M. tuberculosis (MTB) Characterization, Nucleic Acid Amplification Test (NAAT), & Drug Susceptibility Testing (DST)

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Mycobacteriology Testing: Annual Workload

2012
- Primary Isolation
  - 2963 patient samples
  - 807 individual patients
- Reference Culture Identification
  - 656 patients
  - 85 individual patients positive for MTBC
- NAAT Testing (MTD)
  - 34 patients had the GenProbe MTD test performed
  - 30 patients had the MTD detect M. tuberculosis DNA

2013 (6 months)
- Primary Isolation
  - 1455 patient samples
  - 369 individual patients
  - 32 patients positive for MTBC
- Reference Culture Identification
  - 375 patients
  - 67 individual patients positive for MTBC
- NAAT Testing (MTD)
  - 16 patients had the GenProbe MTD test performed
  - 5 patients had the MTD detect M. tuberculosis DNA

MTB Identification & Characterization

- Phenotypic Characterization
  - Microscopy
  - Morphology & Biochemical
  - Drug Susceptibility Testing (DST)

- Genotypic Characterization
  - DNA Fingerprinting
    - Spoligotyping
    - RFLP
    - VNTR analysis
  - Probe hybridization
    - AccuProbe
  - Nucleic Acid Amplification Test (NAAT)
    - GenProbe MTD, Cepheid GeneXpert
    - 16S sequencing

2012 Mycobacteriology Testing: Drug Susceptibility

2012
- First Line
  - 157 1st line DST on all initial M. tuberculosis isolates
- Second Line
  - 40 2nd line DST on all initial M. tuberculosis isolates

2013 (6 months)
- First Line
  - 54 1st line DST on all initial M. tuberculosis isolates
- Second Line
  - 12 2nd line DST on all initial M. tuberculosis isolates

DST for other Mycobacteria spp. available through National Jewish Hospital upon request.
DCLS Current Workflow

- Sputum (raw) sample received digested & decontaminated
- Smear stain complete
- Growth Cultures Established on LJ and MGIT broth
- No AFB growth culture held 8 weeks
- Stain for Acid-Fast Bacteria
- Growth determined to be acid-fast
- Microscopy
- Smear (+)
- Smear (-)
- Biochemical and AccuProbe DNA Probe for four different mycobacteria spp.
- 1st line DST with MGIT
- Send to CDC for additional DST
- Final Report Issued
- 2nd line DST
- Resistant except Strips
- Sensitive

Continuing with the MGIT 960: Primary Isolation, 1st & 2nd Line DST

- Continuous incubation at 37°C & monitoring for fluorescence – based on O₂ concentration
- Growth of any organism is detected
  - Mycobacteria, yeast, other bacteria
- Smears prepared from broth
- Growth determined to be acid-fast

Growth on LJ Slants & Acid Fast Staining

- Pleomorphism and branching often seen in M. avium complex.
- M. avium on LJ slants
- M. tuberculosis on LJ slants
- “Cording” typical of M. tuberculosis

Drug Susceptibility Testing: Performed on MGIT 960

- First line drug testing
  - Isoniazid (INH), Rifampin, Ethambutol, Streptomycin, Pyrazinamide (PZA)
- Results available within 7-12 days after speciation
- Resistant strains - results phoned to submitter
- Second line drug testing
  - Ethionamide, Capreomycin, Ofloxacin, INH at a higher concentration
  - Sent to CDC for additional drug susceptibility testing
FDA approved the GenProbe Amplified *M. tuberculosis* Direct Test for AFB smear (+) respiratory specimens in 1995 and for smear (-) respiratory specimens in 1999

- Amplified molecular assay detects *M. tuberculosis* directly from sputum samples in less than 3.5 hours

- Utilizes a Transcription-Mediated Amplification system (TMA) to detect rRNA

Approved for: Respiratory Specimens
- Testing smear (+) and (-) specimens (NOTE: Smear (-) specimens NOT routinely tested at DCLS)
- Testing patients who have taken TB medications for LESS than 7 days
- Patients with high clinical suspicion of TB

