Xpert MTB/RIF

10th Jan 2013
Points to cover

- Introduction
- Molecular Diagnostics (Rapid Dx)
- Gene Xpert- Advent
- Present Status
  - Pulmonary TB
  - Extrapulmonary TB
  - Pediatric TB
  - PLHIV- TB
- Cost effective analysis
- Conclusions
Introduction

“Reach the three million
A TB test, treatment and cure for all”

World TB day theme for 2014
TB Burden (GTR 2013)

- 8.6 million patients
- 1.1 million TB patients are PLHIV
- 1.3 million deaths
- 4,50,000 MDR (3.6% new, 20% Prev. treated)
- 9.6 % of MDR are XDR TB
- 26% patients from INDIA

- ‘3 million cases missed notification in 2012’
Algorithm for the diagnosis of TB in ambulatory patient

Ambulatory patient with cough 2-3 weeks and no dangeur siens*

AFB HIV test

AFB ++
AFB + -
AFB + - HIV +
AFB + - HIV -
HIV + or unknown*

Treat for TB

CXR**
Sputum AFB & culture**
Clinical assessment**

No TB

Treat for bacterial infection*

HIV -

Treat for bacterial infection*

No or partial response

CXR**
Sputum AFB & culture**

No TB

TB

Refer for HIV care

Reassess for TB

Response*

Reassess for other disease
Diagnosis of MDR TB

Timeline:
- **Microscopy**: Same day
- **Line probe assay**: 48 hours
- **Liquid culture**: 2-3 weeks
- **1st line DST**: 3-4 weeks
- **2nd line DST**: 6-8 weeks

**Steps**:
- **NALC-NaOH processed sample**
- **Microscopy**
  - +
  - -
- **Line probe assay (LPA)**
- **Unsuccessful Amp.**, **INH and/or RIF resist.**
- **INH/RIF susc**
- **Liquid culture**
- **1st line DST**
- **2nd line DST**

**Options**:
- **Option 1**: (std MDR-Rx)
  - Report as MTB, INH and or RIF resistant
  - No culture
  - Sputum for culture at month 3
- **Option 2**: (ind. MDR-Rx)
  - Report as MTB, INH or RIF resistant
  - Culture and 1st and 2nd line DST
  - Rx individualized

**Results**:
- **MDR-TB**
- **XDR-TB**
- **SNRL lab**

**Laboratories**:
- Microscopy center
- LPA lab
- Culture/DST lab
- SNRL lab
Figure: Development pipeline for new tuberculosis diagnostics

## Comparison of Various Diagnostic Tests for Diagnosis of TB

<table>
<thead>
<tr>
<th></th>
<th>Microscopy</th>
<th>LED Microscopy</th>
<th>GeneXpert MTB/RIF</th>
<th>LAMP</th>
<th>Solid Culture</th>
<th>Liquid Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threshold (CFU/ml)</td>
<td>10,000</td>
<td>-</td>
<td>131 (106-176)</td>
<td>-</td>
<td>~100</td>
<td>10-50</td>
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<tr>
<td>Turnaround time</td>
<td>1-2 days</td>
<td>1 day</td>
<td>90 min</td>
<td>-</td>
<td>4-8 week</td>
<td>Days - 2 week</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>50-60 %</td>
<td>10% &gt; than ZN staining</td>
<td>~90 %</td>
<td>88 %</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Specificity</td>
<td>98%</td>
<td>94%</td>
<td></td>
<td></td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Technical expertise</td>
<td>Required</td>
<td>Required</td>
<td>Minimal</td>
<td>Required</td>
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<td>Required</td>
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<tr>
<td>Biosafety</td>
<td></td>
<td></td>
<td>Better than Microscopy</td>
<td></td>
<td></td>
<td>Prone to contamination</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
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</table>

## Comparison of Various Diagnostic Tests for Detection of Drug resistance in TB

<table>
<thead>
<tr>
<th></th>
<th>GeneXpert MTB/RIF</th>
<th>LPA</th>
<th>MODS</th>
<th>CRI</th>
<th>Nitrate reductase A</th>
<th>Solid Culture</th>
<th>Liquid Culture</th>
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</thead>
<tbody>
<tr>
<td><strong>Turnaround time</strong></td>
<td>90 min</td>
<td>1-2 days</td>
<td>7 days</td>
<td>5-10 days</td>
<td>7-14 days</td>
<td>4-8 week</td>
<td>Days - 2 week</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td>~90 %</td>
<td>97 % (R)</td>
<td>92%</td>
<td>&gt;94% (R)</td>
<td>&gt;92% (H)</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td></td>
<td>84% (H)</td>
<td></td>
<td>89- 100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>98%</td>
<td>99%</td>
<td>96%</td>
<td>89- 100%</td>
<td>89- 100%</td>
<td>Reference</td>
<td>Reference</td>
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</tr>
<tr>
<td><strong>Technical expertise</strong></td>
<td>Minimal</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
<td>High</td>
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<tr>
<td><strong>Biosafety</strong></td>
<td>Better than Microscopy</td>
<td>Atleast BSL II</td>
<td></td>
<td></td>
<td></td>
<td>Atleast BSL III</td>
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<tr>
<td><strong>Additional Comments</strong></td>
<td>Can be done in usual lab</td>
<td>Can not replace Culture</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Prone to contamination</td>
</tr>
</tbody>
</table>

