Evaluation of the Cobas TaqMan MTB Test for Direct Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens[▽]

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The Cobas TaqMan MTB test, based on real-time PCR technology, was evaluated for direct detection of *Mycobacterium tuberculosis* complex (MTBC) in respiratory specimens. A total of 1,093 samples from 446 patients, including 118 acid-fast smear-positive and 975 acid-fast smear-negative specimens, were investigated. Diagnostic cultures performed with 7H11 agar, Löwenstein-Jensen medium, and the Bactec MGIT 960 system were considered the reference methods. When discrepant results between the Cobas TaqMan MTB test and culture occurred, additional results from the BD MGIT TBc identification test and the GenoType Mycobacterium CM test performed on growth-positive and acid-fast-stain-positive MGIT tubes and review of the patient's medical history were used for discrepancy analysis. The overall sensitivity, specificity, positive predictive value, and negative predictive value for the Cobas TaqMan MTB test were 91.5%, 98.7%, 91.5%, and 98.7%, respectively. In general, the performance of the new Cobas TaqMan MTB test was comparable to that of the replaced Cobas Amplicor MTB system. The most prominent feature of the new system was its extraordinarily high sensitivity (79.5%) for detecting MTBC in smear-negative specimens; out of 44 smear-negative but culture-positive specimens, 35 were positive by the new system. The Cobas TaqMan MTB assay, including DNA extraction, can be completed within 3 h.

Tuberculosis (TB) is one of the most threatening curable infectious diseases. The disease afflicts approximately 8.6 million patients and causes about 2 million deaths annually (25). The increasing global burden of mycobacteriosis is associated with improper antibiotic therapy and immunocompromised patients, such as those with AIDS (1). Early diagnosis of TB and the prompt use of adequate antibiotics to interrupt transmission remain the top priorities for TB control (5).

The conventional diagnosis of mycobacterial infections is based primarily on demonstration of the presence of acid-fast bacilli (AFB) in the smear, followed by a positive culture and identification of the isolate by biochemical characteristics. In the past decades, several commercial systems are gaining popularity for direct detection of *Mycobacterium tuberculosis* complex (MTBC) in clinical specimens, for instance, the Cobas Amplicor MTB test (Roche, Basel, Switzerland), the amplified

M. tuberculosis direct test (Gen-Probe, San Diego, CA), the BDProbe Tec ET system (Becton, Dickinson and Company, Sparks, MD), and the GenXpert MTB/RIF system (Cepheid, Sunnyvale, CA). These systems, being able to reduce the diagnostic time from weeks to hours, have been acquiring great attention in TB diagnosis. In general, the specificity of these systems is very high while the sensitivity varied widely (16). For most commercial tests, the assay sensitivities (87.5% to 100%) seem to be satisfactory for AFB smear-positive specimens, but the sensitivities (50.0% to 70.8%) varied greatly for AFB smear-negative samples (16).

The Cobas Amplicor MTB assay for direct detection of MTBC in pulmonary specimens is popular in developing and developed countries, and many studies have been conducted worldwide to evaluate the system (9, 13, 16, 20). The Cobas Amplicor MTB assay is based on amplification of a segment of the 16S rRNA gene, followed by colorimetric detection of the PCR product by probe hybridization (16). The Amplicor assay was approved by the FDA for testing on smear-positive respiratory samples. Recently, Roche Diagnostics (Taipei, Taiwan) introduced a new system (Cobas TaqMan MTB test), based on real-time PCR technology, to replace the Cobas Amplicor MTB assay. According to the user's instructions from Roche (19), the total agreement rate was 98.3% (95% confidence interval, 97.1 to 99.1%) between the new and old systems. The new system is recommended for analyzing respiratory samples, including smear-positive and -negative specimens. However, so far, there is only one study published to evaluate the performance of the Cobas TaqMan MTB test by testing a limited number of specimens (11). The aim of this study was to eval-

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TABLE 1. Comparison of the results from the Cobas TaqMan MTB test with those from culture

