Evolution of New TB Diagnostics for Detection and Resistance

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The target TB case detection rate of 70% has not been reached.

WHO, Global Tuberculosis Control 2009
Commonly used TB diagnostic modalities
Overview

• New technologies
  – Xpert MTB/RIF
  – Urine lipoarabinomannan tests
  – Other TB diagnostics on the horizon

• Beyond “accuracy”
Xpert MTB/RIF

- For detection of *M. tuberculosis* and common mutations that confer resistance to rifampin (from respiratory specimens)
- Molecular test: hemi-nested real-time PCR of MTB-specific region of *rpoB* gene, which is then probed with molecular beacons for mutations
- Fully automated; uses GeneXpert platform (Cepheid, Sunnyvale, CA)
- Integrated sample processing and PCR; disposable plastic cartridge contains all reagents
- 2 manual steps: addition of bactericidal buffer to sputum then transfer of defined volume to cartridge
Assay Procedure for the MTB/RIF Test

1. Sputum liquefaction and inactivation with 2:1 sample reagent

2. Transfer of 2 ml material into test cartridge

3. Cartridge inserted into MTB-RIF test platform (end of hands-on work)

4. Sample automatically filtered and washed

5. Ultrasonic lysis of filter-captured organisms to release DNA

6. DNA molecules mixed with dry PCR reagents

7. Seminested real-time amplification and detection in integrated reaction tube

8. Printable test result

Time to result, 1 hour 45 minutes

MTB DETECTED LOW; RIF Resistance NOT DETECTED
Xpert MTB/RIF

• High **analytical specificity** for *M. tuberculosis* conferred through careful selection of amplification target

• **Analytical sensitivity**: LOD 131 cfu/ml (D Helb et al JCM 2010;48:229)
  – Smear microscopy LOD ≈ 10,000 cfu/ml
Evaluation Study of Xpert MTB/RIF
C. Boehme et al. NEJM 2010;363:1005

• Cross-sectional study of diagnostic test accuracy in intended target population using best available reference standard (FIND)

• Population and Procedures
  – Peru, Azerbaijan, South Africa x 2, India
  – Adults with pulmonary TB symptoms
  – 3 sputa obtained (2 spot, 1 morning)

• Lab Methods for each participant
  – 2 of 3 sputa: decontamination then ZN smear microscopy, solid & liquid culture, Xpert MTB/RIF
  – 1 of 3 sputa: direct ZN smear microscopy, Xpert MTB/RIF (no decontamination)
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• Results
  – 1730 eligible participants
    • 976 with HIV status known; 40.2% HIV-positive

  – Final diagnostic category
    • Smear $\text{positive}$, culture positive TB: 32.8%
    • Smear $\text{negative}$, culture positive TB: 10.1%
    • Rifampin-resistant TB: 12.2%
## Evaluation Study of Xpert MTB/RIF

C. Boehme et al. NEJM 2010;363:1005

<table>
<thead>
<tr>
<th># Xpert tests per participant</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All CX POS</td>
<td>SM POS, CX POS</td>
</tr>
<tr>
<td>1 sputum</td>
<td>675/732 92.2%</td>
<td>551/561 98.2%</td>
</tr>
<tr>
<td>3 sputa</td>
<td>723/741 97.6%</td>
<td>566/567 99.8%</td>
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**HIV POS: 93.9%, HIV NEG: 98.4%**
# Evaluation Study of Xpert MTB/RIF

C. Boehme et al. NEJM 2010;363:1005

<table>
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<tr>
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<th>Xpert SENSITIVITY for Rifampin Resistance</th>
<th>Xpert SPECIFICITY for Rifampin Resistance</th>
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<tbody>
<tr>
<td></td>
<td># correct/# total</td>
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<td></td>
<td>%</td>
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<tr>
<td>Phenotypic DST</td>
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<td>505/515</td>
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<td>98.1%</td>
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<tr>
<td>Phenotypic DST and Discrepant Resolution by Sequencing</td>
<td>209/211</td>
<td>506/506</td>
</tr>
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<td></td>
<td>99.1%</td>
<td>100.0%</td>
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Xpert MTB/RIF

• Attributes & Advantages
  – Simple to perform, minimal training required
  – Not prone to cross-contamination
  – Requires minimal biosafety facilities (Banada JCM 2010)
  – “Near-care” (? POC)

