Molecular assays in Tuberculosis

Jatin Yegurla
Junior resident
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Contents

• Introduction
• TB-PCR
• Line Probe assay (LPA)
• GenoType MTBDRs/® test (Second line LPA)
• Xpert MTB/RIF (GeneXpert) (CB-NAAT)
• Xpert MTB/RIF Ultra (Ultra)
• Xpert OMNI
• LAMP
INTRODUCTION
Burden of drug resistance

• Globally in 2016, an estimated 4.1% (95% CI: 2.8–5.3%) of new cases and 19% (95% CI: 9.8–27%) of previously treated cases had MDR/RR-TB.

• India - estimated % of TB cases with MDR/RR-TB 2.8% (2–3.5) of new and 12% (10–13) of Previously treated cases had MDR/RR-TB

WHO global tuberculosis report 2017
Burden of drug resistance

• In 2016, Average proportion of MDR-TB cases with XDR-TB was 6.2% (95% CI: 3.6–9.5%), with the best estimate lower than those based on data available in previous years (9.5% in 2015, 9.7% in 2014)

WHO global tuberculosis report 2017
Diagnostic algorithm for pulmonary TB

Presumptive TB patient

- Smear Examination
  - Smear Positive and CXR suggestive of TB
  - Smear Positive, but CXR not suggestive of TB

- CXR
  - Smear Negative or Not Available & CXR not suggestive of TB or not available

CBNAAT

PMDDT criteria, high MDR settings

- MTB detected
  - Rif sensitive
  - Rif Indeterminate
  - Rif Resistant

- MTB not detected or CBNAAT result not available
  - Refer to management of Rif Resistant

- Consider alternate diagnosis and refer to specialist

Microbiologically Confirmed TB

Recurrent CBNAAT on 2nd sample

*All presumptive TB cases should be offered HIV counseling and testing; however, diagnostic work up for TB must not be delayed.

Technical and operational guidelines for TB control in India, 2016
Diagnostic Algorithm for Extra Pulmonary TB

Presumptive EPTB patient

Appropriate specimen from site

Available

If CBNAAT is not available

CBNAAT

MTB detected

Rif sensitive

Microbiologically Confirmed EPTB

Rif Indeterminate

Repeat CBNAAT on fresh specimen

Rif Resistant

Refer to management of Rif resistance

Indeterminate on 2nd specimen, collect fresh sample for Liquid Culture

MTB not detected.

Not Available

High Clinical suspicion

Use other diagnostic tools

Clinically Diagnosed TB

Alternate diagnosis

Liquid Culture

Culture Positive

Microbiologically Confirmed EPTB

Culture Negative

No TB

*If high clinical suspicion then follow high clinical suspicion flow diagram

Technical and operational guidelines for TB control in India, 2016
CANDIDATES FOR DST

• Failed treatment with first line drugs
• Contacts of MDR-TB (or R resistance)
• Any Positive follow-up sputum smear test during 1st line ATT
• Prior history of anti-TB treatment
• HIV co-infection
• All presumptive TB cases among PLHIV

Ref - Standards for TB Care in India, WHO 2014
Figure 5.2 DR-TB Diagnostic Algorithm

Presumptive TB

Key/Vulnerable populations
- Paediatric age group
- People living with HIV
- EPTB sites
- Smear negative/NA with X-ray suggestive of TB

All diagnosed TB patients

- Non responders to treatment
- DR-TB contacts
- Previously treated TB
- TB-HIV co-infection
- New TB patients

CBNAAT

RR TB
- For discordance on LPA for RR-TB – repeat CBNAAT at LPA lab

RS TB

SL - LPA**

FQ and SLI Sensitive

FQ and/or SLI Resistance

H Resistant

H Sensitive

FL-LPA*

*Offer molecular testing for H mono/poly resistance to TB patients prioritized by risk as per the available lab capacity

**LC DST (Mfx 2.0, Km, Cm, Lzd) will be done only for patients with any resistance on baseline SL-LPA. DST to Z, Cfx, Bdq & Dlm would be considered for policy in future, whenever available, standardized & WHO endorsed.

$ States to advance in phased manner as per PMDT Scale up plan for universal DST based on lab capacity and policy on use of diagnostics

PMDT 2017
Diagnostic Algorithm for Bedaquiline containing and optimized treatment regimen

Presumptive TB Case

- Smear

  Key Population
  PLHIV, Children, EPTB

  - Chest X Ray Abnormal* (Only for presumptive TB)

    - CBNAAT
      - RR or RH resistant
      - Baseline LC DST to E, Z, Km, Cm, Lfx, Mfx (2.0), Lzd, Eto& LPA for H (in presumptive DR TB)
        (In case of resistance to any FQ or SLID)
        Perform extended DST for PAS, Cfx
        BDQ DST (when available)
      - Continue treatment as per category in RNTCP guidelines
    - Only H resistant
      - Baseline LC DST to E, Z, Km, Lfx
      - If resistance to FQ&/or SLID, Perform extended DST (remaining SLD)

Presumptive DR TB Case

- Sm Neg Negative
- Sm Positive

LPA

- R & H sensitive
- Continue treatment as per category in RNTCP guidelines

* Those who do not fit in the definition of presumptive DR-TB case
Phenotypic vs Genotypic DST

<table>
<thead>
<tr>
<th>Phenotypic DST</th>
<th>Genotypic DST</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Evaluation of growth in drug containing media</td>
<td>• Detect genetic mutations</td>
</tr>
<tr>
<td>• Proportion method</td>
<td>• Amplification of specific target of RNA/DNA sequence using NA probe</td>
</tr>
<tr>
<td>• Turn around time:</td>
<td>• Targets- 16S RNA, IS6110</td>
</tr>
<tr>
<td>• Solid LJ media 84 days</td>
<td>• Turnaround time:</td>
</tr>
<tr>
<td>• Liquid Culture(MGIT) - 42 days</td>
<td>• LPA:72 hours</td>
</tr>
<tr>
<td></td>
<td>• CB-NAAT: 2 hours</td>
</tr>
</tbody>
</table>
Phenotypic DST/Culture based tests

