

LABORATORY ALGORITHMS FOR DIAGNOSIS OF TB - QUALITY IMPROVEMENT AWACC 2025

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Outline

Specimen Management

Overview of TB Diagnostic Assays

Diagnostic Algorithms



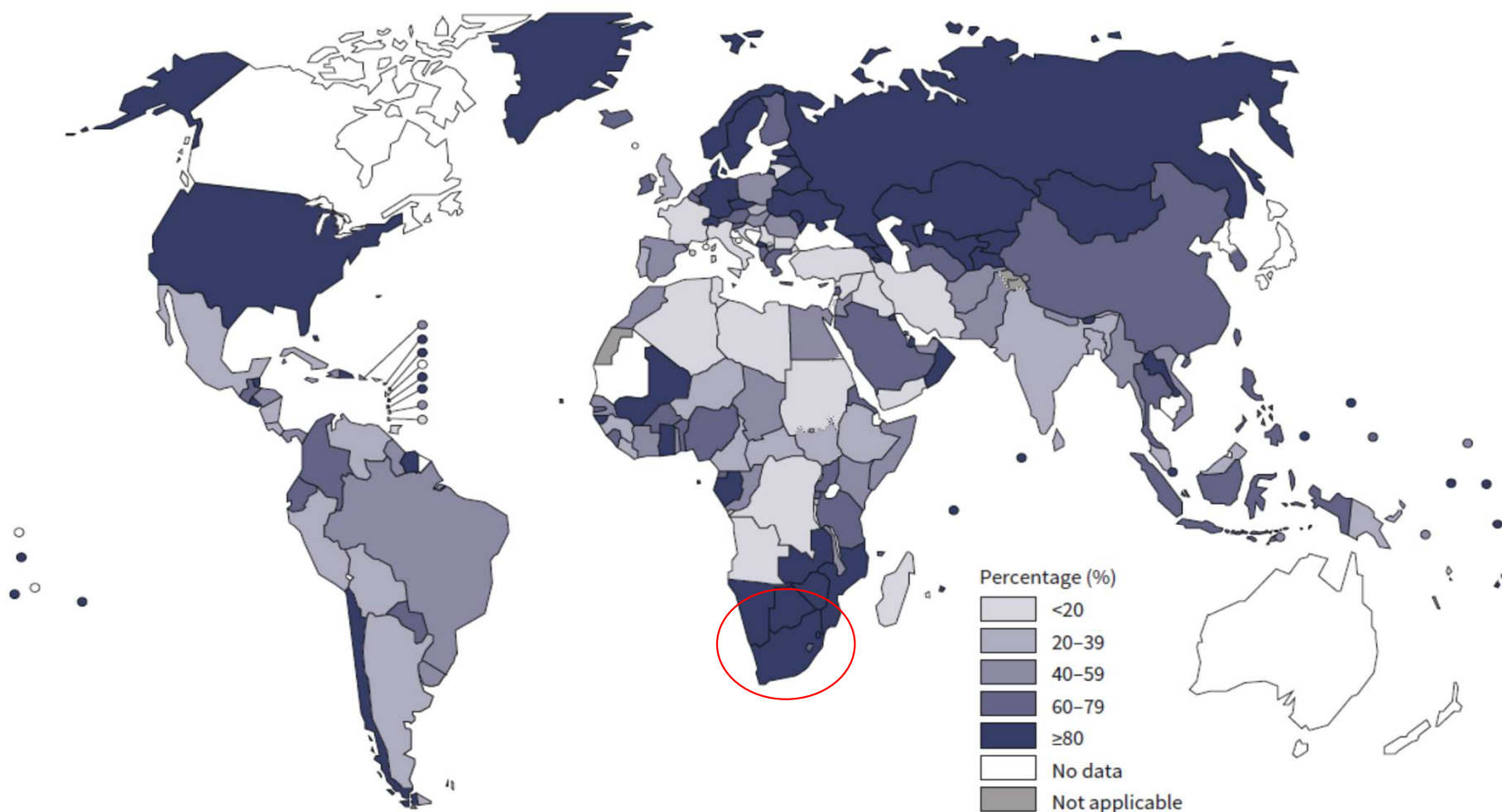
Introduction

- **WHO End TB Strategy (2016-2035)**
 - Early diagnosis of tuberculosis
 - Universal drug-susceptibility testing (DST)
- WHO-recommended rapid diagnostics (WRDs)
 - Accurate and rapid diagnosis
- Universal DST
- Ensure the quality of testing



Global Implementation of WRDs

Percentage of people newly diagnosed with TB who were initially tested with a WHO-recommended rapid diagnostic test (WRD), by country, 2023

