Evaluation of the Cobas TaqMan MTB real-time PCR assay for direct detection of *Mycobacterium tuberculosis* in respiratory specimens

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The Cobas TaqMan MTB assay is a rapid identification tool with a high degree of specificity for the direct detection of *M. tuberculosis* in respiratory specimens. The sensitivity for detecting acid-fast smear-negative respiratory specimens, however, is low.

INTRODUCTION

Pulmonary tuberculosis (TB) remains a global health problem despite strenuous efforts to eradicate this disease (Wright et al., 2009). Rapid and early identification of *Mycobacterium tuberculosis* in sputum samples is essential for diagnosis and management. Non-tuberculous mycobacteria (NTM) pulmonary disease, which is also hard to distinguish from pulmonary TB clinically, is now also on the rise (Lai et al., 2010). Pulmonary diseases caused by NTM, which can arise in patients with prior pulmonary TB and which are difficult to distinguish from active pulmonary TB clinically, are also on the rise (Griffith et al., 2007; Lai et al., 2010; Lee et al., 2012a). Traditionally, the diagnosis of pulmonary TB relies on a direct sputum smear followed by culture, which has a long turn-around time and usually takes more than 2 weeks (Kocagöz et al., 2012). Nucleic acid-based amplification has enabled rapid and sensitive detection, with several commercial kits available (Yang et al., 2011). Nucleic acid amplification methods and rapid culture systems have brought about improvements in the rapid diagnosis of pulmonary TB (Aono et al., 2009). Once a focus of investigation, interferon-γ release assays have not replaced traditional methods due to their limited performance in diagnosing active pulmonary TB (Lai et al., 2011). Real-time quantitative PCR (qPCR) (Bustin et al., 2009, 2010) has been shown to be a rapid and sensitive method for direct detection of *M. tuberculosis* in human specimens (Causse et al., 2011; Gous et al., 2012; Yang et al., 2011). A number of qPCR systems have been developed, including the Roche LightCycler real-time PCR assay and Cobas TaqMan MTB assay (Roche Diagnostics), the Xpert MTB/RIF assay (Cepheid AB), the ‘Care TB’ assay and the ‘SAT-TB’ assay (Chen et al., 2012; Cui et al., 2012; Malbruny et al., 2011; Miller et al., 2011; Yang et al., 2011). Studies have shown that the Cobas TaqMan MTB assay is at least as sensitive as its predecessor, the Cobas Amplicor MTB system (Aono et al., 2009).
et al., 2009; Kim et al., 2004; Lin et al., 2011; Michos et al., 2006; Yang et al., 2011); however, only a limited number of studies have evaluated the diagnostic performance of the Cobas TaqMan MTB assay (Chandran & Kenneth, 2010; Cho et al., 2011; Kim et al., 2011; Yang et al., 2011).

In this prospective study, we evaluated the sensitivity, specificity, positive predictive value and negative predictive value of the Cobas TaqMan MTB assay for direct detection of \textit{M. tuberculosis} in respiratory specimens at two hospitals in Taiwan during the period 1 June 2010 to 30 April 2011. The results of the Cobas TaqMan MTB assay were compared with traditional culture methods.

**METHODS**

Specimen collection and processing. The medical centres participating in this study were the National Taiwan University Hospital (NTUH), a 2900-bed hospital located in northern Taiwan, and the Buddhist Tzu Chi General Hospital (BTCGH), a 1500-bed hospital located in eastern Taiwan. Respiratory specimens (all sputum specimens) were collected for preparation of acid-fast smears and mycobacterial culture from patients with clinically suspected pulmonary TB where anti-TB therapy had not been initiated. The results of the Cobas TaqMan MTB assay were compared with those obtained from conventional culture methods. Physicians’ judgements, clinical manifestations, radiographic findings and the response to anti-TB treatment were used to resolve discrepancies in results between those obtained from the Cobas TaqMan MTB assay and those obtained from conventional culture methods. The study was approved by the Institutional Review Boards of both hospitals.

