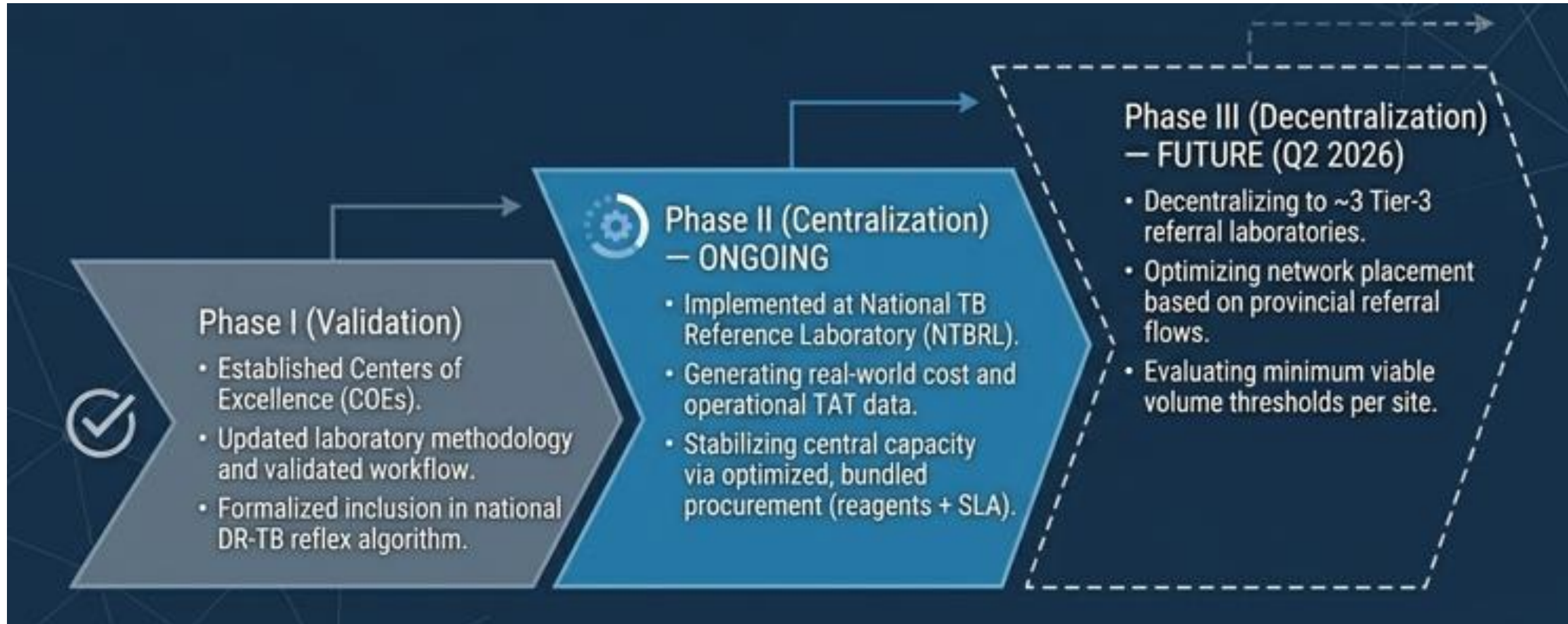


health

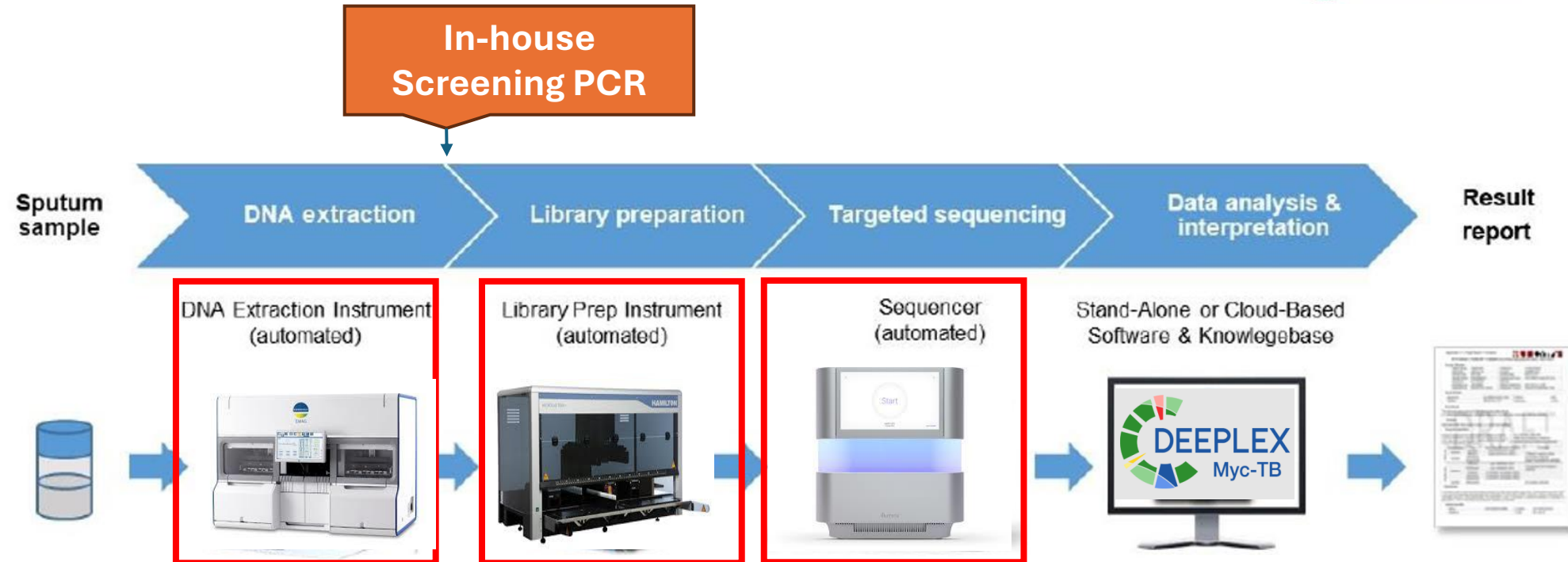
Department:
Health
REPUBLIC OF SOUTH AFRICA



NATIONAL INSTITUTE FOR
COMMUNICABLE DISEASES
Division of the National Health Laboratory Service



