Xpert MTB/RIF Ultra: Understanding this new diagnostic and who will have access to it

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DLS
Disclosures

• None
Objectives

• Provide examples of the steps required for implementation of the MTB/RIF Ultra into the testing algorithm of the local laboratory.

• Describe the different reporting categories for the MTB/RIF Ultra test and how the result will impact the reflex referral or additional local testing.

• Discuss the availability of MTB/RIF Ultra across the region.
Understanding Drug Resistant Tuberculosis

Drug Resistant (DR)-TB

Multidrug Resistant (MDR) TB

Extensively Drug Resistant (XDR) TB

Resistant to Isoniazid + Rifampin

MDR TB + resistant to fluoroquinolone & second-line injectable
The Laboratory is Essential

- Laboratory is a critical partner in the diagnosis of TB
- Rapid, reliable results are essential for early detection of MTBC to prevent ongoing transmission
- Drug susceptibility test results identify drug resistance and help guide the clinician in providing appropriate treatment
- Laboratory results important for monitoring patient response to therapy
Cepheid® Xpert MTB/RIF

- Automated commercial system for identification of MTBC and mutations in \textit{rpoB}
- Uses real-time PCR with molecular beacons
- Decontamination, digestion, DNA extraction, amplification, and detection in same cartridge; limited biosafety requirements
- Results in ~2 hours
- Minimal hands on manipulation - technically simple
Cepheid® Xpert MTB/RIF Ultra

- Higher sensitivity especially for smear negatives & HIV-associated TB
- Improved to eliminate some issues with determination of RIF resistance
- Chemistry and analysis a little different from MTB/RIF
- Phased approach for replacement of MTB/RIF with Ultra
What is different about MTB/RIF Ultra?

- For ID, now has two additional targets
  - IS1081 & IS6110
- Four probes bind to the region of interest in *rpoB* instead of five
- Has a new reporting category of “trace”
- Uses melt analysis
  - Allows for differentiating between wild-type and mutations
    - Wild type is the normal (i.e., predominant) gene sequence at a specific locus

**Principle of detection**

Melting curve analysis: if a mutation is present, dsDNA (probe + TB DNA) dissociates sooner than if ‘normal’ DNA present.
**Xpert MTB/RIF Ultra is Faster and More Sensitive than Xpert MTB/RIF**

<table>
<thead>
<tr>
<th></th>
<th>Xpert MTB/RIF</th>
<th>Xpert MTB/RIF Ultra*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limit of Detection</strong></td>
<td>114 cfu/ ml</td>
<td>16 cfu/ ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>“MTB Detected, trace”</td>
</tr>
<tr>
<td><strong>Sensitivity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All TB</td>
<td>83%</td>
<td>88%</td>
</tr>
<tr>
<td>HIV-Infected</td>
<td>76%</td>
<td>88%</td>
</tr>
<tr>
<td>Pediatric</td>
<td>47%</td>
<td>71%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All TB</td>
<td>98%</td>
<td>95%</td>
</tr>
<tr>
<td>No History of TB</td>
<td>98%</td>
<td>96%</td>
</tr>
<tr>
<td>Any History of TB</td>
<td>97%</td>
<td>92%</td>
</tr>
<tr>
<td><strong>Test Time</strong></td>
<td>&lt; 2 hours</td>
<td>65–77 minutes</td>
</tr>
</tbody>
</table>

*Data from FIND multicenter study of adults with presumptive pulmonary TB & retrospective EPTB/ pediatric evaluations.*
Summarizing Results From FIND Evaluation

- 1,520 persons presumed to have TB
- Ultra non-inferior to MTB/RIF
- Overall Ultra sensitivity 5% higher than MTB/RIF
  - Performance particularly improved for smear negative/culture positive samples and for those from HIV+
- Low limit of detection also allows for detection of low numbers of bacteria
  - Could also detect non-viable bacilli from previous TB episode
- Similar very good performance for detection of RIF resistance
  - Ultra had better differentiation between silent and resistance conferring mutations

Xpert® MTB/RIF
- 25μl PCR reaction
- 5 Probes bind rifampicin-resistance determining region of WT DNA
- Detection based on Cycle Threshold Analysis

Xpert® MTB/RIF Ultra
- 50μl, fully-nested PCR reaction
- 4 Probes bind 2 multi-copy DNA targets & rifampicin-resistance determining region of ALL DNA
- More rapid thermal cycling
- Improved fluidics & enzymes
- Detection based on High Resolution Melt Analysis

Slides courtesy of Patricia Campbell, PhD- CDC DGHT/ILB
Who in the region will have access to Ultra?