All sputum specimens first underwent microscopic acid-fast stain (AFS) examination, culture and the Cobas TaqMan MTB assay. All specimens were processed and pre-treated as described previously (Wang et al., 2004, 2006). Briefly, each specimen was processed by the addition of an equal volume of NaOH/citrate/N-acetyl-L-cysteine at room temperature for 15 min. After centrifugation, the precipitate was resuspended in 1.5 ml PBS (pH 7.4). Cultures were performed by inoculation of sediment from each specimen onto Lowenstein–Jensen slants and a \textit{Mycobacterium} growth indicator tube (MGIT) using the fluorometric BACTEC technique (BACTEC MGIT 960 system; Becton Dickinson Diagnostic Instrument Systems). The Lowenstein–Jensen slants were incubated in a CO2 incubator for the first week and in ambient air at 35 °C for another 7 weeks. Growth in MGITs was checked for 42 days and the presence/absence of growth was recorded. The presence/absence of microcolonies was determined by examination of inoculated Middlebrook 7H11 selective agar with antimicrobials (Remel). This process has been described previously (Lee et al., 2012b; Wang et al., 2004, 2006, 2007). Smears for acid-fast bacilli of the processed specimens were stained with auramine/rhodamine fluorochrome and examined by standard procedures (Wang et al., 2004, 2006, 2007). Fluorochrome stain-positive smears were confirmed by the Kinyoun stain method (Wang et al., 2004, 2006, 2007).

Cobas TaqMan MTB assay. Cobas TaqMan MTB assay tests were conducted according to the manufacturer’s instructions, which could be divided into two major steps: preparation of specimen DNA and qPCR. Positive and negative controls for \textit{M. tuberculosis} were included in each run. Briefly, 100 μl aliquots of the decontaminated respiratory specimens were mixed with 500 μl specimen wash solution and centrifuged. The supernatant was discarded and the pellet lysed. Finally, the mixture was incubated and neutralized. The samples were processed using a Cobas TaqMan 48 Analyser (Roche Diagnostics) with positive and negative controls. The results of the Cobas TaqMan MTB assay were displayed as positive, negative or invalid by Cobas TaqMan analyser interpretation. An invalid result was displayed in cases where the internal control was out of range due to inhibitors or improperly prepared specimens. The detailed process has been described in previous publications (Kim et al., 2011; Yang et al., 2011). The limit of detection determined by the manufacturer (Roche Diagnostics, Doc Rev. 3.1 11/2009) was 0.46 c.f.u. per PCR or approximately 18 c.f.u. ml\textsuperscript{-1} in sputum specimens.

**RESULTS**

During the study period, a total of 592 consecutive clinical respiratory specimens were collected at the NTUH (188 specimens) and BTCGH (404 specimens). The results are shown in Table 1. The results of the Cobas TaqMan MTB assay were invalid in 6 of the 592 samples because of the presence of enzymic amplification inhibitors. Of these six samples, five were AFS-negative specimens and one was an AFS-positive specimen. The Cobas TaqMan MTB assay invalid (inhibitory) but AFS-positive specimen yielded \textit{M. tuberculosis} by culture. Of the remaining 586 specimens, 209 were AFS positive and 377 were AFS negative, and 220 were positive for \textit{M. tuberculosis} in both Middlebrook 7H11 agar and BACTEC MGIT 960 system. Of these 586 specimens, 220 \textit{M. tuberculosis} were isolated overall, and the sensitivity of Cobas TaqMan MTB assay was 82.7 %, the specificity was 96.5 %, the positive predictive value was 93.3 % and the negative predictive value was 90.3 %. For the 209 AFS-positive sputum specimens, the sensitivity and specificity of the Cobas TaqMan MTB assay in detecting \textit{M. tuberculosis} were 94.4 and 78.1 %, respectively. For the 377 AFS-negative sputum specimens, the sensitivity and specificity were 34.9 and 98.2 %, respectively.

Five respiratory specimens, comprising four AFS-positive isolates and one AFS-negative isolate from five patients, were positive for \textit{M. tuberculosis} by the Cobas TaqMan MTB assay but negative for \textit{M. tuberculosis} by conventional culture methods. A diagnosis of pulmonary TB was made based on clinical and radiological findings as well as the response to anti-TB treatment in these five patients. By adding these five specimens with discrepancy results between the two methods but clinically compatible with pulmonary TB, the sensitivity of the Cobas TaqMan MTB assay was 83.1 %.

**DISCUSSION**

A search of the literature in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) on 1 June 2012 found four previous reports primarily describing the performance of the Cobas TaqMan MTB assay system (Chandran & Kenneth, 2010; Cho et al., 2011; Kim et al., 2011; Yang et al., 2011). Cho et al. (2011) evaluated 128 sputum samples and found the Cobas TaqMan MTB test to have 100 % sensitivity and 100 % specificity using culture results and clinical pictures
as the gold standard. Chandran & Kenneth (2010) examined 72 respiratory specimens and found the Cobas TaqMan MTB assay to have 97.2% sensitivity and 100% specificity using culture methods alone as the gold standard. Details regarding AFS status were not provided in the above two studies. Kim et al. (2011) prospectively evaluated the performance of the Cobas TaqMan MTB assay. In 96 respiratory specimens (24 culture positive and 72 culture negative for M. tuberculosis), the Cobas TaqMan MTB assay showed an overall 79.1% sensitivity and 95.8% specificity. In the largest study, Yang et al. (2011) tested 1093 samples from 446 patients and found that the overall sensitivity and specificity of the Cobas TaqMan MTB assay were 91.5 and 98.7%, respectively. The results of these five studies are summarized in Table 2. After pooling the results of the previously published studies with those obtained in this study, we found that the Cobas TaqMan MTB assay had an overall sensitivity of 87.2% and an overall specificity of 98.1%.