National Validation – Workflow Validation



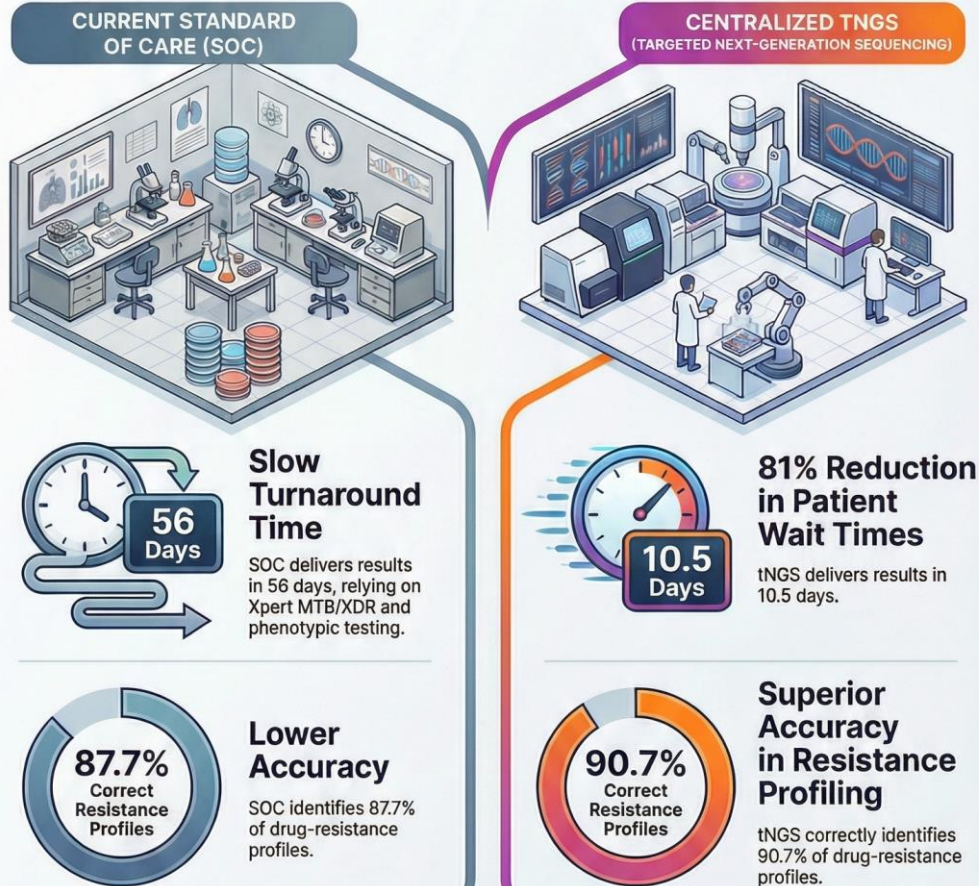
*images of example instruments for illustrative purposes only

Validations performed due to deviation for WHO Recommended END-TO-END Solutions:

- ✓ DNA Extraction System
- ✓ Automated Library Preparation
- ✓ Sequencing Platform

tNGS vs. Standard of Care: A Faster, More Accurate Future for TB Testing in South Africa

« A Paradigm Shift in TB Diagnostics »»



Cost-Effectiveness of tNGS Compared to Standard of Care (South African Context)

\$50

Cost Savings per Patient

20%

Reduction vs SOC

- Economic Analysis: Health system cost-effectiveness study compared tNGS to standard phenotypic DST for rifampicin-resistant TB patients in South Africa
- Targeted NGS showed lower cost per patient (\$195 USD) versus standard phenotypic DST (\$245 USD), achieving \$50 savings per patient tested
- Cost advantage driven by faster turnaround reducing pre-treatment default, fewer repeat tests, and elimination of prolonged culture infrastructure requirements
- Health System Impact: At 7,000 DR-TB patients annually, tNGS adoption could generate estimated \$350,000 USD annual savings while improving diagnostic speed

de Nooy A, Omar SV, Ockhuisen T et al. Cost-effectiveness of Targeted Next-Generation Sequencing for Tuberculosis Drug-resistance Testing as an Alternative to the Standard of Care in South Africa. Clin Infect Dis. 2026 Feb 9;82(1):e119-e125.

National DR-TB Reflex Algorithm with tNGS

15

Xpert XDR Sites

10-Day

tNGS TAT



Algorithm triggers tNGS automatically for rifampicin/isoniazid resistance cases, ensuring rapid comprehensive profiling for critical treatment decisions



