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Performance evaluation of the Cobas TaqMan MTB assay on respiratory specimens according to clinical application

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ABSTRACT

Objective: To evaluate the performance of the Cobas TaqMan MTB assay (Cobas assay) with respect to its clinical application.
Methods: This was a retrospective analysis of 1154 results from 1034 patients for whom mycobacterial cultures and the Cobas assay were performed simultaneously. Based on the patient medical records, two categories of clinical application were defined: (1) the diagnosis of patients with a high probability of pulmonary tuberculosis according to clinical and radiological features ($n = 128$), and (2) the exclusion of tuberculosis in clinically indeterminate patients ($n = 1026$). Standard culture was used as the reference method.
Results: The sensitivity of the Cobas assay for the detection of *Mycobacterium tuberculosis* was 70.4% (95% confidence interval (CI) 49.7–85.5%) for category 1, but only 25.0% (95% CI 4.5–64.4%) for category 2. The specificity was ≥95.0% for both categories. The positive predictive value was 79.2% (95% CI 57.3–92.1%) for category 1 and 33.3% (95% CI 6.0–75.9%) for category 2, while the negative predictive value was 92.3% (95% CI 85.0–96.4%) for category 1 and 99.4% (95% CI 98.7–99.8%) for category 2.
Conclusions: The results of this study indicate that Cobas assay results must be interpreted carefully according to the clinical purpose of the assay.
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Introduction

Tuberculosis (TB) is a major global public health problem and has one of the highest mortality burdens of any infectious disease. According to the 2015 World Health Organization (WHO) Global Tuberculosis Report, South Korea is categorized as a country with an intermediate TB burden. The TB incidence rate was 86 per 100 000 people in 2015, while the TB mortality rate was 3.8 deaths per 100 000 people in 2015 (World Health Organization, 2015). To prevent person-to-person transmission and reduce morbidity and mortality, rapid diagnosis and early treatment are essential (Dye and Williams, 2010). The US Centers for Disease Control and Prevention (CDC) have recommended that nucleic acid amplification (NAA) tests be performed on at least one respiratory specimen from every patient with symptoms or signs of TB (MMWR, 2009).

The Cobas TaqMan MTB assay (Cobas assay) (Roche Diagnostics, Basel, Switzerland) is one of the most widely used real-time PCR assays (UNITAID, 2015). The Cobas assay uses primers and TaqMan hydrolysis probes that bind to specific regions with highly conserved 16S rRNA sequences (UNITAID, 2015). Many studies have characterized the performance of the Cobas assay, with results varying from study to study (Bloemberg et al., 2013; Horita et al., 2015; Huh et al., 2015; Jonsson et al., 2015; Kim et al., 2011; Yang et al., 2011). Some studies have suggested that this variance is due to the acid-fast bacilli (AFB) smear status, variable specimen types, and incidence of TB (Huh et al., 2015; Jonsson et al., 2015). However, no study has yet addressed the performance of this assay in the context of different clinical purposes, and more specifically for diagnosis when TB is strongly suspected versus when TB is considered to be less likely.

This retrospective study was conducted to evaluate the performance of the Cobas assay on respiratory specimens

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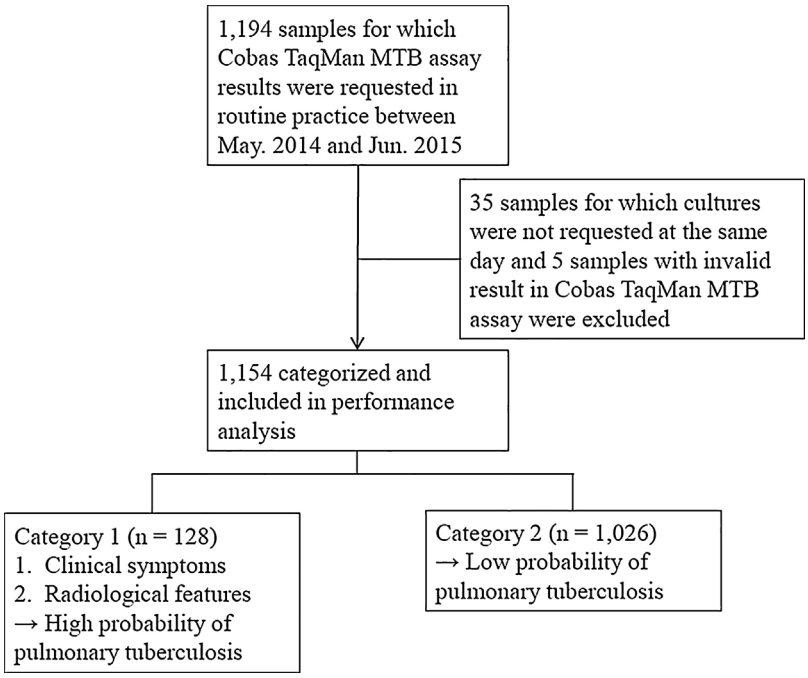


Figure 1. Flow diagram outlining patient enrollment and stratification for the analysis of the diagnostic performance of the Cobas TaqMan MTB assay.

according to the physician's assessment of the likelihood of TB on initial examination and according to clinical and laboratory diagnoses of TB in real clinical settings.

