NATIONAL HEALTH LABORATORY SERVICE

Xpert® MTB/XDR – Considerations for Implementation:

Programmatic & laboratory perspectives South Africa as a case study

NATIONAL HEALTH LABORATORY SERVICE
National Priority Programmes

M. Pedro Da Silva[1], F. Ismail[2], S.V. Omar[2], M.S. Radebe[1], P. Marokane[1], L. Scott[1]

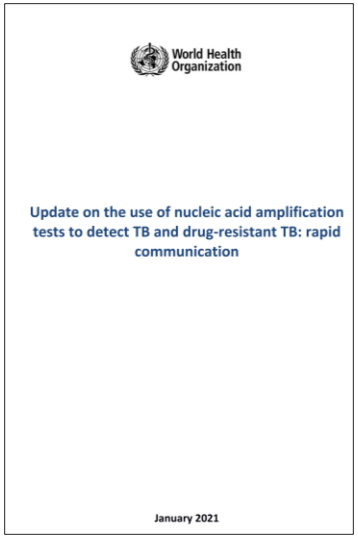
[1] National Priority Programmes, NHLS
[2] Centre for Tuberculosis & WHO TB Supranational Reference Laboratory Network, NICD, NHLS

Conflict of Interest Disclosure

[X] I have no Conflict of Interest to report.

[ ] I have the following Conflict of Interest(s) to report:

WHO Recommendations – NAATs for drug-resistance detection



Classes of technologies and associated products evaluated:

Technology Class	Products included in evaluation
Moderate complexity automated NAATs for detection of TB and resistance to rifampicin and isoniazid	• Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott) • FluoroType MTBDR and FluoroType MTB (Hain Lifescience) • BD MAX™ MDR-TB (Becton Dickinson) • cobas MTB and cobas MTB-RIF/INH (Roche)
Low complexity automated NAATs for detection of resistance to isoniazid and second-line anti-TB agents	• **Xpert MTB/XDR (Cepheid)**
High complexity hybridization-based NAATs for detection of resistance to pyrazinamide	• Genoscholar PZA-TB II (Nipro)

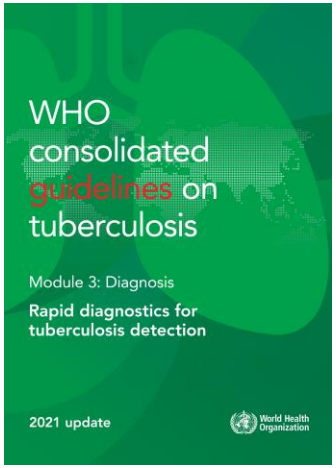
Conclusions:

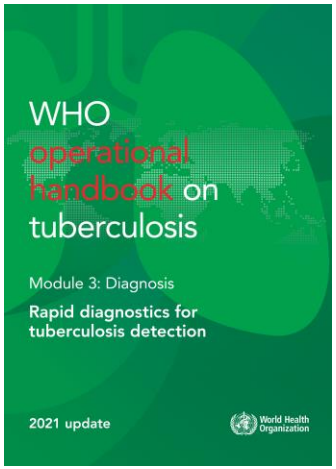
Available evidence **supports the use of**:

1. moderate complexity automated NAATs for the detection of TB and resistance to rifampicin and isoniazid;
2. **low complexity automated NAATs for the detection of resistance to isoniazid and second-line anti-TB agents**; and
3. high complexity hybridization-based NAATs for the detection of resistance to pyrazinamide.

WHO, 2021

WHO Recommendations – Low complexity automated NAATs





Recommendations – Low complexity* NAATs for detection of resistance to isoniazid and second-line anti-TB agents

1. In people with bacteriologically confirmed pulmonary TB, low complexity automated NAATs **may be used on sputum for the initial detection of resistance to isoniazid and fluoroquinolones**, rather than culture-based phenotypic DST. *(Conditional recommendation; moderate certainty of evidence for diagnostic accuracy)*
2. In people with bacteriologically confirmed pulmonary TB and resistance to rifampicin, low complexity automated NAATs **may be used on sputum for the initial detection of resistance to ethionamide**, rather than DNA sequencing of the inhA promoter. *(Conditional recommendation; very low certainty of evidence for diagnostic accuracy)*
3. In people with bacteriologically confirmed pulmonary TB and resistance to rifampicin, low complexity automated NAATs **may be used on sputum for the initial detection of resistance to amikacin**, rather than culture-based phenotypic DST. (*Conditional recommendation; low certainty of evidence for diagnostic accuracy)*