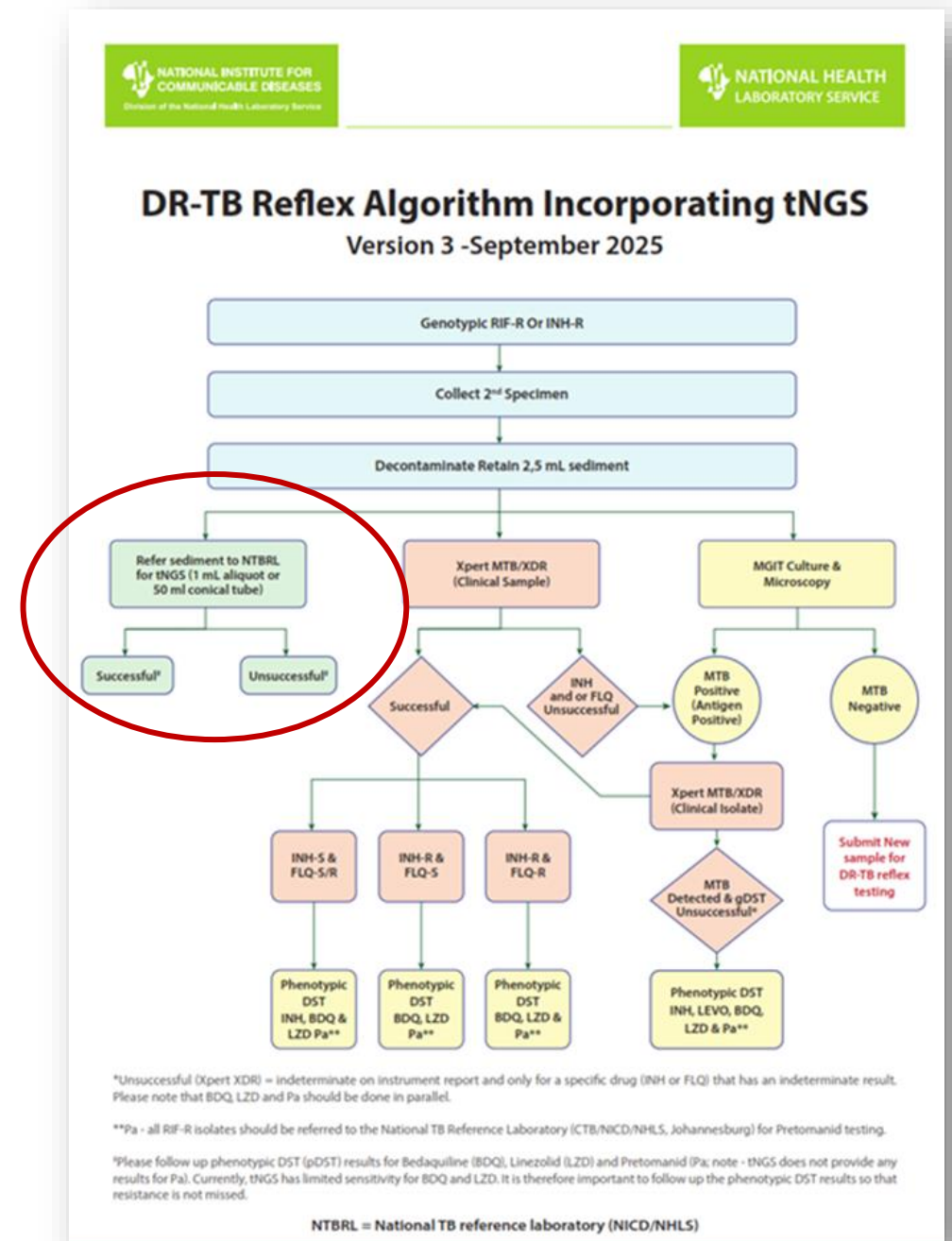
Workflow Required Coordination Across 15 Sites Feeding Samples To Centralized NTBRL With Standardized Tracking Procedures



Reflex Algorithm Eliminated Manual Delays And Ensured Systematic Surveillance For All Eligible Patients



Target 10-day Turnaround Time Represents Substantial Improvement Over 56-day Phenotypic Testing For Treatment Optimization