History of GeneXpert- Platform

- Originally developed by Cepheid Inc. for the detection of anthrax
- Onboard sample preparation with fully-automated rt-PCR amplification and detection
- Cartridge-based system incorporates microfluidics technology and fully automated nucleic acid analysis
- Expanding range of different organisms, genes may be detected using pathogen-specific cartridges within the same platform
- Machines with 1, 4, 16 and 48 modules are available, permitting multiple assays to be run concurrently and independently

Target- Why Rifampicin?

- Amenable to rapid detection- 95% of all rifampicin-resistant mutations localized within the 81 bp core region of rpoB gene
- These mutations are highly predictive of rifampicin resistance
- Core region is flanked by M tuberculosis complex-specific DNA sequences
- M tuberculosis and rifampicin resistance can be tested simultaneously by targeting one amplicon
- Rifampicin resistance is strongly, although not invariably, indicative of MDR tuberculosis

Rifampin susceptible sample

-module Name: S1
-Sample ID: uganda sputum.0
-Assay: MTB Beta
-Assay Version: 2
-Assay Type: Research Use Only
-Reagent Lot ID: 00502
-Cartridge S/N: 0
-Expiration Date: «None»
-Test Type: Specimen
-Notes:

Start Time: 2000/04/16 15:50:33
End Time: 2000/04/16 17:15:31
Status: Done
Error Status: OK
User: support
S/W Version: 2.1
Instrument/Module: S/N: T02206/000013

Views
-Result View
-MTB Beta Assay
-Temperature-Sample
-Optic-All Options
-Optic-Primary
-Pressure
-Optic-Primary-Thres
-2nd Derivative

Legend:
- Probe D: Primary
- Probe E: Primary
- Probe C: Primary
- Probe B: Primary
- Probe A: Primary

Test and Analyte Result
-Assay Name: MTB Beta
-Version: 2
-Test Result: MTB POSITIVE, MEDIUM

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>C1</th>
<th>EndPt</th>
<th>Analyte Result</th>
<th>Probe Check Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe D</td>
<td>19.1</td>
<td>240.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe C</td>
<td>17.7</td>
<td>279.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe E</td>
<td>18.4</td>
<td>174.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe B</td>
<td>18.8</td>
<td>214.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe A</td>
<td>17.3</td>
<td>220.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
</tbody>
</table>

TIMELINE – FROM CONCEPT TO IMPLEMENTATION

May 2006 – FIND and the University of Medicine and Dentistry of New Jersey partner with Cepheid to develop a novel TB test, with funding from the US NIH and the Bill & Melinda Gates Foundation (BMGF)

May 2009 – Demonstration studies underway

September 2010 – Expert Group issues strong recommendation to WHO based on scientific evidence; WHO's Strategic and Technical Advisory Group for TB further reviews evidence and makes policy recommendations

December 2010 – After organization of a Global Consultation, WHO recommends Xpert MTB/RIF

August 2012 – A public-private partnership between the US President’s Emergency Plan for AIDS Relief (PEPFAR), the US Agency for International Development (USAID), UNITAID, and BMGF allows for a drop in price of the Xpert MTB/RIF test cartridge from 16.86 USD to 9.98 USD

May 2013 – Expert Group reviews updated evidence base on use of Xpert MTB/RIF for diagnosis of pulmonary, extrapolmonary and paediatric TB and rifampicin resistance, and issues updated recommendations to WHO

October 2013 – WHO updates recommendations on Xpert MTB/RIF, with an expanded scope of use.
Performance of Xpert MTB/RIF in Clinical setting
Pulmonary TB
**Figure 5.** Forest plots of Xpert sensitivity and specificity for TB detection, Xpert 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 specificity could not be estimated in one study.