• Merits
  – Bacterial growth can be identified visually or by automated detection of its metabolism
  – Provides definitive diagnosis of TB
  – Provides necessary isolates for conventional DST

• Demerits
  – Takes longer time
  – Needs appropriate lab infrastructure and bio safety precautions
### Genes involved in Drug resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Genes involved in resistance</th>
<th>Gene function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>inhA, katG, kasA</td>
<td>Enoyl ACP reductase</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>rpoB gene</td>
<td>Mycobacterial RNA polymerase</td>
</tr>
<tr>
<td>Pyrazinamide</td>
<td>pncA</td>
<td>Pyrazinamidase</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB</td>
<td>Arabinosyl transferase</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>rpsL ,rrs, gidB</td>
<td>rRNA methyltransferase</td>
</tr>
</tbody>
</table>
## Genes involved in drug resistance

<table>
<thead>
<tr>
<th>Drug</th>
<th>Genes involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoroquinolones</td>
<td>gyrA/gyrB</td>
</tr>
<tr>
<td>Kanamycin/amikacin</td>
<td>rrs</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>tlyA</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>inhA</td>
</tr>
<tr>
<td>p-amino salicylic acid</td>
<td>thyA</td>
</tr>
<tr>
<td>PA-824 and OPC-67683</td>
<td>Rv3547 (hypothetical)</td>
</tr>
<tr>
<td>TMC207</td>
<td>atpE</td>
</tr>
</tbody>
</table>
Genotype DST/PCR based tests

- Demerits
  - Detects DNA from **both viable and non-viable** bacteria.
    - Cannot be used for monitoring the progression or successful therapy.
  - Only screens the nucleic acid sequence and **not the amino acid sequence**.
    - Mutations in the probe region that don't cause amino acid exchange (silent mutations) will still produce the absence of one of the wild type bands.
# Probe Based vs Sequence based tests

<table>
<thead>
<tr>
<th>Probe based tests</th>
<th>Sequence based tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detect only if a mutation is present</td>
<td>Provide sequence information and nature of specific mutation</td>
</tr>
<tr>
<td>Also detect silent or missense mutations and signal drug resistance which do not</td>
<td>Hence, can predict drug resistance with greater accuracy</td>
</tr>
<tr>
<td>confer drug resistance in culture</td>
<td></td>
</tr>
<tr>
<td>CB-NAAT LPA</td>
<td>Pyro sequencing</td>
</tr>
<tr>
<td></td>
<td>Sanger sequencing</td>
</tr>
<tr>
<td></td>
<td>Next generation sequencing</td>
</tr>
<tr>
<td>FDA approved</td>
<td>Not FDA approved</td>
</tr>
</tbody>
</table>
Probe Based vs Sequence based tests

**PCR: Polymerase Chain Reaction**

30 - 40 cycles of 3 steps:

1. **Step 1: denaturation**
   - 1 minute 94 °C

2. **Step 2: annealing**
   - 45 seconds 54 °C
   - forward and reverse primers !!!

3. **Step 3: extension**
   - 2 minutes 72 °C
   - only dNTP's

**Sequencing**

30 cycles of 3 steps:

1. **Step 1: denaturation**
   - 1 minute 94 °C

2. **Step 2: annealing**
   - 15 seconds 50 °C
   - 1 primer !!!

3. **Step 3: extension**
   - 4 minutes 60 °C
   - mixture of dNTP's and ddNTP's

(Andy Viarengo 1999)
Probe based: Exponential Amplification

Exponential amplification

1st cycle

2nd cycle

3rd cycle

4th cycle

35th cycle

$2^2 = 4$ copies

$2^3 = 8$ copies

$2^4 = 16$ copies

$2^5 = 32$ copies

$2^{36} = 68$ billion copies

(template DNA)

(wanted gene)
Sequencing: Linear amplification

6 template strands

1st cycle
6 complementary strands

2nd cycle
12 complementary strands

Linear amplification

30 cycles: 180 complementary strands

mixture of strands with different length which end on a fluorescently labelled ddNTP

PCR product

- Primer fits only on one strand

- On incorporation of a fluorescently labelled ddNTP (complementary with the base on the template) the elongation stops

(Andy Vierstraete 1999)
TB PCR
TB PCR

• Multistep in-house PCR
• Amplifies IS 986 or IS 6110 repetitive element specific to M. tuberculosis
• Sensitivity 84 -96 % (Smear positive 96-100%, Smear negative 50-92%)
• Specificity 70-100%
• Provides result in 24-48 hrs

<table>
<thead>
<tr>
<th>Test</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Amplified M. tuberculosis Direct Test (MTD)</td>
<td>AFB smear positive respiratory specimens</td>
</tr>
<tr>
<td>2. Amplicor M. tuberculosis Test</td>
<td>AFB smear positive respiratory specimens</td>
</tr>
<tr>
<td>3. Enhanced MTD test</td>
<td>AFB smear negative respiratory specimens</td>
</tr>
</tbody>
</table>

Ref : PCR For diagnosis of tuberculosis: where are we now? Ind. J Tub. 2000, 47. 79
LINE PROBE ASSAY
# LPA vs CB NAAT

<table>
<thead>
<tr>
<th></th>
<th>LPA</th>
<th>CB-NAAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO endorsed</td>
<td>2008</td>
<td>2010</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Not used</td>
<td>Used</td>
</tr>
<tr>
<td>Resistance</td>
<td>INH and RIF</td>
<td>RIF alone</td>
</tr>
<tr>
<td>Specimens</td>
<td>Smear positive only</td>
<td>Smear positive/negative</td>
</tr>
<tr>
<td>Turnaround time</td>
<td>72 hours</td>
<td>2 hours</td>
</tr>
<tr>
<td>Steps</td>
<td>Separate steps DNA extraction-PCR</td>
<td>Single cartridge for sample processing, amplification and detection</td>
</tr>
<tr>
<td></td>
<td>amplification-Colorimetric detection</td>
<td></td>
</tr>
<tr>
<td>Cross contamination and operator dependence</td>
<td>yes</td>
<td>No</td>
</tr>
</tbody>
</table>
LPA: Commercially available types

