Novel Approaches and New Methods to Increase Case Detection by Microscopy

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Objectives

• Present “hot off the press” findings from 3 systematic reviews concerning sputum microscopy

• Summarize the findings of the reviews using the GRADE approach

Images: CDC, World Lung Foundation, Univ. of Utah Health Sciences Library
Some definitions

- Systematic review is a review of a clearly formulated question that uses systematic and explicit methods to identify, select, and critically appraise relevant research, and to collect and analyse data from the studies that are included in the review.

- Meta-analysis is the use of statistical techniques in a systematic review to integrate the results of included studies.

Glossary of Terms, The Cochrane Collaboration, Version 4.2.5, Updated May 2005
Systematic review questions

• Are front-loaded and standard microscopy strategies comparable for diagnosing pulmonary TB when 2 specimens are examined?

• What is the diagnostic accuracy of LED fluorescence microscopy for pulmonary TB and how does it compare to Ziehl-Neelsen and fluorescence microscopy?
  - What do users think?

• Does bleach centrifugation increase the diagnostic accuracy of sputum smear microscopy for pulmonary TB?
Why carry out these reviews?

• Direct smear microscopy
  – Most widely available test for TB diagnosis
  – Moderate to poor sensitivity
  – High drop-out rate

• Methods to optimize smear microscopy
  – Sputum processing
  – Fluorescence microscopy
  – Diagnostic test strategies

• High quality evidence is important for policy
## Previous microscopy reviews

<table>
<thead>
<tr>
<th>Review (Date of publication)</th>
<th>No. of studies</th>
<th>Median sample size</th>
<th>Principal findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum processing (2006)</td>
<td>83</td>
<td>256</td>
<td>↑ sensitivity (13%) with bleach centrifugation</td>
</tr>
<tr>
<td>Fluorescence microscopy (2006)</td>
<td>45</td>
<td>493</td>
<td>↑ sensitivity (10%) with fluorescence microscopy</td>
</tr>
<tr>
<td>Serial sputum examination (2007)</td>
<td>37</td>
<td>153</td>
<td>2-5% ↑ sensitivity with 3rd sputum specimen</td>
</tr>
</tbody>
</table>
What’s new?

• New studies

• New technique
  – light emitting diode

• New diagnostic strategy
  – “front-loaded” microscopy

• New methods of data analysis and presentation
Standardized approach to systematic reviews of diagnostic accuracy

- Define review questions
- Identify and select studies
- Assess study quality (QUADAS)
- Extract, analyze, and present data
  - Graph results of individual studies
  - Pooled estimates of sensitivity/specificity by hierarchical summary ROC and bivariate random effects methods
  - Visualize and statistically assess heterogeneity
  - Explore reasons for heterogeneity
  - Forest plots, hierarchical summary ROC curves
- Interpret data

Quality assessment of diagnostic accuracy studies (QUADAS)

• Asks reviewers to assess 14 items
• Scores each item as ‘yes’, ‘no’, or ‘unclear’

  – Patient spectrum
  – Selection criteria
  – Appropriate reference test
  – Time between tests
  – Partial verification
  – Differential verification
  – Incorporation bias
  – Index test described
  – Reference test described
  – **Index test blinded**
  – **Reference test blinded**
  – Relevant data available
  – Indeterminate results
  – Study withdrawals

Systematic review questions

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Sputum collection

**Standard Strategy**

**Day 1**
- Spot → Smear preparation: Direct Stain: Ziehl-Neelsen

**Day 2**
- AM → Type of microscopy: Light

**Front-loaded Strategy**

**Day 1**
- Spot → Smear preparation: Direct Stain: Ziehl-Neelsen

**Day 2**
- XSpot → Reference standard: Culture
QUALITY ASSESSMENT (QUADAS)
HSROC curves

Standard Microscopy
Se: 68% (61,74)
Sp: 97% (93, 99)

Front-loaded Microscopy
Se: 66% (61,70)
Sp: 97% (92, 99)
Systematic review questions

- Are front-loaded and standard microscopy strategies comparable for diagnosing pulmonary TB when 2 specimens are examined?

- What is the diagnostic accuracy of LED fluorescence microscopy for pulmonary TB and how does it compare to Ziehl-Neelsen and fluorescence microscopy?
  - What do users think?

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Light Emitting Diode (LED) microscopy

Fluorescence microscopy has been shown to be more sensitive than ZN and more time efficient.

LED fluorescence microscopy uses ultra bright LED bulbs:

- Less expensive
- Require less power (run on batteries)
- Very long half-life
- Lower maintenance
- No toxic components
- No UV production
- Perform equally well without a darkroom
LED fluorescence diagnostic accuracy

- Sensitivity 84% (76, 89); specificity 98% (97,99)

- Head-to head LED versus ZN
  - 6% (0.1, 13) greater sensitivity, comparable specificity (8 studies)
  - 46% less time to examine smears (14 comparisons)

- Head-to head LED versus conventional fluorescence
  - 5% (95% CI 0, 11) greater sensitivity, comparable specificity (7 studies)
  - same time to examine smears (7 comparisons)

- 94-100% of users would recommend implementing an LED system over ZN (FIND)
Systematic review questions

- Are front-loaded and standard microscopy strategies comparable for diagnosing pulmonary TB when 2 specimens are examined?

- What is the diagnostic accuracy of LED fluorescence microscopy for pulmonary TB and how does it compare to Ziehl-Neelsen and fluorescence microscopy? What do users think?

