Democratizing Molecular Diagnostics: The GeneXpert MTB\textsuperscript{r} test

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Some Characteristics of an Ideal TB test

• Direct-specimen detection of MTB
• Sensitivity of culture with goal of eliminating negative cultures
• Simultaneous detection of drug resistance
• On-demand availability (no batching requirement)
• Decentralized Platform technology to reduce or eliminate sample shipping
• Rapid (<2 hours)
• Portable
• Low skill requirements
• Highly reliable

A lack of reliable tests slows fight against TB

By Lawrence K. Altman

In the escalating battle against extensively drug-resistant tuberculosis, conflicting findings from laboratory tests have hampered efforts to control the spread of the disease.

Some of the conflicts come from a lack of standardized testing methods and others from subtle but critical differences in the way the tests are performed.

The most celebrated example of such discordant findings involved Andrew Speaker, the Atlanta lawyer who ceased an international health care after traveling to Europe in May with what was believed to be extensively drug-resistant tuberculosis, known as XDR. That same month, Speaker’s doctors downgraded his type of tuberculosis to multidrug-resistant, or MDR, after retesting showed the bacteria caused different results for such procedures made by a similar agency panel in 2005.

The overwhelming majority of tuberculosis cases are caused by bacterial strains that yield to the standard or first-line, and MTB drugs. Newer second-line drugs are used if a strain of tuberculosis is MDR or XDR, which are resistant to the first-line drugs. If tuberculosis strains are not tested for drug resistance as soon as they are found in a patient, the problem may be detected too late to permit a cure.

Tuberculosis resistance develops when drugs are missed or misused. For example, patients may fail to complete their full course of treatment. Health care providers may prescribe the wrong treatment or the wrong dose or the wrong length of time for taking the drugs. Another problem occurs when drugs are not available, or when the drugs are of poor quality.

What alert health officials in the oratories use pure powders of an antituberculosis drug’s active ingredient. But even such powders can be affected by heat and other factors, leading to inconsistent findings.

Another reason is that the many steps involved in the laboratory process increase chances for human error.

In 2001, the WHO panel said the latest knowledge was “very incomplete regarding how to best perform” resistance tests of second-line drugs and the usefulness of the tests in treating such cases. Since then, experience has shown that the tests are not performed.

If TB strains are not tested for drug resistance as soon as they are found, it may be too late for a cure.

(Oh, and impossibly cheap)
PCR testing in my Mayo lab, circa 1994

- First demonstration of direct-specimen detection of TB and Rif resistance, but it took:
  - A herculean effort involving nested PCR and direct sequencing
  - High Complexity Lab
  - 4000 Square Feet
  - 3 hermetically sealed rooms
  - Specially trained medical technologists, 2 MD fellows, 1 PhD Director
  - HEPA filtered air for each room (in and out)
  - 3-5 day TAT (better than weeks to months of waiting for bugs to grow)
PCR Contamination Control Basics - Dedicated Air Handling
GeneXpert®: PCR Lab in a Disposable Cartridge

- Critical interface between *macrofluidic* requirements of sample processing with *microfluidics* of PCR
- Room-temp stability of reagents within lyophilized beads
- Contamination Control via enclosed, real-time PCR
- Universal sample prep
  - Sputum, stool, blood, BAL, swabs
- Built-in assay controls

Patents: 6,374,684 & 6,391,541
GeneXpert®: Cartridge and Module

- Syringe motor drives fluid movement
- Valve motor directs access to chambers
- Independent thermal cycler allows for random-access design
- Adaptable to different Dx environments
GeneXpert® System Scalability

- GX-I
- GX-IV
- GX-XVI
- GeneXpert Infinity-48 System
Cepheid Systems in the US Post Office for Anthrax

Largest US Biothreat Detection Program (that you probably don’t know about)
- Deployed for 3 years in 265 postal sorting centers throughout the US
- 100 billion letters screened to date
- 7 million cartridges used
- 30-50 spore detection sensitivity
- Zero false positives to date, due to two-target assay design and control of amplicon contamination by containment in the cartridge

How postal germ detectors work

1. Air drawn over mail entering the processing center is diverted to analysis cabinet.
2. Particles leaking from mail are captured and injected into canister of sterile water.
3. Every hour, machine tests the water canisters for anthrax DNA.
4. If anthrax is found, alarm is sounded before contaminated mail leaves the building.

Source: U.S. Postal Service

LaMont W. Harvey | Sun Staff
Gates Foundation/NIAID Support for the GeneXpert TB Project

- Project Initiated in 2006
- FIND Support: $6.2 M
- NIAID support: $4.3 M
- Collaborator: David Alland, MD
Sputum.....the final PCR frontier

- It is usually highly viscous and thus incompatible with microfluidic devices
- It is often purulent
- It is often bloody
- Target organisms require concentration in order to be consistently detected
- Complicated off-line centrifugation and DNA extraction too slow and technically demanding for decentralized testing
DNA molecules are mixed with dry PCR reagents