<table>
<thead>
<tr>
<th>Study</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
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<tr>
<td>Mallbruny 2011</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>46</td>
<td>1.00 [0.74, 1.00]</td>
<td>1.00 [0.92, 1.00]</td>
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<tr>
<td>Boehme 2011e</td>
<td>101</td>
<td>16</td>
<td>0</td>
<td>671</td>
<td>1.00 [0.96, 1.00]</td>
<td>0.98 [0.96, 0.99]</td>
</tr>
<tr>
<td>Boehme 2011b</td>
<td>171</td>
<td>3</td>
<td>6</td>
<td>825</td>
<td>0.97 [0.93, 0.99]</td>
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<tr>
<td>Boehme 2010b</td>
<td>201</td>
<td>0</td>
<td>8</td>
<td>101</td>
<td>0.96 [0.93, 0.98]</td>
<td>1.00 [0.96, 1.00]</td>
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<tr>
<td>Ciffi 2011</td>
<td>24</td>
<td>1</td>
<td>1</td>
<td>59</td>
<td>0.96 [0.80, 1.00]</td>
<td>0.98 [0.91, 1.00]</td>
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<tr>
<td>Boehme 2010e</td>
<td>179</td>
<td>0</td>
<td>8</td>
<td>35</td>
<td>0.96 [0.92, 0.98]</td>
<td>1.00 [0.90, 1.00]</td>
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<td>Bowles 2011</td>
<td>60</td>
<td>2</td>
<td>4</td>
<td>23</td>
<td>0.94 [0.85, 0.98]</td>
<td>0.92 [0.74, 0.99]</td>
</tr>
<tr>
<td>Boehme 2010c</td>
<td>136</td>
<td>1</td>
<td>10</td>
<td>185</td>
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<td>0.99 [0.97, 1.00]</td>
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<tr>
<td>Miller 2011</td>
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<td>2</td>
<td>2</td>
<td>58</td>
<td>0.93 [0.77, 0.99]</td>
<td>0.97 [0.88, 1.00]</td>
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<tr>
<td>Friedrich 2011</td>
<td>117</td>
<td>0</td>
<td>9</td>
<td>9</td>
<td>0.93 [0.87, 0.97]</td>
<td>Not estimable</td>
</tr>
<tr>
<td>Boehme 2011f</td>
<td>136</td>
<td>5</td>
<td>12</td>
<td>234</td>
<td>0.92 [0.86, 0.96]</td>
<td>0.98 [0.95, 0.99]</td>
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<tr>
<td>Ioannidis 2011</td>
<td>29</td>
<td>2</td>
<td>3</td>
<td>32</td>
<td>0.91 [0.75, 0.96]</td>
<td>0.94 [0.80, 0.99]</td>
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<tr>
<td>Teo 2011</td>
<td>56</td>
<td>2</td>
<td>6</td>
<td>42</td>
<td>0.90 [0.80, 0.96]</td>
<td>0.95 [0.85, 0.99]</td>
</tr>
<tr>
<td>Hanif 2011</td>
<td>54</td>
<td>0</td>
<td>6</td>
<td>146</td>
<td>0.90 [0.79, 0.96]</td>
<td>1.00 [0.98, 1.00]</td>
</tr>
<tr>
<td>Marlowe 2011</td>
<td>116</td>
<td>4</td>
<td>14</td>
<td>82</td>
<td>0.89 [0.83, 0.94]</td>
<td>0.95 [0.89, 0.99]</td>
</tr>
<tr>
<td>Boehme 2011a</td>
<td>203</td>
<td>4</td>
<td>26</td>
<td>303</td>
<td>0.89 [0.84, 0.92]</td>
<td>0.99 [0.97, 1.00]</td>
</tr>
<tr>
<td>Zeka 2011</td>
<td>31</td>
<td>0</td>
<td>4</td>
<td>68</td>
<td>0.69 [0.73, 0.97]</td>
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</tr>
<tr>
<td>Scott 2011</td>
<td>58</td>
<td>3</td>
<td>9</td>
<td>104</td>
<td>0.67 [0.76, 0.94]</td>
<td>0.97 [0.92, 0.99]</td>
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<tr>
<td>Boehme 2011c</td>
<td>201</td>
<td>2</td>
<td>32</td>
<td>669</td>
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<td>1.00 [0.99, 1.00]</td>
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<tr>
<td>Rachow 2011</td>
<td>49</td>
<td>1</td>
<td>9</td>
<td>101</td>
<td>0.84 [0.73, 0.93]</td>
<td>0.99 [0.95, 1.00]</td>
</tr>
<tr>
<td>Boehme 2010d</td>
<td>36</td>
<td>3</td>
<td>7</td>
<td>215</td>
<td>0.84 [0.69, 0.93]</td>
<td>0.99 [0.96, 1.00]</td>
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<tr>
<td>Boehme 2010a</td>
<td>123</td>
<td>1</td>
<td>24</td>
<td>68</td>
<td>0.84 [0.77, 0.89]</td>
<td>0.99 [0.92, 1.00]</td>
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<tr>
<td>Boehme 2011d</td>
<td>121</td>
<td>0</td>
<td>24</td>
<td>144</td>
<td>0.83 [0.76, 0.89]</td>
<td>1.00 [0.97, 1.00]</td>
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<td>Helb 2010</td>
<td>67</td>
<td>0</td>
<td>15</td>
<td>25</td>
<td>0.82 [0.72, 0.89]</td>
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<td>Theron 2011</td>
<td>111</td>
<td>19</td>
<td>30</td>
<td>320</td>
<td>0.79 [0.71, 0.85]</td>
<td>0.94 [0.84, 0.97]</td>
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<td>Moura 2011</td>
<td>61</td>
<td>0</td>
<td>17</td>
<td>29</td>
<td>0.78 [0.67, 0.87]</td>
<td>1.00 [0.88, 1.00]</td>
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<td>Lawn 2011</td>
<td>42</td>
<td>2</td>
<td>30</td>
<td>320</td>
<td>0.58 [0.46, 0.70]</td>
<td>0.99 [0.98, 1.00]</td>
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</tbody>
</table>

Sn- 88%

-Cochrane Review 2013
Follow-up to Negative smear

Figure 9. Forest plot of Xpert sensitivity for TB detection in smear-positive subgroup. The squares represent the sensitivity and specificity of one study, the black line its CI. TP = true positive; FP = false positive; FN = false negative; TN = true negative. Xpert specificity could not be estimated in these studies.