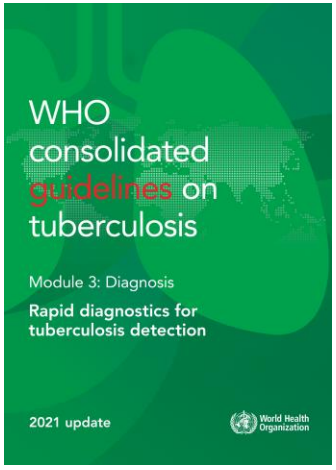
Subgroups to be considered:

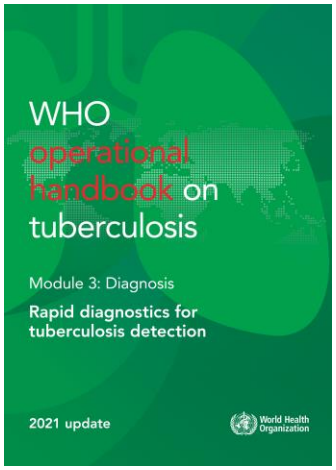
- Recommendations based on evidence of accuracy in sputum of adults with confirmed PTB.
- Extrapolated to adolescents and children and to people with EPTB.
- Apply to PLHIV.

* LOW COMPLEXITY: no specialized biosafety infrastructure is required; only basic laboratory skills to perform the test and equipment is required.

WHO, 2021

WHO Recommendations – Low complexity automated NAATs





Diagnostic performance - pooled data:

Resistance detection to:	Pooled Sensitivity	Pooled Specificity	Comparator
Isoniazid	94.2% (95% CI: 89.3-97.0%)	98.0% (95% CI: 95.2-99.2%)	Culture-based DST
Fluoroquinolones	93.1% (95% CI: 88.0-96.1%)	98.3% (95% CI: 94.5-99.5%)	Culture-based DST
Ethionamide	98.0% (95% CI: 74.2-99.9%)	99.7% (95% CI: 83.5-100.0%)	Sequencing of *inhA* promoter
Amikacin *	86.1% (95% CI: 75.0-92.7%)	98.9% (95% CI: 93.0-99.8%)	Culture-based DST

* The review did not include molecular DST for kanamycin and capreomycin as WHO does not currently recommend these second-line injectable agents for use in RR-TB or MDR-TB treatment regimens.

WHO, 2021

Xpert® MTB/XDR: Assay characteristics and operational requirements

1. Nested real-time PCR for detection of drug-resistant TB
- Self-contained cartridge minimising risk of cross-contamination
- Simultaneous detection of MTB complex, mutations associated with INH, ETH, FQ, SLID resistance

Drug	Gene Target
Isoniazid	*inhA* promoter
	katG
	fabG1
	oxyR-ahpC intergenic region
Ethionamide	*inhA* promoter
Fluoroquinolones	*gyrA*
	gyrB
Amikacin, kanamycin, and capreomycin	*rrs*
Amikacin and kanamycin	*eis* promoter

2. Built-in controls
- Sample processing control (monitor PCR inhibition)
- Probe check control (internal cartridge mechanics)

3. Time to result
- Random access
- <90 minutes: Xpert® MTB/XDR
- <80 minutes: Xpert® MTB/RIF Ultra
- Throughput: 5 tests per module/8-hour

4. Limit of detection
- Xpert® MTB/XDR: 136 cfu/mL (raw sputum); 86 cfu/mL (sediment)
- Xpert® MTB/RIF: 112.6 cfu/mL
- Xpert® MTB/RIF Ultra: 15.6 cfu/mL

Xpert® MTB/XDR: Assay characteristics and operational requirements

5. Assay intended for use as a reflex once MTB detected/rifampicin-R

- Either off residual sputum, or
- Concentrated sputum sediments

6. Operational specifications: Temperature

- Storage temperature: 2-28°C
- Unprocessed sputum: up to 7 days, 2-35°C
- Sputum sediments: up to 7 days, 2-8°C