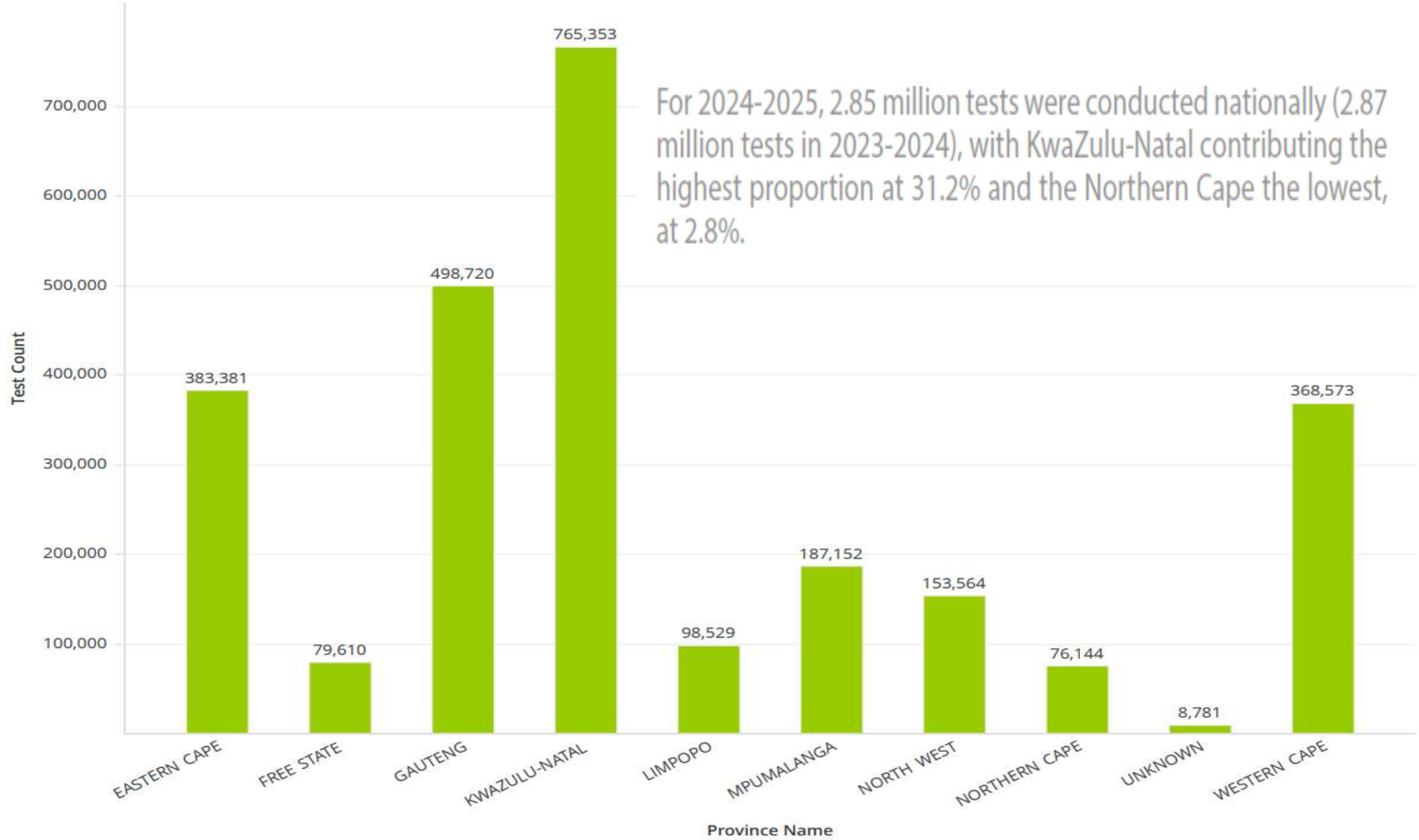


WRDs for initial diagnosis of TB and drug resistance

Purpose of classes	Technology class	Included products ^a
Initial tests for TB diagnosis with drug-resistance detection	NEW: Low-complexity automated nucleic acid amplification tests (LC-aNAATs) for detection of TB and resistance to RIF	Xpert® MTB/RIF Ultra (Cepheid)
		Truenat® MTB Plus and Truenat MTB-RIF Dx (Molbio)
	Moderate-complexity automated nucleic acid amplification tests (MC-aNAATs) for detection of TB, and resistance to RIF and INH	RealTime® MTB and RealTime MTB RIF/INH (Abbott)
		BD MAX™ MDR-TB (Becton Dickinson)
		cobas® MTB and cobas MTB-RIF/INH (Roche)
Initial tests for TB diagnosis without drug-resistance detection	NEW: Low-complexity manual nucleic acid amplification tests (LC-mNAATs) for detection of TB	FluoroType® MTB and FluoroType MTBDR (Bruker-Hain)
	Antigen detection in a lateral flow format (biomarker-based detection; LF-LAM) for detection of TB	Loopamp™ MTBC Detection Kit (TB-LAMP) (Eiken Chemical)
		Determine™ TB LAM Ag (Alere/Abbott)