National tNGS performance

Total samples

541

Sites

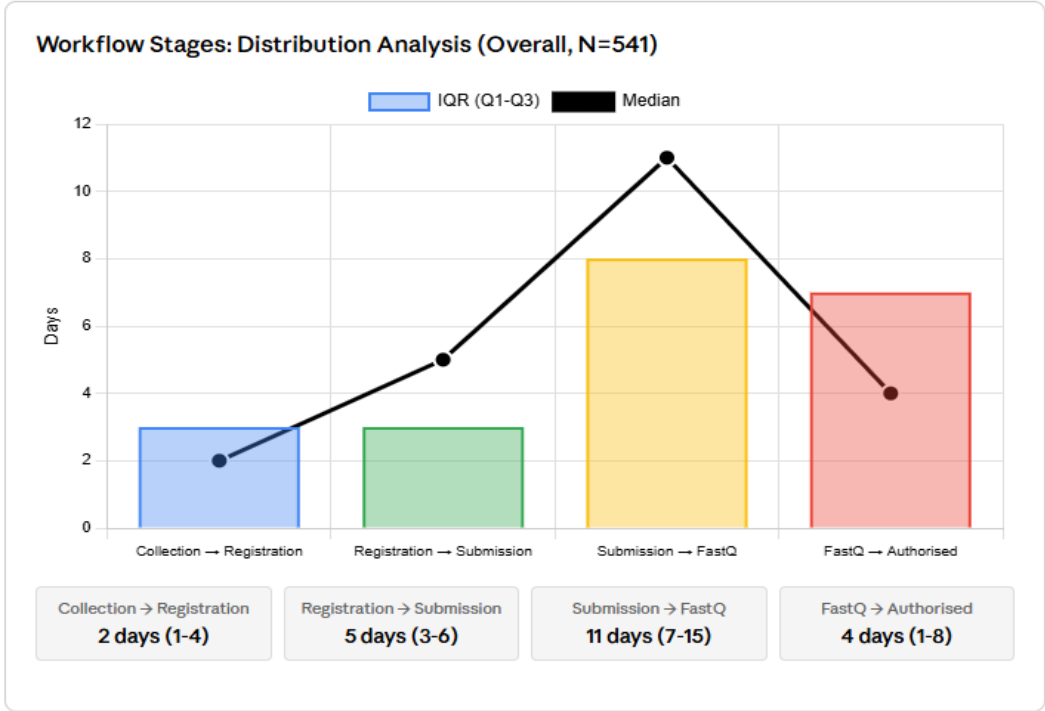
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End-to-end median

23 days

Processing median

11 days



Drug Target	Reference	N	Anti-TB Drug Prevalence (95% CI)	Sensitivity(95% CI)	Specificity(95% CI)
Rifampicin	WGS	255	86.7% (81.9%–90.6%)	98.2% (95.4%–99.5%)	76.5% (58.8%–89.3%)
	pDST	224	65.6% (59.0%–71.8%)	94.6% (89.6%–97.6%)	92.2% (83.8%–97.1%)
Isoniazid	WGS	224	67.4% (60.8%–73.5%)	91.4% (85.7%–95.3%)	95.9% (88.5%–99.1%)
	pDST	224	27.8% (21.9%–34.3%)	86.7% (75.4%–94.1%)	95.5% (91.0%–98.2%)
Fluoroquinolones	WGS	216	21.5% (16.5%–27.3%)	94.1% (83.8%–98.8%)	84.4% (78.4%–89.3%)
	pDST	237	32.1% (26.2%–34.3%)	80.3% (69.5%–88.5%)	88.8% (82.9%–93.2%)
Bedaquiline	WGS	237	1.3% (0.3%–3.8%)	100.0% (29.2%–100.0%)	100.0% (98.4%–100.0%)
	pDST	227	1.3% (0.3%–3.8%)	100.0% (29.2%–100.0%)	100.0% (98.4%–100.0%)
Linezolid	WGS	230	1.3% (0.3%–3.8%)	100.0% (29.2%–100.0%)	100.0% (98.4%–100.0%)
	pDST	227	1.3% (0.3%–3.8%)	100.0% (29.2%–100.0%)	100.0% (98.4%–100.0%)

Overview of tNGS samples processed

2,476

samples processed

73% (1,614)

