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Comparison of AdvanSure TB/NTM PCR and COBAS TaqMan MTB PCR for Detection of *Mycobacterium tuberculosis* Complex in Routine Clinical Practice

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The AdvanSure tuberculosis/non-tuberculous mycobacterium (TB/NTM) PCR (LG Life Science, Korea) and COBAS TaqMan *Mycobacterium tuberculosis* (MTB) PCR (Roche Diagnostics, USA) are commonly used in clinical microbiology laboratories. We aimed to evaluate these two commercial real-time PCR assays for detection of MTB in a large set of clinical samples over a two-year period. AdvanSure TB/NTM PCR and COBAS TaqMan MTB PCR were performed on 9,119 (75.2%) and 3,010 (24.8%) of 12,129 (9,728 respiratory and 2,401 non-respiratory) MTB specimens, with 361 (4.0%) and 102 (3.4%) acid-fast bacilli (AFB)-positive results, respectively. In MTB culture, 788 (6.5%) MTB and 514 (4.2%) NTM were identified. The total sensitivity and specificity of the AdvanSure assay were 67.8% (95% confidence interval [CI], 63.9-71.6) and 98.3% (95% CI, 98.0-98.6), while those of the COBAS TaqMan assay were 67.2% (95% CI, 60.0-73.8) and 98.4% (95% CI, 97.9-98.9), respectively. The sensitivities and specificities of the AdvanSure and COBAS TaqMan assays for AFB-positive and AFB-negative samples were comparable. Furthermore, the AdvanSure assay showed fewer invalid results compared with the COBAS TaqMan assay (5.0 vs. 20.4 invalid results/1,000 tests, $P<0.001$). AdvanSure assay represents a comparable yet more reliable method than COBAS TaqMan for the identification of mycobacteria in routine clinical microbiology.

Key Words: AdvanSure TB/NTM PCR, COBAS TaqMan MTB PCR, *Mycobacterium tuberculosis*

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Tuberculosis (TB) is a global public health concern. According to the World Health Organization's Global Tuberculosis Report, 2013, the incidence of TB in Korea was 161 per 100,000 individuals in 2011 [1]. Rapid and accurate diagnosis of TB is vital to reduce morbidity and mortality rates and the risk of person-to-person transmission of TB. The advent of molecular methods for *Mycobacterium tuberculosis* (MTB) detection has reduced the time to diagnosis to a few days, whereas diagnosis by conventional culture systems required several weeks [2, 3]. The COBAS TaqMan MTB assay (Roche Diagnostics, Branchburg, NJ, USA) is a reliable method for MTB identification [4]. The AdvanSure tuberculosis/non-tuberculous mycobacteria (TB/NTM) real-time PCR kit (LG Life Sciences, Seoul, Korea) also allows for the identification of clinically important MTB and NTM [5]. Several studies have shown the efficiency of this kit; however, these evaluations were performed in a limited number of cases [5-8]. We compared the clinical performance of AdvanSure TB/NTM real-time PCR and COBAS TaqMan MTB PCR assays in a wide spectrum of clinical specimens obtained over a two-year period (2011-2012).

A total of 12,129 specimens, including 9,728 (80.2%) respiratory and 2,401 (19.8%) non-respiratory specimens, were ex-

amined between January 2011 and December 2012 at Chonnam National University Hospital. The study protocol was approved by the Institutional Review Board of the hospital. All clinical specimens were liquefied and decontaminated with N-acetyl-L-cysteine-sodium hydroxide. After centrifugation at 3,000 g for 20 min, the sediment from each specimen was used for acid-fast bacilli (AFB) staining. The specimens were cultured on 2% Ogawa medium (Asan Pharmaceutical Co., Seoul, Korea) and BACTEC MGIT 960 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA). The culture-positive clinical isolates were identified with multiplex PCR for MTB and NTM (Seegene, Seoul, Korea).