Several factors are associated with false-negative results of qPCR assays for M. tuberculosis, including a relatively low bacterial load, an unequal distribution of bacteria among test specimens, the presence of enzymic amplification inhibitors and the sample volume effect (Reischl et al., 1998; Yang et al., 2011). The sample volume effect refers to the relatively small volume of tested specimens sent for qPCR steps compared with the large volume of tested specimens sent for culture (Reischl et al., 1998; Yang et al., 2011). Although increasing the volume of the original specimens sent for qPCR steps can theoretically decrease the number of false negatives, it may not be practical because qPCR is usually performed as a second-line method using the remains of tested specimens after AFS and culture methods have been performed (Reischl et al., 1998).

The greatest competitor to the Cobas TaqMan MTB assay in the field of qPCR is probably the Xpert MTB/RIF assay, a widely marketed technique that targets the rpoB gene (Causse et al., 2011). The Xpert MTB/RIF assay is an automated system that requires very little handling by laboratory staff, which ensures minimal sample contamination. The assay is also able to detect rifampicin resistance (Causse et al., 2011). Results from a meta-analysis of 10 224 suspected TB specimens showed that the Xpert MTB/RIF test had a sensitivity of 94.1% and a specificity of 97.0% at detecting TB (Chang et al., 2012).

To the best of our knowledge, no studies have compared the sensitivity and specificity of the Cobas TaqMan MTB assay with the sensitivity and specificity of the Xpert MTB/RIF test for detecting TB in pulmonary specimens. Yang et al. reported that the high sensitivity (80%) of the Cobas TaqMan MTB assay for detecting M. tuberculosis in AFS-negative sputum was due to the very low limits of detection (0.33–0.83 c.f.u.) (Yang et al., 2011). Furthermore, eight of the respiratory specimens that were AFS negative as well as culture negative, but which were positive for M. tuberculosis according to the Cobas TaqMan MTB assay, were classified as true positive results based on clinical diagnosis. In our study, however, the sensitivity of the assay for AFS-negative specimens was only 34.9%.

Table 1. Comparison of the diagnostic performance of the Cobas TaqMan MTB assay with that of conventional culture methods for direct detection of M. tuberculosis in respiratory specimens at two hospitals in Taiwan

<table>
<thead>
<tr>
<th>Hospital</th>
<th>AFS results (no. of specimens)</th>
<th>Cobas TaqMan MTB assay (no. of specimens)</th>
<th>No. of specimens</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTUH (182)</td>
<td>Positive (57)</td>
<td>Positive (44)</td>
<td>42</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Negative (13)</td>
<td>Negative (13)</td>
<td>0</td>
<td>13</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Positive (2)</td>
<td>Negative (123)</td>
<td>1</td>
<td>122</td>
<td>97.7</td>
</tr>
<tr>
<td>BTCGH (404)</td>
<td>Positive (152)</td>
<td>Positive (130)</td>
<td>125</td>
<td>5</td>
<td>92.6</td>
</tr>
<tr>
<td></td>
<td>Negative (22)</td>
<td>Negative (22)</td>
<td>10</td>
<td>12</td>
<td>34.2</td>
</tr>
<tr>
<td></td>
<td>Positive (19)</td>
<td>Negative (233)</td>
<td>14</td>
<td>206</td>
<td>79.0</td>
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<tr>
<td></td>
<td>Negative (232)</td>
<td>All (404)</td>
<td>27</td>
<td>328</td>
<td>82.7</td>
</tr>
<tr>
<td>Two hospitals (586)</td>
<td>Positive (209)</td>
<td>Positive (174)</td>
<td>167</td>
<td>7</td>
<td>94.4</td>
</tr>
<tr>
<td></td>
<td>Negative (35)</td>
<td>Negative (35)</td>
<td>10</td>
<td>25</td>
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<tr>
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<td>Negative (356)</td>
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<tr>
<td></td>
<td>Negative (377)</td>
<td>All (586)</td>
<td>28</td>
<td>328</td>
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</tr>
</tbody>
</table>

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In conclusion, the Cobas TaqMan MTB assay is a rapid identification tool with a high degree of specificity for the direct detection of *M. tuberculosis* in respiratory specimens. The sensitivity (34.9%) for detecting AFS-negative respiratory specimens, however, is low.

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