Webinar

Targeted Next-Generation Sequencing:

A game changer in drug resistance detection

Date: 24 April 2025

Time: 13h00 – 14h30

health
Department:
Health
REPUBLIC OF SOUTH AFRICA

2030 NDP

30 YEARS OF FREEDOM
South Africa 1994 - 2024

Thank you for your interest in this webinar

- The chat has been disabled for the attendees.
- **Please use the Q&A box to post questions for our panel of experts.**
- The session is recorded and will be shared with all the presentations on the Knowledge Hub – www.knowledgehub.health.gov.za/lms

Prof N Ndjeka

Prof Ndjeka serves as the Chief Director TB Control and Management, under the National Department of Health in South Africa.

Under his leadership, there has been a decline in the number of cases of DR -TB in South Africa and a remarkable improvement in proportion of patients successfully treated for DR- TB.

Programme Director: Prof N Ndjeka

Time	Duration	Topic	Presented By:
13h00 - 13h05	5 min	Opening & Welcome	Prof. Norbert Ndjeka
13h05 - 13h15	10 min	Aims and objectives of webinar	Prof. Norbert Ndjeka
13h15 - 13h55	40 min	The introduction of targeted Next-Generation Sequencing (tNGS)	Dr Shaheed V Omar
13h55 - 14h25	30 min	Discussion (Q&A)	Prof. Norbert Ndjeka
14h25 - 14h30	5 min	Vote of thanks	Prof. Norbert Ndjeka

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Dr Shaheed Vally Omar is a Medical Scientist, currently serving as the Head of the Centre for Tuberculosis at the National Institute for Communicable Diseases a division of the National Health Laboratory Service in South Africa.

Dr Shaheed has facilitated the seamless implementation of cutting-edge TB diagnostics into the routine laboratory, thereby strengthening standard practices and augmenting the efficacy of tuberculosis control measures.

Panellist	Facility
Dr Samantha Zealand-Smith	Jose Pearson TB Hospital (EC)
Dr Xavier Padanilam	Sizwe Hospital (GP)
Dr Nalini Singh	King Dinizulu Hospital (KZN)
Dr Hannetjie Ferreira	Tshepong Hospital (NW)
Dr C Duran	West End TB Hospital (NC)
Dr Neelum Mohamood	Brooklyn Chest TB Hospital (WC)

Thank you for attending this webinar

The session recording and all the presentations will be shared on the Knowledge Hub – www.knowledgehub.health.gov.za

THANK YOU

Implementation of targeted Next-Generation Sequencing [tNGS] to enhance management of DR-TB patients In South Africa

24th April 2025

Dr Shaheed V Omar
Centre Head
Centre for Tuberculosis
National & Supranational TB Reference Laboratory
NICD/NHLS

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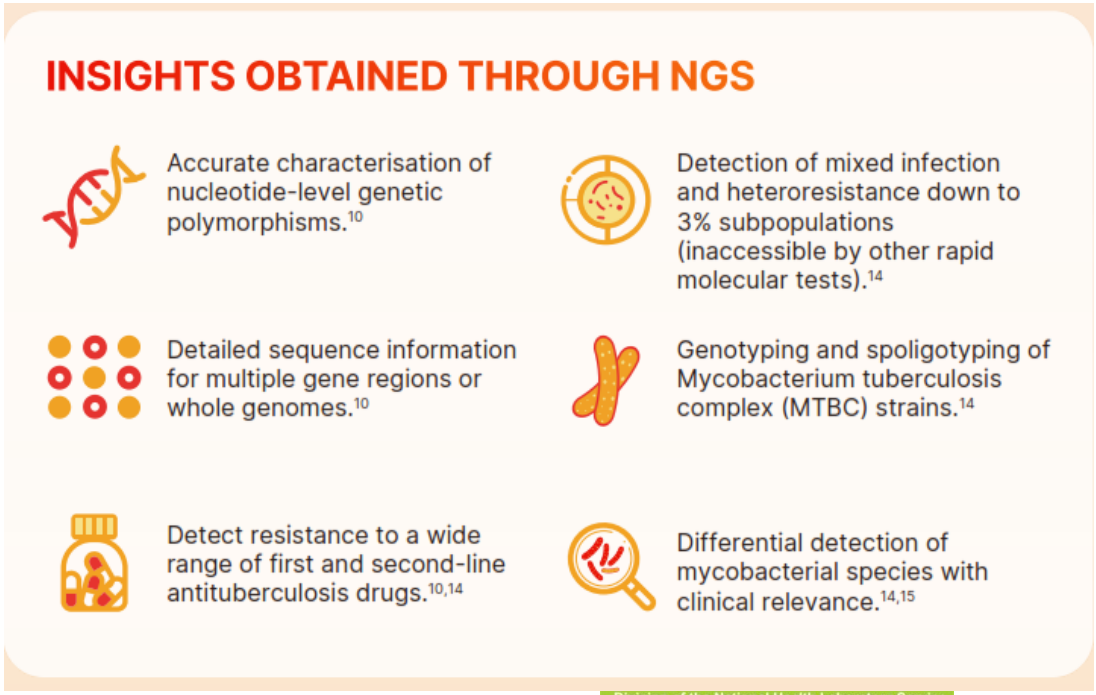
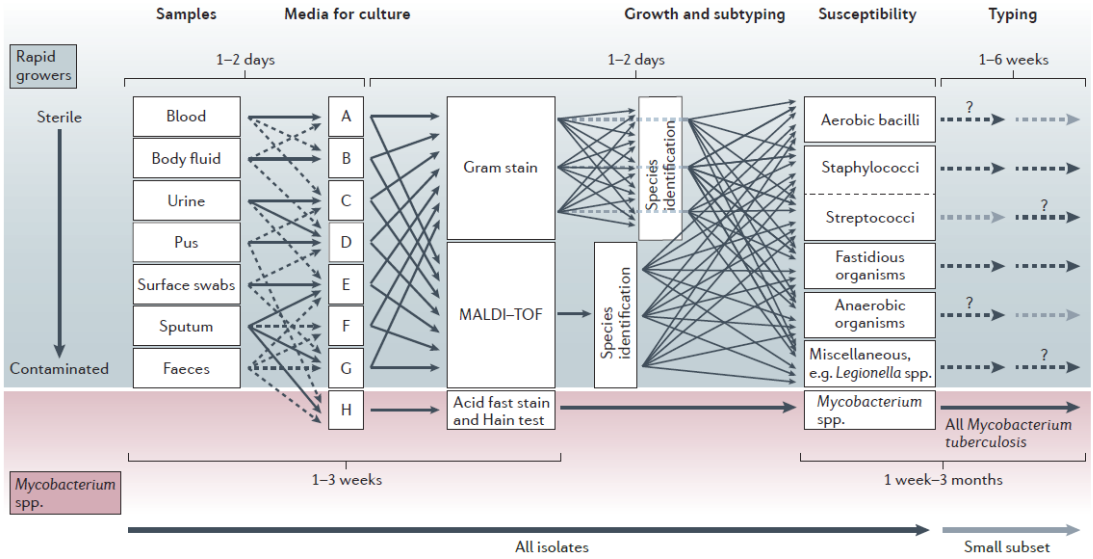
NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