Moreover, we randomly selected and applied either AdvanSure or COBAS TaqMan assay to the clinical samples. The AdvanSure TB/NTM real-time PCR and COBAS TaqMan MTB PCR assays were performed according to the manufacturers' recommendations by using the SLAN real-time PCR detection system (LG Life Sciences) and COBAS TaqMan 48 Analyzer (Roche Diagnostics), respectively. The sensitivities, specificities, positive predictive values (PPVs), and negative predictive values (NPVs) were calculated on the basis of the results of concurrently performed cultures. The invalid results obtained from both systems were analyzed over a year (2012). The rate of invalid results obtained by using both systems was defined as the number of invalid results per 1,000 PCR tests. The results were deemed to be invalid when the AdvanSure assay result was "retest required" or when the COBAS TaqMan assay result was "invalid." Such invalid results were re-tested by either AdvanSure or COBAS TaqMan assay. The differences in specimen distributions or AFB positivity, analytical performances of both systems, and the proportions of invalid results were determined by using Mantel-Haenszel corrected chi-square test, Fisher's exact test, and the corresponding *P* values. A *P* value of <0.05 was considered statistically significant in both analyses.

Among 12,129 samples, we evaluated 9,119 samples (75.2%; 7,344 respiratory and 1,775 non-respiratory specimens) by AdvanSure assay and 3,010 samples (24.8%; 2,384 respiratory and 626 non-respiratory specimens) by COBAS TaqMan assay. Of these, 463 (3.8%) were AFB-positive and 11,666 (96.2%) were AFB-negative. There were no significant differences in the distribution of respiratory and non-respiratory specimens and AFB positivity between the two systems (Table 1). A total of 713

Table 1. Distribution of specimens examined by the AdvanSure TB/NTM PCR and COBAS TaqMan MTB PCR assays according to specimen type and AFB status

Specimens	Subtotal N (% among total submitted specimens to the individual systems)			N (%) of submitted specimens with			
				AFB-positive		AFB-negative	
	Total	AdvanSure	COBAS TaqMan	AdvanSure	COBAS TaqMan	AdvanSure	COBAS TaqMan
Respiratory specimens	9,728 (80.2)	7,344 (80.5)	2,384 (79.2)	345 (4.7)	99 (4.2)	6,999 (95.3)	2,285 (95.8)
Sputum	7,643 (63.0)	5,725 (62.8)	1,918 (63.7)	263 (4.6)	76 (4.0)	5,462 (95.4)	1,842 (96.0)
Endotracheal aspirate	850 (7.0)	656 (7.2)	194 (6.5)	41 (6.3)	16 (8.2)	615 (93.8)	178 (91.8)
Bronchial washing	648 (5.3)	500 (5.5)	148 (4.9)	23 (4.6)	5 (3.4)	477 (95.4)	143 (96.6)
BAL	581 (4.8)	459 (5.0)	122 (4.1)	18 (3.9)	2 (1.6)	441 (96.1)	120 (98.4)
Throat swab	6 (0.1)	4 (0.0)	2 (0.1)	0 (0.0)	0 (0.0)	4 (100.0)	2 (100.0)
Non-respiratory specimens	2,401 (19.8)	1,775 (19.5)	626 (20.8)	16 (0.9)	3 (0.5)	1,759 (99.1)	623 (99.5)
Pus	790 (6.5)	592 (6.5)	198 (6.6)	8 (1.4)	1 (0.5)	584 (98.6)	197 (99.5)
Pleural fluid	789 (6.5)	578 (6.3)	211 (7.0)	2 (0.3)	0 (0.0)	576 (99.7)	211 (100.0)
CSF	342 (2.8)	257 (2.8)	85 (2.8)	0 (0.0)	0 (0.0)	257 (100.0)	85 (100.0)
Urine	277 (2.3)	192 (2.1)	85 (2.8)	3 (1.6)	1 (1.2)	189 (98.4)	84 (98.8)
Tissues	121 (1.0)	91 (1.0)	30 (1.0)	3 (3.3)	1 (3.3)	88 (96.7)	29 (96.7)
Peritoneal fluid	69 (0.6)	55 (0.6)	14 (0.5)	0 (0.0)	0 (0.0)	55 (100.0)	14 (100.0)
Other fluid	13 (0.1)	10 (0.1)	3 (0.1)	0 (0.0)	0 (0.0)	10 (100.0)	3 (100.0)
Total (%)	12,129 (100.0)	9,119 (100.0)	3,010 (100.0)	361 (4.0)	102 (3.4)	8,758 (96.0)	2,908 (96.6)

There were no significant differences in the distribution of submitted specimen type (respiratory or non-respiratory) and AFB positivity between the two systems. Statistical analysis was performed by Mantel-Haenszel corrected chi-square test or Fisher's exact test.
Abbreviations: AFB, acid-fast bacilli; BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid.

