TB Diagnostics: Progress, needs, pipeline

Catharina Boehme
EDCTP Stakeholder Meeting: Tuberculosis and mycobacterial infections
28 – 29 October 2013
Paris
## Advancements in TB diagnostics as per WHO recommendations, 2006-2013

<table>
<thead>
<tr>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smear-positive TB case definition</td>
<td>Liquid culture</td>
<td>LPA</td>
<td>LED-FM</td>
<td>Automated NAAT (Xpert MTB/RIF)</td>
<td>Xpert in extrapulmonary TB</td>
</tr>
<tr>
<td>Number of smears</td>
<td>Rapid speciation</td>
<td>Front-loading microscopy</td>
<td>Against Serology</td>
<td>Against IGRA</td>
<td></td>
</tr>
<tr>
<td>FM</td>
<td></td>
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</tbody>
</table>

### Key Advancements:
- **2006**: Smear-positive TB case definition
- **2007**: Liquid culture
- **2008**: LPA
- **2009**: LED-FM
- **2010**: Automated NAAT (Xpert MTB/RIF)
- **2013**: Xpert in extrapulmonary TB
Uptake of new TB diagnostics

A. iLED

- WHO Endorsement
- Year to Q3 2013
- # Modules

B. Xpert

- WHO Endorsement
- Year to Q3 2013
- Cartridges (in thousands)

C. MGIT

- WHO Endorsement
- Fiscal Year to Q3 2013
- # Strips (in thousands)

D. MTBDRplus

- WHO Endorsement
- Year to Q3 2013
- # Strips (in thousands)
Translate opportunities into impact

New WHO endorsed TB tools offer real opportunity to
• Enhance case detection & Rapidly identify drug resistance & Reduce time to treatment & default

Let’s learn how to use these and future tools optimally
• New insights into broader problems within the health system
• Use this evidence and do more OR to improve infrastructure & linkage to care
• Innovative partnerships help to maximize impact (across diseases; across sectors)
• Preparing the ground for speedy uptake of optimized tools

Supply, customer support, QA, data management, lab integration
Linkage to public and private treatment providers: acceptance, data access, logistics, incentives
Linkage to patients: alerts, incentives, health insurance schemes
Potential impact of new tools greater than actual impact (– where known...)

- Patient drop out rates and remaining tx delays
- Patient access limited (tech limitations, coverage, linkage to care)

CAVEAT: Impact measurement challenging: Not only depending on dx intervention; overtreatment at baseline

Nicol et al., unpublished, Results from RCT Khayelitsha/Paarl
Major unmet needs remain

Screening at first point of contact

1. Triaging test
   - Incl. for childhood TB & EPTB
   - Passive / active screening
2. TB infection with high risk of disease progression

Work up & choice of treatment at dedicated unit

1. TB confirmation with rapid DST to critical drugs
   - Incl. for childhood TB & EPTB
2. Treatment monitoring
3. Multiplex test to manage TB-neg

Support, surveillance, QA at specialized unit

1. Comprehensive, rapid DST

M-Health supported network
Multiplex antibody array (mBio)

**Antibody detection**
- Early development
  - High complexity assays
    - Molecular DST
      - TruArray MDR-TB (Akkoni)
      - INFINITIMTB (AutoGenomics)
      - B-SMART (Sequella)
    - Culture-based technologies
      - BNP Middlebrook (NanoLogix)
      - Rapid colorimetric DST
    - Molecular detection and DST
      - Xpert MTB/RIF Enhanced Sensitivity / XDR (Cepheid)
      - Alere Q (Alere)
      - Enigma ML (Enigmadiagnostics)
      - Q-POC (QuantuMDx)
      - DiagCORE (Stat-Diagnostica)
      - EOSCAPE (Wave80)
      - RT-PCR Testing Platform (NWGHF)
      - iCubate 2.0 (iCubate)
    - Volatile organic compounds
      - BreathLink (Menssana)
      - Prototype breathalyzer (Next Dimensions)
      - TB Breathalyser (Rapid Biosensor Systems / Ortho Clinical)
    - Microscopy
      - TBdx (Signature Mapping)
      - Fluorescent microscopy with molecular probes (ID-FISH Technology)
    - Antigen detection
      - LAM in sputum (Standard Diagnostics)
    - Enzymatic detection and DST
      - β-lactamase reporter (Global BioDiagnostics)
    - Volatile organic compounds
      - Breath analysis instrument (Metabolomx)
  - Moderate complexity assays
    - Culture-based technologies
      - TREK Sensitive MYCOTB (Trek)
    - Molecular detection and DST
      - Xpert MTB/RIF Enhanced Sensitivity / XDR (Cepheid)
      - Alere Q (Alere)
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      - DiagCORE (Stat-Diagnostica)
      - EOSCAPE (Wave80)
      - RT-PCR Testing Platform (NWGHF)
      - iCubate 2.0 (iCubate)
    - Volatile organic compounds
      - Giant African Pouch Rats (Apopo)
    - Microscopy
      - Microimager (BD)
    - Imaging
      - CAD4TB (Delft Imaging Systems)
    - Antigen detection
      - Alere Determine TB-LAM in urine (Alere)
  - Low complexity assays
    - Culture-based technologies
      - LPA second-line (Hain)
      - LPA first line followers (Nipro, YD)
    - Molecular DST
      - LPA second-line (Hain)
      - LPA first line followers (Nipro, YD)
      - TB LAMP (Eiken)
    - Molecular detection
      - TruArray MDR-TB (Akkoni)
      - INFINITIMTB (AutoGenomics)
      - B-SMART (Sequella)
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    - Antigen detection
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Key approaches to fill TB diagnostics gaps

1. Molecular detection

State of science

- >10 NAAT platforms in development
- Development of fast follower platforms slower than expected.
- Promising amplification & detection technologies, but no proof-of-principle for point-of-care.