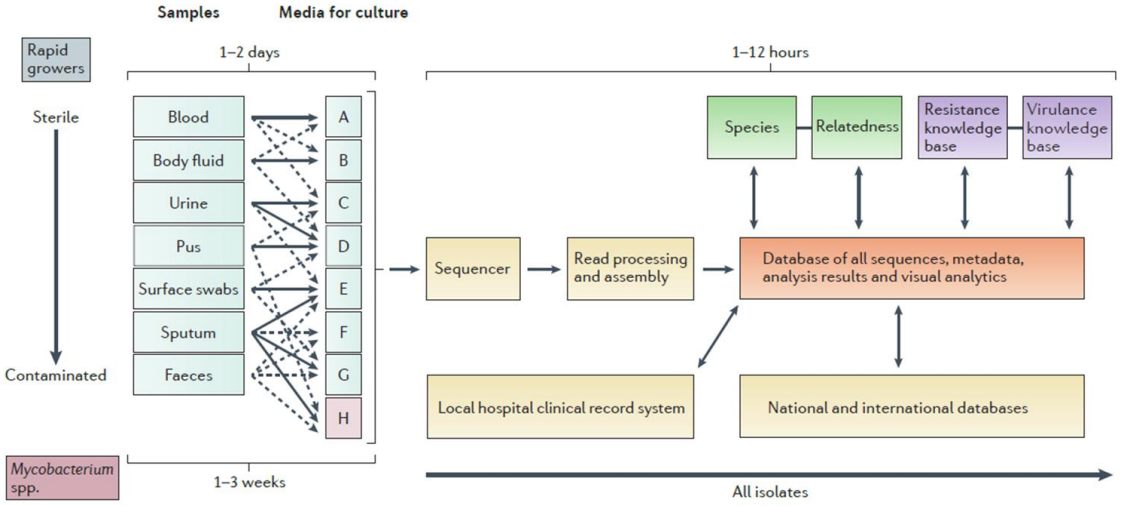
Next-Generation Sequencing

- Over the past few years Next-generation Sequencing has established itself in the infectious disease's domain
  - *Feasibility of equipment – minimal infrastructural requirements*
  - *Uncomplicated workflows*
  - *Reasonable cost and improved turnaround times*
- Next-Generation Sequencing (NGS) is a technology that rapidly reads and analyses genetic material to identify changes or patterns in DNA or RNA, helping us understand diseases, traits, or organisms at a molecular level.
- NGS is essentially NAATS on steroids, since it is able to explore larger portions of the genome compare to PCR

