Ann Clin Microbiol Vol. 18, No. 2, June, 2015
http://dx.doi.org/10.5145/ACM.2015.18.2.44
pISSN 2288-0585 · eISSN 2288-6850

Performance Evaluation of Anyplex plus MTB/NTM and AdvanSure TB/NTM for the Detection of *Mycobacterium tuberculosis* and Nontuberculous Mycobacteria

Wonho Choe, Ehwa Kim, Seo Yeon Park, Jeong Don Chae

Department of Laboratory Medicine, Eulji General Hospital, Eulji University College of Medicine, Seoul, Korea

Background: Polymerase chain reaction (PCR) methods from direct specimen are widely used for the rapid and accurate detection of mycobacteria infection. In this study, we evaluated two domestically developed detection kits for *Mycobacterium tuberculosis* (MTB) and nontuberculous mycobacteria (NTM) using real-time PCR.
Methods: A total of 348 samples from patients with suspected tuberculosis were tested with real-time PCR over seven months. We performed real-time PCR using the recently developed Anyplex plus MTB/NTM Detection kit (Seegene) with the CFX 96TM Real-time PCR System (Bio-Rad Laboratories) and the conventional AdvanSure TB/NTM real-time PCR kit (LG Life Sciences) with the SLAN Real-time PCR detection system (LG Life Sciences) to evaluate their performance for detecting MTB and NTM.
Results: The two real-time PCR systems showed 96.8% concordance rate for MTB-positive, NTM-positive, and negative results. Based on culture results, the sensitivity and specificity for the detection of MTB using PCR were 71.0% and 94.9% for Anyplex plus, and 78.1% and 93.9% for AdvanSure, respectively. For the detection of NTM, the sensitivity and specificity were 33.3% and 98.4% for Anyplex plus, and 51.7% and 97.9% for AdvanSure. Both PCR systems showed high MTB positive results in bronchial washing and sputum samples.
Conclusion: In detecting MTB and NTM, Anyplex plus MTB/NTM (Seegene) and AdvanSure TB/NTM real-time PCR (LG Life Sciences) showed high concordance rate with each other in all samples. Therefore both detection kits can be used as rapid and reliable detection tool for MTB. **(Ann Clin Microbiol 2015;18:44-51)**

Key Words: Nontuberculous mycobacteria, Real-time PCR, Tuberculosis

INTRODUCTION

Diagnosis of tuberculosis (TB) is often very difficult due to many different clinical presentations. When acid-fast bacillus (AFB) is detected in a sample obtained from TB suspected lesion and is identified as *Mycobacterium tuberculosis* (MTB), the diagnosis can be made easily. However, more often the organism is not detected, and TB culture requires very long time, thus, the diagnosis is made after comprehensive interpretation of clinical presentation, radiographic findings, and many other examinations. TB nucleic acid amplification (NAT) test is a molecular biological test which amplifies and identifies TB specific DNA using polymerase chain reaction (PCR). Although molecular biological test cannot replace the conventional smear and culture methods, it can be used as a useful supplementary test because it has many advantages including high sensitivity and specificity, and short result processing time [1].

Almost all of the molecular diagnostic kits used in Korea were domestic products except for COBAS Taqman MTB Test (Roche, Basel, Swiss). AdvanSure TB/NTM real-time PCR kit (LG Life Sciences, Seoul, Korea) was most widely used, followed by products from MTB Real-time PCR (Bioneer, Daejeon, Korea), NTM & MTB PCR Kit (Biosewoom, Seoul, Korea), and Anyplex MTB/NTM Real-time Detection (Seegene, Seoul, Korea) [2]. AdvanSure TB/NTM real-time PCR (LG Life Sciences) uses IS6110 primer for the detection of MTB complex

