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Original Article

Comparison of the Three Molecular Diagnostic Assays for Molecular Identification of *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria Species in Sputum Samples

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Mycobacterium tuberculosis (MTB) continues to be one of the main causative agents of tuberculosis (TB); moreover, the incidence of nontuberculous mycobacteria (NTM) infections has been rising gradually in both immunocompromised and immunocompetent patients. Precise and rapid detection and identification of MTB and NTM in respiratory specimens are thus important for MTB infection control. Molecular diagnostic methods based on the nucleic acid amplification test (NAAT) are known to be rapid, sensitive, and specific compared to the conventional acid-fast bacilli (AFB) smear and mycobacterial culture methods. In the present study, the clinical performances of three commercial molecular diagnostic assays, namely TB/NTM PCR (Biocore), MolecuTech Real MTB-ID® (YD Diagnostics), and REBA Myco-ID® (YD Diagnostics), were evaluated with a total of 92 respiratory specimens (22 AFB smear positives and 67 AFB smear negatives). The sensitivity and specificity of TB/NTM PCR were 100% and 75.81%, respectively. The corresponding values of MolecuTech Real MTB-ID® and REBA Myco-ID® were 56.52% and 90.32%, and 56.52% and 82.26%, respectively. TB/NTM PCR showed the highest sensitivity; however, the concordant rate was 10% compared with sequence analysis. Although MolecuTech Real MTB-ID® showed lower sensitivity, its specificity was the highest among the three methods. REBA Myco-ID® allowed accurate classification of NTM species; therefore, it was the most specific diagnostic method. Of the three PCR-based methods, MolecuTech Real MTB-ID® showed the best performance. This method is expected to enable rapid and accurate identification of MTB and NTM.

Key Words: *Mycobacterium tuberculosis* (MTB), Nontuberculous mycobacteria (NTM), Molecular identification, TB/NTM PCR, MolecuTech Real MTB-ID®, REBA Myco-ID®

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INTRODUCTION

Tuberculosis (TB) remains a major health problem worldwide. TB is one of the top 10 causes of death globally and a major cause of death from a single infectious agent, ranking higher than even human immunodeficiency virus (HIV). Globally, 1.2 million and 251,000 deaths due to TB were reported among HIV-negative and HIV-positive people, respectively, in 2018. Moreover, 10.4 million people suffered from TB in 2016, of whom 89% were adults and 57% were male (World Health Organization, 2019).

Over 170 species of nontuberculous mycobacteria (NTM), the other members of the *Mycobacterium tuberculosis* complex (MTBC), and *M. leprae* have been classified to date (Otchere et al., 2017). They are ubiquitous organisms that have been isolated from several sources, such as water, sewage, soil, air, dust, food, animals, and humans (Chin'ombe et al., 2016; Kasa et al., 2015). Some species are opportunistic pathogens that cause diseases in high-risk individuals, namely those who are immunocompromised or infected with HIV, especially in developed countries (Mohammadi et al., 2017). The isolation rate of NTM has increased and now ranges from 20 to 50%, which is in contrast with the reduction in *M. tuberculosis* (MTB) infections (Wang et al., 2014; Kazumi and Mitarai, 2012). It is necessary to differentiate between patients with NTM infections and those with TB to provide optimal therapy and ensure appropriate patient management. Additionally, accurate identification of NTM species is required, because antimicrobial susceptibilities and courses of treatment differ for each NTM organism (Chae et al., 2017).

The most recent and commonly used TB diagnostic method involves detection of acid-fast bacilli (AFB) by smear or mycobacterial culture (Wang et al., 2017). While smear microscopy is rapid, it has a detection limit of approximately 5,000 bacilli/mL, and the recovery rate of NTM species in the sputum from Korean AFB smear positive patients is about 10% (Sutantangjai et al., 2014; Kim et al., 2015). Mycobacterial culture is considered the gold standard method in the identification of mycobacteria isolates; however, culture tests usually require about four to eight weeks because of the slow growth of the mycobacteria (Chae and Shin, 2018; Rakotosamimanana et al., 2019). Although automated liquid culture systems have complemented the limitations, classical diagnostic methods still have low sensitivity and are time-consuming (Wang et al., 2014; Zhu et al., 2010; Şamlı and İlki, 2016; Wang et al., 2015). Advancements in molecular diagnostic techniques have overcome these disadvantages. Various nucleic acid amplification test (NAAT)-based assays, including conventional polymerase chain reaction (PCR), real-time PCR, PCR-restriction fragment length polymorphism (PCR-RFLP) assays, line probe assays, oligonucleotide arrays, and sequence analysis, have been developed for differential identification of MTBC and NTM species, improving the speed and analytical sensitivity of diagnosis (Jung et al., 2016; Maruthai et al., 2015; Wang et al., 2015).

