Advances in Diagnostic Tests for Tuberculosis
Overview of the Diagnostics Pipeline

Richard O’Brien
3rd Global Laboratory Initiative (GLI) Meeting
Veyrier-du-Lac, France
4-5 October 2010
Potential Impact of New TB Diagnostics
TB Diagnostics Pathway*

*Stop TB New Diagnostics Working Group: A blueprint for the development of TB Diagnostics, 2009
WHO Policy on Implementing New Tools*

- Identifying the need for a policy change
  - WHO monitoring of technical developments
  - Requests from interested outside parties
- Reviewing the evidence
  - Use of standardized criteria for assessing available data
  - Systematic review and meta-analyses
  - GRADE approach for rating the strength of a recommendation
- Convening an expert panel to review evidence and draft recommendations
- Assessing draft policy and evidence by STAG-TB
- Formulating and disseminating policy

**Abbreviations**

- **DST**: Drug Susceptibility Test
- **NAAT**: Nucleic Acid Amplification Test
- **LTBI**: Latent TB Infection
- **POC**: Point of Care
- **MODS**: Microscopic observation drug-susceptibility
- **NRA**: Nitrate reductase assay
- **CRI**: Colorimetric redox indicator assay
- **LED**: Light-emitting diode
- **LPA**: Line probe assay

Technologies or processes endorsed by STAG/WHO

Technologies for which WHO review is in process
Manual Solution: Eiken

Loop-mediated Isothermal Amplification (LAMP)

Target DNA

3’ F3c F2c F1c B1 B2 B3 5’

5’ F3 F2 F1 B1c B2c B3c 3’

- Closed system
- Isothermal
- Rapid
- Multiprimer
- Visible readout
# LAMP Feasibility Studies V.1.0
Peru, Tanzania & Bangladesh*

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity in sS+, LJ+</th>
<th>Sensitivity in sS-, LJ+</th>
<th>Specificity in LJ-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lima</td>
<td>97.7% (75/78)</td>
<td>51.8% (14/27)</td>
<td>99.3% (152/153)</td>
</tr>
<tr>
<td>Dhaka</td>
<td>98.4% (61/62)</td>
<td>50% (2/4)</td>
<td>97.8% (181/185)</td>
</tr>
<tr>
<td>Mbeya</td>
<td>100% (37/37)</td>
<td>41.7% (5/12)</td>
<td>100% (167/167)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>97.7% (173/177)</strong></td>
<td><strong>48.8% (21/43)</strong></td>
<td><strong>99.0% (500/505)</strong></td>
</tr>
</tbody>
</table>

“The assay was **robust**, with high end-point stability and low rates of test failure. Technicians with no prior molecular experience easily performed the assay after 1 week of training, and opportunities for further simplification of the assay were identified”.

sputum

Heat at 90°C for 5min

Collect 40ul using transfer device

Heat at 67°C for 40min

Detect fluorescence signal

Add 25 - 35 µl (between lines)

Mix

Close caps and let it stand upside down for 2 min

Dried reagent

Dropper cap

Reaction tube

Absorbent tube

LAMP reaction at 67°C 40min

Heating container

Transfer device
**MDR-XDRTB Color Test for Regional Laboratories**

1. **Liquefaction & decontamination in transport medium at room temperature**

2. **Direct application of 2 drops to selective thin layer agar for incubation in room air for MDRTB testing & XDRTB screening**

3. **Color growth detection & microscopy confirmation of morphology**

Biosafety similar to sputum microscopy because sputum is smeared directly onto the plate which is then permanently double-sealed until autoclaving.

*Carlton Evans, Welcome Trust, Peru*
MDR-XDRTB Color Test Performance (n=214)

Gold standard = culture positive in any test (n=84/214)

TB diagnostic sensitivity

- ZN microscopy: 51%
- Centrifuge decontamination & thin layer agar Culture (TLA): 74%
- MDR-XDRTB COLOUR TEST: 89%
- Centrifuge decontamination & low-volume MODS: 94%

51% sensitivity vs. any positive (+95%CI)