Specimen group (no. of specimens)	Result for Cobas TaqMan MTB test[a]	No. of specimens with culture result		% (95% CI) for Cobas TaqMan MTB test[b]			
		Positive	Negative	Sensitivity	Specificity	PPV	NPV
Smear positive (118)	Positive	94	0	96.9 (93.5–1.0)	100 (1.0–1.0)	100 (1.0–1.0)	87.5 (74.3–1.0)
	Negative	3	21				
Smear negative (975)	Positive	35	12	79.5 (67.6–91.5)	98.7 (98.0–99.4)	74.5 (62.0–86.9)	99.0 (98.4–99.7)
	Negative	9	919				
All (1,093)	Positive	129	12	91.5 (86.9–96.1)	98.7 (98.0–99.4)	91.5 (86.9–96.1)	98.7 (98.0–99.4)
	Negative	12	940				

[a] The results were adjusted after discrepancy analysis.
[b] CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

uate the new system by using a large number of respiratory specimens.

MATERIALS AND METHODS

Clinical specimens and processing. In an open prospective study in the spring of 2010, a total of 1,093 respiratory specimens (1,036 sputum, 39 bronchial and tracheal aspirate, and 18 bronchial alveolar lavage samples) were sent to the Division of Clinical Microbiology, Department of Laboratory Medicine, Kaohsiung Medical University Hospital (KMUH), for mycobacterial testing. The collection of these clinical samples for this study was approved by the Review Board Committee of KUMH. The specimens were collected from 446 patients with clinical signs of pulmonary TB or in order to exclude the possibility of TB infection. Specimens were digested and decontaminated by the N-acetyl-L-cysteine-NaOH method, neutralized with phosphate buffer (67 mM; pH 6.8), and centrifuged (10). The sediment was resuspended in 2.0 ml of the same phosphate buffer. An aliquot of the suspension was stained with an auramine fluorescent stain. The status of those deemed positive was confirmed by the Kinyoun acid-fast staining method and classified as AFB +/−, 1+, 2+, 3+, or 4+ based on standard procedures (2). Portions (0.5 ml) of the sediment from each specimen were used to inoculate a Löwenstein-Jensen (LJ) tube, a 7H11 agar plate, and a Bactec MGIT 960 tube (Becton, Dickinson and Company, Taipei, Taiwan) supplemented with oleic acid, albumin, dextrose, catalase (BBL MGIT OADC) and PANTA Plus (both products from Becton, Dickinson and Company) (12).

Culture conditions and MTBC identification. The LJ tubes and 7H11 agar plates were incubated at 37°C for 8 weeks and examined weekly for positive cultures. The identification of the mycobacterial isolates as MTBC is based mainly on routine morphological and biochemical assays (24) and confirmed by amplification of the 65-kDa heat shock protein followed by restriction enzyme analysis (23). The MGIT tubes were incubated at 35°C and monitored automatically every 60 min for fluorescence intensity. The tubes were incubated until they were positive or for 42 days. Positive tubes were removed from the MGIT 960 instrument, and smears for AFB stain were prepared. The smears were first screened with the auramine fluorescent stain and confirmed by the Kinyoun acid-fast stain. If the smear was AFB positive, subculture was made on the 7H11 agar plate and LJ slant for recovery of mycobacteria, and an aliquot (0.1 ml) of the broth was used for testing of the presence of MTBC-specific protein (MPT64) using the BD MGIT TBc identification test (Becton, Dickinson and Company) according to the manufacturer's instructions. The immunochromatographic test provides results in 15 min. In addition, an 0.2-ml aliquot of the growth-positive and AFB-positive broth from each MGIT tube was used for DNA extraction using a Gentra Puregene DNA extraction kit (Qiagen, Valencia, CA) in accordance with the manufacturer's instructions, except that a preceding step of heat inactivation (80°C for 5 min) was included. The DNA was kept for further testing by using a line probe hybridization kit (GenoType Mycobacterium CM; Hain Lifescience, GmbH, Germany). In addition to MTBC, the GenoType kit can also detect more than 10 species of nontuberculous mycobacteria (14). The results for the BD MGIT TBc identification and Mycobacterium CM assays were used as supporting evidence for the presence of MTBC when discrepant results between the Cobas TaqMan MTB test and culture occurred.

