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Evaluation of Diagnostic Performance of Three Real-Time Polymerase Chain Reaction Assays for the Detection of Mycobacteria Species

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Abstract

Background: The disease burden caused by the *Mycobacterium tuberculosis* (Mtb) complex (MTC) continues to decrease in most countries. However, the diseases caused by nontuberculous mycobacteria (NTM) become a public health problem. This study aimed to compare the diagnostic accuracy of three real-time PCR assays: AdvanSure™ TB/NTM real-time PCR kit (AdvanSure; LG Chem., Korea), Genedia® MTB/NTM detection kit (Genedia; Green Cross MS, Korea), and PowerChek™ MTB/NTM real-time PCR kit (Power Chek; Kogenebiotech, Korea) for the detection of MTC and NTM. *Methods:* A total of 102 acid-fast bacilli (AFB) smear-positive and 177 smear-negative specimens from Korea University Medical Center, Guro Hospital, were enrolled. The AFB smear-positive and negative specimens were collected from November 2016 to October 2017 and November to December 2018, respectively. DNA extraction was performed using Genedia Mycobacteria DNA Prep Kit (Green Cross MS, Korea). The statistical analysis was performed using MedCalc18.11.6 (MedCalc Software, Belgium). *Results:* Among 261 specimens, 64 showed MTC growth and 28 exhibited NTM growth. The sensitivity, specificity, positive predictive value, and negative predictive value of AdvanSure/Genedia/PowerChek kits for Mtb were 96.9%/95.3%/96.9%, 98.5%/99.5%/98.5%, 58.9%/80.9%/58.9%, and 99.9%/99.9%/99.9%, respectively; whereas those for NTM detection were 81.5%/44.4%/88.9%, 99.6%/100.0%/98.7%, 57.3%/100.0%/32.8%, and 99.9%/99.6%/99.9%, respectively. The area under the receiver operating characteristic curve of AdvanSure and PowerChek for NTM detection was statistically different from that of Genedia ($P < 0.0001$). *Conclusion:* Three real-time PCR assays were reliable for Mtb detection in AFB-positive and-negative specimens. There was a difference between these three reagents for the accuracy of NTM detection.

Keywords

Mycobacterium tuberculosis
Nontuberculous mycobacteria
Real-time PCR

1. Introduction

Tuberculosis (TB) is one of the top 10 causes of death worldwide, surpassing the death toll from human immunodeficiency virus (HIV) and infecting millions of people each year. The World Health Organization (WHO) has launched the Stop TB Strategy to eliminate TB by 2035 and is calling for global collaboration, which has resulted in a steady decline in global TB incidence [1]. Nontuberculous mycobacteria (NTMs) are common in nature and cause chronic lung disease, lymphadenitis, skin disease, disseminated disease, etc. in immunocompromised patients such as HIV-infected patients and patients with malignancies, as well as in immunocompetent individuals, and their incidence is increasing [2-4]. In addition, pulmonary disease accounts for more than 90% of nontuberculous antibacterial infections, and fibrocavitary nontuberculous antibacterial pulmonary disease closely resembles pulmonary tuberculosis [5]. Isolation and identification of the causative organism are important for the diagnosis of nontuberculous antibacterial lung disease, as therapeutic agents vary depending on the isolate, and treatment is difficult to initiate, with treatment discontinuation due to adverse events being common [5]. Therefore, it is important to differentiate between TB and nontuberculous antibacterial lung disease in order to prevent transmission of TB and avoid unnecessary TB treatment [6].

Acid-Fast Bacilli (AFB) smears, which are commonly ordered to screen for AFB infection, are inexpensive and can provide results relatively quickly, and are currently recommended for use in suspected cases [7]. Nucleic acid amplification testing methods have shown good sensitivity in detecting *Mycobacterium tuberculosis* (Mtb), so a positive AFB smear with a negative nucleic acid amplification test result can be provisionally diagnosed as NTM [5,8].

