

Evaluation of the Rapid MGIT TBc Identification Test for Culture Confirmation of *Mycobacterium tuberculosis* Complex Strain Detection[∇]

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A culture confirmation test for the detection of *Mycobacterium tuberculosis* complex strains that uses a lateral-flow immunochromatographic assay to detect the MPB64 antigen, the MGIT TBc identification (TBc ID) test, has been developed. We evaluated the performance of the TBc ID test in the detection of the *M. tuberculosis* complex in 222 primary-positive liquid cultures. We compared these results to those of nucleic acid-based identification and conventional biochemical tests. The validity of the TBc ID test was determined, and all of the nontuberculous mycobacteria (NTM) and *Nocardia* species tested were found to be negative. The detection limit of the TBc ID test was 5×10^5 CFU/ml, and for IS6110 real-time PCR it was 5 CFU/ml. All of the *M. tuberculosis* and *M. africanum* cultures were found to be positive, while *M. bovis* and *M. bovis* BCG cultures were negative. With the exception of 1 contaminated culture, the 221 culture-positive isolates contained 171 (77.5%) *M. tuberculosis* isolates, 39 (17.6%) NTM species, and 11 (5.0%) unidentified species. Two culture-positive isolates harbored a 63-bp deletion at position 196 of the *mpb64* gene. The sensitivity, specificity, positive predictive values, and negative predictive values of the TBc ID test were 98.8, 100, 100, and 95.1%, respectively. Furthermore, the approximate turnaround time for real-time PCR was 4 h (including buffer and sample preparation), while for the TBc ID test it was less than 1 h. We suggest an algorithm for the primary identification of *M. tuberculosis* in liquid culture using the TBc ID test as an alternative to conventional subculture followed by identification using biochemical methods.

In 2007, the World Health Organization (WHO) adopted a policy that recommended the use of liquid culture methods for culture and drug susceptibility tests as a standard for tuberculosis (TB) diagnosis and case management (28). The Taiwan Centers for Disease Control (CDC) recommended that liquid and solid media be used simultaneously for mycobacterial culture (7), and approximately 90% of clinical mycobacteriology laboratories in Taiwan use a liquid culture system for the isolation of the *Mycobacterium tuberculosis* complex from clinical specimens. Acid-fast bacillin (AFB) smear tests then are performed on positive cultures to dismiss contamination (4, 20). The turnaround time (TAT) for the recovery of the *M. tuberculosis* complex thus is reduced to 10 to 14 days (8, 18). Although the recovery of mycobacteria can be accelerated by using liquid culture systems, this practice provides only partial benefits if it is not accompanied by a rapid species identification test (16). Differentiating *M. tuberculosis* from nontuberculous mycobacteria (NTM) as soon as possible is important,

particularly in situations in which NTM strains represent a considerable share of the clinical isolates.

The identification of *M. tuberculosis* is time-consuming using conventional biochemical methods. The subculturing of isolated mycobacteria from liquid cultures onto solid media and their subsequent identification using conventional biochemical methods requires an additional 3 to 5 weeks (25). In addition, ambiguous biochemical reactions can confuse the test results. Thus, the identification of *M. tuberculosis* using biochemical methods is a complex, labor-intensive, and time-consuming process. Nucleic acid amplification (NAA) methods, such as real-time PCR, are both rapid and specific but are technically challenging, and they require the use of sophisticated instruments. For this reason, the WHO also recommends the use of rapid and affordable methods for the identification to the species level of the *M. tuberculosis* complex and NTM organisms (<http://www.who.int/tb/dots/laboratory/policy/en/index.html>).

The Taiwan CDC conducted a TB laboratory diagnosis survey in 2009. Their results showed that there are 36 clinical mycobacteriology laboratories in the country that perform four conventional bacteriological tests (smear, culture, identification, and drug susceptibility testing) for TB diagnosis. Of these 36 laboratories, 19 (52.8%) use biochemical tests, 9 (25%) use NAA, and 8 (22.2%) use both methods for the culture identification of *M. tuberculosis* complex strains. Among these same laboratories, 13 use commercial molecular diagnosis assays,

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and 10 perform in-house PCR using IS6110 (11) or other probes. Therefore, to strengthen TB laboratory services and facilitate the timely management of TB cases, the implementation of a simple and reliable test is needed in Taiwan.