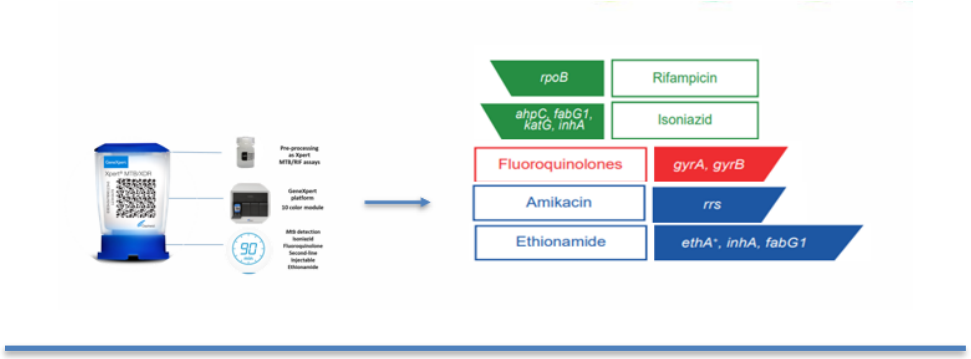


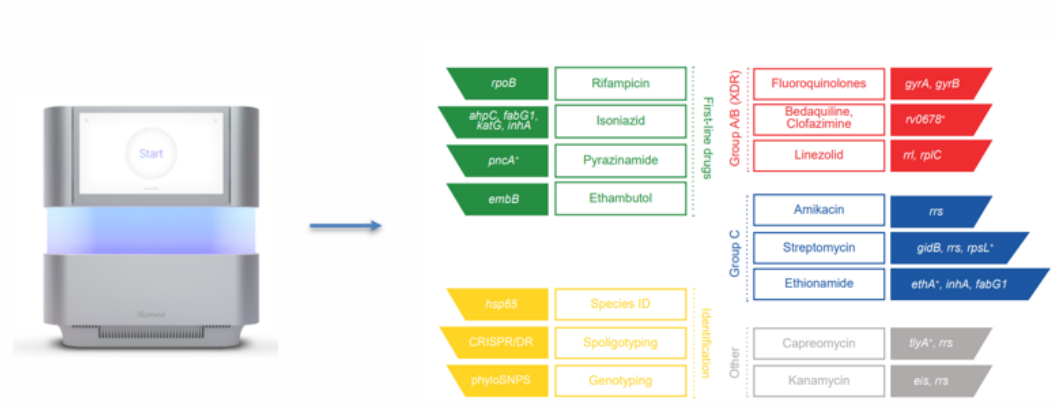
NGS Changing the paradigm





Current TB Diagnostics vs tNGS for Resistance Detection





Drug	pDST	Genes associated with resistance	Rapid Diagnostic	NGS
RIF	Yes Suboptimal sensitivity for disputed mutations	*rpoB, rpoA, rpoC*	*yes*	High sensitivity and identification of silent and disputed mutations
INH	Yes	*katG, inhA, oxyR-ahpC, fpbC, fabD, kasA, accD, ndh, fadE24, nat, iniA, iniB, iniC, mabA, srmR, nhoA*	*yes*	High sensitivity
EMB	Yes - Poor accuracy	*embB, embC, embA, embR iniA, iniB, iniC, manB, rmlD, rmlD*	*yes*	High sensitivity
SLI	Yes	*rrs, tlyA, rpsL, gidB, eis*	*yes*	High sensitivity
FLQ	Yes	*gyrA, gyrB*	*yes*	High sensitivity
PZA	Not recommended	*pncA, rpsA*	*yes (high complexity)*	High sensitivity
ETH	Not recommended	*inhA, ethA, ethR, ndh,mshA, fabG1*	*Yes (limited to shared target with INH)*	Moderate sensitivity
BDQ	yes	*atpE, Rv0678 (pepQ, Rv1979c)*	*no*	Moderate sensitivity
PAS	Limited information	*folC, thyA, ribD*	*no*	Limited information
LZD		*23SrRNA, rplC*		
DLM		*ddn, fbiA/b/c, fgd1/2*		
Pa		*ddn?*		

WHY SOUTH AFRICA plans to implement tNGS?

NEED: Only available RAPID technology to support laboratory guidance for BPAL/L Regimen with emerging BDQ resistance (~6.5%)

Direct sequencing for samples

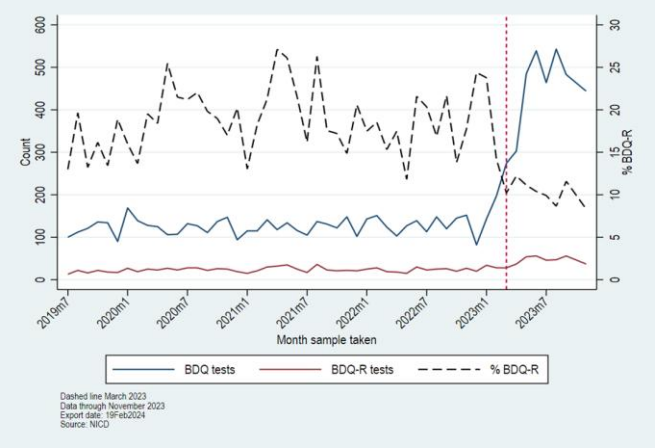
CURRENT DST TAT = 56 days

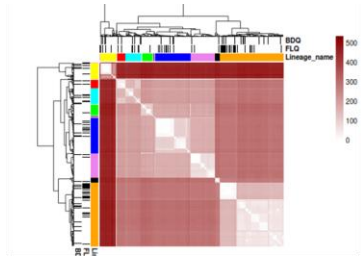
ENVISAGED TAT = 7-10 days

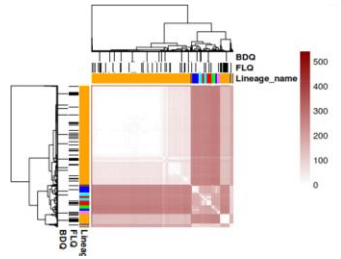
Cost benefit

Reduced labor cost

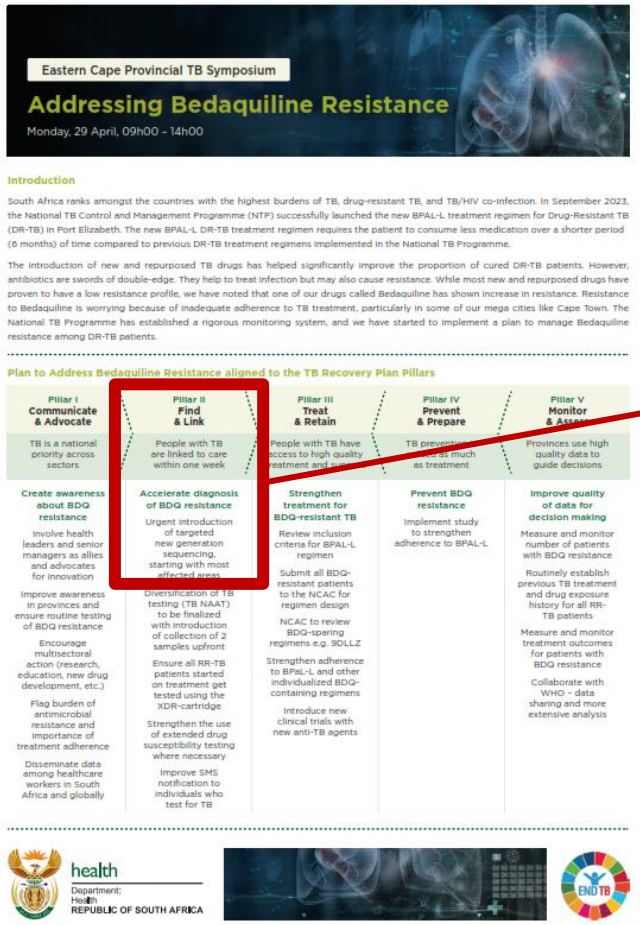
Optimized laboratory space vs pDST







National Plan to Address emerging BDQ resistance



Eastern Cape Provincial TB Symposium

Addressing Bedaquiline Resistance

Monday, 29 April, 09h00 - 14h00

Introduction

South Africa ranks amongst the countries with the highest burdens of TB, drug-resistant TB, and TB/HIV co-infection. In September 2023, the National TB Control and Management Programme (NTP) successfully launched the new BPAL-L treatment regimen for Drug-Resistant TB (DR-TB) in Port Elizabeth. The new BPAL-L DR-TB treatment regimen requires the patient to consume less medication over a shorter period (6 months) of time compared to previous DR-TB treatment regimens implemented in the National TB Programme.

