Rapid Serological Based TB Test Evaluation: Prelim Analysis

Jane Cunningham
Medical Officer
WHO/CDS/TDR/PDE

S. Arloing, P. Courmont: *Technique et résultats du séro-diagnostic de la tuberculose*. Zeitschrift für Tuberkulose, 1901; 2:530
Humoral Response to *M. tuberculosis* is heterogeneous

ELISA readouts from 52 seroreactive TB patients using a variety of antigens

88% have detectable antibody response to at least one antigen; but < 50% responded to any single antigen
In 2005……..

- Over 40 commercially available rapid serologic tests for TB based on antibody detection using native and recombinant antigens including 38kDa, 16KDa, 6kDa, LAM, ESAT-6, CFP-10
- Formats – lateral flow immunochromatographic assays
- Aggressive marketing in developing countries where TB is endemic ……and lack of regulatory policies
- Antigen discovery, seroreactivity and development of antigen detection systems continues
Rationale

- Simple, rapid, point of care tests are now available and may be appropriate for use in primary health care settings but limited data on their performance characteristics in both HIV infected and non-infected patient populations.
- An objective evaluation would provide National TB Programs with critical preliminary information required to guide appropriate use.
Objectives

• To compare the performance and reliability of rapid M. tuberculosis specific antibody detection tests using banked serum samples from the WHO/TDR TB Specimen Bank

• To assess the operational characteristics of rapid M. tuberculosis tests, including ease of use, technical complexity and inter-reader variability
Test Inventory

• Inventory of serological tests for TB via internet searches; international conferences (MEDICA, AACCC); key contacts in countries, TDR correspondence

• All tests identify anti-mycobacterial antibodies in serum.

• Only 6/19 companies provided the antigen type.
Inclusion Criteria

- Rapid – test result available in less than 30 mins
- Simple* – can be performed in 1 or 2 steps, requiring minimal training and no equipment
- Easy to interpret – card or strip format with visual readout

* - 2 exceptions
Invitation

• 27 letters of invitation to test manufacturers (not distributors) – to reduce testing of identical products under different labels
• Manufacturers requested to donate tests, provide detailed product information and sign an agreement permitting the publication of results in a WHO/TDR report.
• 19 companies agreed, 7 declined, 1 withdrawn
Participating Companies

1. ABP Diagnostics
   Focus Sure Check TB
2. Advanced Diagnostics
   Tuberculosis Rapid Test
3. American Bionostica
   Rapid Test for TB
dBest One Step TB Test
4. Ameritek
   TB Rapid Screen Test
5. BioMedical Products Corp
   TB Stat-Pak II
6. Chembio
   TB Antibody onsite Rapid
7. CTK Biotech
   Screening Test Kit
8. Hema Diagnostic
   Rapid 1-2-3 TB Test
9. Millenium Biotechnology
   Immuno-Sure TB Plus
10. Minerva Biotech
    V Scan
11. Mossman Associates
    MycoDot
12. Pacific Biotech
    Bioline TB
13. Premier Medical Corporation
    First Response Rapid TB
14. Princeton BioMeditech
    BioSign M. tuberculosis
15. Span Diagnostics
    TB Spot ver. 2.0
16. Standard Diagnostics
    SD Rapid TB
17. Veda Lab
    TB Rapid Test
18. UniMED International Inc.
    FirstSign MTB Card Test
19. Silanes
    TB-Instantest
Source of Sera

- Banked serum samples from the WHO/TDR TB Specimen Bank. Frozen on site at – 70°C and transferred to a central repository and study center without thawing. One freeze-thaw cycle for aliquoting.
- Sera is collected from patients presenting to collaborating health clinics* and showing symptoms of pulmonary TB.
- TB diagnosed or excluded on the basis of smear microscopy, culture, radiography and clinical follow up (2-3 months).
  - TB confirmed: sm+/culture +; sm-/culture+
  - TB negative: sm-/culture-; no treatment for TB and negative repeat smear and improved clinical exam after 2-3 months.
Sample Size and Reliability

- Each test evaluated using a panel of 400 serum samples, allowing a determination of sensitivity and specificity with 95% +/- 10% confidence interval.
- 4 diagnostic categories (100x4=400):
  - TB+, HIV+.................................61
  - TB+, HIV-.................................100
  - TB-, HIV+.................................40
  - TB-, HIV-.................................99
  - Total 300 (prelim analysis)

- Test reliability: will the test give the same results…..
  - With tests of different manufacturing lots using the same specimens (lot to lot reproducibility)
  - On the same specimen if it is performed by two different operators (operator reproducibility)
  - On the same specimen on different days (run to run variability)
# Geographic Distribution

