Briefing:

Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB

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21 February 2011
GeneXpert

Xpert MTB/RIF

5 20 80 500-1000 Samples per shift
### Table 34: Existing test cartridges on the GeneXpert platform

<table>
<thead>
<tr>
<th>Staphylococcus aureus colonization</th>
<th>Bordetella pertussis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin resistance</td>
<td>Bordetella parapertussis</td>
</tr>
<tr>
<td>Clostridium difficile</td>
<td>HSV Type 1</td>
</tr>
<tr>
<td>MRSA from tissue or blood</td>
<td>HSV Type 2</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>RSV Type A</td>
</tr>
<tr>
<td>Enteroviral meningitis</td>
<td>RSV Type B</td>
</tr>
<tr>
<td>Coagulation disorders</td>
<td>Norovirus GI</td>
</tr>
<tr>
<td>Anthrax</td>
<td>Norovirus GII</td>
</tr>
<tr>
<td></td>
<td>Flu A</td>
</tr>
<tr>
<td></td>
<td>Flu B</td>
</tr>
<tr>
<td></td>
<td>Leukemia (BCR-ABL)</td>
</tr>
</tbody>
</table>

>2010: **CT/NG***; Vaginitis panel (CT/NG/Trich); **HPV***; Noro/Rotavirus; Group A Strep; Fungal Sepsis; HSV-CSF; Mycology Panel; CMV; EBV Quant.; **HIV Quant***
Conventional NAAT

- Extraction and purification DNA/RNA
- Amplification
- Detection

Xpert MTB/RIF

- Extraction and purification DNA/RNA
- Amplification
- Multiplex detection (automated)
### Table 1: Pooled values (95% CI) of sensitivity and specificity of five commercial NAATs for pulmonary TB in 60 published studies (Greco, Girardi et al. 2006)

<table>
<thead>
<tr>
<th>Test</th>
<th>AFB+</th>
<th></th>
<th>AFB-</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sensitivity</td>
<td></td>
<td>Sensitivity</td>
</tr>
<tr>
<td>Amplicor (PCR)</td>
<td>96 (94-97)</td>
<td>83 (80-86)</td>
<td>61 (57-65)</td>
<td>97 (96.8-97.4)</td>
</tr>
<tr>
<td>Cobas Amplicor (PCR)</td>
<td>96 (95-97)</td>
<td>74 (68-8)</td>
<td>64 (59-69)</td>
<td>99 (99.2-99.4)</td>
</tr>
<tr>
<td>BDP (SDA)</td>
<td>98 (96-99)</td>
<td>89 (84-93)</td>
<td>71 (66-76)</td>
<td>97 (96.4-97.4)</td>
</tr>
<tr>
<td>E-MTD (TMA)</td>
<td>97 (95-98)</td>
<td>96 (93-97)</td>
<td>76 (70-80)</td>
<td>97 (96.6-97.4)</td>
</tr>
<tr>
<td>LCx (LCR)</td>
<td>96 (94-98)</td>
<td>71 (64-78)</td>
<td>57 (50-64)</td>
<td>98 (97.8-98.5)</td>
</tr>
</tbody>
</table>

PCR: polymerase chain reaction; SDA: strand displacement amplification; TM: transcription mediated amplification; LCR: ligase chain reaction.
Xpert MTB/RIF

1. Sputum liquefaction & inactivation with 2:1 SR
2. Transfer of 2 ml after 15 min
3. End of hands on work
4. Sample is automatically filtered & washed
5. Ultrasonic lysis of filter-captured organisms to release DNA
6. DNA molecules are mixed with dry PCR reagents
7. Semi-nested real-time amplification & detection in integrated reaction tube

Concentrates bacilli & removes inhibitors

Time-to-result: 1 h 45 min

Printable test result
Simple Sample Processing – Direct Sputum

1. Add 2:1 Sample Buffer to sample
2. Shake then stand 10 minutes
3. Shake then stand further 5 minutes
4. Transfer 2ml to cartridge

Begin Test...
Cartridge Design and Operating Principle
Inside the cartridge
Rifampin susceptible sample
Limits of detection

Analytic sensitivity based on 20 replicates per concentration

5 genome copies of purified DNA

131 cfu/ml MTB spiked in sputum
Detection of rifampicin resistance

- >99.5% rifampicin resistance mutations located within rpoB detected
- No cross reactivity with NTM
- Rifampicin resistance detected in mixed strains when >65% presence of mutant strain
# Inactivation procedure

