Contemporary diagnostics: moving towards integrated technology platforms

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FIND CEO

GLI Conference
Les Pensiéres
15 October 09
Maputo Declaration on Strengthening of Laboratory Systems

• Call on donors and implementing partners to ensure that in supporting laboratory strengthening that proper consideration is given to fostering national ownership.

• Call on donors and development partners to commit to work collaboratively with each other and with coordination from the national governments to support strengthening of laboratory systems in order to create one unified, integrated national laboratory network. These laboratory strengthening efforts should seek to build public private partnerships.
Integration of public health laboratory services

- Infrastructure upgrade and maintenance
- Linked referral services and timely reporting
- Training and retention – Human Resources
- Logistics and Commodity Management
- Quality Assurance
- Integrated laboratory network

TB, HIV, Malaria, Opportunistic infections
The nightmare scenario for service, maintenance and training...
Evolution of TB diagnostics in the public sector

Fundamental diagnostic: 1882

Fundamental diagnostic: 2007
Evolution of TB diagnostics in the public sector

Fundamental diagnostic: 1882

Fundamental diagnostic: 2008
An extraordinary act of will
Complexity of conventional sputum decontamination in reference labs

1. Liquefaction
2. Sample decanted
3. Decontamination NaOH
4. Vortex
5. Phosphate Buffer

6. Centrifugation
7. Decant
8. Re-suspend
9. Inoculation

Simplicity of MDR-XDRTB COLOUR TEST for regional labs

Combined optimizations: single-step decontamination (Vasanthakuri et al 1987), microscopic observation of growth, direct susceptibility testing for MDRTB testing & XDRTB screening, selective culture media (Mitchison et al), colour indication of culture positivity
<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Liquefaction &amp; decontamination in transport medium at room temperature</strong></td>
<td><strong>Direct application of 2 drops to selective thin layer agar for incubation in room air for MDRTB testing &amp; XDRTB screening</strong></td>
<td><strong>Colour growth detection &amp; microscopy confirmation of morphology</strong></td>
</tr>
</tbody>
</table>

Biosafety similar to sputum microscopy because sputum is smeared directly onto the plate which is then permanently double-sealed until autoclaving.
MDR-XDRTB Colour Test Performance (n=214)
Gold standard=culture positive in any test (n=84/214)

**TB diagnostic sensitivity**

- P<0.01
- P<0.001
- ns P=0.3

- 51% (74%) (89%) (94%)

**Concurrent Drug Susceptibility Testing**

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

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

*Colour test had 2% contamination (all fungal) & median time to positive result was 16 days*
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
Serodiagnosis of TB

S. Arloing, P. Courmont: Technique et résultats du séro-diagnostic de la tuberculose. Zeitschrift für Tuberkulose, 1901; 2:530
Figure 4. ROC curve of commercial rapid tests for the diagnosis of pulmonary tuberculosis (all patients, n=355)

Sensitivity of selected antigens at >95% specificity level compared to healthy controls

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Europe, HIV– (n=71)</th>
<th>Africa, HIV– (n=79)</th>
<th>Africa, HIV+ (n=77)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB9.7</td>
<td>35%</td>
<td>79 %</td>
<td>91%</td>
</tr>
<tr>
<td>CFP10:ESAT6*</td>
<td>25%</td>
<td>64%</td>
<td>49%</td>
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<tr>
<td>TB10.2</td>
<td>21%</td>
<td>45%</td>
<td>48%</td>
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<tr>
<td>TB15.3</td>
<td>41%</td>
<td>75%</td>
<td>65%</td>
</tr>
<tr>
<td>TB16.3</td>
<td>55%</td>
<td>81%</td>
<td>88 %</td>
</tr>
<tr>
<td>TB 51</td>
<td>31%</td>
<td>76%</td>
<td>48%</td>
</tr>
<tr>
<td>TB51.7</td>
<td>57%</td>
<td>83%</td>
<td>78%</td>
</tr>
<tr>
<td>aCry:MPT83</td>
<td>26%</td>
<td>83%</td>
<td>58%</td>
</tr>
<tr>
<td>38 kDa</td>
<td>19%</td>
<td>29%</td>
<td>15%</td>
</tr>
</tbody>
</table>
Whole proteome screening of *M. tuberculosis* for diagnostic antigens
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

Xpert™ MTB/Rif
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%)

