Recent Approaches in Detection of Drug-Resistant Tuberculosis

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New Delhi Tuberculosis Centre
Newer Diagnostic Methods for MDR TB

- Rapid Culture and DST using automated liquid media system (MGIT 960)
- Resazurin Microtitre Assay (REMA)
- Colorimetric DST assay
- Nitrate Reductase assay (NRA assay)
- Malachite Green Triphenyl Methane assay (MGMT assay)
- Microscopic Observation Drug Susceptibility assay (MODS assay)
- Phage Based assay
- DNA sequencing
- Single Strand Confirmation Polymorphism- PCR (PCR-SSCP)
- High Performance Liquid Chromatography (HPLC)
- Nucleic Acid Amplification Test (NAAT assay)
- Loop Mediated Isothermal Amplification assay (LAMP assay)
- Line Probe assay (LPA) for 1st line drugs
- Cartridge based Nucleic acid amplification test (CBNAAT): Xpert MTB/Rif
- DNA Microarray
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WHO recommendation for culture and DST using alternative methods

Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug-resistant tuberculosis

Policy statement

2011

Microscopic observation drug susceptibility (MODS)

Colorimetric DST assay

Nitrate reductase assay (NRA)
Mycobacteria Growth Indicator Tube (MGIT 960)

- Manufacturer: Becton Dickinson, USA
- Endorsed in 2007 by WHO
- Non radioactive Liquid system
- Diagnosis and DST pattern
- MGIT culture vials can be stored up to 2 years
Mycobacteria Growth Indicator Tube (MGIT 960)

Specimen Processing

• Digestion and decontamination of the sputum specimens by N-Acetyl-L-cysteine (NALC) - sodium hydroxide method
• Inoculation in MGIT vial containing growth supplement mixture
• Enter MGIT tubes into the instrument
**Mycobacteria Growth Indicator Tube (MGIT 960)**

**Reading of results**

- Indicator lamp on drawer illuminates
- Audible alert sounds
- Generates reports in printed format

![LCD screen display](image)
Mycobacteria Growth Indicator Tube (MGIT 960)

MGIT DST

- Inoculation of pure culture into MGIT vial with and without drugs (SIRE)
- Place tube in AST carrier set and Load in the Instrument
- Instrument reports automatically as Sensitive or Resistant

<table>
<thead>
<tr>
<th>Drug</th>
<th>Critical µg/mL</th>
</tr>
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<tbody>
<tr>
<td>S</td>
<td>1.0</td>
</tr>
<tr>
<td>I</td>
<td>0.1</td>
</tr>
<tr>
<td>R</td>
<td>1.0</td>
</tr>
<tr>
<td>E</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Mycobacteria Growth Indicator Tube (MGIT 960)

**STRENGTHS**
- Capacity to incubate 960 tubes
- System alerts when tube +ve,
- Increased sensitivity*
- Reduced turn around time
- Results; Culture: 7-14 days, DST: 4-13 Days

**WEAKNESS**
- Continuous Electricity
- Reagent costs
- High Contamination rate

*Dongsi Lu et al, Am J Clin Pathol 2002;118:542-545*
Molecular Techniques

- Line Probe Assay (1st line drugs)
  - Genotype MTBDRplus, Hain Lifesciences, Nehren, Germany

- Cartridge based Nucleic Acid Amplification Technique (CBNAAT)
  - Xpert MTB/RIF, Cepheid Inc, Sunnyvale, CA, USA
Use of LPA under the program

• Use of LPAs on smear-positive sputum and cultures (insufficient evidence on smear negatives)

• Commercial assays recommended
Line Probe Assay

- Line-probe assay is designed to identify *M. tuberculosis* complex and simultaneously detect mutations associated with drug resistance to Rif and INH.
- Many studies reported Sensitivity: 82-100% and Specificity 92-100% *

* Morgan M et al, BMC Infect Dis 2005. 4: 62
Line Probe Assay

DNA strip-based test that use multiplex PCR and reverse hybridization method for the rapid detection of mutations associated with drug resistance to Rif and INH

Procedure involves three steps:

1) DNA isolation from the clinical specimen

2) Multiplex amplification of DNA with primers

3) Reverse hybridization and detection
Hain’s test – MTBDRplus
LPA: Advantages & Disadvantages

Advantages

• Faster results
• High-throughput testing
• Improved yield of results relative to culture
• Less biohazard risk

Disadvantages

• DNA cross-contamination risk
• Not evaluated for smear-negative specimens
• Limited number of drugs for which assays standardized
Xpert MTB/ RIF

Endorsed in 2010
Xpert MTB/ RIF

- Fully automated with 1-step external (sample preparation)

- Assay targets the rpoB gene, which is critical for identifying mutations associated with rifampicin resistance
Xpert MTB/ RIF

Procedure

1. Pour Sample Reagent into sample tube.
   Incubate for 15 minutes at room temperature.
   (Acceptable sample types: unprocessed sputum or sediment from concentrated specimen.)