In August 2009, BD Diagnostics (a division of Becton, Dickinson, and Company) launched the BD MGIT TBc identification (TBc ID) test for the identification of the *M. tuberculosis* complex from liquid culture in Africa and the European Union. The TBc ID test is a lateral-flow immunochromatographic assay based on the detection of MPB64 in liquid cultures using an MPT64-specific monoclonal antibody. MPB64 is a mycobacterial protein that is secreted by *M. tuberculosis* and certain strains of *M. bovis* (1, 22, 29). However, some substrains of *M. bovis* BCG in the *M. tuberculosis* complex produce no MPT64 antigen (19). This qualitative test is rapid (readable in 15 min), easy to use, and requires no processing or additional instrumentation. No clinical trial of the TBc ID test was ever reported. In this study, we evaluated the performance of the TBc ID, NAAs (such as IS6110 real-time PCR), and biochemical tests for the identification of culture-positive mycobacteria in liquid media.

MATERIALS AND METHODS

Reference strains, clinical specimens, and mycobacterial culture. The validation of the TBc ID test was conducted using 24 NTM strains, 18 mixtures of *M. tuberculosis* and NTM strains, 2 *M. bovis* strains, 1 *M. africanum* strain, and 1 *Nocardia* strain. For prospective analysis, clinical respiratory specimens were digested and decontaminated using *N*-acetyl-L-cysteine (Sigma Chemical Company, St. Louis, MO) and 2% sodium hydroxide (NaLC-NaOH). The detection limits of both the TBc ID test and real-time PCR were evaluated using serial dilutions of an *M. tuberculosis* stock (5×10^7 CFU/ml). We performed 10-fold serial dilutions from stocked H37Rv DNA (5×10^7 CFU/ml) to 0.05 CFU/ml as the lowest concentration, and we separately evaluated the limit of each assay. The procedures were repeated twice. The processed specimens were concentrated using centrifugation, inoculated in Bactec MGIT 960 culture tubes, and incubated in the BD Bactec MGIT system (Becton Dickinson Microbiology Systems, Cockeysville, MD) (10). Once a positive signal was detected using the system, a smear microscopy test was performed to screen the deposit in the culture tubes for AFB using an auramine fluorescent stain. The results were confirmed after staining using the Ziehl-Neelsen method, and samples were classified as either scanty, 1+, 2+, 3+, or 4+ (3). From January to February 2010, consecutive culture-positive MGIT samples recovered from 3,214 clinical specimens were included in this study.

Assays for culture-positive MGIT media. Both TBc ID and conventional biochemical tests were performed in the TB Laboratory at the Taipei Medical University-Wan Fang Hospital, one of the contracted mycobacteriology laboratories of the Taiwan CDC. NAA tests and sequencing were performed in the Reference Laboratory of Mycobacteriology at the Taiwan CDC.

(i) **Conventional biochemical tests.** Bactec cultures that were AFB positive were subcultured onto solid Löwenstein-Jensen (L-J) slants and incubated at 37°C to obtain colonies for identification. Conventional biochemical tests, including niacin production, nitrate reduction, 3-day acrylsulfatase, 3-day Tween 80 hydrolysis, urease, semiquantitative catalase, and tolerance to 5% NaCl, were performed to identify the *M. tuberculosis* complex (6).

(ii) **NAA tests.** For the identification of *M. tuberculosis* complex strains in AFB-positive Bactec cultures (2 to 3 days after a positive signal was detected), IS6110 real-time PCR was performed according to the method of Cleary et al. (9). The primers used for this assay were IS6 (5'-GGCTGTGGGTAGCAGAC C-3') and IS7 (5'-CGGGTCCAGATGGCTTGC-3'), as well as an internal oligonucleotide probe (5'-[6-carboxyfluorescein]-TGTCGACCTGGGCAGGG TTCG-[6-carboxytetramethylrhodamine]-3').