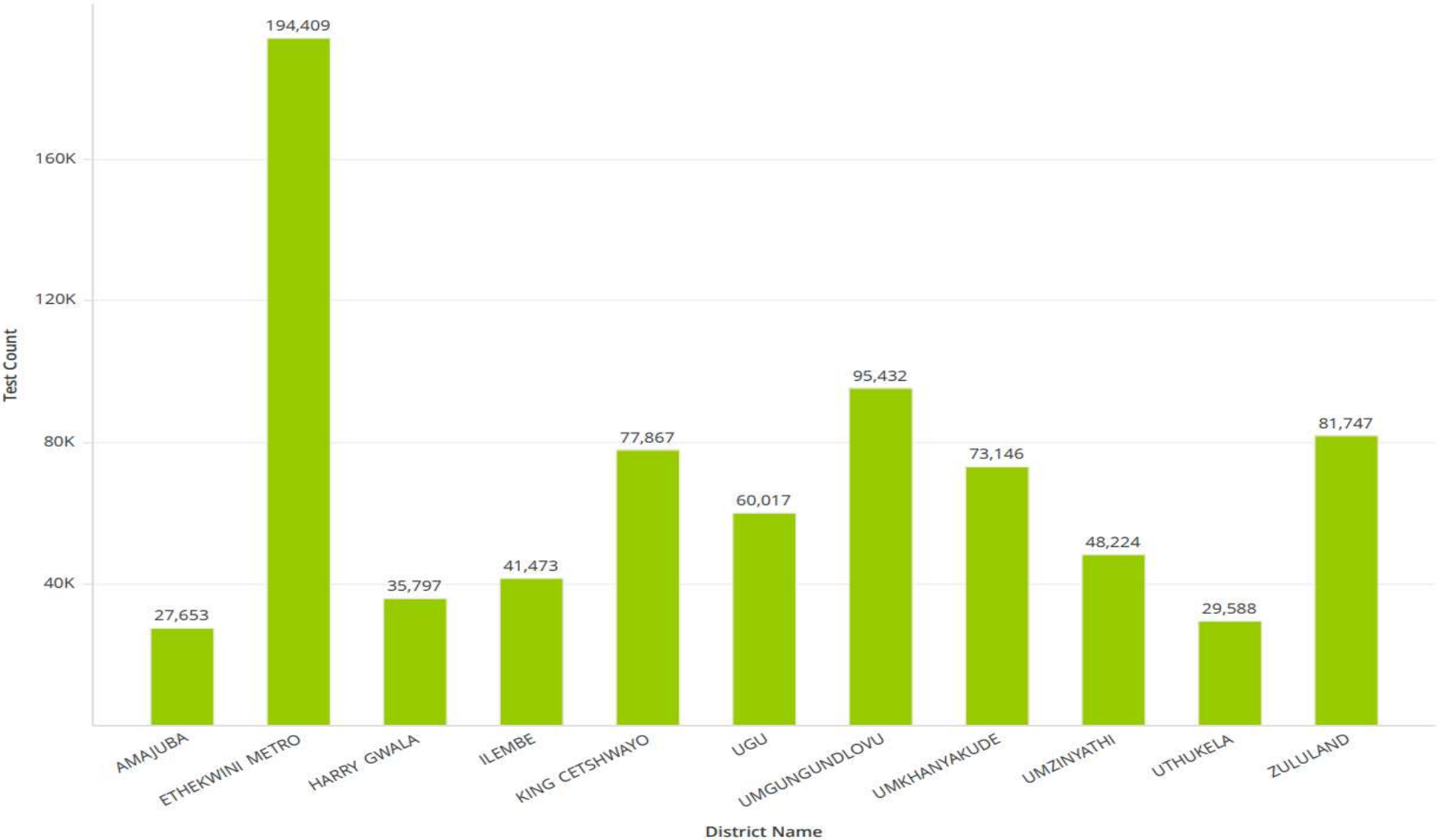


TB NAAT National Uptake Q1- Q3 2025



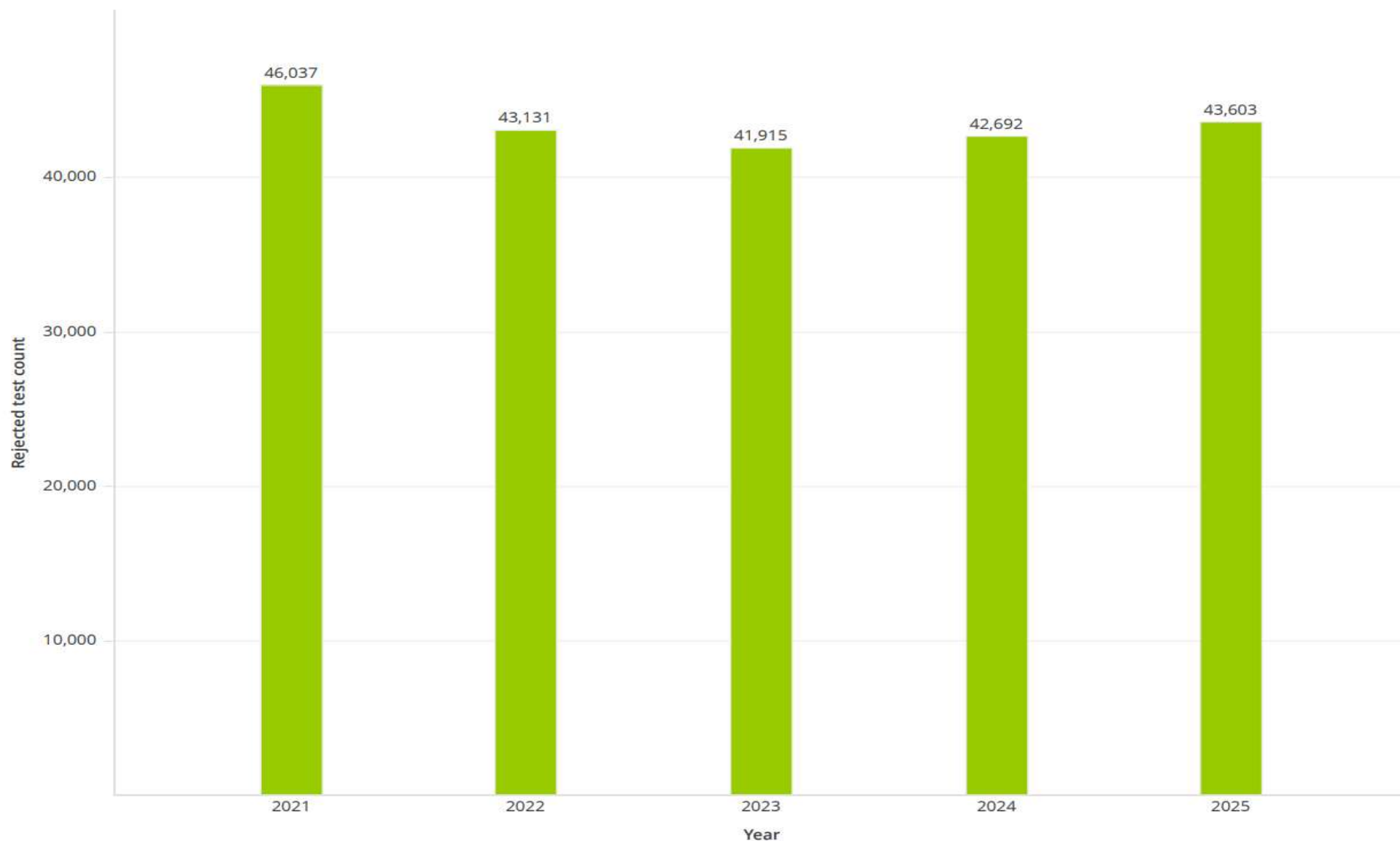


TB NAAT KZN District Uptake Q1- Q3 2025

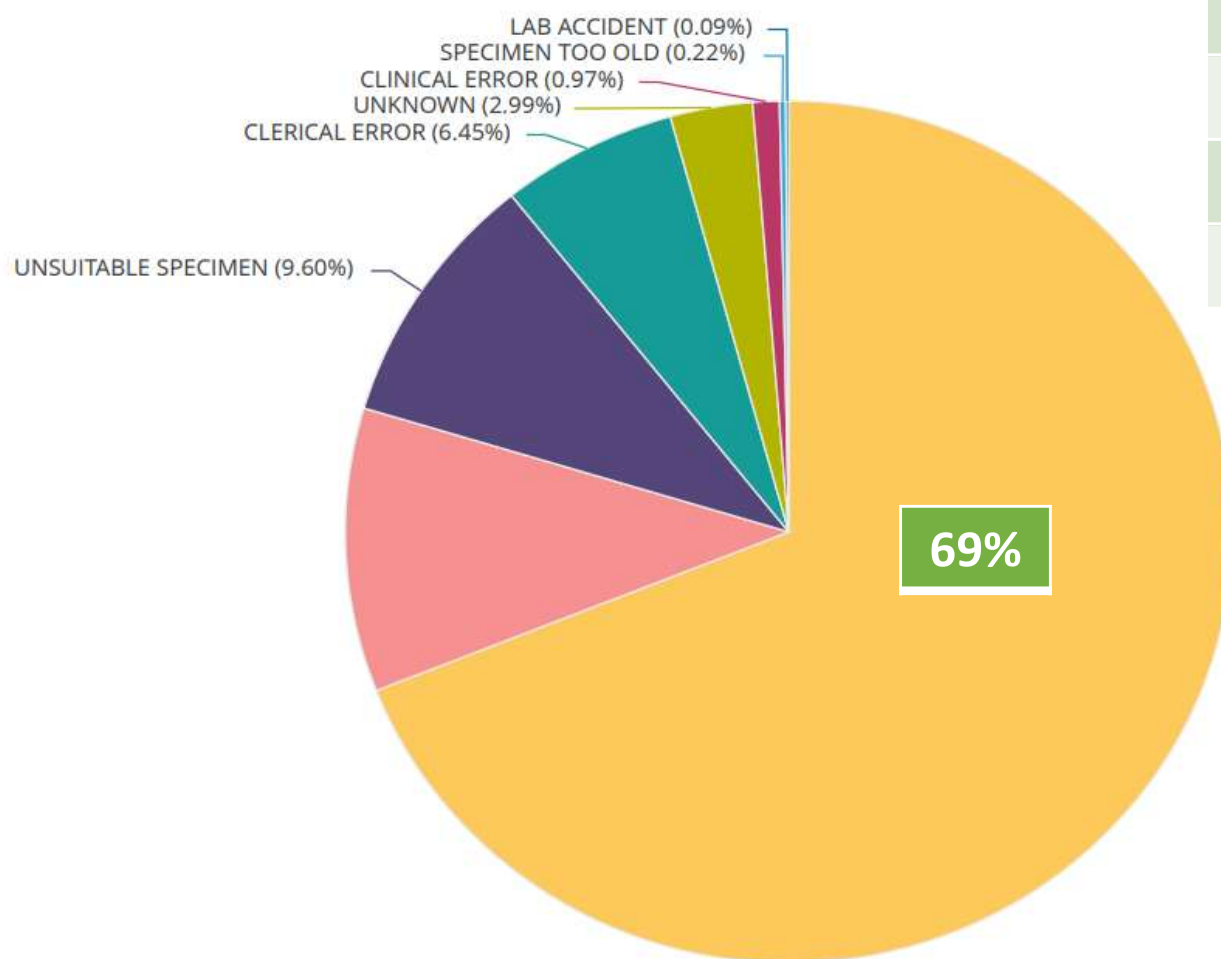




TB NAAT Rejected Specimens Q1-Q3: 2021-2025



TB NAAT Rejection Reasons: Q1-Q3 2025



Insufficient specimen

Leaked	52%
Insufficient	31%
Container empty	15%
Not received	2%

Rejection Reason

- CLERICAL ERROR
- CLINICAL ERROR
- INSUFFICIENT SPECIMEN
- LAB ACCIDENT
- NO VALID RESULT
- SPECIMEN TOO OLD
- UNKNOWN
- UNSUITABLE SPECIMEN