1. INNO-LiPA Rif.TB (Innogenetics, Ghent, Belgium)
2. Genotype MTBDR (Hain LifeScience, Germany)
3. Genotype MTBDRplus (Hain LifeScience, Germany)
4. Genotype MTBDRsl (Hain LifeScience, Germany)
## Mutations detected

<table>
<thead>
<tr>
<th>Mutations detected</th>
<th>Drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. INNO-LiPA Rif.TB</td>
<td>rpoB</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
</tr>
<tr>
<td>2. Genotype MTBDR</td>
<td>rpoB</td>
</tr>
<tr>
<td></td>
<td>katG</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
</tr>
<tr>
<td></td>
<td>Isoniazid</td>
</tr>
<tr>
<td>3. Genotype MTBDRplus</td>
<td>rpoB</td>
</tr>
<tr>
<td></td>
<td>katG</td>
</tr>
<tr>
<td></td>
<td>inhA</td>
</tr>
<tr>
<td></td>
<td>Rifampicin</td>
</tr>
<tr>
<td></td>
<td>Isoniazid</td>
</tr>
<tr>
<td>4. Genotype MTBDRsl version 1</td>
<td>rrs</td>
</tr>
<tr>
<td></td>
<td>gyrA</td>
</tr>
<tr>
<td></td>
<td>EMB</td>
</tr>
<tr>
<td></td>
<td>SLID</td>
</tr>
<tr>
<td></td>
<td>Fluroquinolones</td>
</tr>
<tr>
<td></td>
<td>Ethambutol</td>
</tr>
<tr>
<td>5. Genotype MTBDRsl version 2</td>
<td>rrs, eis</td>
</tr>
<tr>
<td></td>
<td>gyrA, gyrB</td>
</tr>
<tr>
<td></td>
<td>SLID</td>
</tr>
<tr>
<td></td>
<td>FQ</td>
</tr>
</tbody>
</table>

Principle and procedure

1. **DNA extraction** from the clinical specimens (pulmonary, decontaminated) or the cultured material (solid/liquid medium)

2. Multiplex **amplification** with biotinylated primers

3. **Reverse hybridization.**
   - Membrane strips are coated with specific probes complementary to the amplified nucleic acids.
   - After chemical denaturation, the single stranded amplicons bind to the probes (hybridization)

Principle and procedure

GenoType® MTBDRplus for MDR TB

Specimen requirements

• Pulmonary **smear positive** specimens such as sputum (induction or expectorated), bronchial material (bronchoalveolar lavage) or aspirates (pleural aspirates).

• **Cultivated samples** (solid/liquid medium).

Storage and transport

1. Collected in a sterile container and stored at a temperature of 2-8 °C.

2. The transport of specimens at room temperature has to be carried out as soon as possible and should be done within 1-2 days.

3. Specimens used for decontamination should not be older than 4 days.

4. After decontamination & subsequent resuspension of the bacteria pellet with phosphate buffer, the samples can be stored at -20 °C or -80 °C for a maximum of 5 days until DNA extraction is performed.

## LPA Performance - WHO 2016

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rifampicin resistance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Direct-sputum specimens)</td>
<td>97.1 (93.3–99.0) (166/171)</td>
<td>97.1 (94.3–98.7) (267/275)</td>
</tr>
<tr>
<td></td>
<td>98.2 (95.0–99.6) (168/171)</td>
<td>97.8 (95.3–99.2) (269/275)</td>
</tr>
<tr>
<td>(Indirect-culture isolates)</td>
<td>91.3 (86.0–95.0) (157/172)</td>
<td>97.1 (94.3–98.7) (267/275)</td>
</tr>
<tr>
<td></td>
<td>91.3 (86.0–95.0) (157/172)</td>
<td>97.1 (94.3–98.7) (267/275)</td>
</tr>
<tr>
<td><strong>INH resistance</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Direct testing)</td>
<td>94.4 (90.2–97.2) (186/197)</td>
<td>95.4 (91.5–97.9) (188/197)</td>
</tr>
<tr>
<td></td>
<td>96.4 (93.2–98.3) (240/249)</td>
<td>98.8 (96.5–99.8) (246/249)</td>
</tr>
<tr>
<td>(Indirect testing)</td>
<td>89.4 (84.3–93.3) (178/199)</td>
<td>98.9 (96.0–99.9) (175/177)</td>
</tr>
<tr>
<td></td>
<td>89.4 (84.3–93.3) (178/199)</td>
<td>98.9 (96.0–99.9) (175/177)</td>
</tr>
</tbody>
</table>

V1:MTBDRTB plus version 1 (Hain version 1)
V2:MTBDRTB plus version 2 (Hain version 2)

WHO/HTM/TB/2016.12
LPA Performance- WHO 2016

• Patients with signs and symptoms consistent with TB and a positive LPA result can be treated with confidence.
• Strong correlation with Phenotypic resistance
• Similar diagnostic accuracy for direct or indirect tests

LPA – WHO recommendations 2016

• Sputum smear-positive specimens (direct testing)
• Cultured isolates of MTBC (indirect testing) from both pulmonary and extra pulmonary sites
• Not recommended for the direct testing of sputum smear-negative specimens
• Do not eliminate the need for conventional culture-based DST

LPA – WHO recommendations 2016

• Culture based DST will still be necessary in addition to LPA
  – to determine resistance to other anti-TB agents
  – to monitor the emergence of additional drug resistance
  – to detect INH resistance, when the LPA result is negative but high pre test probability present
  – to identify false positives from LPA (dead bacilli)

• Applied to the use of LPA in children based on the generalization of data from adults

LPA

• Merits :
  - Lower contamination rates than culture
  - Detects resistance genes for Rifampicin and INH
  - Results available in 48-72 hours

