Drug resistant TB and new diagnostics for people living with HIV: emerging results from FIND

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FIND CEO

IAS Conference
Catalysing HIV/TB research
Cape Town
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Outline

• Evolution of TB diagnostics as per WHO endorsement and relevance with respect to HIV & MDR/XDR

• Evolution of molecular diagnostics platforms and their potential for integration of various diseases

• Update on new molecular tools (Xpert MTB/Rif, LPA XDR, LAMP)

• Update on POC (antigen, antibody detection)
Evolution of technologies for TB & M(X)DR as per WHO endorsement

**Relevance for HIV/TB:**
- Importance of:
  a) *early diagnosis & care*;
  b) *smear-negative TB*;
  c) *rapid MDR/XDR detection*

<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 Smart Culture</td>
<td>2-3 days 30-60 days</td>
<td>Baseline</td>
</tr>
<tr>
<td>2007</td>
<td>Liquid Culture Smart 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>Expected 2009</td>
<td>LED-based FM</td>
<td>1-2 days</td>
<td>+10% compared to ZN</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 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

* Development completed
Abbreviating delay through better sensitivity and better access

Where delay contributes greatest to morbidity, mortality, transmission

Implement molecular test with sensitivity similar to culture
Decentralization of molecular diagnostics

1st generation MDR

2nd generation automated MDR

LPA

Xpert

1st generation manual detection

2008

2010

2011

2015

LAMP

POC test

Less complexity, more robustness
Integrated NAAT for TB/Rif: An update

Workflow

- fully automated with 1-step external sample prep.
- time-to-result < 2 h (walk away test)
- throughput: up to 1-48 tests / run
- no bio-safety cabinet
- closed system (no contamination risk)

A technology platform for
- TB & Rif Resistance
- TB Quinolone resistance
- Potential for HIV viral load
Good performance during feasibility studies led to Xpert MTB/Rif design lock

<table>
<thead>
<tr>
<th>TB CASE DETECTION</th>
<th>Xpert sensitivity in smear+, cul+</th>
<th>Xpert sensitivity in smear-, cul+</th>
<th>Xpert specificity in smear-, cul-</th>
</tr>
</thead>
<tbody>
<tr>
<td>LATVIA</td>
<td>93.3% (14/15)</td>
<td>92.3% (12/13)</td>
<td>98.6% (71/72)</td>
</tr>
<tr>
<td>PERU</td>
<td>100% (99/99)</td>
<td>81.8% (9/11)</td>
<td>96.7% (148/153)</td>
</tr>
<tr>
<td>TOTAL 95% CI</td>
<td>99.1% (113/114)</td>
<td>87.5% (21/24)</td>
<td>97.3% (219/225)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RIF RESISTANCE DETECTION</th>
<th>Xpert sensitivity in Rif resistant</th>
<th>Xpert specificity in Rif sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LATVIA</td>
<td>100% (8/8)</td>
<td>100% (18/18)</td>
</tr>
<tr>
<td>PERU</td>
<td>100% (14/14)</td>
<td>100% (94/94)</td>
</tr>
<tr>
<td>TOTAL 95% CI</td>
<td>100% (22/22)</td>
<td>100% (112/112)</td>
</tr>
</tbody>
</table>

*1 follow-up visit at 2-4 m

• Rate of invalid Xpert results: 3% (24/786 cartridges)
Xpert MTB/Rif: FIND Evaluation studies

Rigorous performance evaluation at 5 sites (>1500 TB suspects)  
Included 2 sites with high HIV prevalence (80%) & 2 with high MDR prevalence (>30%)
Xpert Evaluation Study

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity S+C+</th>
<th>Sensitivity S-C+</th>
<th>Specificity Non-TB</th>
<th>Sensitivity in phenotypic Rif resistant cases</th>
<th>Specificity in phenotypic Rif sensitive cases</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>99.5% (564/567)</td>
<td>90.2% (157/174)</td>
<td>98.1% (604/616)</td>
<td>97.5% (199/204)</td>
<td>98.1% (504/514)</td>
</tr>
<tr>
<td>[95% CI]</td>
<td>[98.5 – 99.8]</td>
<td>[84.9 – 93.8]</td>
<td>[96.6 – 98.9]</td>
<td>[94.4 - 99.0]</td>
<td>[96.5 - 98.9]</td>
</tr>
</tbody>
</table>

Xpert™ MTB performance for case detection and Rifampicin resistance detection compared to conventional methods

Including results from 5 sites: Peru, Azerbaijan, India and South Africa (2)