AFB culture is the gold standard for TB diagnosis and is essential for diagnosis, but it has the disadvantage of taking two to eight weeks. However, culture is necessary to obtain bacterial isolates for drug susceptibility testing and to guide treatment [9]. Unlike Mtb, whose reagent performance is well known, there are relatively few studies evaluating the performance of the three nucleic acid amplification test reagents included in this study on Mtb [10,11]. In addition, the performance of diagnostic reagents for Mtb is relatively good, more than 90% for AFB smear-positive specimens, but for AFB smear-negative specimens, there are differences in performance depending on the diagnostic reagent [12].

Therefore, the authors compared the performance of three real-time polymerase chain reaction (PCR) reagents for detecting Mtb and NTM currently used in Korea: AdvanSure™ TB/NTM real-time PCR kit (AdvanSure; LG Chem, Korea), Genedia® MTB/NTM detection kit (Genedia; Green Cross MS, Eumseong, Korea), and PowerChek™ MTB/NTM real-time PCR kit (PowerChek; Kogenebiotech, Seoul, Korea), were evaluated for their diagnostic accuracy according to AFB smear results, including a large number of AFB-negative specimens, and to determine whether they differed.

2. Materials and methods

This study was conducted at the Department of Diagnostic Laboratory Medicine, Guro Hospital, Korea University, using 102 AFB smear-positive residual specimens received from November 2016 to October 31, 2017. For specificity evaluation, 177 nucleic acids with negative AFB smear results were analyzed among specimens with culture referrals received from November 10 to December 3, 2018. All specimens were tested from specimens that had been frozen at -70°C.

AFB smears were made by smearing the obtained respiratory or non-respiratory specimen directly onto a slide glass or by centrifugation at 3000×g for 15 minutes to smear saliva onto a slide glass. Potentially contaminated specimens, such as sputum, were smeared with an equal volume of 4% N-Acetyl-L-Cysteine-sodium hydroxide (NALC-NaOH) mixture to dissolve

the specimen. Staining was performed by auramine-rhodamin (AR) staining using Aerospray TB model 7722 equipment (ELITechGroup, Signes, France) followed by fluorescence microscopy, and positive results were confirmed by carbol fuchsin (Ziehl-Neelsen) staining. Smears for homeostasis were judged according to the American Thoracic Society/Centers for Disease Control and Prevention (CDC) criteria [13].

AFB cultures were pretreated in the same way as smears and inoculated into liquid medium (VersaTREK Myco, ThermoFisher Scientific, Lenexa, KS, USA) and 2% Ogawa medium (The Korean Institute of Tuberculosis, Cheongju, Korea). The liquid and solid media were incubated in a Versa TREK system (ThermoFisherScientific, Waltham, MA, USA) and a non-CO_2 incubator for 2 and 6 weeks, respectively. The grown strains were subjected to Ziehl-Neelsen carbol fuchsin staining and, if positive, were reported as Mtb using the SD Bioline Ag MPT64 Rapid (SD, Yongin, Korea) antigen test [14], and, if negative, were identified as NTM using a real-time PCR test with the Genedia MTB/NTM detection kit (Green Cross MS). Identification of NTM was based on the results of the same specimen referred to the Institute of Tuberculosis Research.

The real-time PCR test was performed with three different kits: AdvanSure, Genedia, and PowerChek. Nucleic acid extraction was performed manually using the Genedia Mycobacterial DNA Extraction Kit (Green Cross MS, Eumseong, Korea), and the same nucleic acid was used to evaluate each of the three reagents. All three reagents had the same target gene, IS6110 for Mtb and ITS for mycobacterial identification. AdvanSure amplified nucleic acids using the SLAN96 real-time PCR system (LG Chem., Seoul, Korea), while Genedia and PowerChek amplified nucleic acids using the CFX96 system (Bio-Rad, Hercules, CA, USA). PowerChek gave intermediate results, which were borderline between positive and negative and were considered negative in the analysis of the evaluation results.