NOT Approved for:
- Specimens from patients receiving TB medications in the past 12 months
  - NOT a test of cure
- Testing children or patients unable to produce sputum

Performed on sputum samples (raw or processed) in ~2h with little hands on time
In combination with Smear results, detects within 1-2 days of receiving sample:
- Smear +/-
- MTBC present
- Mutation indicative of RIF resistance
GenProbe Amplified MTD will serve as a backup method
Methodology Comparison

<table>
<thead>
<tr>
<th></th>
<th>Gen-Probe Amplified MTD Test</th>
<th>Hains Genotype MTBDRplus Test</th>
<th>Cepheid GeneXpert MTB/RIF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FDA Approval?</td>
<td>YES</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Detection of:</td>
<td>MTB only</td>
<td>MTB &amp; Resistance</td>
<td>MTB &amp; Resistance</td>
</tr>
<tr>
<td>Method:</td>
<td>Transcription-Mediated Amplification of rRNA</td>
<td>PCR + DNA-Strip hybridization</td>
<td>Nested real-time PCR</td>
</tr>
<tr>
<td>Sample type:</td>
<td>Sputum Sediment and bronchial specimens</td>
<td>Pulmonary specimens &amp; isolates</td>
<td>Raw Sputum or Sputum Sediment</td>
</tr>
<tr>
<td>Time-to-Result</td>
<td>2.5 - 3.5 h</td>
<td>9 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Labor Intensive</td>
<td>YES</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Detection of MTBC in Smear (+) Sensitivity/Specificity</td>
<td>87.5%/100%</td>
<td>120%/NA</td>
<td>99.7%/98.5%</td>
</tr>
<tr>
<td>Detection of MTBC in Smear (-) Sensitivity/Specificity</td>
<td>64.0%/100%</td>
<td>60%-95%/98.4%</td>
<td>76%-95%/98.8%</td>
</tr>
<tr>
<td>RIF Sensitivity/Specificity vs. Conventional DST</td>
<td>NA</td>
<td>NA (low sample volume)</td>
<td>94%/99.3%</td>
</tr>
<tr>
<td>INH Sensitivity/Specificity vs. Conventional DST</td>
<td>NA</td>
<td>NA (low sample volume)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*GeneXpert MTB/RIF users guide 301-1404 Rev. A July 2013
†GenProbe Amplified MTD users guide IN0014 Rev. P
‡Hains GenoType MTBDRplus users guide v2.0 IFU-304A-02. 02/2012

What is the GeneXpert MTB/RIF Assay?

- Nucleic acid amplification test (NAAT)
- Detects both MTBC and RIF resistance
- Test takes 2h from start to finish

Figure 6. An Example of a MTB DETECTED; Rif Resistance DETECTED Result

Principle of the GeneXpert Test

- Uses a hemi-nested PCR to amplify the rpoB gene in MTB
- Simultaneously probes the PCR amplicon for antibiotic resistance markers.


Principle of the GeneXpert Test part II

Fluorescent! = No mutation = NOT RESISTANT
Not Fluorescent! = Mutation = RESISTANT

81 bp region that 5 molecular beacons target
**GeneXpert MTB/RIF Process**

- Samples should be stored & transported at 2-8°C
- Test is only approved for induced or expectorated sputa
- Samples from patients on antituberculosis drugs for >3 days are NOT acceptable.

**Sample Requirements**

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Minimum Volume for One Test</th>
<th>Minimum Total Volume for Test and Retest – See Section 11.2, Retest Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum sediment</td>
<td>0.6 mL</td>
<td>1 mL</td>
</tr>
<tr>
<td>Raw sputum</td>
<td>1 mL</td>
<td>2 mL</td>
</tr>
</tbody>
</table>

- Test has not been evaluated for pediatric patients
- Performance of assay relative to HIV infection status is not known
- Positive MTBC result ≠ viable organisms
- Test does not differentiate between species of MTBC
- Limit of Detection