• Shortcomings & Disadvantages
  – Complex instrument (calibration, electrical supply)
  – Platform well-suited to detecting limited # of mutations
  – Cost for instrument and cartridges
Xpert MTB/RIF

• WHO evidence review to policy announcement, Sept to Dec 2010

• WHO expert group recommendations:
  – “Xpert should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB” (strong recommendation)

  – “Xpert may be used as a follow-on test to microscopy where MDR and/or HIV is of lesser concern, especially in smear-negative specimens” (conditional recommendation, recognizing resource implications)

  Courtesy of Dr. K. Weyer
Urine Assays for Mycobacterial Lipoarabinomannan (LAM)

• Background
  – LAM
    • 17.5 kd lipopolysaccharide component of MTB cell wall; heat stable
    • Released from metabolically active or degraded MTB
    • Prelim data animal models & some humans: in urine

  – Urine-based test
    • Urine easy to obtain
    • Lacks infection control issues of blood, sputum
    • Inverness: ELISA format; lateral flow under development
Sensitivity of Inverness LAM ELISA, by CD4 Count in HIV/TB Patients

- Lawn et al
  AIDS 2009;23:1875
- Shah et al
  JCM 2010;48:2972
- Gounder et al
  CROI 2011
Beyond the current LAM ELISA...

• Lateral flow format
  – Dheda et al, 301 HIV+ hospitalized adults
  – Sensitivity LF 71% (61, 79) vs ELISA 61% (52, 70)
  – LF sensitivity if CD4<100: 86% (77, 93)

• This test performs best in those patients (advanced HIV) in whom conventional TB tests perform least well
• Expanding interest in the biology of mycobacterial products in clinical specimens
• Bringing new detection platforms, experts into TB field
• Prompting discussion of/strategies for integrating non-sputum tests into TB dx algorithms
Other TB Diagnostic Tests

• Smear microscopy improvements: LED; concentration of bacilli; automated reading

• Culture improvements: Novel detection systems; novel media

• Nucleic acid amplification for MTB detection: Isothermal “near care”

• Molecular for DST: Expanded mutation capacity

• Serology: Proteomic approaches

• Detection of volatiles: Giant pouched rats; electronic noses
Beyond “accuracy” as measured in the lab...
Accuracy in the lab is only one step of a complex process.

- **Pre-analytical**
  - Identifying “TB suspects”
  - Obtaining the specimen
  - Labeling the specimen
  - Handling the specimen
  - Transporting the specimen
  - Tracking the specimen

- **Analytical**
  - Logging in the specimen
  - Processing the specimen
  - Performing “the test”
  - Transmitting the results
  - Quality assurance

- **Post-Analytical**
  - Receiving the test result
  - Conveying the test result
  - Interpreting the result
  - Using result to treat the pt
“near care” or “POC” tests will simplify the process BUT...

Pre-analytical Analytical Post-Analytical

Identifying “TB suspects” Obtaining the specimen

Performing “the test” Quality assurance

Interpreting the result Using result to treat the pt

Still many steps.... opportunities & needs for operational research around diagnostic and clinical care processes
Impact: on Whom, What?

The Patient

- Time to tx?
- Morbidity?
- Survival?

The TB/HIV Program(s)

- The Health System

- # patients reached?
- Costs ($ and opportunity)?

The Community

- TB rates?
- Rates of drug resistant TB?
Infection Control
(e.g. in healthcare settings)

• TB case-finding is essential for infection control

• How can sensitive POC/near-care diagnostics open up new approaches to infection control esp. in healthcare settings?
“Deliverability”

• Can the test be rolled out to the places that matter?
• Will there be uptake?
• What is needed to sustain uptake?
• How to ensure that capacity for successful TB treatment keeps pace with case detection?

Important work already underway: Global Laboratory Initiative, TB REACH, PEPFAR, others
From accuracy to access: a role for advocacy and activism

- HIV/AIDS
  - Pre-2000: high ARV prices and patents limited access in developing countries
  - Post-2000: expansion started (e.g. WHO “3 by 5”)
    - Special terms in international trade laws allow manufacture of generic drugs
    - Some countries allow purchase of generic drugs from abroad
    - Brazil: legislation for free access to tx
  - Common theme: role of civil society

- Parallels between HIV treatment access and TB diagnostic access
“HIV TESTING IS THE ANSWER — WHAT’S THE QUESTION?”
Acknowledgements

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