Evaluation of the Cepheid Xpert MTB/RIF Assay for Direct Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens

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A total of 217 specimens submitted for routine smear and culture from three different sites within the western United States were used to evaluate the GeneXpert MTB/RIF assay (for research use only) (Cepheid, Sunnyvale, CA). Overall agreement compared to culture was 89% (98% for smear positives and 72% for smear negatives) for detection of *Mycobacterium tuberculosis*.

The ability to accurately detect *Mycobacterium tuberculosis* directly from clinical specimens with nucleic acid amplification (NAA) tests provides significant advantages for the management and infection control of the disease (6). In the United States, only two FDA-approved NAA tests, the Roche Amplicor Mtb PCR (PCR) and Gen-Probe MTD (MTD) assays have been available. The Roche Amplicor Mtb PCR assay was discontinued in early 2010, leaving many laboratories with only one commercially available option. While both assays have provided benefits, neither has offered a practical solution for all levels of clinical and public health laboratories; hence, specimens are often referred to reference laboratories.

Cepheid has recently introduced the GeneXpert MTB/RIF assay (for research use only) (Cepheid, Sunnyvale, CA) (4). The GeneXpert assay is a real-time PCR test that will simultaneously identify *M. tuberculosis* and detect rifampin resistance directly from clinical specimens (10). Rifampin resistance can serve as a marker for multidrug-resistant tuberculosis (MDR-TB) and has been reported in >95% of the multidrug-resistant *M. tuberculosis* isolates (2, 8, 12). The GeneXpert assay detects an 81-bp "core" region of the *rpoB* gene. The test utilizes five molecular beacons that detect mutations in the core region that are associated with rifampin resistance.

To date, studies assessing the GeneXpert assay have focused entirely on specimens from patients tested outside the United States (3, 4). The objectives of this multisite study were to evaluate the performance of the GeneXpert assay for the direct detection of *M. tuberculosis* in respiratory specimens from patients from within the United States and to evaluate the ability of the assay to detect rifampin resistance in a low-prevalence population.

Two hundred seventeen specimens (126 acid-fast smear positive and 91 smear negative) ordered for routine mycobacterial testing were included in the study. Specimens were collected and tested at three different sites in the western United States. Specimens that could not be tested immediately after processing were stored at −80°C.

The N-acetyl-l-cysteine–NaOH (NALC-NaOH) method was used to digest, decontaminate, and concentrate respiratory specimens (11). At the Southern California Permanente Regional Laboratories, NAC-Attack (Remel, Lenexa, KS) was used according to manufacturer’s instructions to digest and decontaminate specimens. Kaiser NW and San Francisco Department of Public Health Laboratories reagents were prepared in house according to standard methods (11). A smear of the processed sediment was prepared, stained, and read, and results were reported according to published standards (11). *Mycobacterium* isolates were identified by using high-performance liquid chromatography, gas-liquid chromatography, DNA probes (Gen-Probe, Inc., San Diego, CA), and/or biochemical tests. All molecular testing was performed on processed sediment. Antimycobacterial susceptibility testing was performed by a broth microdilution method.

The GeneXpert assay was run according to the manufacturer’s instructions on all 217 samples. Alternative nucleic acid amplification testing by the Roche Amplicor Mtb PCR (Roche Diagnostics, Indianapolis) (67 samples) and Gen-Probe MTD (Gen-Probe, Inc., San Diego, CA) (51 samples) assays was performed according to the manufacturer’s instructions on a subset of specimens as specimen volume and resources permitted. Processed specimens were diluted 1:1 prior to testing with the MTD assay to bring the final concentration of NaOH in the sample down to a level of approximately 1%. This was done to minimize the inhibitory effect of the NaOH, which according to the manufacturer if >1.5% can inhibit detection of *M. tuberculosis* (9).

A summary of the performance data is shown in Table 1. Of the 217 specimens tested, 130 were positive by culture (liquid or solid), resulting in an 89% agreement with the GeneXpert assay. The GeneXpert assay had a 98% agreement for culture-positive, smear-positive specimens, and 72% agreement for culture-positive, smear-negative specimens for the detection of *M. tuberculosis*. When the culture-positive results were evalu-
ated by smear result, there was 96% agreement for specimens with smear results of 1+/2+ (i.e., “rare/few”) and 100% agreement with those that had smear results of 3+/4+ (“moderate/numerous”). One bronchial specimen, which was smear, culture, and MTD negative, demonstrated inhibition with the GeneXpert assay and PCR and is not included in Table 1.

Previously, the sensitivity of the GeneXpert system in detecting smear-positive specimens has been reported to be 98 to 100% and detection of rifampin resistance to be 98% in populations outside the United States with high rifampin resistance (4, 10). Overall in this study, the sensitivity was 99% for the direct identification of *M. tuberculosis* from sputum and bronchial specimens, which rose to 98% in smear-positive specimens. Fourteen specimens that were culture positive tested negative by the GeneXpert assay (Table 1). Twelve of these 14 specimens were smear negative, and two had a smear result of “rare” or “few.” This is an understandable result as it has been shown that smear results, which are indicative of organism burden, are less sensitive than culture and require 5 × 10^5 to 1 × 10^6 bacilli/ml of specimen to generate a positive result (1). With regard to specificity, four specimens that were GeneXpert positive and culture negative (Table 1) were true positives. Three of four were from patients treated for tuberculosis and likely represented the detection of nonviable organisms. The other specimen was from a patient that had subsequent positive cultures. Three of four of these specimens were positive by at least one other NAA test.