Sn- 98%

-Cochrane Review 2013
Follow-up to Negative smear

Figure 7. Forest plots of Xpert for TB detection. Xpert used as an add-on test following a negative smear microscopy result. TP = True Positive; FP = False Positive; FN = False Negative; TN = True Negative. Between brackets 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).

Sn- 68%
Xpert in Diagnosis of PTB

• Pooled sensitivity in HIV Negative- 89% (95% CI 81-94%)

• Pooled sensitivity in PL-HIV 80% (95% CI 67% - 88%)
  • PLHIV Smear +ve, Culture +ve – 100% (82-100%)
  • PLHIV Smear – ve , Culture +ve- 43% (30-58%)

• Fresh specimen > Frozen

-Cochrane Review 2013
Xpert MTB/RIF for Rif Detection in PTB

Figure 12. Forest plots of Xpert sensitivity and specificity for detection of rifampicin resistance, Xpert used as an initial test replacing conventional drug susceptibility testing as the initial test. The individual studies are ordered by decreasing sensitivity and decreasing number of true positives. The squares represent the sensitivity and specificity of one study, the black line its CI. TP = true positive; FP = false positive; FN = false negative; TN = true negative.
Xpert in Diagnosis of PTB

Systematic review and Meta-analysis
• Studies published up to October 2011
• 18 studies, 10,224 patients
• 15 reported on Dx of PTB,
• Pooled sn- 90.4% (95% CI 89.2–91.4), sp- 98.4% (98.0–98.7).
  • Sm –ve- 75.0%
  • Sm +ve- 98.7%

Similar to Cochrane review, however current G4 version, launched in Dec 2011 might differ in performance

• G4 version in Africa- Specificity of 99.5%

-Chang K, J Infect 2012; 64: 580–88
- Muhammad Osman et al. JCM, Nov 2013
Extrapulmonary TB
<table>
<thead>
<tr>
<th>Study</th>
<th>Tissue</th>
<th>Lymph node</th>
<th>CSF</th>
<th>Gastric</th>
<th>Pleural</th>
<th>Urine</th>
<th>Cavitary fluid</th>
<th>Pericardial fluid</th>
<th>Stool</th>
<th>Pus</th>
<th>Other</th>
<th>Total sensitivity</th>
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<tbody>
<tr>
<td>Ligthalm et al</td>
<td>28/29</td>
<td>28/29 (97%)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>28/29 (97%)</td>
</tr>
<tr>
<td>Hillemann et al</td>
<td>20/29</td>
<td>20/29 (68%)</td>
<td>7/8</td>
<td>7/8 (87.5%)</td>
<td>5/5 (100%)</td>
<td>2/2 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35/44 (77.3%)</td>
</tr>
<tr>
<td>Teo et al</td>
<td>2/3 (66%)</td>
<td>2/3 (66%)</td>
<td>4/4</td>
<td>4/4 (100%)</td>
<td>1/1 (100%)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>7/7 (100%)</td>
</tr>
<tr>
<td>Vadwai et al</td>
<td>54/70 (77%)</td>
<td>1/3 (33%)</td>
<td>Body fluids 16/21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>125/150 (83%)</td>
</tr>
<tr>
<td>Miller et al</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>7/8 (88%)</td>
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<td>Zeka et al</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>21/31 (68%)</td>
</tr>
<tr>
<td>Causse et al</td>
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<td></td>
<td></td>
<td></td>
<td>39/41 (95%)</td>
</tr>
<tr>
<td>Friedrick et al</td>
<td>5/20 (25%)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
<td>5/20 (25%)</td>
</tr>
<tr>
<td>Hanif et al</td>
<td></td>
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<td></td>
<td></td>
<td>12/12 (100%)</td>
</tr>
<tr>
<td>Armand et al</td>
<td>3/5 (60%)</td>
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<td></td>
<td></td>
<td></td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>Moure et al</td>
<td>5/12 (42%)</td>
<td>5/12 (42%)</td>
<td>2/2</td>
<td>2/2 (100%)</td>
<td>7/26 (27%)</td>
<td>2/3 (67%)</td>
<td>1/1 (100%)</td>
<td>2/2 (100%)</td>
<td></td>
<td></td>
<td></td>
<td>63/108 (58%)</td>
</tr>
<tr>
<td>Tortoli et al</td>
<td>11/13 (85%)</td>
<td></td>
<td>45/58</td>
<td>5/15 (33%)</td>
<td>11/13 (85%)</td>
<td>40/47 (85%)</td>
<td>5/10 (50%)</td>
<td>71/82 (87%)</td>
<td></td>
<td></td>
<td></td>
<td>188/238 (79%)</td>
</tr>
</tbody>
</table>

