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更新封面

Evaluation of the AdvanSure TB/NTM Plus Real-Time PCR Assay for the Simultaneous Detection of *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria from Clinical Specimens

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Abstract

Background: The AdvanSure TB/NTM plus real-time PCR (AdvanSure plus PCR; LG Chem., Korea) assay has been developed to increase the diagnostic sensitivity of nontuberculous mycobacteria (NTM) compared with the existing AdvanSure TB/NTM real-time PCR (AdvanSure PCR; LG Chem., Korea) assay. This study aimed to evaluate the performance of the AdvanSure plus PCR comparing the results with mycobacterial culture and the AdvanSure PCR. *Methods:* Patients (n = 199) with suspected NTM or *Mycobacterium tuberculosis* complex (MTC) were tested using AdvanSure plus PCR assay, AdvanSure PCR assay, acid-fast bacilli staining, and mycobacteria culture. Additionally, 200 DNA samples (MTC, n = 100, and NTM, n = 100) were obtained from positive MTC or NTM cultures for evaluation using the AdvanSure plus PCR assay. *Results:* The two real-time PCR systems showed a 94.0% (n = 187/199) concordance rate (Kappa = 0.94). Based on culture results, the sensitivity and specificity for the detection of MTC were 100% (n = 45/45) and 83.8% (n = 129/154) using AdvanSure plus PCR, and 100.0% (n = 45/45), and 99.3% (n = 153/154) using AdvanSure PCR, respectively. The sensitivity and specificity for NTM detection were 68.0% (n = 17/25) and 90.2% (n = 157/174) using AdvanSure plus PCR, and 64.0% (n = 16/25) and 95.4% (n = 166/174) using AdvanSure PCR, respectively. With culture-positive samples, AdvanSure plus PCR tested positive for 100% of both the MTC and NTM specimens. Seven (out of 200) culture-positive samples tested positive for both MTC and NTM using the AdvanSure plus PCR. *Conclusion:* AdvanSure plus PCR had increased sensitivity but decreased specificity compared with AdvanSure PCR for the detection of NTM. The AdvanSure plus PCR assay can be used for the simultaneous detection of MTC and NTM in direct specimens and cultures.

Keywords

Mycobacterium
Polymerase chain reaction
Tuberculosis

1. Introduction

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb). In addition to the lungs, Mtb can infect all organs, including lymph nodes, pleura, osteoarticular joints, kidneys, meninges, peritoneum, small intestine, large intestine, ovaries, and skin, and clinical manifestations vary. Common symptoms are fatigue, weakness, weight loss, and fever, with a chronic cough in the lungs and bloody sputum in more advanced cases. On the other hand, nontuberculous mycobacteria (NTM) have been recognized as non-pathogenic bacteria widely present in living environments such as soil and water [1]. However, with the epidemic of acquired immunodeficiency in the 1980s, NTM-induced diseases increased, and with the recognition of their clinical significance and the development of culture methods, they have been reported to cause lung diseases, ulcerative diseases, and inflammatory bowel diseases even in patients not infected with human immunodeficiency virus (HIV). According to domestic reports, the prevalence of NTM-induced lung disease is increasing rapidly, from 19.0 per 100,000 population in 2007 to 39.6 in 2016 [2]. In addition, most of these NTMs are resistant to anti-TB drugs. Therefore, as the number of NTM-induced diseases increases every year in Korea, a diagnostic method that accurately distinguishes between Mtb and NTM is needed to reduce unnecessary treatment, apply different treatments according to the strain, and prevent the waste of money and resources on infection control [3].

Although the prevalence of TB in the country is gradually decreasing, it is still high compared to Organization for Economic Co-operation and Development (OECD) countries, accounting for 51 cases per 1 million people in 2018 [4]. Therefore, efforts are being made as a national priority to rapidly diagnose and treat TB.

