Xpert MTB/RIF Training

Indira Soundiram

2012
A Better Way to Platform Design
Defining Molecular Diagnostics

**Any Test  Any Time  Any Sample  Any Place**

The GeneXpert Family of Systems

ICORE® Module  SmartCycler® System  GeneXpert® Module  GeneXpert® Cartridge  SmartCycler® Tube
Delivering a Better Way to Realize the Benefits of Molecular Diagnostics

ACCURATE
Results

FAST
Answers

EASY
To Use
GeneXpert Dx System Components

GeneXpert System includes:

- **Modules**
  - Thermal and optical system

- **Cartridge**
  - Self-Contained
  - Disposable

- **Computer System**
  - Software
  - Barcode scanner

- **Optional Accessories**
  - Printer
  - UPS
A micro lab: GeneXpert module + the cartridge

- Syringe Motor
- Motherboard
- I-CORE
- Module Door
- Valve Drive Motor
- Processing Chambers
- Reaction tube
- Valve Body
Automated Xpert MTB/RIF Protocol

1. Put the eluted sample in the cartridge, and then put the cartridge in the instrument.

2. The sample combines with the SPC.

3. A filter captures the sample and the SPC.

4. Ultrasonically lysed cells release nucleic acid.

5. Eluted DNA mixes with dried-down bead reagents.

6. Simultaneous PCR amplification and detection occurs.

7. Results are ready to view and print in less than 2h.
Intended Use

• Detection of *Mycobacterium tuberculosis* (MTB) and Rifampicin resistant *Mycobacterium tuberculosis*.

• Semi Quantitative in vitro Diagnostic test

• Specimen :
  - Sputum sample
  - induced sputum

• Detects
  - *rpoB* gene responsible for the resistance to Rifampicin for the wild type of *Mycobacterium tuberculosis*

• 10 cartridges per kit
The Xpert MTB/RIF Molecular Beacon Assay

5-Probes bind to wild type (do not bind to mutant sequence)
1-Probe for SPC (*Bacillus globigii*)
6-fluorescent dyes detected simultaneously

**rpoB** GENE 81 bp RIF RESISTANCE DETERMINING REGION

5'- GCACCAGCCAGCTGAGCCAAATTCATGGACCAGAACACACCGCTGCGGGTTGACACCACAAGCGGCGACTGTCGGCGCTG - 3'

3'- CGTTGTCGGTCGACTCGGTAAAGTACCTGTGGTGTTGGGCGACAGCCAACCTGGGTTCGCGGCCTGACAGCCGGAC - 5'

Molecular Beacon

Target

Hybrid
All Common \textit{rpoB} Mutations Detected

- No signal from Probe B
- Delayed signal from Probe A
- Wild-type strains - all probe signals tightly grouped
- Representative selection of wild-type strains
- Xpert MTB/RIF detects the most prevalent \textit{rpoB} mutations
- Representative selection of known mutations
Specimen collection, transport, and storage
Sputum Samples

- Subject must be seated or standing

- Rinse the patient’s mouth twice with water

- Learn the patient how to:
  - open and close the collection device
  - produce good sputum (collecting real sputum, not saliva; deep inhalation and exhalation of breath followed by cough from as deep inside the chest as possible)
  - avoid contamination of the exterior of the container (carefully spitting and closing the container);
  - collect and safely deliver the sputum to the laboratory

_Sputum is usually thick and mucoid. It may be fluid and contain pieces of purulent material. Color varies from opaque white to green. Bloody specimens will appear reddish or brown. Clear saliva or nasal discharge is not suitable as a TB specimen._

Source: CDC [www.cdc.gov/dls/ila/.../ParticipantModule3.doc](http://www.cdc.gov/dls/ila/.../ParticipantModule3.doc)
Recommendations for Xpert MTB/RIFF

- Do not collect less than 1 mL of sputum per specimen.

- Do not accept specimens with obvious food particles or other solid particulates.

- Do not leave the specimen at Room Temperature more than 3 days and (stable 4 to 10 days at 4 °C).

- Specimens should be held at 2–8 °C whenever possible including during transport to the laboratory.
Kit storage and handling
Description
Warnings and Precautions

• The performance of Xpert MTB/RIF for the detection of MTB complex has not been demonstrated from non-respiratory specimens such as blood, CSF, stool or urine.