<table>
<thead>
<tr>
<th>Category</th>
<th>Uganda</th>
<th>Gambia</th>
<th>Canada</th>
<th>Tanzania</th>
<th>Brazil</th>
<th>Spain</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB+ HIV +</td>
<td>37</td>
<td>5</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>0</td>
<td>61</td>
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<tr>
<td>TB+ HIV -</td>
<td>39</td>
<td>45</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>11</td>
<td>100</td>
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<td>TB- HIV +</td>
<td>0</td>
<td>17</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>9</td>
<td>40</td>
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<tr>
<td>TB- HIV -</td>
<td>0</td>
<td>37</td>
<td>42</td>
<td>0</td>
<td>17</td>
<td>3</td>
<td>99</td>
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<tr>
<td><strong>Total</strong></td>
<td>76(25%)</td>
<td>94(31%)</td>
<td>42(14%)</td>
<td>33(11%)</td>
<td>22(7%)</td>
<td>23(8%)</td>
<td>300</td>
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</table>
Implementation

• Pilot phase: each test evaluated with 2 TB positive and 1 TB negative serum sample
• 2 Technicians blinded to reference material status
• Kits evaluated in groups of 2 (1 group of 3); 40 patient samples per day.
• Each test result read by 2 technicians
Results

• Performance = sensitivity & specificity
  – All samples (n=298; 158 culture +, 140 TB neg)
    • HIV -: n= 197 (66%); 97 culture +, 100 culture & f/u –
    • HIV+: n= 101 (34%); 61 culture +, 40 culture & f/u -
  – Sm-/culture + samples excluded (n=28)
    • Smear +/-culture + (n=130)
    • HIV -: n= 172 (64%); 72 culture +, 100 culture & f/u –
    • HIV+: n= 98 (36%); 58 culture +, 40 culture & f/u –
ROC TB All Samples n=298

Sens range: 0.63%-61%
Spec range: 53%-99%
ROC TB HIV Negative Samples n=197

Sens range: 0%-70%
Spec range: 51%-100%
ROC TB HIV Positive Samples  n=97

Sens range: 0%-54%
Spec range: 45%-100%
ROC Smear +/Culture + only  n=270

Sens range: 0.77%-62%
Spec range: 53%-99%
ROC Smear+/culture + only; HIV Negative Samples n=172

Sens range: 0%-78%
Spec range: 51%-100%
ROC Smear+/culture + only; HIV Positive Samples  n=98

Sens range: 0%-55%
Spec range: 45%-100%
Test Reliability

• Number of discrepant replications/the total number of replications of the three reliability tests (operator-to-operator, lot-to-lot and day-to-day)
<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Reader to Reader (n=48)</th>
<th>Lot to Lot (n=48)</th>
<th>Day to Day (n=96) Consecutive</th>
<th>Day to Day (n=96) Alternative</th>
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## TEST RELIABILITY

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Limitations

- Testing done retrospectively using stored frozen sera that passed through one freeze-thaw cycle. *Fresh serum may increase sensitivity.*
- Limited geographic diversity amongst TB+ and HIV+ patients. *Variations in specific antibody responses to mycobacterial antigens in different human populations may limit sensitivity.*
- Potentially inadequate follow-up of symptomatic patients. *Determining true specificity requires prolonged and careful follow-up; in other studies inclusion of healthy control subjects improves specificity.*
- Duration of illness unknown. Patients with relatively "early disease." *Greater duration or severity of illness has been correlated with likelihood of positive serologic test.*
- Nontuberculous mycobacterial infections causing cross reactivity and loss of specificity.
Conclusion - Testing of archived reference materials shows that:

- Currently marketed TB ICTs vary widely in performance
- Some products show high lot-to-lot and reader-to-reader variability
- Specificity is poor (<80%) in the majority of products when tested in TB suspects from endemic settings
- Tests with specificity over 90% detected <40% of TB patients
- HIV co-infection diminishes performance of existing assays
- None of the assays perform well enough to replace microscopy
Acknowledgements:

Anandi Martin, IMT, Antwerp Belgium
Francoise Portaels. IMT, Antwerp Belgium
Andrew Ramsay, WHO/TDR
Carl-Michael Nathanson, WHO/TDR
Mary Cheang, University of Manitoba
Mark Perkins, FIND
Freddie Poole, US FDA
Industry collaborators

Donors: USAID, Bill & Melinda Gates Foundation