Evaluation of Cobas TaqMan MTB PCR for Detection of *Mycobacterium tuberculosis*[▽]

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Nucleic acid-based amplification tests allow the rapid detection of *Mycobacterium tuberculosis*. Recently, a real-time PCR assay for *M. tuberculosis* complex, the Cobas TaqMan MTB test (Roche Diagnostics, Basel, Switzerland), was introduced. We performed a prospective study to evaluate the diagnostic performance of the Cobas TaqMan MTB test system. A total of 406 specimens collected from 247 patients were simultaneously tested by conventional culture, Cobas Amplicor MTB PCR, and TaqMan MTB PCR. The cross-reactivity with other *Mycobacterium* species and the detection limit were also evaluated. Among 406 specimens, a total of 24 specimens (5.9%) were culture positive: 14 specimens were positive by both TaqMan and Amplicor MTB PCRs, while 5 specimens were positive by only TaqMan PCR. The remaining five specimens were negative by both PCR methods. Seven specimens with negative culture results were positive by TaqMan PCR, but five of these were negative by Amplicor MTB PCR. The sensitivity, specificity, and positive (PPV) and negative (NPV) predictive values were 79.1%, 98.2%, 73.1%, and 98.7% for TaqMan and 58.3%, 99.5%, 87.5%, and 97.4% for the Amplicor MTB PCR test, respectively. There was no cross-reactivity with *M. tuberculosis* and nontuberculous mycobacterial species. The detection limit for the Cobas TaqMan MTB PCR test was 4.0 copies/μl. The Cobas TaqMan MTB PCR test showed higher sensitivity for detection of the *M. tuberculosis* complex without disturbing the specificity and NPV than the Amplicor MTB PCR test.

Tuberculosis is a global public health problem involving significant morbidity, and early diagnosis followed by adequate treatment is essential for the prevention of morbidity and mortality (12, 17). The reemergence of tuberculosis due to the appearance of multidrug-resistant strains of *Mycobacterium tuberculosis* has intensified the need for rapid detection of *M. tuberculosis* (19). Over the past several decades, diagnostic methods for *M. tuberculosis* have improved, and nucleic acid-based amplification techniques now allow rapid and sensitive detection in clinical settings (1, 7, 10).

Currently, several assays are commercially available for the detection of the *M. tuberculosis* complex, including the Cobas Amplicor MTB PCR test (Roche Diagnostics, Basel, Switzerland), the RealArt Mycobact Diff Kit (Qiagen, Hamburg, Germany) (2), AMTD (Gen-Probe, Inc. San Diego, California) (14), the Inno-LiPA line probe assay (Innogenetics, Ghent, Belgium) (8), and the GenoType MTBC assay (Hain Lifescience, Nehren, Germany) (15). These commercial assays are divided into two methods: real-time PCR and probe hybridization. In particular, real-time PCR technology has replaced the methodology of microbiological diagnosis using an automated system based on increased sensitivity. More recently, the Cobas TaqMan MTB PCR test, which uses a real-time PCR technique, was introduced.

The aim of this study was to evaluate the performance of the Cobas TaqMan MTB Test by comparing the results to those from the Amplicor MTB PCR. The cross-reactivity with nontuberculous mycobacterial species and the detection limit were also evaluated.

MATERIALS AND METHODS

Study design. A total of 406 clinical specimens were prospectively collected from 247 patients with suspected tuberculosis infection between June and August 2008 at a tertiary care hospital in Seoul, South Korea. Clinical data, including history and radiologic and laboratory findings, were collected for each patient. All clinical specimens were examined blindly by direct microscopy, conventional culture, and Cobas Amplicor and TaqMan MTB PCR tests for the detection of *M. tuberculosis*. To evaluate cross-reactivity between *M. tuberculosis* and nontuberculous mycobacteria (NTM), 40 NTM and 2 *M. tuberculosis* colonies were tested by Cobas TaqMan MTB PCR. The detection limit for the TaqMan MTB PCR was measured by 1:10 serial dilutions of a cultured colony of *M. tuberculosis*. To evaluate the sensitivity and specificity, the overall diagnosis of *M. tuberculosis* was defined by positive culture results.

Processing of specimens. All specimens were decontaminated with *N*-acetyl-L-cysteine-sodium hydroxide and concentrated by 20 min of centrifugation at 3,000 × *g*. After resuspension of the sediments in phosphate buffer, smears with Ziehl-Neelsen staining were prepared and examined by an experienced laboratory technologist using the technique described by Stewart in 1953 (18). Mycobacterial cultures were prepared by the inoculation of a 200-μl aliquot of the decontaminated samples onto 3% Ogawa agar. The cultures were incubated for 8 weeks at 36°C and were examined for growth weekly. The culture results were divided into the following 5 classifications: no growth; trace, <50 colonies; 1+, 50 to 1,000 colonies; 2+, 100 to 200 colonies; 3+, nearly fused colonies; and 4+, fused colonies.

