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Multicenter Evaluation of Seegene Anyplex TB PCR for the Detection of *Mycobacterium tuberculosis* in Respiratory Specimens

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Culture is the gold standard for diagnosis of tuberculosis, but it takes 6 to 8 weeks to confirm the result. This issue is complemented by the detection method using polymerase chain reaction, which is now widely used in a routine microbiology laboratory. In this study, we evaluated the performance of the Seegene Anyplex TB PCR to assess its diagnostic sensitivity and specificity, and compared its results with the Roche Cobas TaqMan MTB PCR, one of the most widely used assays in the world. Five university hospitals located in the Chungcheong area in South Korea participated in the study. A total of 1,167 respiratory specimens ordered for acid-fast bacilli staining and culture were collected for four months, analyzed *via* the Seegene Anyplex TB PCR, and its results were compared with the Roche Cobas TaqMan MTB PCR. For detection of *Mycobacterium tuberculosis*, the diagnostic sensitivity and specificity of the Anyplex TB PCR were 87.5% and 98.2% respectively, whereas those of the Cobas TaqMan were 92.0% and 98.0% respectively (p value > 0.05). For smear-positive specimens, the sensitivity of the Anyplex TB PCR was 95.2%, which was exactly the same as that of the Cobas TaqMan. For smear-negative specimens, the sensitivity of the Anyplex TB PCR was 69.2%, whereas that of the Cobas TaqMan TB PCR was 84.6%. For detection of MTB, the Seegene Anyplex TB PCR showed excellent diagnostic performance, and high sensitivity and specificity, which were comparable to the Roche Cobas TaqMan MTB PCR. In conclusion, the Anyplex TB PCR can be a useful diagnostic tool for the early detection of tuberculosis in clinical laboratories.

Keywords: *Mycobacterium tuberculosis*, tuberculosis, real-time PCR

Introduction

Tuberculosis (TB) caused by the bacillus *Mycobacterium tuberculosis* is a global public health problem related with significant morbidity and mortality in developing and industrialized countries [12]. Traditionally, acid fast bacilli (AFB) smear microscopy has been the initial method for diagnosis of tuberculosis, due to its speed, simplicity, and low cost. However, its low sensitivity as well as the fact that a significant percentage of bacillus transmission is due to smear-negative pulmonary cases, limits the usefulness of this technique [2, 7]. Another conventional detection method, culture, which is a gold standard for diagnosis, takes 3 to 8 weeks for confirmation [4]. To overcome these problems of conventional methods, diagnostic methods for *M. tuberculosis* using nucleic acid based amplification have improved TB diagnosis [1], thus making early diagnosis and treatment for tuberculosis possible [3].

The recently developed Seegene Anyplex plus MTB Detection kit (Seegene, Seoul, South Korea) uses Dual Priming

Oligonucleotide (DPO) technology to prevent nonspecific priming, Real Amplicon Detection (READ) technology to accommodate high-throughput multiplexing in real-time PCR, and 8-methoxypsoralen (8-MOP) technology to minimize the contamination of DNA.

In this present study, we evaluated the diagnostic performance of the Anyplex plus MTB Detection kit and compared its results with the Cobas TaqMan MTB test (Roche Diagnostics, Basel, Switzerland), which is one of the most widely used assays.

Materials and Methods

Collection of Specimens

A total of 1,167 clinical samples from sputum and bronchial alveolar lavage (BAL) were collected prospectively after approval by the institutional review board (IRB number; 12-003). The samples were collected from April to July 2012 from five university hospitals in Chungcheong area in South Korea. All clinical samples were examined blindly by direct microscopy, conventional culture, and Cobas TaqMan MTB test. The specimens collected from each institution were processed and studied at each institution.

Processing of Specimen for AFB and Culture

All specimens were decontaminated with 4% *N*-acetyl-L-cysteine-sodium hydroxide and concentrated by 15 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 the laboratory technicians according to the technique used by Stewart in 1953 [9]. 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.

Processing of Specimen for Seegene Anyplex TB PCR and Roche Cobas TaqMan MTB PCR

All specimens were evaluated by both Seegene Anyplex TB PCR and the Cobas TaqMan MTB PCR tests in accordance with the manufacturers' instructions. Positive and negative controls were included in each run for both instruments. For DNA extraction, 100 µl of DNA extraction solution was added and mixed with vortexing. Then the sample was allowed to boil at a heat block of 100°C for 20 min and the mixtures were subjected to 5 min of centrifugation at 15,000 ×*g*. A 5 µl aliquot of supernatant was added to 15 µl of master mix solution and then processed for PCR according the manufacture's protocol. For Cobas TaqMan, 100 µl aliquots of the decontaminated samples were mixed with 500 µl of specimen wash solution. The mixtures were subjected to 10 min of centrifugation at 12,000 ×*g*. The supernatant was discarded, and the pellet was resuspended in 100 µl of lysis reagent; then, the mixture was incubated at 60°C for 45 min, and 100 µl of neutralization reagent was added. A 50 µl portion was added to 50 µl of the master mix solution, including the internal controls. The samples were processed with positive and negative controls. The results of the Cobas 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 owing to inhibitors or improperly prepared specimens.

Statistical Analysis

The sensitivity, specificity, positive and negative predictive values, and diagnostic efficiency were calculated based on culture as the gold standard. Statistical comparisons were performed using McNemar's test. The calculations were done using Analyse-it (Microsoft, Leeds, UK).