Cobas TaqMan MTB test. The Cobas TaqMan MTB test is used for detection of MTBC in liquefied, decontaminated, and concentrated respiratory specimens. The test utilizes the TaqMan 48 analyzer for automated amplification and detection. The test includes two major steps: (i) preparation of specimen DNA and

(ii) real-time PCR. The assay permits the detection of amplified MTBC amplicon and internal control DNA, which is amplified and detected simultaneously with the specimen. A 100-µl aliquot of the liquefied, decontaminated, and concentrated respiratory specimen from each specimen was used for testing. One *Mycobacterium*-negative [MYCO (−)] control and one *M. tuberculosis*-positive [MTB (+)] control were included in each test run. The TaqMan 48 analyzer determined the cycle threshold value (C_T) for the DNA of MTBC and checked whether the C_T values of the internal control DNA, the MTB (+) control, and the MYCO (−) control were within the normal ranges. The internal control DNA was used to detect polymerase inhibitors that might be present in specimens. In this study, PCR inhibitors were found in about 1% of the specimens (10 samples), and these specimens were 1:10 diluted and retested as recommended (19).

Analysis of discrepant results. Conventional culture was primarily considered the "gold standard" for performance calculation. When discrepant results between the Cobas TaqMan MTB test and culture occurred, the test results from the adjunct BD MGIT TBc identification and GenoType Mycobacterium CM assays were taken into consideration for resolving discrepancies. For example, if a specimen was TaqMan MTB test positive but culture negative, the specimen was considered to contain MTBC if the results for the BD MGIT TBc identification assay and/or the GenoType Mycobacterium CM test were also positive for MTBC. In addition, the patient's clinical pictures, including the chest symptoms, X ray, and history of antibiotics administered, whenever those data were available, were taken into account for discrepancy analysis. The patient's clinical history was classified into five groups according to the recommendations of the American Thoracic Society (3), with small modifications. The five groups were as follows: group 1 (exclusion of TB), negative result for tuberculin skin test, smear and culture negative, and definitive other diagnosis obtained by culture or on the basis of clinical presentation; group 2 (TB infection), smear and culture negative and not clinically active (positive tuberculin skin test and/or history of tuberculosis); group 3 (TB infection), smear positive or negative, culture negative, and clinically active (positive result for tuberculin skin test, history of TB, radiological or clinical signs of active TB, exclusion of other definitive cultures, and improvement under antimycobacterial chemotherapy); group 4 (TB infection), smear negative or positive and culture positive; and group 5 (TB infection), smear and culture positive. If a specimen was positive by the Cobas test but was negative by culture and the two adjunct tests, the specimen was still determined to contain MTBC if the patient clinical history was classified in group 3.

Performance analysis. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the Cobas TaqMan MTB test were calculated after discrepancy analysis. The 95% confidence interval was calculated according to the method of Gardner and Altman (8).

RESULTS

Smear-positive specimens. A total of 118 specimens from 52 patients were AFB smear positive. For these samples, the Cobas TaqMan MTB assay yielded 115 concordant results (94 positives and 21 negatives) with culture after discrepancies were resolved (Table 1). The TaqMan MTB assay produced three false negatives (T03092, T03609, and T04270), since MTBC was isolated from these samples by culture. In addition,

TABLE 2. Discrepancy analysis of 42 respiratory specimens (38 patients) for *M. tuberculosis* complex detection