<table>
<thead>
<tr>
<th>Study</th>
<th>Starting cfu/mL</th>
<th>Diluent</th>
<th>Average cfu/plate for each replicate</th>
<th>Average cfu/plate</th>
<th>Average cfu/mL</th>
<th>Average log Reduction</th>
<th>Percent Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study 1</td>
<td>$3.5 \times 10^7$ BCG</td>
<td>7H9 media</td>
<td>&lt;10</td>
<td>&lt;10</td>
<td>$&gt;3.5 \times 10^6$</td>
<td>&gt;99.9</td>
<td></td>
</tr>
<tr>
<td>Study 2</td>
<td>$3.5 \times 10^7$ BCG</td>
<td>sputum</td>
<td>1.5</td>
<td>1.5</td>
<td>$2.3 \times 10^7$</td>
<td>&gt;99.9</td>
<td></td>
</tr>
<tr>
<td>Study 3</td>
<td>$3.3 \times 10^7$ H37Rv</td>
<td>sputum</td>
<td>12, 13, 21</td>
<td>15.3</td>
<td>153</td>
<td>$2.15 \times 10^5$</td>
<td>99.9</td>
</tr>
<tr>
<td>Study 4</td>
<td>$3.63 \times 10^8$ H37Rv</td>
<td>sputum</td>
<td>4, 6, 2</td>
<td>4</td>
<td>40</td>
<td>$9.1 \times 10^6$</td>
<td>~99.9</td>
</tr>
<tr>
<td>Study 5</td>
<td>$4.0 \times 10^8$ H37Rv</td>
<td>sputum</td>
<td>2, 2, 3</td>
<td>2.33</td>
<td>23.3</td>
<td>$1.7 \times 10^7$</td>
<td>&gt;99.9</td>
</tr>
</tbody>
</table>

Average log kill: $1.06 \times 10^7$
Aerosol viability during manual steps

5 $\times 10^8$ cfu BCG spiked into sputum. SR added and sample immediately pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)

SR added 15 min wait then sample pipetted in and out of three Xpert TB cartridge over 15 min time period (equivalent to loading >30 cartridges)

Sputum smeared/layered on 10 microscope slides over 10 min period.

Mean cfu/m³ air detected over 3 experiments

<table>
<thead>
<tr>
<th>Method</th>
<th>Anderson impactor</th>
<th>BioSampler</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>324</td>
<td></td>
</tr>
</tbody>
</table>
Aerosol viability during automated assay

Total cfu detected over all three runs

<table>
<thead>
<tr>
<th>Sample type placed into Xpert TB cartridge (3 runs with 3 cartridge per condition)</th>
<th>Anderson impactor</th>
<th>BioSampler</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 $\times$ 10^8 cfu BCG spiked into water</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 $\times$ 10^8 cfu BCG spiked into sputum then treated with SR in standard protocol</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5 $\times$ 10^8 cfu M. smegmatis spiked into sputum then treated with SR in standard protocol</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
During conventional slide preparation

- BSC air flow on
- BSC air flow off, background measurement
- BSC air flow off, during slide preparation
- BSC airflow on
Concentration of airborne particles when GeneXpert is in operation
(Sample - *Mycobacterium bovis* BCG + Sputum+SR)

![Graph showing concentration of airborne particles](image)
Xpert MTB/RIF evaluations

Three groups of studies

1. Multi-centre clinical validation studies (FIND co-ordinated) ➢ 1,730 subjects in five evaluation sites (four countries)

2. Demonstration studies (FIND co-ordinated) ➢ 6,673 subjects in nine evaluation sites (six countries)

3. Single-centre evaluation studies (investigator-driven) ➢ 4,575 subjects in 12 studies (nine countries)
Overall sensitivity of a single Xpert test 92.2%

Smear-negative/ Culture-positive:
single Xpert test - 72.5% sensitivity;
Two tests 85.1%; Three tests 90.2%

Rifampicin resistance detection
sensitivity 98%; specificity 99%
<table>
<thead>
<tr>
<th>Location</th>
<th>HIV</th>
<th>TB (C+)</th>
<th>MDR TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manila, Philippines</td>
<td>&lt;1%</td>
<td>20.3%</td>
<td>53.7%</td>
</tr>
<tr>
<td>Kampala, Uganda</td>
<td>100%</td>
<td>42.3%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Lima, Peru</td>
<td>3%</td>
<td>16.8%</td>
<td>8.1%</td>
</tr>
<tr>
<td>Vellore, India</td>
<td>&lt;1%</td>
<td>9.8%</td>
<td>6.7%</td>
</tr>
<tr>
<td>Baku, Azerbaijan</td>
<td>6%</td>
<td>47.5%</td>
<td>22.4%</td>
</tr>
<tr>
<td>Cape Town, South Africa</td>
<td>77% (K), 30% (P)</td>
<td>26.4%</td>
<td>3.9%</td>
</tr>
</tbody>
</table>

**Sensitivity**

**PULMONARY TB**
Smear pos. /Culture pos. 99 %
Smear neg. / Culture pos. >80%

HIV-positive 86% sensitivity
HIV-negative 92% sensitivity

**RIFAMPICIN RESISTANCE**
Sensitivity 95.1%
Specificity 98.4%
Single-centre evaluation studies

Varying study designs and study populations, pulmonary and extrapulmonary samples