PCR test. All specimens were evaluated by both the Cobas Amplicor and TaqMan MTB PCR tests. The Cobas Amplicor and TaqMan MTB PCR tests were conducted in accordance with the manufacturer's instructions. Positive and negative controls were included in each run. Briefly, 100-μl aliquots of the decontaminated samples were mixed with 500 μl specimen wash solution. The mixtures were subjected to 10 min of centrifugation at 12,000 × *g*. The super-

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TABLE 1. Performance of the Cobas TaqMan and Amplicor PCRs based on culture results

Specimens	Test	Culture-positive (n)		Culture-negative (n)		Sensitivity/specificity (%)	PPV/NPV ^a (%)
		PCR ⁺	PCR ⁻	PCR ⁺	PCR ⁻		
All	TaqMan	19	5	7	375	79.1/98.2	73.1/98.7
	Amplicor	14	10	2	380	58.3/99.5	87.5/97.4
Respiratory	TaqMan	19	5	3	69	79.1/95.8	86.4/93.2
	Amplicor	14	10	1	71	58.3/98.6	93.3/87.6

^a PPV, positive predictive value; NPV, negative predictive value.

natant was discarded, and the pellet was resuspended in 100 μ l of lysis reagent; then, the mixture was incubated at 60°C for 45 min, and 100 μ l of neutralization reagent was added. A 50- μ l portion was added to 50 μ l of the master mix solution, including the internal controls. The samples were processed using the Cobas TaqMan 48 Analyzer (Roche Diagnostics, Basel, Switzerland) with positive and negative controls. The results of the TaqMan PCR test were displayed as positive, negative, and invalid using software provided in the Cobas TaqMan analyzer. An invalid result was displayed in cases where the internal control was out of range due to inhibitors or improperly prepared specimens.

Statistical analysis. The sensitivity, specificity, and positive and negative predictive values were calculated based on the results of concurrently performed cultures. To evaluate the differences in sensitivity between the TaqMan and Amplicor PCR methods, McNemar's test was used. The calculations were performed using the statistical software SPSS for Windows (version 12; SPSS, Chicago, IL).

RESULTS

A total of 406 samples from 247 patients were processed, and all specimens were tested by culture, staining, and nucleic acid tests. The specimens included 96 respiratory and 310 non-respiratory specimens. The respiratory specimens were sputum and pleural and bronchoalveolar lavage (BAL) fluids, and the nonrespiratory specimens were body fluids, including joint fluids, ascites, and drainage and tissue specimens. A total of 24 specimens (5.9%) were culture positive. Of these, 14 (58.3%) were positive by both TaqMan and Amplicor PCRs, while five (20.8%) were positive by only TaqMan MTB PCR, and the remaining five were negative by both PCR tests. The other 382 specimens were culture negative, and two of them showed positive results by both PCRs while five showed positive results only by TaqMan MTB PCR (Table 1). The two specimens that were culture negative and PCR positive were from patients with a clinical history of *M. tuberculosis* infection. Of the 10 specimens that showed discrepant results between the TaqMan and Amplicor PCR tests, 5 were identified as true positive by positive culture results, 1 was regarded as true positive by a history of *M. tuberculosis* infection, and 4 were regarded as false positive.

TABLE 2. Positive, negative, and invalid results according to sample types by TaqMan MTB PCR

Sample	No. positive	No. negative	No. (%) invalid	Total
Sputum	22	53	3 (3.8)	78
Tissue	3	123	14 (10.7)	140
Body fluid	1	167	2 (1.2)	170
BAL fluid	0	14	1 (6.7)	15
Pleural fluid	0	3	0	3
Total	26	360	20 (4.9)	406

With regard to the types of specimens, positive results by TaqMan MTB PCR were detected in 22 samples of sputum, three tissue samples, and one body fluid sample. Among the nonrespiratory specimens, three were false negatives and one was a true positive.

The amplified patterns of the graphs were reviewed for all specimens. Twenty cases yielded invalid results: 14 tissue samples, 3 sputum samples, 2 joint fluid samples, and 1 BAL fluid. All of these specimens showed negative results by Amplicor PCR, acid-fast bacillus (AFB) stain, and culture assessments. The percentages of invalid results according to sample type were as follows: 6.7% for BAL fluid, 1.2% for body fluid, 3.8% for sputum, and 10.7% for tissue samples (Table 2).

In the cross-reactivity test between cultured colonies of *M. tuberculosis* and NTM, none of the colonies showed false positives or false negatives (Table 3). However, 13 (30.9%) of the 42 colonies showed invalid results. In tests for the detection limit of the TaqMan MTB PCR, the measured limit was 4 copies/ μ l.

The sensitivity, specificity, and positive and negative predictive values were analyzed in comparison with the culture results for concordant specimens as reference standards (Table 1). The overall sensitivity and specificity were 79.1% and 98.2% for TaqMan MTB PCR and 58.3% and 99.5% for Amplicor MTB PCR, respectively. The corresponding values for respiratory specimens were 79.1% and 95.8% for TaqMan MTB PCR and 58.3% and 98.6% for Amplicor MTB PCR. Thus, for the respiratory specimens, the sensitivity of the TaqMan MTB PCR was 20.8% higher ($P = 0.002$) and the specificity was 2.8% lower than those for the Amplicor MTB PCR.