For concordance analysis, percent positive agreement, percent negative agreement, and overall percent agreement were calculated between each of the three reagents and AFB culture according to CLSI EP12-A2 guidelines, and Cohen's kappa test was performed [15-16]. To evaluate the diagnostic accuracy, sensitivity, specificity, positive predictive value, and negative predictive value were calculated based on the standard test method of AFB culture. The area under the ROC curve (AUC) was compared to determine whether there was a difference in performance between the test reagents. The prevalence required to obtain the predictive value was calculated based on the mean positive rate of all specimens referred to in the same period.

For Genedia, which showed a difference in sensitivity with the other two reagents, an inter-assay comparison between the CFX96 (Bio-Rad) and the Applied Biosystems 7500 Real-Time PCR Instrument system (AB7500; Thermo Fisher Scientific, Waltham, MA, USA) was performed on 89 specimens to determine if there was a difference in positivity rate based on the nucleic acid amplification instrument.

Statistical analysis was performed according to the formula of CLSI EP12-A2 for concordance rate analysis, and MedCalc 18.11.6 (MedCalc Software, Ostend, Belgium) was used for diagnostic accuracy evaluation.

3. Results

3.1. Culture results of AFB smear specimens used in the analysis

Of the 102 AFB smear-positive specimens, nucleic acid was extracted from 85 specimens, excluding 8 specimens with negative culture results and 9 specimens that were consistently referred from the same patient. Sputum was collected from 60 specimens and bronchial aspirate from 25 specimens, of which 63 grew Mtb and 22 grew NTM. Among the NTM, *Mycobacterium avium* was identified in 4 weeks, *Mycobacterium abscessus* in 3 weeks, *Mycobacterium intracellulare* and *Mycobacterium massiliense* in 2 weeks each, and *Mycobacterium szulgai* in 1 week. There were 5 cases

in which two or more NTM grew together, and 5 cases were not referred for identification.

Of the total 177 specimens reported as negative for AFB smear and conventional MTB/NTM detection real-time PCR, 170 were negative, one grew Mtb and six grew NTM. Of the six specimens that grew NTM, all but one were referred for species identification, with *M. avium* identified in three weeks and *M. intracellulare* in two weeks (**Table 1**).

3.2. Accuracy assessment of AFB smear positive and negative specimens

AFB smear-positive specimens were amplified in 85 cases; culture revealed 63 cases of Mtb and 22 cases of NTM. For PCR, AdvanSure results were 62 Mtb, 20 NTM, and 3 negative; Genedia results were 61 Mtb, 12 NTM, and 12 negative. PowerChek had 61 cases of Mtb, 20 cases of NTM, 2 negative, and 2 simultaneously positive for Mtb and NTM.

Discrepancies between culture and PCR were most common with AdvanSure and PowerChek being positive but Genedia being negative and culture growing NTM in 13 cases (**Table 2**). There were seven cases where all PCR tests were positive for Mtb but culture did not grow, all in patients with previously diagnosed and treated TB.

Of the 177 negative AFB smears, AdvanSure had 170 negatives, 3 Mtb positives, 3 NTM positives, and 1 requiring repeat testing. Genedia had 175 negatives, 1 Mtb positive, and 1 requiring retest. PowerChek had 169 negatives, 2 Mtb positives, 5 NTM positives, and 1 recall. The difference in accuracy between the

Table 1. The AFB culture results for the specimens of AFB smear-negative and MTB/NTM real-time PCR negative

Specimen	Results				
	Mtb	NTM	Negative	Subtotal	(%)
Sputum	0	3	68	71	(40.1)
Bronchial aspirate	0	2	46	48	(27.1)
Pleural fluid	1	1	25	27	(15.3)
Cerebrospinal fluid (CSF)	0	0	16	16	(9.0)
Ascites	0	0	9	9	(5.1)
Bronchoalveolar lavage (BAL)	0	0	2	2	(1.1)
Pericardial fluid	0	0	1	1	(0.6)
Tissue (biopsy)	0	0	1	1	(0.6)
Urine	0	0	1	1	(0.6)
Joint (synovial fluid)	0	0	1	1	(0.6)
Total	1	6	170	177	(100.0)

Abbreviation: AFB, acid-fast bacilli; MTB, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria.

