Update on Advances in TB Diagnostics with Special Reference to Progress in Drug Susceptibility Testing Methods and Smear Negative TB

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Inefficiency of global TB case detection

- Smear-positive reported: 2,216,108
- Smear-positive undetected/unreported: 1,722,892
- Smear-negative reported: 2,304,640
- Smear-negative undetected/unreported: 2,674,360

Total: 8,918,000

2006 Global TB Report, WHO
The slow road to TB diagnosis

Number of TB bacilli per millilitre of sputum

- Infection of healthy patient
- Patient feels unwell
- Night cough begins
- Cough worsens: patient returns to clinic
- Blood appears in sputum; infant daughter infected with TB
- Too weak to work

Threshold for visibility of AFB by smear microscopy

Patient returns to clinic

AFB+: TB diagnosis made

first month  second month  third month  fourth month  fifth month
Importance of early diagnosis:
Sensitivity (cfu/ml) of pulmonary TB tests in portfolio

- iLED* fluorescent microscope: 10,000/ml
- LAMP-TB: 50-150/ml
- Xpert MTB*: 50-150/ml
- MGIT*: 10-100/ml
- Line-probe*: 10,000/ml
- Capilia* speciation dipstick (of culture): 1,000,000/ml

Target sensitivity range of FIND antigen detection tests

* Development completed
### Achievements as per WHO endorsements 2007-2010

<table>
<thead>
<tr>
<th>Year</th>
<th>Technology</th>
<th>Turnaround time</th>
<th>Sensitivity gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before 2007</td>
<td>ZN microscopy, Solid Culture</td>
<td>2-3 days, 30-60 days</td>
<td>Baseline</td>
</tr>
<tr>
<td>2007</td>
<td>Liquid Culture / DST Rapid speciation</td>
<td>15-30 days</td>
<td>+10% compared to LJ</td>
</tr>
<tr>
<td>2008</td>
<td>Line Probe Assay (1st line, Rif &amp; INH)</td>
<td>2-4 days</td>
<td>At this time for S+ only</td>
</tr>
<tr>
<td>2009</td>
<td>LED-based FM</td>
<td>1-2 days</td>
<td>+10% compared to ZN</td>
</tr>
<tr>
<td>Conditional 2010</td>
<td>In house DST (MODS, CRI, NRA)</td>
<td>15-30 days</td>
<td>1st line only</td>
</tr>
<tr>
<td>Expected 2010</td>
<td>Integrated NAAT (TB, Rif)</td>
<td>90 minutes</td>
<td>+40% compared to ZN</td>
</tr>
</tbody>
</table>

**Importance of**

- a) early diagnosis & care;
- b) smear-negative TB;
- c) rapid MDR/XDR detection
Line probe assay
(WHO endorsement: 2008)

- DNA extraction + PCR + reverse hybridization of amplified DNA to oligonucleotide probes
- Identifies *M. tb* and detects Rif & INH resistance in 1-2 days
- Detection of rpoB gene for Rifampicin & inhA and catG gene for INH
LPA for XDR: An update

- Overall sensitivity for OFL, AM, CM and EMB was 90.2 %, 83.3 %, 86.8 % and 59.0 %, respectively.

- Specificity was 100 % for FLQ, AM, and EMB, and 99.1 % for CM.

- The rapid detection of XDR strains is possible from DNA isolates and directly from sputum specimens.

A technology platform for
- TB MDR (Rif & INH resistance)
- TB XDR (Quinolone resistance)
- Potential for knowledge and know how transfer for Early Infant Diagnosis for HIV
Introducing high tech in low tech settings

Development completed / Demonstration ongoing

Major advantages in workflow

- fully automated with 1-step external sample prep.
- time-to-result 1 1/2 h (walk away test)
- throughput: up to 16 tests / module / run
- no bio-safety cabinet
- closed system (no contamination risk)

Performance

- specific for MTB
- sensitivity close to culture
- detection of rif-resistance via rpoB gene

Automated Sample Prep, Amplification and Detection <120 minutes

A technology platform:
- TB & Rif Resistance
- Potential for HIV viral load
- Potential for HPV / STD /
## Demonstration project - interim review

### Case detection

<table>
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<tr>
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<th>Sensitivity All C+</th>
<th>Sensitivity S+C+</th>
<th>Sensitivity S-C+</th>
<th>Specificity Non-TB</th>
</tr>
</thead>
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<tr>
<td><strong>Single Xpert</strong></td>
<td>90.3% [87.5-92.5]</td>
<td>99.0% [97.0 – 100.0]</td>
<td>67.2% [61.0-73.2]</td>
<td>95.0% [93.2 – 96.1]</td>
</tr>
</tbody>
</table>

### Evaluation study results:
- July 2008 - March 2009 at 5 sites; 1462 patients (4386 samples)

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<td><strong>Three Xpert tests</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (Correct / total)</td>
<td>97.6 (723/741)</td>
<td>99.8 (566/567)</td>
<td>90.2 (157/174)</td>
<td>98.1 (604/616)</td>
</tr>
<tr>
<td>[CI]</td>
<td>96.2 – 98.5</td>
<td>99.0 – 100.0</td>
<td>84.9 – 93.8</td>
<td>96.6 – 98.9</td>
</tr>
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| Two Xpert tests      |                    |                  |                  |                   |
| %                    | 96.0 [94.6 - 97.1] | 99.4 [98.6 – 99.7] | 85.1 [79.7 – 89.2] | 98.6 [97.5 – 99.2] |
| [CI]                 |                     |                  |                  |                   |

| One Xpert test       |                    |                  |                  |                   |
| %                    | 92.2 [90.0 – 93.9] | 98.2 [96.8 – 99.0] | 72.5 [65.4 – 78.7] | 99.2 [98.1 – 99.6] |
| [CI]                 |                     |                  |                  |                   |
Demonstration studies