P<0.01

ns P=0.3

P<0.001

Concurrent Drug Susceptibility Testing

<table>
<thead>
<tr>
<th>COLOUR TEST</th>
<th>Direct MODS</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR</td>
<td>9</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>not-MDR</td>
<td>1</td>
<td>68</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>71</td>
<td>81</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>COLOUR TEST</th>
<th>Indirect TEMA</th>
<th></th>
<th></th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDR</td>
<td>8</td>
<td>4</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>not-MDR</td>
<td>1</td>
<td>51</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>55</td>
<td>64</td>
<td></td>
</tr>
</tbody>
</table>

Color test had 2% contamination (all fungal)
Median time to positive result was 16 days
New Point-of-Care Technologies

- Antigen and antibody detection
- VOC detection systems
Lipoarabinomannan (LAM)-A (not so) low hanging fruit

- Major cell wall glycolipid of *M. tuberculosis*
- Described by several groups as diagnostic for TB
- Measurement in urine (+ sputum)
- FIND evaluation of ChemBio / Inverness ELISA
- FIND’s collaboration with various partners to prove LAM as diagnostic marker
Point of care TB antigen detection:  
*Lipoarabinomannan (LAM) as candidate marker*

Chemogen assay results (FIND Tanzania study 2006)

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity in ss+ TB</th>
<th>Sensitivity in ss- TB</th>
<th>Specificity Non-TB</th>
<th>Specificity healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV+</td>
<td>62% (75/119)</td>
<td>28% (22/80)</td>
<td>93% (240/258)</td>
<td>99% (222/224)</td>
</tr>
<tr>
<td>HIV-</td>
<td>79%</td>
<td>42%</td>
<td>32%</td>
<td></td>
</tr>
</tbody>
</table>

- Chemogen / Inverness assay suggested for urine testing
- Claims not supported by study data (4+ independent studies)

Tessema, Svenson et al., 2001

Inverness Medical Innovations Launches Clearview TB ELISA at IAS Meeting 2008  
(New York Times Business, August 8, 2008)
On the way to a POC: The search for TB antigens

**Clearview MTB ELISA: Result overview for completed studies (accepted or submitted)**

<table>
<thead>
<tr>
<th>Site</th>
<th>Overall sensitivity</th>
<th>Sensitivity in HIV-infected</th>
<th>Sensitivity in HIV-uninfected</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mbeya</td>
<td>51%</td>
<td>65%</td>
<td>34%</td>
<td>93%</td>
</tr>
<tr>
<td>Dar Es Salaam</td>
<td>65%</td>
<td>65%</td>
<td></td>
<td>86%</td>
</tr>
<tr>
<td>Harare</td>
<td>44%</td>
<td>52%</td>
<td>21%</td>
<td>89%</td>
</tr>
<tr>
<td>Cape Town 1</td>
<td>59%</td>
<td>67%</td>
<td>14%</td>
<td>96%</td>
</tr>
<tr>
<td>Cape Town 2</td>
<td>38%</td>
<td>38%</td>
<td></td>
<td>100%</td>
</tr>
</tbody>
</table>

Box & Whisker plot of LAM concentrations in TB patients with and without HIV coinfection.

**Urinary LAM assay: Potential application in HIV-positive patients?**

What’s next:

- Alternative LAM AB pairs in clinical validation studies in Zimbabwe/SA
- Several LAM prototype POC assays in development
- Promising new AG leads followed-up in several collaborations
Towards TB Serology: Anti-M.tb Antibody Profiling

- Antigen array chip with ~4,000 proteins
- Whole M.tb proteome screen to identify a set of diagnostic antigens for seroprofiling
- >1,000 sera selected for WPS (FIND sample repository)
**M. tuberculosis** Whole Proteome Screen: *profiling 927 serum samples from 10 countries* (FIND sample repository)
Establishment of database

Data from different sites have been reconciled and re-coded in a common semantic framework to create an Oracle database that integrates microarray measurements and clinical data.

Original Sources  →  Standardized  →  Oracle Database w/ custom query interface
Ranking of Proteins from Whole Proteome Screen

- All confirmed TB vs. endemic non-TB controls included in analysis
- Two independent statistical analyses employed
- 19 top rank proteins targeted for purification
- Validation studies to be initiated
Giant pouched rats have been trained to detect TB in sputum specimens by smell alone. Preliminary results of a blinded study using 2 rats for 67 AFB+ and 752 AFB- samples found a sensitivity of 86% and a specificity of 89%
Application of E-Nose to Detection of *M. tuberculosis* in Culture*