Received 20 November, 2014, Revised 26 May, 2015, Accepted 12 June, 2015

Correspondence: Jeong Don Chae, Department of Laboratory Medicine, Eulji General Hospital, Eulji University College of Medicine, 68, Hangeulbiseok-ro, Nowon-gu, Seoul 139-711, Korea. (Tel) 82-2-970-8324, (Fax) 82-2-970-8524, (E-mail) jdchae@eulji.ac.kr

specific gene, and *rpoB* gene specific primer and probe for the detection of nontuberculous mycobacteria (NTM). It can detect MTB and 70 other NTMs from human sputum, bronchoalveolar lavage (BAL), stool, cerebrospinal fluid (CSF), urine, and blood. MTB Real-time PCR (Bioneer) uses IS6110 primer for detecting MTB DNA from human sputum, BAL, and urine within 2 hours of analysis. NTM & MTB PCR Kit (Biosewoom) uses TB TaqMan probe targeted for IS6110 of MTB, and NTM TaqMan probe targeted for *rpoB* gene of NTM by real-time PCR. Anyplex MTB/NTM Real-time Detection Kit (Seegene) uses IS6110 and mpb64 specific primers for the detection of MTB, and 16S rRNA gene specific primer for the detection of NTM from sputum, BAL, body fluid, and fresh tissue.

The conventional PCR uses only the primer and not the probe. Thus the amplification reaction specificity can be decreased compared to real-time PCR, and it also has the risk of contamination or carry-over by the amplified products. As a consequence, there is a recent trend of increase in using real-time PCR for the detection of MTB/NTM [3]. In Korea, more than 10% of AFB positive sputum are being identified as NTM instead of MTB [4], thus the use of methods that can detect both MTB and NTM, instead of MTB alone, is on the rise.

In this study, we evaluated and compared the performances of two real-time PCR systems. The most widely used MTB molecular diagnostic kit, AdvanSure TB/NTM real-time PCR (LG Life Sciences) and the recent MTB/NTM diagnostic kit, Anyplex plus MTB/NTM Detection (Seegene).

MATERIALS AND METHODS

1. Samples

We selected a total of 348 samples that were transferred to the Department of Laboratory Medicine in Eulji General Hospital for MTB PCR test between May 2012 and December 2012. Among 348 samples, TB culture was performed on 279 samples which were comprised of 210 sputum samples, 57 BAL samples, 7 CSF samples, 3 open pus, 1 colon sample and 1 tissue sample (Fig. 1). There were 92 DNA samples stored frozen. Five samples were from patients under MTB treatment. There were no duplicates among the referred samples. This study was approved by the institutional review board of Eulji University Eulji General Hospital.

2. Acid fast bacilli (AFB) stain

Ziehl-Neelsen method using Carbol-fuchsin was performed

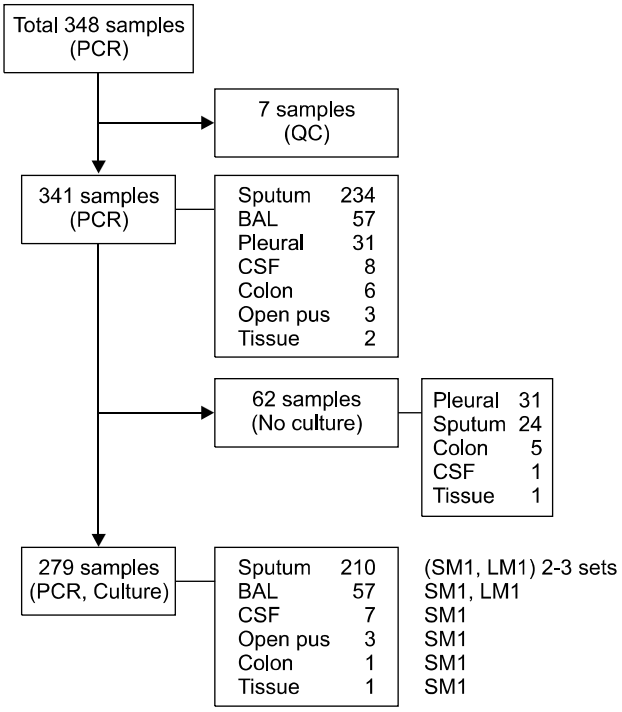

Fig. 1. Type distributions of samples according to the tests performed. Abbreviations: BAL, bronchoalveolar lavage; Pleural, pleural fluid; CSF, cerebrospinal fluid, SM, solid media; LM, liquid media.

and interpretations were made according to American CDC standards [5].