Table 2. Discrepant results among three real-time PCR assays and AFB culture in AFB smear-positive specimens

Specimen type (no.)	AdvanSure	Genedia	PowerChek	No. of specimens	AFB culture results
Sputum (4), Bronchial aspirate (9)	NTM	Negative	NTM	13	NTM
Sputum (6), Bronchial aspirate (1)	Mtb	Mtb	Mtb	7	No growth
Sputum (1)	Mtb	Negative	Mtb	1	Mtb
Bronchial aspirate (1)	Mtb	Mtb	Mtb, NTM	1	Mtb
Sputum (1)	Negative	Negative	Negative	1	Mtb
Sputum (1)	Negative	Negative	Negative	1	NTM (*M. intracelullare* + *M. massiliense*)
Bronchial aspirate (1)	Negative	Negative	NTM	1	NTM (*M. avium*)
Bronchial aspirate (1)	NTM	NTM	NTM, Mtb	1	NTM (non-ID)

Abbreviation: AFB, acid-fast bacilli; Mtb, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria, ID; identification.

reagent results was small, with the highest number of results positive for AdvanSure and Power Chek and negative for Genedia being four, one of which had Mtb detected but only grew NTM, and sample was obtained from a patient being treated for Mtb. Two results were negative on AdvanSure and Genedia but positive on Power Chek, with two NTM, one of which was also NTM on culture. The other was retested with the same nucleic acid and was negative, indicating a non-specific reaction or contamination. There was one sample that required simultaneous retesting by all three reagents; Genedia was tested with a 10-fold dilution of the nucleic acid and was negative, while Advan Sure and Power Chek could not be retested and were excluded from the final performance analysis (**Table 3**).

3.3. Concordance analysis of the three reagents with *M. tuberculosis* and non-tuberculous mycobacteria cultures

Concordance with antibacterial culture results for the detection of Mtb was similar for AdvanSure and PowerChek, with a positive concordance rate (95% confidence interval) of 96.9% (89.3%–99.1%) and 95.3% (87.1%–98.4%) for the Genedia reagents, all of which were above 95% (**Table 4**). The negative concordance rate was also identical for AdvanSure and PowerChek at 98.5% (95.6%–99.5%), and Genedia had a negative concordance rate of 99.5% (97.2%–99.9%). The overall concordance rate was equal to or greater than 98% for all three reagents. The kappa test was greater than 0.95 for all three reagents, indicating almost perfect agreement with culture results.

Table 3. Discrepant results between three real-time PCR assays and AFB culture in AFB smear-negative specimens

Specimen type	AdvanSure	Genedia	PowerChek	AFB culture
Sputum	NTM	Negative	NTM	No growth
Bronchial aspirate	NTM	Negative	NTM	NTM
Sputum	NTM	Negative	NTM	NTM
Pleural fluid	Mtb	Negative	Mtb	NTM
Sputum	Negative	Negative	NTM	NTM
Ascites	Negative	Negative	NTM	No growth
Sputum	Mtb	Negative	Negative	NTM
Pleural fluid	Negative	Negative	Negative	Mtb
Bronchial aspirate	Negative	Negative	Negative	NTM
Bronchial aspirate	Mtb	Mtb	Mtb	No growth
Sputum	Retest is required	Retest is required	Retest is required	No growth

Abbreviation: AFB, acid-fast bacilli; Mtb, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria.

Table 4. Agreement between AFB culture tests and three real-time PCR assays for MTB and NTM detection (n = 261)

Parameter	Target organism	AdvanSure		Genedia		PowerChek	
		Result	95% CI	Result	95% CI	Result	95% CI
PPA (%)	Mtb	96.9	89.3–99.1	95.3	87.1–98.4	96.9	89.3–99.1
	NTM	81.8	65.6–91.4	44.4	27.6–62.7	88.9	71.9–96.1
PNA (%)	Mtb	98.5	95.6–99.5	99.5	97.2–99.9	98.5	95.6–99.5
	NTM	99.6	97.6–99.9	100.0	98.3–100.0	98.7	96.2–99.6
Overall percent agreement (%)	Mtb	98.1	95.6–99.2	98.5	96.1–99.4	98.1	95.6–99.2
	NTM	97.3	94.6–98.7	94.3	90.7–96.5	97.3	94.6–98.7
Kappa	Mtb	0.95	0.90–0.99	0.96	0.92–1.00	0.95	0.90–0.99
	NTM	0.88	0.78–0.97	0.59	0.39–0.79	0.88	0.78–0.97

Abbreviation: PPA, percent positive agreement; PNA, percent negative agreement, Mtb; *Mycobacterium tuberculosis*, NTM; nontuberculous mycobacteria; CI, confidence interval.