<table>
<thead>
<tr>
<th></th>
<th>HIV</th>
<th>TB (C+)</th>
<th>MDR TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>UPCH</td>
<td>2%</td>
<td>61%</td>
<td>7%</td>
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<tr>
<td>UCT</td>
<td>77%</td>
<td>39%</td>
<td>10%</td>
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<tr>
<td>SAMRC</td>
<td>72%</td>
<td>13%</td>
<td>9%</td>
</tr>
<tr>
<td>Hinduja</td>
<td>5%</td>
<td>60%</td>
<td>67%</td>
</tr>
<tr>
<td>STI</td>
<td>5%</td>
<td>42%</td>
<td>31%</td>
</tr>
<tr>
<td>Germany</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Azerbaijan</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>South Africa</td>
<td></td>
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Xpert MTB/Rif: FIND evaluation studies

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

High accuracy for Rif detection
Sequencing data for discrepant results suggest Xpert correct
Simple, manual NAAT

Loop-mediated Isothermal Amplification (LAMP)

- Closed system
- Isothermal
- Rapid
- Multiprimer
- Visible readout
LAMP TB steps

1. Collect 40ul using the device
2. Heat at 90°C for 5 min
3. Shake
4. Absorbent tube
5. Dribble out within two lines.
6. Dried reagent
7. Detect fluorescence signal
8. LAMP reaction at 67°C for 40 min
9. NC, 1, 5 genomes/μl
### Evaluation of PURE device performance

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Sample</th>
<th>Device (Tt)</th>
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<tbody>
<tr>
<td>1</td>
<td>scanty S1 (4/100)</td>
<td>14.0</td>
</tr>
<tr>
<td></td>
<td>S2 (2/100)</td>
<td>12.1</td>
</tr>
<tr>
<td></td>
<td>purulent P1 (1+)</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>bloody B2 (3+)</td>
<td>15.3</td>
</tr>
<tr>
<td>2</td>
<td>scanty S3 (5/100)</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>S4 (7/100)</td>
<td>13.1</td>
</tr>
<tr>
<td></td>
<td>S5 (6/100)</td>
<td>12.0</td>
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<td></td>
<td>S6 (4/100)</td>
<td>19.3</td>
</tr>
<tr>
<td></td>
<td>S7 (7/100)</td>
<td>13.2</td>
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<tr>
<td></td>
<td>S8 (6/100)</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>P2 (1+)</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>P3 (2+)</td>
<td>13.4</td>
</tr>
<tr>
<td></td>
<td>P4 (1+)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>P5 (1+)</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>P6 (1+)</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>P7</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>bloody B1 (1+)</td>
<td>10.5</td>
</tr>
<tr>
<td></td>
<td>B3 (6/100)</td>
<td>12.1</td>
</tr>
<tr>
<td>3</td>
<td>scanty S9 (5/100)</td>
<td>18.4</td>
</tr>
<tr>
<td></td>
<td>S10 (8/100)</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>P8 (1+)</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>purulent P9 (2+)</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>P10 (1+)</td>
<td>11.4</td>
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<tr>
<td></td>
<td>bloody B4 (6/100)</td>
<td>14.4</td>
</tr>
<tr>
<td></td>
<td>B5 (5/100)</td>
<td>12.5</td>
</tr>
</tbody>
</table>
Decentralization of molecular diagnostics

1st generation MDR

2nd generation automated MDR

1st generation manual detection

2nd generation manual detection

LPA

Xpert

LAMP

POC test

Less complexity, more robustness
Patient-centered approach / technological platforms

- Surveillance
- Reference methods
- Network supervision

- Resolution testing (screening-test negative, drug resistance)

- Passive case finding
- Detect and treat

- Screening
- Primary care

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**TB**
- Liquid Culture/LPA
- 1st/2nd Generation Molecular/Viral Load

**Malaria**
- Lot Testing

**Microscopy**
- TB Solid Culture
- iLED
  - Sensitivity
  - Speed
- Manual Molecular
  - Infant HIV Malaria HAT TB

**RDTs**
- Second Generation Molecular/Viral Load
- Reference Labs
- Regional Labs
- District Level

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**MALARIA**
- Positive Second Control Wells
- Rx

**TB Symptoms**
- Referral

**HAT**
- Screening-Met
- RDT
- Referral

**HIV**
- Symptoms RDT Referral
Integrating HIV-TB diagnostics platforms: Lesotho

MDR-TB LPA → EID for HIV by PCR

Demonstration (Evidence) → WHO (Policy) → Implementation (Practice)

March 2007 LC April 2008 LPA
June 2007 LC June 2009 LPA
November 2007 LC November 2008 LPA April 2009 EID for HIV by PCR

Partners
- WHO
- PIH
- MOH (Lesotho)

Molecular laboratory in Maseru
Thank you