Validation of a Two Week Agar Proportion Drug Susceptibility Test for INH & Rifampin

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APHL 10th National Conference on Laboratory Aspects of Tuberculosis, April 18-20, 2017
Background (1)

- CDC recommends rifampin DST results be reported within 17 days after MTBC culture positive ID
  - Most labs use a rapid commercial broth-based system (MGIT or VersaTREK)
  - Agar Proportion (AP) is the “gold standard” method for phenotypic DST
  - AP is not a rapid method; conventional AP method takes 21 days (3 weeks)

- CDC MPEP surveys & literature reports show commercial broth DST methods miss some clinically significant rifampin and ethambutol resistance detected by AP
Background (2)

• A rapid report of INH & rifampin susceptible is the single most common & important DST report issued by the TB lab
  – ~90% of U.S. cases are RIPE-S
  – INH & RMP susceptible predicts RIPE Rx efficacy (Rx often already started empirically)
  – INH & RMP susceptible allows discontinuation of relatively toxic EMB & PZA in treatment continuation phase
Hypothesis

MTBC drug susceptibility to highly tuberculocidal drugs like isoniazid & rifampin can be determined at 2 weeks by microscopic observation of growth on drug-free control & drug-containing Middlebrook 7H10 agar.
Agar Proportion

**Pro**
- Multiple dilutions plated
  - Reduces under- or over-inoculated tests
- Colonial growth visualized
  - Confirm resistance
  - Confirm purity
- Detects RMP and EMB resistance missed by broth methods
- Concurrent 2\textsuperscript{nd} line DST easily added (FQNs & injectables)

**Con**
- Some drugs like EMB & FQNs require 3 weeks to declare susceptibility
- Some MTBC strains do not grow on 7H10 agar
- LDT Validation required
- Labor intensive/Subjective
  - Requires expert personnel
Texas DSHS 7H10 Agar Proportion Plate Panel

Drug Free Control
0.2 INH Ethambutol

Rifampin

Ofloxacin
1.0 INH

Ethionamide
Kanamycin
Two & Three Week 0.2 mcg/ml INH

<table>
<thead>
<tr>
<th>Two Week 0.2 mcg/ml INH Result</th>
<th>Three Week 0.2 mcg/ml INH Result</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inconclusive</td>
<td>Resistant</td>
</tr>
<tr>
<td>Inconclusive</td>
<td>20*</td>
<td></td>
</tr>
<tr>
<td>Resistant</td>
<td></td>
<td>52</td>
</tr>
<tr>
<td>Susceptible</td>
<td>1**</td>
<td></td>
</tr>
<tr>
<td>Wait for 3 week result</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>24</td>
<td>59</td>
</tr>
</tbody>
</table>

* Tests were inconclusive due to insufficient control growth or contamination
**Repeat test was susceptible at two and three weeks

<table>
<thead>
<tr>
<th>Incubation</th>
<th>No. of Conclusive Results</th>
<th>% of Total Results</th>
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</thead>
<tbody>
<tr>
<td>Two Week</td>
<td>52(R) + 469(S) = 521</td>
<td>94.2%</td>
</tr>
<tr>
<td>Three Week</td>
<td>59(R) + 470(S) = 529</td>
<td>95.7%</td>
</tr>
</tbody>
</table>
Typical MTBC 7H10 Agar Proportion Growth at 50X

Two Weeks Incubation

Three Weeks Incubation
Summary

2 week AP was an accurate, practical, & rapid method for INH and RMP DST

– >= 98.5% of INH & RMP tests could be declared S or R at 2 weeks with 100% accuracy

– Only a small proportion of tests required a third week of incubation

– 2 Week AP allowed CDC DST TAT guidelines to be met
Thank You!