(iii) **TBc ID.** TBc ID devices were inoculated with 0.1 ml of Bactec cultures within 3 days of the detection of AFB-positive growth using an MGIT 960 instrument. Cultures were used directly for the TBc ID assay according to the manufacturer's recommendations (5). All inoculated devices were incubated for 15 min at room temperature before the results were visually assessed for positive detection (i.e., a visible test line) and reagent function (i.e., a visible control line).

TABLE 1. Validation of the BD MGIT TBc identification test using reference mycobacterial strains

Mycobacterium sp. (no.)	TBc ID	Real-time PCR
<i>M. tuberculosis</i> (3)	+	+
<i>M. africanum</i> TMC 5122 [Rist 3414]	+	+
<i>M. bovis</i> TMC1011 [BCG Pasteur]	-	+
<i>M. bovis</i> [BCG Pasteur]	-	+
<i>M. kansasii</i> TMC 1201	-	-
<i>M. intracellulare</i> TMC 1411 [P-54; Wilson]	-	-
<i>M. avium</i> 1982 [McKee 1]	-	-
<i>M. simiae</i> 3055 [N14]	-	-
<i>M. haemophilum</i> 1 [TMC 804]	-	-
<i>M. gordonae</i> TMC 1325 [Kowal]	-	-
<i>M. fortuitum</i> [TMC 1529]	-	-
<i>M. malmhoense</i> Mo 816 [CIP 105775; TMC 802]	-	-
<i>M. marinum</i> Aronson [TMC 1218]	-	-
<i>M. chelonae</i> TMC 1544 [Friedmann]	-	-
<i>M. abscessus</i> CDC T-2366-6 [PS 308]	-	-
<i>M. flavescens</i> D-25 [TMC 1541]	-	-
<i>M. triviale</i> [V subgroup of Runyon group III]	-	-
<i>M. xenopi</i> TMC 1482	-	-
<i>M. tuberculosis</i> and NTM (18)	+	+
<i>Nocardia</i> sp.	-	-
<i>Gordonia</i> sp.	-	-

(iv) **PCR-RFLP.** To identify mycobacteria at the species level, we performed PCR-restriction fragment length polymorphism (PCR-RFLP) analysis of a 65-kDa protein according to the method described by Telenti et al. (26). We compared the obtained patterns to those in the database at <http://app.chuv.ch/prasite/index.html> (Prasite; Centre Hospitalier Universitaire Vaudois Lausanne).

Analysis of discrepant results. Conventional confirmation tests were considered the gold standard for performance calculations. When discrepant results between the NAA, TBc ID assays, and conventional biochemical confirmation tests were observed, the results from *mpb64* gene sequencing were used to resolve the discrepancies. Mutations in the *mpb64* gene were analyzed using PCR amplification, and the resulting product was sequenced using the following oligonucleotide primers: AMS-50-F (5'-TCGATCTGCTAGCTTGAGTCTGGT-3') and AMS-51-R (5'-ACCACCGCACCAAGCTGCTGTCTA-3') (23). The PCR products were sequenced using an ABI 3730 automated sequencer (Applied Biosystems, Life Technologies Corporation, CA) under standardized conditions. The data were analyzed using Sequencing Analysis 5.2.0 software (Applied Biosystems, Life Technologies Corporation, CA).

Performance analysis. Using the results of the conventional biochemical identification methods as the gold standard, the performance of the TBc ID and IS6110 real-time PCR tests was analyzed. To assess the performance of these identification tests, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated after discrepant analysis (2). We also evaluated the TAT of the tests based on the protocols suggested by the manufacturers and the Taiwan CDC.

RESULTS

Validity of the TBc ID test. The detection limits of the TBc ID and IS6110 real-time PCR tests were 5×10^5 and 5 CFU/ml, respectively. The results of the identification of reference mycobacterial strains are summarized in Table 1. The analytical specificity of the TBc ID test was assessed using reference strains. Using the TBc ID test, 3 *M. tuberculosis* strains, 1 *M. africanum* strain, and 18 mixtures of *M. tuberculosis* and NTM strains were identified as positive, while 18 NTM strains, 1 *Nocardia* strain, 1 *Gordonia* sp., 1 *M. bovis* strain, and 1 *M. bovis* BCG strain were identified as negative.