Minimum respiratory specimen volume for TB NAAT

- BD Max MDR-TB and Xpert MTB/RIF: 1 mL
- Roche cobas MTB and MTB-RIF/INH assay: 2.5 mL



Specimen collection

- Label container and form clearly
- Good quality specimen
- At least 2ml
- Container must be properly closed to prevent leakage of sample
- Transport to laboratory as soon as possible
 - If not same day store in fridge

Specimen collection



Diagnostic tools available in the NHLS for initial diagnosis of TB: Molecular Assays (TB NAAT)

Low-volume laboratories

Cepheid



10%

Medium-volume laboratories

Becton Dickinson



60%

High-volume laboratories

Roche Diagnostics



30%



Diagnostic tools available in the NHLS for initial diagnosis of TB

- Nucleic acid amplification test (NAAT)
- Detect presence of *M. tuberculosis* complex DNA
 - **Specific for *M. tuberculosis* complex**
- Detect changes (mutations) in the DNA that may be associated with drug resistance.
- Resistance to rifampicin (*rpoB*)
- Resistance to isoniazid (*katG* gene and *inhA*)
- Cannot be used to monitor treatment



Diagnostic tools available in the NHLS for initial diagnosis of TB

	Xpert Ultra	BD Max	Roche Cobas
Technology Class	Low complexity	Moderate complexity	Moderate complexity
MTBC Target	Multicopy target: IS6110 and IS1081	Multicopy target: IS6110 and IS1081	16S rRNA, <i>esxJ</i> , <i>esxK</i> , <i>esxM</i> , <i>esxP</i> , <i>esxW</i>
Specimens	Respiratory and extra-pulmonary	Respiratory only	Respiratory only
Resistance Testing	Rifampicin (<i>rpoB</i> gene)	Rifampicin and INH (<i>rpoB</i> , <i>katG</i> , <i>inhA</i>)	Rifampicin and INH (<i>rpoB</i> , <i>katG</i> , <i>inhA</i>)
LoD	MTB: 16 cfu/mL	0.5 & 6.0 cfu/mL	7.6–8.8 cfu/mL



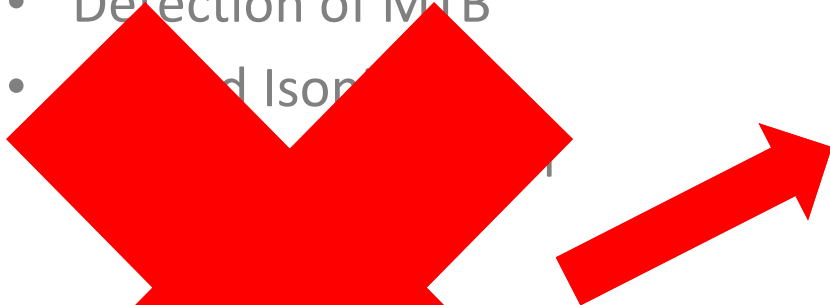


TB NAAT MTBC Positive BUT Drug Susceptibility Testing Unsuccessful

- Low bacterial load
- Based on detection of multi-copy amplification targets
- Not based on amplification of the *rpoB/katG/inhA*
 - No results for RIF/INH susceptibility or resistance.



Follow-on diagnostic tests for detection of additional drug resistance

- First line LPA
 - Detection of MTB
 - Isoniazid resistance detection
 - Second line tests
 - Fluoroquinolone and second line injectable resistance detection
 - Xpert XDR
 - Detection of MTB
 - Isoniazid resistance detection
 - Fluoroquinolone resistance detection
 - Ethionamide resistance detection
 - Second-line injectable resistance (Amikacin)
- 



Xpert MTB/XDR (Cepheid)

- Low complexity automated NAATs for detection of resistance to INH and FLQ and second-line injectable agents
- Directly on clinical specimens or culture isolates
- LoD 136 CFU/mL
- **Intended for use: Reflex or follow up test for patients with a bacteriologically confirmed pulmonary TB diagnosis**



TB culture

- **Gold standard** for the bacteriological confirmation of TB
- Diagnosis of **paucibacillary** TB: HIV+, paediatric and extra-pulmonary
- Distinguish **live from dead** organisms (monitoring of DR TB treatment)
- Diagnosis of non-tuberculous mycobacteria (**NTM**) infection.
- DST for **additional drugs** not tested by molecular assays
- Provides an **isolate** for further tests
- **Time to reportable result long** (2-6 weeks for TB isolation)





TB Diagnostic algorithms

- Initial Test: TB NAAT (Xpert Ultra/BD Max/Roche cobas)
- Three algorithms based on the TB NAAT Results:



**1. MTB detected – No Treatment
within the last 2 years**



**2. MTB detected – Treatment
within the last 2 years**



**3. MTB not detected, Test
unsuccessful, Trace results**



Rationale for the two year cut off period

Clinical Infectious Diseases

MAJOR ARTICLE



Xpert MTB/RIF Results in Patients With Previous Tuberculosis: Can We Distinguish True From False Positive Results?