• Demerits : Requires
  - Skilled man power (Training)
  - Specialized equipment
  - Dedicated space to avoid cross-contamination between specimens
  - Manual processing of specimen
  - Complexity & no. of steps preclude use in peripheral settings
  - Do not perform well on pauci-bacillary specimen
GENOTYPE MTBDR\textsuperscript{SL} ® TEST
GenoType MTBDRs/®

• Endorsed by the WHO.
• Field validation of the MTBDRs/ assay in smear-positive patients completed in India.
• To detect additional resistance to second line drugs in confirmed MDR-TB/RR-TB
<table>
<thead>
<tr>
<th>New grouping of drugs</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. Fluoroquinolones</strong></td>
<td></td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>Lfx</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>Mfx</td>
</tr>
<tr>
<td>Gatifloxacin</td>
<td>Gfx</td>
</tr>
<tr>
<td><strong>B. Second-line injectable agents</strong></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>Am</td>
</tr>
<tr>
<td>Capreomycin</td>
<td>Cm</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>Km</td>
</tr>
<tr>
<td>(Streptomycin)</td>
<td>(S)</td>
</tr>
<tr>
<td><strong>C. Other second-line agents</strong></td>
<td></td>
</tr>
<tr>
<td>Ethionamide / Prothionamide</td>
<td>Eto/Pto</td>
</tr>
<tr>
<td>Cycloserine / Terizidone</td>
<td>Cs/Trd</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Lzd</td>
</tr>
<tr>
<td>Clofazimine</td>
<td>Cfz</td>
</tr>
<tr>
<td><strong>D. Add-on agents (not part of the core MDR-TB regimen)</strong></td>
<td></td>
</tr>
<tr>
<td>D1 Pyrazinamide</td>
<td>Z</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>E</td>
</tr>
<tr>
<td>High-dose isoniazid</td>
<td>H^h</td>
</tr>
<tr>
<td>D2 Bedaquiline</td>
<td>Bdq</td>
</tr>
<tr>
<td>Delamanid</td>
<td>Dlm</td>
</tr>
<tr>
<td>D3 p-aminosalicylic acid</td>
<td>PAS</td>
</tr>
<tr>
<td>Imipenem-cilastatin</td>
<td>Ipm / Cls</td>
</tr>
<tr>
<td>Meropenem</td>
<td>Mpm</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>Amx-Clv</td>
</tr>
<tr>
<td>(Thioacetazone)</td>
<td>(T)</td>
</tr>
</tbody>
</table>
## Mutations detected

<table>
<thead>
<tr>
<th>1. INNO-LiPA Rif.TB</th>
<th>rpoB</th>
<th>Rifampicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Genotype MTBDR</td>
<td>rpoB katG</td>
<td>Rifampicin Isoniazid</td>
</tr>
<tr>
<td>3. Genotype MTBDRplus</td>
<td>rpoB katG inhA</td>
<td>Rifampicin Isoniazid</td>
</tr>
<tr>
<td>4. Genotype MTBDRsl version 1</td>
<td>rrs gyrA EMB</td>
<td>Fluroquinolones Ethambutol</td>
</tr>
<tr>
<td>5. Genotype MTBDRsl version 2</td>
<td>rrs,eis gyrA,gyrB</td>
<td>SLID FQ</td>
</tr>
</tbody>
</table>

# SL LPA: Commercially available types

Table 1. Characteristics of Genotype MTBDRsl versions 1.0 and 2.0 as per manufacturer

<table>
<thead>
<tr>
<th>Detection</th>
<th>Version 1</th>
<th>Version 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detects resistance to</td>
<td>FQ, SLID, Ethambutol</td>
<td>FQ, SLID</td>
</tr>
<tr>
<td>Samples</td>
<td>Smear +, cultures</td>
<td>Smear- and smear +, cultures</td>
</tr>
<tr>
<td>FQ</td>
<td>gyrA</td>
<td>gyrA, gyrB</td>
</tr>
<tr>
<td>SLID</td>
<td>rrs</td>
<td>rrs, eis</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>embB</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

Procedure

- Decontamination (e.g. with sodium hydroxide) and concentration of a sputum specimen by centrifugation
- Isolation and amplification of DNA
- Detection of the amplification products by reverse hybridization
- Visualization using a streptavidin-conjugated alkaline phosphatase colour reaction

GenoType MTBDRs®

- Turn around time: 24-48 hours
- Allows quick triage of MDR-TB patients into either the shorter MDR-TB regimen or the conventional longer regimen.
- If Positive SL-LPA is treated with shorter regimen  
  – treatment outcome jeopardised  
  – risk of development of XDR-TB
- XDR-TB + by the SL-LPA: carefully designed individual regimen
## Accuracy of MTBDRsl

<table>
<thead>
<tr>
<th>Sr. No</th>
<th>Name of Drug</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fluoroquinolone</td>
<td>97%</td>
<td>98%</td>
</tr>
<tr>
<td>2</td>
<td>SLID (Second line injectable dug)</td>
<td>89%</td>
<td>90%</td>
</tr>
<tr>
<td>3</td>
<td>XDR TB</td>
<td>79%</td>
<td>97%</td>
</tr>
</tbody>
</table>

## Accuracy of MTBDRsI

<table>
<thead>
<tr>
<th></th>
<th>Pooled sensitivity (95% CI)</th>
<th>Pooled specificity (95% CI)</th>
<th>Pooled sensitivity (95% CI)</th>
<th>Pooled specificity (95% CI)</th>
<th>Pooled sensitivity P value¹</th>
<th>Pooled specificity P value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fluoroquinolones, indirect testing</strong></td>
<td>85.6% (79.2 to 90.4)</td>
<td>98.5% (95.7 to 99.5)</td>
<td>86.2% (74.6 to 93.0)</td>
<td>98.6% (96.9 to 99.4)</td>
<td>0.932</td>
<td>0.333</td>
</tr>
<tr>
<td><strong>Second-line injectable drugs, indirect testing</strong></td>
<td>76.5% (63.3 to 86.0)</td>
<td>99.1% (97.3 to 99.7)</td>
<td>87.0% (38.1 to 98.6)</td>
<td>99.5% (93.6 to 100.0)</td>
<td>0.547</td>
<td>0.664</td>
</tr>
<tr>
<td><strong>XDR-TB, indirect testing</strong></td>
<td>70.9% (42.9 to 88.8)</td>
<td>98.8% (96.1 to 99.6)</td>
<td>69.4% (38.8 to 89.0)</td>
<td>99.4% (95.0 to 99.3)</td>
<td>0.888</td>
<td>0.855</td>
</tr>
</tbody>
</table>

¹ Likelihood ratio test for evidence of a significant difference between accuracy estimates.
WHO Recommends the use of the SL-LPA for patients with MDR-TB (or RR-TB) as the initial test to detect resistance to fluoroquinolones and the second-line injectable drugs, instead of phenotypic culture-based drug-susceptibility testing (DST).