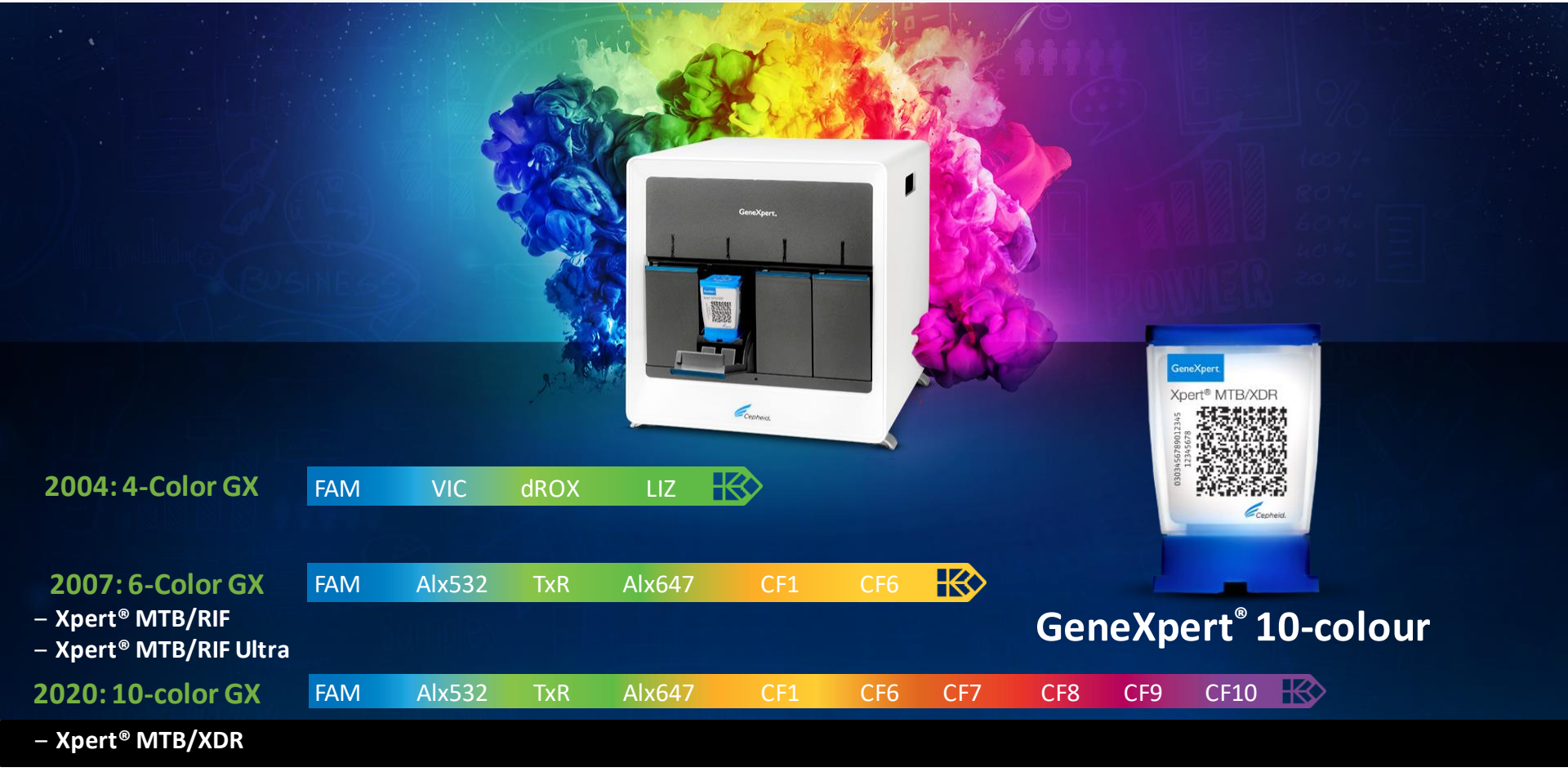
7. Reflexing to Xpert® MTB/XDR requires

- Once MTB detected/rifampicin-R then reflex off SR-treated specimen
- If SR-treated residual stored at 2-8°C, then 4 hour processing window
- If SR-treated residual stored unrefrigerated (up to 35°C), then 2.5 hour processing window

8. Minimum specimen volume

- Starting volume of raw sputum: ≥2.2ml
- ≥2ml SR-treated specimen required for reflex