• The performance of the Xpert MTB/RIF test has not been evaluated with specimens processed by methods other than those described in this package insert.

• Do not open the Xpert MTB/RIF cartridge lid except when adding sample.

• Do not use a cartridge that has been dropped or shaken after you have added the treated sample.
Warnings and Precautions

• Do not use a cartridge if it appears wet or if the lid seal appears to have been broken.

• Do not use a cartridge that has a damaged reaction tube.

• Each single-use Xpert MTB/RIF cartridge is used to process one test. Do not reuse spent cartridges.

• Dispose of used Xpert MTB/RIF cartridges according to your institution’s and country’s safety guidelines for hazardous material.
Materials Required but Not Provided

- Use clean, translucent, screw-capped specimen collection containers (Single use disposable plastic containers (50 ml capacity) are preferred)
- Disposable Gloves/ Coat/ Respiratory mask
- Timer

Optional

- Printer
- Additional 2 ml sterile transfer pipettes
Our results suggest that the Xpert MTB/RIF assay poses equivalent biohazard risk than the performance of a direct AFB smear.

References:
Biosafety requirements for Xpert MTB/RIF

---------Smear preparation area---------

Work on well ventilated area.
Wear gloves and lab coat.
Use BSL2 with laminar flow hood if available

Direct Sputum

1. Raw Sputum Sample
2. Add Sample Reagent & Recap
3. Decontamination Process
4. Decontaminate Container Exterior

10-20x

Decontaminated, concentrated sediment (pellet)

1. Resuspended Sediment Sample (NACL-NaOH)
2. Add Sample Reagent & Recap
3. Decontamination Process
4. Decontaminate Container Exterior

10-20x

--Cartridge preparation--

General Laboratory

Run on GeneXpert® System

Results < 2 hr

Xpert is a low risk procedure and requires the same level of precaution as for performing direct AFB sputum smear microscopy.
Preparing cartridge
Procedure

Cartridge contains all necessary reagents: Just add the sample treated with the Sample Reagent

1. Mix “Sample Reagent” with the Sample
2. Incubate for 15 minutes at room temperature (Samples are direct sputum or concentrated sediment)
3. Transfer sample into the open port of the Xpert MTB/RIF cartridge

Insert the cartridge and start the test

Handling Time = 2 MINUTES

Once cartridge is ready, test should be launched within 30 minutes.
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…
1. Inoculate media and prepare smear from deposit

2. Add 1.5ml Sample Reagent to 0.5ml deposit

3. Shake then stand 10 min.

4. Shake then stand further 5 minutes

5. Transfer the 2ml to cartridge

Begin Test…
Control Materials

- ATCC strains

- **External Positive Controls:**
  - QC organisms such as ATCC 27290 as BCG Copenhagen,

- **External Negative Controls:**
  - ATCC 2500 as *Mycobacterium avium*
  - ATCC 35776 as *Mycobacterium intracellulare*
  - ATCC 2278 as *Mycobacterium xenopii*
  - ATCC 35776 as *Mycobacterium kansasii*

External controls should be used in accordance with local, state accrediting organizations, as applicable.
Result Interpretation
Algorithm

Probe Check
  Pass
  At least 2 probes Pos
    Yes
    All positives
    MTB Detected
    Riff resistance not Detected
  No
    NB probes Pos
      1 Neg or more
      MTB Detected
      Riff resistance Detected
    No Value
  Fail
    Error
  Test Stopped
  No Value

SPC
  Pos
  Negative
  Invalid
### Semi Quantative Results

<table>
<thead>
<tr>
<th>MTB result</th>
<th>Ct range</th>
</tr>
</thead>
<tbody>
<tr>
<td>High</td>
<td>&lt;16</td>
</tr>
<tr>
<td>Medium</td>
<td>16–22</td>
</tr>
<tr>
<td>Low</td>
<td>22–28</td>
</tr>
<tr>
<td>Very Low</td>
<td>&gt;28</td>
</tr>
</tbody>
</table>

Table 1. MTB result name and $C_t$ value range
Xpert MTB/RIF Positive Result

MTB DETECTED RIF NOT DETECTED
Xpert MTB/RIF Positive Result

MTB DETECTED RIF DETECTED

Sample ID: 124967
Assay: Xpert MTB-RIF G3
Version: 3
Reagent Lot ID: 02808
Test Type: Specimen
Sample Type: Other