Grant Theron,^{1,2*} Rouxjeane Venter,² Greg Calligaro,¹ Liezel Smith,¹ Jason Limberis,¹ Richard Meldau,¹ Duncan Chanda,^{1,3} Aliasgar Esmail,¹ Jonny Peter,¹ and Keertan Dheda^{1,4}



Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study



Susan E Dorman*, Samuel G Schumacher*, David Alland, Pamela Nabeta, Derek T Armstrong, Bonnie King, Sandra L Hall, Soumitesh Chakravorty, Daniela M Cirillo, Nestani Tukvadze, Nino Bablishvili, Wendy Stevens, Lesley Scott, Camilla Rodrigues, Mubin I Kazi, Moses Joloba, Lydia Nakiyingi, Mark P Nicol, Yonas Ghebrekristos, Irene Anyango, Wilfred Murithi, Reynaldo Dietze, Renata Lyrio Peres, Alena Skrahina, Vera Auchynka, Kamal Kishore Chopra, Mahmud Hanif, Xin Liu, Xing Yuan, Catharina C Boehme, Jerrold J Ellner, Claudia M Denking, on behalf of the study team†



AGORA
RESEARCH LETTER

Effect of history of tuberculosis on specificity of Xpert MTB/RIF

Frederick Haraka^{1,2,3}, Samuel G. Schumacher⁴, Amanda Ross^{2,3}, Anna Mantsoki⁴, Sebastien Gagneux^{1,2,3}, Klaus Reither^{1,2,3,6} and Claudia M. Denking^{4,5,6}

This study represents the largest dataset to date to assess the effect of TB history on the specificity of Xpert for TB. The specificity of Xpert was lower in those with TB history compared to those without it and increased as time since previous TB increased (figure 1). In patients with TB history within 2 years, specificity was reduced. False-positive was associated with negative



Laboratory Samples Submitted for TB-NAAT**

MTB Detected

No Treatment within the last 2 years

Rifampicin Unsuccessful

- Collect sample for smear, culture & DST.
- Start DS-TB treatment.

Follow-up and review culture and DST results.

Rifampicin-S

- Start DS-TB treatment.
- Collect sample for baseline smear.

Follow-up review and record smear results.

Rifampicin-S & Isoniazid-R

- Start treatment for Isoniazid-R TB.
- Collect sample for DR-TB Reflex testing.

Follow-up and review Reflex testing results.

Fluoroquinolone-S

Continue Isoniazid-R treatment

Fluoroquinolone-R

Refer to DR-TB treatment initiation site or if DR-TB treatment initiation site, consult with *PCAC or *NCAC

Rifampicin-R OR Rifampicin-R & Isoniazid-R

Refer to DR-TB treatment initiation site or if DR-TB treatment initiation site, treat for RR/MDR-TB as per DR-TB guidelines.

DR-TB Reflex:

- Smear microscopy
- Xpert MTB/XDR
- Culture & Phenotypic DST

* NCAC - National Clinical Advisory Committee.

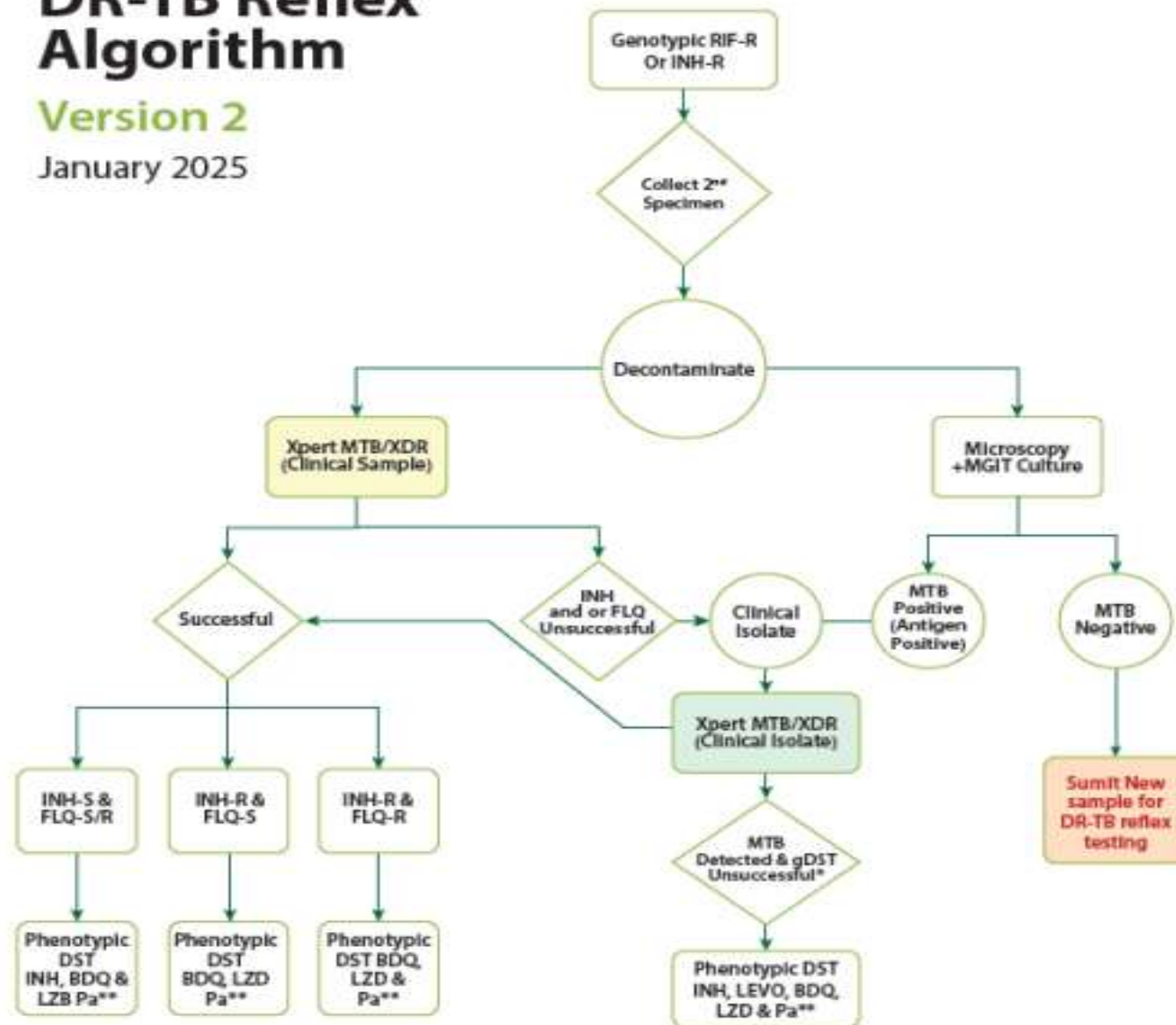
* PCAC - Provincial Clinical Advisory Committee.