Source: Based on literature published by September 2012 and categorized by sample type.
Evaluation of GeneXpert MTB/RIF for Diagnosis of Tuberculous Meningitis

Nguyen Thi Quynh Nhu,a Dorothée Heemskerk,a Do Dang Anh Thu,a Tran Thi Hong Chau,a Nguyen Thi Hoang Mai,b Ho Dang Trung Nghia,b Pham Phu Loc,b Dang Thi Minh Ha,a,c Laura Merson,a Tran Thi Van Thinh,a Jeremy Day,a Nguyen Van Vinh Chau,a,b Marcel Wolbers,a Jeremy Farrar,a,b Maxine Caws,a

Oxford University Clinical Research Unit, Hospital for Tropical Diseases, Wellcome Trust Major Overseas Programme, Ho Chi Minh City, Vietnam; Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam; Pham Ngoc Thach Hospital for Tuberculosis and Lung Diseases, Ho Chi Minh City, Vietnam

Tuberculous meningitis (TBM) is the most severe form of tuberculosis. Microbiological confirmation is rare, and treatment is often delayed, increasing mortality and morbidity. The GeneXpert MTB/RIF test was evaluated in a large cohort of patients with suspected tuberculous meningitis. Three hundred seventy-nine patients presenting with suspected tuberculous meningitis to the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, between 17 April 2011 and 31 December 2012 were included in the study. Cerebrospinal fluid samples were tested by Ziehl-Neelsen smear, mycobacterial growth indicator tube (MGIT) culture, and Xpert MTB/RIF. Rifampin (RIF) resistance results by Xpert were confirmed by an MTBDR-Plus line probe assay and all positive cultures were tested by phenotypic MGIT drug susceptibility testing. Overall, 182/379 included patients (48.0%) were diagnosed with tuberculous meningitis. Sensitivities of Xpert, smear, and MGIT culture among patients diagnosed with TBM were 59.3% (108/182 [95% confidence interval [CI], 51.8 to 66.5%]), 78.6% (143/182 [95% CI, 71.9 to 84.3%]) and 66.5% (121/182 [95% CI, 59.1 to 73.3%]), respectively. There was one false-positive Xpert MTB/RIF test (99.5% specificity). Four cases of RIF resistance (4/109; 3.7%) were identified by Xpert, of which 3 were confirmed to be multidrug-resistant (MDR) TBM and one was culture negative. Xpert MTB/RIF is a rapid and specific test for the diagnosis of tuberculous meningitis. The addition of a vortexing step to sample processing increased sensitivity for confirmed TBM by 20% ($P = 0.04$). Meticulous examination of a smear from a large volume of cerebrospinal fluid (CSF) remains the most sensitive technique but is not practicable in most laboratories. The Xpert MTB/RIF represents a significant advance in the early diagnosis of this devastating condition.
Pediatric TB
<table>
<thead>
<tr>
<th>Country</th>
<th>Summary of findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>South Africa</td>
<td>Prospective study of inpatients (n=452) with median age 19 months (maximum 15 years) and suspected TB: from two induced sputum samples, the Xpert MTB/RIF assay diagnosed 75.9% (44 of 58) of culture-positive cases (specificity 98.8%) compared with 37.9% using smear microscopy; in smear-negative cases, the incremental yield of the second Xpert MTB/RIF test was 27.8%</td>
</tr>
<tr>
<td>Tanzania</td>
<td>Prospective study of 154 children aged &lt;14 years (median 5.8 years): of 28 microbiologically confirmed cases, the Xpert MTB/RIF assay diagnosed 100% (7 of 7) smear-positive cases and 66.6% (14 of 21) smear-negative cases with 100% specificity; the incremental yields of testing second and third samples were 20% and 16%, respectively</td>
</tr>
<tr>
<td>South Africa</td>
<td>Prospective study of inpatients (n=535) with median age 19 months (maximum 15 years) and suspected TB: the yield of two Xpert MTB/RIF assay tests on nasopharyngeal aspirates from culture-confirmed cases was 65% (41 of 63) compared with 33% (21 of 63) by smear microscopy</td>
</tr>
<tr>
<td>Zambia</td>
<td>Prospective study of inpatients (n=930) with median age 24 months (maximum 15 years) and suspected TB: in culture-positive cases (n=58), the Xpert MTB/RIF assay was more sensitive than smear microscopy when testing sputum samples, (90.0% vs 30.0%) or gastric lavage aspirates (68.8% vs 25.0%) and specificity was 99.3%</td>
</tr>
<tr>
<td>Italy</td>
<td>Study of the diagnosis of extrapulmonary TB in adults and children with a wide range of different sample types (tissue biopsies, pleural fluid, gastric aspirates, pus, CSF, and urine) that used a composite reference standard of culture, radiology, histology, and treatment response: the sensitivity in samples from children (86.9%) tended to be higher than that in samples from adults (77.6%), possibly as a result of the types of clinical samples in each group</td>
</tr>
</tbody>
</table>

TB=tuberculosis. MTB=Mycobacterium tuberculosis. RIF=rifampicin.