* C: culture; S: AFB smear
**Xpert MTB/Rif: FIND evaluation studies**

<table>
<thead>
<tr>
<th></th>
<th>AFB-</th>
<th>AFB+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Culture Positive</strong></td>
<td><strong>Culture Negative</strong></td>
<td><strong>Culture Positive</strong></td>
</tr>
<tr>
<td>MTB Detected</td>
<td>70</td>
<td>3</td>
</tr>
<tr>
<td>MTB Not Detected</td>
<td>7</td>
<td>171</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined Xpert MTB/RIF</td>
<td>98.0%</td>
<td>98.3%</td>
</tr>
</tbody>
</table>

**Sensitivity for in S+/C+ = 100%, in S-/C+ = 91%**

<table>
<thead>
<tr>
<th></th>
<th>DST RIF Resistant</th>
<th>DST Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Combined Xpert MTB/RIF</strong></td>
<td>58</td>
<td>4</td>
</tr>
<tr>
<td>RIF Resistance Detected</td>
<td>2</td>
<td>280</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIF Resistance Not Detected</td>
<td>96.7%</td>
<td>98.6%</td>
</tr>
</tbody>
</table>

**High accuracy for Rif detection**
**Sequencing data for discrepant results suggest Xpert correct**
Xpert MTB/Rif: Low biosafety requirements

- Biosampler measurements (10^8 cfu/ml):
  1. No aerosol release during sample preparation
  2. Aerosol generation < than during preparation of direct smear
- Spiking studies: 7 log killing after 15 min SR incubation
- Clinical studies: >90% MGIT neg after 15 min SR incubation

✓ Closed system
✓ Sample treatment buffer (SR) high inactivation activity
Early & decentralized diagnosis with Xpert MTB/Rif: What’s next?

• Highly promising technology for DECENTRALIZED case detection (smear-negative TB & relevance in HIV prevalent settings) and for MDR screening
  ✓ Sensitivity for S-/C+ = 90% across sites (78% with a single test)
  ✓ Equal sensitivity in HIV+ (80% co-infection at 2 sites)
  ✓ Specificity in symptomatic Non-TB = 98%
  ✓ Very high accuracy for Rif detection
• Demonstration projects at microscopy centers / district hospitals underway in 7 countries (on the spot diagnosis!)
• WHO submission planned for 2010
Manual NAAT: An update

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

Registration study in Japan to start July 09
Evaluation study to start Dec 09
Molecular POC: the long awaited golden bullet?

- Candidate Technologies
  - LAMP
  - bDNA
  - Los Alamos Labs
  - PSS
  - Claros
  - Label free systems
  - Microfluidics

- Numerous new leads awaiting assessment

Expected results from the UNITAID project (1st phase) GLI FIND GDF

74 000 MDR-TB patients diagnosed (and provided with treatment)

• Cover 15% of global MDR-TB burden (16 Countries).
• At least 3-fold increase over current situation
• 2nd Phase starting with additional 11 Countries with total coverage of 35% of global burden

Molecular laboratory in Maseru
Evolution of **XDR** testing: decentralizing

- **2006**: Phenotyping: Solid Culture
- **2007**: Phenotyping: Liquid Culture
- **2010**: Phenotyping & Genotyping: Simplified culture & LPA XDR
- **2013**: Integrated Genotyping: Xpert XDR
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.

Using MTBDRsl to detect quinolone, aminoglycosid & ethambutol resistance

Evaluation GenoType® MTBDRsl assay
Doris Hillemann, Sabine Rüsch-Gerdes, and Elvira Richter
The urgent need for a POC test

WHY

• 4 M undiagnosed cases
  WHO Global TB Report 2008

• Diagnostic delays fuel transmission & severity
  Liam, 1997, Int J Tub & Lung Dis

WHAT

• Simple &
• Accurate &
• Robust &
• Rapid Test
• For qualitative TB case detection
• At the lowest level of health system: the health posts
On the way to a POC: The search for TB antigens

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

<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>Harare</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>

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
On the way to a POC – The search for AB sets

What's next:

- Validation of identified Ab patterns w Luminex / overlapping peptide approach
- Final Ab sets will require confirmatory testing in large sample set
- Prototype assays for first field studies expected for July 2010

Whole proteome screen completed
- 1000 samples from 10 countries
- Target protein set identified for HIV+/HIV-
- IP filed
FIND RFA for POC test development: An update

- 5 M USD set aside
- 50 high-quality proposals
- Scientific Review committee: Cliff Barry, David Alland, Stewart Cole, Mickey Urdea, Stephen Stroupe, Robert Wilkinson
- Scientific review meeting 21/22 July 09
- Groups with highest scores will be invited for individual presentations / discussions
Thank you