Xpert® MTB/XDR – Considerations for Implementation:

Programmatic & laboratory perspectives
South Africa as a case study

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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:

<table>
<thead>
<tr>
<th>Technology Class</th>
<th>Products included in evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Moderate complexity</strong></td>
<td>• Abbott RealTime MTB and Abbott RealTime MTB RIF/INH (Abbott)</td>
</tr>
<tr>
<td></td>
<td>• FluoroType MTBDR and FluoroType MTB (Hain Lifescience)</td>
</tr>
<tr>
<td></td>
<td>• BD MAX™ MDR-TB (Becton Dickinson)</td>
</tr>
<tr>
<td></td>
<td>• cobas MTB and cobas MTB-RIF/INH (Roche)</td>
</tr>
<tr>
<td><strong>Low complexity</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Xpert MTB/XDR (Cepheid)</td>
</tr>
<tr>
<td><strong>High complexity</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Genoscholar PZA-TB II (Nipro)</td>
</tr>
</tbody>
</table>

## 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 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 Recommendations – Low complexity automated NAATs

## Diagnostic performance - pooled data:

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

* 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.
**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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene Target</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td><em>inhA</em> promoter</td>
</tr>
<tr>
<td>Ethionamide</td>
<td><em>katG</em></td>
</tr>
<tr>
<td></td>
<td><em>fabG1</em></td>
</tr>
<tr>
<td></td>
<td><em>oxyR-ahpC intergenic region</em></td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td><em>gyrA</em></td>
</tr>
<tr>
<td>Amikacin, kanamycin, and capreomycin</td>
<td><em>rrs</em></td>
</tr>
<tr>
<td>Amikacin and kanamycin</td>
<td><em>eis</em> promoter</td>
</tr>
</tbody>
</table>

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-CE IVD. In vitro diagnostic medical device. May not be available in all countries. Not available in the United States.
**Xpert® MTB/XDR: Diagnostic pathway for accurate results**

- **Xpert® MTB/RIF Ultra (or Xpert® MTB/RIF)**
  - 80 min
  - MTB Detected
  - RIF Susceptible
    - High risk mono-INH or MDR-TB
  - MTB Detected
  - RIF Resistance
    - High risk XDR or pre-XDR TB
  - MTB Negative

- **MTB Detected RIF Susceptible**
  - 90 min
  - Same day
    - Xpert® MTB/XDR
    - Initiate or optimise treatment

- **MTB Detected RIF Resistance**
  - 90 min
  - Same day
    - Xpert® MTB/XDR
    - Culture specimen and perform phenotypic DST

- **MTB Negative**
  - 80 min
  - Xpert® MTB/RIF Ultra (or Xpert® MTB/RIF)

**Adjust treatment if needed**

**Note:**
- Xpert® MTB/XDR-CE-IVD. In vitro diagnostic medical device. May not be available in all countries. Not available in the United States.
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:
    o Lead to more/fewer individuals diagnosed compared to current
    o 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
- China
- DR Congo
- Ethiopia
- India
- Indonesia
- Kenya
- Mozambique
- Myanmar
- Nigeria
- Papua New Guinea
- SOUTH AFRICA

Largest gaps between incident TB cases and TB incidence, 2019:
1. India
2. Nigeria
3. Indonesia
4. Pakistan
5. Philippines
6. SOUTH AFRICA
7. China
8. DR Congo
9. Bangladesh
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:

- Reflex to Xpert® MTB/XDR if sufficient volume of treated residual specimen
- Would require additional specimen for pDST
- If insufficient volume to reflex to Xpert® MTB/XDR, then additional 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

Presumptive TB cases
TB and DR-TB contacts, non-contact symptomatic individuals, re-treatment after relapse, poor treatment response, and lost-to-follow up

All Provinces - Except WC
Collection of 1 sputum specimen

Western Cape
Collection of 2 sputum specimens

Decentralised Xpert® MTB/RIF Ultra

MTB Detected
Rifampicin-R Detected

Collection of additional specimen

Centralised
Drug-Resistance TB-Reflex at TB-culture laboratories
- Digestion & decontamination
- Smear microscopy, TB-culture, MTBDRplus, and MTBDRsl
- Phenotypic drug susceptibility testing (pDST): Either just LVX; or LZD + bedaquiline + CFZ ± LVX

MTB Detected
Rifampicin-R Detected

2nd specimen retained by laboratory

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

Xpert® MTB/XDR – Considerations for implementation: A laboratory perspective, ASLM Webinar, July 2021
**Current diagnostic algorithm – other than rifampicin resistance:**

**Presumptive TB cases**
TB and DR-TB contacts, non-contact symptomatic individuals, re-treatment after relapse, poor treatment response, and lost-to-follow up

**All Provinces - Except WC**
Collection of 1 sputum specimen

**Western Cape**
Collection of 2 sputum specimens

Decentralised Xpert® MTB/RIF Ultra

- MTB Not Detected, MTB Detected, rifampicin unsuccessful, MTB 'trace' Detected, rifampicin unsuccessful
- Collection of additional specimen

**MTB-culture laboratories**
- Refer additional/retained specimen for TB-culture
- If culture positive, then Xpert® MTB/RIF Ultra and reflex to Xpert® MTB/XDR if rifampicin-R detected.
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)

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Xpert® MTB/XDRCE-IVD. In vitro diagnostic medical device. May not be available in all countries. Not available in the United States.
Integrating Xpert® MTB/XDR – South African context

Programmatic considerations:

- Testing & GeneXpert instrumentation:
- Costs

Current Xpert® MTB/RIF Ultra infrastructure:

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

% Rifampicin Resistance by District:

- Variability in rifampicin-R rate detection:
  - Capacitation of regions with higher rifampicin-R rates
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 MTBDRplus/MTBDRsl**
  - Minimal change to existing DR-TB reflex
  - Same targets: gyrA/B (FLQ); rrs/eis (SLID); inhA (ETH)
  - INH-result/Higher sensitivity than MTBDRplus
    (2 additional targets: fabG1 and oxyR-ahpC region)
  - Culture performed in parallel, for pDST
  - Performance not smear-status dependent: ↓TAT
  - No subjective interpretation with MTBDRplus/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 paucibacillary 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

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- Department of Molecular Medicine and Haematology, Wits University, NPP, R&D team: Anura David, Graeme Dor, Prof Lesley Scott
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