Specimen(s) (acid-fast stain score)	No. of specimens	Detection of *M. tuberculosis* complex (other mycobacterium detected) by:				Medical group	Final interpretation of Cobas TaqMan MTB test
		Cobas TaqMan MTB test	GenoType CM test[a]	BD MGIT TBc test	Culture[b]		
T03092 (2+), T03609 (3+)	2	−	ND[c]	+	+	5	False negative
T04270 (1+)	1	−	ND	−	+	5	False negative
T03876 (1+)	1	+	+	−	− (*M. intracellular*)	4	True positive
T03951 (2+)	1	+	+ (*M. abscessus*)	+	− (*M. abscessus*)	4	True positive
T03148 (+−), T03587 (2+) T04256 (1+), T05990 (+−)	4	+	ND	ND	−	3	True positive
T04869 (2+)	1	+	ND	−	− (*M. fortuitum*)	3	True positive
T05989 (1+)	1	+	−	−	−	3	True positive
T06228 (1+), T06229 (1+)	2	+	ND	−	−	3	True positive
T03266 (−), T03960 (−) T04270 (−)	3	−	ND	−	+	4	False negative
T03281 (−), T03323 (−), T03434 (−), T03577 (−), T03919 (−), T04265 (−)	6	−	ND	+	+	4	False negative
T03116 (−), T03218 (−) T03258 (−), T03949 (−) T04077 (−), T04302 (−)	6	+	ND	ND	−	3	True positive
T04118 (−)	1	+	ND	ND	− (*M. kumamotonense*)	3	True positive
T04332 (−)	1	+	− (*M. abscessus*)	−	− (*M. abscessus*)	3	True positive
T03090 (−), T03389 (−)	2	+	ND	−	−	1	False positive
T03215 (−)	1	+	ND	ND	− (an unidentified mycobacterium)	1	False positive
T03329 (−), T03508 (−) T03798 (−), T03897 (−) T04043 (−), T04058 (−) T04193 (−)	7	+	ND	ND	−	1	False positive
T03464 (−)	1	+	−	−	−	1	False positive
T04277 (−)	1	+	− (*M. gordonae*)	−	− (*M. gordonae*)	1	False positive

[a] Nontuberculous mycobacteria detected by the GenoType CM test are indicated in parentheses.
[b] Nontuberculous mycobacteria isolated by culture are indicated in parentheses.
[c] ND, not determined, because there was no growth in the MGIT tube or the tube was growth positive but AFB negative.

clinical history indicated that the three specimens were from patients in clinical group 5. Before discrepancy resolution, 10 specimens were found to be false positive by the Cobas TaqMan MTB assay (Table 2). Of the 10 samples, specimens T03876 and T03951 were determined to be true positives by the Cobas system, since both samples were MTBC positive by the GenoType CM test and specimen T03951 was also BD MGIT TBc test positive. Clinical history also indicated that the two specimens were from patients with TB infection (group 4). However, *Mycobacterium abscessus* was detected in specimen T03951 by culture and by the GenoType CM test. Therefore, specimen T03951 was recognized as a mixed culture of MTBC and *M. abscessus*, with MTBC being detected by the Cobas TaqMan, GenoType, and BD MGIT TBc assays and *M. abscessus* being detected by culture and the GenoType assay.

The remaining eight false-positives (T03148, T03587, T04256, T04869, T05989, T05990, T06228, and T06229) produced by the Cobas TaqMan MTB test were also considered to be true positives after the patient's medical history was reviewed, although these samples were MTBC negative as determined by three other methods (culture and the GenoType and BD MGIT TBc assays). The eight specimens were from patients who had TB history and/or typical signs of TB and were classified in clinical group 3 (TB infection) (Table 2). After the discrepancies were resolved, the sensitivity, specificity, PPV, and NPV for the Cobas system for smear-positive specimens were 96.9%, 100%, 100%, and 87.5%, respectively

(Table 1). It was noted that specimens T05989 and T05990 were from the same patient, while specimens T06228 and T06229 were from another patient.