(conditional recommendation)
WHO Recommendation 2016

- Both direct as well as indirect testing
- Both pulmonary and extra pulmonary samples
- For second-line injectable results, resistance conferring mutations detected by SL-LPA are highly correlated with culture-based phenotypic resistance.
- For fluoroquinolones, ofloxacin/levofloxacin better correlated than moxifloxacin
  - Inclusion of moxifloxacin in a RR or MDR-TB regimen: best guided by phenotypic testing
- Need phenotypic DST
# LPA vs CB NAAT

<table>
<thead>
<tr>
<th></th>
<th>LPA</th>
<th>CB-NAAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO endorsed</td>
<td>2008</td>
<td>2010</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>Not used</td>
<td>Used</td>
</tr>
<tr>
<td>Resistance</td>
<td>INH and RIF</td>
<td>RIF alone</td>
</tr>
<tr>
<td>Specimens</td>
<td>Smear positive only</td>
<td>Smear positive/negative</td>
</tr>
<tr>
<td>Turnaround time</td>
<td>72 hours</td>
<td>2 hours</td>
</tr>
<tr>
<td>Steps</td>
<td>Separate steps DNA extraction-PCR</td>
<td>Single cartridge for sample processing, amplification and detection</td>
</tr>
<tr>
<td></td>
<td>amplification-Calorimetric detection</td>
<td></td>
</tr>
<tr>
<td>Cross contamination and operator dependence</td>
<td>yes</td>
<td>No</td>
</tr>
</tbody>
</table>
Xpert MTB/RIF (GeneXpert)

- Single-use disposable Cartridge containing all necessary elements
  - Automated sample preparation, amplification & detection
- Provides results from unprocessed sputum samples
- Limit of detection (LOD) of 133 CFU/ml sputum
- Digital read outs within 2 hours
- Minimal specimen handling & bio-safety requirements
- Technicians trained in 2-3 hrs
- In-built quality control
GeneXpert Dx System Components

- **Catridge**
  - Self contained
  - Disposable

- **Computer system**
  - Software
  - Barcode scanner

- **Optional accessories**
  - Printer
  - UPS

- **Modules**
  - Thermal and optical system
GeneXpert - Procedure

1. Add 2:1 Sample Buffer to sample

2. Shake then stand 10 minutes

3. Shake then stand further 5 minutes

4. Transfer 2ml to cartridge

Begin Test...
GeneXpert- Procedure

1. Sputum liquefaction and inactivation with 2:1 sample reagent
2. Transfer of 2 ml material into test cartridge
3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)
4. Sample automatically filtered and washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules mixed with dry PCR reagents
7. Seminested real-time amplification and detection in integrated reaction tube
8. Printable test result
CBNAAT Result algorithm

- **MTB and Rif Res detected**
  - H/o Previous Treatment
    - Refer to DR TB Centre
      - Rif Res
        - Refer to DR TB Centre
        - Decide treatment as per result of DST
  - No H/o Previous treatment
    - Confirmatory Repeat CBNAAT

- **MTB detected and Rif Sen**
  - H/o of previous treatment
    - Initiate Regimen for PT TB patient
      - Test with LPA/liquid
      - Decide treatment as per result of DST

- **MTB Detected and Rif Ind / invalid**
  - Resend fresh sample
    - Send fresh sample for C&DST

- **MTB not detected**
  - Rule out other Diff Diag

- **Error/ no result**
  - Repeat test on same sample if available

- **Invalid**
  - Repeat test in second sample

(Guidance document for use of CB-NAAT under RNTCP, Sept 2013)
GeneXpert Performance for pulmonary samples

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity % (95% CI)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Culture positive</td>
<td>Smear negative-culture positive</td>
<td>Smear positive-culture positive</td>
</tr>
<tr>
<td>N = 1437</td>
<td>95.7 (93.4–97.2)</td>
<td>77.7 (66.9–85.8)</td>
<td>99.2 (97.6–99.7)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Specificity % (95% CI)</th>
<th>PPV % (95% CI)</th>
<th>NPV % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99.6 (98.9–99.8)</td>
<td>99.0 (97.6–99.6)</td>
<td>98.1 (97.0–98.7)</td>
</tr>
</tbody>
</table>

Diagnostic performance of Xpert MTB/RIF assay in different respiratory samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Expectorated sputum [n = 1092]</th>
<th>Endotracheal tube aspirate [n = 143]</th>
<th>Bronchoalveolar lavage [n = 127]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity %</td>
<td>96.9 (94.7–98.2)</td>
<td>87.5 (63.9–96.5)</td>
<td>90.0 (69.9–97.2)</td>
</tr>
<tr>
<td>Specificity %</td>
<td>99.8 (99.2–99.9)</td>
<td>98.4 (94.4–99.5)</td>
<td>100 (96.5–100)</td>
</tr>
<tr>
<td>PPV %</td>
<td>99.7 (98.5–99.9)</td>
<td>87.5 (63.9–96.5)</td>
<td>100 (82.4–100)</td>
</tr>
<tr>
<td>NPV %</td>
<td>98.3 (97.0–99.0)</td>
<td>98.4 (94.4–99.5)</td>
<td>98.1 (93.5–99.5)</td>
</tr>
</tbody>
</table>

PPV-Positive predictive value, NPV- Negative predictive value. Values in parentheses are 95% confidence intervals

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Induced sputum [n = 71]</th>
<th>Bronchial wash [n = 4]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity %</td>
<td>84.2 (62.4–94.4)</td>
<td>100 (34.2–100)</td>
</tr>
<tr>
<td>Specificity %</td>
<td>98.0 (89.0–99.6)</td>
<td>100 (34.2–100)</td>
</tr>
<tr>
<td>PPV %</td>
<td>94.1 (73.0–98.9)</td>
<td>100 (34.2–100)</td>
</tr>
<tr>
<td>NPV %</td>
<td>94.4 (84.8–98.0)</td>
<td>100 (34.2–100)</td>
</tr>
</tbody>
</table>

Rifampin susceptibility testing by Xpert MTB/RIF and phenotypic DST.

