• Overview
• History & Use Today
• Principles of the Test
• Sensitivity & Specificity
• Samples / Specimens
• Advantages & Disadvantages
• Considerations of Implementation
• New Developments
GeneXpert MTB/Rif

- Said to have revolutionized TB diagnosis
- Xpert MTB/Rif used with Cepheid GeneXpert® Dx system
- Tests for:
  1. Detection of MTB DNA
  2. Detection of rifampicin resistance – rpoB gene
GeneXpert MTB/Rif

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
Established Methods of TB Diagnosis
History

- Originally developed by Cepheid for detection of anthrax
- Deployed by United States Postal Service in mail sorting facilities (early 2000s)
• Endorsed by WHO in 2010
Principles of the Test

- **Nucleic acid amplification testing (NAAT)** searches genetic information in test sample to find MTB
  - Can analyze both DNA and RNA

- Able to analyze genetic mutations that cause drug resistance – i.e. Rifampicin resistance

- Fast testing method because genetic information is extracted and amplified, rather than waiting for bacteria to grow

- Limitations of NAAT:
  - Identifies genetic material – cannot differentiate if bacteria are alive or dead
  - Not a useful diagnostic tool if patient received TB treatment in previous 2 years
Principles of the Test

- Nested PCR

- Real-time PCR
**Principles of Detection**

- A normal PCR is conducted to produce multiple copies of the RRDR.
- 5 probes (A to E) or molecular beacons are designed to bind at the amplified RRDR causing an extended distance between fluorophore and quencher.
- Definitions of binding:
  - 5 probes: MTB positive, no rifampicin resistance
  - 4, 3, 2 probes: MTB positive, rifampicin resistance
  - 1 and 0 probes: MTB not detected
Principles of Detection

6 probes:
• 5 probes spanning rpoB gene
• >2 probes positive (Ct <38) confirm MTB

Estimates bacterial burden by measuring threshold-cycle (Ct)

Semi-quantitative cut-offs:
• High → Ct <16
• Medium → Ct 16-22
• Low → Ct 22-28
• Very low → Ct 28-38

### Sensitivity & Specificity

A good medical screening test has a high sensitivity to identify all sick subjects. False positive (healthy) subjects can be ruled out by a high specificity (second test).

<table>
<thead>
<tr>
<th>Test result</th>
<th>“Gold standard” positive</th>
<th>“Gold standard” negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>True positive</td>
<td>False positive</td>
</tr>
<tr>
<td>Negative</td>
<td>False negative</td>
<td>True negative</td>
</tr>
</tbody>
</table>

Sensitivity = \( \frac{\text{True positive}}{(\text{True positive} + \text{False negative})} \)

Specificity = \( \frac{\text{True negative}}{(\text{True negative} + \text{False positive})} \)
Sensitivity & Specificity

- **Sensitivity:**
  - 83 – 88 %, smear positive + negative
  - 46 – 63%, if smear negative
  - Can improve sensitivity by testing 3 samples

- **Specificity:**
  - 96 – 96%

Correlations between:
- Microscopy and Ct ($r = -0.77$)
- TTP and Ct ($r = 0.68$)
- CFU and Ct ($r = -0.56$)
Evaluation of the Xpert MTB/RIF assay for rapid identification of *Mycobacterium tuberculosis* DNA as a sputum biomarker of response to tuberculosis treatment: a prospective cohort study

Sven Friedrich, Andrea Rachow, Elmar Saathoff, Kasha Singh, Chacha Mangu, Rodney Dawson, Patrick Phillips, Amour Venter, Anna Bateson, Catharina Boehme, Norbert Heinrich, Robert Hunt, Alimuddin Zumla, Timothy McHugh, Stephen Gillespie, Andreas Diacon, and Michael Hoelscher
**Sensitivity & Specificity in Xpert MTB/Rif Ultra Assay**

