Evaluating the Significance of Equivocal Mycobacterial Smear Results

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• August 28, 2014—both TB analysts retire (on the same day!)
• August 29, 2014—new analyst results a smear as equivocal
  • Previous analysts had always reported a smear as negative if they observed less than four AFB per slide
  • Which way is “correct”?
    • Is it preferable to err on the side of caution, or to wait until you see enough bacilli to be really sure?
What Do Other Labs Do?

- Informally surveyed several other PHLs:
  - Prepare a new smear from same specimen
  - Report as negative
  - Report as suspicious
  - Report as doubtful
  - Perform NAAT
  - Request a new specimen
Study Objective and Design

• Objective: To determine the significance of mycobacterial smear results when very few acid fast bacilli are observed in the concentrated smear

• Sputa were digested, decontaminated, and concentrated using standard methods

• Smears were fixed using 70% ethanol with 5% phenol and stained using Auramine O-phenol

• Smears were examined with a fluorescent microscope at 200x magnification, and AFB were counted at 500x

• All slides read by one analyst, and blindly verified by a second analyst

• Reading discrepancies resolved using a third analyst
## Total Specimens Processed

<table>
<thead>
<tr>
<th>Smear Result</th>
<th>Number</th>
<th>% of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>516</td>
<td>24.6%</td>
</tr>
<tr>
<td>Equivocal</td>
<td>119</td>
<td>5.7%</td>
</tr>
<tr>
<td>Negative</td>
<td>1462</td>
<td>69.7%</td>
</tr>
<tr>
<td>TOTAL</td>
<td>2097</td>
<td></td>
</tr>
</tbody>
</table>

September 1, 2015-August 31, 2016
Culture Results, Stratified by Smear

- Smear Positive: 150 Culture Positive, 352 Culture Negative
- Equivocal Smear: 74 Culture Positive, 42 Culture Negative
- Smear Negative: 1252 Culture Positive, 200 Culture Negative
Smear-to-Culture Correlation Rates

% Culture Correlation

- Smear Positive
- Equivocal Smear
- Smear Negative
Discordant Smears

Patients categorized into three groups:
1. Sputa collected from a known TB patient
2. One or more NTMs were isolated from another sputa
3. All cultures for the patient were negative
Negative Cultures, by Patient

Equivocal Smears

Positive Smears

- TB Patient
- NTM Patient
- True False Positive
Smear Specificity

- Smear Positive: 97.41%
- Equivocal Smear: 81.90%
Discussion

• The majority of culture discordant equivocal and positive smears were collected from previously diagnosed MTBC patients or were accompanied by one or more positive NTM cultures, 71.6% and 91.3%, respectively. It seems likely that the majority of these smear results represent either non-viable MTBC bacteria or low levels of NTM present in the specimen.

• Although none of the equivocal results in this limited data set led to a newly identified case of MTBC (all were follow up samples) the results suggest that additional follow up and testing should be considered.
Practical Considerations

• Analysts should be experienced in reading smears in order to reduce the reporting of “artifacts” as AFB

• Crucial to work with TB Control—how will a new result be used for case management?

• Important to educate submitters about results interpretation
Conclusions

• Equivocal smear results are more often predictive of a positive culture than are negative smear results

• For patients with an unknown TB status, NAAT testing should be performed to rule out MTBC

• For patients with known TB infection, equivocal smears are more likely to indicate infectiousness than negative smears
Questions?

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