2. Pipette diluted sample into cartridge.

3. Insert cartridge and start assay.

TOTAL HANDS-ON TIME = 2 MINUTES
Advantages

• Time-to-result: ~2 hrs
• Throughput: up to 4 tests / module / run
• Closed system

Performance

• Specific for MTB: 99.2%
• Sensitivity similar to culture*
  • Smear +ve: 98.2%
  • Smear -ve: 72.5%

# Comparison of LPA and Xpert MTB/ RIF

<table>
<thead>
<tr>
<th>Performance Parameter</th>
<th>LPA</th>
<th>Xpert MTB/ RIF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Automation</td>
<td>Semi-automated with multiple manual steps</td>
<td>fully automated with one external step</td>
</tr>
<tr>
<td>Turn around time</td>
<td>2 - 4 days</td>
<td>2 - 3 hours</td>
</tr>
<tr>
<td>Throughput</td>
<td>High throughput: up to 45 samples per run</td>
<td>up to 4 tests / module / run</td>
</tr>
<tr>
<td>Infrastructure</td>
<td>Need for 3 rooms</td>
<td>Need one room</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>For Smear +ve cases only</td>
<td>Applicable for smear –ve cases also</td>
</tr>
<tr>
<td>DST results</td>
<td>Rifampicin and Isonaizid</td>
<td>Rifampicin only</td>
</tr>
</tbody>
</table>
Diagnostic Technologies – Sensitivity (cfu/ml)

- iLED fluorescent microscope: 10,000/ml
- Line-probe: 10,000/ml
- Xpert MTB: 50-150/ml
- MGIT: 10-100/ml

Log cfu/ml
## WHO approved diagnostic options

<table>
<thead>
<tr>
<th>Year</th>
<th>Technology</th>
<th>Turn around time</th>
<th>Sensitivity gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td><strong>Before 2007</strong></td>
<td>ZN microscopy Solid Culture</td>
<td>1-2 days 30-60 days</td>
<td>Baseline</td>
</tr>
<tr>
<td><strong>2007</strong></td>
<td>Liquid Culture Rapid speciation</td>
<td>15-30 days</td>
<td>+10% compared to LJ</td>
</tr>
<tr>
<td><strong>2008</strong></td>
<td>Line Probe Assay (1st line, Rif &amp; INH)</td>
<td>2-4 days</td>
<td>At this time for S+ only</td>
</tr>
<tr>
<td><strong>2009</strong></td>
<td>LED-based FM</td>
<td>1-2 days</td>
<td>+10% compared to ZN</td>
</tr>
<tr>
<td><strong>2010</strong></td>
<td>Integrated NAAT (TB, Rif)</td>
<td>2 hours</td>
<td>+40% compared to ZN</td>
</tr>
</tbody>
</table>
### Choice of Diagnostic Technology of Drug Resistant TB under RNTCP

<table>
<thead>
<tr>
<th>MDR Diagnostic Technology</th>
<th>Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular DST (e.g. LPA DST or CB-NAAT)</td>
<td>First</td>
</tr>
<tr>
<td>Liquid culture isolation and LPA DST</td>
<td>Second</td>
</tr>
<tr>
<td>Solid culture isolation and LPA DST</td>
<td>Third</td>
</tr>
<tr>
<td>Liquid culture isolation and Liquid DST</td>
<td>Fourth</td>
</tr>
<tr>
<td>Solid culture isolation and DST</td>
<td>Fifth</td>
</tr>
</tbody>
</table>
Suspect Criteria

Criteria A
• All failures of new TB cases
• Smear +ve previously treated cases who remain smear +ve at 4th month onwards
• All pulmonary TB cases who are contacts of known MDR TB case

Criteria B
• All smear +ve previously treated pulmonary TB cases at diagnosis
• Any smear +ve follow up result in new or previously treated cases

Criteria C
• All smear -ve previously treated pulmonary TB cases at diagnosis
• HIV TB co-infected cases at diagnosis
Algorithm for diagnosis of MDR-TB

MDR TB suspects

Sputum smear Microscopy (ZN / Fluorescent)

Smear +ve

Smear -ve

Specimen transport

DST (1st line) by LPA / Xpert MTB/Rif

Specimen transport
Algorithm for diagnosis of MDR-TB (C-DST Lab)

MDR TB suspects referred along with Request form from MO-PHC via DTO to RNTCP C-DST Lab