Xpert® MTB/XDR: Assay characteristics and operational requirements

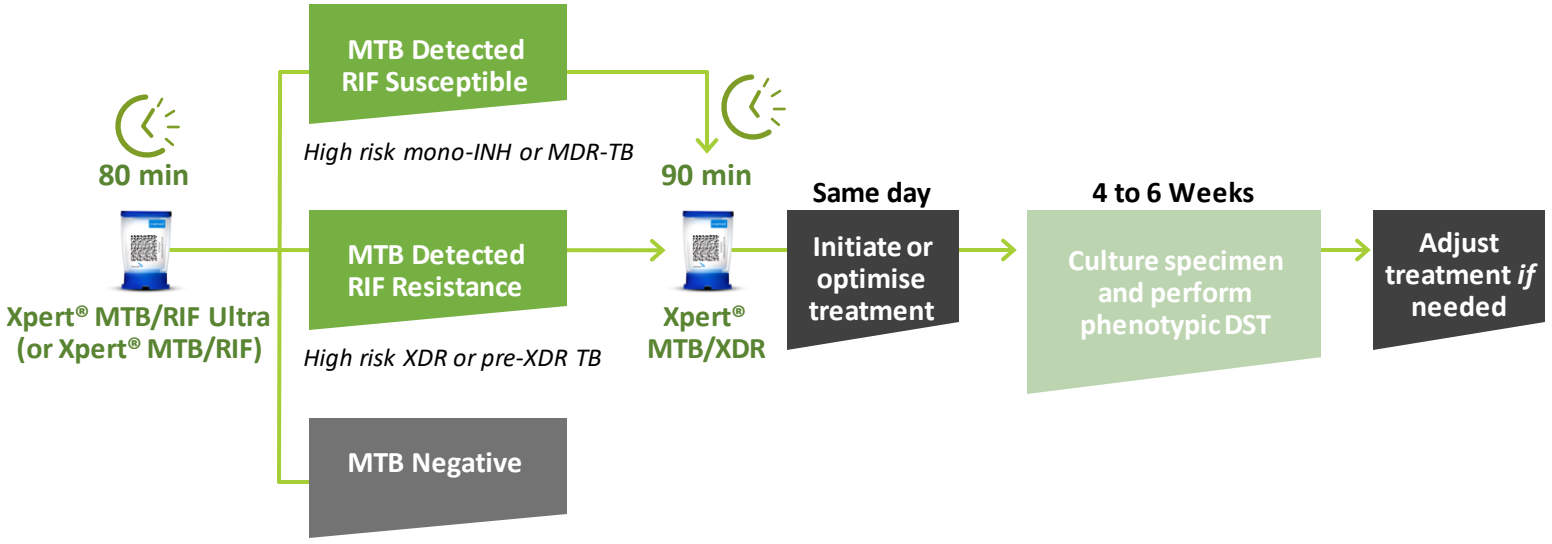


9. 10-colour GeneXpert® Technology
- Higher degree of multiplexing targeting >6 genes
- Independently monitor ≥10 signals
- Procured as new modules; new systems; or as satellites

10. 10-colour GeneXpert® Technology: Compatibility
- Supported systems: GXI, II, IV, and XVI
- Supported assays: Compatible with all Xpert® tests
- Supported software: 10-colour on Dx4,7b and higher; Xpert® MTB/XDR on Dx6,2 and higher

GENEXPERT® 10-COLOUR GRAPHIC COURTESY OF CEPHEID

Xpert® MTB/XDR: Diagnostic pathway for accurate results



DIAGNOSTIC PATHWAY GRAPHIC COURTESY OF CEPHEID (ADAPTED)

Considerations for integration of Xpert® MTB/XDR

Programmatic:

- **Epidemiology**:
  - Isoniazid, rifampicin, other drug resistance rates
- **Diagnostic algorithms**:
  - For detection of TB-disease
  - Detection of drug-resistant TB/additional resistance
  - Number of baseline-specimens collected
- **Testing & GeneXpert instrumentation**:
  - Centralised/decentralized
  - Number of modules/6-colour/10-colour
  - Operating software version
  - Testing using Xpert® MTB/RIF or MTB/RIF Ultra
- **TB program**:
  - Drug-resistant TB treatment regimens
  - Consultation and placement
- **Costs**:
  - Test cartridge costs; Instrumentation/equipment
  - Additional/repeated testing – 'indeterminate' or 'invalid' results
  - With ↑ identification, ↑ total resources to treat
  - Local costing
  - Cost-effectiveness:
    - Lead to more/fewer individuals diagnosed compared to current
    - Diagnostic algorithm and placement

Diagnostic value-chain:

- **Pre-analytical**:
  - Specimen volume
  - PTB/EPTB specimens
  - Testing volumes
  - Supply chain management
- **Analytical**:
  - Laboratory workflow
  - Refrigeration capacity
  - Technical aspects
  - ? Replacement of existing technologies
- **Post-analytical**:
  - Clinician training/guidance
  - Reporting
  - Actions and additional testing
  - Definitions