TB/NTM PCR (one tube; Biocore, Seoul, Korea) is extensively used as a rapid diagnostic tool in the conventional PCR method. It targets the *IS6110* sequence of MTB and the *rpoB* gene sequence of NTM. Another molecular diagnostic method, called real-time PCR, is widely used due to its higher sensitivity and rapid processing time compared to those of the conventional PCR and other diagnostic methods (Wang et al., 2015). MolecuTech Real MTB-ID® (YD Diagnostics, Yongin, Korea) is designed to simultaneously detect and differentiate MTBC and NTM species using real-time PCR on direct sputum, body fluid, tissue, and cultured isolates. This molecular diagnostic kit employs real-time PCR using a specific target probe for maximum sensitivity and specificity. A commercial diagnostic kit based on PCR-reverse blot hybridization assay (REBA), REBA Myco-ID® (YD Diagnostics, Yongin, Korea), is employed for rapid detection and accurate identification of 20 mycobacterial species, including *M. tuberculosis*, *M. avium*, *M. intracellulare*, *M. scrofulaceum*, *M. abscessus*, M. *massiliense*, *M. chelonae*, *M. fortuitum* complex, *M. ulcerans*/*M. marinum*, *M. kansasii*, *M. genavense*/*M. simiae*, *M. terrae*/*M. nonchromogenicum*, *M. celatum*, *M. gordonae*, *M. szulgai*, *M. mucogenicum*, and *M. aubagnense* (Wang et al., 2015).

The aim of the present study was to evaluate the performance of the three above-mentioned molecular diagnostic assays for the rapid and accurate detection and molecular identification of MTB and NTM species in a total of 92

respiratory specimens containing 22 AFB smear positives and 67 AFB smear negatives.

MATERIALS AND METHODS

Clinical specimens

A total of 92 sputum samples were provided by the Department of Laboratory Medicine, Maryknoll Medical Center, Busan, Republic of Korea. Identification tests, including AFB smear and mycobacterial cultures, were conducted. All sputum samples were decontaminated by treatment with 3% NaOH trisodium citrate N-acetyl-L-cysteine for 15 min at 24℃ and neutralized with sterile 0.067 M phosphate buffer. After centrifugation at 3,000×g for 15 min, each pellet was resuspended in 1.0 mL of sterile 0.067 M phosphate buffer. Then, a 600 μL aliquot of the suspension was inoculated onto Löwenstein-Jensen culture media and used for acid-fast staining. The remainder of the aliquot was directly processed for the three molecular assays.

Genomic DNA extraction from sputum samples

To prepare genomic DNA (gDNA) samples for the testing, 500 μL of each specimen was centrifuged, and the pellet was washed twice with 500 μL of Tris-EDTA buffer (10 mM Tris, 1 mM EDTA, pH: 8.0) and then resuspended with 200 μL of the same buffer. The samples were then incubated in a boiling water bath for 20 min and centrifuged. The supernatants containing gDNA were transferred to clean microcentrifuge tubes and kept at -20℃ until use. All the centrifugation steps were performed at 14,000×g in microcentrifuge tubes.

Conventional PCR assay

To evaluate the conventional PCR assay, TB/NTM (one tube) PCR was used according to the manufacturer's instructions. In brief, the PCR was performed with 20 μL of the total reaction mixture containing 10 μL of the TB/NTM PCR reactions mixture, 6 μL of the TB/NTM PCR primer mixture, and 4 μL of the template gDNA. The PCR amplification was also performed according to the manufacturer's instructions as follows: initial denaturation at 94℃ for 10 min, followed by 40 cycles each of 94℃ for 30s, 65℃ for 30s, and 72℃ for 45s. After the final cycle, the samples were maintained at 72℃ for 5 min to complete the synthesis of all strands. The PCR products were electrophoresed and visualized on a 2% agarose/tris-borate-EDTA gel.