3. Culture

The result was positive when there was growth in at least one of either liquid or solid culture media. The MTB confirmation of positive culture was performed using MTB Real-time PCR kit (Bioneer). According to the types of samples, we used 2 to 3 pairs of solid and liquid media for sputum, 1 pair of solid and liquid media for BAL, and 1 solid media for CSF, open pus, colon, and tissue samples.

4. Real-time PCR

1) Pre-analytic processing

(1) Anyplex plus

Sputum

1. The same volume of sputum and NALC-NaOH (0.5% NALC, 2% NaOH, 1.47% trisodium citrate) were mixed with 1 minute of vortexing
   Note: 4% NaOH (1 N NaOH) can be used for NALC-NaOH
2. Let it stand for 15 minutes under room temperature
3. Transfer the 1.5 mL of mixture into new sterilized tube

and centrifuge for 5 minutes at 15,000 ×g (13,000 rpm).
4. Remove the supernatant, add 1X PBS 1 mL and mix well.
5. Centrifuge for 5 minutes at 15,000 ×g (13,000 rpm) and remove the supernatant using the pipette.
6. Add 1X PBS 1 mL and mix well.
7. Centrifuge for 5 minutes at 15,000 ×g (13,000 rpm) and remove the supernatant using the pipette.
8. Use the precipitate at the bottom of the tube for DNA extraction.

BAL

1. Without the addition of NALC-NaOH, centrifuge the tube that contains 1.5 mL of sample for 5 minutes at 15,000 ×g and remove the supernatant.
2. Add 1mL of 1X PBS and mix.
3. Centrifuge for 5 minutes at 15,000 ×g (13,000 rpm) and remove the supernatant.
4. Use the precipitate at the bottom of the tube for DNA extraction.

(2) AdvanSure

Sputum, BAL

1. Add sputum or BAL (more than 500 uL recommended) into 1.5 mL microtube, and add the same volume of pre-analytic processing reagent (10 times concentrated NaOH), and vortex the mixture. For 5 minutes at 15-30°C, strongly mix the solution for 30 seconds for every 1 minute interval using vortex mixer.
2. Centrifuge for 20 minutes at 3,000 rpm or for 3 minutes at 13,000 rpm, and remove the supernatant
3. If the viscosity was completely resolved after step 2, skip steps 4 to 6.
4. Add 500 uL of Pre-analytic processing reagent (NaOH) and mix the precipitate using the pipette
5. For 5 minutes at 15-30°C, strongly mix the solution for 30 seconds for every 1 minute interval using vortex mixer.
6. Centrifuge for 3 minutes at 13,000rpm, and remove the supernatant
7. Use the precipitate at the bottom of the tube for DNA extraction.

2) Interpretation guideline for positive result (Ct value)

(1) Anyplex plus guideline: Primers specific for IS6110 and mpb64 of MTB, and 16S rRNA gene of NTM were used. For the detection of PCR reaction, 3 channels (MTB, Mycobacteria, internal control) were used to detect FAM, Cal Red 610, Quasar 670 wave signals. When the Ct value of MTB and internal control were below 45, and that of Mycobacteria was below 35, the result was interpreted as 'positive'. According to the manufacturer's instruction, MTB positive was given when results were either MTB positive or both MTB and Mycobacteria positive. Co-infection of MTB and NTM was given when results were both positive for MTB and Mycobacteria in which the Ct value of MTB was greater than that of Mycobacteria. NTM positive was given when result was only positive for Mycobacteria.

(2) AdvanSure guideline: The nucleotide sequences of the much conserved region of MTB and NTM (IS6110 region for MTB, *rpoB* gene for NTM) were used. For the detection of PCR reaction, 3 channels (MTB, Mycobacteria, internal control) were used to detect FAM, HEX, and Cy5 wave signals. When the Ct value of MTB and internal control were below 35, and that of Mycobacteria was below 35, the result was interpreted as 'positive'. According to the manufacturer's instruction, when the results were positive only for MTB or for both MTB and NTM, co-infection of MTB and NTM was given when HEX Ct − FAM Ct≤0, MTB positive was given when HEX Ct − FAM Ct >0, and NTM positive was given when only Mycobacteria was positive.