(7.3%) MTB and 505 (5.2%) NTM were confirmed on culture from 9,728 respiratory specimens, while 75 (3.1%) MTB and 9 (0.4%) NTM were confirmed from 2,401 non-respiratory specimens. There were no significant differences between AdvanSure and COBAS TaqMan assays with regard to the culture rates of MTB (6.6% vs. 6.3%, P=0.573) and NTM (4.2% vs. 4.4%, P=0.502) (data not shown).

Using mycobacterial culture as the reference method, both systems showed comparable performances, in that the overall sensitivity, specificity, PPV, and NPV were 67.8% (95% confidence interval [CI], 63.9-71.6), 98.3% (95% CI, 98.0-98.6), 73.7% (95% CI, 69.9-77.4), and 97.8% (95% CI, 97.4-98.1) for AdvanSure assay; and 67.2% (95% CI, 60.0-73.8), 98.4% (95% CI, 97.9-98.9), 74.3% (95% CI, 67.0-80.6), and 97.8% (95% CI, 97.2-98.3) for COBAS TaqMan assay, respectively (Table 2). Higher positive rates of PCR results of 7.0% (514/7,344) and 6.6% (157/2,384) were obtained using AdvanSure and COBAS TaqMan assays for respiratory specimens, compared with 2.1% (38/1,775) and 2.2% (14/626) for non-respiratory specimens (respiratory vs. non-respiratory; P<0.001, in both systems). Both

Table 2. Diagnostic performance of the AdvanSure TB/NTM PCR and COBAS TaqMan MTB PCR assays for detection of *Mycobacterium tuberculosis* complex

Instrument	Specimens	Diagnostic performances, % (95% CI)					*P* values for			
		Prevalence	Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity	PPV	NPV
AdvanSure	Respiratory specimens	7.4 (6.8-8.0)	70.9 (66.9-74.7)	98.1 (97.8-98.4)	74.9 (70.9-78.6)	97.7 (97.3-98.0)	<0.001	0.505	0.011	>0.999
	Non-respiratory specimens	3.2 (2.4-4.1)	38.6 (26.0-52.4)	99.1 (98.5-99.5)	57.9 (40.8-73.7)	98 (97.2-98.6)				
	AFB-positive specimens	64.8 (59.7-69.7)	97.4 (94.5-99.1)	85 (77.6-90.7)	92.3 (88.3-95.3)	94.7 (88.9-98.0)	<0.001	0.001	<0.001	0.31
	AFB-negative specimens	4.2 (3.8-4.6)	48.9 (43.7-54.2)	98.5 (98.2-98.8)	58.7 (52.9-64.3)	97.8 (97.5-98.1)				
COBAS TaqMan	Respiratory specimens	7.2 (6.2-8.3)	70.8 (63.3-77.5)	98.4 (97.8-98.9)	77.1 (69.7-83.4)	97.8 (97.15-98.3)	<0.001	>0.999	<0.001	>0.999
	Non-respiratory specimens	2.9 (1.7-4.5)	33.3 (13.4-59.0)	98.7 (97.4-99.4)	42.9 (17.8-71.1)	98 (96.6-99.0)				
	AFB-positive specimens	68.6 (58.7-77.5)	98.6 (92.3-99.8)	81.3 (63.6-92.8)	92 (83.4-97.0)	96.3 (81.0-99.4)	<0.001	<0.001	<0.001	0.196
	AFB-negative specimens	4.1 (3.4-4.9)	48.7 (39.5-58.1)	98.6 (98.1-99.0)	60.4 (49.9-70.3)	97.8 (97.2-98.3)				
Total	Respiratory specimens	7.3 (6.8-7.9)	70.9 (67.4-74.2)	98.2 (97.9-98.4)	75.4 (72.0-78.6)	97.7 (97.4-98.0)	<0.001	>0.999	0.001	>0.999
	Non-respiratory specimens	3.1 (2.5-3.9)	37.3 (26.4-49.3)	99 (98.5-99.3)	53.9 (39.5-67.8)	98 (97.4-98.5)				
	AFB-positive specimensw	65.7 (61.1-70.0)	97.7 (95.3-99.1)	84.3 (77.7-89.6)	92.2 (88.8-94.9)	95 (90.0-98.0)	<0.001	<0.001	<0.001	0.464
	AFB-negative specimens	4.2 (3.8-4.5)	48.9 (44.3-53.4)	98.5 (98.3-98.8)	59.1 (54.1-64.0)	97.8 (97.5-98.1)				
AdvanSure (Total)		6.6 (6.1-7.1)	67.8 (63.9-71.6)	98.3 (98.0-98.6)	73.7 (69.9-77.4)	97.8 (97.4-98.1)	>0.999	0.956	0.923	>0.999
COBAS TaqMan (Total)		6.3 (5.4-7.2)	67.2 (60.0-73.8)	98.4 (97.9-98.9)	74.3 (67.0-80.6)	97.8 (97.2-98.3)				