Detection of TB
• Pooled crude sensitivity 92%
• Pooled crude specificity 98%

Detection of rifampicin resistance
• Pooled crude sensitivity 98%
• Pooled crude specificity 99%
Time to detection of TB

- **Xpert**: Mean: 0.7, Median: 0
- **Microscopy**: Mean: 1, Median: 1
- **Liquid culture**: Mean: 17.2, Median: 15
- **Solid culture**: Mean: 35.7, Median: 29
Time to rifampicin detection

- **Xpert**: Mean: 0.7, Median: 0
- **Molecular DST (LPA)**: Mean: 19.1, Median: 20
- **Conventional DST**: Mean: 74.8, Median: 93.5

(Day)

- Xpert (861)
- Molecular DST (LPA) (19)
- Conventional DST (110)
Operational aspects

• **Waste management** – as for sputum containers

• **Storage & supply** – cartridges fairly bulky, 2 - 28°C, 12month shelf life

• **Operating temperature** – currently approved 15-30°C; >40°C error message

• **Electrical supply** – UPS (400 VA) or battery packs

• **Training** – minimal (computer use, prevention of contamination)

• **Biosafety** – as for smear microscopy

• **Annual calibration** – module replacement/swop out

• **Security** – against theft (computer)
Cost-effectiveness: CPCD* & LSHTM

*CPCD – Center for Poverty-related Communicable Diseases / LSHTM – London School of Hygiene and Tropical Medicine

- **Modelling** based on demonstration findings in India, South Africa and Uganda

- 3 scenarios for use: i) as smear-replacement test; ii) as smear add-on test (S-); and iii) as replacement for culture (S-C+); and both ii) and iii) for retreatment cases

- Unit costs were established through in-depth costing assessments in each country

- FIND-negotiated prices applied for LC, LPA and LED reagents and equipment

- Current per test costs applied (20 USD per test) to make a conservative estimate

- Main limitations: reduction in transmission and costs to the patient were not yet factored in (planned in collaboration with Harvard Medical School)
Cost-effectiveness: Conclusions

• According to WHO criteria*, Xpert MTB/RIF appeared cost-effective in scenarios in which it complements (for S-TB) or replaces smear examination when compared to current practice
• The “in-addition-to” had the best incremental cost-effectiveness ratio (but also less impact)
• Findings were consistent across settings (South Africa, Uganda, India)
• Caution warranted:
  – Actual costs to the health system will increase, additional funding will be required
  – Placement in low utilisation environments or in low TB prevalence settings with caution until we have more data on reduced transmission, morbidity & mortality associated with early detection

* WHO cost effectiveness: Incremental cost-effectiveness ratios (ICERs) should be less than the country per capita GDP
# GRADE summary

<table>
<thead>
<tr>
<th>Xpert MTB/RIF</th>
<th>Absolute difference per 1000 persons</th>
<th>Quality of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
<td>TN</td>
</tr>
<tr>
<td>Pre-test prevalence 10%</td>
<td>92</td>
<td>891</td>
</tr>
<tr>
<td>TB detection</td>
<td>92</td>
<td>891</td>
</tr>
<tr>
<td>R detection</td>
<td>95</td>
<td>891</td>
</tr>
<tr>
<td>Overall quality of evidence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Desirable vs undesirable effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient values and preferences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost and requirements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Added value to conventional methods</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Recommendations

1. Xpert MTB/RIF should be used as the initial diagnostic test in individuals suspected of having MDR-TB or HIV-associated TB (strong recommendation)

2. Xpert MTB/RIF 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, recognising major resource implications)
Remarks

• **Recommendations also apply to children**, based on generalisation of data from adults and acknowledging the limitations of microbiological diagnosis of TB (including MDR-TB) in children;

• **Access to conventional microscopy, culture and DST is still needed** for monitoring of therapy, for recovering isolates for drug susceptibility testing other than rifampicin (including second-line anti-TB drugs); and for prevalence surveys and/or surveillance;

• **Recommendations apply to Xpert MTB/RIF use in sputum specimens** (including pellets from decontaminated specimens), as data on the utility of Xpert MTB/RIF in extra-pulmonary specimens are still limited;

• **Recommendations support the use of one sputum specimen for diagnostic testing**, acknowledging that multiple specimens increase the sensitivity of Xpert MTB/RIF but have major resource implications.
Positioning in tiered health system

Expected 2012 (Gen 1) / 2014 (Gen 2)

- Surveillance
- Reference methods
- Network supervision

Resolution testing (screening-test negative drug resistance)