- Oct 2009 – Nov 2010
- 9 routine district / subdistrict laboratories
  - SA (2x), Peru (2x), Philippines (2x), India (1x), Uganda (1x), Azerbaijan (1x)
- Enrolment status: >5000 patients

WHO endorsement in May 2010
A technology platform for:
- TB (microscopy center)
- Malaria
- HAT
- HIV Early Infant Diagnosis
- Leishmania / Chagas

**FIND feasibility studies**
- Performance improvement for new version
- Assay further simplified
- Optimization of direct sputum transfer ongoing (biosafety & precision)

Registration study in Japan started in July 2009
Evaluation study in 2010
Decentralization of molecular diagnostics

1st generation MDR

2nd generation automated MDR

LPA

Xpert

LAMP

POC test

2008

2009

2010

2011

2015

Less complexity, more robustness
Integration of new tools in the tiered health system

Expected 2012 (Gen 1) / 2014 (Gen 2)

- Surveillance
- Reference methods
- Network supervision

- Resolution testing (screening-test negative drug resistance)

- Screening
- Passive case finding
- Detect and treat

- Clinical screening
- Primary care

Community Level

Microscopy Level

SubDistrict Level

District Level

Regional Labs

Reference Labs

• Surveillance
• Reference methods
• Network supervision

• Resolution testing (screening-test negative drug resistance)

• Screening
• Passive case finding
• Detect and treat

• Clinical screening
• Primary care

RDT Gen1 / Gen 2

Manual NAAT +25%

Integrated NAAT +40% / 2h

LC / DST
15d / 30d

LED FM +10%

Integrated NAAT +40% / 2h

ZN 2-3d

LC / DST
15d / 30d

In house DST (MODS, NRA, CRI) Special settings and conditions

LPA Rif / INH 2d

LC / DST
15d / 30d

LPA Rif / INH 2d

Special settings and conditions

Manual NAAT +25%
Evaluation of Serological TB Rapid Tests

**Figure 4. ROC curve of commercial rapid tests for the diagnosis of pulmonary tuberculosis**
(all patients, n=355)  
*WHO / TDR Report, Sept. 2008*

POC generation 2 + X
- Orchestrating the Gears -

❖ Building on 1st Gen. technology

❖ Next Gen. Challenges
  ➢ Improved biomarkers (microbial and host)
  ➢ Heat stable reagents
  ➢ Label-free detection technology
  ➢ Molecular POC
  ➢ Robust and affordable devices (bioterrorism model)
  ➢ Solar power supply
  ➢ Data transmission
  ➢ Waste management

➢ Clinical subpopulations (ethnicity, TB strains, coinfections)
➢ Multiplexing for disease panels and host markers
Closing the Gaps in TB POC Diagnostics

• WHAT to detect and HOW to do it?

• Biology meets Technology
  – Validation of candidate diagnostic molecules vs. biomarker discovery
  – Established platforms vs. new detection technologies

• Focus 1: TB antigen and antibody detection
• Focus 2: POC enabling technologies
Knowledge Gaps in TB Biomarker Research
(Presented by FIND at US Congressional Hearing, 2008)

- Lack of systematic approaches to marker discovery: no consensus on TB biomarkers
- Lack of reproducibility of preliminary biomarker results (e.g., antibodies, sputum antigens)

Probable causes of failure and bias:
- opportunistic approaches (TB serology) and / or
- poor understanding of in vivo antigen processing (actual marker presence in sample) and / or
- lack of appropriate detection technology (insufficient sensitivity; e.g. EIA / LFI) and / or
- inefficient sample preparation procedures (degradation; sputum, urine) and / or
- poorly documented clinical samples in addition to
- limited statistical power of studies (small patient numbers)
TB Antibody POC

• Whole Proteome Array
  – 927 sera tested
  – Target proteins identified
  – Protein purification initiated
  – Validation study plans in place

• Candidate peptides
  – First data back from NYU on FIND sera

• Overlapping Peptide Arrays
  – Agreement in place
  – Must wait for WPS IP submission before start
On the way to a Rapid TB Antibody Test
Profiling the TB Immunoproteome

Search for TB Vaccines & Diagnostics

- Whole proteome screen completed (> 800 samples from 10 countries)
- Target protein set identified

Next steps:

- Validation of identified Ab patterns w Luminex / overlapping peptide approach
- Final Ab sets will require confirmatory testing in large sample set
- Prototype assays expected for end 2010 / early 2011
Better TB Biomarkers – The Options

• Starting material = clinical sample
  - Classic MS-MS
  - Protein depletion / hs-MS
  - Highly characterized samples needed
  - Immunization cycle required to generate ABs

• Starting material = target molecules
  - Bulk immunization / affinity capture
  - Fast pace (Fab)2 production
  - Protein supply is key issue
  - Direct use of ABs for antibody arrays
TB Biomarker Discovery & Evaluation
Focus on “omics“ Discovery Technologies

- 4 urine candidate peptides in validation studies
- Several new peptide signatures discovered in urine - immunoassays in development
- Sputum lipidomics discovery yielded markers giving >90% dx accuracy
- LAM (Lipoarabinomannan) studies ongoing
Point of Care Enabling Technologies

- Reality and Promises

• Lateral Flow Immunoassays
  – Real and vital
  – Limited multiplexing

• Microfluidics
  – Real but awaiting market penetration

• Label-free detection methods
  – Some real, many promises
  – No marketed POC devices yet

• Other assay formats…
  – Many promises
  – Continuous technology scouting
  – Data base established

• Advances in antibody engineering
  – Very real; still advancing rapidly

• Alternative binding agents
  – Pharma driven; most awaiting dx validation