Results

Diagnostic Performance of Seegene Anyplex TB PCR and its Comparison with Roche Cobas TaqMan MTB PCR

A total of 1,167 samples were processed and tested for AFB stain, culture, Seegene Anyplex TB PCR, and Roche Cobas TaqMan MTB PCR. The specimens were mainly from the respiratory tract (sputum: 1,021, BAL: 146). A total of 88 specimens (7.5%) were positive in culture, among which 77 specimens (87.5%) showed positive results in Seegene Anyplex TB PCR. Compared with the routine

Table 1. Performance of the Seegene Anyplex TB PCR based on the culture results.

	Assays	Culture positive		Culture negative		Sensitivity/specificity	PPV/NNP/Efficiency
		PCR +	PCR -	PCR +	PCR-		
All	Anyplex	77	11	19	1,060	87.5/98.2	80.2/99.0/97.4
	Cobas	81	7	21	1,058	92.0/98.0	97.4/99.3/97.6
Smear positive	Anyplex	59	3	3	8	95.2/72.7	95.2/72.7/91.8
	Cobas	59	3	3	8	95.2/72.7	95.2/72.7/91.8
Smear negative	Anyplex	18	8	16	1,052	69.2/98.5	52.9/99.2/97.8
	Cobas	22	4	18	1,050	84.6/98.3	55.0/99.6/98.0

PCR = polymerase chain reaction; PPV = positive predictive value; NNP = negative predictive value.

culture method, the Seegene Anyplex TB PCR showed an overall sensitivity of 87.5% and specificity of 98.2%. The positive and negative predictive values were 80.2%, and 99.0% respectively. Overall, the agreement of PCR with culture (efficiency) was 97.4%. The sensitivity, specificity, positive predictive value, and negative predictive value of Cobas TaqMan MTB PCR were 92.0%, 98.0%, 97.4%, and 99.3%, respectively. For smear-positive specimens, the diagnostic sensitivity and specificity of Seegene Anyplex TB PCR were 95.2% and 72.7%, respectively, which are exactly the same as those of Roche Cobas TaqMan MTB PCR. For smear-negative specimens, the diagnostic sensitivity and specificity of Seegene Anyplex TB PCR were 69.2% and 98.5% respectively, whereas for Roche Cobas TaqMan MTB PCR, those were 84.6% and 98.3%, respectively (p value > 0.05) (Table 1).

Analysis of Discrepant Specimens

Thirty specimens showed discordant results between PCR and culture. Among them, 11 specimens were culture positive and PCR negative, and 19 specimens were culture negative and PCR positive. In the investigation of 15 culture-negative isolates of which the patient's medical history was reviewed, seven patients did not show clinical features of tuberculosis, three patients turned out to not have tuberculosis, and five patients showed clinical features of tuberculosis on computerized tomography and clinical symptoms of tuberculosis for which TB medications were started. In addition, cases of culture positive while PCR negative showed CT values were all negative.

Discussion

Early diagnosis and starting treatment of tuberculosis are paramount in terms of public health concerns. To make early detection possible, nucleic acid amplification methods are widely used because of their significant potential added value for clinicians as well as TB control officials [3]. In this regard, we evaluated Seegene Anyplex TB PCR using newly developed technology for its applicability to clinical laboratories and compared its results with the widely used Roche Cobas TaqMan MTB PCR. According to the results, for respiratory specimens, the sensitivity and specificity of Anyplex TB PCR were 87.5% and 98.2%, and those of Cobas TaqMan MTB PCR were 92.0% and 98.0%, respectively. Both results did not show statistical significance.

According to the recent literatures, the diagnostic sensitivity of Roche Cobas TaqMan for TB detection ranged from 77.3% to 91.5% [5, 11, 13]. Although Yang *et al*. [13] dealt with a much higher proportion of culture-positive samples (11.2% versus our 7.5%), our diagnostic sensitivity of Roche Cobas TaqMan was quite similar (91.5% versus our 92.0%) [13]. In other reports, the overall pooled diagnostic sensitivity of Xpert MTB/RIF (Cepheid, Sunnyvale, USA) ranged from 88% to 90.4% [6, 8]. Thus, the sensitivity of Seegene Anyplex TB PCR showed similar performance to other assays that were studied extensively over several years.

The AFB smear test is the most commonly used method for *M. tuberculosis* diagnosis, but its low sensitivity and specificity values were the main limitations for use. In our study, the sensitivity of AFB smear was 70.5%, which is lower than that of PCR. For smear-negative specimens, the sensitivities of Seegene Anyplex TB PCR and the Cobas TaqMan MTB PCR were 69.2% and 84.6%, and for smear positive specimens, the sensitivity of both assays was 95.2%. This finding is compatible with the fact that the PCR method is reliable in smear-positive specimens, from the previous studies [10]. Therefore, for patients with a positive AFB smear, the chances of early treatment and prevention of further transmission can be enhanced by the nucleic acid amplification method. For smear-negative specimens, the sensitivity and specificity of Seegene Anyplex TB PCR (69.2% and 98.5%, respectively) were similar to those of Xpert (67% and 99% respectively) [8], but the sensitivity was lower than that of Roche Cobas TaqMan MTB PCR (84.6%). However, smear-negative but positive-culture specimens were only 26, and so further validation is needed for the evaluation of the diagnostic sensitivity for smear-negative specimens.

In terms of specimen processing, less volume of specimen is required for the assay, but the need for incubation at a heat block for 30 min makes the process complex and time consuming.

In summary, the Seegene Anyplex TB PCR has demonstrated a high capacity for detecting *M. tuberculosis* and showed similar performance compared with the Roche Cobas TaqMan MTB PCR. It can be a good candidate for routine use in many clinical laboratories. However, only respiratory specimens were evaluated, so a study to evaluate the performance using other types of specimens is needed in the near future.

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