Concordance with culture results for the detection of NTM differed between the three reagents, with positive concordance rates of 81.8%, 44.4%, and 88.9% for AdvanSure, Genedia, and PowerChek, respectively, and negative concordance rates not significantly different, resulting in no statistical difference in overall concordance rates. The kappa test was 0.59 for Genedia and 0.88 for AdvanSure and Power Chek, showing moderate agreement and almost perfect agreement, respectively.

3.4. Diagnostic accuracy of the three reagents for detecting *M. tuberculosis*

The final diagnostic accuracy for detecting Mtb in AFB smear-positive specimens (n = 85) and total specimens (n = 261) was not significantly different among the three assays in terms of sensitivity, specificity, positive predictive value, and negative predictive value (**Table 5**). In addition, AdvanSure/Genedia/PowerChek showed excellent results with AUC and 95% confidence intervals of 0.98 (0.95–0.99)/0.97 (0.94–0.99)/0.98 (0.95–0.99), respectively. However, the positive predictive value of PowerChek was affected by the prevalence rate and was 98.4% when applying the *M. tuberculosis* positive rate of 74.1% in the study, which was not significantly different, but 32.8% when assuming the average positive rate of 2.2% during the study period, which was significantly different from other reagents (**Table 5**).

3.5. Diagnostic accuracy of the three reagents for detection of nontuberculous mycobacteria

Diagnostic accuracy for detection of NTM varied among the kits, with PowerChek having the highest AUC of 0.97 for AFB smear positives, followed by AdvanSure with similar results, but Genedia was significantly lower at 0.77 (**Table 6**). Specificity was good for all of them, which is due to the difference in sensitivity between negative and positive AFB smears. When diagnostic accuracy was assessed in all AFB smear positive and negative specimens, the AUC (95% CI) for AdvanSure and PowerChek were 0.91 (0.86–0.94) and 0.94 (0.90–0.96), respectively, compared to 0.72 (0.66–0.78) for Genedia, which was statistically significantly different (P < 0.0001) (**Table 6**).

Table 5. Diagnostic accuracy of three real-time PCR assays for the MTB detection stratified by AFB smear results (n = 261)

Parameter	AFB smear	AdvanSure		Genedia		PowerChek	
		Result	95% CI	Result	95% CI	Result	95% CI
Sensitivity (%)	Positive	98.4	91.5–100.0	96.8	89.0–99.6	98.4	91.5–100.0
	Negative	0.0	0.0–79.3	0.0	0.0–79.3	0.0	0.0–79.3
	Total	96.9	89.2–99.6	95.3	86.9–99.0	96.9	89.2–99.6
Specificity (%)	Positive	100.0	84.6–100.0	100.0	84.6–100.0	95.5	77.2–99.9
	Negative	98.3	95.1–99.4	99.4	96.8–99.9	98.9	95.9–99.7
	Total	98.5	95.6–99.7	99.5	97.2–100.0	98.5	95.6–99.7
PPV (%)	Positive	100.0	-	100.0	-	32.8	6.7–76.8
	Negative	Incomputable		Incomputable		Incomputable	
	Total	58.9	31.7–81.5	80.9	37.4–96.8	58.9	31.7–81.5
NPV (%)	Positive	100.0	99.8–100.0	99.9	99.7–100.0	100.0	99.7–100.0
	Negative	Incomputable		Incomputable		Incomputable	
	Total	99.9	99.7–100.0	99.9	99.7–100.0	99.9	99.7–100.0
AUC	Positive	0.99	0.94–1.00	0.98	0.93–1.00	0.97	0.91–1.00
	Negative	Incomputable		Incomputable		Incomputable	
	Total	0.98	0.95–0.99	0.97	0.94–0.99	0.98	0.95–0.99

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ROC curve; CI, confidence interval.