5. Statistics

Sensitivity, specificity, positive predictive rate, and negative predictive rate were obtained using Microsoft Excel 2010 software (Microsoft, Redmond, WA, USA). Chi-square test ($\chi 2$ test) was used to compare the sensitivity between two methods. *P* value of less than 0.05 was considered statistically significant.

RESULTS

Among 279 samples referred for culture, a total of 79 samples were MTB or NTM positives. Of 79 positive samples, 57 were positive for MTB (53 respiratory and 4 non-respiratory samples) and 22 were positive for NTM (all respiratory samples). No sample was cultured positive for both MTB and NTM. The detection rates of MTB for respiratory samples and non-respiratory samples according to each diagnostic kit were 86.8% (46/53) and 0% (0/4) respectively for Anyplex plus MTB/NTM Detection Kit (Seegene), and 88.7% (47/53) and 25% (1/4) respectively for Advansure TB/NTM real-time PCR (LG Life Sciences).

Based on the culture result, the sensitivity, specificity, pos-

itive predictive rate, and negative predictive rate in detecting MTB for Anyplex plus MTB/NTM Detection Kit (Seegene) were 71.0%, 94.9%, 83.1%, and 90.3% respectively, and for that of AdvanSure TB/NTM real-time PCR (LG Life Sciences) were 78.1%, 93.9%, 80.6%, and 92.9%, respectively (Table 1). For AFB stain positive samples, the sensitivity and specificity of detecting MTB by Anyplex plus MTB/NTM Detection (Seegene) were 95.8% and 100%, and for that of AdvanSure TB/NTM real-time PCR (LG Life Sicences) were both 100%. For AFB stain negative samples, the sensitivity and specificity of Anyplex plus MTB/NTM Detection (Seegene) were 62.5% and 94.9%, and for that of AdvanSure TB/NTM real-time PCR (LG Life Sciences) were 69.4%, and 93.8%. The detection rates of MTB from pulmonary samples and extrapulmonary samples were 19.9% and 33.3% by culture, 21.7% and 8.3% by Anyplex plus MTB/NTM Detection (Seegene), and 22.5% and 16.7% by AdvanSure TB/NTM real-time PCR (LG Life Sciences) (Table 2).

Based on the culture result, the sensitivity, specificity, positive predictive rate, and negative predictive rate in detecting NTM for Anyplex plus MTB/NTM Detection Kit (Seegene) were 33.3%, 98.4%, 76.9%, and 90.3% respectively, and for those of AdvanSure TB/NTM real-time PCR (LG Life Sciences) were 51.7%, 97.9%, 78.9%, and 92.9%, respectively. For AFB stain positive samples, the sensitivity and specificity of detecting NTM by Anyplex plus MTB/NTM Detection Kit (Seegene) were 90.0% and 100.0%, and those of AdvanSure TB/NTM real-time PCR (LG Life Sciences) were both 100%.

Based on the culture result, the overall concordance rate of two methods was 96.8% (270/279). For discordant results, 6 samples were positive for both culture and AdvanSure TB/NTM real-time PCR (LG Life Sciences) but were negative using Anyplex plus MTB/NTM Detection Kit (Seegene), and 3 samples were negative for both culture and Anyplex plus

Table 1. Results of two real-time PCR systems according to AFB smear and culture results

Target	AFB smear	Assays	Culture + (79)		Culture − (200)		Sensitivity/Specificity	PPV/NPV
			PCR+	PCR−	PCR+	PCR−		
MTB	Positive	Anyplex plus	23	1	0	2	95.8/100.0	100.0/66.7
		AdvanSure	23	0	0	2	100.0/100.0	100.0/100.0
	Negative	Anyplex plus	25	15	10	185	62.5/94.9	71.4/92.5
		AdvanSure	25	11	12	182	69.4/93.8	67.6/94.3
	All*	Anyplex plus	49†	20§	10	187	71.0/94.9	83.1/90.3
		AdvanSure	50‡	14‖	12	184	78.1/93.9	80.6/92.9
NTM	Positive	Anyplex plus	9	1	0	2	90.0/100.0	100.0/66.7
		AdvanSure	10	0	0	2	100.0/100.0	100.0/100.0
	Negative	Anyplex plus	1	15	3	185	6.3/98.4	25.0/92.5
		AdvanSure	5	11	4	182	31.3/97.8	55.6/94.3
	All*	Anyplex plus	10	20§	3	187	33.3/98.4	76.9/90.3
		AdvanSure	15	14‖	4	184	51.7/97.9	78.9/92.9