- Does bleach centrifugation increase the diagnostic accuracy of sputum smear microscopy for pulmonary TB?
### Direct microscopy

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angeby (a) 2000</td>
<td>303</td>
<td>0.57</td>
<td>0.99</td>
</tr>
<tr>
<td>Bruchfeld 2000</td>
<td>510</td>
<td>0.54</td>
<td>0.97</td>
</tr>
<tr>
<td>Daley 2009</td>
<td>178</td>
<td>0.72</td>
<td>0.97</td>
</tr>
<tr>
<td>Eyangoh (a) 2008</td>
<td>936</td>
<td>0.68</td>
<td>0.99</td>
</tr>
<tr>
<td>Eyangoh (b) 2008</td>
<td>936</td>
<td>0.68</td>
<td>1.00</td>
</tr>
<tr>
<td>Gebre (a) 1995</td>
<td>100</td>
<td>0.31</td>
<td>1.00</td>
</tr>
<tr>
<td>Merid (c) 2009</td>
<td>497</td>
<td>0.51</td>
<td>0.97</td>
</tr>
<tr>
<td>Mutha (a) 2005</td>
<td>297</td>
<td>0.65</td>
<td>0.98</td>
</tr>
<tr>
<td>Wilkinson 1997</td>
<td>166</td>
<td>0.43</td>
<td>0.95</td>
</tr>
</tbody>
</table>

### Bleach centrifugation

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<td>Eyangoh (b) 2008</td>
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<td>0.72</td>
<td>0.97</td>
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<td>Merid (c) 2009</td>
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<td>0.64</td>
<td>0.81</td>
</tr>
<tr>
<td>Mutha (a) 2005</td>
<td>297</td>
<td>0.65</td>
<td>0.94</td>
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<tr>
<td>Wilkinson 1997</td>
<td>166</td>
<td>0.44</td>
<td>0.97</td>
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Forest Plot: Sensitivity Difference

<table>
<thead>
<tr>
<th>Study</th>
<th>Sensitivity Difference (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eyangoh SI (a) (2008)</td>
<td>0.05 (0.03, 0.08)</td>
</tr>
<tr>
<td>Eyangoh SI (b) (2008)</td>
<td>0.04 (0.02, 0.06)</td>
</tr>
<tr>
<td>Mutha A (b) (2005)</td>
<td>0.00 (-0.06, 0.06)</td>
</tr>
<tr>
<td>Wilkinson D (1997)</td>
<td>0.01 (-0.02, 0.04)</td>
</tr>
<tr>
<td>Angeby KA (a) (2000)</td>
<td>0.09 (-0.02, 0.19)</td>
</tr>
<tr>
<td>Bruchfeld J (2000)</td>
<td>0.09 (0.04, 0.14)</td>
</tr>
<tr>
<td>Daley P (a) (2009)</td>
<td>-0.06 (-0.16, 0.05)</td>
</tr>
<tr>
<td>Merid Y (c) (2009)</td>
<td>0.13 (0.08, 0.17)</td>
</tr>
<tr>
<td>Gebre N (a) (1995)</td>
<td>0.38 (0.23, 0.54)</td>
</tr>
</tbody>
</table>

Favors Bleach Centrifugation
SPECIFICITY

SENSITIVITY

HSROC curves

Direct Microscopy
Se: 56% (49, 63)
Sp: 98% (97, 99)

Bleach Centrifugation Microscopy
Se: 65% (59, 71)
Sp: 96% (93, 98)
Strengths and limitations

- **Strengths**
  - Standardized systematic review protocol
  - Comprehensive search strategy
  - Rigorous data analysis methods

- **Limitations**
  - Variability in diagnostic accuracy estimates for sputum processing
  - Limited data in HIV-infected patients
Concerns

- **Front-loaded**
  - risk of TB transmission in health care settings
  - loss of morning specimen for culture

- **LED versus conventional fluorescence**
  - increased cost of EQA because of fading of slides

- **Sputum processing**
  - primary analysis presented included only studies with culture reference
Arriving at a Recommendation

ARE WE THERE YET !?!
The Grading of Recommendations Assessment, Development and Evaluation - GRADE

“The GRADE approach provides a system for rating quality of evidence and strength of recommendations that is explicit, comprehensive, transparent, and pragmatic and is increasingly being adopted by organisations worldwide.”

www.gradeworkinggroup.org
## GRADE and Patient-Important Outcomes

<table>
<thead>
<tr>
<th></th>
<th>With TB</th>
<th>Without TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test positive</td>
<td>True Positive (TP)</td>
<td>False Positive (FP)</td>
</tr>
<tr>
<td>Test negative</td>
<td>False Negative (FN)</td>
<td>True Negative (TN)</td>
</tr>
</tbody>
</table>

TP - benefit from earlier diagnosis and treatment
TN - spare patients unnecessary treatment
FP - likely anxiety, possible morbidity from additional testing and treatment; may halt further diagnostic evaluation
FN - increased risk of severe disease from delayed diagnosis; continued TB transmission in the community
## GRADE Summary of Findings - Microscopy

<table>
<thead>
<tr>
<th>Review Question (studies, participants)</th>
<th>Absolute Difference per 1000 persons (Prevalence 20%)</th>
<th>Quality of Evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
<td>TN</td>
</tr>
<tr>
<td>Standard versus two-specimen front-loaded (7, 7308)</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>LED versus ZN light (8, 20155)</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>Bleach centrifugation versus direct (9, 3923)</td>
<td>18</td>
<td>-16</td>
</tr>
</tbody>
</table>
Acknowledgments

Evidence-Based Tuberculosis Diagnosis
A comprehensive resource for evidence syntheses, policies, guidelines and research agendas on TB diagnostics

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Public Health Agency of Canada (PHAC)
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McGill TB Research Group

www.tb.evidence.org
Merci!