Real-time PCR assay

To evaluate the real-time PCR assay, MolecuTech Real MTB-ID® was used according to the manufacturer's instructions. In brief, 12.5 μL of 2X Real-time PCR Premix was added in MicroAmp® Fast Reaction Tubes (Thermo Fisher Scientific, Waltham, MA, USA). Then, 7.5 μL of primer mixture and 5 μL of template gDNA were added. The PCR amplification was performed as per the manufacturer's instructions: initial denaturation at 94℃ for 3 min, followed by 45 cycles each of 94℃ for 20 s and 60℃ for 40s. The value of the cycle threshold was positive, namely 34 or less, for each channel (internal control-CY5 (668 nm), MTB-FAM (520 nm), and NTM-TEXAS RED (617 nm)).

PCR-reverse blot hybridization assay (PCR-REBA)

For evaluate PCR-REBA, REBA Myco-ID® was used according to the manufacturer's instructions. In brief, the PCR was performed using 20 μL of reaction mixture (Genet Bio, Korea) containing 2X master mix, 1X biotinylated primer mixture, and 5 μL of template gDNA, and purified distilled water was added to obtain a final volume of 20 μL. Forty PCR cycles comprised an initial denaturation at 95℃ for 30 s, followed by annealing and extension at 65℃ for 30 s. After the final cycle, the samples were maintained at 72℃ for 10 min to complete the synthesis of all strands. The amplified PCR products were subjected to REBA. Downstream applications (including hybridization and washing) were performed by an automated system, the MolecuTech REBA processor HybREAD 480 (YD Diagnostics, Yongin, Korea). In brief, biotinylated PCR products were denatured at 25℃ for 5 min in denaturation solution, diluted in 970 μL of hybridization solution, and added to the REBA membrane strip in the provided blotting tray. Denatured single-stranded PCR products were used to hybridize with the probes on the strip at 55℃ for 30 min. The strips were then washed twice with gentle shaking in 1 mL of washing solution for 10 min at 55℃, incubated at 25℃ with 1:2,000 diluted streptavidin-alkaline phosphatase conjugate (Roche

Table 1. Characteristics of the sputum samples used in this study

Characteristics		No. (%)
AFB smear	Positive	22 (23.9)
	Negative	67 (72.8)
	Unidentified	3 (3.3)
Mycobacterial culture	MTB	23 (25.0)
	NTM	3 (3.3)
	Co-infected	1 (1.1)
	Negative	62 (67.3)
	Unidentified	3 (3.3)

AFB: Acid-fast bacilli

Diagnostics, Mannheim, Germany) in conjugate diluent solution for 30 min, and washed twice with 1 mL of conjugate diluent solution at room temperature for 1 min. The colorimetric hybridization signals were visualized by adding 1:50 diluted alkaline phosphatase (AP)-mediated staining solution and NBT/BCIP (Roche Diagnostics, Mannheim, Germany), which was subsequently incubated until color was detected. The patterns of each membrane were interpreted by MolecuTech REBA SCAN (YD Diagnostics, Yongin, Korea).

rpoB gene sequence analysis

The mycobacterial *rpoB* gene was sequenced to confirm mismatched samples with the three molecular assays used for the identification of the MTB and NTM species. The obtained sequences were compared using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information.

RESULTS

Characteristics of the study subjects

Of the 92 patients included in this study, 65 were males and 27 were females. The number of AFB smear positives and negatives were 22 (23.91%) and 67 (72.83%), respectively, and three specimens could not be identified. The number of MTB-positive, NTM-positive, co-infected, and negative samples for the mycobacterial cultures were 23 (25%), 3 (3.26%), 1 (1.09%), and 62 (67.39%), respectively, and three specimens could not be identified (Table 1).

