TB diagnostics - needs, achievements and future ambitions

European Society of Mycobacteriology
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Claudia Denkinger, MD, PhD, MSc, DTMH
Head of Tuberculosis and Hepatitis Program, FIND, Geneva
Beth Israel Deaconess Medical Center, Harvard, Boston
Thank you
What is needed?
WHO END TB Goals and Milestones

**TB Deaths**

- **Phase 1:** + Universal health coverage
  - -75%

- **Phase 2:** + Prevent reactivation
  - -95%

**TB Incidence**

- **EARLY diagnosis** + DST for ALL TB cases
  - -50%

- Diagnosis of AT RISK pts among pool of infected
  - -90%

Courtesy of Global TB Program
Target Product Profiles

Iterative process with input from many stakeholders

WHO Consensus Meeting
- Delphi process leading up to the meeting
- > 75% agreement amongst stakeholders

Prioritized TPPs:
1. Point-of-care sputum based test for microscopy replacement
2. Point-of-care DST-microscopy center
3. Point-of-care, non-sputum based test
4. Point-of-care triage test
Sputum-based microscopy replacement test
Greatest progress - NAAT for smear replacement and DST

New NAAT platforms

- USTDR
- Alere
- molbio
epistem
- QuantaMDx
- Roche
- Cepheid
- ABBOTT
- BD
- Insilixa HYDRA
- GenePOC

Needs to be addressed

- Decentralization
- Improving time to diagnosis
- Improving MTB detection
- Higher throughput, multiplexing
- Extended, timely DST
- Detection of EPTB
Moving TB diagnosis closer to the patient

<table>
<thead>
<tr>
<th>Cartridge</th>
<th>Description</th>
<th>Sample</th>
<th>Status</th>
</tr>
</thead>
</table>
| MTB/RIF   | • Tuberculosis detection  
            • Rifampicin resistance detection | • Sputum | On the market |
| Ultra     | • Increased sensitivity for TB detection/ culture-replacement  
            • Rifampicin resistance detection  
            • New sample types (children) | • Sputum  
            • Stool  
            • Urine | Available Dec 2016 |
| XDR       | • Drug resistance detection (isoniazid, fluoroquinolones, aminoglycylsides) | • Sputum | Development  |
Diversification of market

**Analytical and Clinical Evaluation of the Epistem Genedrive Assay for Detection of Mycobacterium tuberculosis.**

Shenai S1, Armstrong DT2, Valli E3, Dolinger DL3, Nakayangi L4, Dietze R5, Dalcolmo MP6, Nicol MP7, Zemanay W7, Manabe Y8, Hadad DJ9, Marques-Rodrigues P5, Palaci M5, Peres RL5, Gaedert M9, Armakovitch S9, Nonyane BA10, Denkinger CM11, Banada P1, Joloba ML11, Eliyav J10, Boehme C3, Alland D1, Dorman SE12.

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**ABBOTT TO ACQUIRE ALERE, BECOMING LEADER IN POINT OF CARE TESTING AND SIGNIFICANTLY ADVANCING GLOBAL DIAGNOSTICS PRESENCE**

Abbott CEO Creates Doubt Surrounding $5.8B Alere Deal

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**Evaluation of the Indian TrueNAT micro RT-PCR device with GeneXpert for case detection of pulmonary tuberculosis.**

Nikam C1, Kazi M1, Nair C2, Jaggannath M2, M M2, RV2, Shetty A1, Rodrigues C3.
Is there a limit to the sensitivity we can achieve with molecular tests?

Figure 5. Forest plots of Xpert MTB/RIF sensitivity and specificity for TB detection, Xpert MTB/RIF used as an initial test replacing smear microscopy. The individual studies are ordered by decreasing sensitivity. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Between brackets are the 95% CI of sensitivity and specificity. The figure shows the estimated sensitivity and specificity of the study (blue square) and its 95% CI (black horizontal line). Xpert MTB/RIF specificity could not be estimated in one study.