<table>
<thead>
<tr>
<th></th>
<th>Xpert MTB/Rif</th>
<th>Xpert MTB/Rif Ultra</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity: all culture positive</td>
<td>83%</td>
<td>88%</td>
<td>5.4%</td>
</tr>
<tr>
<td>Sensitivity: smear-negative</td>
<td>46%</td>
<td>63%</td>
<td>17%</td>
</tr>
<tr>
<td>Sensitivity: HIV-negative</td>
<td>90%</td>
<td>91%</td>
<td>1.3%</td>
</tr>
<tr>
<td>Sensitivity: HIV-positive</td>
<td>77%</td>
<td>90%</td>
<td>13%</td>
</tr>
<tr>
<td>Specificity</td>
<td>98%</td>
<td>96%</td>
<td>-2.7%</td>
</tr>
</tbody>
</table>

- Xpert Ultra higher sensitivity in smear-negative PTB
- May improve early diagnosis in:
  - HIV+
  - Children
- Loss in specificity
- If used as single diagnostic method in medium-prevalence settings, 2.7% difference in specificity may lead to considerable over-treatment

Xpert MTB/RIF Ultra for detection of *Mycobacterium tuberculosis* and rifampicin resistance: a prospective multicentre diagnostic accuracy study
Susan E Dorman, Samuel G Schumacher, David Alland et al. 2017
*The Lancet Infectious Diseases*
Samples

- Pulmonary TB:
  - Sputum
  - Tracheal aspirate
  - Gastric washings – paediatrics

- Extra-pulmonary TB:
  - Tissue
  - Cerebrospinal fluid (CSF)
  - Pleural fluid
  - Stool
  - Urine

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>Xpert assay</th>
<th>Culture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indeterminate</td>
<td>Negative</td>
</tr>
<tr>
<td>Tissue (245)</td>
<td>6 (2.4)</td>
<td>216</td>
</tr>
<tr>
<td>CSF (19)</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Gastric fluid (30)</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Pleural fluid (113)</td>
<td>7 (6.2)</td>
<td>103</td>
</tr>
<tr>
<td>Stool (23)</td>
<td>3 (13.0)</td>
<td>15</td>
</tr>
<tr>
<td>Urine (91)</td>
<td>4 (4.4)</td>
<td>81</td>
</tr>
</tbody>
</table>

* A total of 521 specimens were tested. The numbers in parentheses are the number of each specimen type.
## Advantages & Disadvantages

### Advantages

<table>
<thead>
<tr>
<th>Advantage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>One step process – automated</td>
<td></td>
</tr>
<tr>
<td>Quick (results &lt; 2 hours)</td>
<td></td>
</tr>
<tr>
<td>Requires fewer biosafety measures than culture/LPA, so can be used in lower-level laboratories</td>
<td></td>
</tr>
<tr>
<td>High sensitivity</td>
<td></td>
</tr>
<tr>
<td>High specificity</td>
<td></td>
</tr>
<tr>
<td>Can detect rifampicin resistance</td>
<td></td>
</tr>
<tr>
<td>Same machine can also be used for HIV, hepatitis C diagnoses / viral load monitoring</td>
<td></td>
</tr>
<tr>
<td>Can work on many extrapulmonary TB samples</td>
<td></td>
</tr>
</tbody>
</table>

### Disadvantages

<table>
<thead>
<tr>
<th>Disadvantage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Reliant on electricity</td>
<td></td>
</tr>
<tr>
<td>Expensive</td>
<td></td>
</tr>
<tr>
<td>Cannot be used to track treatment progress</td>
<td></td>
</tr>
<tr>
<td>Requires annual calibration</td>
<td></td>
</tr>
<tr>
<td>Cartridge shelf-life only 18 months</td>
<td></td>
</tr>
</tbody>
</table>
For Different Test Volumes

- **1 module**
- **4 module**
- **16 module**
- **48 module**

- **I**
  - Primary care setting
  - Health care professionals but no trained lab personnel, self-testing