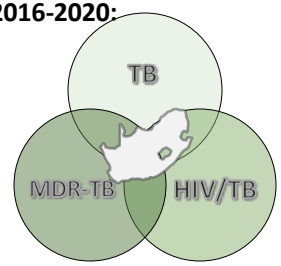
Integration of Xpert® MTB/XDR – South African context

Programmatic considerations:

– **Epidemiology**:

High-burden TB, TB/HIV, and MDR-TB, 2016-2020:

– Angola	– Mozambique
– China	– Myanmar
– DR Congo	– Nigeria
– Ethiopia	– Papua New Guinea
– India	– **SOUTH AFRICA**
– Indonesia	– Thailand
– Kenya	– Zimbabwe



Largest gaps between incident TB cases and TB incidence, 2019:

1. India	**6. SOUTH AFRICA**
2. Nigeria	7. China
3. Indonesia	8. DR Congo
4. Pakistan	9. Bangladesh
5. Philippines	10. Vietnam

Estimates of TB burden, 2019:

– Incidence (including HIV+) per 100'000:	615 (981 in 2010)
– MDR/RR-TB incidence per 100'000:	23
– HIV+ TB mortality per 100'000:	62

Estimated proportion of TB cases with MDR/RR-TB, 2019:

– New cases:	3.4%
– Previously treated cases:	7.1%

Treatment success rate, 2018:

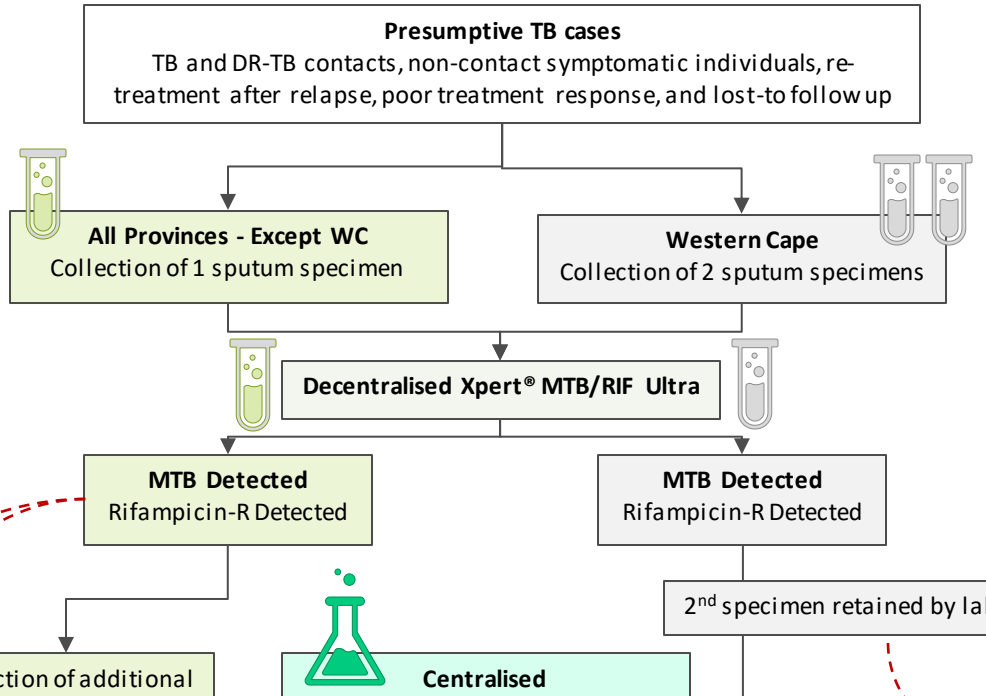
– New and relapse cases:	71%
– HIV+ TB cases:	79%
– MDR/RR- cases started on 2nd-line treatment:	60%
– XDR-TB cases started on 2nd-line treatment:	60%

Integration of Xpert® MTB/XDR – South African context

Programmatic considerations:

– **Diagnostic algorithms**:

Current diagnostic algorithm – rifampicin resistance:



- Reflex to Xpert® MTB/XDR if sufficient volume of treated residual specimen
- Would require <u>additional</u> specimen for pDST

- If insufficient volume to reflex to Xpert® MTB/XDR, then <u>additional</u> specimen required
- Channel this specimen to TB-culture laboratory to process for Xpert® MTB/XDR and culture in parallel (pDST)

- Consider losses in TB-care cascade with additional specimen requests: inability to reflex to Xpert® MTB/XDR due to insufficient volume