Mycobacterial species identification. We obtained 221 (6.9%) AFB smear-positive Bactec cultures out of 3,214 dif-

TABLE 2. Comparison of the IS6110 real-time PCR and BD MGIT TBc identification tests to culture confirmation tests

Mycobacterial species identified using biochemical methods and <i>hsp65</i> PCR-RFLP ^a (no. of specimens)	IS6110 real-time PCR (no. of specimens = 210)		BD MGIT TBc identification test (no. of specimens = 210)	
	Positive	Negative	Positive	Negative
<i>M. tuberculosis</i> (171)	171	0	169	2
NTM (39)	0	39	0	39
<i>M. abscessus</i> (19)		19		19
<i>M. kansasii</i> (6)		6		6
<i>M. goodii</i> (4)		4		4
<i>M. intracellulare</i> (3)		3		3
<i>M. arupense</i> (1)		1		1
<i>M. avium</i> (1)		1		1
<i>M. chelonae</i> (1)		1		1
<i>M. fallax</i> (1)		1		1
<i>M. fortuitum</i> (1)		1		1
<i>M. holsaticum</i> (1)		1		1
<i>M. nonchromogenicum</i> (1)		1		1

^a A total of 222 specimens were primary positive in liquid culture, 11 were culture negative on L-J medium, and 1 was contaminated.

ferent clinical specimens. However, 11 cultures (5.0%) yielded no colonies after being subcultured onto L-J slants; thus, no identification results were obtained for these cultures. We identified 210 L-J culture-positive samples using standard biochemical tests: 171 (81.4%) were culture positive for *M. tuberculosis*, and 39 (18.6%) were culture positive for NTM. The most prominent NTM species identified using PCR-RFLP were *M. abscessus* (48.7%), *M. kansasii* (15.4%), *M. goodii* (10.3%), and *M. intracellulare* (7.7%) (Table 2). None of the 210 culture-positive samples collected from patients were identified as containing mixed cultures of *M. tuberculosis* and NTM.

TBc ID and NAA tests. The results of the NAA and TBc ID tests were compared to the culture results, and the results are summarized in Table 2. The NAA test performed as well as gold-standard biochemical methods. In contrast, the TBc ID test successfully yielded 39 true-negative results for 39 NTM cultures and 2 false-negative results for *M. tuberculosis* cultures. The gene sequencing of the two false-negative samples revealed a 63-bp deletion at position 196 in the *mpb64* gene in both. In addition, of the 11 AFB smear-positive Bactec cultures with no L-J growth, the results of all of the TBc ID tests were negative, while one true-positive NAA test result was observed. This false-negative TBc ID result also was seen in tests of two other specimens from the same patient that yielded culture-positive *M. tuberculosis*. Of the 39 samples that were identified as negative by the TBc ID and NAA tests, each tested positive for NTM (Table 2).

Correlation between tests. Compared to conventional biochemical methods, the sensitivity of the TBc ID test was 98.8%, the specificity was 100%, the positive predictive value was 100%, and the negative predictive value was 95.1% (Table 3). In contrast, the same four performance indexes all were 100% for the NAA method. For the primary identification of *M. tuberculosis* from liquid culture, the TAT was 1 h using the TBc ID test and 1 to 3 days using the NAA test; in contrast, the average TAT for conventional biochemical tests was 24 days (range, 7 to 49 days).