<table>
<thead>
<tr>
<th></th>
<th>Rif resistant by DST</th>
<th>RIF sensitive by DST</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF resistant by Xpert</td>
<td>104</td>
<td>7</td>
<td>111</td>
</tr>
<tr>
<td>RIF sensitive by Xpert</td>
<td>6</td>
<td>305</td>
<td>311</td>
</tr>
<tr>
<td>Total</td>
<td>110</td>
<td>312</td>
<td>422</td>
</tr>
</tbody>
</table>

Sensitivity- 94.5% (88.6–97.4)
Specificity- 97.7% (95.4–98.9)
Positive Predictive Value- 93.6% (87.5–96.9)
Negative Predictive Value- 98.0% (95.8–99.1)

Data are presented as whole numbers. RIF- Rifampin, DST- Drug susceptibility testing

EPTB – Tubercular Pleural effusion (TPE)

<table>
<thead>
<tr>
<th>Diagnostic test</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thoracoscopic pleural biopsy</td>
<td>93 - 100%</td>
</tr>
<tr>
<td>PF ADA</td>
<td>47-100%</td>
</tr>
<tr>
<td>PF microscopy</td>
<td>10%</td>
</tr>
<tr>
<td>PF culture</td>
<td>20%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ADA</th>
<th>Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;40</td>
<td>Thoracoscopic pleural biopsy</td>
</tr>
<tr>
<td>40-70</td>
<td>ATT given if Pretest probability high*</td>
</tr>
<tr>
<td>&gt;70</td>
<td>Most patients receive ATT</td>
</tr>
</tbody>
</table>

*Age of <45 years, nonsmoker, straw-colored effusion, and high tuberculosis prevalence area

Gene Xpert in TPE

<table>
<thead>
<tr>
<th></th>
<th>Inderpaul et al. (2016)</th>
<th>Denkinger et al. (2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>22.7 to 51.4 %</td>
<td>46.4%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98.6 to 99.8 %</td>
<td>99.1%</td>
</tr>
</tbody>
</table>

- Low sensitivity: Cannot be used alone for the diagnosis of TPE
- High specificity: Obviate the need for an invasive procedure such as pleural biopsy in patients with high Pretest probability


# CBNAAT in Non respiratory specimen

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph Node Biopsy</td>
<td>93 (70-99)</td>
<td></td>
</tr>
<tr>
<td>Lymph Node FNAC</td>
<td>96 (72-79)</td>
<td>98 (all combined)</td>
</tr>
<tr>
<td>Tissues all types</td>
<td>88 (76-94)</td>
<td></td>
</tr>
<tr>
<td>CSF</td>
<td>85 (75-100)</td>
<td></td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>34 (24-44)</td>
<td></td>
</tr>
<tr>
<td>Other fluid samples (Pericardial, Ascitic, Synovial)</td>
<td>67 (0-100)</td>
<td></td>
</tr>
<tr>
<td>Gastric aspirates</td>
<td>78% (68-85)</td>
<td>99%</td>
</tr>
</tbody>
</table>

Maynard et al. BMJ Inf Dis 2014:14:709

- A positive test provides useful confirmation so that ATT can be started promptly
- A negative test does not always rule out TB
<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Comparison (No. of studies, No. of samples)</th>
<th>Median (%) pooled sensitivity (pooled 95% CrI)</th>
<th>Median (%) pooled specificity (pooled 95% CrI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lymph node tissue and aspirate</td>
<td>Xpert MTB/RIF compared against culture [14 studies, 849 samples]</td>
<td>84.9 (72–92)</td>
<td>92.5 (80–97)</td>
</tr>
<tr>
<td></td>
<td>Xpert MTB/RIF compared against a composite reference standard [5 studies, 1 unpublished]</td>
<td>83.7 (74–90)</td>
<td>99.2 (88–100)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>Xpert MTB/RIF compared against culture [16 studies, 709 samples]</td>
<td>79.5 (62–90)</td>
<td>98.6 (96–100)</td>
</tr>
<tr>
<td></td>
<td>Xpert MTB/RIF compared against a composite reference standard [6 studies, 512 samples]</td>
<td>55.5 (51–81)</td>
<td>98.8 (95–100)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>Xpert MTB/RIF compared against culture [17 studies, 1385 samples]</td>
<td>43.7 (25–65)</td>
<td>98.1 (95–99)</td>
</tr>
<tr>
<td></td>
<td>Xpert MTB/RIF compared against a composite reference standard [7 studies, 698 samples]</td>
<td>17 (8–34)</td>
<td>99.9 (94–100)</td>
</tr>
<tr>
<td>Gastric lavage and aspirate</td>
<td>Xpert MTB/RIF compared against culture [12 studies, 1258 samples]</td>
<td>83.8 (66–93)</td>
<td>98.1 (92–100)</td>
</tr>
<tr>
<td>Other tissue samples</td>
<td>Xpert MTB/RIF compared against culture [12 studies, 699 samples]</td>
<td>81.2 (68–90)</td>
<td>98.1 (87–100)</td>
</tr>
</tbody>
</table>

Crl, credible interval; the CrI is the Bayesian equivalent of the confidence interval.
GeneXpert in EPTB: INDEX TB guidelines 2017