Table 2: Studies of the Xpert MTB/RIF assay for diagnosis of tuberculosis in children

TB in PLHIV
<table>
<thead>
<tr>
<th>Country</th>
<th>Clinical population</th>
<th>Patient selection</th>
<th>Sensitivity of smear microscopy, % (95% CI)</th>
<th>Sensitivity of single Xpert MTB/RIF assay test, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Studies of outpatients</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Boehme et al, 2011³⁸</td>
<td>South Africa, Uganda, India, Peru, Azerbaijan, Philippines</td>
<td>Outpatients (HIV+ and HIV−)</td>
<td>Presentation with suspected TB with cough ≥2 weeks</td>
<td>HIV+: 44.6% (37.7–51.6); HIV−: 68.6% (63.5–73.3); p&lt;0.001</td>
</tr>
<tr>
<td>Theron et al, 2011⁴⁰</td>
<td>South Africa</td>
<td>Outpatients (HIV+ and HIV−)</td>
<td>Presentation with suspected TB</td>
<td>HIV+: 50.0% (36.1–63.9); HIV−: 73.2% (62.7–81.6); p=0.01</td>
</tr>
<tr>
<td>Scott et al, 2011⁵⁰</td>
<td>South Africa</td>
<td>Outpatients (mostly HIV+) with suspected TB with cough for ≥2 weeks</td>
<td>Presentations with suspected TB with cough ≥2 weeks</td>
<td>HIV+: 54% (38–69)</td>
</tr>
<tr>
<td>Lawn et al, 2011²⁵</td>
<td>South Africa</td>
<td>Outpatients (HIV+) enrolling in an antiretroviral treatment clinic</td>
<td>Unselected patients screened for TB irrespective of symptoms before antiretroviral therapy</td>
<td>HIV+: 22.2% (13.3–33.6)</td>
</tr>
<tr>
<td><strong>Studies of hospital inpatients</strong></td>
<td></td>
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</tr>
<tr>
<td>O’Grady et al, 2012³²</td>
<td>Zambia</td>
<td>Hospital medical inpatient admissions (HIV+ and HIV−)</td>
<td>All who could produce sputum samples</td>
<td>HIV+: 52.8% (45.1–60.4); HIV−: 48.6% (33.0–64.4); p=0.71</td>
</tr>
<tr>
<td>Balcells et al, 2012³³</td>
<td>Chile</td>
<td>Hospital medical inpatients (HIV+)</td>
<td>Admission with suspected TB and symptoms &gt;10 days</td>
<td>HIV+: 66.7% (39.1–86.2)</td>
</tr>
<tr>
<td>Carrquiry et al, 2012³⁴</td>
<td>Peru</td>
<td>Hospital medical inpatients (HIV+)</td>
<td>Admission with suspected TB and cough &gt;10 days plus abnormal chest radiograph plus additional symptoms</td>
<td>HIV+: 68.9% (54.3–80.6)</td>
</tr>
</tbody>
</table>
GeneXpert MTB/RIF for EBA/Response to ATT
The early bactericidal activity of antituberculosis agents is usually determined by measuring the reduction of the sputum mycobacterial load over time on solid agar medium or in liquid culture. This study investigated the value of a quantitative PCR assay for early bactericidal activity determination. Groups of 15 patients were treated with 6 different antituberculosis agents or regimens. Patients collected sputum for 16 h overnight at baseline and at days 7 and 14 after treatment initiation. We determined the sputum bacterial load by CFU counting (log CFU/ml sputum, reported as mean ± standard deviation [SD]), time to culture positivity (TTP, in hours [mean ± SD]) in liquid culture, and Xpert MTB/RIF cycle thresholds (Ct, n [mean ± SD]). The ability to discriminate treatment effects between groups was analyzed with one-way analysis of variance (ANOVA). All measurements showed a decrease in bacterial load from mean baseline (log CFU, 5.72 ± 1.00; TTP, 116.0 ± 47.6; Ct, 19.3 ± 3.88) to day 7 (log CFU, −0.26 ± 1.23, P = 0.2112; TTP, 35.5 ± 59.3, P = 0.0002; Ct, 0.55 ± 3.07, P = 0.6030) and day 14 (log CFU, −0.55 ± 1.24, P = 0.0006; TTP, 54.8 ± 86.8, P < 0.0001; Ct, 2.06 ± 4.37, P = 0.0020). The best discrimination between group effects was found with TTP at day 7 and day 14 (F = 9.012, P < 0.0001, and F = 11.580, P < 0.0001), followed by log CFU (F = 4.135, P = 0.0024, and F = 7.277, P < 0.0001). Ct was not significantly discriminative (F = 1.995, P = 0.091, and F = 1.203, P = 0.316, respectively). Culture-based methods are superior to PCR for the quantification of early antituberculosis treatment effects in sputum.
We report an innovative approach to selectively amplify DNA derived from viable *Mycobacterium tuberculosis* in clinical specimens, which is useful for monitoring mycobacterial load in pulmonary TB patients during anti-TB treatment.