Strategic approach

New generation NAAT platforms
- Simplifying & minimizing macrofluidics

Lab-on-a-chip
- Isothermal amplification & solving problem of sample volume
Extending utility of molecular tools

Improving detection of Extrapulmonary & Pediatric TB

David Alland, UMDNJ, US

Treatment monitoring

Expanding drug menu

<table>
<thead>
<tr>
<th>Target</th>
<th>Targeted strand</th>
<th>Target primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>gyrA QRDR</td>
<td>Sense</td>
<td>gyrA-F</td>
</tr>
<tr>
<td>gyrB 500-543</td>
<td>Sense</td>
<td>gyrB-F1, gyrB-F2</td>
</tr>
<tr>
<td>katG 315-316</td>
<td>Sense</td>
<td>katG-F</td>
</tr>
<tr>
<td>inhA -8 to -16</td>
<td>Antisense</td>
<td>inhA-R</td>
</tr>
<tr>
<td>embB 306</td>
<td>Sense</td>
<td>embB-F</td>
</tr>
<tr>
<td>Rrs 1400-1484</td>
<td>Antisense</td>
<td>rrs-R</td>
</tr>
<tr>
<td>B. globii (control)</td>
<td>Sense</td>
<td>Bg-R</td>
</tr>
</tbody>
</table>
### Key approaches to fill TB diagnostics gaps

#### 2. Antigen detection

<table>
<thead>
<tr>
<th>Author/Year</th>
<th>Sample</th>
<th>Sensitivity ss+ TB</th>
<th>Sensitivity ss-TB</th>
<th>Specificity Non-TB</th>
<th>Specificity healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cho, 1990</td>
<td>Sputum</td>
<td>62% (75/119)</td>
<td>28% (22/80)</td>
<td>93% (240/258)</td>
<td>99% (222/224)</td>
</tr>
<tr>
<td>Sada, 1992</td>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lee, 1997</td>
<td>Sputum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pereira, 2000</td>
<td>Sputum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hamasur, 2001</td>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tessema 2001</td>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Boehme 2005</td>
<td>Urine</td>
<td></td>
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Chemogen prototype ELISA evaluation in Tanzania, 2006.

- LAM in urine only validated marker

### State of science

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*LAM early data - Literature overview*

### Strategic approach

**Novel targets**

Apply new tools for AG discovery

**Better detection technology**

Such as fluorescence labeled LFI
Urinary LAM (Alere Determine) as a screening test for TB in HIV positives with low CD4 cell counts?

**Lawn, SD et al 2012**

Sensitivity of TB LAM in HIV-TB co-infected patients

<table>
<thead>
<tr>
<th>CD4 Cell Count</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 200</td>
<td>4.0%</td>
</tr>
<tr>
<td>&lt; 200</td>
<td>39.0%</td>
</tr>
<tr>
<td>&lt; 150</td>
<td>45.7%</td>
</tr>
<tr>
<td>&lt; 100</td>
<td>51.7%</td>
</tr>
<tr>
<td>&lt; 50</td>
<td>66.7%</td>
</tr>
</tbody>
</table>

**Peter, JG et al 2012**

<table>
<thead>
<tr>
<th>Against composit RS</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>45</td>
<td>96</td>
</tr>
<tr>
<td>CD4 &gt; 200</td>
<td>29</td>
<td>100</td>
</tr>
<tr>
<td>CD4 ≤ 200</td>
<td>52</td>
<td>94</td>
</tr>
</tbody>
</table>

**Dorman, S et al 2012**

Performance of TB LAM in HIV-TB co-infected patients

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<thead>
<tr>
<th>CD4 Cell Count</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>36.8%</td>
<td>96</td>
</tr>
<tr>
<td>≤ 100</td>
<td>63.9%</td>
<td></td>
</tr>
<tr>
<td>&gt; 100</td>
<td>19.6%</td>
<td></td>
</tr>
</tbody>
</table>

**Shah, M et al 2012**

Performance of TB LAM, Xpert alone, and Xpert + TB LAM in HIV-TB co-infected patients

<table>
<thead>
<tr>
<th>Xpert + TB LAM</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TB LAM* alone</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Xpert alone</td>
<td>77%</td>
<td></td>
</tr>
<tr>
<td>Xpert + TB LAM*</td>
<td>87%</td>
<td></td>
</tr>
</tbody>
</table>
LAM in sputum: Prototype accuracy similar to smear microscopy