Xpert (G4) specificity in patients with past history of TB (from Theron et al).

Steingart Cochrane 2014; Theron CID 2016
Expansion of the utility beyond sputum

Xpert for MTB detection on stool

<table>
<thead>
<tr>
<th>Xpert vs. Culture</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiratory</td>
<td>71.4% (29.0%, 96.3%)</td>
<td>98.1% (94.5%, 99.6%)</td>
</tr>
<tr>
<td>Swab rectal</td>
<td>42.9% (9.9%, 81.6%)</td>
<td>100.0% (97.7%, 100.0%)</td>
</tr>
<tr>
<td>0.6 g stool</td>
<td>71.4% (29.0%, 96.3%)</td>
<td>98.7% (95.5%, 99.8%)</td>
</tr>
</tbody>
</table>

FIND unpublished; Banada PLOSone 2016; Denkinger ERJ 2014
Drug susceptibility testing
What about the DST?

**TPP: Current drug prioritization:** RIF > FQ (incl. Mox) > INH = PZA

- **ReMox Trial was not successful → Are Fluoroquinolones still relevant for treatment of TB?**
  - Yes, in MDR regimen, STREAM regimen, PaMZ

- **PaMZ trial was on hold but will be restarted → Is PZA relevant independent of PaMZ?**
  - Yes, in HRZE, STREAM, NC-005, PaMZ

- **Are there drugs that have become more important?**
  - Bedaquiline: NC-005, Nix-TB
  - Linezolid: Nix-TB, XDR regimens

**Revised drug prioritization?:** RIF > FQ (incl. Mox) > PZA = BDQ > LZD
How well do we understand the genotypic basis of resistance?

- **RIF**: molecular methods for SNP detection in rpoB might be the best single reference standard.

- **INH**: molecular methods for SNP detection in katG/inhA ~82% sensitive and very specific; added sensitivity of kasA, ndh, Rv1584c, oxyR, ahpC and others, but not close to 100%.

- **FQ**: molecular methods for SNP detection in the QRDR for gyrA:
  - Are ~75-90% sensitive and very specific for LVX/OFX;
  - Problem: MFX limited understanding of best clinical cut points for phenotypic DST → limited cross-resistance with high cut-points.

- **PZA**: molecular methods for SNP detection within pncA might be the best single reference standard.

- **What about new drugs**: Pretomanid, Bedaquiline, Linezolid? – incomplete understanding of the basis of resistance.

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But can we prioritize based on the drug pipeline?
Recommendations apply to testing of patients with confirmed rifampicin-resistant TB or MDR-TB

- SL-LPA may be used as the initial test, instead of phenotypic culture-based DST, to detect resistance to fluoroquinolones and second-line injectable drugs
- Testing from sputum specimens (direct testing) and cultured isolates
- Does not eliminate the need for conventional phenotypic DST both for confirmation and also testing of SL-LPA negative high-risk patients

High specificity. Variable sensitivity
Caveat: interpretation of resistance for Moxifloxacin
### What is the best next DST?

#### Integrated Platform
- Define number of target resistance mutations
- Sample to result
- Changes to targets require revalidation of entire assay

#### Sequencing
- Modular with extraction, PCR, sequencer
- Possible directly from sputum
- Targeted or whole genome
- Flexible targets

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**Regimen selection at level I/II**

**Individualized care at level III**
Towards a better understanding of the genotypic basis of resistance

Contributed Data

- WGS SNP reports
- New SNP reports from Unified Pipeline
- Original WGS SNP reports
- Surveillance data
- DR study data
- Clinical trial data
- Phenotypic data
- Genotypic data

RDST Consortium

CURATED AND AGGREGATED DATA
- Genotypic Data
- Phenotypic Data
- Clinical Data
- Drug Resistance Data
- SNP Reports