**Results & Potential Reporting**

<table>
<thead>
<tr>
<th>Xpert® MTB/RIF Readout</th>
<th>Interpretation</th>
<th>Report* (Suggested Minimal Language)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTB DETECTED; RIF Resistance DETECTED</td>
<td>A mutation in the rpoB gene has been detected. A full first and second line drug panel should be conducted.</td>
<td>rpoB mutation detected; likely rifampin resistance. Confirmatory testing in progress. OR isolate has been forwarded to a reference laboratory for confirmatory testing.</td>
</tr>
<tr>
<td>MTB DETECTED; RIF Resistance NOT DETECTED</td>
<td>A mutation in the rpoB gene has not been detected.</td>
<td>No rpoB mutation detected; likely rifampin susceptible.</td>
</tr>
<tr>
<td>MTB DETECTED; RIF Resistance INDETERMINATE</td>
<td>A mutation in the rpoB gene could not be determined due to insufficient signal detection.</td>
<td>Insufficient MTB in the sample to allow determination of rpoB mutation result.</td>
</tr>
<tr>
<td>MTB NOT DETECTED</td>
<td>The conserved sequences up- and downstream of the 81bp region were not detected.</td>
<td>MTB not detected; Confirmatory testing in progress.</td>
</tr>
</tbody>
</table>

*GeneXpert MTB/RIF users guide 301-1404 Rev. A July 2013

**Limitations**

- Test has not been evaluated for pediatric patients
- Performance of assay relative to HIV infection status is not known
- Positive MTBC result ≠ viable organisms
- Test does not differentiate between species of MTBC
- Limit of Detection

APHL Fact Sheet: Sept. 2013
Sputum (raw or processed) sample received and decontaminated

Smear stain complete

Sample process with GeneXpert MTB/RIF

Preliminary Report Issued

Growth Cultures Established on LJ and MGIT broth

Stain for Acid-Fast Bacteria

1st line DST with MGIT

Resistant (except Strips)

2nd line DST with MGIT

Send to CDC for additional DST

Final Report Issued

Samples can be sent to CDC for NAAT for drug resistance if requested by TB control

False positive/negative results are possible

Therefore, this test provides preliminary results only

Smear & MTB/RIF results will be sent as a preliminary report

Culture and growth based DST will still be conducted

MGIT 960 used to confirm

Final report will be a culmination of all preliminary reports

Molecular Detection of Drug Resistance (MDDR) from CDC will be included as an attachment.

DNA Sequencing to Detect 1st and 2nd line Drug Resistance (2-3 day TAT).

<table>
<thead>
<tr>
<th>Gene Loci</th>
<th>Associated Antibiotic Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB (81bp region)</td>
<td>Rifampicin</td>
</tr>
<tr>
<td>inhA (promoter region)</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>katG</td>
<td>Isoniazid</td>
</tr>
<tr>
<td>embB</td>
<td>Ethambutol</td>
</tr>
<tr>
<td>pncA</td>
<td>Pyrazinamide</td>
</tr>
<tr>
<td>gyrA</td>
<td>fluoroquinolones</td>
</tr>
<tr>
<td>rrs</td>
<td>Kanamycin, Amikacin, Capreomycin</td>
</tr>
<tr>
<td>tlyA</td>
<td>Capreomycin</td>
</tr>
<tr>
<td>eis (promoter region)</td>
<td>Kanamycin</td>
</tr>
</tbody>
</table>

Known RIF MTBC specimen received

Conventional & Pyrosequencing of RIF&INH Regions

Mutation

Resistant specimen*

Sanger Sequencing of Comprehensive Panel

Non-resistant specimen*

*Based on NAAT. Confirmation by growth still necessary
Limitations of ALL Molecular Testing

- Gaps in Knowledge
  - What/when do mutations REALLY confer resistance
  - Not all mechanisms of resistance are known
- Limits of Detection
  - Absence of mutation does not necessarily mean susceptible
  - Mutation does not necessarily mean resistant
  - Conventional detection & DST still required

Conclusions

- DCLS will implement the Cepheid GeneXpert technology in the 1st quarter 2014
- Culture and growth based DST are still the gold standard
- CDC MDDR service can help with early detection of drug resistance. Requests for testing must go through TB Control in collaboration with DCLS
- DCLS and TB Control are available to assist with patient consultations and interpretation of results

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