Statistical analysis was performed by Mantel-Haenszel corrected chi-square test or Fisher's exact test.
Abbreviations: AFB, acid-fast bacilli; CI, confidence interval; MTB, *Mycobacterium tuberculosis*; NPV, negative predictive value; PPV, positive predictive value.

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systems showed higher sensitivity and PPV, and lower specificity in AFB-positive specimens than in AFB-negative specimens. The sensitivity, specificity, PPV, and NPV of AdvanSure assay were 97.4% (95% CI, 94.5-99.1), 85.0% (95% CI, 77.6-90.7), 92.3% (95% CI, 88.3-95.3), 94.7% (95% CI, 88.9-98.0), whereas those of COBAS TaqMan assay were 98.6% (95% CI, 92.3-99.8), 81.3% (95% CI, 63.6-92.8), 92.0% (95% CI, 83.4-97.0), 96.3% (95% CI, 81.0-99.4), respectively, for AFB-positive samples. The corresponding values for AFB-negative samples were 48.9% (95% CI, 43.7-54.2); 98.5% (95% CI, 98.2-98.8); 58.7% (95% CI, 52.9-64.3); and 97.8% (95% CI, 97.5-98.1), and 48.7% (95% CI, 39.5-58.1); 98.6% (95% CI, 98.1-99.0); 60.4% (95% CI, 49.9-70.3); and 97.8% (95% CI, 97.2-98.3) for AdvanSure and COBAS TaqMan assay, respectively.

AdvanSure assay produced 5.0 invalid results per 1,000 tests, whereas COBAS TaqMan assay produced 20.4 invalid results per 1,000 tests (AdvanSure vs. COBAS TaqMan, $P<0.001$) (See Supplemental Data Table S1). When re-tested, all 27 invalid results obtained using AdvanSure were revealed as negative, while 5 valid results (three NTM, two MTB) were found in the 37 invalid results previously obtained using COBAS TaqMan. Compared with the results of MTB culture, all invalid results of AdvanSure assay were negative, while five NTM and two MTB specimens showed invalid results by COBAS TaqMan assay (Table 3).

The introduction of PCR-based diagnostic techniques is crucial for early detection of MTB in clinical laboratories. When other clinical data raise suspicion of TB, it is recommended to submit the samples for AFB smear, culture, and MTB PCR analyses. This study was designed to assess the diagnostic performance of AdvanSure and COBAS TaqMan assays in clinical settings by using a large quantity of routine samples (12,129 samples) collected over two years. We observed that AdvanSure assay showed a performance comparable with that of COBAS TaqMan assay and might have fewer invalid results.

Several studies have assessed the performance of COBAS TaqMan assay [4, 8-12]; however, only a few studies have assessed the performance of AdvanSure assay [5-8]. In general, other studies have found greater sensitivity estimates than those observed in our study, which could probably be attributed to the limited number of samples and different sample compositions used in those studies. Several researchers assessed the clinical performance of the two PCR systems in respiratory specimens and observed a high sensitivity of >90% for both systems [5, 10]. In our study, both systems showed lower sensitivity (70.9% and 70.8% for AdvanSure and COBAS TaqMan assay, respectively) in respiratory specimens than that observed in previous studies (91.5%) [10]. This might be attributed to the lower proportion of AFB-positive specimens (4.7%) in our study compared with that in the previous study that showed 10.8% of AFB-positive specimens, with 91.5% sensitivity [10].

However, we did not notice any significant development in the detection of MTB in AFB-negative respiratory specimens. In our study, the sensitivity of the AdvanSure assay with smear-negative respiratory specimens (43.4%) was found to be lower than that documented in a previous report (74.5%) [5]. This might be explained by the inclusion of gastric aspirates and other respiratory fluids among the smear-negative respiratory specimens, for which nucleic acid amplification assays are less sensitive than they are for sputum specimens and bronchial aspirates [13].