Traditionally, the diagnosis of TB is based on bacteriological confirmation from respiratory specimens, which may include clinical presentation, chest X-ray, acid-fast bacilli (AFB) smear, sputum culture, and molecular biological testing. The AFB smear is a simple and rapid diagnostic test but has the disadvantage of low sensitivity and the inability to differentiate between Mtb and NTM. Mtb culture is the standard diagnostic method for confirming Mtb, but it is time-consuming, taking up to 6–8 weeks [5,6]. Polymerase chain reaction (PCR) has been widely used to diagnose TB and is a useful adjunctive test because it is rapid, can differentiate between Mtb and NTM and is more sensitive than antibacterial smears [7,8]. Recently, the real-time polymerase chain reaction (real-time PCR) method has been widely used because of its high sensitivity and good reproducibility compared to the traditional PCR method [9-11].

The recently developed AdvanSure TB/NTM plus real-time PCR (AdvanSure plus PCR; LG Chem., Seoul, Korea) kit has changed the detection site compared to the existing AdvanSure TB/NTM real-time PCR (AdvanSure PCR; LG Chem., Seoul, Korea) kit, enabling the detection of small amounts of NTM in overlapping infections, thereby increasing the sensitivity of NTM diagnosis.

Therefore, this study aimed to compare the recently developed AdvanSure plus PCR kit, which has increased the sensitivity of NTM diagnosis, with the existing AdvanSure PCR kit, and to evaluate the results of antibacterial smear and Mtb culture.

2. Materials and methods

2.1. Study population

From January 2016 to July 2017, TB was detected in various clinical specimens (140 sputa, 17 pleural fluid, 18 tracheal aspirates, 17 abscesses, 17 lymph nodes, 17 tissues, 3 urine, 2 cerebrospinal fluid, 1 feces, 1 other body fluid) that were referred for TB/NTM PCR testing and stored in a -70°C freezer, and 199 DNA samples (100 Mtb and 100 NTM isolates) that were referred for TB culture and stored in a -70°C freezer from January 2012 to August 2016. This study was approved by the Institutional Review Board of Mokdong Hospital,

Ewha Womans University (IRB No. 2016-12-015-003).

2.2. Specimen pretreatment

Respiratory specimens including sputum were placed in a 15 mL conical tube with an equal volume of 4% sodium hydroxide/standard N-acetyl-L-cysteine (NaOH/NALC), mixed well in a vortex mixer, left at room temperature for 15 min, centrifuged at 3,000 rpm for 15 min, and the supernatant was discarded, and the remaining precipitate was used for testing.

2.3. AFB smear staining test

Ziehl-Neelsen staining with carbol fuchsin or fluorescence staining with auramine-rhodamine fluorescence were used to read according to the standards of the Centers for Disease Control and Prevention (CDC).

2.4. Mycobacterium tuberculosis culture test

Pretreated specimens were simultaneously cultured in solid and liquid media, respectively. Solid medium cultures were inoculated in 3% Ogawa broth and incubated at 37° C for 8 weeks. Liquid media cultures were inoculated into BBL MGIT mycobacteria growth indicator tubes (Beckton Dickinson, Sparks, MD, USA) and incubated for 6 weeks using MGIT 960 (Beckton Dickinson, Sparks, MD, USA). Culture-positive strains were differentiated into Mtb and NTM by molecular diagnostic methods using MoleculTech MTBID (Molecular & Diagnostic Inc., Gangwon, Korea), and NTM strain identification was performed by PCR-restriction fragment length polymorphism analysis (PCR-RFLP) using GenoType Mycobacterium CM & AS (Hain Lifescience GmbH, Nehren, Germany). All methods were performed according to the manufacturer's instructions.

2.5. Nucleic acid extraction

To the sediment remaining after the pretreatment procedure, 200 μL of 2× sample preparation was added, the sediment was mixed using a pipette, and DNA was extracted using an automated nucleic acid extraction system of TANBead Smart LabAssist-32 (SLA-32) (TAN Bead, Taoyuan, Taiwan) using a TANBead Optipure viral auto plate (TAN Bead, Taoyuan, Taiwan) according to the manufacturer's instructions.