Evaluation of three molecular assays compared to AFB smears

Among the 22 positive samples, 20 (90.91%) were MTB- and 2 (9.09%) were NTM-positive as per the results of the conventional PCR using TB/NTM PCR. Moreover, 16 (72.73%) were MTB- and 4 (18.18%) were NTM-positive, whereas 2 (9.09%) were MTB- and NTM-negative as per the results of the real-time PCR using MolecuTech Real MTB-ID®. Fourteen (63.64%) samples were MTB-positive, 4 (18.18%) were NTM-positive, 2 (9.09%) showed co-infection, and 2 (9.09%) were MTB- and NTM-negative according to the results of PCR-REBA using REBA Myco-ID®. The analytical sensitivities of the conventional PCR, real-time PCR, and PCR-REBA compared to the AFB smears were 90.91, 72.73, and 63.64%, respectively. Among the 67 negative samples, 19 (28.36%) were MTB-positive, 2 (9.09%) were NTM-positive, and 47 (70.15%) were negative for both infections according to the results of the conventional PCR using TB/NTM PCR. Three (4.48%) of the samples were MTB-positive, 3 (4.48%) were NTM-positive, and 61 (91.04%) were negative for both infections as per the results of the real-time PCR using MolecuTech Real MTB-ID®. Six (8.96%) were MTB-positive, 4 (8.96%) were NTM-positive, 2 (2.99%) were co-infected, and 53 (79.10%) were negative for both infections as per the results of PCR-REBA using REBA Myco-ID®. Among the three unknown samples, two were MTB-positive while one was NTM-positive according to the results of the conventional PCR using TB/NTM PCR. The results of the real-time PCR using MolecuTech Real MTB-ID® indicated that two samples were MTB-positive and one was MTB-negative. The results of PCR-REBA using REBA Myco-ID® showed that one sample each was MTB-positive, co-infected, and negative. The analytical specificities of the conventional PCR, real-time PCR, and PCR-REBA compared to the AFB smears were 70.15, 91.04, and 79.10%, respectively (Table 2).

Evaluation of three molecular assays compared to mycobacterial cultures

The results of the conventional PCR using TB/NTM PCR were completely concordant with the 23 positive myco-

Table 2. Results of three molecular assays compared to AFB smears

		Conventional PCR			Real-time PCR			PCR-REBA			
		MTB	NTM	Negative	MTB	NTM	Negative	MTB	NTM	Co-infected	Negative
AFB smears (*n* = 92)	Positive (*n* = 22)	20 (90.9%)	2 (9.1%)	0 (0.0%)	16 (72.7%)	4 (18.2%)	2 (9.2%)	14 (63.6%)	4 (18.2%)	2 (9.1%)	2 (9.1%)
	Negative (*n* = 67)	19 (28.4%)	1 (1.5%)	47 (70.2%)	3 (4.5%)	3 (4.5%)	61 (91.0%)	6 (9.0%)	6 (9.0%)	2 (3.0%)	53 (79.1%)
	Unidentified (*n* = 3)	2 (66.7%)	1 (33.3%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	1 (33.3%)	1 (33.3%)	0 (0.0%)	1 (33.3%)	1 (33.3%)
	Total	41	4	47	21	7	64	21	10	5	56

MTB: *Mycobacterium tuberculosis*, NTM: Nontuberculous mycobacteria

Table 3. Results of three molecular assays compared to mycobacterial cultures

		Conventional PCR			Real-time PCR			PCR-REBA			
		MTB	NTM	Negative	MTB	NTM	Negative	MTB	NTM	Co-infected	Negative
Mycobacterial cultures (*n* = 92)	Positive (*n* = 23)	23 (100.0%)	0 (0.0%)	0 (0.0%)	13 (56.5%)	3 (13.0%)	7 (30.4%)	13 (56.5%)	4 (17.4%)	2 (8.7%)	4 (17.4%)
	NTM (*n* = 3)	1 (33.3%)	2 (66.7%)	0 (0.0%)	0 (0.0%)	3 (100.0%)	0 (0.0%)	0 (0.0%)	3 (100.0%)	0 (0.0%)	0 (0.0%)
	Co-infection (*n* = 1)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	Negative (*n* = 62)	14 (22.6%)	1 (1.6%)	47 (75.8%)	5 (8.1%)	1 (1.6%)	56 (90.3%)	6 (9.7%)	3 (4.8%)	2 (3.2%)	51 (82.3%)
	Unidentified (*n* = 3)	2 (66.7%)	1 (33.3%)	0 (0.0%)	2 (66.7%)	0 (0.0%)	1 (33.3%)	1 (33.3%)	0 (0.0%)	1 (33.3%)	1 (33.3%)
	Total	41 (44.6%)	4 (4.4%)	47 (51.1%)	2 (22.8%)	7 (7.6%)	64 (69.6%)	21 (22.8%)	10 (10.9%)	5 (5.4%)	56 (60.9%)