The introduction of new and repurposed TB drugs has helped significantly improve the proportion of cured DR-TB patients. However, antibiotics are swords of double-edge. They help to treat infection but may also cause resistance. While most new and repurposed 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 mega cities like 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 & Retain	Pillar IV Prevent & Prepare	Pillar V Monitor & Asse
TB is a national priority across sectors	People with TB are linked to care within one week	People with TB have access to high quality treatment and su	TB prevention as much as treatment	Provinces use high quality data to guide decisions
Create awareness about BDQ resistance Involve health leaders and senior managers as allies and advocates for innovation Improve awareness in provinces and ensure routine testing of BDQ resistance Encourage multisectoral action (research, education, new drug development, etc.) Flag burden of antimicrobial resistance and importance of treatment adherence Disseminate data among healthcare workers in South Africa and globally	**Accelerate diagnosis of BDQ resistance** Urgent introduction of targeted new generation sequencing, starting with most affected areas Diversification of TB testing (TB NAAT) to be finalized with introduction of collection of 2 samples upfront Ensure all RR-TB patients started on treatment get tested using the XDR-cartridge Strengthen the use of extended drug susceptibility testing where necessary Improve SMS notification to individuals who test for TB	**Strengthen treatment for BDQ-resistant TB** Review inclusion criteria for BPAL-L regimen Submit all BDQ-resistant patients to the NCAC for regimen design NCAC to review BDQ-sparing regimens e.g. 9DLLZ Strengthen adherence to BPaL-L and other individualized BDQ-containing regimens Introduce new clinical trials with new anti-TB agents	**Prevent BDQ resistance** Implement study to strengthen adherence to BPAL-L	**Improve quality of data for decision making** Measure and monitor number of patients with BDQ resistance Routinely establish previous TB treatment and drug exposure history for all RR-TB patients Measure and monitor treatment outcomes for patients with BDQ resistance Collaborate with WHO - data sharing and more extensive analysis

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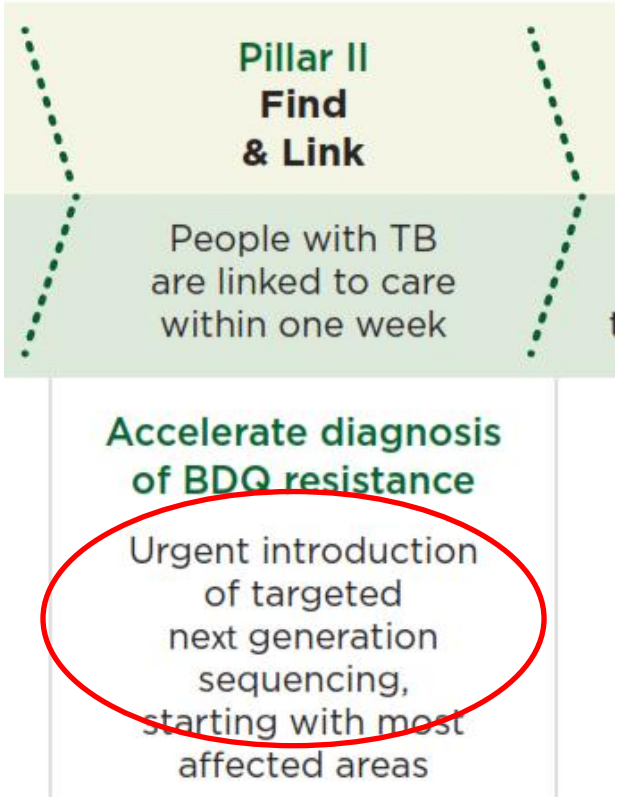
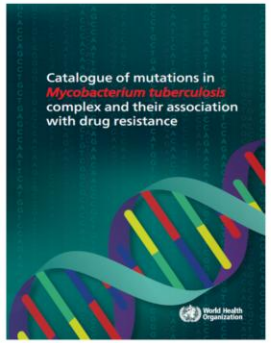
END TB



RAPID Diagnostics – Next GENERATION

*images of example instruments for illustrative purposes only



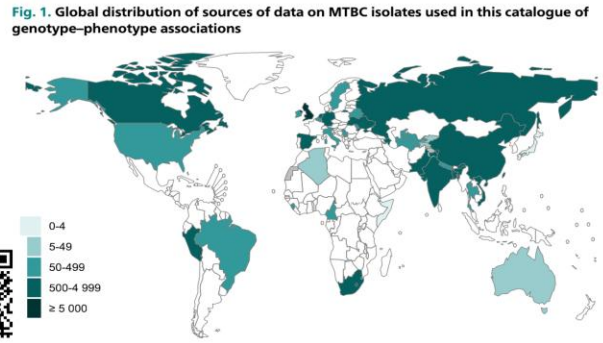


Fig. 1. Global distribution of sources of data on MTBC isolates used in this catalogue of genotype–phenotype associations

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

41137 clinical isolates with pDST and WGS

World Health Organization

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

Rapid communication, July 2023

Capability

- Sputum & Culture based
- 3-5 day turnaround time
- Comprehensive resistance profiling – upto 15 TB drugs
- Supported by WHO Mutation Catalogue
- Limitation – high complexity