2.6. AdvanSure TB/NTM real-time PCR

The AdvanSure PCR kit was used and performed according to the manufacturer's instructions. The precipitate remaining after the pretreatment was transferred to a 1.5 mL tube and 1.0 mL of sample preparation solution was added, centrifuged at 13,000 rpm for 3 min, the supernatant was removed, and the procedure was repeated twice. After shaking well to mix, 50 μL of extraction buffer was added to a 1.5 mL tube, heated at 100°C for 20 min, centrifuged at 13,000 rpm for 1 min, mixed well, centrifuged again at 13,000 rpm for 3 min, and 5 μL of the supernatant was used for PCR. In a 200 μL tube containing 10 μL of the 2× PCR mixture, 5 μL of the TB/NTM primer/probe mixture, and 5 μL of the extracted DNA sample were added, centrifuged, and loaded into the SLAN real-time quantitative PCR detection system (Shanghai Hongshi Medical Technology Co., Ltd, Shanghai, China). A threshold cycle (Ct) value of less than 35 was considered positive, and the NTM Ct value minus the TB Ct value was judged as the presence of Mtb alone if the value was greater than 0, and as NTM co-infection if the value was less than 0.

2.7. AdvanSure TB/NTM plus real-time PCR

Compared with the previous AdvanSure PCR kit, the recently developed AdvanSure plus PCR kit has changed the detection site of NTM from internal transcribed spacer (ITS) to 16s rRNA, enabling the detection of a small amount of NTM in the case of co-infection, thereby increasing the sensitivity of NTM diagnosis.

The AdvanSure plus PCR kit was used and performed according to the manufacturer's instructions.

To a 200 μL tube containing 10 μL of 2× PCR mixture, 5 μL of TB/NTM primer/probe mixture and 5 μL of extracted DNA sample were added, centrifuged, and mixed well by vortex mixer or finger tapping. After centrifugation, it was placed in the SLAN real-time quantitative PCR detection system (Shanghai Hongshi Medical Technology Co., Ltd) for amplification and testing.

A total of three channels (TB, NTM, and internal control, IC) were used to detect the PCR reaction, and the formation of signals of FAM, HEX, and Cy5 wavelengths was checked in each channel. A positive result was defined as a Ct value of less than 35 for TB and IC, and less than 40 for NTM. A positive result in the TB channel was considered TB, a positive result in the NTM channel was considered NTM, a simultaneous positive result in the TB and NTM channels was considered a duplicate infection, a positive result in the IC channel alone was considered negative, and a negative result in all channels was tested again.

2.8. Comparison of results

Sensitivity and specificity for the detection of Mtb and NTM were calculated based on the culture of 199 clinical specimens and 200 isolates. The results of AdvanSure plus PCR on clinical specimens were compared with the results of conventional AdvanSure PCR to obtain the percentage of agreement. Discrepant results were retested, and specimens with discordant culture results from AdvanSure plus PCR were subjected to additional AdvanSure PCR.

3. Results

3.1. Mycobacterium species distribution of cultured strains

The Mycobacterium species identification results included 100 (50.0%) Mtb complex (MTC) and 100 (50.0%) NTM, with NTM comprising *M. intracellulare* 37 weeks (16.4%), *M. avium* spp. 28 weeks (12.4%), *M. abscessus* 14 weeks (6.2%), *M. gordonae* 6 weeks (2.7%), *M. fortuitum* 5 weeks (2.2%), *M. kansasii* 4 weeks (1.8%), *M. chelonae* 2 weeks (0.9%), *M. heckeshornense* 3 weeks (1.3%), and *M. lentiflavum* 1 week (0.4%) (**Table 1**).