- CE marked—not yet FDA approved
- Not yet available for CLIA-regulated labs (Guam, CNMI, and American Samoa)
  - Labs will still be able to purchase and use MTB/RIF (1st generation test)
- RMI, FSM, and Palau should have it soon
  - Available through HBDC program
  - Pricing remains the same ($9.98/test)
Intended Use of Xpert MTB/RIF Ultra

- Use with specimens from untreated patients for whom there is clinical suspicion of TB
- Should have not yet received anti-tuberculosis therapy, or less than 3 days of therapy in the last 6 months
- Aids in diagnosis of pulmonary tuberculosis
- Serves as one tool that should be used in conjunction with clinical and other laboratory findings (e.g., culture from DLS)
What do I need to know about the Ultra kits?

- Kits have 10 or 50 test cartridges
- Kits come with a CD, test cartridges, transfer pipettes, and the sample reagent
- Similar to the MTB/RIF, the maximum storage temperature is 28°C
Considerations for Performing the Test

- Only open the lid of the cartridge when you are ready to add the sample
- Close the lid after sample addition
- Test must be performed within 4 hours of adding the sample
- Do not shake the cartridge
- Do not use a cartridge that appears damaged or has been dropped
Considerations for Performing the Test (2)

- Reminder: Test cartridges and transfer pipettes can only be used one time
- Do not use expired reagents
- Avoid stock outs
  - Keep sufficient stocks on hand for 3 months of testing
- Good quality specimen of sufficient volume is key
Performing the Test

**Cartridge Preparation – Unprocessed Sputum**

1. Obtain one Xpert cartridge, sample reagent (SR), and sputum collection container for each sample.
   - Note: Minimum sputum volume for each test is 1 mL

2. Estimate volume of sputum. Add 2 volumes of SR to 1 volume of Sputum. Replace container lids.
   - Shake vigorously 10 to 20 times or vortex for at least 10 seconds.
   - Incubate at room temperature for 10 minutes.
   - Shake or vortex again vigorously 10 to 20 times.
   - Incubate for another 5 minutes. **See Note below.**
   - *One back and forth motion equals one shake*

3. Close the lid firmly. Start the test within the time frame specified in the package insert.
   - Sputum volume for each test is 1 mL

4. Bring the cartridge to room temperature. Write on the side of the cartridge or affix an ID label. Open the cartridge lid.

5. Aspirate the liquefied sample just above the line on the pipette.

6. Gently empty the sample into the sample chamber of the cartridge.

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www.Cepheid.com (slides adapted from Cepheid Training Center)
How do I prepare for using this test in my lab?

- Uses same instrument already in place
- Procure Ultra cartridges
- Upgrade software (4.7b or higher) and upload a new assay definition file before performing Ultra for the first time
  - Contact Cepheid for assistance
- Retrain all staff on new test and reporting characteristics
How do I prepare for using this test in my lab? (2)

- Finish using MTB/RIF cartridges already in stock (within expiry date)
- Update SOPs and job aids
- Will need to perform a verification study before testing patient samples with Ultra
  - Can be a limited set of samples for non-CLIA regulated labs
  - Could use proficiency testing panels or external controls that are commercially available
  - A verification protocol and final report should be prepared
What are the potential results for MTB/RIF Ultra?

- Semi-quantitative value just like MTB/RIF with one exception

![Table showing MTB/RIF Ultra results](https://www.slideshare.net/SystemOneUS/xpert-mtbrif-ultra)
What are the potential results for MTB/RIF Ultra?

- Semi-quantitative value just like MTB/RIF with one exception

![MTB not detected = neither of multi-copy target probes are positive but SPC is positive (valid)

MTB detected = one or both probes for multi-copy target are positive and at least two rpoB probes positive](https://www.slideshare.net/SystemOneUS/xpert-mtbrif-ultra)

<table>
<thead>
<tr>
<th>Category</th>
<th>MTB/RIF</th>
<th>Xpert Ultra</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not detected</td>
<td>X</td>
<td>X</td>
<td>No TB detected</td>
</tr>
<tr>
<td>High</td>
<td>X</td>
<td>X</td>
<td>TB detected</td>
</tr>
<tr>
<td>Med</td>
<td>X</td>
<td>X</td>
<td>TB detected</td>
</tr>
<tr>
<td>Low</td>
<td>X</td>
<td>X</td>
<td>TB detected</td>
</tr>
<tr>
<td>Very low</td>
<td>X</td>
<td>X</td>
<td>TB detected</td>
</tr>
<tr>
<td>Trace</td>
<td></td>
<td>X</td>
<td>Trace amounts MTB detected</td>
</tr>
</tbody>
</table>
The MTB target is present within the sample:

- IS1081-IS6110 signal is detected.
- RIF resistance cannot be determined due to insufficient signal detection.
- SPC: NA (not applicable). An SPC signal is not required because MTB amplification can compete with this control.
- Probe Check: PASS

A Trace result call means that low levels of MTB are detected but no RIF resistant result is detected. The increased sensitivity

A TRACE result is always RIF Resistance INDETERMINATE
Understanding the “trace” result

- Increased sensitivity but also reduced specificity
  - More likely to pick up paucibacillary contamination (i.e., cross contamination)
  - Found as possible cause of false-positive results in FIND study
  - To avoid potential cross contamination, labs must thoroughly clean work surfaces and the instrument.

- Result is detection of lowest bacillary burden but could be false positive
  - If trace result is detected, repeat testing with a new specimen
    - If 2\textsuperscript{nd} test is positive with trace or higher semi-quantitative result= TB positive
Proposed Ultra Algorithm

1. Collect 3 sputa (spot, morning, spot)
2. Perform AFB by ZN
3. Perform MTB/RIF Ultra on first specimen
4. Record and report all results.
5. Ship the 2 remaining (not tested by Xpert) sputa to DLS for all from high risk & those testing positive from low risk category
6. If Xpert result is “trace” for MTB detected, request a new sample and perform MTB/RIF again
Interpreting the “Trace” Result

- For PLHIV and children who are being evaluated for pulmonary TB, or when extrapulmonary specimens (CSF, lymph nodes and tissue specimens) are tested ‘MTB detected trace’ results in one or both samples should be considered as bacteriological confirmation of TB (i.e., true positive results)
Interpreting the “Trace” Result (2)

- For adults being evaluated for pulmonary TB who are not at risk of HIV, need to know prior history of TB
  - If no history, ‘MTB detected trace’ results in both Ultra tests should be considered as bacteriological confirmation of TB (i.e., true positive results)
    - If the second Ultra test result is ‘MTB not detected’, consider the possibility that the first Ultra test result was a false positive-use clinical judgement
  - If known history of prior TB, possibility of false positive Ultra result- use clinical judgement

Will results be reported differently for Ultra?

- No differences with exception of “trace” result
- Clinician should know the semi-quantitative result of “trace” because of potential for false-positive
- Reporting form does not currently have an area for reporting this value
- Until reporting forms are modified, write “trace” when detected on the right-hand side of the form beside the sample number

<table>
<thead>
<tr>
<th>Lab Serial No</th>
<th>Spec Type</th>
<th>Date of Collection</th>
<th>Date of Exam</th>
<th>Vol of Specimen (ml)</th>
<th>Visual Appearance</th>
<th>AFB Smear Result</th>
<th>Xpert MTB/RIF Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**RESULTS** (to be completed by local laboratory)

Example: Ohio State University

trace

```
0.5 ml
```
### Results for Rifampin Resistance

<table>
<thead>
<tr>
<th>Semi-quantitative category</th>
<th>Result Xpert MTB/RIF</th>
<th>Result Xpert MTB/RIF Ultra</th>
</tr>
</thead>
<tbody>
<tr>
<td>High, medium, low</td>
<td>Rif resistance detected/Rif resistance not detected</td>
<td>Rif resistance detected/ Rif resistance not detected/indeterminate</td>
</tr>
<tr>
<td>Very low</td>
<td>Rif resistance indeterminate</td>
<td>Rif resistance detected, not detected/ indeterminate</td>
</tr>
<tr>
<td>Trace</td>
<td></td>
<td>Rif resistance indeterminate</td>
</tr>
</tbody>
</table>
Training and Education

- Plan and prepare before implementation
  - Revised algorithm
  - New reporting categories
  - Interpretive criteria- especially for trace result for Mtb detected
  - Revised reporting form
- Include everyone- laboratorians, program, nurses, clinicians
GeneXpert Omni: A Point-of-Care Device!

- Single-module GeneXpert machine
- Compatible with all Xpert tests (and times!)
- Portable with locking dock for security
- Battery-powered
- Resistant to dust, vibration, motion
- Locked mobile phone interface
  - Device set-up and control
  - Test result display
  - Automatic connection to Cepheid’s cloud-based connectivity solution, C360
- Projected Release: After Q3 2017

Photo Credit: FINDDx.org
Acknowledgements

- Patricia Campbell, PhD/ CDC DGHT, ILB
- Heather Alexander, PhD/ CDC DGHT, ILB