**FIGURE 1.** Comparison between the mean difference in threshold cycle (ΔCt) (propidium monoazide (PMA) treated minus PMA untreated) obtained from sputum samples collected before starting treatment (t0) and 10–20 days after the beginning of anti-tuberculosis therapy (t1). Error bars represent ± SEM values. ***: p<0.001.
Impact Of GeneXpert MTB/RIF
## Impact Of GeneXpert MTB/RIF

<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Setting</th>
<th>Comparison</th>
<th>Sample size</th>
<th>Impact</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boehme CC, 2011</td>
<td>Multicentric, 9 Centres</td>
<td>Baseline vs Implementation group</td>
<td>6648</td>
<td>Early Dx 0/1/16/30 days Median time tp Rx 5 vs 56 days</td>
<td>Outcome variables not evaluated</td>
</tr>
<tr>
<td>Yoon C, 2012</td>
<td>Inpatients, Uganda</td>
<td>Baseline vs implementation group</td>
<td>477</td>
<td>Improved % microbiological Dx</td>
<td>No improvement in 2 month mortality</td>
</tr>
<tr>
<td>Hanrahan CF, 2013</td>
<td>OPD, South Africa, POC Xpert</td>
<td>Xpert +ve vs -ve</td>
<td>641</td>
<td>Early Dx 0/14/14/144 days</td>
<td>No improvement in outcomes at 6 months</td>
</tr>
<tr>
<td>Menzies NA, 2012</td>
<td>Multicentric, 5 African countries</td>
<td>Status quo vs introduction of Xpert</td>
<td>Calibrated dynamic mathematical model</td>
<td>Avert 13200 TB Cases Avert 182000 TB Deaths Prevalence will decrease by 28%, 2022 Cost effective over 20 yrs</td>
<td></td>
</tr>
<tr>
<td>Dowdy DW, 2013</td>
<td>Africa</td>
<td>Same day Microscopy vs Xpert vs Both</td>
<td>Compartmental Model for Africa</td>
<td>Micro- 11% inci, 11.8% Death Red^n Xpert- 9.3% incid, 23.8 % Death Red^n Combined- 18.7 %, 33.1%</td>
<td></td>
</tr>
</tbody>
</table>
Figure 2: Proportion of tuberculosis cases detected by each method in culture-positive patients. Percentages are the maximum proportion of cases detected by every method. (A) Tuberculosis case detection. (B) Detection of rifampicin resistance. Time to detection was defined as time between date of sputum sample collection and date of positive result. MTB=Mycobacterium tuberculosis, RIF=rifampicin.
Impact of GeneXpert MTB/RIF

Figure 2. Projected Trajectory of TB Incidence in Africa, 2013–2022. Panel A shows the TB incidence rate (per 100,000 population per year), while Panel B shows the projected number of TB cases per year in an area with an adult population of 10 million in 2002, assuming constant 2.25% population growth.
doi:10.1371/journal.pone.0070485.g002
## Impact of Xpert on PTB Rx and Outcome

<table>
<thead>
<tr>
<th></th>
<th>Smear microscopy (N=758)</th>
<th>Xpert MTB/RIF (N=744)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients with a positive result (by any means)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>By day 1</td>
<td>99/758 (13%)</td>
<td>178/744 (24%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>By day 2</td>
<td>107/758 (14%)</td>
<td>183/744 (25%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>By day 3</td>
<td>109/758 (14%)</td>
<td>185/744 (25%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>By day 14</td>
<td>165/758 (22%)</td>
<td>196/744 (26%)</td>
<td>0.0380</td>
</tr>
<tr>
<td>By day 28</td>
<td>199/758 (26%)</td>
<td>212/744 (29%)</td>
<td>0.33</td>
</tr>
<tr>
<td>By day 56</td>
<td>204/758 (27%)</td>
<td>215/744 (29%)</td>
<td>0.39</td>
</tr>
</tbody>
</table>

Feasibility, accuracy, and clinical effect of point-of-care Xpert MTB/RIF testing for tuberculosis in primary-care settings in Africa: a multicentre, randomised, controlled trial

*Article In Press: Corrected Proof*

Grant Theron, Lynn Zijenah, Duncan Chanda, Petra Clowes, Andrea Rachow, Maia Lesosky, Wilbert Bara, Stanley Mungofa, Madhukar Pai, Michael Hoelscher, David Dowdy, Alex Pym, Peter Mwaba, Peter Mason, Jonny Peter, and Keeran Dheda

Lancet, The
Anticipated benefits

• Increase case detection, esp. smear-ve
• Reduction in time to diagnosis and treatment
• Reduced patient default during investigation
• Reduced morbidity, mortality, and tuberculosis transmission
• Increased detection and Rx of MDR TB
• Reduced need of culture
• Reduced biohazard
Limitations

- Lack of a battery-operated system
- Annual calibration
- Limited range of operating temperatures
- Detects Dead bacilli, Not suitable for monitoring Rx response

- Rif resistance >15% PPV- >90%
- Rif Resistance <5% PPV <70%
- Does not detect INH resistance