*Includes samples without AFB smear results: †, n=1; ‡, n=2; §, n=4; ‖, n=3.
Abbreviations: MTB, *mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria; AdvanSure, AdvanSure TB/NTM real-time PCR; Anyplex plus, Anyplex plus MTB/NTM Detection; AFB, acid-fast bacilli; PCR, polymerase chain reaction; PPV, positive predictive value; NPV, negative predictive value.

Table 2. MTB positive rates according to sample types in culture and two real-time PCR systems

Specimen	No. MTB positive (%)		
	Culture	Anyplex plus	AdvanSure
Pulmonary specimen	53/267 (19.9%)	58/267 (21.7%)	60/267 (22.5%)
Extrapulmonary specimen*	4/12 (33.3%)	1/12 (8.3%)	2/12 (16.7%)
Total	57/279 (20.4%)	59/279 (22.1%)	65/279 (22.2%)

*CSF (7), Open pus (3), Colon (1), Tissue (1).
Abbreviations: AdvanSure, AdvanSure TB/NTM real-time PCR; Anyplex plus, Anyplex plus MTB/NTM Detection; MTB, *Mycobacterium tuberculosis*.

MTB/NTM Detection Kit (Seegene) but were positive by AdvanSure TB/NTM real-time PCR (LG Life Sciences) (Table 3). The overall concordance rate between two methods was 96.8% (330/341) (Table 4). A total of 11 samples showed discordant results (Table 5). For discordant results, 4 were positive for MTB and 5 were positive for NTM only by AdvanSure TB/NTM real-time PCR (LG Life Sciences), 1 was positive for MTB only by Anyplex plus MTB/NTM Detection (Seegene), and 1 was MTB positive using Anyplex plus MTB/NTM Detection (Seegene) but was NTM positive using AdvanSure TB/NTM real-time PCR (LG Life Sciences) (Table 4, 5).

DISCUSSION

CDC recommended that NAT should be performed on at least one respiratory specimen from each patient with signs and symptoms of pulmonary TB for whom a diagnosis of TB is being considered [6]. Also in Korean MTB protocol, MTB NAT is recommended 1) if pulmonary MTB is suspected but showed negative smear result or 2) if AFB smear is positive and there is a possibility of NTM, and 3) it is considered helpful for the diagnosis of extra-pulmonary MTB [1]. The number of new mycobacterial species has increased over the years and since there are no distinct phenotypic differences to identify new species, molecular tests were mostly being used [3]. The molecular biological method used in this study is the real-time PCR method, through which the fluorescent dye is bound to the amplified genetic material and the real-time measurement of fluorescent dye is analyzed. This PCR method does not require electrophoresis or hybridization processes, thus the contamination rates had improved and test time was shortened compared to conventional PCR methods [7,8]. In Korea, the percentage of institutions using the real-time PCR for molecular biological test of MTB gradually rose from 66% in 1999 to 94% in 2013 [2].

In a previous study, Anyplex plus MTB/NTM Detection Kit (Seegene) on pulmonary samples showed the sensitivity of

Table 3. Results of two real-time PCR systems according to culture results

	Anyplex plus PCR	AdvanSure PCR	Number
Culture + (79)	+	+	59
	+	−	0
	−	+	6
	−	−	14
Culture − (200)	+	+	13
	+	−	0
	−	+	3
	−	−	184

Abbreviations: AdvanSure, AdvanSure TB/NTM real-time PCR; Anyplex plus, Anyplex plus MTB/NTM Detection.

Table 4. Result correlations of two real-time PCR systems

		Anyplex plus			
		MTB	NTM	Negative	Total
AdvanSure	MTB	61	0	4	65
	NTM	1	13	5	19
	Negative	1	0	256	257
	Total	63	13	265	341

Abbreviations: AdvanSure, AdvanSure TB/NTM real-time PCR; Anyplex plus, Anyplex plus MTB/NTM Detection; MTB, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria.