The overall specificities of both systems were comparable to those documented in previous studies [4, 7, 8, 11]. The high specificity indicated a low overall chance of obtaining false positives. The PPVs of both systems for smear-positive specimens (92.0-92.3%) demonstrated the superiority of the systems for MTB detection in AFB-positive samples. The lower sensitivity (48.7-48.9%) was accompanied by a relatively poor PPV (58.7-60.4%) for smear-negative specimens. The overall NPV (97.7-98.0%) of both systems in our study was comparable with that

Table 3. Confirmative analysis of invalid data obtained from the AdvanSure TB/NTM PCR and COBAS TaqMan MTB PCR assays by re-test and TB culture system

Confirmative results obtained from		N of invalid results obtained using		Total N
Re-test	TB culture	AdvanSure (n=27)	COBAS TaqMan (n=37)	(n=64)
Negative	No acid-fast bacilli	27 (100%)	29 (78.4%)	56 (87.5%)
NTM	NTM		3 (8.1%)	3 (4.7%)
Positive	MTB		2 (5.4%)	2 (3.1%)
Negative	NTM		2 (5.4%)	2 (3.1%)
Invalid	No acid-fast bacilli		1 (2.7%)	1 (1.6%)

Abbreviations: MTB, *Mycobacterium tuberculosis*; NTM, non-tuberculous mycobacteria; TB, tuberculosis.

reported in other studies (97.0-98.7%) [10-12]. This also indicates the reliability of both systems for excluding non-TB cases. Notably, these two systems tend to produce a higher rate of false negatives in smear-negative specimens than in smear-positive specimens.

This study highlighted that AdvanSure assay could reduce the necessity of a re-test by one-fourth, compared to COBAS TaqMan assay. This would be advantageous for promoting the cost-effectiveness of AdvanSure assay in clinical laboratories. Notably, all invalid results produced by the AdvanSure assay showed negative results on re-test and culture, while some of the invalid results produced by the COBAS TaqMan assay were revealed to be NTM or MTB. Some researchers also reported that COBAS TaqMan assay yielded a high rate of invalid results (4.1%), which could be attributed to the presence of inhibitors [14]. This study showed that re-testing might be sufficient to confirm the invalid results obtained by the AdvanSure assay; however, invalid results obtained by the COBAS TaqMan assay should be compared with the culture results for confirmation. Further studies to evaluate the high rate of invalid results shown by the COBAS TaqMan assay are warranted.

This study also had some limitations. First, the two assays have not been performed in the same specimens. Because our preliminary data showed similar diagnostic performance when both assays were compared using the same specimens (data not shown), thus clinical samples have been randomly examined by using either assay in the routine practice. The selection bias according to the preference of the laboratory personnel could be existed, however, it was thought that the large number of cases (12,129) with similar compositions of specimen types could compensate this bias. Second, the invalid results have been available just for a one-year period due to limitation in the data storage system. To overcome this issue, the rate of invalid results instead of the number could be compared between the two assays. Taken together, our data indicate that the AdvanSure TB/NTM PCR assay provides a clinical performance comparable with that of the COBAS TaqMan MTB system and may have fewer invalid results. Both AdvanSure TB/NTM and COBAS TaqMan MTB PCR assays can be useful identification tools in routine clinical microbiology.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Table S1. Distribution of specimens among invalid results obtained from the AdvanSure TB/NTM PCR and COBAS TaqMan MTB PCR assays at Chonnam National University Hospital (2012)

Specimens	AdvanSure	COBAS TaqMan	Total N
Respiratory specimens	22 (81.5%)	30 (81.1%)	52 (81.3%)
Sputum	19	25	44
Bronchial washing	3	1	4
BAL		2	2
Transtracheal aspirate		1	1
Pleural fluid		1	1
Non-respiratory specimens	5 (18.5%)	7 (18.9%)	12 (18.8%)
Blood	1	2	3
CSF	3		3
Pus		4	4
Pericardial fluid	1		1
Random urine		1	1
Total N of result*	27	37	64
Incidence of invalid results	5.0	20.4	
(N of invalid results per 1,000 tests)			

*$P<0.001$. Statistical analysis was performed by Mantel-Haenszel corrected chi-squared test.
Abbreviation: CSF, cerebrospinal fluid.