<table>
<thead>
<tr>
<th>Recommendations: Diagnosis of EPTB using the Xpert MTB/RIF test</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymph node TB</strong></td>
</tr>
<tr>
<td>Xpert MTB/RIF should be used as an additional test to conventional smear microscopy, culture and cytology in fine-needle aspiration cytology (FNAC) specimens.</td>
</tr>
<tr>
<td>Strong recommendation, low quality evidence for sensitivity estimate, high quality evidence for specificity estimate.</td>
</tr>
<tr>
<td><strong>TB meningitis</strong></td>
</tr>
<tr>
<td>Xpert MTB/RIF may be used as an adjunctive test for tuberculous meningitis (TBM). A negative Xpert MTB/RIF result on a cerebrospinal fluid (CSF) specimen does not rule out TBM. The decision to give anti-tuberculosis treatment (ATT) should be based on clinical features and CSF profile.</td>
</tr>
<tr>
<td>Conditional recommendation, low quality evidence for sensitivity estimate, high quality evidence for specificity estimate.</td>
</tr>
<tr>
<td><strong>Pleural TB</strong></td>
</tr>
<tr>
<td>Xpert MTB/RIF should not be routinely used to diagnose pleural TB.</td>
</tr>
<tr>
<td>Strong recommendation, low quality evidence for sensitivity estimate, high quality evidence for specificity estimate.</td>
</tr>
</tbody>
</table>

WHO recommendation 2013

• Xpert MTB/RIF should be used rather than conventional microscopy, culture and DST as the initial diagnostic test in adults and children suspected of having MDR-TB or HIV-associated TB (strong recommendation, high-quality evidence).

• Xpert MTB/RIF may be used rather than conventional microscopy and culture as the initial diagnostic test in all adults and children suspected of having TB (conditional recommendation acknowledging resource implications, high-quality evidence).

WHO recommendation 2013

• Xpert MTB/RIF may be used as a follow-on test to microscopy in adults suspected of having TB but not at risk of MDR-TB or HIV-associated TB, especially when further testing of smear negative specimens is necessary (conditional recommendation acknowledging resource implications, high-quality evidence).
RNTCP recommendations 2013

- For MTB +, Rif sensitive results, treat the patient with first line drug regimen
- For MTB +, Rif resistance results-
  - In re-treatment TB cases: 2nd line DST (Rx as MDR-TB)
  - In new TB cases, treat with regimen for MDR-TB after reconfirming Rif resistance with another technology.
    - In smear positive cases, reconfirm with LPA
    - In smear negative cases, offer liquid or solid culture and if culture is positive, the culture isolates must be subjected to LPA as per RNTCP PMDT guidelines

Guidance document for use of CB-NAAT under RNTCP, Sept 2013
RNTCP recommendations 2013

MTB+, Rif

Resistant

Retreatment

2nd line ATT

New TB cases

Sputum +
LPA

Sputum –
Solid/liquid

Culture +

Sensitive

1st line ATT

Reconfirm with another technology

Guidance document for use of CB-NAAT under RNTCP, Sept 2013
Advantages

1. Better sensitivity and specificity than smear microscopy
2. Good accuracy for tuberculosis diagnosis
3. Diagnosis and rifampicin resistance
4. Rapid - 2 hours
5. Simple to use
6. Operators do not need formal laboratory training
7. Does not need advanced biosafety equipment
8. Closed system with low risk of cross-contamination
9. Could potentially be used to test a broad range of samples from extrapulmonary sites

Lancet Infect Dis March 2013
Disadvantages

1. Expensive
2. Yearly calibration and maintenance
3. Continuous electrical power supply
4. Relatively short shelf life of cartridges (18 months)
5. Cannot be used to monitor treatment success or failure, or relapse
6. Use with extrapulmonary samples is not yet fully defined
XPERT MTB/RIF ULTRA
Xpert MTB/RIF Ultra assay (Ultra)

- Xpert MTB/RIF sensitivity is imperfect, particularly in smear negative and HIV-associated TB
# XpertUltra vs Xpert

<table>
<thead>
<tr>
<th></th>
<th>Xpert MTB/RIF Ultra</th>
<th>Xpert MTB/RIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mechanism of rifampicin</td>
<td>Melting temperature based PCR</td>
<td>Real time PCR</td>
</tr>
<tr>
<td>resistance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplification targets</td>
<td>IS6110 and IS1081, 4rpoB</td>
<td>rpoB</td>
</tr>
<tr>
<td>DNA chamber</td>
<td>50µl PCR reaction</td>
<td>25µl PCR reaction</td>
</tr>
<tr>
<td>Amplification</td>
<td>Fully nested</td>
<td>Hemi nested</td>
</tr>
<tr>
<td>Thermal cycling</td>
<td>More rapid</td>
<td></td>
</tr>
<tr>
<td>Fluidics and enzymes</td>
<td>Improved</td>
<td></td>
</tr>
<tr>
<td>Limit of detection (LOD)</td>
<td>16 cfu/ml</td>
<td>114 cfu/ml</td>
</tr>
</tbody>
</table>

Interpretation of results

- New semiquantitative category “trace call”-lowest bacillary burden for MTB detection

## Ultra Performance

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity of Ultra vs GeneXpert</th>
<th>Specificity of Ultra vs GeneXpert</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N = 1520</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>5% higher (95%CI +2.7, +7.8)</td>
<td>3.2% lower (95%CI -2.1, -4.7)</td>
</tr>
<tr>
<td>Smear – Culture +</td>
<td>+17% (95%CI +10, +25)</td>
<td></td>
</tr>
<tr>
<td>HIV infected</td>
<td>+12% (95%CI +4.9, +21)</td>
<td></td>
</tr>
<tr>
<td>Pediatric EPTB: CSF</td>
<td>95% vs 45%</td>
<td></td>
</tr>
<tr>
<td>Pediatric PTB:</td>
<td>71% vs 47%</td>
<td></td>
</tr>
</tbody>
</table>