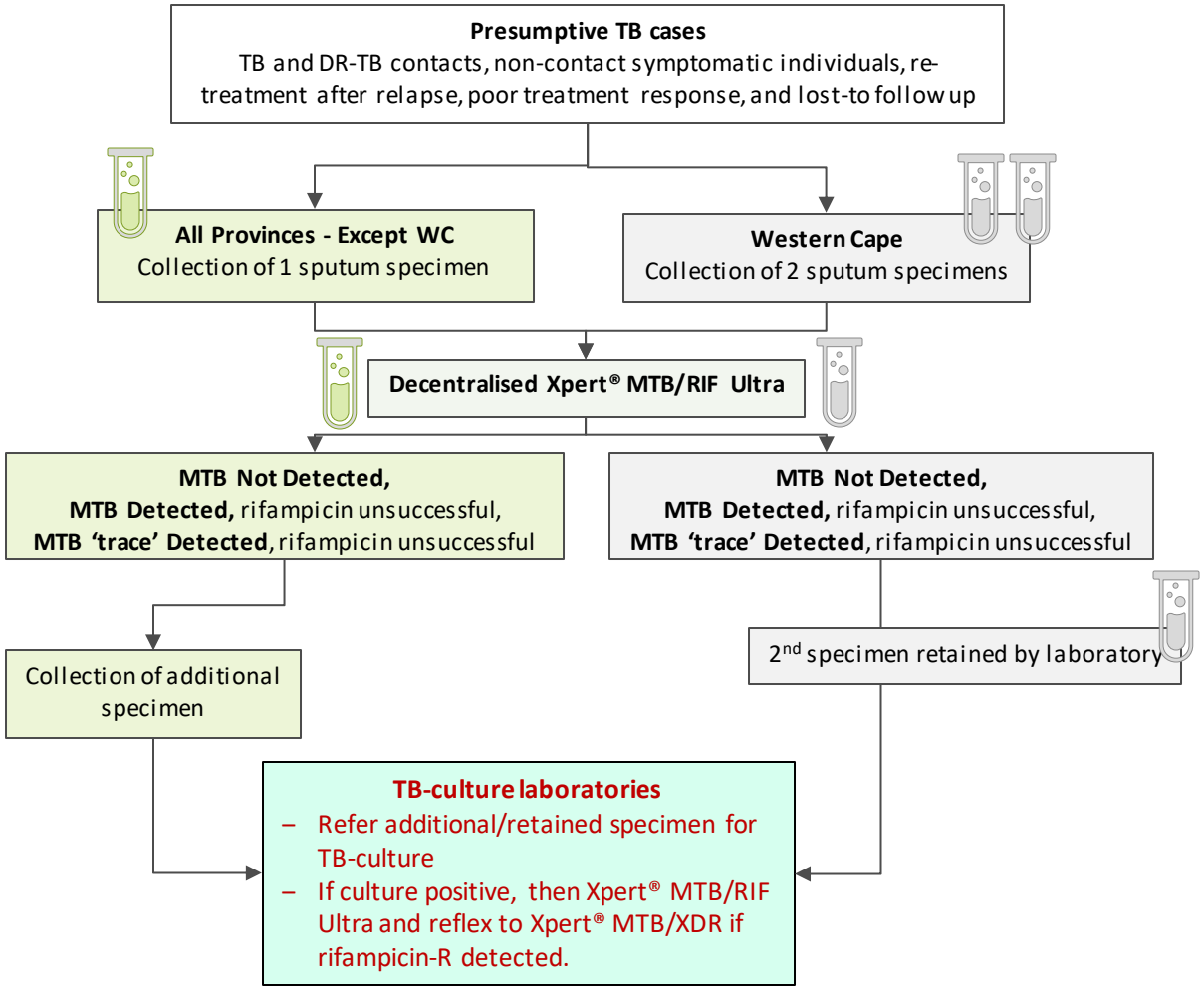
- If insufficient volume to reflex to Xpert® MTB/XDR, then refer <u>second retained</u> specimen to TB-culture laboratory to process for Xpert® MTB/XDR and culture in parallel (pDST)

Integration of Xpert® MTB/XDR – South African context

Programmatic considerations:

– **Diagnostic algorithms**:

Current diagnostic algorithm – other than rifampicin resistance:



Integration of Xpert® MTB/XDR – South African context

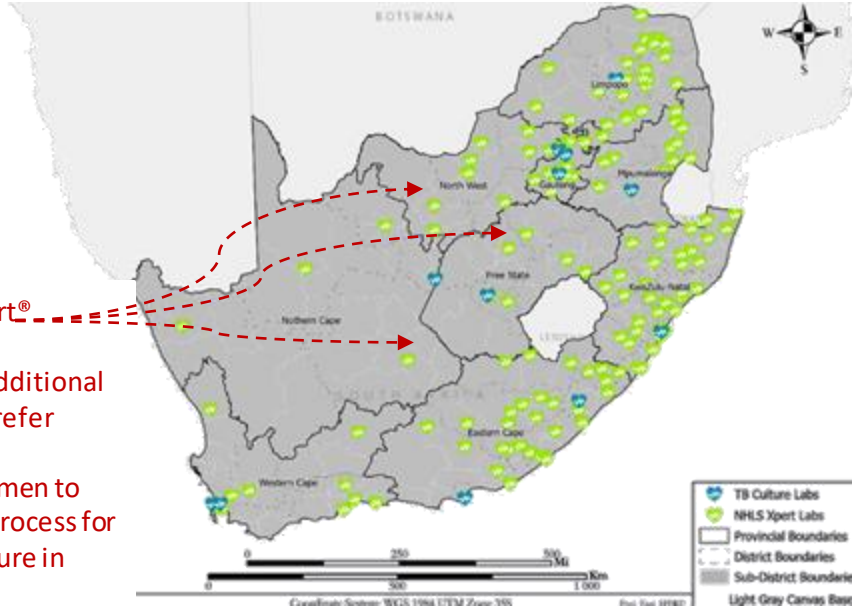
Programmatic considerations:

- **Testing & GeneXpert instrumentation**:
- **Costs**

Current Xpert® MTB/RIF Ultra infrastructure:

– Decentralised across 165 laboratories • Catering for low-, medium-, & high-throughput needs	
– Annual capacity:	>4 million tests
– Actual tested/annual:	~2 million TB tests
– MTB detection rate:	~9%
– Rifampicin-R rate:	~5%

- Decentralised reflexing to Xpert® MTB/XDR:
  - If insufficient volume or additional specimen required, then refer either second retained or additional collected specimen to TB-culture laboratory to process for Xpert® MTB/XDR and culture in parallel (pDST)



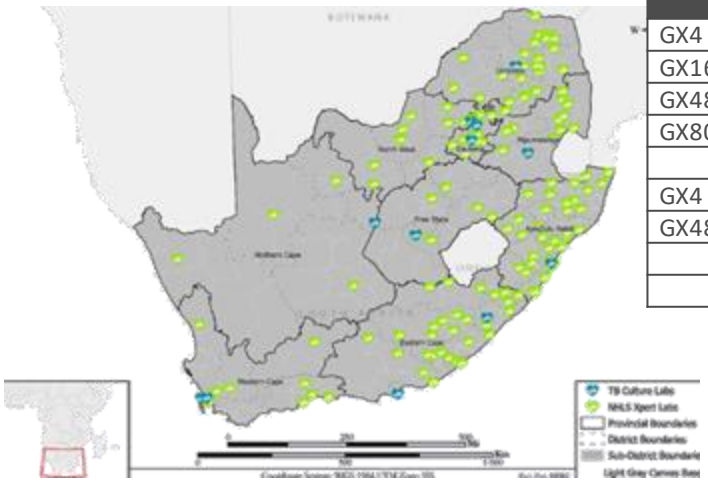
GRAPHIC COURTESY OF GRAEME DOR, DMMH, WITS UNIVERSITY

Integration of Xpert® MTB/XDR – South African context

Programmatic considerations:

- **Testing & GeneXpert instrumentation**:
- **Costs**

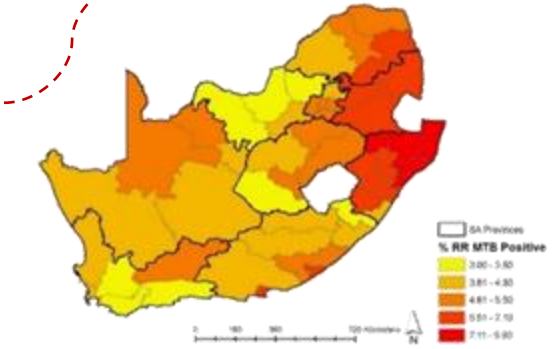
Current Xpert® MTB/RIF Ultra infrastructure:



Type	Number of Instruments	Modules per instrument type
GX4	140	560
GX16	189	3,024
GX48	1	48
GX80	8	640
Pre-COVID-19		**4,272 (all 6-colour)**
GX4	160	640
GX48	3	144
COVID-19		**784 (440 10-colour)**
Total to date		**5,056 (440 10-colour)**