ANALYTICS TOOLS
- “R” Statistical Analysis
- Misc. Integrated Analysis

RECOGNITION
- Individual Recognition
- Institutional Recognition
- Global Impact

Unified Pipeline

Expert Panel Review

Stop TB Partnership
New Diagnostics Working Group
CRITICAL PATH INSTITUTE
FIND
BILL & MELINDA GATES FOUNDATION
WHO
Next Generation Sequencing

- Directly from sputum
- Simplified library prep
- Targeted NGS or WGS
- Initial focus on reference laboratories and surveillance
- Easy interpretation for inform clinical decision-making
Centralized testing – necessary links

- Optimized sample transport
  - Improves upon the current routine methods in terms of recovery of mycobacteria on liquid and solid culture
  - Reduction of contamination
  - Simplification of laboratory workflows (cold chain requirements), biohazard

- Optimized communication solutions

  A patient gets notified via SMS that a test result is ready
  DOtS programme gets notified and ensures patients is started on therapy
  A clinician gets notified that an HIV patient tested + for TB in TB clinic
  A national programme manager identifies stock-outs early and organizes the shipment from another site
  A high error rate of diagnostic system is detected. A new system is sent.
Connectivity of diagnostics: Example India

- **SMS Notification**
- **Voice massage**
- **Real-time spread-sheet**
- **Customizable dashboards**
- **Nikshay integration (proof-of-concept)**

**Consumer API**

**Diagnostis API**

- **GeneXpert**
- **Rapid Speciation test reader**
- **LPA test reader**
- **BACTEC MGIT Liquid Culture**

**«Cloud» Aggregator**

**Connected diagnostics platform - CDx**

FIND unpublished
Triage/biomarker test
> 80% of Global TB testing is done at the microscopy center level

- **Microscopy Centers**: 60 million tests (smear, rapid serology)
- **District Labs**: 5 million tests (Xpert, ELISA)
- **Reference Labs**: 6 million tests (LPA/PCR)

**Global TB testing need**
“Sensitivity and point-of-care amenability are equally important considerations when developing novel diagnostic tests for TB.”
WHO triage/biomarker test targets

TPP 1: Rapid biomarker-based non-sputum test for detecting TB

TPP2: Triage test (Rule-out)

WHO reviewed 19 commercial rapid diagnostic tests for TB detection in 2008

<table>
<thead>
<tr>
<th>TPPs</th>
<th>TB Detection</th>
<th>TB Triage (Rule out)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Opt</td>
<td>Min</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>&gt;80%</td>
<td>&gt;65%</td>
</tr>
<tr>
<td>Specificity</td>
<td>≥98%</td>
<td>≥98%</td>
</tr>
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</table>
Current LAM assay – a niche test

Absolute reduction in mortality – 4%

Adjusted HR 0.82 (95% CI 0.70–0.96); p=0.015

<table>
<thead>
<tr>
<th>Number at risk</th>
<th>Following from enrolment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No LAM</td>
<td>1271 1069 997 953 923 896 690 263</td>
</tr>
<tr>
<td>LAM</td>
<td>1257 1093 1028 994 970 941 696 246</td>
</tr>
</tbody>
</table>
Mortality: Absence of evidence ≠ evidence of absence

LAM RCT
0.82 (95%CI 0.70, 0.96)
[1 RCT, 8,728 participants, 578 deaths]

IPD-MA of Xpert RCTs
0.82 (95%CI 0.64, 1.05)
[4 RCTs, 8,567 participants, 360 deaths]
Promising biomarkers for POC assays: Host proteins

**SomaLogic - FIND**

- Combination of SomaLogic Host Markers and FIND Antibody Detection
- Number of required markers reduced from 9 to 6
- 6-marker model showed promising performance to distinguish TB from non-TB close to minimal targets as defined in TPPs in well-characterized samples (N=480) from FIND
- Potential to measure biomarkers on a relatively simple, and patient near platform

<table>
<thead>
<tr>
<th>Sensitivity: 93 %</th>
</tr>
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<tbody>
<tr>
<td>Specificity: 75%</td>
</tr>
<tr>
<td>AUC: 0.91</td>
</tr>
</tbody>
</table>

Preliminary Data

- **Model:**
  - 5 Host markers
  - 1 IgG marker

Hybridization slides

- Agilent anti-sense probe array
- Fluorescent read-out (cyanine on 5' end)

FIND unpublished
**Promising biomarkers: Host transcription signatures**

**Transcriptional Blood Signatures Distinguish Pulmonary Tuberculosis, Pulmonary Sarcoidosis, Pneumonias and Lung Cancers**  
C. Bloom et al.