** NAAT: Nucleic Acid Amplification Test.

DR-TB Reflex Algorithm

Version 2

January 2025



*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 referred to the National TB Reference Laboratory (CTB/NICD/NHLS, Johannesburg) for Pretomanid testing



NATIONAL HEALTH
LABORATORY SERVICE

Laboratory Samples Submitted for TB-NAAT

MTB Detected

Treatment within the last 2 years

Rifampicin-S

- Collect sample for smear, culture & DST.
- If symptomatic, start DS-TB treatment.
- If asymptomatic defer TB treatment.

Follow-up and review culture and DST results.

Rifampicin Unsuccessful

Previous DS-TB

- If symptomatic start DS-TB treatment.
- If asymptomatic defer TB treatment.

Previous DR-TB

Defer TB treatment

Follow-up and review culture and DST results.

Rifampicin-S & Isoniazid-R

Previous Isoniazid-R

Defer TB treatment

Fluoroquinolone-S

Continue Isoniazid-R treatment

Previous Isoniazid-S OR Unknown

Start Isoniazid-R treatment

Fluoroquinolone-R

Refer to DR-TB treatment initiation site or if DR-TB treatment initiation site, consult with *PCAC or *NCAC

Rifampicin-R OR Rifampicin-R & Isoniazid-R

Previous Rifampicin-S

Refer to DR-TB Treatment initiation site or If DR-TB treatment initiation site, treat as per DR-TB Guidelines

Previous Rifampicin-R

Refer to DR-TB Treatment initiation site or If DR-TB treatment initiation site, consult with *PCAC or *NCAC

* NCAC - National Clinical Advisory Committee.

* PCAC - Provincial Clinical Advisory Committee.

** NAAT: Nucleic Acid Amplification Test.

Laboratory Samples Submitted for TB-NAAT

MTB Not Detected

Clinically assess the patient.
- Symptoms, physical examination, chest x-ray (where available)

Clinical presentation not consistent with TB.

No TB symptoms, clinically well and or normal chest x-ray

Clinical presentation consistent with TB.

Symptoms and signs suggestive of TB and or chest x-ray suggestive of TB.

Consider HIV status

- Start DS-TB treatment.
- Collect sample for culture and DST

HIV Negative

HIV positive

Continue routine care

- Collect sample for culture and DST.
- Defer TB treatment.
- Continue routine care.

Follow-up and review culture and DST results.

Test Unsuccessful

Collect another sample for TB-NAAT

Trace (Xpert only)

Treatment within the last 2 years

No treatment within the last 2 years

- Clinically assess the patient. Symptoms, physical examination
- Collect sample for culture and DST.

Clinically assess the patient.
Symptoms, physical examination, chest x-ray (where available)

Clinical presentation not consistent with TB.
No TB symptoms and clinically well.

Continue routine care

Clinical presentation consistent with TB.
Symptoms and signs suggestive of TB.

Start DS-TB treatment.

Follow-up and review culture and DST results.

Clinical presentation consistent with TB.
Symptoms and signs suggestive of TB and or chest x-ray suggestive of TB