*tNGS successful

tNGS SUCCESS*

In-progress

13%

Library prep failed

5%

Successful-MTB Detected

70%

Unsuccessful-MTB Not Detected

12%

Successful-NTM

BATCHES REPORTED

37

in window

AVG BATCH SIZE

39

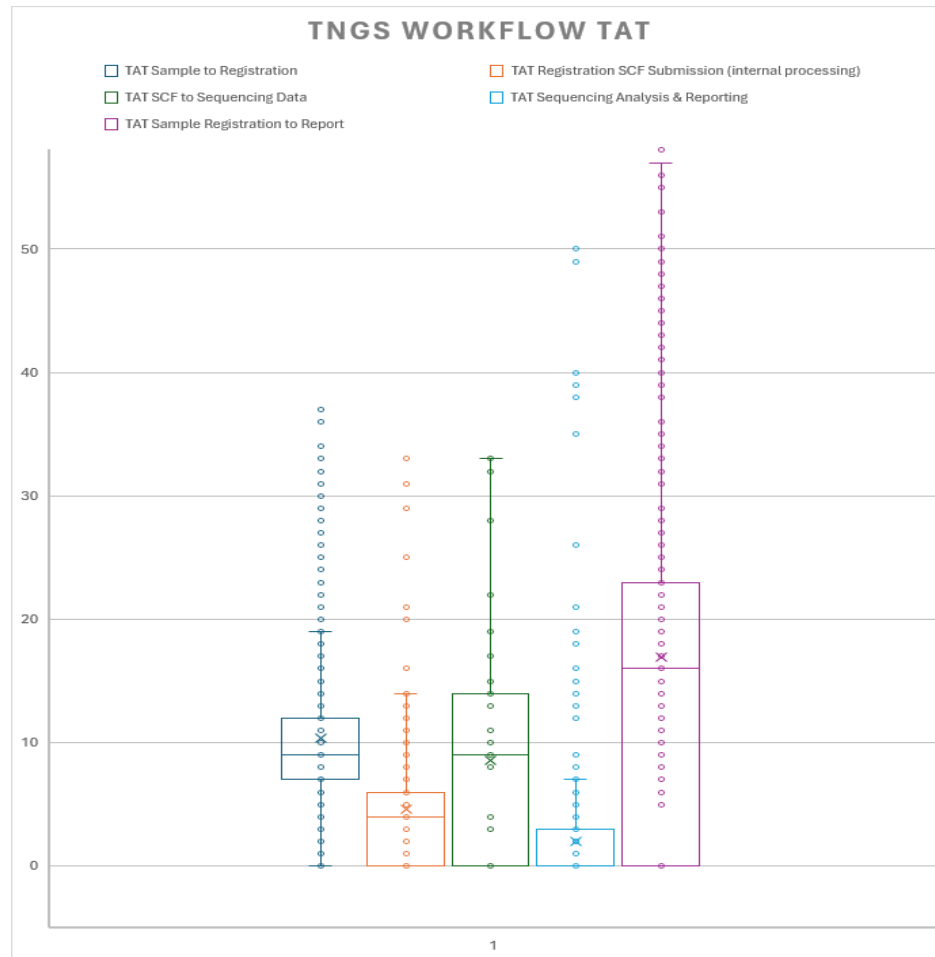
samples

BATCH SIZE & TAT COMPLIANCE OVER TIME



*EXCLUDES SAMPLES NOT MEETING THE CT-THRESHOLD, REJECTED OR NOT ELIGIBLE

Measured tNGS TAT by workflow stage



Sequencing analysis & reporting

~3 DAYS

Fastest stage — a consistent, efficient process once sequencing is complete.

SCF submission (internal)

~4 DAYS

Lowest median but moderate variability, with occasional processing delays.

SCF → sequencing data

~9 DAYS

Occasional inconsistency in sequencing throughput or batching delays.

Sample registration → report

~16 DAYS

End-to-end TAT and greatest variability — the cumulative effect of upstream delays.

Benchmark: half of all samples fall within a 2-week window — a useful target for turnaround.