PCR: polymerase chain reaction, REBA: reverse blot hybridization assay, MTB: *Mycobacterium tuberculosis,* NTM: nontuberculous mycobacteria

bacterial culture samples. Thirteen (56.52%) samples were MTB-positive, 3 (13.04%) were NTM-positive, and 7 (30.43%) were negative for both infections as per the results of the real-time PCR using MolecuTech Real MTB-ID®. Thirteen (56.52%) samples were MTB-positive, 4 (17.39%) were NTM-positive, 2 (8.70%) were co-infected, and 4 (17.39%) were negative for both MTB and NTM according to the results of PCR-REBA using REBA Myco-ID®. The analytical sensitivities of the conventional PCR, real-time PCR, and PCR-REBA compared to the mycobacterial cultures were 100, 56.52, and 56.52%, respectively. Of the three NTM-positive samples in the mycobacterial cultures, one was MTB-positive and two were NTM-positive as per the results of the conventional PCR using TB/NTM PCR. The results of the real-time PCR using MolecuTech Real MTB-ID® and PCR-REBA using REBA Myco-ID® were completely concordant. The results of all the three molecular assays were MTB-positive for the one co-infected sample in the mycobacterial cultures. Among 62 negative samples in the mycobacterial cultures, 14 (22.58%) were MTB-positive, one (1.61%) was NTM-positive, and 47 (75.81%) were negative for both MTB and NTM as per the results of the conventional PCR using TB/NTM PCR. The results of the real-time PCR using MolecuTech Real MTB-ID® indicated that 5 (8.06%) samples were MTB-positive, 1 (1.61%) was NTM-positive, and 56 (90.32%) were negative for both infections. Moreover, 6 (9.68%) samples were MTB-positive, 3 (4.84%) were NTM-positive, 2 (3.23%) were co-infected,

and 51 (82.26%) were negative for both infections as per the results of PCR-REBA using REBA Myco-ID®. Regarding the three unknown samples in the mycobacterial cultures, two were MTB-positive and one was NTM-positive as per the results of the conventional PCR using TB/NTM PCR. Two of the samples were MTB-positive and one was negative for both infections according to the results of the real-time PCR using MolecuTech Real MTB-ID®. The results of PCR-REBA using REBA Myco-ID® indicated that one sample each was MTB-positive, co-infected, and negative for both infections. The analytical specificities of the conventional PCR, real-time PCR, and PCR-REBA compared to the AFB smears were 75.81, 90.32, and 82.26%, respectively (Table 3).

Comparison of three molecular assays with *rpoB* gene sequence analysis

All the samples showing different results for the three molecular diagnostic methods were analyzed using mycobacterial *rpoB* gene sequence analysis. The five samples that

Table 4. Comparison of three molecular assays with *rpoB* gene sequence analysis

Sample	Conventional PCR	Real-time PCR	PCR-REBA	*rpoB* gene PCR	*rpoB* sequence analysis
1	MTB	MTB	MTB complex *M. gastri/M. haemophilum*	Single-band	MTB
2	MTB	MTB	MTB complex *M. gastri/M. haemophilum*	Single-band	MTB
3	MTB	MTB	MTB complex *M. mucogenicum*	Multi-band	ND (mixed peak)
4	MTB	MTB	MTB complex *M. mucogenicum*	NA	Negative
5	MTB	MTB	Negative	Multi-band	ND (mixed peak)
6	MTB	NTM	MTB complex	NA	Negative
7	MTB	NTM	MTB complex	NA	Negative
8	MTB	NTM	*M. chelonae*	Single-band	*M. chelonae*
9	MTB	NTM	Negative	NA	Negative
10	MTB	Negative	MTB complex	NA	Negative
11	MTB	Negative	MTB complex	NA	Negative
12	MTB	Negative	MTB complex	NA	Negative
13	MTB	Negative	MTB complex	NA	Negative
14	MTB	Negative	MTB complex *M. mucogenicum*	NA	Negative
15	MTB	Negative	*M. genavense/M. simiae*	NA	Negative
16	MTB	Negative	*Mycobacterium* species	Multi-band	ND (mixed peak)
17	MTB	Negative	*Mycobacterium* species	NA	Negative
18	MTB	Negative	*Mycobacterium* species	NA	Negative
19	MTB	Negative	*Mycobacterium* species	NA	Negative
20	MTB	Negative	*Mycobacterium* species	NA	Negative
21	MTB	Negative	Negative	Single-band	*Corynebacterium striatum*
22	MTB	Negative	Negative	Multi-band	ND (mixed peak)
23	MTB	Negative	Negative	Multi-band	ND (mixed peak)
24	MTB	Negative	Negative	NA	Negative
25	MTB	Negative	Negative	NA	Negative

NA: not amplified, ND: not determined, MTB: *Mycobacterium tuberculosis*

were undetermined due to multi-bands in the *rpoB* gene PCR results were excluded. Comparing a total of 20 results revealed that the coincidence rate with the conventional PCR was 10% (2/20), while the corresponding values for the real-time PCR and PCR-REBA were 75% (15/20) and 20% (4/20), respectively. The detailed results of the three molecular assays compared with the *rpoB* gene sequence analysis are shown in Table 4.