- **II**
  - District hospital/laboratory
  - Technicians and assistants

- **III**
  - Regional/provincial/specialized laboratories
  - Specialists/senior technicians

- **IV**
  - National reference laboratory
  - Senior health specialists
Samples per Shift

- 5
- 20
- 80
- 500-1000
## Costs

<table>
<thead>
<tr>
<th>Item</th>
<th>Unit Cost (USD)</th>
<th>Shipping Cost (USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneXpert 2 Module with: Laptop / Desktop</td>
<td>12,280 / 11,780</td>
<td>600</td>
</tr>
<tr>
<td>GeneXpert 4 Module with: Laptop / Desktop</td>
<td>17,500 / 17,000</td>
<td>600</td>
</tr>
<tr>
<td>GeneXpert 16 Module with: Laptop / Desktop</td>
<td>71,500 / 71,100</td>
<td>700</td>
</tr>
<tr>
<td>Annual Calibration kit (XPERTCHECK-CE-5)</td>
<td>450</td>
<td></td>
</tr>
<tr>
<td>Test Cartridge</td>
<td>9.98 *</td>
<td>1.28</td>
</tr>
</tbody>
</table>

*9.98 USD is concessional cost. Market price is 50 - 150 USD.*
Xpert Omni

- Same test platform as Xpert MTB/Rif
- Single-module
- Battery powered
- Robust, for use in remote settings
- Cost of unit = $5315
- Surcharge of $1.50 / cartridge = $11.50
Xpert XDR / Extended Platform

- Tests for resistance to:
  - $rpoB \rightarrow$ Rifampicin
  - $katG \rightarrow$ High level isoniazid
  - $inhA \rightarrow$ Low level isoniazid and ethionamide
  - $gyrA$ & $gyrB \rightarrow$ Fluoroquinolones
  - $rrs \rightarrow$ Kanamycin, amikacin and capreomycin
  - $eis \rightarrow$ Kanamycin and amikacin

<table>
<thead>
<tr>
<th>Gene target</th>
<th>DNA Sequencing</th>
<th>Mutation</th>
<th>No mutation</th>
<th>Sensitivity % (95% CI)</th>
<th>Specificity % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$katG$¹</td>
<td>Invest assay</td>
<td>Mutation</td>
<td>120</td>
<td>0</td>
<td>97.5 (92.4-99.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No Mutation</td>
<td>3 (2)</td>
<td>181</td>
<td></td>
</tr>
<tr>
<td>$inhA$ promoter</td>
<td></td>
<td>Mutation</td>
<td>33</td>
<td>0</td>
<td>97.1 (82.9-99.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No mutation</td>
<td>1</td>
<td>271</td>
<td></td>
</tr>
<tr>
<td>$gyrA$²</td>
<td>Invest assay</td>
<td>Mutation</td>
<td>89</td>
<td>0</td>
<td>95.7 (88.7-95.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No mutation</td>
<td>4 (4)</td>
<td>211</td>
<td></td>
</tr>
<tr>
<td>$gyrB$³</td>
<td>Invest assay</td>
<td>Mutation</td>
<td>5</td>
<td>2 (1)</td>
<td>71.4 (34.0-92.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No mutation</td>
<td>2 (2)</td>
<td>290</td>
<td></td>
</tr>
<tr>
<td>$rrs$⁴</td>
<td>Invest assay</td>
<td>Mutation</td>
<td>30</td>
<td>0</td>
<td>96.7 (81.5-99.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No mutation</td>
<td>1</td>
<td>268</td>
<td></td>
</tr>
<tr>
<td>$eis$ promoter⁵</td>
<td>Invest assay</td>
<td>Mutation</td>
<td>9</td>
<td>1</td>
<td>81.8 (47.7-96.8)</td>
</tr>
</tbody>
</table>
GeneXpert rolled out as first-line diagnostic for TB in South Africa - 31 March 2011

"If a minister can do it, it can’t be that hard," said South African Health Minister Aaron Moatsoaledi, demonstrating GeneXpert test for TB.