Improvements in Efficiency and Safety: Phenol/Alcohol Fixing of AFB Smears

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Background

- Approx. 100 cases of TB annually (2014=108)
  - Largest Burmese population outside of Burma
- State PHL tests approximately 2000 specimens/year
  - Majority of samples submitted by county health departments
Background, (con’t.)

- TB Control regulated by the Indiana Communicable Disease Rule (CDR)
  - Investigation must be performed immediately by local health officer
  - Infectiousness determined by set of 3 sputa
    - 3 smear negative sputa = release from isolation

- As a direct result of the CDR, timeliness of smear results is critical
**AFB Testing at ISDH**

- AFB and culture performed for every specimen
  - Heat fixed on slide warmer for 2 hours
  - Stained with Auramine o-phenol
  - AFB rated with CAP scale
    - $>50/\text{field}$, $>10/\text{field}$, $1-10/\text{field}$, $<1/\text{field}$, $<1/\text{smear}$ (very few)
- PCR performed for smear positives and high risk smear negatives
False-Positive and false-negative smear and culture results—Ken Jost

- Acceptable methods
  - 2 hours at 65-75 °C
  - 20 minutes at 80 °C
  - 5% phenol in 70% ethanol for 5 minutes
80 °C Validation

- Attempted in August 2012
- Panel of 20 smear positive sputa specimens
- Tested in duplicate
  - 14/20 specimens exhibited lower AFB counts at 80 °C
    - AFB not adhering sufficiently to slide?
- Additionally, problems observed with hot and cold spots on slide warmer
Chedore et al.

Journal of Clinical Microbiology

Method for Inactivating and Fixing Unstained Smear Preparations of Mycobacterium tuberculosis for Improved Laboratory Safety

Pamela Chedore, Cecelia Th'ng, Dennis H. Nolan, George M. Churchwell, David E. Sieffert, Yvonne M. Hale and Frances Jamieson

Phenol/Alcohol Validation

- August 2012
- Panel of 40 samples tested in duplicate
  - 30 previously tested sputa
    - 20 smear positives with a range of AFB counts
    - 10 smear negatives
  - 10 AFB cultures
    - 5 MGITs
    - 5 7H11 plates

<table>
<thead>
<tr>
<th>Number of Samples</th>
<th>Previous AFB Smear Results</th>
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<tbody>
<tr>
<td>7</td>
<td>&gt;10/field</td>
</tr>
<tr>
<td>1</td>
<td>8-9/field</td>
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<td>2</td>
<td>5-6/field</td>
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<td>3-4/field</td>
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<td>3</td>
<td>1-2/field</td>
</tr>
<tr>
<td>4</td>
<td>&lt;1/field</td>
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<tr>
<td>10</td>
<td>AFB negative</td>
</tr>
<tr>
<td>3</td>
<td>MGIT pos</td>
</tr>
<tr>
<td>2</td>
<td>MGIT pos w/cording</td>
</tr>
<tr>
<td>5</td>
<td>7H11 solid medium</td>
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</tbody>
</table>
Smear Fixing Procedure

- **Step 1:** Pipette a few drops of processed sputa onto the slide
- **Step 2:** Place the slide on the slide warmer for 10 minutes to dry
- **Step 3:** Place the slides in a staining rack over absorbent paper
- **Step 4:** Flood the slide with 5% phenol in 70% ethanol
- **Step 5:** Let the slide soak for 5 minutes
- **Step 6:** Immediately stain the slide

*Step 4: Flooding the slide with phenol/alcohol*
Validation Results

- 100% correlation
- Slightly improved smear counts for 13% of smear positive samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Sample Type</th>
<th>65-75 °C</th>
<th>Phenol/alcohol</th>
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<tr>
<td>7</td>
<td>Sputa</td>
<td>&lt;1/field</td>
<td>1-2/field</td>
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<tr>
<td>14</td>
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<tr>
<td>18</td>
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<td>&gt;10/field</td>
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<tr>
<td>26</td>
<td>7H11 plate</td>
<td>Pos (very few)</td>
<td>Positive</td>
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</table>

- Could potentially result in the detection of AFB in samples with very low bacillary loads—the difference between a positive and a negative result
Improvement in Safety

- TB infection is one of the most common forms of lab acquired infection (Harding et al., 2000)
- Reducing the risk of infection is very desirable
- All steps of procedure are performed in the BSC until stain is applied
- Chedore et al. paper demonstrated that flaming slides was insufficient to kill MTBC (10/10 were viable)
  - After 5 minutes in 5% phenol in 70% ethanol, all samples were nonviable

*Applying Auramine o-phenol stain*
AFB Smear Turnaround Time

Time of Day of AFB Result

<table>
<thead>
<tr>
<th>Year</th>
<th>Time of Day</th>
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<tr>
<td>2012</td>
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<tr>
<td>2014</td>
<td>12:28</td>
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</table>
PCR Turnaround Time

Turnaround Time in Days

2012
2014

Average
Median
Acknowledgements

Ed Harris
Senior Microbiologist

ISDH TB Lab Staff
Questions?

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