Table 1. Frequency of *Mycobacterium* species in this study

Species	No.	%
MTC	100	50.0
NTM	100	50.0
M. abscessus	14	6.2
M. avium spp.	28	12.4
M. kansasii	4	1.8
M. intracellulare	37	16.4
M. chelonae	2	0.9
M. fortuitum	5	2.2
M. gordonae	6	2.7
M. heckeshornense	3	1.3
M. lentiflavum	1	0.4
Total	200	100.0

Abbreviation: MTC, *M. tuberculosis* complex; NTM, nontuberculous mycobacteria

3.2. Results of AdvanSure plus PCR performed on cultured strains

AdvanSure plus PCR detected all 100 isolates of Mtb and 100 isolates of NTM, resulting in a detection rate of 100%; however, seven of the 100 isolates of Mtb and 100 isolates of NTM showed duplicate detection of Mtb and NTM, resulting in a concordance rate with antimicrobial culture of 96.5% (193/200) (**Table 2** and **3**). Of the three weeks with overlapping MTC and NTM infections in the AdvanSure plus PCR for 100 MTC isolates, only one week showed mixed MTC and NTM, and two weeks were read as MTC. Of the 100 NTM isolates, 4 isolates (1 *M. intracellulare*, 1 *M. kansasii*, and 2 *M. gordonae*) with mixed Mtb and NTM on AdvanSure plus PCR were tested with AdvanSure PCR, and 1 isolate was found to be NTM on AdvanSure PCR and 3 isolates were found to be co-infected with Mtb and NTM.

Table 2. Performance of AdvanSure plus PCR in 200 mycobacterial strains

Culture	AdvanSure plus PCR		
	MTC (*n* = 97)	MTC and NTM (*n* = 7)	NTM (*n* = 96)
MTC	97	3	
NTM		4	96
M. abscessus	0	0	14
M. avium	0	0	28
M. chelonae	0	0	2
M. fortuitum	0	0	5
M. gordonae	0	2	4
M. heckeshomense	0	0	3
M. intracellulare	0	1	36
M. kansasii	0	1	3
M. lentiflavum	0	0	1

Abbreviations: MTC, *M. tuberculosis* complex; NTM, nontuberculous mycobacteria; AdvanSure plus PCR, AdvanSure TB/NTM plus real-time PCR

Table 3. Results of AdvanSure plus PCR and AdvanSure PCR in 7 cases of MTC and NTM mix

Species	AdvanSure plus PCR				AdvanSure PCR			
	MTC Ct	NTM Ct	IC Ct	Result	MTC Ct	NTM Ct	IC Ct	Result
M. intracellulare	33.57	17.87	24.06	MTC and NTM positive	31.99	18.37	28.92	MTC and NTM positive
M. kansasii	33.32	20.48	24.03	MTC and NTM positive	33.19	22.92	27.76	MTC and NTM positive
M. gordonae	34.66	19.98	24.10	MTC and NTM positive	No Ct	21.16	27.97	NTM positive
M. gordonae	29.15	17.90	23.98	MTC and NTM positive	29.25	19.01	29.39	MTC and NTM positive
MTC	21.16	17.25	24.34	MTC and NTM positive	21.81	18.56	No Ct	MTC and NTM positive
MTC	16.31	25.8	28.02	MTC and NTM positive	16.94	21.88	No Ct	MTC positive
MTC	14.16	19.84	No Ct	MTC and NTM positive	13.71	17.91	No Ct	MTC positive

Abbreviation: Ct, threshold cycle; IC, internal control; MTC, *M. tuberculosis* complex; NTM, nontuberculous mycobacteria; AdvanSure PCR, AdvanSure TB/NTM real-time PCR; AdvanSure plus PCR, AdvanSure TB/NTM plus real-time PCR.