Rifampicin resistance by Ultra

- Ultra performed similarly well as Xpert MTB/RIF
- ‘Trace call’: Indeterminate rifampicin result
- If low risk for rifampicin resistance (e.g. new TB cases not at risk for MDR-TB) a positive rifampicin resistance result should be repeated

Advantages

• Better sensitivity in **paucibacillary specimens** (Smear negative culture+, HIV+, pediatric, EPTB esp CNS TB)

• **Better differentiate silent mutations** (such as Q513Q or F514F) from resistance conferring mutations

Limitations

• **False positives:** If past h/o TB +
  – Picks non replicating and non viable bacilli also
WHO Recommendation

• Use as initial diagnostic test for all adults and children with signs and symptoms of TB.
• Use in the testing of selected extrapulmonary specimens (CSF, lymph nodes and tissue specimens)
XPERT OMNI
GeneXpert Omni

- 9 inches (23 cm) tall 2.2 pounds (1kg)
- Proven Cartridge Technology similar to GeneXpert
- Battery operated, wireless, web enabled
- Able to transmit instrument and time information in real time
Loop Mediated Isothermal Amplification (LAMP)

- Developed by Eiken chemical, Tokyo, Japan
- Manual NAAT, DNA can be amplified $10^{10}$ times in 15-60 min
- Targets gyrB gene (M. tuberculosis, M. avium, and M. intracellulare)
- Sensitivity (smear positive)- 97%
  (smear negative)-62
- Specificity-96.3%
- Advantages: 1.High speed (35 mins for solid media, 60 mins for liquid media and sputum)
  2.No use of thermal cycler (isothermic - $63^\circ$ C)
  3.Can be used in peripheral level

Steps

Collect 60µl using Eiken disposable pipette

Sputum cup ➔ Heating tube ➔ Heating block

Heating 90°C 5 min ➔ Adsorbent tube ➔ Injection cap

Add 30-35µl (between lines)

Dried reagents

Mix ➔ Close lids and let stand upside down for 2 min ➔ Reaction tubes

LAMP reaction, 67°C 40 min

Fluorescent signal detection

WHO/HTM/TB/2016.11

The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis
Detection

- Double-stranded DNA binding dyes, such as SYBR green detect turbidity caused by precipitating magnesium pyrophosphate
Molecular principles

• Loop primers contain sequences complementary to the ss loop region on the 5’-end of the hairpin structure, speeds the reaction by providing a greater number of starting points for DNA synthesis

• Using loop primers, amplification by 109 to 1010 times can be achieved within 15–30 minutes.
Molecular principles

- Requirement for homogeneous sequences at multiple binding sites preserves the specificity of the assay even in the absence of a probe
- LAMP method is relatively insensitive to the accumulation of DNA and DNA by-products (pyrophosphate salts), so the reaction proceeds until large amounts of amplicon are generated
Molecular principles
Table 4. TB-LAMP as a replacement test for smear microscopy: estimates of pooled sensitivity and specificity

<table>
<thead>
<tr>
<th>Reference standard(^a)</th>
<th>Pooled sensitivity(^b)</th>
<th>Pooled specificity(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 1</td>
<td>77.7 (71.2-83.0)</td>
<td>98.1 (95.7-99.2)</td>
</tr>
<tr>
<td>Standard 2</td>
<td>76.0 (69.9-81.2)</td>
<td>98.0 (96.0-99.0)</td>
</tr>
<tr>
<td>Standard 3</td>
<td>80.3 (70.3-87.5)</td>
<td>97.7 (96.1-98.7)</td>
</tr>
</tbody>
</table>

\(^a\) All reference standards classify patients as having TB if ≥ 1 positive culture was confirmed as *M. tuberculosis* by speciation testing. To be classified as not having TB, patients had to have no positive and at least (i) two negative cultures on two different sputum specimens (Standard 1), (ii) two negative cultures on the same or different sputum specimens (Standard 2), or (iii) at least one negative culture (Standard 3).

\(^b\) Values are percentages (95% confidence intervals).
WHO recommendations 2016

• TB-LAMP may be used as a replacement test for sputum-smear microscopy to diagnose pulmonary TB in adults with signs and symptoms consistent with TB (conditional recommendation, very low-quality evidence).

• TB-LAMP may be used as a follow-on test to smear microscopy in adults with signs and symptoms consistent with pulmonary TB, especially when further testing of sputum smear-negative specimens is necessary (conditional recommendation, very low-quality evidence).

World Health Organization 2016 The use of loop-mediated isothermal amplification (TB-LAMP) for the diagnosis of pulmonary tuberculosis WHO/HTM/TB/2016.11
Mycobacteriology Lab Workflow – PGIMER

Presumptive EPTB, Presumptive Ped TB, PLHIV

Presumptive TB, Presumptive DR TB, Confirmed TB

Smear Microscopy

CBNAAT

Positive

Negative

Culture

Positive

Negative

Culture

SL-DST(MGIT 960) – Levofloxacin, Kanamycin
PGIMER- Available lab equipment

• DMC (Designated Microscopy Centre) (New OPD/1/1031)
• Culture & DST Laboratory (Research Block A/ 2/221)
• Auramine stain is used under LED based microscope since 2011
• BACTEC 460 and MGIT are available
• Gene Xpert and LPA are also available
• Solid culture and DST for First Line DST (RIF + INH + STR + ETM) - 2011
• Line Probe Assay For First Line DST (RIF+ INH) - April 2013
• Liquid culture and DST for First Line DST (RIF + INH + STR + ETM) – Feb 2015
• Liquid culture and DST for Second Line DST (OFLx + AMK + KAN + CAP) – Sept 2015
• LAMP and second line LPA are also available and is currently used for research purposes
Take home points

• Rapid molecular tests donot eliminate the need for conventional Culture and DST
• GeneXpert (R): poor sensitivity in body fluids (PF)
• GeneXpert Ultra (R): better sensitivity in paucibacilli specimens (sputum- and EPTB)
• LPA (H&R): only in sputum + specimens
• Second line LPA: In all MDR-TB, to rule out resistance to FQ/SLID
Thank You