Methods

Study design

This study was approved by the Institutional Review Board of Samsung Medical Center, Seoul, South Korea (#2016-08-170). A total of 1194 respiratory specimens were evaluated using the Cobas assay between May 2014 and June 2015, and the results were analyzed retrospectively. Thirty-five samples for which cultures were not requested on the same day and five samples with invalid Cobas assay results were excluded (Figure 1).

Patient medical records, AFB smear findings, and mycobacterial culture results were reviewed. The clinical application of the Cobas assay was identified in the patient medical records and the samples were divided into two categories, as outlined below. All cases were categorized independently by two doctors. All disagreements in data interpretation required final agreement between doctors.

Category 1 consisted of samples from patients with a high probability of pulmonary TB for whom a rapid diagnosis of TB was needed. High-probability pulmonary TB was defined as the presence of clinical symptoms (cough, sputum, fever, night sweats, or weight loss) and radiological features highly indicative of pulmonary TB on chest X-ray or a chest computed tomography, such as patchy or nodular shadows and cavitation (Jeong and Lee, 2008).

Category 2 consisted of samples from patients with a clinically low probability of pulmonary TB, i.e., patients for whom a diagnosis of pulmonary TB was not considered to be highly probable, but for whom a diagnosis of pulmonary TB could not be reliably excluded by clinicians. The patients in this clinical low-probability group were either asymptomatic or did not have any radiological features highly suggestive of TB. This group also included samples from patients with an AFB smear-positive specimen, for whom *Mycobacterium tuberculosis* (MTB) needed to be differentiated from non-tuberculous mycobacteria (NTM).

Specimen processing

All respiratory specimens were decontaminated with 2% *N*-acetyl-l-cysteine–sodium hydroxide (NALC–NaOH), followed by centrifugation at 3000 *g* for 20 min. After resuspension of the resulting sediments in phosphate buffer, acid-fast staining smears were prepared. Each mycobacterial culture was prepared by

Table 1
Performance of the Cobas TaqMan MTB assay according to smear status; results are presented as No./total No., % (95% CI).

	Total (n = 1154)	Smear result	
		Smear-positive (n = 38)	Smear-negative (n = 1116)
Sensitivity	21/35 60.0 (42.2–75.6)	13/13 100.0 (71.7–100.0)	8/22 36.4 (18.0–59.2)
Specificity	1110/1119 99.2 (98.4–99.6)	24/25 96.0 (77.7–99.8)	1086/1094 99.3 (98.5–99.7)
PPV	21/30 70.0 (50.4–84.6)	13/14 92.9 (64.2–99.6)	8/16 50.0 (25.5–74.5)
NPV	1110/1124 98.8 (97.9–99.3)	24/24 100.0 (82.8–100.0)	1086/1100 98.7 (97.8–99.3)

CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

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Table 2
Performance of the Cobas TaqMan MTB assay according to the clinical application; results are presented as No./total No., % (95% CI).

	Total (n = 1154)	Clinical application		
		Category 1 (n = 128)	Category 2 (n = 1026)	p-Value
Sensitivity	21/35 60.0 (42.2–75.6)	19/27 70.4 (49.7–85.5)	2/8 25.0 (4.5–64.4)	0.039
Specificity	1110/1119 99.2 (98.4–99.6)	96/101 95.0 (88.3–98.2)	1014/1018 99.6 (98.9–99.9)	0.001
PPV	21/30 70.0 (50.4–84.6)	19/24 79.2 (57.3–92.1)	2/6 33.3 (6.0–75.9)	0.049
NPV	1110/1124 98.8 (97.9–99.3)	96/104 92.3 (85.0–96.4)	1014/1020 99.4 (98.7–99.8)	<0.001

Category 1, high-probability of pulmonary tuberculosis; Category 2, low-probability of pulmonary tuberculosis; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

inoculating 500 μl of the decontaminated sample into a mycobacterial growth indicator tube (MGIT 960 system; Becton Dickinson, Sparks, MD, USA) and 300 μl onto a 3% Ogawa agar plate (Shinyan, Seoul, Korea).