- Boehme CC et al. Semin Respir Crit Care Med 2013;34:17 – 31
Challenges

• Increase in budget
• Use of the assay in centralized laboratories might blunt the potential effect
• Increased diagnostic capacity should be matched by increases in treatment capacity
• Diagnostic algorithms need to be redefined
• Need for robust supply chains and storage facilities for bulky cartridges with short shelf-life
Cost Effective analysis- Global

**FIGURE 1** Global estimates of the annual cost of tuberculosis (TB) and multidrug-resistant (MDR)-TB diagnosis using Xpert MTB/RIF, compared with the costs of conventional diagnostics following World Health Organization-recommended algorithms in US$ millions using 2011 prices. Estimates include costs for solid and/or liquid media for culture and drug susceptibility testing (C-DST). The light grey section of the bar depicts the additional cost for liquid media of culture and/or DST. S: smear microscopy; X: radiograph; c: culture; TB-SS: people with signs and symptoms of tuberculosis; HIV+: HIV-positive.
Cost Effective analysis- India

Methods and Findings: We estimate the impact of the introduction of Xpert on the costs and cost-effectiveness of TB care using decision analytic modelling, comparing the introduction of Xpert to a base case of smear microscopy and clinical diagnosis in India, South Africa, and Uganda. The introduction of Xpert increases TB case finding in all three settings; from 72%–85% to 95%–99% of the cohort of individuals with suspected TB, compared to the base case. Diagnostic costs (including the costs of testing all individuals with suspected TB) also increase: from US$28–US$49 to US$133–US$146 and US$137–US$151 per TB case detected when Xpert is used “in addition to” and “as a replacement of” smear microscopy, respectively. The incremental cost effectiveness ratios (ICERs) for using Xpert “in addition to” smear microscopy, compared to the base case, range from US$41–$110 per disability adjusted life year (DALY) averted. Likewise the ICERS for using Xpert “as a replacement of” smear microscopy range from US$52–$138 per DALY averted. These ICERS are below the World Health Organization (WHO) willingness to pay threshold.

Conclusions: Our results suggest that Xpert is a cost-effective method of TB diagnosis, compared to a base case of smear microscopy and clinical diagnosis of smear-negative TB in low- and middle-income settings where, with its ability to substantially increase case finding, it has important potential for improving TB diagnosis and control. The extent of cost-effectiveness gain to TB programmes from deploying Xpert is primarily dependent on current TB diagnostic practices. Further work is required during scale-up to validate these findings.
Scale up so far
As of 30 September 2013, a total of 1,843 GeneXpert instruments (comprising 9,625 modules) and 4,214,990 Xpert MTB/RIF cartridges had been procured in the public sector in 95 of the 145 countries eligible for concessional pricing.
Pilot of Xpert-MTB-Rif (CB-NAAT) in India

- 10 CB-NAAT sites under EXPANDx TB project to supplement the routine DST capacity

- RNTCP-WHO-FIND CB NAAT assessing feasibility of introducing CBNAAT for TB suspects in RNTCP across 18 TU sites in 12 states from March 2012. Interim Results expected shortly and will be placed before National Technical Committee

- 950 CBNAAT machines planned for every district and Medical College in India by 2017
## Choice of Diagnostic Technology

<table>
<thead>
<tr>
<th>MDR Diagnostic Technology</th>
<th>Choice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular DST (e.g. CBNAAT or LPA DST)</td>
<td>First</td>
</tr>
<tr>
<td>Liquid culture isolation and LPA DST</td>
<td>Second</td>
</tr>
<tr>
<td>Solid culture isolation and LPA DST</td>
<td>Third</td>
</tr>
<tr>
<td>Liquid culture isolation and Liquid DST</td>
<td>Fourth</td>
</tr>
<tr>
<td>Solid culture isolation and DST</td>
<td>Fifth</td>
</tr>
</tbody>
</table>
For diagnosis of pulmonary TB and rifampicin resistance:

**Strong recommendation:**

- Xpert MTB/RIF should be used as the initial diagnostic test in adults and children presumed to have MDR-TB or HIV-associated TB

**Conditional recommendations** (recognising major resource implications):

- Xpert MTB/RIF may be used as the initial diagnostic test in adults and children presumed to have TB
- Xpert MTB/RIF may be used as a follow-on test to microscopy in adults presumed to have TB but not at risk of MDR-TB or HIV-associated TB, especially in further testing of smear-negative specimens

For diagnosis of extrapulmonary TB and rifampicin resistance:

**Strong recommendation:**

- Xpert MTB/RIF should be used as the initial diagnostic test in testing cerebrospinal fluid specimens from patients presumed to have TB meningitis

**Conditional recommendation:**

- Xpert MTB/RIF may be used as a replacement test for usual practice (including conventional microscopy, culture, and/or histopathology) for testing of specific non-respiratory specimens (lymph nodes and other tissues) from patients presumed to have extrapulmonary TB

For all WHO TB diagnostics policy documents: http://www.who.int/tb/laboratory/policy_statements/