Table 5. Result summary for samples with discrepant results among test methods including culture, AFB smear, and PCR

No	Sample	AdvanSure	Ct	Anyplex plus	Ct	Culture	Culture media	NTM identification	AFB smear	Treatment
1	BAL	MTB	33.5	-		-			−	Done
2	BAL	MTB	33.5	-		-			−	Done
3	Sputum	MTB	34.5	-		-			−	
4	Colon	MTB	34.0	-		MTB	SM 1		NT	Done
5	BAL	NTM	31.8	MTB	42.2	NTM	SM 1, LM 1	*M. avium*	−	Done
6	Pleural fluid	-		MTB	43.6	NT			−	Done
7	BAL	NTM	33.8	-	37.2	NTM	SM 2, LM 2	*M. intracellulare*	−	
8	Sputum	NTM	25.6	-		NTM	SM 1, LM 1	*M. intracellulare*	+ (few)	
9	Sputum	NTM	23.6	-		NTM	SM 2, LM 2	*M. intracellulare*	−	
10	BAL	NTM	32.6	-	35.4	NTM	SM 1, LM 1	NT	−	
11	Sputum	NTM	33.9	-	36.5	-			−	

Abbreviations: AdvanSure, AdvanSure TB/NTM real-time PCR; Anyplex plus, Anyplex plus MTB/NTM Detection; AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; Ct, cycle threshold; MTB, *Mycobacterium tuberculosis*; NTM, nontuberculous mycobacteria; SM, solid media; LM, liquid media; NT, not tested.

87.5% and specificity of 98.2%, and for AFB stain positive samples, the sensitivity was 95.2% and specificity was 72.7% [9]. In another study which included extrapulmonary samples, Anyplex plus MTB/NTM Detection Kit (Seegene) showed the sensitivity of 82.9% and specificity of 99.4%, and for AFB stain positive samples, the sensitivity was 91.7% [10]. In this study of Anyplex plus MTB/NTM Detection Kit (Seegene), we included both pulmonary and extrapulmonary samples and showed the sensitivity of 71.0% and specificity of 94.9%, and for AFB stain positive samples, the sensitivity was 95.8%, and specificity was 93.9%. Overall sensitivity was lower than previous studies but sensitivity for AFB stain positive samples was similar. AdvanSure TB/NTM real-time PCR (LG Life Sciences) showed the sensitivity of 78.1% and specificity of 93.9% which had higher sensitivity than Anyplex plus MTB/NTM Detection Kit (Seegene), but did not show any statistical significance between the two methods ($\chi 2$ test, P=0.35).

For the detection of NTM, the sensitivity and specificity of Anyplex plus MTB/NTM Detection kit (Seegene) were 33.3% and 98.4% respectively, and those of AdvanSure TB/NTM real-time PCR (LG Life Sciences) were 51.7% and 97.9%, respectively. There were no statistically significant difference between the two methods ($\chi 2$ test, P=0.15). One previous study showed sensitivity of NTM detection as 76.5% by Anyplex plus MTB/NTM Detection kit (Seegene), and 41.2% by AdvanSure TB/NTM real-time PCR (LG Life Sciences) [10], which was different from our result. This difference in result could be due to differences in the positive cut-off value of Ct. Our study gave positive result when Ct value was below 35 for Anyplex plus MTB/NTM Detection Kit (Seegene); however, in a study where the sensitivity of NTM detection was 76.5%, positive result was given when Ct value was below 40. This could have yielded higher sensitivity. When we used Ct value of 40 as our positive cut-off for Anyplex plus MTB/NTM Detection Kit (Seegene), 3 of 5 samples that showed discrepant result between two NTM detection methods become positive results (Table 5). In the manufacturer's instruction of Anyplex plus MTB/NTM Detection Kit (Seegene), Ct value for NTM positive was set as below 38 and this cut-off would also give positive results for 3 of 5 samples that showed discrepant results. According to the manufacturer's instruction, the positive cut-off Ct value of AdvanSure TB/NTM real-time PCR (LG Life Sciences) for MTB detection was Ct $<$35; however, one study reported the best predicted stain positivity with a sensitivity of 95.0% and a specificity of 32.0% when the cut-off Ct value of $<$33.2 was used [11]. Therefore, further studies involving the optimal cut-off Ct value for the detection of MTB and NTM would be needed.