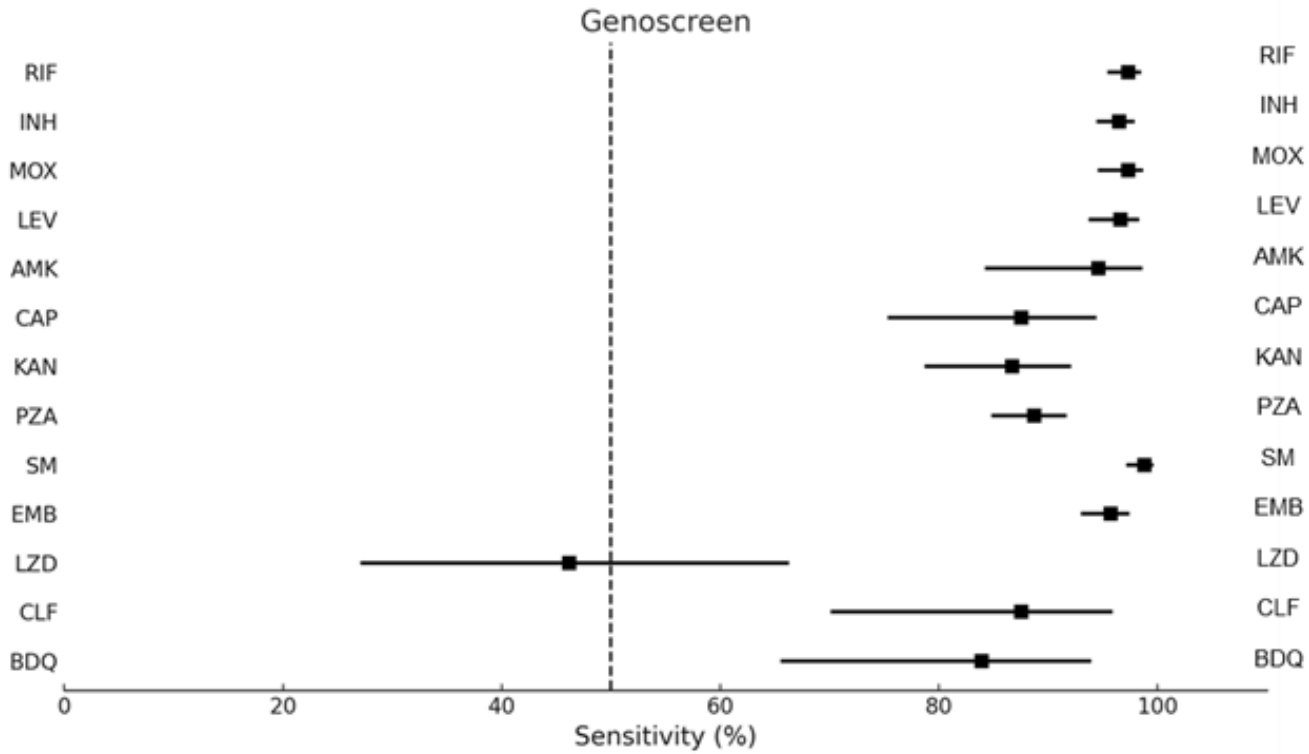
Performance

- Pooled sensitivity > 95%
  - RIF, INH, FLQ, EMB, PZA
- Acceptable sensitivity ~70%
  - BDQ, LZD & CFZ

NGS solutions recommended (selected drugs for each)

- Deeplex Myc-TB (Genoscreen) – Illumina platform
- NanoTB (Oxford Nanpore) – Minion platform
- Tbseq (Sheng Ting Biotech)

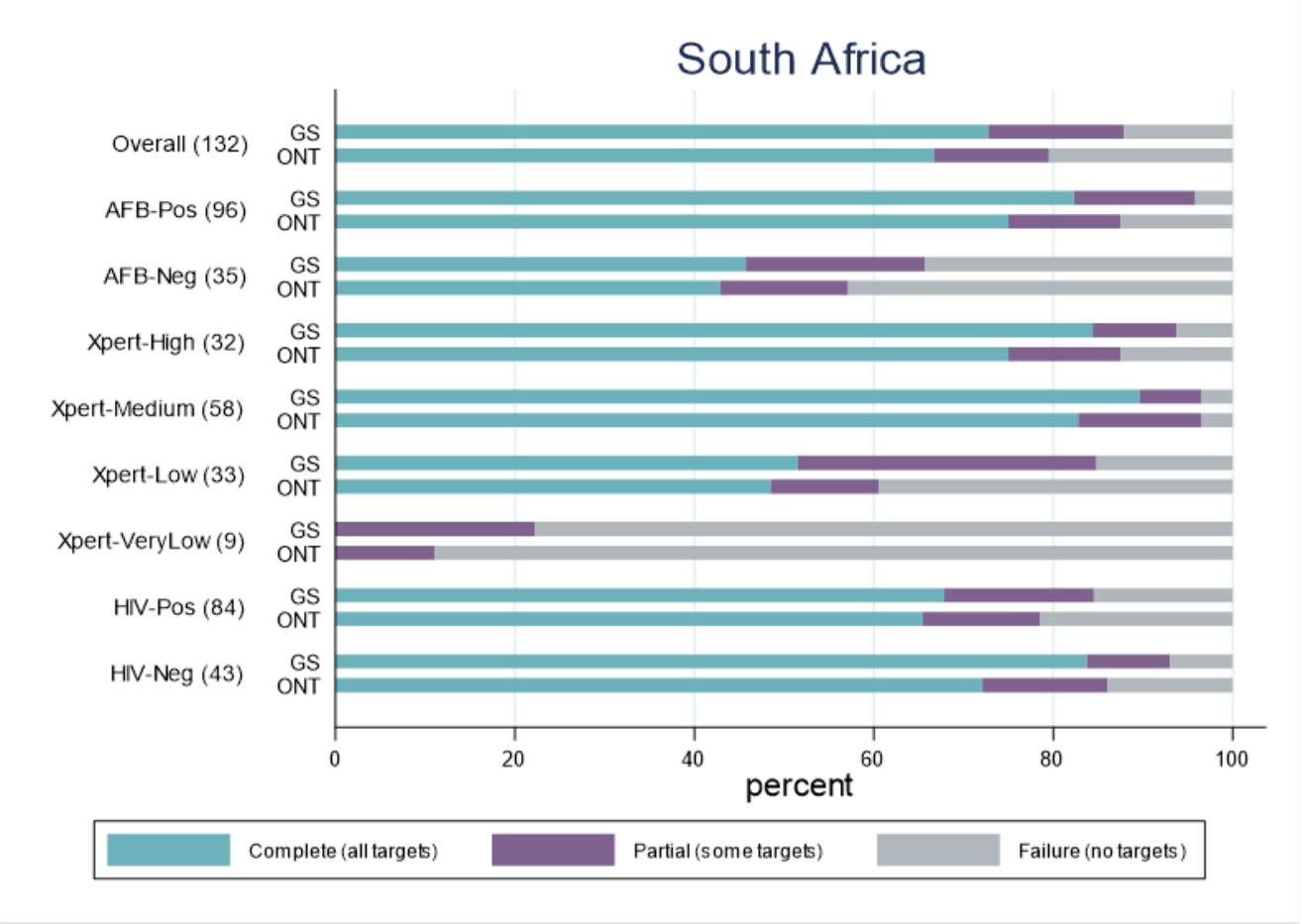
tNGS Performance (Seq&Treat multi-country evaluation)