DISCUSSION

In mycobacterial laboratories in Taiwan, the average recovery rate of the *M. tuberculosis* complex in all clinical specimens has decreased from approximately 70% in 2000 to 50% in 2009 (unpublished data). Therefore, a fast and cost-effective method that allows for the qualitative detection of the *M. tuberculosis* complex from acid-fast bacillus (AFB)-positive cultures is necessary. We conducted and reported the first clinical trial of the TBc ID test. We evaluated the use of a simple MPB64-based lateral-flow immunochromatographic assay, the TBc ID test, to enhance TAT and facilitate the rapid identification of the *M. tuberculosis* complex directly from liquid culture. The lateral-flow immunochromatographic assay is useful because of its specificity and ease of use. Thus, several such tests are commercially available, including the Capilia TB assay (Taunus Laboratories, Inc., Numazu, Japan), the Tbilisa rapid test (Hangzhou, China), the SD Bioline TB Ag MPT64 rapid test (Standard Diagnostics, South Korea), and the MGIT TBc ID test. Previous studies have demonstrated that these assays all had outstanding performance, exhibiting 92.4 to 100% sensitivity and 94 to 100% specificity in identifying the *M. tuberculosis* complex (Table 4) (13, 14, 21, 23, 24, 27, and M. Warns et al., presented at the 30th Annual Congress of the European Society of Mycobacteriology, Porto, Portugal, July 2009). In this study, we found that the TBc ID test exhibited 98.8% sensitivity and 100% specificity compared to biochemical tests. However, to improve the accuracy of the identifications and confirm negative TBc ID test results, a secondary NAA test must be employed after reculturing MGIT-positive cultures onto solid medium.

The specificity of the TBc ID test was shown to be 100% in two studies. However, cross-reactivity with *M. avium* complex (MAC), *M. chelonae*, *M. intracellulare*, *M. marinum*, and *M. goodii* was observed with the Capilia test, and cross-reactivity was observed with *M. gastri* with the Bioline device (Table 4). The sensitivity of the TBc ID test ranged from 98.8 to 100%, while the sensitivity of the Capilia test ranged from 92.4 to 99.6% in similar studies. In our study, the TBc ID test

TABLE 3. Correlation between culture confirmation assays and culture results

Assay (no. of samples)	No. of cultured specimens with indicated result						Sensitivity (%)	Specificity (%)	Predictive value (%)	
	<i>M. tuberculosis</i>			NTM					Positive	Negative
	All	Assay (+)	Assay (-)	All	Assay (+)	Assay (-)				
IS6110 real-time PCR (210)	171	171	0	39	0	39	100	100	100	100
BD MGIT TBc identification test (210)	171	169	2	39	0	39	98.8	100	100	95.1

TABLE 4. Summary of the performance of MPPB64-based lateral-flow immunochromatographic assays in the detection of *Mycobacterium tuberculosis* in different studies

Category	Study method	No. of NTM with positive results	Species (no.)	No. of MTB with negative results	Mutation(s) in <i>mpb64</i> gene (no. of isolates)	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Reference	Country(ies)
1	Capilia TB assay	0		3	63-bp deletion at 196 (3), 1-bp deletion at 266 (1), G→A at 402 (2), IS6110 insertion at 501 (1), 176-bp deletion at 512 (5)	99.2	100				Japan
2	Accuprobe Capilia TB assay	0 0		0 3	C insertion at 287 (1), A→T at 388 (1), IS6110 insertion at 177 (1)	100 92.4	100 100				Germany
3	Capilia TB assay BD ProbeTec ET system	2 2	MAC (1), <i>M. chelonae</i> (1) <i>M. fortuitum</i> (1), <i>M. triviale</i> (1)	2 3		98.6 97.3	97.9 97.1	98.6 98.2	97.9 95.8		Taiwan Thailand
4	Capilia TB assay	1	MTBC and NTM mix	6	63-bp deletion at 196 (5), 2-bp insertion at 436 (1)	97	100				
5	Capilia TB assay BD ProbeTec ET system	1 2	<i>M. intracellulare</i> (1) <i>M. intracellulare</i> (1), <i>M. terrae</i> (1)	5 1		96.9 99.4	98.6 97.3	99.4 98.8	93.5 98.6	24	Taiwan
6	MGIT TBc ID Test	0		0		100	100			M. Warns et al., presented at the 30th Annual Congress of the European Society of Mycobacteriology, Porto, Portugal, July 2009	
7	Capilia TB assay SID bioline TB Ag MPT64 Rapid Test	1 1	<i>M. marinum</i> (1) <i>M. gastr</i> (1)	0 0		100 100	94 94				Zambia, South Africa
8	MGIT TBc ID Test IS6110 real-time PCR	0 0	<i>M. goodii</i> (1), unidentified NTM (1)	1 2 (from 1 patient)	G/C insertion (1) 63-bp deletion at 196, downstream 50 A→G (2)	99.6 98.8	99.5 100	100 100	95.1	7 This study	Taiwan