Based on the culture results, the concordance rate of two methods was 96.8% (270/279). The overall concordance rate of two methods was 96.8% (330/341). Of 11 overall discrepant samples, 10 samples showed discrepant results based on MTB culture results and one sample did not have culture result. Among the samples that showed positive NTM results by only AdvanSure, 3 were identified as *M. intracellulare*. A previous study also reported 2 true-positive outcomes from *M. intracellulare* and one false positive outcome by AdvanSure [12]. Whether AdvanSure system is more sensitive in detecting *M. intracellulare* or other NTM species would need further evaluation using NTM samples identified of their species using multiple measures such as DNA sequencing, and/or culture methods. One patient who was MTB positive by culture were treated for the infection, and one of 5 patients who were NTM positive by culture were treated for MTB. A patient who was not tested by culture and 2 patients who were negative by culture were treated for MTB (Table 5). Thirteen patients, who were clinically suspected of MTB but showed negative results in all TB culture, AFB stain, and PCR tests, were still treated for MTB because they were clinically diagnosed as active MTB (data not shown).

There were several limitations of this study. First, we were not able to compare the detection performance of two methods according to AFB grade because the reporting of AFB stain had recently been changed from WHO standard to CDC standard. Second, the samples for TB culture, AFB stain, and PCR were performed using samples from separate containers. Because the samples for TB culture, AFB stain, and PCR were separately delivered in several containers, the results might have been affected. Also, culture and PCR test were not simultaneously performed which could have influenced the results as well. Third, the overall number of positive samples was very small and this could have given us rather low positive predictive value. Especially, there were too few NTM positive samples to give us a chance for any satisfactory comparison. Fourth, due to the lack of positive samples in the last phase of the study, the tests were performed after gathering the samples. Using the stored DNA (92 samples) for the testing could have affected the results. Fifth, we should have confirmed the results using other methods such as DNA sequencing in cases of discrepancies between two methods, but we could not perform the further confirmation because samples tested for PCR were inconsistent

(collected at different times from same patients) and the study was retrospectively designed. Lastly, there was a change of automated liquid culture system in our laboratory which could have also influenced our results.

In conclusion, Anyplex plus and AdvanSure real-time PCR assays for MTB and NTM detection showed high concordance rate of performance. Both methods showed high sensitivity and specificity in detecting MTB with favorable cost and time efficiency. Thus both real-time PCR assays can be used in rapid and reliable diagnosis of MTB.

ACKNOWLEDGMENTS

This work was financially supported by Seegene (Seegene, Seoul, Korea). The authors have no conflicting interests regarding this work.