TABLE 3. Cross-reactivity between NTM and *M. tuberculosis* in cultured colonies

Species	Cobas TaqMan MTB PCR results (n)		
	Negative	Positive	Invalid
<i>M. tuberculosis</i>	0	2	0
NTM	27	0	13
<i>M. abscessus</i>	10	0	2
<i>M. avium</i>	4	0	4
<i>M. fortuitum</i>	1	0	5
<i>M. intracellulare</i>	10	0	2
<i>M. szulgai</i>	2	0	0

DISCUSSION

In this study, 10 specimens yielded discrepant results between TaqMan and Amplicor PCRs, where all samples were positive only by TaqMan PCR. Among these, five of the specimens were culture positive for *M. tuberculosis*, and the other four were culture negative. The one remaining sample was a culture-negative sputum specimen obtained from a patient with a history of *M. tuberculosis* infection and treatment 6 months before. Thus, this specimen could be considered a true positive, owing to the presence of nonviable organisms. Additionally, the one specimen that generated positive results on both PCR tests but negative culture results was continuously detected using an AFB stain and PCR tests; accordingly, the TaqMan PCR result was considered a true positive.

Four specimens were identified as false positives on the TaqMan PCR assay. They were tissue, joint fluid, bone marrow, and respiratory specimens with C_T values of 46.2, 44.0, 33.3, and 20.7, respectively. The tissue and joint fluid samples were collected from two patients with no signs suggesting tuberculosis, and the bone marrow was collected from a patient suffering from Kikuchi disease. All three specimens evidenced weak but increasing fluorescence patterns and adequate C_T values for internal controls that were consistent with the positive results. However, the respiratory sample with a C_T value of 20.7, obtained from a patient with pneumonia, exhibited a premature increase in fluorescence, followed by a rather flat amplification pattern with a C_T value of 43.6 for the internal control. Therefore, this case could be regarded as negative considering the unusual amplification pattern observed; however, the software identified it as a positive sample. Notably, among the 4 specimens for which false-positive results were generated, 3 were nonrespiratory specimens and the other was a sputum specimen that was culture positive for NTM.

On the basis of the culture results, 5 specimens that were false negative by the TaqMan PCR all showed "trace" results, which indicates low-grade growth in culture. Additionally, the specimens that showed more than 50 colonies in culture, referenced as 1+ to 4+, were all positive by TaqMan MTB PCR (10/10). These results demonstrate that the culture method still has an advantage in *M. tuberculosis* detection, especially for low-grade infections.

In our study, there was a relatively high proportion of nonrespiratory specimens. The TaqMan PCR test limits valid specimens to respiratory specimens, but the experimental data for nonrespiratory specimens have not been provided by any research. In the clinical field, nonrespiratory specimens represent a high proportion in the detection of *M. tuberculosis*. Our specimens were evaluated regardless of the specimen type; therefore, our study provides information about nonrespiratory specimens, including the cultured colonies. In our data, 3 nonrespiratory specimens were false positive by TaqMan MTB. Also, the number of invalid results was higher in the nonrespiratory specimens than in the respiratory specimens (16% versus 4.8%, respectively). Although the cross-reactivity test between cultured colonies of *M. tuberculosis* and NTM yielded 0% false positives, there was a higher proportion of invalid results (32.5%). These data indicate that positive results for the TaqMan MTB PCR are not sufficient for the

detection of *M. tuberculosis* infection in nonrespiratory specimens, including cultured colonies.

Over the course of the PCR, some specimens were not amplified because of inhibitors, such as heparin, hemoglobin, and phenol (3, 4, 16). The TaqMan MTB PCR test uses an internal control that has a different fluorescence from the target probe; thus, failure of amplification can be readily detected. Although the Amplicor PCR includes an internal control, there are some restrictions, in that an additional step is required to detect invalid results. In this study, 20 (4.9%) samples yielded invalid results, and 14 (70%) of these were tissue specimens. The internal controls were not amplified in 13 (32.5%) of the 27 cultured NTM colonies. The substantial proportion of invalid results in the PCR test confirmed that a detection system with an internal control is essential.

Our study showed lower sensitivity than the previously reported data, especially for the Cobas Amplicor PCR. Until now, the diagnostic sensitivity of commercially available kits, including the Cobas Amplicor PCR, for detection of *M. tuberculosis* had been reported to be over 80% (3, 5, 11, 13). However, in Korea, the sensitivity had been reported to be as low as about 62 to 88% (6, 9), which might be due to the high prevalence of *M. tuberculosis* infection. Therefore, the performance of the Cobas TaqMan MTB PCR must be evaluated based on simultaneously tested specimens. In this study, we were able to evaluate both PCR techniques with the same specimens and conditions, thus allowing a direct comparison of the techniques. In comparison with the Cobas Amplicor PCR, the sensitivity of the TaqMan PCR increased 20% ($P < 0.005$) and the specificity decreased 2.8%.

In conclusion, the Cobas TaqMan MTB PCR system yielded improved sensitivity for detection of the *M. tuberculosis* complex from respiratory specimens compared with the Amplicor PCR.

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