Mycobacterial stains and cultures

Acid-fast staining of decontaminated specimens was performed with an auramine–rhodamine fluorescent stain, followed by confirmation with Ziehl–Neelsen staining. Staining results were graded according to the American Thoracic Society (ATS)/CDC recommendations (American Thoracic Society, 2000). Specimens with no AFB detected and trace grades were defined as smear-negative, whereas specimens graded 1 to 4 were defined as smear-positive. Mycobacterial cultures in solid and liquid media were incubated for 6 weeks. All positive cultures were subjected to AFB staining to confirm the presence of AFB and to exclude the possibility of contamination. All positive cultures were confirmed using a Genedia MTB/NTM Detection Kit (Green Cross Medical Science Corp., Chungbuk, South Korea), to differentiate between MTB and NTM.

Cobas TaqMan MTB assay

A total 100 μl of decontaminated sample sediment was used for the Cobas assay. The Cobas assay was performed according to the manufacturer's instructions, as described previously (Kim et al., 2011).

Statistical analysis

The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the assay were calculated for each category. To evaluate these parameters, concurrent conventional culture was performed and served as the reference method. The clinical characteristics and analytical performance of the Cobas assay were compared between the two groups with Fisher's exact test using IBM SPSS Statistics version 23.0 software (IBM Corp., Armonk, NY, USA). The 95% confidence intervals (CIs) were calculated using the VassarStats website (http://vassarstats.net/).

Results

A total of 1154 respiratory specimens (762 sputum, 303 bronchial washing fluid, 68 bronchoalveolar lavage fluid, and 21 endotracheal aspirate) from 1034 patients were used to analyze the diagnostic performance of the assay. The median patient age was 64 years (interquartile range 54–72 years); 685 (59.4%) patients were male. A total of 128 (11.1%) samples were classified as category 1, whereas the other 1026 (88.9%) were category 2. Based on conventional culture results, 21.1% in category 1 and 0.8% in category 2 were MTB-positive ($p < 0.001$).

Thirty-eight of the 1154 samples (3.3%) were smear-positive, while 1116 (96.7%) were smear-negative, including 25 trace results and 1091 negative results. Thirty (2.6%) samples were MTB-positive according to the Cobas assay, and 35 (3.0%) samples gave MTB-positive culture results.

The overall performance of the Cobas assay and its performance according to sample smear status are shown in Table 1. The sensitivity, specificity, PPV, and NPV (95% CI) of the Cobas assay were 60.0% (42.2–75.6%), 99.2% (98.4–99.6%), 70.0% (50.4–84.6%), and 98.8% (97.9–99.3%), respectively. The sensitivity in smear-positive specimens was 100.0% (95% CI 71.7–100.0%), while it was only 36.4% (95% CI 18.0–59.2%) in smear-negative specimens. All of the smear-positive and Cobas assay-negative samples were confirmed as NTM-positive on conventional culture.

The overall performance of the Cobas assay and its performance according to clinical application are shown in Table 2. The sensitivity of the Cobas assay was 70.4% (95% CI 49.7–85.5%) for

Table 3
Performance of the Cobas TaqMan MTB assay according to the clinical application, excluding samples from patients who were receiving anti-tuberculosis treatment; results are presented as No./total No., % (95% CI).

	Total (n = 1120)	Clinical application		
		Category 1 (n = 106)	Category 2 (n = 1014)	p-Value
Sensitivity	18/31 58.1 (39.3–74.9)	16/23 69.6 (47.0–85.9)	2/8 25.0 (4.5–64.4)	0.043
Specificity	1085/1089 99.6 (99.0–99.9)	82/83 98.8 (92.5–99.9)	1003/1006 99.7 (99.1–99.9)	0.272
PPV	18/22 81.8 (59.0–94.0)	16/17 94.1 (69.2–99.7)	2/5 40.0 (7.3–83.0)	0.024
NPV	1085/1098 98.8 (97.9–99.3)	82/89 92.1 (83.9–96.5)	1003/1009 99.4 (98.6–99.8)	<0.001

Category 1, high probability group for pulmonary tuberculosis; Category 2, low probability group for pulmonary tuberculosis; CI, confidence interval; NPV, negative predictive value; PPV, positive predictive value.

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category 1, while it was 25.0% (95% CI 4.5–64.4%) for category 2. The specificity was ≥95.0% in both categories. Category 1 yielded higher PPV compared to category 2 (79.2% vs. 33.3%), while NPV was higher in category 2 than in category 1 (99.4% vs. 92.3%). Significantly different performance was found for all measured parameters of the analytical performance of the Cobas assay according to the intended clinical application ($p < 0.05$).

When the 34 samples from patients who had been receiving TB treatment for more than 7 days during the 3 months prior to testing were excluded from analysis (Catanzaro et al., 2000), PPV was improved to 94.1% (95% CI 69.2–99.7%) in category 1 and 40.0% (95% CI 7.3–93.0%) in category 2 (Table 3). The sensitivity, PPV, and NPV of the Cobas assay differed significantly between category 1 and category 2. When stratified by smear status, good diagnostic performance was obtained in smear-positive cases regardless of the category, although there were limitations associated with the small positive sample number (**Supplementary Material** Tables S1–4).