DISCUSSION

Despite the recent fall in TB incidence, the disease is the ninth leading cause of death globally and is caused by a major single infectious agent, ranked even higher than HIV /AIDS (World Health Organization, 2019). In addition to MTB infections, a significant proportion of patients, especially those with HIV, are infected with NTM. The symptoms of NTM-infected patients are often similar to those suffering from the MTB infection (Kumar et al., 2014; Singh et al., 2007). Therefore, rapid and accurate detection of MTB and NTM species is crucial for both optimal drug therapy and appropriate disease control (Chae et al., 2017; Kim at al., 2018). AFB smears and mycobacterial cultures can be used to distinguish between MTB and NTM species, but these methods have limitations, as false positives are common and they are time-consuming. Although semi-automated culture systems have reduced the time required for the tests, traditional tests for identification between MTB and NTM species still suffer from disadvantages (Kazumi and Mitarai, 2012; Şamlı and İlki, 2016; Gholoobi et al., 2014). The development of molecular diagnostic techniques has significantly increased sensitivity and reduced diagnosis time (Jung et al., 2016; Lin et al., 2015). PCR-based assays have been used to detect mycobacterial DNA with high sensitivity and specificity (Mackay, 2004; Kwon and Koh, 2014).

In this study, we evaluated the clinical performance of three molecular assays, namely conventional PCR, real-time PCR, and PCR-REBA. A total of 92 respiratory specimens were used, and the results of the three molecular assays were compared with the findings of AFB smears and mycobacterial cultures. Samples showing mismatched results among the three molecular assays were then analyzed using *rpoB* gene sequence analysis.

The conventional PCR assay showed 100% sensitivity and 75.81% specificity. Its sensitivity was the highest among the three methods. It is expected that conventional PCR using the TB/NTM (one tube) kit determines the multi-band as MTB-positive in the gel electrophoresis result, thus increasing its sensitivity to 100%. Table 4 shows that the concordant rate was 10% compared with the sequence analysis. This result may be due to the low specificity of this assay compared to those of the other methods. The sensitivity and specificity of the real-time PCR assay compared to the AFB cultures were 56.52% and 90.32%, respectively. Since this study was conducted on direct samples of clinical sputum, the estimated sensitivity was expected to be lower. However, a confirmation of the sequence analysis and sample discrepancy results showed that the sensitivity was in fact 100%, and thus, the specificity of this assay was the highest among the three methods. Therefore, considering the execution time and cost, this assay is expected to be the most efficient. For REBA Myco-ID, the sensitivity and specificity compared to the AFB cultures were 56.52% and 82.26%, respectively. Although the sensitivity of this method is lower, it was possible to accurately distinguish the NTM species. Also, it offered the advantage of identifying complex NTM infections without sequence analysis. Compared with the NTM results of the mycobacterial cultures, both real-time PCR and PCR-REBA were perfectly matched. Their specificities also exceeded that of the conventional PCR. Of the three methods, the conventional PCR had the highest sensitivity to AFB cultures, but it suffered from a disadvantage; it was difficult to accurately differentiate MTB and NTM infections. The sensitivity of real-time PCR compared to AFB cultures was relatively low, although it was higher when compared to the results of the sequence analysis. The specificity of real-time PCR was the highest among the three methods, and thus, it is considered to be the most efficient method. However, complementary analytical sensitivity will be required for accurate diagnosis. REBA Myco-ID allowed accurate classification of NTM species; therefore, it is expected to be the most specific diagnostic method.

This study suffered from a few limitations. All of the sequence analyses could not be performed due to the lack of

sample volume. Also, it is necessary to perform additional experiments on an increased number of samples to more accurately determine the sensitivities and specificities of the three methods.

In conclusion, of the three PCR-based methods, real-time PCR showed most effective performance, and complementary analytical sensitivity will be required. This method is expected to enable rapid and accurate identification of MTB and NTM. It is also essential to conduct additional studies with larger sample sizes to increase the reliability of the results among the methods.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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