Table 6. Diagnostic accuracy of three real-time PCR assays for NTM detection stratified by AFB smear results (n = 261)

Parameter	AFB smear	AdvanSure		Genedia		PowerChek	
		Result	95% CI	Result	95% CI	Result	95% CI
Sensitivity (%)	Positive	90.9	70.8–98.9	54.6	32.2–75.6	95.5	77.2–99.9
	Negative	40.0	5.3–85.3	0.0	0.0–52.2	60.0	14.7–94.7
	Total	81.5	61.9–93.7	44.4	25.5–64.7	88.9	70.8–97.6
Specificity (%)	Positive	100.0	94.3–100.0	100.0	94.3–100.0	98.4	91.5–100.0
	Negative	99.4	96.8–100.0	100.0	97.9–100.0	98.8	95.8–99.9
	Total	99.6	97.6–100.0	100.0	98.4–100.0	98.7	96.3–99.7
PPV (%)	Positive	100.0	73.5–100.0	100.0	73.5–100.0	29.8	5.7–74.8
	Negative	32.5	4.9–81.8	Incomputable		26.6	7.1–63.1
	Total	57.3	15.9–90.5	100.0	73.5–100.0	32.8	13.6–60.3
NPV (%)	Positive	99.9	99.8–100.0	99.7	99.5–99.8	100.0	99.8–100.0
	Negative	99.6	99.1–99.8	99.3	99.3–99.3	99.7	99.2–99.9
	Total	99.9	99.7–99.9	99.6	99.5–99.7	99.9	99.8–100.0
AUC	Positive	0.96	0.89–0.99	0.77	0.67–0.86	0.97	0.91–1.00
	Negative	0.70	0.62–0.76	0.50	0.42–0.58	0.79	0.73–0.85
	Total	0.91	0.86–0.94	0.72	0.66–0.78	0.94	0.90–0.96

Abbreviation: PPV, positive predictive value; NPV, negative predictive value; AUC, area under the ROC curve; CI, confidence interval.

3.6. Inter-instrumental evaluation of Genedia reagents

In Mtb-positive specimens (n = 69), the threshold cycle (Ct) value was consistently delayed by 2 cycles when tested on the AB7500 instrument compared to the CFX96 instrument (P < 0.0001). In NTM-positive specimens (n = 22), there was little difference in accuracy, but on average, the AB7500 resulted in a 0.6 cycle delay (P = 0.02). Only specimens within the cut-off value of Ct were tested using the CFX96 instrument, and although qualitative results did not show significant performance differences between instruments for Genedia, there is a need to validate the cut-off value for Mtb and NTM detection on Genedia.

4. Discussion

This study evaluated the diagnostic accuracy of three real-time PCR tests for the simultaneous detection of Mtb and NTM by comparing them to a co-referred AFB culture. Previous studies have shown that the diagnostic accuracy of AdvanSure, Genedia, and PowerChek varied with AFB smear results and specimen type, and the authors' results also showed these differences [11,17,18].

In addition, we evaluated the specificity of the test in specimens with low concentrations of Mtb and Mtb nucleic acid by using a large number of specimens with negative AFB smears but positive culture results for Mtb/NTM real-time PCR. We found seven cases that were negative by nucleic acid amplification and AFB smear but positive by culture, six of which grew NTM and one of which grew Mtb. Compared with the standard test and antibacterial culture, the test showed excellent sensitivity of more than 95% for Mtb, but a difference in performance could be seen for NTM. However, it was difficult to evaluate the performance for Mtb as there was only one sample that grew Mtb in a negative AFB smear.