3.3. Sensitivity and specificity of AFB smear stains with AdvanSure PCR and AdvanSure plus PCR in clinical specimens

Sensitivity and specificity were calculated for 199 clinical specimens based on the AFB smear results. The sensitivity for Mtb was 51.1% (23/45) with AFB smear staining and 100% (45/45) with both AdvanSure PCR and AdvanSure plus PCR, and the specificity for Mtb was 99.3% (153/154) with AFB smear staining and 83.8% (129/154) with both AdvanSure PCR and AdvanSure plus PCR. Sensitivity for NTM was 20.0% (5/25) for AFB smear staining, 64% (16/25) for AdvanSure PCR, and 68% (17/25) for AdvanSure plus PCR, and specificity for NTM was 99.4% (173/174) for AFB smear staining, 95.4% (166/174) for AdvanSure PCR, and 90.2% (157/174) for AdvanSure plus PCR (**Table 4** and **5**).

3.4. Comparison of AdvanSure PCR and AdvanSure plus PCR in clinical specimens

In 199 clinical specimens, the agreement between the results of AdvanSure PCR and AdvanSure plus PCR was 94.0% (187/199) [κ = 0.9478, standard error (SE) 0.01472, 95% confidence interval (CI) 0.91855–0.97625; **Table 6**].

The 12 discordant cases were: 3 of 70 cases with Mtb on AdvanSure PCR showed overlapping infection with Mtb and NTM on AdvanSure plus PCR; 1 of 24 cases with NTM on AdvanSure PCR was negative on AdvanSure plus PCR; and 8 cases that were negative on AdvanSure plus PCR were positive for NTM on AdvanSure plus PCR.

Table 4. Results of AdvanSure plus PCR, AdvanSure PCR assay, and AFB staining according to the results of culture

	Culture		
	MTC (*n* = 45) no. (%)	**Negative (*n* = 129) no. (%)**	**NTM (*n* = 25) no. (%)**
AdvanSure PCR			
MTC	45 (100.0)	25 (19.4)	0
Negative	0	96 (74.4)	9 (36.0)
NTM	0	8 (6.2)	16 (64.0)
AdvanSure plus PCR			
MTC	42 (93.3)	25 (19.4)	0
MTC and NTM	3 (6.7)	0	0
Negative	0	90 (69.8)	8 (32.0)
NTM	0	14 (10.9)	17 (68.0)
AFB stain			
Negative	22 (48.9)	128 (99.2)	20 (80.0)
Positive	23 (51.1)	1 (0.8)	5 (20.0)

Abbreviation: MTC, *M. tuberculosis* complex; NTM, nontuberculous mycobacteria; AdvanSure PCR, AdvanSure TB/NTM real-time PCR; AdvanSure plus PCR, AdvanSure TB/NTM plus real-time PCR.

Table 5. The performance of AdvanSure plus PCR, AdvanSure PCR assay, and AFB stain for detection of MTC/NTM compared with mycobacterial culture

	Sensitivity % (no.)	**Specificity % (no.)**	**PPV[§] %**	**NPC[§] %**
AdvanSure PCR				
MTC	100.0 (45/45)	83.8 (129/154*)	32.1	100.0
NTM	64.0 (16/25)	95.4 (166/174[‡])		
AdvanSure plus PCR				
MTC	100.0 (45/45[†])	83.8 (129/154*)	32.1	100.0
NTM	68.0 (17/25)	90.2 (157/174[‡])		
AFB staining	40.0 (28/70)	99.2 (128/129)		

*Included negative culture samples (*n* = 129) and NTM positive culture samples (*n* = 25). [†]Included MTC & NTM co-positive samples (*n* = 3) by AdvanSure plus PCR. [‡]Included negative culture samples (*n* = 129) and MTC positive culture samples (*n* = 45). [§]Calculated using Korean tuberculosis prevalence of 0.0768%. Abbreviation: MTC, *M. tuberculosis* complex; NTM, nontuberculous mycobacteria; AdvanSure PCR, AdvanSure TB/NTM real-time PCR; AdvanSure plus PCR, AdvanSure plus TB/NTM real-time PCR.