<table>
<thead>
<tr>
<th>Bloom et al. 2013</th>
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<tbody>
<tr>
<td>• Unbiased system biology revealed type I IFN/inflammatory transcriptional signature in blood during pulmonary TB (Berry et al. 2010)</td>
</tr>
<tr>
<td>• Class prediction of 144 transcripts (validation set, TB vs. Non-TB)</td>
</tr>
<tr>
<td>• The signature was validated in different populations (Lu et al. 2011; Maertzdorf et al. 2011, 2012, Bloom et al. 2013)</td>
</tr>
<tr>
<td>• The signature also increases with disease activity and diminishes with treatment (Bloom 2012).</td>
</tr>
</tbody>
</table>

Sensitivity: 88%  
Specificity: 92%
Promising biomarkers: Host RNA signatures

- Using host RNA
  - Signature

- 51-transcript signature

- Assessed across
  - Cohorts of children:
    - South Africa (655)
    - Malawi (701)
    - Kenya (1599)

Sensitivity = 83% (Cx-confirmed TB)
Specificity = 84% (Cx-confirmed TB)
Detection on breath/VOCs

Overview of the processes involved in breath testing.

- Metal-oxide-based olfactory sensor [Bruins et al. 2013]
- Portable GC coupled to surface acoustic wave (SAW) detector [Phillips et al. 2013]
- New sensors based on array of chemical films: VOC leads to changes in electrical conduction
- Cough/aerosol collection combined with immunoassay based antigen detection [McNerney et al. 2010]
- 

Proof-of-principle data from feasibility studies available for some technologies (performance thus far not meeting TPP, limited independent study data)
Detecting TB before it can be transmitted

- **Primary or secondary M. tuberculosis infection**
  - TST+/IGRA+
  - Immunological equilibrium (Latency)
  - X-ray
  - Mtb culture
  - Multi-gene RNA expression (IFN, IL-13, etc.)
  - Activation markers
  - Specific CD8
  - M/L ratio

- **Subclinical TB disease/ Incipient TB disease**
  - TST+/IGRA+
  - Progression?
  - X-ray
  - Mtb culture
  - Multi-gene RNA expression (IFN, IL-13, etc.)
  - Activation markers
  - Specific CD8
  - M/L ratio

- **Active Clinical TB disease**
  - TST+/IGRA+
  - Progression
  - X-ray
  - Mtb culture
  - Multi-gene RNA expression (IFN, IL-13, etc.)
  - Activation markers
  - Specific CD8
  - M/L ratio

- **Transmission**
  - X-ray
  - Mtb culture
  - Multi-gene RNA expression (IFN, IL-13, etc.)
  - Activation markers
  - Specific CD8
  - M/L ratio

- **Petruccioli, Goletti unpublished**

- **TST/IGRA-**
  - No M. tuberculosis infection

- **TST+/IGRA-**
  - TST-/+IGRA-
Latent to active progression – How good is good enough