In the case of Mtb, there is a risk of unnecessary treatment in case of false positive results. In particular, in the case of a positive AFB smear, it is difficult to exclude recurrence of TB if there is a history of TB in the past, and there is a possibility of taking TB medication. In this study, the PowerChek resulted in a grey zone with an intermediate result, which is ambiguous to interpret. However, one out of three

Mtb intermediate results and one out of five NTM intermediate results grew NTM in culture, and there was one case of a patient with a history of TB who consistently grew NTM but reported a simultaneous positive Mtb and NTM result. This was likely due to the use of Ct values to differentiate between the simultaneous detection of Mtb and NTM and the detection of M. tuberculosis alone, leading to a high false-positive rate. Because of the potential for unnecessary medication in the event of a false-positive result, this study counted all of them as negative and calculated the accuracy result, so laboratories need to create their own interpretation criteria. In addition, it also had the highest sensitivity for detecting NTM, making it useful for detecting NTM in conjunction with AdvanSure.

In recent years, it has become important not only to treat confirmed cases but also to detect latent TB and carriers early to prevent transmission [1], and it has become essential for medical institutions to quickly preemptively isolate suspected TB patients who are capable of transmission. As TB and non-TB lung diseases have similar clinical manifestations and are difficult to differentiate based on imaging findings, a method to quickly distinguish between them is essential, and the detection rate of non-TB lung diseases in Korea is increasing year by year [19]. On the other hand, the Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA) test detects Mtb by detecting the rpoB gene and its mutations directly from the specimen, which requires less time to detect Mtb and determine multidrug resistance, and has relatively good sensitivity and specificity, and is recommended by the World Health Organization for initial diagnosis in patients with suspected multidrug-resistant TB or HIV-associated TB [20]. The three Mtb/NTM real-time PCR assays evaluated in this study have similar AUCs to the previously reported Xpert MTB/RIF (Cepheid) in detecting Mtb, which is thought to be helpful for early diagnosis of TB [21,22].

The authors evaluated the diagnostic accuracy of three real-time PCR assays for the detection of Mtb and NTM. All three assays showed better sensitivity and specificity for the detection of Mtb and NTM compared to previous literature [17,18]. The performance of each assay did not differ for the detection of Mtb, but the sensitivity of Genedia was significantly different from the other two assays for the detection of NTM.

Limitations of this study include the different collection periods for positive and negative AFB smears and the insufficient number of samples evaluated (102 positive and 177 negative AFB smears) to determine the superiority of performance among the three reagents. However, there was a significant difference in results for the detection of NTM, including several specimens that were thought to have low concentrations. For AdvanSure, the manufacturer's limit of detection was 1.4 copies/μL and varied from 8.5 to 538.2 copies/μL for the major NTM (*M. avium*, *M. fortuitum*, *M. abscessus*, *M. kansasii*, and *M. intracellulare*); for Genedia, the limit of detection was 0.2 copies/μL for Mtb and 10 copies/μL for NTM (*M. fortuitum*). For Power Chek, only the lowest detection limit for the target gene is given, which is the same for both IS6110 and ITS at 2.5 copies/μL. The three real-time PCR reagents target the same gene but amplify different sites, and the difference in the limit of detection between the reagents shows the difference in test performance. In the future, when introducing new reagents in the laboratory, it is recommended to consider these characteristics and verify the manufacturer's limit of detection (LOD) for each target gene or strain.

Despite its high sensitivity and specificity, nucleic acid amplification testing cannot completely replace AFB smear and culture testing because of the epidemiological nature of Mtb strains and the need for antituberculosis drug-susceptibility testing, and the possibility of false negatives and false positives exists, so results should be interpreted in relation to clinical presentation [23].

In conclusion, this study evaluated the diagnostic

accuracy of three real-time polymerase chain reaction reagents for the detection of Mtb and NTM. The diagnostic accuracy of the three reagents, AdvanSure™ TB/NTM real-time PCR kit (LG Chem.), Genedia® MTB/NTM detection kit (Green Cross MS), and PowerChek™ MTB/NTM Real-time PCR kit (Kogenebiotech), was equivalent for the detection of Mtb and different for the detection of NTM.

Disclosure statement

The authors declare no conflict of interest.

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