Table 6. Concordance of AdvanSure plus PCR and AdvanSure PCR assays for detection of MTC and NTM

MTC		**AdvanSure PCR**				
		MTC and NTM	**Negative**	**NTM**	**Total no. (%)**	
AdvanSure plus PCR	MTC	67	0	0	0	67 (33.7)
	MTC and NTM	3	0	0	0	3 (1.5)
	Negative	0	0	97	1	98 (49.2)
	NTM	0	0	8	23	31 (15.6)
	Total	70 (35.2)	0	105 (52.8)	24 (12.1)	199 (100.0)

Abbreviation: MTC, *M. tuberculosis* complex; NTM, nontuberculous mycobacteria; AdvanSure PCR, AdvanSure TB/NTM real-time PCR; AdvanSure plus PCR, AdvanSure TB/NTM plus real-time PCR.

4. Discussion

Early diagnosis and initiation of treatment for TB are critical from a public health and healthcare perspective, and the use of PCR for early diagnosis is beneficial to clinicians and patients [11]. AdvanSure plus PCR was developed to increase the sensitivity of NTM detection compared to the original AdvanSure PCR kit. The original AdvanSure PCR uses IS*6110* as the detection site for Mtb and ITS as the detection site for NTM. Therefore, since Mtb possesses both IS*6110* and ITS, signals from both Mtb and NTM appear simultaneously when detecting Mtb. Since Mtb possesses 10 times more copies of IS*6110* than ITS, the combination of the Ct values of the Mtb and NTM channels is used to determine the co-infection of Mtb and NTM only when the NTM Ct value is greater than the MTC Ct value. However, if a small amount of NTM is co-infected, it may be difficult to detect NTM because ITS is detected due to Mtb. AdvanSure plus PCR has the same detection site for Mtb as IS*6110*, but changes the detection site for NTM to 16s rRNA to detect NTM independently, increasing the possibility of detecting NTM even in Mtb and NTM co-infection with small amounts of NTM.

In this study, AdvanSure plus PCR was performed on cultured strains and detected all 100 strains of Mtb and 100 strains of NTM, resulting in a detection rate of 100%, but 7 of the 100 strains of Mtb and 100 strains of NTM were found to be mixed with Mtb and NTM, resulting in a concordance rate with culture of 96.5% (193/200). Of the three weeks that showed mixed Mtb and NTM in the AdvanSure plus PCR with 100 isolates of Mtb, only one week showed mixed Mtb and NTM and two weeks showed Mtb when tested with the AdvanSure PCR, likely due to differences in the reading criteria of the two tests. Of the 100 NTM isolates, 4 isolates (1 *M. intracellulare*, 1 *M. kansasii*, and 2 *M. gordonae*) were tested by AdvanSure PCR, 1 isolate was found to be NTM by AdvanSure PCR, 3 isolates were found to be Mtb and NTM, and 1 isolate had an MTC Ct value of 34. 66, a value near the cut-off, which is thought to be negative on AdvanSure PCR, and most of the cases showed MTC Ct values near the cut-off value of 29.15–33.57.

In this study, DNA extracted from cultured strains was tested by AdvanSure PCR and AdvanSure plus PCR after storage, and MoleculTech MTC-ID (Molecular & Diagnostic Inc.) was used for the differentiation of Mtb and NTM. The MoleculTech MTC-ID (Molecular & Diagnostic Inc.) differs from the AdvanSure PCR kit by adding a region of difference (RD) gene, which is present only in Mtb [12,13], to the detection site to reduce negative results caused by Mtb that does not possess the IS*6110* gene and to increase sensitivity by amplifying two genes [14]. In a study using MoleculTech MTC-ID (Molecular & Diagnostic Inc.), the test was performed after mixing the DNA of Mtb and NTM in various ratios, and Mtb could be accurately detected even when the ratio of Mtb and NTM was 1:9, showing excellent performance in detecting Mtb even when the DNA of Mtb and NTM is duplicated [15]. In addition, in this experiment, NTM identification was performed using GenoType Mycobacterium CM & AS (Hain Lifescience GmbH), which contains a Mtb-specific band, and all strains with a Mtb-specific band were excluded if NTM was detected. According to the literature, GenoType Mycobacterium CM (Hain Lifescience GmbH) was compared with DNA sequencing using cultured strains, and 74 out of 75 cases were correctly identified as Mtb and only one case was negative, indicating that it is sensitive for detecting Mtb [16].