The analytical limit of detection of the GeneXpert assay is reported to be 131 CFU/ml of specimen, based on spiked-sputum studies (3). Culture of concentrated specimens can detect very low concentrations of organisms—as low as 10 to 100 CFU/ml (1). When testing at the lower limits of any assay, variability is to be expected due to factors such as sampling. As has been suggested previously, because we used frozen archived specimens, the freeze-thaw cycle may have altered sputum viscosity, improving mycobacterial nucleic acid recovery (10).

When the culture-positive results are stratified by smear results, the GeneXpert assay demonstrated a sensitivity of 72% among the smear-negative results. The limited data set shown herein suggests that the GeneXpert assay is comparable to the MTD assay for the direct detection of *M. tuberculosis* from smear-negative specimens (9). Our results are consistent with those reported by others (4, 5, 10). A limitation to our analysis is the lack of complete information as to when in the course of disease the respiratory specimens were collected.

A total of 41 nontuberculosis mycobacteria (MOTT) grew from the specimens tested, which included *Mycobacterium avium* (n = 18), *Mycobacterium chelonae* (n = 9), *Mycobacterium kansasii* (n = 6), *Mycobacterium gordonae* (n = 2), *Mycobacterium fortuitum* (n = 4), *Mycobacterium xenopi* (n = 1), and *Mycobacterium abscessus* (n = 1). No cross-reactivity was observed in any of the 41 specimens. One specimen contained *Mycobacterium bovis* and was correctly identified as *M. tuberculosis* complex by the GeneXpert assay.

MDR-TB is defined as resistance to at least the two major drugs used to treat tuberculosis, isoniazid and rifampin. The incidence of MDR-TB is low in the United States (7). For all specimens tested, no rifampin-resistant isolates were identified by previous culture-based antimycobacterial susceptibility testing. However, the GeneXpert assay reported three specimens as containing rifampin-resistant *M. tuberculosis*. Two of these specimens were subsequently subjected to liquid culture and were again tested with the GeneXpert assay. Both liquid cultures tested as rifampin susceptible by the GeneXpert assay. The third specimen repeated as rifampin resistant on GeneXpert testing, despite sequencing of the *rpoB* gene and repeat susceptibility testing that revealed the isolate was consistent with the wild-type strain (rifampin susceptible).

All three false rifampin-resistant GeneXpert results exhibited a delay in the threshold cycle (*C*<sub>T</sub>) value of probe B. Since there are five probes involved in this assay and a sophisticated algorithm to interpret results, further evaluation of probe B by the manufacturer is warranted. Additional investigation into this issue is necessary before conclusions can be drawn regarding the accuracy of rifampin resistance detection in a low-prevalence population like that in the United States.

Hands-on time to process specimens with the random access GeneXpert assay is less than 5 min per specimen, with a turnaround time (TAT) of less than 2 h. The MTD assay is run in a batch mode, with a hands-on time of less than 2 h and a TAT of 4 to 6 h. With a 10-specimen batch, the hands-on time per specimen would be less than 12 min per specimen. Since MTD does not have an internal control, some laboratories have opted to split samples to spike for inhibition. Spiking for inhibition increases assay run size and costs. The list price cost of the GeneXpert assay (RUO) is $60, and that of the MTD is $50. Overall, the GeneXpert assay is simple, fast, accurate, and

### Table 1. Comparison of GeneXpert MTB/RIF-positive, MTB culture-positive results with smear results

<table>
<thead>
<tr>
<th>Smear result (n = 216)</th>
<th>MTB culture&lt;sup&gt;+&lt;/sup&gt;, GeneXpert&lt;sup&gt;+&lt;/sup&gt;</th>
<th>MTB culture&lt;sup&gt;+&lt;/sup&gt;, GeneXpert&lt;sup&gt;-&lt;/sup&gt;</th>
<th>MTB culture&lt;sup&gt;-&lt;/sup&gt;, GeneXpert&lt;sup&gt;+&lt;/sup&gt;</th>
<th>MTB culture&lt;sup&gt;-&lt;/sup&gt;, GeneXpert&lt;sup&gt;-&lt;/sup&gt;</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Numerous (score, 4+)</td>
<td>21</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>Moderate (score, 3+)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Rare/few (score, 1+/2+)</td>
<td>52</td>
<td>2</td>
<td>3</td>
<td>20</td>
<td>77</td>
</tr>
<tr>
<td>Negative (no acid-fast bacilli seen)</td>
<td>31</td>
<td>12</td>
<td>0</td>
<td>47</td>
<td>90</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>14</td>
<td>4</td>
<td>82</td>
<td>216</td>
</tr>
</tbody>
</table>

<sup>a</sup> Smear results represent sputum and bronchial specimens combined. Note that one bronchial specimen is not included in the table. This specimen was GeneXpert MTB/RIF assay inhibitory, smear negative, culture negative, PCR inhibitory, and MTD negative.
cost-comparative to other commercially available PCR assays for the direct detection of *M. tuberculosis*.

Cepheid supplied the GeneXpert MTB/RIF assays and Gen-Probe supplied the MTD kits for this study.

REFERENCES