Sample is automatically filtered & washed

Concentrates bacilli & removes inhibitors

Ultrasonic lysis of filter-captured organisms to release DNA

DNA molecules are mixed with dry PCR reagents

Sputum liquefaction & inactivation with 1:1 SR

Transfer of 2 ml after 15 min

End of hands on work

Time-to-result: 1 h 45 min

Nested real-time amplification & detection in integrated reaction tube
Genetics of Rifampin Resistance in *M. tuberculosis*

**rpoB**

- **Deletion**
  - AATTCATGG
  - GACCAG
  - GAACAA
- **Deletion**
  - CCATTC
  - CAGAAC
- **Insertion**
  - TTC
  - TTCATG
- **Deletion**
  - GGCACC
- **Deletion**
  - AAC
- **Deletion**
  - GACCAG
- **Deletion**
  - AATTCATGG
- **Deletion**
  - GAACAA

81 base pair core region
Example of Rif-Sensitive Profile – 5 probes are positive

The MTB assay target is the 81 bp region (RRDR) of the rpoB gene.

Each probe is labeled with a different fluorophore, permitting simultaneous detection of the presence of wild type.
Potential Advantages of Nested PCR

- Replenishment of enzyme activity when it is most needed (late cycles)
- Reduced nonspecific amplification (especially important when genetic background is complex)
- Dilution of reaction inhibitors, resulting in:
  - 2-3 log sensitivity improvement
  - Consistent, high level product accumulation for more robust product analysis (sequencing, Beacons)
- However, almost all of these advantages are outweighed by the extreme risk of false positivity
Fully enclosed, nested PCR amplification in the GeneXpert
Performance Improvement with Self-Contained Nested PCR

Fluorescence

PCR Cycles
Limit of detection (LOD) of *M. tuberculosis* DNA

Based on 20 replicates per concentration tested
All common \textit{rpoB} mutations detected by a Ct delay* in at least one probe

*For Rifampicin-resistant mutants, delta Ct between the earliest & latest Ct values is greater than 3.5 Ct.
Assay specificity. No NTM detected as *M. tuberculosis* using at least two probes positive with “2 cycle” rule.

10⁶ cfu/mL of each NTM spiked into 1 mL sputum
Relationship between number of added *M. tuberculosis* cells and *rpoB* signal (A) or internal control signal (B)
Semi-Quantitative results on the GeneXpert

- **MTB NEG**: 18 samples
- **VERY LOW**: 16 samples
- **LOW**: 10 samples
- **MEDIUM**: 6 samples
- **HIGH**: 4 samples

Legend:
- Smear Neg.
- Scanty
- 1+
- 2+
- 3+
Evaluation partner sites

Trial sites:
- Evaluation
  - Germany
  - Azerbaijan
  - India
  - South Africa
  - Peru

Evaluation partner sites: NRL, STI, Hinduja, UCT, SAMRC, STELLENBOSCH UNI.
## Development study in Peru & Latvia

### Per patient analysis (4 cultures, 3 ZN, 3 Xpert)

<table>
<thead>
<tr>
<th>TB CASE DETECTION</th>
<th>Xpert sensitivity in smear+, cul+</th>
<th>Xpert sensitivity in smear-, cul+</th>
<th>Xpert specificity in smear-, cul- *</th>
</tr>
</thead>
<tbody>
<tr>
<td>LATVIA</td>
<td>93.3% (14/15)</td>
<td>92.3% (12/13)</td>
<td>98.6% (71/72)</td>
</tr>
<tr>
<td>PERU</td>
<td>100% (99/99)</td>
<td>81.8% (9/11)</td>
<td>96.7% (148/153)</td>
</tr>
<tr>
<td>TOTAL 95% CI</td>
<td>99.1% (113/114)</td>
<td>87.5% (21/24)</td>
<td>97.3% (219/225)</td>
</tr>
<tr>
<td></td>
<td>[95.2, 99.8]</td>
<td>[69.0, 95.7]</td>
<td>[94.3, 98.8]</td>
</tr>
</tbody>
</table>

- Rate of invalid Xpert results: 3% (24/786 cartridges)

*1 follow-up visit at 2-4 m
## Development study in Peru & Latvia

*Per patient analysis (4 cultures, 3 ZN, 3 Xpert)*

<table>
<thead>
<tr>
<th>RIF RESISTANCE DETECTION</th>
<th>Xpert sensitivity in Rif resistant</th>
<th>Xpert specificity in Rif sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LATVIA</td>
<td>100% (8/8)</td>
<td>100% (18/18)</td>
</tr>
<tr>
<td>PERU</td>
<td>100% (14/14)</td>
<td>100% (94/94)</td>
</tr>
<tr>
<td>TOTAL 95% CI</td>
<td>100% (22/22) [85.1, 100]</td>
<td>100% (112/112) [96.7, 100]</td>
</tr>
</tbody>
</table>

- Rate of invalid Xpert results: 3% (24/786 cartridges)
Preliminary Conclusions – Xpert TB Project

• The GX MDR TB assay generates TB ID results in less than 2 hours directly from sputum
• It simultaneously detects rifampin resistance
• Sputum processing is simple and does not require a centrifuge
• It can be performed by personnel with minimal training
• It does not require dedicated lab space
• It can be run on-demand (not batched) to generate real-time results
• It will be available outside the US in April/May 2009 (CE mark)
“What works for the third world, also works for the third shift”