	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
RIF	510	0	14	123	97.3 (95.4, 98.5)	100 (96.2, 100)
INH	472	3	17	126	96.5 (94.4, 97.9)	97.7 (92.8, 99.4)
MOX	287	5	8	337	97.3 (94.5, 98.7)	98.5 (96.4, 99.5)
LEV	287	5	10	334	96.6 (93.7, 98.3)	98.5 (96.4, 99.5)
AMK	53	1	3	525	94.6 (84.2, 98.6)	99.8 (98.8, 100)
CAP	49	2	7	533	87.5 (75.3, 94.4)	99.6 (98.5, 99.9)
KAN	98	1	15	483	86.7 (78.7, 92.1)	99.8 (98.7, 100)
PZA	306	4	39	264	88.7 (84.8, 91.7)	98.5 (96.0, 99.5)
SM	421	7	5	163	98.8 (97.1, 99.6)	95.9 (91.4, 98.2)
EMB	377	1	17	212	95.7 (93.0, 97.4)	99.5 (97.0, 100)
LZD	12	1	14	589	46.2 (27.1, 66.3)	99.8 (98.9, 100)
CLF	28	12	4	590	87.5 (70.1, 95.9)	98.0 (96.4, 98.9)
BDQ	26	14	5	589	83.9 (65.5, 93.9)	97.7 (96.0, 98.7)

Figure 4. Sensitivity and specificity of Genoscreen end-to-end tNGS solutions for detection of drug resistance compared with a composite reference of phenotypic drug suceptibility testing and whole-genome.

tNGS Success Rate (Seq&Treat: South Africa ONLY)



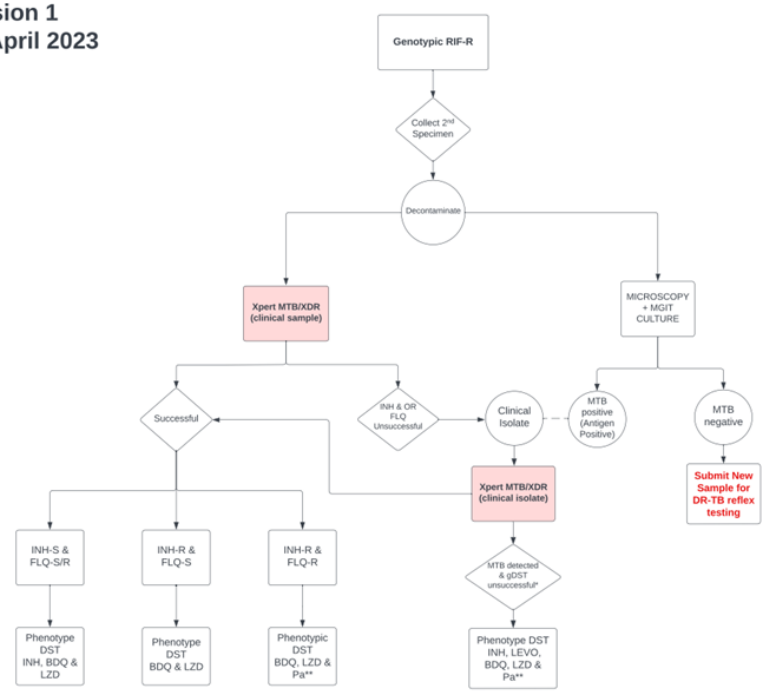
Sequencing Success on RSA samples by bacterial load proxies (AFB Smear status, GeneXpert Results Category, and HIV status). Total number of samples in each category are indicated in parentheses on Y-axis.

Current vs Improved DR-TB Reflex Algorithm incorporating tNGS

NATIONAL HEALTH LABORATORY SERVICE

DR-TB Reflex Algorithm

Version 1
24 April 2023



*unsuccessful = indeterminate on instrument report and only for a specific drug (INH or FLQ) that has an indeterminate result. Please note that BDQ, LZD and Pa should be done in parallel.

**Pa - all FLQ-R isolates should be reference to the National TB Reference Laboratory (CTB/NICD/NHLS, Johannesburg) for Pretomanid testing

NATIONAL HEALTH LABORATORY SERVICE

DR-TB Reflex Algorithm

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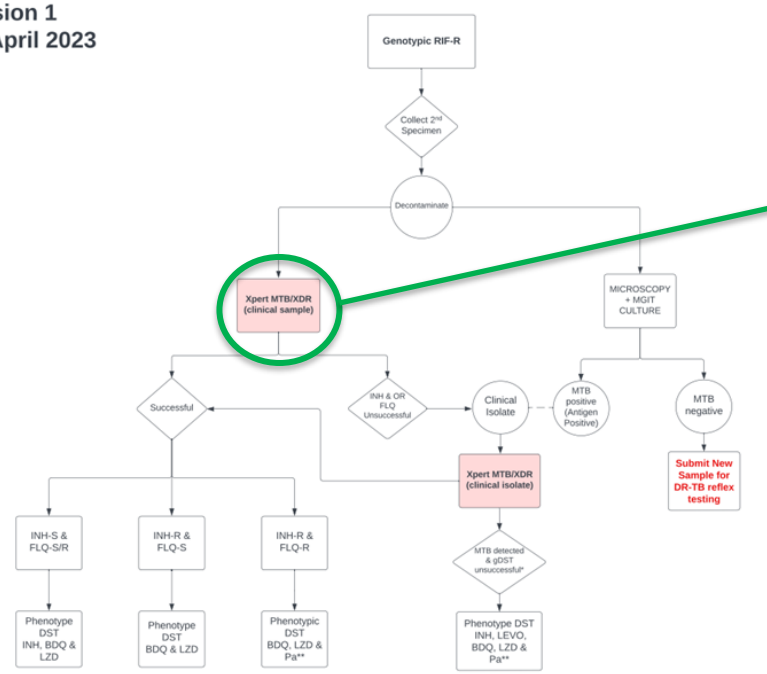

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tNGS

Laboratory Reporting: Current vs tNGS

CURRENT

Extended Drug Sensitivity Testing - MGIT Culture Based:	
Amikacin	Sensitive
Bedaquiline	Resistant
Clofazimine	Resistant
Ethambutol	Sensitive
Isoniazid High	Resistant
Levofloxacin	Sensitive
Linezolid	Sensitive
Moxifloxacin Low	Sensitive
Moxifloxacin High	Sensitive
p-aminosalicylic acid	Sensitive
Pretomanid	Sensitive
Rifabutin	Sensitive

tNGS

Targeted Next-Generation Sequencing Mycobacterium tuberculosis complex

Drug:	gDST interpretation:
Rifampicin	Resistant
Isoniazid	Resistant
Pyrazinamide	Resistant
Ethambutol	Resistant
Fluoroquinolone	Sensitive
Linezolid	Sensitive
Bedaquiline	Sensitive
Clofazimine	Sensitive
Amikacin	Uncertain significance

tNGS Implementation (Summary of Activities)