Smear-negative specimens. A total of 975 specimens from 394 patients were AFB smear negative. For these specimens, the Cobas TaqMan MTB assay yielded 954 concordant results (35 positives and 919 negatives) with culture after the discrepancies were resolved. Before discrepancy analysis, the Cobas assay produced 9 false negatives and 20 false positives (Table 2). The nine false negatives (specimens T03266, T03281, T03323, T03434, T03577, T03919, T03960, T04265, and T04270) were true false negatives, as MTBC was recovered from these samples by culture; six of these specimens were also positive by the BD MGIT TBc assay (Table 2). The false-positive number was reduced from 20 to 12 after the discrepancies were resolved, and 8 false positives (T03116, T03218, T03258, T03949, T04077, T04118, T04302, and T04332) were considered to be true positives, as clinical data revealed that these specimens were from patients with TB history or from patients highly suspected of having TB (medical group 3) (Table 2). Twelve specimens (T03090, T03215, T03329, T03389, T03464, T03508, T03798, T03897, T04043, T04058, T04193, and T04277) were from patients without any sign of TB (medical group 1), and these specimens were considered to be true false positive by the Cobas TaqMan MTB assay. Therefore, for smear-negative specimens, the sensitivity, specificity, PPV, and NPV for the Cobas TaqMan MTB test were 79.5%, 98.7%,

74.5%, and 99.0%, respectively, after discrepancy resolution. The most prominent feature of the Cobas TaqMan MTB test was its extraordinary high sensitivity (79.5%) for detecting MTBC in smear-negative samples. Out of 44 specimens that were culture positive, 35 were also positive by the Cobas system. The less satisfactory PPV (74.5%) for smear-negative specimens was due to the fact that among the 47 Cobas TaqMan MTB-positive specimens, 12 were false positives (Table 1).

Overall performance of the Cobas TaqMan MTB test. With smear-positive and smear-negative specimens taken together, a total of 1,093 specimens were analyzed. The overall sensitivity, specificity, PPV, and NPV of the Cobas system were 91.5%, 98.7%, 91.5%, and 98.7%, respectively (Table 1).

DISCUSSION

The Cobas TaqMan MTB test is a new system used to substitute for the Cobas Amplicor MTB assay, which was the first automated nucleic acid amplification test for direct detection of MTBC in respiratory specimens. Since the introduction of nucleic acid-based test systems, the most concerning question has been that regarding the sensitivity of these assays, especially with smear-negative samples (16). In this study, we evaluated the performance of the new Cobas system by testing 1,093 respiratory samples. During the evaluation, the BD MGIT TBc identification test and the GenoType Mycobacterium CM assay were also performed on growth-positive and AFB-positive MGIT broth. The two adjunct tests were intended to compensate culture results that were not available due to an absence of visible growth or outgrowth by other bacteria (contamination) on the LJ slants and 7H11 agar plates. The sensitivities of the new Cobas system were 96.9%, 79.5%, and 91.5% for smear-positive, smear-negative, and overall specimens, respectively (Table 1). For smear-positive samples, the sensitivity (96.9%) was comparable to those (87.5% to 100%) of the Cobas Amplicor MTB test (4, 6, 7, 16–18). For detection of MTBC in smear-negative specimens, the sensitivity (79.5%) of the new system was the highest among those (51% to 71.7%; average, 59.5%) reported for the Cobas Amplicor MTB system (Table 2). In other words, the newly launched Cobas system has a prominent ability to detect lower loads of MTBC in smear-negative samples. However, the performance determined in this study may vary according to the prevalence of tuberculosis in different countries, especially the positive and negative predictive values.

In this study, the new system was able to detect MTBC in 35 out of 44 smear-negative specimens that were culture positive (Table 1). According to the manufacturer's instructions (19), the detection limit of the Cobas TaqMan MTB assay is 0.33 to 0.83 CFU (95% confidence interval) per PCR. The high sensitivity for smear-negative specimens seems to be a great improvement for the new system, since smear-negative samples normally represent a major portion (>90%) of clinical specimens sent to the routine laboratory for initial diagnosis or follow-up of mycobacterial infections. Recently, a study was conducted to compare the performances of the Cobas TaqMan MTB test and the Cobas Amplicor MTB system (11). The sensitivity, specificity, PPV, and NPV were 79.1%, 98.2%, 73.1%, and 98.7%, respectively, for the Cobas TaqMan MTB

test. The performance was less satisfactory than those obtained in this study (Table 1); this might be due to the small sample size (406 specimens, with only 24 being MTBC positive), the fact that the discrepant results were not resolved by other molecular methods, and the fact that the patient's medical data were not taken into consideration for discrepancy resolution.