The sensitivity and specificity of nucleic acid amplification tests for the diagnosis of Mtb have been studied in several studies, and another study evaluating the existing AdvanSure PCR in respiratory specimens reported sensitivity and specificity of 97.3% and 95.5% for detecting Mtb and 75.0% and 100% for detecting NTM, respectively, based on AFB smear results [17]. In this study, based on the AFB smear results of 199 clinical specimens, the sensitivity and specificity for Mtb were 100% (45/45) and 83.8% (129/154) for AdvanSure PCR and AdvanSure plus PCR,

respectively. Sensitivity for NTM was 64% (16/25) and 68% (17/25) for AdvanSure PCR and AdvanSure plus PCR, respectively, and specificity for NTM was 95.4% (166/174) for AdvanSure PCR and 90.2% (157/174) for AdvanSure plus PCR. Using real-time PCR, both tests were highly sensitive for Mtb but somewhat less sensitive for NTM. AdvanSure plus PCR was more sensitive than the original AdvanSure PCR for detecting NTM, but less specific.

Comparing the results of AdvanSure PCR and AdvanSure plus PCR in 199 clinical specimens, the concordance rate was 94.0% (187/199). In the 12 discordant cases, 3 of the 70 cases that were Mtb on AdvanSure PCR showed a mixture of Mtb and NTM on AdvanSure plus PCR, which is a possible false positive for NTM with a strain that is Mtb in culture. Of the 24 cases that were NTM on AdvanSure PCR, 1 case was negative on AdvanSure plus PCR, which is thought to be a true negative for NTM in culture and has the potential for a false positive result on AdvanSure PCR. Eight cases that were negative on the AdvanSure PCR were positive for NTM on the AdvanSure plus PCR, seven of which were negative for NTM on culture, suggesting that the AdvanSure plus PCR may be a false positive, and one case was positive for NTM on culture, suggesting a true positive. False positives in Mtb/NTM PCR tests can be caused by carry-over or cross-contamination, and also by the colonization of small numbers of Mtb bacteria in specimens from patients without active TB in epidemiologically high prevalence areas, which can be detected by PCR [18,19]. However, in patients on treatment with some anti-TB drugs, Mtb or NTM may not be detected in culture but only by PCR, and it cannot be excluded that NTM with challenging growth conditions may not be detected in culture [20,21].

The AdvanSure PCR and AdvanSure plus PCR kits used in this study use real-time PCR, which allows for analysis without additional steps such as post-amplification electrophoresis or hybridization reactions, thus reducing the possibility of false positives due to cross-contamination by paying attention to sample contamination during nucleic acid extraction. False negatives can also occur due to technical errors, low strain counts, non-uniform distribution due to the tendency of Mtb to aggregate or form strings, the presence of inhibitors of the amplification process in the specimen, and mutations or deficiencies in the target gene being amplified [22]. Because of these problems of false positives and false negatives with molecular biological methods, AFB smear staining and culture should be used in combination, and the results should be interpreted in light of clinical information.

The AFB smear is the most widely used basic test for the detection of Mtb, but it has the disadvantage of low sensitivity and specificity. In this study, the sensitivity of the AFB smear test was only 51.1%, which is somewhat lower than the various sensitivities of 52%–97% reported previously. The specificity of the AFB smear test was 99.3% in this study, which is similar to previously reported studies. To summarize the results of this study, both AdvanSure PCR and AdvanSureplus PCR using real-time PCR were highly sensitive for detecting Mtb but less sensitive for detecting NTM. AdvanSure plus PCR was more sensitive than AdvanSure PCR for the detection of NTM, confirming that it can be used as a method for the simultaneous detection of Mtb and NTM in clinical specimens and cultures.

Disclosure statement

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