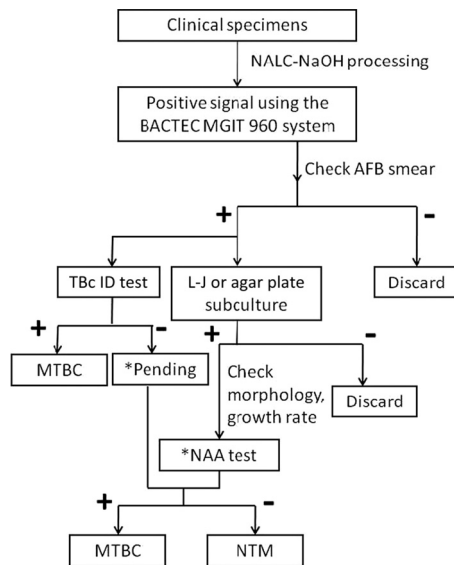


FIG. 1. Suggested algorithm for the culture and identification of the *Mycobacterium tuberculosis* complex. The asterisk indicates that for pending samples, a nucleic acid amplification (NAA) test should be performed when the morphology and growth rate suggest the presence of the *M. tuberculosis* complex.

yielded two false-negative results for two *M. tuberculosis* complex cultures from the same patient, using biochemical tests as a gold standard. These two isolates were confirmed as true positives using IS6110 real-time PCR and were found to contain a 63-bp deletion at position 196 of the *mpb64* gene. Mutations (13, 14), deletions (14, 23), and IS6110 or CG insertions (13, 14, 21, 23) in the coding region (22, 29) of the *mpb64* gene that result in false-negative test results have been reported previously.

A recent clinical evaluation performed in South Africa revealed that the TBc ID test exhibited excellent performance compared to that of the niacin test (21) (Table 4). Similar results were obtained using the BD ProbeTec ET system (BD Diagnostic Systems, Sparks, MD) in two studies performed in Taiwan (24, 27). However, both the Gen Probe AccuProbe and IS6110 real-time PCR tests outperformed the TBc ID, which occasionally was cross-reactive with NTM strains or yielded false negatives for *M. tuberculosis* complex species with mutations in the *mpb64* gene (15). Furthermore, the immunochromatographic assay was less labor-intensive than other identification methods. In this study, the TAT for identification was 24 days in the clinical setting using standard biochemical tests, and it was 1 day for the TBc ID test. Thus, to avoid false-negative results and shorten the TAT of routine clinical practices, we suggest a new algorithm for the primary identification of *M. tuberculosis* strains from liquid culture that uses the TBc ID test as an alternative to the currently available biochemical methods (Fig. 1).

Taiwan is considered a country with moderate TB prevalence, with an incidence rate of 62 per 100,000 in 2008 (<http://www.cdc.gov.tw/public/Data/9123117221971.pdf>). However, an increased incidence of NTM infections, from 32.3% in 2000 to 49.8% in 2008, was reported in a recent hospital-based survey; this rise in NTM infections was due primarily to *M.*

avium and *M. abscessus* infections (17). Therefore, to avoid inappropriate antituberculous treatment decisions, the accurate differential diagnosis of *M. tuberculosis* is imperative. The increase in the number of NTM isolations has been attributed to the implementation of liquid culture systems (12) and the use of improved identification/differentiation techniques. In this study, we proved that the TBc ID test could unambiguously differentiate *M. tuberculosis* from NTM strains and mixtures of both in a clinical setting.

In conclusion, the use of liquid culture systems together with a rapid, simple identification test has the potential to increase the speed of diagnosis and, most importantly, aid in the identification of drug-resistant TB cases. Our findings suggest that lateral-flow immunochromatographic assays for the detection of MPB64, such as the TBc ID test, are crucial for the rapid and accurate diagnosis of TB to facilitate early treatment and reduce the spread of infections.

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