Sputum smear Microscopy (Fluorescent)

- Smear +ve
  - DST (1st line) by LPA
    - MDR/ Mono Rif Res.
    - Mono INH Res.
    - Sensitive
    - LPA –ve / Invalid
- Smear –ve
  - Culture +ve
    - Culture (Liquid / Solid)
  - Culture –ve / CT
Algorithm for diagnosis of MDR-TB (Xpert MTB/Rif)

MDR TB suspects referred along with Request form from MO-PHC via DTO to RNTCP C-DST Lab

Xpert MTB/Rif

- Rif Res.
- Sensitive
- Mtb Negative / Invalid
  - Repeat Xpert MTB/Rif
    - LPA –ve / Invalid
      - Refer for C-DST
# Annexure I

## RNTCP Request for Culture and Drug Sensitivity Testing
(Required for Culture and DST laboratory to conduct testing; please send copy to District TB Officer)

<table>
<thead>
<tr>
<th>Patient information</th>
<th>Molecular TB/DST result</th>
<th>LAB NO</th>
<th>Date of Receiving</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient Name:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Address with landmark</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Mobile No. or other Contact No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age:</td>
<td>Sex: Male ☐ Female ☐</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sputum – Date of collection (DD/MM/YY):</td>
<td>Sample 1:</td>
<td>Sample 2:</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Notes:</th>
<th>Date tested:</th>
<th>Reported by (Name &amp; Signature):</th>
</tr>
</thead>
</table>

| Name referring facility (PHI/DMC/DR-TB Center / other): | |
|--------------------------------------------------------| |
| District | Tuberculosis Unit (TU) | |

| Reason for Testing | FOLLOW-UP | |
|--------------------|-----------| |
| □ MDR Suspect Criteria | PMDT Registration Number | |
| □ Failure | Treatment month of Follow-up | |
| □ Re-treatment case S+ at 4th month | | |
| □ Contact of known MDR TB case | | |
| □ S+ at diagnosis, re-treatment case | | |
| □ Any follow up S+ | | |
| □ S- at diagnosis, re-treatment case | | |
| □ HIV TB case | | |
| □ RNTCP TB Reg No. Type Cat | DR-TB Centre Name | |

| LJ/ Liquid Culture results: | |
|-----------------------------| |
| Date received Specimen No. Specimen Smear Neg Pos 1-19 col | + ++ +++ Contaminated | |
| A | | |
| B | | |

<table>
<thead>
<tr>
<th>Notes:</th>
<th>Result Date:</th>
<th>Reported by (Name &amp; Signature):</th>
</tr>
</thead>
</table>

| LJ/ Liquid culture DST Results: (Note: ‘S’ if susceptible, ‘R’ if resistant) | |
|--------------------------------| |
| Date DST Initiated Specimen No S H R E Z Km Ofx Eto Other | |

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<tr>
<th>Result Date:</th>
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Diagnosis of extra-pulmonary & Paediatric cases

• Same algorithm as of pulmonary cases
• Specimen subjected to Xpert MTB/Rif or Liquid culture and DST

• Project “Accelerating access to quality TB diagnosis for paediatric cases” from April 2014
DST for 2\textsuperscript{nd} line Drugs

- Choice of Technology: Liquid / Solid culture & DST (OF, KM)
## Infrastructure Flexibility

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Facility</th>
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<tbody>
<tr>
<td>NITRD</td>
<td>• Xpert MTB/ Rif</td>
</tr>
<tr>
<td></td>
<td>• LPA</td>
</tr>
<tr>
<td></td>
<td>• Liquid &amp; Solid Culture - DST</td>
</tr>
<tr>
<td>Lok Nayak Hospital</td>
<td>Xpert MTB/ Rif</td>
</tr>
<tr>
<td>RBIPMT</td>
<td>Xpert MTB/ Rif</td>
</tr>
<tr>
<td>Safdarjung</td>
<td>Xpert MTB/ Rif</td>
</tr>
<tr>
<td>AIIMS</td>
<td>LPA</td>
</tr>
<tr>
<td>NDTBC</td>
<td>• LPA</td>
</tr>
<tr>
<td></td>
<td>• Liquid &amp; Solid Culture - DST</td>
</tr>
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Future prospects

- Truelab/TrueNAT MTB, Molbio/bigtec Diagnostics, India
- iCubate System, iCubate, USA
- TB drug resistance array, Capital Bio, China
- EasyNAT TB Diagnostic kit, Ustar Biotechnologies, China
- Prototype breath analyser device, Next Dimensions Technology, USA
- Alere Q, Alere, USA
- MDR-XDR TB color test, FIND, Switzerland/Imperial College, UK
THANK YOU