Notes:

Start Time: 08/12/11 10:24:01
End Time: 08/12/11 12:11:29
Status: Done
Upload Status: NA
User: TB laboratory technicia

Test Result

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Ct</th>
<th>EndPt</th>
<th>Analyte Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe D</td>
<td>0.0</td>
<td>15.0</td>
<td>NEG PASS</td>
</tr>
<tr>
<td>Probe C</td>
<td>32.5</td>
<td>104.0</td>
<td>POS PASS</td>
</tr>
<tr>
<td>Probe E</td>
<td>33.8</td>
<td>37.0</td>
<td>POS PASS</td>
</tr>
<tr>
<td>Probe B</td>
<td>32.5</td>
<td>77.0</td>
<td>POS PASS</td>
</tr>
<tr>
<td>SPC</td>
<td>28.0</td>
<td>369.0</td>
<td>NA PASS</td>
</tr>
<tr>
<td>Probe A</td>
<td>32.9</td>
<td>63.0</td>
<td>POS PASS</td>
</tr>
</tbody>
</table>
### Xpert MTB/RIF Positive Result

**MTB DETECTED RIF INDETERMINATED**

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**GeneXpert® Dx System**

**Views**

- Sample ID
- Assay: Xpert MTB-RIF O3
- Version: 3
- Reagent Lot ID: 02806
- Test Type: Specimen
- Sample Type: Other
- Notes
- Start Time: 13/12/11 06:20:07
- End Time: 13/12/11 06:06:53
- Status: Done
- Upload Status: NA
- User: TB laboratory technician

**Result View**

- **Primary Curve**

**Test Result**

<table>
<thead>
<tr>
<th>Analyte Name</th>
<th>Ct</th>
<th>EndPt</th>
<th>Analyte Result</th>
<th>Probe Check Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probe D</td>
<td>37.9</td>
<td>49.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe C</td>
<td>36.8</td>
<td>99.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe E</td>
<td>39.1</td>
<td>32.0</td>
<td>NEG</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe B</td>
<td>38.3</td>
<td>52.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
<tr>
<td>SPC</td>
<td>35.4</td>
<td>211.0</td>
<td>NA</td>
<td>PASS</td>
</tr>
<tr>
<td>Probe A</td>
<td>36.4</td>
<td>72.0</td>
<td>POS</td>
<td>PASS</td>
</tr>
</tbody>
</table>

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**Legend**

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

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### Chart

- **Fluorescence vs Cycles**

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Xpert MTB/RIF Invalid Result

Test Result: INVALID

Analyte Name | Ct  | EndPt | Analyte Result |
-------------|-----|-------|----------------|
Probe D      | 0.0 | 1.0   | INVALID        |
Probe C      | 0.0 | 0.0   | INVALID        |
Probe E      | 0.0 | 0.0   | INVALID        |
Probe B      | 0.0 | 2.0   | FAIL           |
SPC          | 0.0 | 2.0   | PASS           |
Probe A      | 0.0 | 0.0   | PASS           |

Legend:
- ✔ Probe D; Primary
- ✔ Probe C; Primary
- ✔ Probe E; Primary
- ✔ Probe B; Primary
- ✔ SPC; Primary
- ✔ Probe A; Primary

Notes:
- Inhibited

Start Time: 1/6/2010 15:04:16
End Time: 1/6/2010 16:32:16
Status: Done
Error Status: OK
User: [User Name]
SW Version: [Version Number]
Xpert MTB/RIF Troubleshooting
Reasons to Repeat the Assay

- **INVALID**: Result indicates that the SPC (Internal Control) failed. The PCR was inhibited due to PCR inhibitors (pus, blood or food particles presence).

- **ERROR 5006/5007/5008**: Result indicates that the Probe Check control failed: This is mainly linked to the sputum viscosity and/or volume; the reaction tube being filled improperly, or probe integrity problem detected.

- **ERROR 2008**: Pressure exceeds the maximum pressure allowed or GeneXpert module failure. If this happens randomly, this is mostly linked to the sample viscosity.

- **NO RESULT**: indicates that insufficient data were collected. For E.g, the test in progress has been stopped voluntarily or due to electrical failure.