MICs in TB Susceptibility Testing:
Challenges and Solutions for Implementation

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MICs in TB Susceptibility Testing: Challenges and Solutions for Implementation

Goals

• Describe antimicrobial susceptibility testing (AST) for M. tuberculosis complex using the TREK Sensititre® MIC Microtitre Plate method

• List some of the challenges and solutions for implementing TREK Sensititre® MIC Microtitre Plate method

• Discuss the reporting and interpretation of MIC results
BPHL-Jacksonville Mycobacteriology Laboratory

Staff: 19 FTEs (17 technical staff)

Yearly Workload: 21,082 (2012 total specimens)

Testing:
- AFB Smear Microscopy
- Nucleic Acid Amplification Test
- Molecular Detection of Drug Resistance
- Culture
  - PCR Restriction Analysis
  - GenProbe Accuprobe
- Conventional Antimicrobial Susceptibility Testing
Antimicrobial Susceptibility Testing (AST)

**Molecular:**
- Hain GenoType® MTBDRplus Assay
  - Workload: ~800/year
- GeneXpert MTB/RIF Assay
  - Workload: ~20/year
- DNA Sequencing (Sanger)
  - Workload: ~1000/year (pncA)

**Conventional:**
- TREK Sensititre® MIC Plate Method
  - Workload: ~1000/year

**Algorithm:** BPHL performs AST on all first-time MTBC-positive patients and those who are positive after 60 days of treatment
BPHL Mycobacteriology Laboratory
Antimicrobial Susceptibility Testing

No more BACTEC 460TB!

Say it isn't so!

Never forget.
Alternatives for AST from Culture

Options:  
- MGIT 960  
- Agar Proportion Method (APM)  
- TREK Sensititre® Microtitre  
- MYCOTB Plate Method

BPHL: Evaluated the TREK method in 2011-2012 and compared to BACTEC 460TB and decided to move forward with this method. The TREK method was fully implemented January 2013
Method

Company: TREK Diagnostic Systems – Thermo Scientific

Equipment:  
- Nephelometer
- Sensititre AIM™ – Automated Inoculation Delivery System
- Sensititre Vizion System® (PC, Software, Plate Reader)

Supplies: MYCOTB 96-well plates, Dosing Heads, Media, Plate Seal, ATCC Control Strain

Workflow: We batch test and set up approx. 2x/week
Antimicrobial Susceptibility Testing
TREK Sensititre® MIC Microtiter Plate Method

Workflow

1. TB Culture
2. Inoculate Saline-Tween with Glass Beads, Vortex
3. McFarland Standard 0.5
4. Nephelometer or manual method
5. Inoculate Middlebrook 7H9 with 100µl suspension
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1. Place MYCOTB Plate in Autoinoculator
2. Inoculate plate (100µl/well)
3. Seal plate, place in bag and incubate at 37°C
4. Read in Vizion Plate Reader
5. Read at 14 and 21 days
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Inoculate Blood Agar Plate
Incubate at 30°C

Blood Agar Plate – check for purity

• Inoculate with 50µl of bacterial suspension

Inoculate Middlebrook 7H11 Biplate
Incubate at 37°C

Middlebrook 7H11 Biplate
- check for purity and perform colony count (6-10 colonies)

• Inoculate with 50µl of bacterial suspension
• Streak with 10µl loop
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TREK Sensititre® MIC Microtiter Plate Method

Results

<table>
<thead>
<tr>
<th>OFL</th>
<th>MXF</th>
<th>RIF</th>
<th>AMI</th>
<th>STR</th>
<th>RFB</th>
<th>PAS</th>
<th>ETH</th>
<th>CYC</th>
<th>INH</th>
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OFL = OFL oxacillin
MXF = Moxifloxacin
RIF = Rifampin
AMI = Amikacin
STR = Streptomycin
RFB = Rifaximin
PAS = PAS 60
ETH = Ethambutol
CYC = Cycloserine
INH = Isoniazid
KAN = Kanamycin
EMB = Erythromycin
### Result Report

<table>
<thead>
<tr>
<th>Drug</th>
<th>MIC (µg/ml)</th>
<th>Interpretation (Tentative breakpoint)</th>
</tr>
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<tbody>
<tr>
<td>Rifampin</td>
<td>16</td>
<td>Resistant (Susceptible ≤1 Resistant ≥2)</td>
</tr>
</tbody>
</table>
Validation