Phase 1 (October 2024) –National Validation – updated methodology

Phase 2 (Q2 2025) – National Implementation – Centralized Model (NTBRL)

Phase 3 (Q2 2026) – Decentralization (Tier 3 Labs – potentially 3)

PHASE 1 - *Laboratory validation of next-generation sequencing for routine genotypic Drug Susceptibility Testing in South Africa for Drug Resistant Tuberculosis – October 2024*

Objective	Endpoint
PRIMARY	
1. To validate and compare diagnostic accuracy of next generation sequencing including test success rate against the current recommendation and standard of care	Point estimates of sensitivity and specificity with 95% confidence interval for resistance detection to each drug
2. To assess technical performance, including non-determinant rates, ease of use and other system operational characteristics i.e. turnaround time	Non-determinate rates (errors, failed sequencing etc) for resistance detection to each drug. Summary of technical performance characteristics such as ease of use metrics, and other operational characteristics (including TAT).
SECONDARY	
3. To evaluate whether the NGS approach is cost-effective and decentralization capable to selected laboratories to service national demand from a health care system perspective.	Conduct a cost outcomes analysis that estimated the cost per person and cost per successful result for susceptibility testing in those diagnosed with RR-TB under the intervention arm compared to the standard of care.

- Ethics Approval – DEC 2024
- NHRD Approval – JAN 2025
- Study Sites
  - Selected sites
    - Jose Pearson
    - Nkqubela
    - Sizwe
    - King Dinizulu
    - West End
    - Tshepong
    - Brooklyn Chest
- Testing site preparedness Complete
- Logistics being finalized

PHASE 2 - *Next-Generation Tuberculosis Care: Programmatic implementation of WHO recommended targeted Next Generation Sequencing (tNGS) for rapid genotypic drug susceptibility profiling in patients diagnosed with rifampicin/isoniazid resistance (CENTRALIZED MODEL) – Q2 2025*

1. **Develop infrastructure and human resource capacity** for programmatic implementation of a WHO-recommended end-to-end tNGS solution (Deeplex)
2. **Conduct phased provincial implementation** of tNGS at the National TB Reference Laboratory as part of the DR-TB reflex algorithm for all RR-TB patients and specific groups of rifampicin susceptible (RS)-TB patients.
3. **Determine the most optimal network placement** of the technology to support DR-TB reflex testing with **decentralization to tier 1 referral laboratories** (currently performing phenotypic DST n=6)
4. **Evaluate the impact of including tNGS as part of the SOC in the DR-TB reflex testing** algorithms compared with standard of care alone (Xpert MTB/XDR & pDST) in terms of:
    a. **Time to resistance detection**
    b. **Indeterminate rates (failure** per gene target per drug **/**culture-pDST **contamination)**.
    c. Determine the **impact of tNGS vs. pDST on patient-important outcomes**, including:
        i. **Time to treatment with at least 3 effective drugs** with susceptibility documented for BDQ or LZD-resistant patients. Susceptibility as defined by the reference method
        ii. **Time to culture conversion and proportion** achieving culture conversion **by month3**
        iii. Proportion with an **unsuccessful outcome or died**

tNGS Costing and Performance

tNGS IS LIKELY TO COST LESS THAN THE SOC DST ALGORITHM

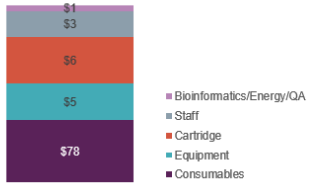
- This is the cost per test for 10,000 RIF+ tests, and for tNGS, for only 1 site

	Shrestha/Zwerling	Updated
pDST	$95 ($68 - $120)	$97
Xpert XDR	$39 ($37-$41)	$40
Total SOC	**$134**	**$137**

MGIT 960 instruments contribute significantly to the equipment cost.

115 instruments

	Shrestha/Zwerling	Updated
tNGS	$197 ($134 - $257)	$102



NextSeq 1000

	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Inputs				
Annual tNGS testing volumes	10000	10000	10000	10000
Number of sites	1	3	4	6
Types of instruments	NextSeq 1000	NextSeq 1000	NextSeq 1000	NextSeq 1000
Objective	Goal 2: minimise cost	Goal 1: minimise TAT	Goal 1: minimise TAT	Goal 1: minimise TAT
Currency for results	$	$	$	$
Results				
Number of instruments	1	3	4	6
% sequencer loading capacity (per run)	100%	18%	13%	9%
Total annual cost	$1,017,883	$2,023,445	$2,669,657	$5,410,462
Sequencing reagent cost	$6	$78	$128	$373
Sequencing TAT (days)	7	7	7	7
Cost per test	**$102**	**$202**	**$267**	**$541**
SOC comparator				
Total cost	$1,368,110	$1,368,110	$1,368,110	$1,368,110
Cost per test	**$137**	**$137**	**$137**	**$137**

