Evaluation of the Cepheid Xpert MTB/RIF assay for the Direct Detection of Mycobacterium tuberculosis Complex from Respiratory Specimens

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Running Title: Mycobacterium tuberculosis direct assay
Abstract:
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 [RUO] [Cepheid, Sunnyvale, CA]. Overall agreement compared to culture was
89% [98% for smear-positives and 72% smear negatives] for detecting *M. tuberculosis*.

(Abstract word count 50)
The ability to accurately detect *M. tuberculosis* directly from clinical specimens using 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 (RUO) (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 MDR-TB and has been reported in > 95% of the multi-drug resistant *M. tuberculosis* (MDR-TB) isolates (2,8,12). The GeneXpert assay detects an 81 base pair “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 of the United States (3,4). The objectives of this multi-site study were to evaluate the performance of the GeneXpert assay for the direct detection of *M. tuberculosis* in respiratory specimens of 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 and 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, read, and results reported according to published standards (11). *Mycobacterium* isolates were identified using high performance liquid chromatography, gas liquid chromatography, DNA probes (Gen-Probe Incorporated, San Diego, CA), and/or biochemical tests. All molecular testing was performed on processed sediment. Anti-mycobacterial susceptibility testing was performed by a broth microdilution method.

The GeneXpert assay was run according to manufacturer’s instructions on all 217 samples. Alternative nucleic acid amplification testing by the Roche Amplicor Mtb PCR (Roche Diagnostics, Indianapolis) (67 samples) or Gen-Probe MTD (Gen-Probe Incorporated, San Diego, CA) (51 samples) assays were performed according to 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 performance data are shown in Tables 1. Of the 217 specimens tested, 130 were positive by culture (liquid or solid) resulting in an 89% agreement with the GeneXpert. The GeneXpert 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 evaluated by smear result there was a 96% agreement for specimens with smear results of 1+/2+ (i.e., “rare”/“few”) and 100% agreement with those that were smear 3+/4+ (“moderate”/“numerous”). One bronchial specimen, which was smear, culture, and MTD negative, demonstrated inhibition with the GeneXpert 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 98-100% and detection of rifampin resistance to be 98% in population outside the United States with high rifampin resistance (4,10). Overall in this study the sensitivity was 89% 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 (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 requires $5 \times 10^3$ to $1 \times 10^4$ 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 non-viable 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 10-100 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 demonstrated a sensitivity of 72% among the smear-negative results. The limited data set shown herein suggests that the GeneXpert is comparable to the MTD assay for the direct detection of \textit{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 non-tuberculosis mycobacteria (MOTT) grew from the specimens tested, which included: \textit{M. avium} (n=18), \textit{M. chelonae} (n=9), \textit{M kansasii} (n=6), \textit{M. gordonae} (n=2), \textit{M. fortuitum} (n=4), \textit{M. xenopi} (n=1) and \textit{M. abcessus} (n=1). No cross reactivity was observed in any of the 41 specimens. One specimen contained \textit{M. bovis} and was correctly identified as \textit{M. tuberculosis} complex by the GeneXpert.

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 anti-mycobacterial susceptibility testing. However, the GeneXpert reported three specimens as containing rifampin-resistant *M. tuberculosis*. Two of these specimens were subsequently subjected to liquid culture and were again tested by the GeneXpert. Both liquid cultures tested as rifampin-susceptible by the GeneXpert. The third specimen repeated as rifampin resistant on the GeneXpert, 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 Ct 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 is less than 5 minutes per specimen with a turn around time (TAT) of less than two hours. The MTD assay is run in a batch mode with hands on time of less than 2 hours and a TAT of 4-6 hours. With a 10 specimen batch the hands on time per specimen would be less than 12 minutes 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 (RUO) is $60, and MTD is $50. Overall, the GeneXpert assay is simple, fast, accurate and cost comparative to other commercial available PCR assays for the direct detection of *M. tuberculosis*.

Cepheid supplied the GeneXpert MTB/RIF assays and Gen-Probe for supplied the MTD kits for this study.
Table 1. Xpert MTB/RIF positive, MTB culture positive results by smear result (sputum and bronchial specimens combined).

<table>
<thead>
<tr>
<th>Smear positive - numerous (4+)</th>
<th>TB culture + Xpert +</th>
<th>TB culture + Xpert -</th>
<th>TB culture - Xpert +</th>
<th>TB culture - Xpert -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td></td>
<td>35</td>
</tr>
<tr>
<td>Smear positive - moderate (3+)</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td>Smear positive – rare / few (1+ / 2+)</td>
<td>52</td>
<td>2</td>
<td>3</td>
<td>20</td>
<td>77</td>
</tr>
<tr>
<td>Smear negative (no AFB seen)</td>
<td>31</td>
<td>12</td>
<td>0</td>
<td>47</td>
<td>90</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>116</td>
<td>14</td>
<td>4</td>
<td>82</td>
<td>216</td>
</tr>
</tbody>
</table>

Note one bronchial specimen is not included in the table. This specimen was Xpert MTB/RIF inhibitory, smear negative, culture negative, PCR inhibitory and MTD negative.
Reference List