Instead of the Ziehl-Neelsen acid-fast staining method that is popular worldwide, the cold staining technique (Kinyoun staining) was used in this study. A recent paper (21) indicated that the positive yields of the Ziehl-Neelsen (14.2%) and Kinyoun (13.8%) staining techniques were comparable. Therefore, the use of the Kinyoun staining method might not be able to alter the proportion of smear-positive to smear-negative specimens in this study (Table 1).

In this study, a total of 12 false negatives were produced by the Cobas TaqMan MTB assay (Table 2), with 9 of these specimens being smear negative. Of the 12 specimens, 10 exhibited positive culture results after a period of ≥28 days of incubation, indicating the low counts of mycobacteria in these samples. Negative results obtained from culture-positive specimens by molecular amplification assays are normally explained by a low load of mycobacteria, the presence of polymerase inhibitors, and an unequal distribution of mycobacteria in the test specimens (18). A total volume of 1.5 ml of the processed sample sediment was inoculated to an LJ tube, a 7H11 agar plate, and a Bactec MGIT 960 tube (each 0.5 ml). If MTBC was finally found in any of the three media, the sample was declared to be "culture positive." Therefore, the total volume used for culture was 1.5 ml; this volume was 15 times that (0.1 ml) processed for real-time PCR. Therefore, "sample volume effect" may contribute to some false-negative results for the Cobas TaqMan MTB system.

The overall specificity of the Cobas TaqMan MTB system was 98.7% (Table 1); this value was comparable to those (91.3% to 100%) reported for the Cobas Amplicor MTB assay (4, 6, 7, 15–18, 22). The high specificity indicated that the chance of production of false positives in overall samples by the new system was low. The PPV of the Cobas TaqMan MTB assay for smear-positive specimens was 100% (Table 1), demonstrating the superiority of the new system for detection MTBC in AFB-positive samples. It was noted that the extraordinary high sensitivity (79.5%) of the new system for smear-negative samples was accompanied with a relatively poor PPV (74.5%) for smear-negative specimens, suggesting the possibility of cross-reactions caused by nonmycobacterial microorganisms. The overall PPV (91.5%) of the new assay was, in general, comparable to the performance (73.3% to 100%) of the Cobas Amplicor MTB system (4, 6, 7, 16–18).

The overall NPV (98.7%) of the Cobas TaqMan MTB assay was satisfactory in comparison with those (80.8% to 99.2%) obtained by the Cobas Amplicor MTB system (4, 6, 7, 16–18). This indicates that the new system is also reliable for excluding non-TB cases. It should be noted that the new Cobas system tends to produce a higher rate of false negatives in smear-positive specimens than in smear-negative samples. For smear-positive specimens, the NPV was 87.5%, versus 99.0% for smear-negative specimens (Table 1). A possible explanation of the result is that some nontuberculous mycobacteria might compete for the primers used to amplify the DNA of MTBC during the assay and thus caused false negatives. But the am-

plified nontuberculous mycobacterial DNA was unable to react with the hybridization probes used in the Cobas TaqMan MTB system, since 100% specificity was obtained by the Cobas system for smear-positive specimens.

In conclusion, the performance of the Cobas TaqMan MTB test was comparable to that of the replaced Cobas Amplicor MTB system. The most important characteristic of the new system is its high sensitivity (79.5%) for smear-negative specimens. From the results of this study and previous reports, it is obvious that molecular methods are still not as sensitive as culture. A commercially automatic system for MTBC detection should always be performed in conjunction with microscopy and culture, and the results should be interpreted alongside the patient's clinical data, as recommended by other authors (16). Moreover, the reagents used in the Cobas TaqMan MTB kit are packaged in 12-test, single use vials. For the most efficient use of reagents, specimens and controls should be processed in batches that are multiples of 12. The use of an expensive and automatic instrument should depend on several issues, such as the daily samples processed, the engineering and technical support from the hospital, and the prevalence of TB or other mycobacterium-related diseases (16). The major strengths and weaknesses of an automatic system should be fully understood before the system is adopted in the routine laboratory.

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