Workflow-level contributors to extended TAT

TAT reflects cumulative constraints across pre-analytical, analytical and post-analytical stages.



Biological (Bacillary Load)

Constraint

Sensitivity drops significantly in smear-negative, paucibacillary specimens

Impact

Library generation failure highly correlates with Ct-scores >30.

Systemic Solution

Implement qPCR Ct-score **pre-screening** to triage low-load specimens directly to WGS rescue



Bioinformatic Pipeline

Constraint

Instability in newer automated interpretation pipelines

Impact

Version 4.03 iterative status yielded poor concordance for BDQ and LZD resistance detection

Systemic Solution

Retain validated v3.0.1 pipeline (Extended Catalogue) while negotiating updates with manufacturer.



Workforce Capacity

Constraint

High-throughput automation is gated by human infrastructure

Impact

Current NHLS staffing cannot absorb projected national volumes (~200 samples/week)

Systemic Solution

Targeted Workforce Injection:
2 Receiving Clerks, 2 Technologists, 6 Scientists, and Data Clerks

Evaluating the optimal network design for DR-TB tNGS

OBJECTIVE

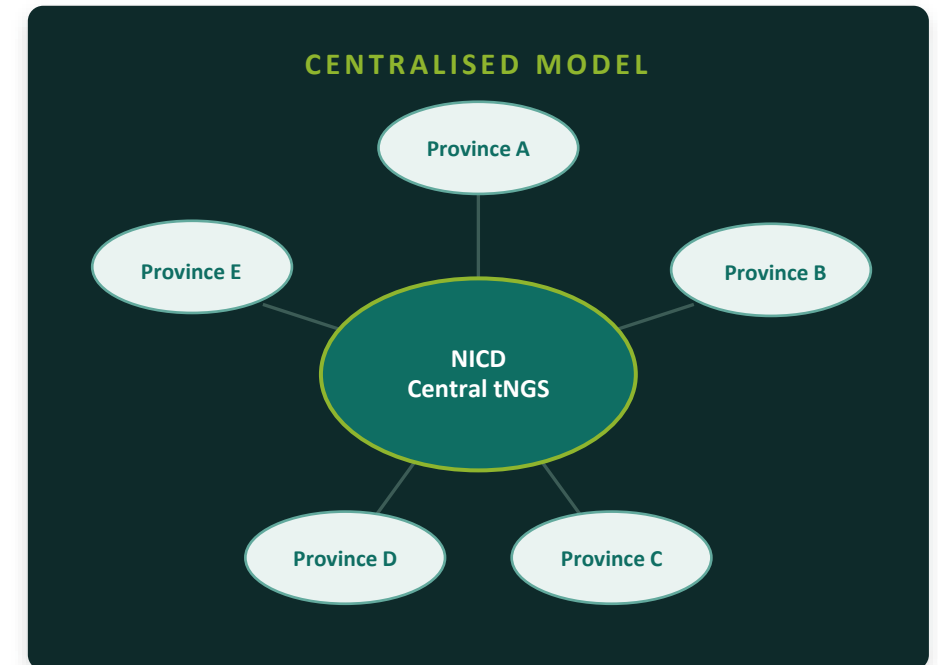
- What is the optimal balance between centralised efficiency and provincial access?
 - Centralised model — all testing at NTRL
 - Decentralised model — targeted platform decentralisation

ANALYTICAL APPROACH

- Calculate full end-to-end centralised cost (including transport)
- Map provincial volumes and referral flows
- Estimate minimum viable volume thresholds per site
- Model incremental HR, infrastructure and logistics requirements

EXPECTED OUTPUT

- Define the minimum viable volume threshold for provincial placement
- Quantify the incremental cost of decentralisation
- Inform optimal network placement



Key question: does targeted decentralisation improve system performance relative to cost?



Findings will guide sustainable scale-up decisions and inform broader global guidance on tNGS implementation models.



YES! WE CAN
#ENDTB
Commit, Invest, Deliver!