- Screening
- Passive case finding
- Detect and treat

- Clinical screening
- Primary care

Reference Labs

Regional Labs

District Level

SubDistrict Level

Microscopy Level

Community Level

In house DST (MODS, NRA, CRI) Special settings and conditions

Integrated NAAT +40% /2h

LED FM +10%

ZN 2-3d

LPA Rif / INH 2d

LC / DST 15d / 30d

LPA Rif / INH 2d 160d 30d

Integrated NAAT +40% /2h

LED FM +10%
Changing TB control dynamics

- Changes in diagnostic and screening algorithms
- Increased capacity needed to treat TB and MDR-TB
- Need to re-define TB case and outcome definitions
- Monitoring of impact on case detection and cure
- Resource awareness by donors/funders
- Use in non-traditional TB settings (HIV, private sector)
- Innovative new partnerships needed

Global Consultation: 30 Nov - 2 Dec 2010
Future R&D needs

- Assess feasibility of decentralizing further in rural areas
- Evaluation in extrapulmonary and pediatric TB (ongoing)
- Study best positioning in existing diagnostic algorithms on a per-country basis
- Accelerate development and complete evaluation of 2nd generation RIF/INH assay (ongoing)
- Accelerate development of a 1st generation FQ/AG assay (ongoing)
Pricing

• FIND everaging its investments in development of Xpert MTB/RIF
• Current situation (private sector, Europe):
  - Instrument: €40,000 – €45,000 ($55,000 – $62,000)
  - Test cartridge: €40 – €60 ($55 – $82)
  - Pricing: country-specific (up to $120/cartridge)
• FIND-negotiated pricing is volume dependent
• Full cost-reduction programme underway
• Royalty reductions due to patent expiry: RPCR (2011, 4%); Bead (2011, 3%); rpoB (2011, 1%); Instrumentation (2012/13, 20%)
• Exploring creative ways to cover losses linked to immediate introduction of technology at the lowest price
### FIND-negotiated volume/price relationship

<table>
<thead>
<tr>
<th>Applicable global volumes (cartridges per annum)</th>
<th>FIND Demonstration study price</th>
<th>FIND-negotiated price</th>
<th>FIND-negotiated price</th>
<th>FIND-negotiated price</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 150,000</td>
<td>&gt; 600,000</td>
<td>&gt; 1,700,000</td>
<td>&gt; 3,700,000</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Estimated year</th>
<th>Now</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
</table>

| Ave % Reduction over US/EU | 70% | 73% | 75% | 86% |
# FIND-negotiated instrument cost

<table>
<thead>
<tr>
<th>Instrument cost for FIND markets</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneXpert 4-module with desktop</td>
<td>US$ 17,000</td>
</tr>
<tr>
<td>GeneXpert 4-module with laptop</td>
<td>US$ 17,500</td>
</tr>
</tbody>
</table>

- Price (FOB)
- >60% reduction over EU/US
## Maintenance and calibration costs

Standard: one-year warranty; 24-hour hotline and e-mail support

### Scenarios for after-sales service, support, maintenance and calibration

<table>
<thead>
<tr>
<th>Model 1: Cepheid Toulouse</th>
<th>Model 2: Distributor</th>
<th>Model 3: NTP staff</th>
<th>Model 4: Web-based</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated year</td>
<td>Now</td>
<td>Now</td>
<td>2012</td>
</tr>
<tr>
<td>Calibration (4 modules)</td>
<td>US$ 1,400</td>
<td>US$ 1,400</td>
<td>US$ 1,000</td>
</tr>
<tr>
<td>Description</td>
<td>In Toulouse (requires 2 shipments: site-Toulouse)</td>
<td>Local distributor basis (requires 2 local shipments: site-distributor)</td>
<td>On-site (no swap out)</td>
</tr>
<tr>
<td>Shipment (4 modules)</td>
<td>US$ 400</td>
<td>US$ 200</td>
<td>None</td>
</tr>
<tr>
<td>Total</td>
<td>US$ 1,800</td>
<td>US$ 1,600</td>
<td>US$ 1,000</td>
</tr>
</tbody>
</table>
Test volume as function of capacity

Annual test volumes (million cartridges)

Conservative

Agressive

2011 2012 2013 2014 2015

$10.7/test price achieved, if further UNITAID and Global Fund scale-up

$14.5/test price achieved, if UNITAID-funded scale-up, and early Global Fund grants

Collecting evidence for scale-up

Expansion to greater number of countries, and introduction of other funding mechanisms
Moving forward

WHO endorsement 2010
- Global Consultation
- WHO Policy Guidance
- Roadmap for implementation

Phased implementation 2011
- Through UNITAID, TBREACH, TBCARE, PEPFAR
- Selected countries, different health service levels

Scale up 2012
- EXPAND-TB, Global Fund R11, TBREACH, TBCARE, PEPFAR, country budgets, etc