- Variability in rifampicin-R rate detection:
  - Capacitation of regions with higher rifampicin-R rates

% Rifampicin Resistance by District:



GRAPHIC COURTESY OF GRAEME DOR, DMMH, WITS UNIVERSITY

Integration of Xpert® MTB/XDR – South African context

Diagnostic value-chain considerations:

– **Pre-analytical**:
  - Specimen volume
  - PTB/EPTB specimens
  - Testing volumes
  - Supply chain management

Adequate starting specimen volume to reflex to Xpert® MTB/XDR
– Majority of specimens <<2ml raw volume

Currently only validated for pulmonary TB
– Verifications required to cater for EPTB specimen types

Integration of Xpert® MTB/XDR – South African context

Diagnostic value-chain considerations:

– **Analytical**:
- Laboratory workflow
- Refrigeration capacity
- Technical aspects
- ? Replacement of existing technologies

Processing as reflex test: workflow changes
- Refrigeration of SR-treated residual to maximise 4hr processing window
- Labelling & ensuring correct retrieval
- Meeting 4hr window in high-throughput laboratories (High rifampicin-R rates)
- Reflex processing window: ? extension and impact

Technical aspects
- If no additional R-detected, no confirmation of susceptibility
- Heteroresistance/ mixed populations – proportions important

Replacement strategy for MTBDR*plus*/MTBDR*sl*
- Minimal change to existing DR-TB reflex
- Same targets: *gyrA*/*B* (FLQ); *rrs*/*eis* (SLID); *inhA* (ETH)
- INH-result/Higher sensitivity than MTBDR*plus* (2 additional targets: *fabG1* and *oxyR-ahpC* region)
- Culture performed in parallel, for pDST
- Performance not smear-status dependent: ↓TAT
- No subjective interpretation with MTBDR*plus*/*sl*
- ↓ workload
- ↓ user training/expertise required.

Integration of Xpert® MTB/XDR – South African context

Diagnostic value-chain considerations:

– **Post-analytical**:
  • Clinician training/guidance
  • Reporting
  • Actions and additional testing
  • Definitions

Introduction of a new test
– Training for clinicians on interpretation and treatment decisions
– Guideline updates

Clinical management
– Earlier clinical decision if Xpert® MTB/XDR result successful:
  • Hours *versus* days/weeks
  • ↑ patients initiating treatment
  • ↓ loss to follow-up
  • ↑ survival rates
– When not reflexed and testing off an additional specimen:
  • Delay if MTB-not detected

Updated WHO pre-XDR/XDR definitions
– Alignment within the TB-program

In conclusion

Provision of molecular DST by Xpert® MTB/XDR has numerous advantages:

- Of low complexity allowing for decentralisation and nearer to point of care testing
- Faster time to results than current standards of care
- Rapid and early detection of fluoroquinolone resistance for oral, shortened drug-R treatment regimens
- Simultaneous detection of resistant to isoniazid, fluoroquinolones, ethionamide, and amikacin
- Detection of isoniazid resistance
- Potential impact on clinical care: early initiation, reduced loss to follow up, etc.
- Better performance in paucibacilliary specimens, compare to current technologies
- Can be used to rule-in resistance

Key considerations for laboratory implementation:

- Local epidemiology
- Placement in the context of local algorithms/guidelines and existing infrastructure
- Additional investment/costs and costing analysis
- Starting specimen volume to be able to reflex following Xpert® MTB/RIF or MTB/RIF Ultra testing
- Culture-based DST still required
- Training aspects and results interpretation

Acknowledgements

- **Commercial collaborators:** Cepheid
- **FIND**
- **Clinical Laboratory Services:** Mycobacteriology laboratory staff
- **NPP Xpert Programme:** Puleng Marokane, Mbuti Samuel Radebe
- **Department of Molecular Medicine and Haematology, Wits University, NPP:** Prof Wendy Stevens
- **Department of Molecular Medicine and Haematology, Wits University, NPP, R&D team:** Anura David, Graeme Dor, Prof Lesley Scott
- **NPP Data Analyst:** Silence Ndlovu
- **Centre for Tuberculosis, NICD:** Dr Farzana Ismail, Dr Shaheed V Omar
- **NHLS, TB Subcommittee of the Microbiology Expert Committee and Wider TB-Forum**
- **NHLS Xpert and TB-culture laboratories**
- **Clinical partners**
- **Department of Health (TB Cluster)**
- **ASLM**