REFERENCES

1. Joint Committee for the Revision of Korean Guidelines for Tuberculosis Korea Centers for Disease Control and Prevention. Korean Guidelines for Tuberculosis. 2nd ed. Korea Centers for Disease Control and Prevention; 2014:30-1.
2. Jang MA, Shin SY, Park S, Seong MW, Park SS, Kim SH; Diagnostic Genetics Subcommittee, The Korean Association of Quality Assurance for Clinical Laboratory. Annual report on the external quality assessment of diagnostic genetics in Korea (2013). J Lab Med Qual Assur 2014;36:71-83.
3. Korea Centers for Disease Control and Prevention. Manual of Laboratory Tests for Tuberculosis. Korea Centers for Disease Control and Prevention; 2013:75-80.
4. Joint Committee for the Revision of Korean Guidelines for Tuberculosis Korea Centers for Disease Control and Prevention. Korean Guidelines for Tuberculosis. 2nd ed. Korea Centers for Disease Control and Prevention, 2014:227-8.
5. Shinnick TM, Iademarco MF, Ridderhof JC. National plan for reliable tuberculosis laboratory services using a systems approach. Recommendations from CDC and the Association of Public Health Laboratories Task Force on Tuberculosis Laboratory Services. MMWR Recomm Rep 2005;54:1-12.
6. Centers for Disease Control and Prevention (CDC). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. MMWR Morb Mortal Wkly Rep 2009;58:7-10.
7. Broccolo F, Scarpellini P, Locatelli G, Zingale A, Brambilla AM, Cichero P, et al. Rapid diagnosis of mycobacterial infections and quantitation of *Mycobacterium tuberculosis* load by two real-time calibrated PCR assays. J Clin Microbiol 2003;41:4565-72.
8. Chang HE, Heo SR, Yoo KC, Song SH, Kim SH, Kim HB, et al. Detection of *Mycobacterium tuberculosis* complex using real-time polymerase chain reaction. Korean J Lab Med 2008;28:103-8.
9. Lim J, Kim J, Kim JW, Ihm C, Sohn YH, Cho HJ, et al. Multicenter evaluation of Seegene Anyplex TB PCR for the detection of *Mycobacterium tuberculosis* in respiratory specimens. J Microbiol Biotechnol 2014;24:1004-7.
10. Lee JH, Kim BH, Lee MK. Performance evaluation of anyplex plus MTB/NTM and MDR-TB detection kit for detection of mycobacteria and for anti-tuberculosis drug susceptibility test. Ann Clin Microbiol 2014;17:115-22.
11. Lee H, Park KG, Lee G, Park J, Park YG, Park YJ. Assessment of the quantitative ability of AdvanSure TB/NTM real-time PCR in respiratory specimens by comparison with phenotypic methods. Ann Lab Med 2014;34:51-5.
12. Cho SY, Kim MJ, Suh JT, Lee HJ. Comparison of diagnostic performance of three real-time PCR kits for detecting *Mycobacterium* species. Yonsei Med J 2011;52:301-6.

=국문초록=

결핵균 및 비결핵항산균 검출을 위한 Anyplex plus MTB/NTM과 AdvanSure TB/NTM의 검사능력 평가

을지대학교 의과대학 을지병원 진단검사의학교실

최원호, 김이화, 박서연, 채정돈

배경: 결핵균 감염의 신속 정확한 진단을 위해 중합효소연쇄반응법(PCR)이 널리 사용되고 있다. 본 연구에서는 결핵균 및 비결핵항산균 검출을 위해 국내에서 개발된 두 가지 실시간 PCR (real-time PCR) 법을 비교 평가하였다.

방법: 2012년 5월부터 12월까지 을지대학교 을지병원 진단검사의학과에 결핵균 PCR 검사가 의뢰된 348개의 검체를 대상으로 최근 개발된 Anyplex plus MTB/NTM Detection kit (Seegene)와 기존의 AdvanSure TB/NTM real-time PCR kit (LG Life Sciences)를 적용하여 결핵균 및 비결핵항산균 검출능력을 평가하였다.

결과: 시행된 2개의 실시간 PCR에서 결핵균 양성, 비결핵항산균 양성 및 모두 음성 결과의 일치도는 96.8%로 나타났다. 결핵균 검출에서 Anyplex plus의 민감도 및 특이도는 각각 71.0%와 94.9%였고 AdvanSure의 민감도 및 특이도는 각각 78.1%와 93.9%였다. 비결핵항산균 검출에서 Anyplex plus의 민감도 및 특이도는 각각 33.3%와 98.4%였고 AdvanSure의 민감도 및 특이도는 각각 51.7%와 97.9%였다. 2가지 PCR 모두 기관지세척액 및 객담 검체에서 높은 결핵균 양성률을 보였다.

결론: 결핵균과 비결핵항산균을 검출하는데 있어 Anyplex plus MTB/NTM (Seegene) 및 AdvanSure TB/NTM (LG Life Sciences) 실시간 PCR이 모든 검체에서 높은 일치율을 보였다. 이 2가지 실시간 PCR법은 결핵균 검출에 있어 높은 민감도, 특이도, 양성예측도 및 음성예측도를 나타냈다. 이 2가지 PCR법은 결핵균을 신속 정확하게 검출하는 도구로서 유용하게 사용될 수 있을 것으로 사료된다. [Ann Clin Microbiol 2015;18:44-51]

교신저자 : 채정돈, 139-711, 서울시 노원구 한글비석로 68
을지대학교 의과대학 진단검사의학교실
Tel: 02-970-8324, Fax: 02-970-8524
E-mail: jdchae@eulji.ac.kr