<table>
<thead>
<tr>
<th>Description of the test</th>
<th>Biomarker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial or traditional tests for LTBI diagnosis</td>
<td>RD1-specific immune response in IGRA, immune sensitization to PPD in TST</td>
</tr>
<tr>
<td>Molecular tests</td>
<td>mRNA expression signature of 16 IFN response genes, IL-13 and AIRE mRNA expression signature, Elevated expression signatures of IFN response and T cell genes, Elevated expression signatures of inflammation, myeloid and glucose metabolism genes</td>
</tr>
<tr>
<td>Antigen-specific T cells</td>
<td>Increased HLA-DR-expressing CD4 T cells, Increased IFNg expressing Ag85A-specific T cells, Increased Th1-cytokine expressing BCG-specific CD4 T cells</td>
</tr>
<tr>
<td>Cell activation markers</td>
<td>Increased levels of anti-Ag85A binding IgG</td>
</tr>
<tr>
<td>Cell differentiation markers</td>
<td>Down-modulation of CD27 in CD4 T cells</td>
</tr>
<tr>
<td>Antigen-specific Antibodies</td>
<td>Elevated IFNg response to in vitro RD1 stimulation of PBMC</td>
</tr>
<tr>
<td>Responses to latency antigens</td>
<td>IFNg response to in vitro stimulation of PBMC with HBHA, IFNg response to in vitro stimulation of whole blood with Rv2628</td>
</tr>
<tr>
<td>Blood cell counts</td>
<td>Elevated monocyte/lymphocyte ratio</td>
</tr>
<tr>
<td>CD8 T-cell response</td>
<td>IFNg response to in vitro RD1 stimulation of PBMC</td>
</tr>
</tbody>
</table>

A blood RNA signature for tuberculosis disease risk: a prospective cohort study


66% sens; 81% spec

Optimum TPP - NNT: ~13
COR signature - NNT: ~37
Minimum TPP - NNT: ~40
TST / IGRA

Petruccioli/Goletti/FIND unpublished; Zak 2016 Lancet
Accelerating path to success - TB Biomarker Database

**CHALLENGE**

Biomarker Discovery | Biomarker Verification | Clinical Validation | Regulatory Approval | Evidence Assessment & WHO Recommendation

**APPRAOCH**

A *biomarker database* with an evidence-based *TB biomarker scoring system*:

- Up-to-date biomarker information
- Prioritize of the most promising candidates in the pipeline
- Explore combinations of biomarkers with a higher potential to meet TPPs

**Issues:**

- Rapidly increasing number of publications
- Mostly exploratory studies with limited sample size
- Limited knowledge sharing among researchers
- Lack of quality standards
- Undefined use cases

*StopTB Partnership*
New Diagnostics Working Group
It is not the test alone!
Process innovation necessary as much as product innovation
Impact of tests considering the diagnostic cascade

The impact of novel tests for tuberculosis depends on the diagnostic cascade

Amanda Y. Sun¹, Claudia M. Denkinger²,³ and David W. Dowdy⁴
¹Johns Hopkins University School of Medicine, Baltimore, MD, USA. ²Foundation for Innovative New Diagnostics, Geneva, Switzerland. ³Beth Israel Deaconess Medical Center, Boston, MA, USA. ⁴Dept of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA.

FIGURE 1. The diagnostic cascade in tuberculosis. Shown are the expected reductions in tuberculosis incidence and mortality 10 years after full replacement of sputum smear microscopy with Xpert MTB/RIF. Each sequential step to the right of the graph incorporates an additional element of tuberculosis diagnosis that may reduce the number of individuals benefiting from a more sensitive diagnostic test.
Vision for TB diagnostics in 2020 - new tools/approaches across the healthcare system

**Triage/case finding**
- First point of contact

1. **Triage test**
   - incl. for childhood TB & EPTB
2. **Active case finding**
   - Highly sensitive, portable
3. ** Syndromic test (Bact vs viral)**

**Further work up & treatment**
- Dedicated units

1. **TB confirmation with rapid DST**
   - for critical drugs
   - incl. for childhood TB & EPTB
   - Using platform synergies (e.g. HIV)
2. **Treatment monitoring**
3. **Disease progression**

**Coordination, Surveillance, QA, M&E**

1. **Real-time monitoring of network and integrated care**
2. **Comprehensive, rapid DST**

**E-Health supported solutions**
Thank you – Questions?

Tim Rodwell
Tobias Broger
David Dolinger
Samuel Schumacher
Catharina Boehme
Seda Yerlikaya
Chris Isaacs