Sample volume: 0.5 ml

- 2+/3+ smear +
  - Sensitivity = 22/26 = 84.6%
- 1+ smear +
  - Sensitivity = 10/20 = 50%
- Scanty smear culture +
  - Sensitivity = 0/16 = 0%
- Smear -, culture +
  - Sensitivity = 1/32 = 3%
- Smear- /culture-
  - Specificity = 44/49 = 89.8%

4/5 smear+ HIV+ and 6/6 smear-cult- HIV+ classified correctly

Cutoff = 0.25 OD
Automating smear microscopy
Rapid point-of-care detection of the tuberculosis pathogen using a BlaC-specific fluorogenic probe

Hexin Xie¹, Joseph Mire², Ying Kong³, MiHee Chang³, Hany A. Hassounah³, Chris N. Thornton⁴, James C. Sacchettini², Jeffrey D. Cirillo³ and Jianghong Rao¹*

Reported Enzyme Fluorescence

BlaC Enzyme

M. tb

CDG-OMe
Key approaches to fill TB diagnostics gaps

3. Volatile Organic Compound detection

Olfactory sensing
- e.g. Apopo
- Enoses
  VOC interact with polymer to produce change in electrical resistance.
Analytical noses
- Miniature mass spec/chromatographic devices.

- MTB-specific VOC
  Use of high end MS devices

- Field-applicable detection technology
  Miniaturization of sensitive MS

State of science

<table>
<thead>
<tr>
<th>VOC in</th>
<th>Author/Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breath</td>
<td>Phillips, 2007</td>
</tr>
<tr>
<td>HS of culture</td>
<td>Trevejo, 2007</td>
</tr>
<tr>
<td>HS of culture</td>
<td>Syhre, 2008</td>
</tr>
</tbody>
</table>

- Proof-of-principle data with inadequate performance in feasibility studies
Key approaches to fill TB diagnostics gaps

4. Antibody detection

State of science

- >30 tests based on narrow set of AB with inadequate performance
- WHO recommendation against use

Report TDR, Sep 2008

Commercial serological antibody detection: Highly inconsistent sensitivity & specificity

- >30 tests based on narrow set of AB with inadequate performance
- WHO recommendation against use

Strategic approach

Identify diagnostic AB pattern

Microarray-based screening using high-throughput expression systems
Biomarker efforts critical to fill POC gaps

- Systematic approaches
- Large sample repositories
- More resources

Confidence in Biomarker

- Antibodies (b)
  - Campos
  - Dobos
  - Moritz
  - Feldheim
  - Ochsner
  - Kaufmann
- Proteins (b, u)
  - Anderson
  - Lowary
  - Add even
- Enzymes (s, b, u)
  - Alland
  - Whole Bug (s)
- Mycolic Acids (s)
- Nucleic Acids (u, b)
- Sugars (s)
- Metabolites (s, b, u)
- VOC-patterns (breath)
- Non-Ab Host (b)
  - Ochsner
  - Freidland
- Proteins (s)
  - Feldheim
  - Belisle
- Nucleic Acids (u, b)
  - CD, LMU
  - fast followers
- Mycolic Acids (u)
  - Belisle
- MicroRNA (b, u)
  - Graham
  - VOC-specific (breath, u)
Reexamining the role of radiography in tuberculosis case finding
Diagnostics trial needs are substantial

>90,000 study participants recruited in TB diagnostic trials in 2012

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Demonstration</th>
<th>Post-WHO validation</th>
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<tbody>
<tr>
<td>~9,500</td>
<td>~1,500</td>
<td>~80,000</td>
</tr>
</tbody>
</table>
Clinical pathway to WHO for TB

- WHO endorsement is key to public sector uptake
- Solid evidence base required for WHO expert review

**Evaluation studies**
- 2000 subjects, 3-6 sites
- 6 mo – 1 y

**Demonstration studies**
- 4000-6000 subjects, 5-20 sites
- 1 – 1.5 y

**In-country validation**

**New technologies**

**Follower technologies**

**Product registration**

**World Health Organization**

**Continued adoption into national policy**

**Clinical performance**

**Patient important outcomes**
- (Cost-) Effectiveness
- Requirements for implementation

**Algorithm of use**

**Non-inferiority studies**
- 800-1000 subjects, 2-3 sites
- 6 months

**In-county validation**
Access to a flexible clinical feasibility platform key for technology selection and development

**Down-select fast and terminate early**

1 out of 5 into clinical feasibility
1 out of 5 through feasibility

**Effective development**

- Specimens and field studies
- Specimens and early field studies

[Graph showing investment and development stages]
Strengthening the enabling infrastructure…

... to accelerate access to the diagnostics tools we need

Effective development and informed policy decisions:

- Access to specimen
- Trial platform (certified trial sites; reference lab networks)
- Expert guidance / training around WHO-defined needs and standards

Maximize impact of new tools

- Track impact of diagnostic-guided interventions
- Pragmatic operational research & in country capacity building
- Post-market quality assurance (sentinel sites; product and lot testing initiatives)
Global trial site and laboratory network