• *Performed validation 2011-2012*

• *230 clinical specimens*

• *Compared TREK to BACTEC 460TB method*

• *Evaluated discrepant samples with analysis of the rpoB and inhA and katG genes, when possible (Hain GenoType MTBDRplus assay or DNA sequencing)*
Validation - Results

- Analysis of Rifampin and Isoniazid: 95.7% agreement
  - 10/230 discrepant isolates

- Analysis of four drugs (Rifampin, Isoniazid, Ethambutol, Streptomycin): 91.7% agreement
  - 19/230 discrepant isolates

- Analysis of Ethionamide, Kanamycin, Rifabutin, Ofloxacin: 60.5% agreement
  - 15/38 discrepant isolates

- Amikacin, Moxifloxacin, PAS and Cycloserine are on the MYCOTB plate but not performed on BACTE 460TB and therefore could not be compared
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Validation Results - Rifampin

![Graph showing validation results for Rifampin]

- S ≤1
- R ≥2
Validation Results - Isoniazid

### Isoniazid

<table>
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<tr>
<th>MIC</th>
<th>Number of Isolates</th>
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<td>0.5</td>
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<tr>
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<tr>
<td>2</td>
<td></td>
</tr>
<tr>
<td>≥4</td>
<td></td>
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</tbody>
</table>

- **S**: ≤0.12
- **I**: 0.25 - 1
- **R**: ≥2
Validation Results - Streptomycin

- **S** ≤1
- **I** 2 - 4
- **R** ≥8

**Antimicrobial Susceptibility Testing**
**TREK Sensititre® MIC Microtiter Plate Method**
Validation Results - Ethambutol
Antimicrobial Susceptibility Testing
TREK Sensititre® MIC Microtiter Plate Method

Validation – Challenges

• On a time crunch to implement a new test
• Have to compare two different methods that provide a different result factor (MIC vs. CC)
• BACTEC perform first line drugs and then second line drugs if first-line are resistant so limited data for second line drugs
• Not all drugs tested by both methods
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Validation – Solutions

- **Started evaluating TREK in 2011 to give plenty of time before discontinuation of BACTEC**
- **Determined tentative MIC breakpoint:**
  - **Susceptible** - equivalent to or lower than critical concentration by BACTEC
  - **Resistant** - higher than the critical concentration by BACTEC
- **For drugs with limited data or not evaluated**
  - **Disclaimer**
  - Correlate with clinical picture/Educate physicians
  - Alternative testing – Molecular, CDC?
Implementation – October 2012

- Implemented TREK method in November to allow for a 2-month overlap with BACTEC
- Ensured up-to-date training of staff in the laboratory
- Provided information and education to TB Control Program, Providers in the State of Florida
  - Discussed with our TB Control Program and TB Physician’s Network
  - Fact sheet/Notification of change faxed out with every report
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Implementation

- Adapted our molecular AST algorithm
  - Pyrazinamide testing – pncA DNA Sequencing
  - Hain GenoType® MTBDRplus Assay – reflex test
    - First-time TB-suspect, NAAT positive specimen (irrespective of AFB smear result)
    - First-time TB-suspect, culture for identification, M. tuberculosis complex ID

- Developed reporting format for TREK results with our TB Physician’s Network (and other appropriate stakeholders)

Full implementation January 2013
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TREK Sensititre® MIC Microtiter Plate Method

Pros
• Simple, cost-effective test method
• Minimal/inexpensive test equipment
• All drugs reported at same time
• Result is an MIC, which can be useful for clinicians

Cons
• Slow turnaround time (requires solid culture)
• Cannot test PZA
• Interpretation of results a challenge
• Not an FDA-approved test
Future

- **Develop method for broth inoculation of MYCOTB plates to reduce TAT**
- **Develop customized MYCOTB plate**
  - Kanamycin off, Capreomycin on?
  - Bedaquiline?
- **Continued evaluation of tentative breakpoint determination and of reporting**
  - Example 1. Interpretation for Amikacin, Moxifloxacin, PAS and Cycloserine
  - Example 2. Intermediate determination for some drugs e.g. Isoniazid, Ethambutol, Streptomycin
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Thank You!

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