Discussion

Many recent studies have assessed the diagnostic performance of the Cobas assay (Bloemberg et al., 2013; Huh et al., 2015; Jonsson et al., 2015; Lee et al., 2013; Yang et al., 2011). Various results have been obtained in these studies. One recent meta-analysis confirmed that the Cobas assay exhibits heterogeneous performance (Horita et al., 2015). This heterogeneity may be due to AFB smear status, variable specimen types, and baseline incidence of TB (Huh et al., 2015; Jonsson et al., 2015). In particular, the present authors have previously found that the performance of the Cobas assay depends on smear status (Huh et al., 2015). Concordant with this previous report, the Cobas assay had higher sensitivity in smear-positive specimens than in smear-negative specimens in the present study. Other measurements of analytical performance (specificity, PPV, and NPV) also showed trends similar to those observed in previous studies.

The performance of the Cobas assay can also be evaluated in the context of its intended clinical application, e.g., according to the level of clinical suspicion of TB. Such an evaluation has been performed using the Enhanced Amplified Mycobacterium Tuberculosis Direct Test (E-MTD assay; Gen-Probe Inc., San Diego, CA, USA) and the Xpert MTB/RIF assay (Xpert assay; Cepheid, Sunnyvale, CA, USA) (Catanzaro et al., 2000; Huh et al., 2014). Catanzaro et al. evaluated the performance of the E-MTD assay according to the level of clinical suspicion: low, intermediate, and high probability of TB. They reported that the sensitivity was over 75% and the specificity over 97% in each group, without significant differences between the clinical suspicion groups. However, variable PPVs and NPVs were observed among the groups. A lower PPV (59%) and higher NPV (99%) were noted in the low probability group compared to the high probability group (100% PPV and 55% NPV). A previous evaluation of the Xpert assay by the present authors demonstrated that the performance varied according to its clinical application (Huh et al., 2014). The sensitivity was 89.8% and PPV was 91.4% in the high probability group (category 1), compared to sensitivity of 66.7% and PPV of 33.3% in the low probability group (category 2). The specificity was over 91.4% and NPV was over 90.0% in categories 1 and 2. In the present study, significant differences in the performance of the Cobas assay according to the physician's assessment of the likelihood of TB were confirmed. In accordance with previous studies, the Cobas assay showed a trend towards lower sensitivity and PPV in category 2 compared to category 1. However, the performance of NAA tests according to clinical application has varied depending on the study, which might be due to differences in the testing method and incidence of TB. Since clinical suspicion is more likely to reflect physician decision-making, an evaluation of the performance of NAA tests in different settings is needed.

In the present study, the Cobas assay showed higher specificity and NPV in category 2 than in category 1. Although the current guidelines recommend that a negative NAA test result should not be used to rule out TB (Alvarez et al., 2014; American Thoracic Society, 2000; Dinnes et al., 2007), the Cobas assay could be helpful for the exclusion of TB or release from isolation of patients with a low probability of pulmonary tuberculosis (Floe et al., 2015). Since tests with high specificity can satisfactorily distinguish between MTB and NTM in specimens from patients with a clinically low probability of pulmonary TB (including NTM) at a relatively early stage, the Cobas assay might be useful for increasing the identification of NTM (Koh et al., 2013). Indeed, all AFB smear-positive and Cobas assay-negative specimens in the present study (nine cases in category 1 and five cases in category 2) were eventually confirmed to be NTM-positive by conventional culture. It is of note that the NPV of the Cobas assay for category 2 was significantly higher than that for category 1, after the exclusion of patients receiving TB treatment.

This study had some limitations. First, this was a retrospective study performed in routine clinical practice at a single institution. However, the retrospective design allowed a more complete review of sequential specimens tested with the Cobas assay in routine clinical laboratories. Second, these findings might be somewhat limited by the small number of positive results, which was a consequence of the relatively low prevalence of TB. Prospective studies in these patient groups are warranted to strengthen the results. However, compared with previous studies of assay performance according to clinical application (Catanzaro et al., 2000; Huh et al., 2014), the present study was a large-scale clinical review in an intermediate pulmonary TB burden setting.

In conclusion, this retrospective study revealed that the Cobas assay shows significantly different performance when used on respiratory specimens from high versus low TB probability groups. The findings indicate that careful interpretation of Cobas assay results is needed and that the clinical application of the assay must be considered. This study has important implications for physicians who frequently interpret Cobas assay results.

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None.

Conflict of interest

None declared.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijid.2017.08.014.

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