Proposed Workflow & Considerations

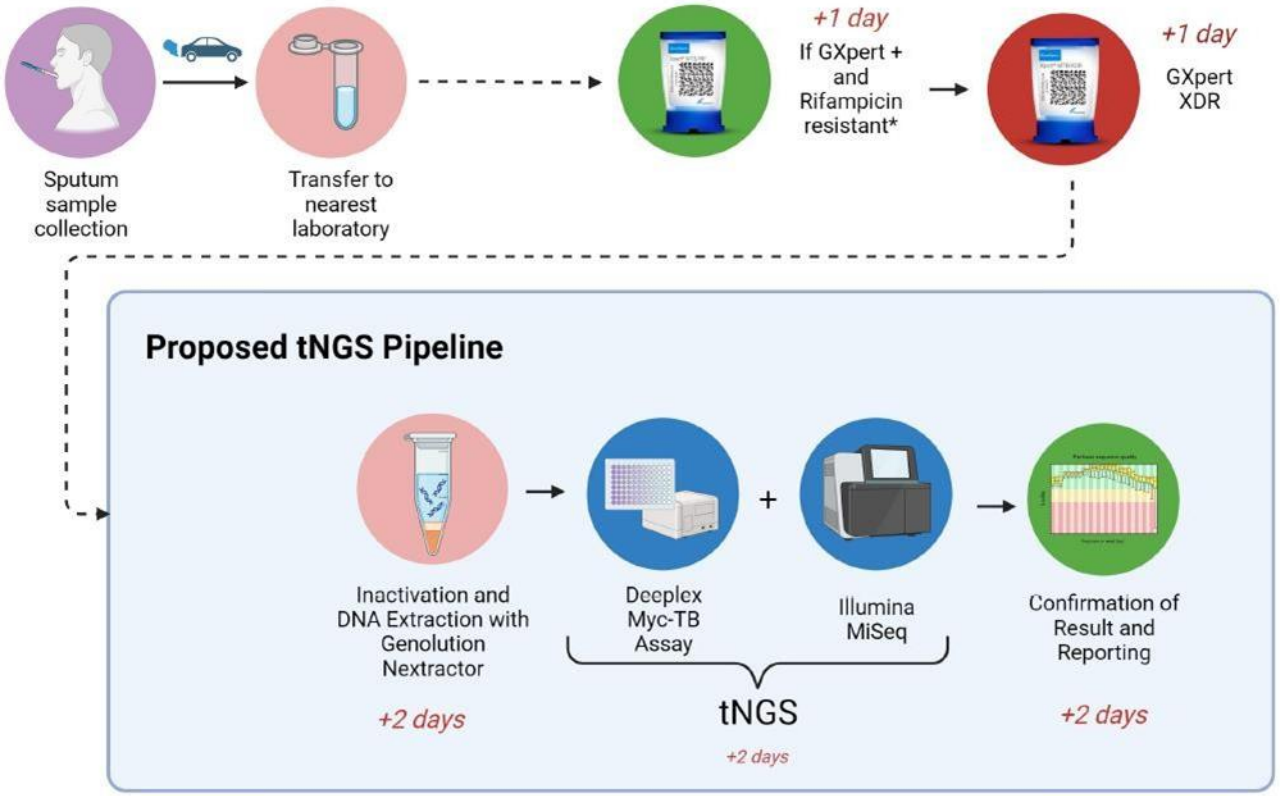


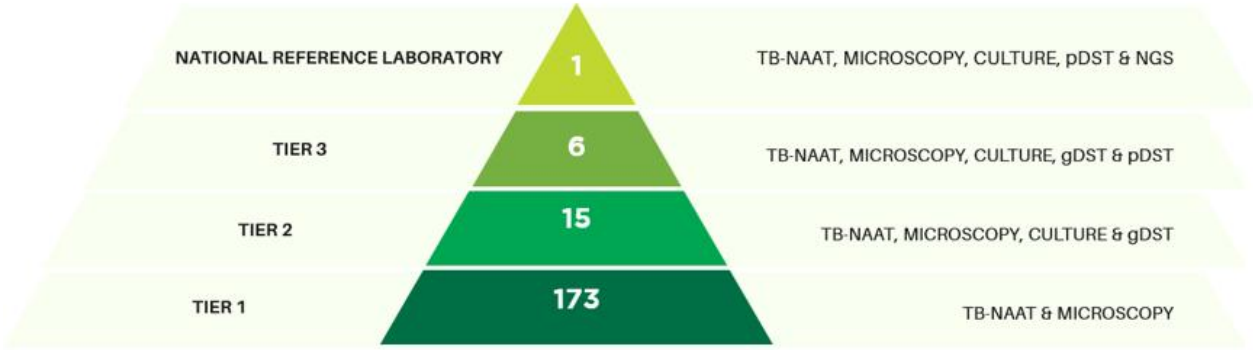
Table 2. Costs associated with the setup of tNGS in South Africa

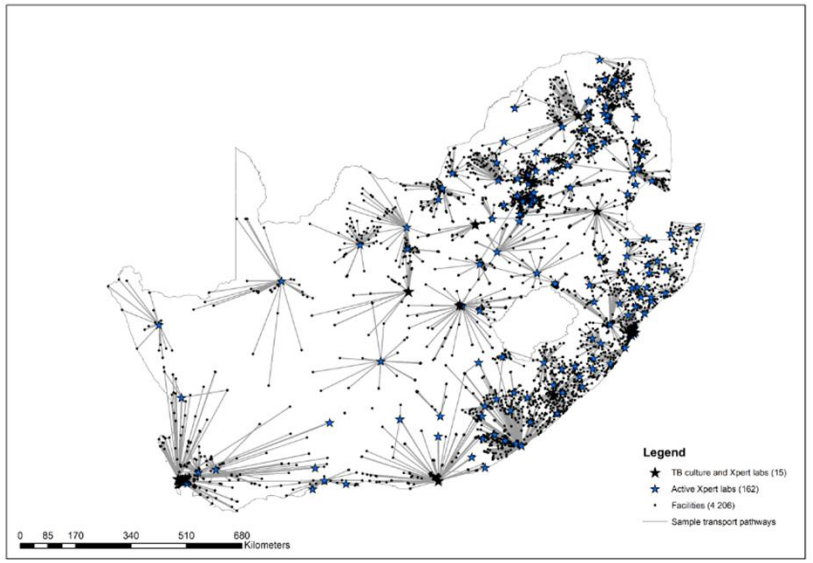
Workforce	• Training of staff • Continued employment of staff and salary packages
Infrastructure	• Electricity and backup power • Maintenance and service • Internet and phoneline services • Cloud services and storage • Floor/building/office space
Sequencing	• Initial purchase of Illumina MiSeq or iSeq • Service-level agreement for sequencing platform • Deeplex Myc-Tb Kits • Miscellaneous consumables
Location	• Ideal rollout would cover nine labs (one for each province).

PHASE 3 - *Next-Generation Tuberculosis Care: Programmatic implementation SCALE UP (DECENTRALIZATION) – Q4 2025*

Decentralization to ~3 sites for National testing

1. Decentralize and replicate infrastructure to support National routine diagnostic testing for all RR-TB
2. Staff capacitation and training





Take Home Messages

- The implementation of targeted NGS is an exciting step forward in how we diagnose and manage drug-resistant TB.
- tNGS improves the time of resistance detection considerably compared to culture-based DST
- tNGS addresses important gaps in the diagnosis of resistance to new/repurposed drugs such as Bedaquiline
- This will inturn provide Health Care Workers with comprehensive resistance information to adequately of DR-TB patients

YES! WE CAN #ENDTB

Commit, Invest, Deliver!