- Start DS-TB treatment.
- Collect sample for culture and DST

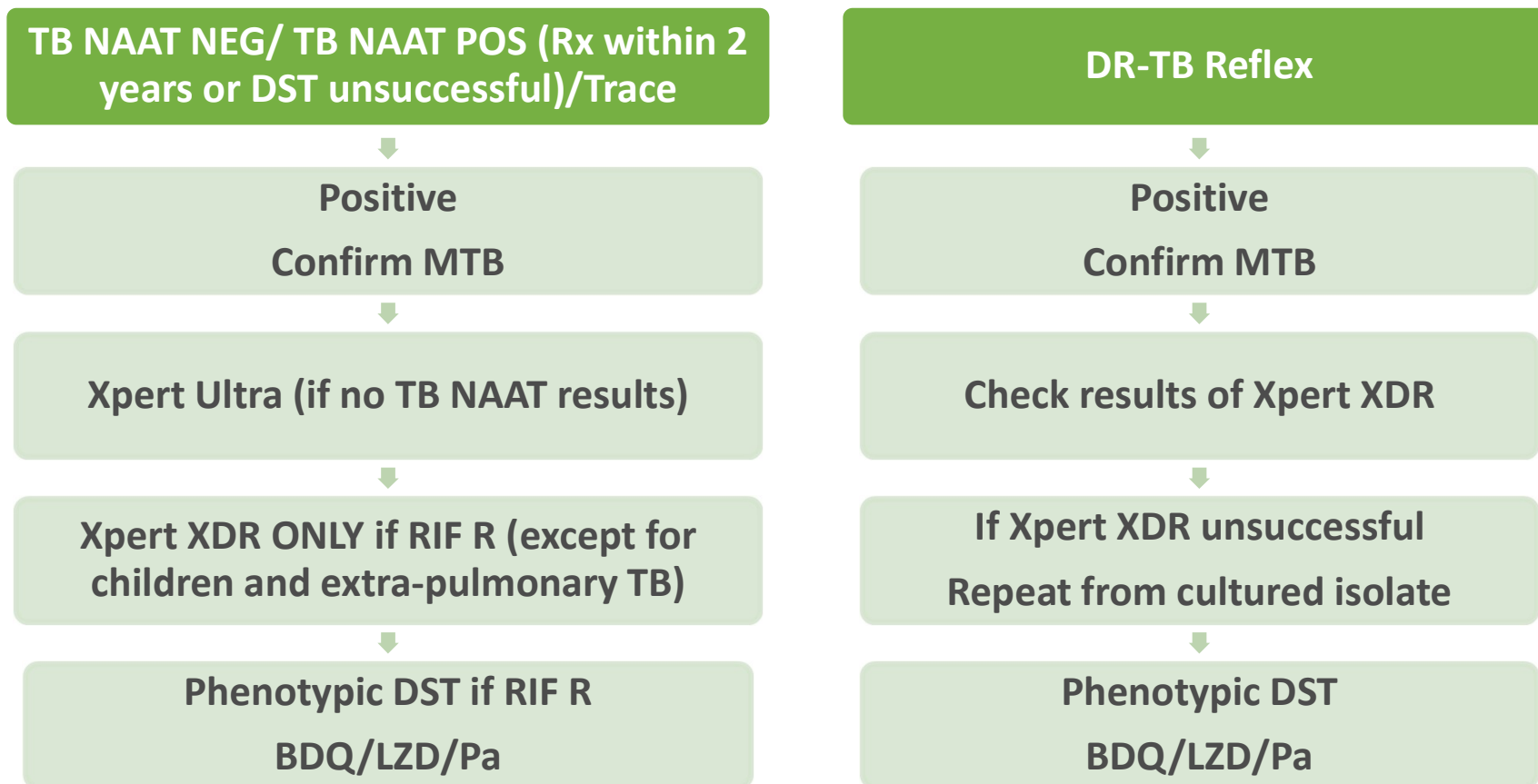
Follow-up and review culture and DST results.

Clinical presentation not consistent with TB.
No TB symptoms, clinically well and or normal chest x-ray

Continue routine care



Two scenarios for TB culture in the Laboratory





DR-TB Reflex Specimens August 2024 - July 2025 (1 year)

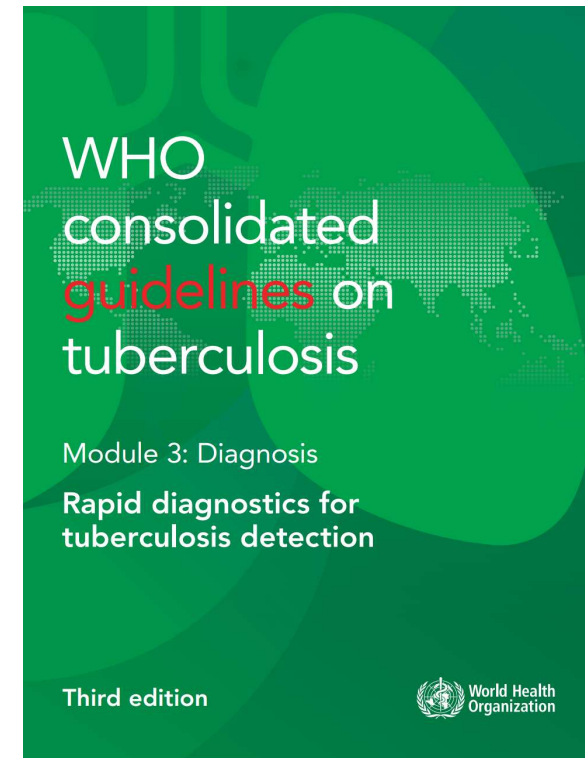
	Total	Positive (%)	Negative (%)
Xpert MTB/XDR	7434	3728 (50.2%)	3699 (49.8%)
Culture	6969	3491 (50.1%)	3478 (49.9%)
Auramine	6534	1822 (27.9%)	4712 (72.1)

During 2024-2025, 16 082 tests were conducted nationally (66.7% from concentrated specimen sediment and 33.3% from cultured isolate), with KwaZulu-Natal contributing 48.0% of tested volumes and Limpopo contributing 1.6%. Testing on concentrated sediment yielded an MTBC detection rate of 62.2%. All tests entering the drug-resistant TB-reflex workflow are specimens collected from clients diagnosed with rifampicin-resistant TB. The lower detection rate for sediment is explained by the higher detection limit of the Xpert® MTB/XDR assay compared to Xpert® MTB/RIF Ultra. The lower detection rate



Future Plans

- Targeted next generation sequencing (tNGS)
 - Deeplex[®] Myc-TB assay (Genoscreen, Lille, France)
 - Ten drugs: Rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, **bedaquiline**, **linezolid**, clofazimine, amikacin.
 - **No Pa or DLM**



WHO 2024