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Early detection of *Mycobacterium tuberculosis* complex in BACTEC MGIT cultures using nucleic acid amplification

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Abstract We evaluated the application of nucleic acid amplification (NAA) in liquid cultures for the early detection of *Mycobacterium tuberculosis*. The Cobas TaqMan MTB test, IS*6110* real-time PCR, and *hsp65* PCR-restriction fragment length polymorphism (RFLP) analysis were used to detect BACTEC MGIT 960 (MGIT) cultures on days 3, 5, 7, and 14. The procedure was initially tested with a reference strain, H37Rv (ATCC 27294). Subsequently, 200 clinical specimens, including 150 Acid Fast bacillus (AFB) smear-positive and 50 AFB smear-negative samples, were examined. The Cobas TaqMan MTB test and IS*6110*-based PCR analysis were able to detect *M. tuberculosis* after 1 day when the inoculum of H37Rv was >3 x 10^{-2} CFU/ml. After a 5-day incubation in the MGIT system, all three NAA assays had a positive detection regardless of the inoculum size. After a 1-day incubation of the clinical specimens in the MGIT system, the sensitivity, specificity, positive predictive value (PPV), and negative pre-dictive value (NPV) for the Cobas TaqMan MTB assay were 70.2%, 100%, 100%, and 82.3% respectively. For IS*6110*-based PCR analysis, these values were 63.1%, 100%, 100%, and 78.9%, and were 88.1%, 100%, 100%, and 92.1% respectively for *hsp65* PCR-RFLP analysis. After a 3-day incubation, the specificity and PPV were 100% for all three NAA tests; the Cobas TaqMan MTB assay had the best sensitivity (97.6%) and NPV (98.3%). The sensitivity, specificity, PPV, and NPV for conventional culture analysis were 98.8%, 100%, 100%, and 99.1%. Thus, NAA may be useful for the early detection of *M. tuberculosis* after 3 days in MGIT.

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Introduction

In 2007, the World Health Organization (WHO) issued a policy that recommended use of liquid culture methods for culture and drug susceptibility testing as a standard for tuberculosis (TB) diagnosis and case management [1]. However, *Mycobacteria tuberculosis* grows slowly, requiring time to be detected by conventional culture methods. BACTEC MGIT 960 liquid culture tubes that contain 7 ml of Middlebrook 7H9 broth supplemented with oleic acid, albumin, dextrose and catalase (BBL MGIT OADC) and BBL MGIT PANTA (an antibiotic mixture of polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin) enhance the growth of *Mycobacteria* [2]. This system can efficiently shorten the turnaround time for growth detection of *Mycobacteria* from clinical specimens [3]. In Taiwan, approximately 90% of clinical mycobacteriology laboratories use a combination of solid and liquid culture methods for the isolation of *Mycobacteria* from clinical specimens [4]. Although the time to positivity for mycobacterial detection can be accelerated using liquid culture systems, it did not shorten the time for species identification [5].

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Nucleic acid amplification (NAA) methods, which detect targeted sequences directly from clinical specimens, shorten the time of identification of TB [6, 7]. A number of commercial systems have been developed, including the Probe Tec ET system, the Cobas Amplicor MTB test, the CobasTaqMan MTB test, the amplified *M. tuberculosis* direct test, and Xpert®MTB/RIF. These molecular diagnostic assays are used to test clinical specimens. Although the specificity of the NAA is high in Acid Fast bacillus (AFB) smear-positive specimens (87.5% to 100%), the sensitivity is relatively low (50.0% to 70.8%) in AFB smear-negative specimens [8]. Detecting TB with NAA in a liquid culture medium, which may have the advantages of amplifying nucleic acid targets while diluting inhibitors of amplification, may provide rapid, accurate diagnosis of TB before there is a positive culture signal from the MGIT system. The aim of this study was to evaluate this procedure to determine whether it improves the sensitivity and specificity of NAA tests after a period of incubation in liquid culture. In the first part of the study, we used various inoculum concentrations of a reference strain of *M. tuberculosis* to determine the smallest inoculum of bacilli for three NAA methods. In the second part of the study, we used clinical samples to evaluate the performance of applying NAA in liquid culture specimens before a positive culture signal was obtained by the MGIT system.

Materials and methods

Evaluation of the culture-enhanced NAA tests with an in-vitro-prepared *M. tuberculosis* reference strain

A reference strain of *M. tuberculosis*, H37Rv (ATCC 27294), was cultured on a Lowenstein–Jensen (LJ) slant, and colonies were dispersed in sterile water to obtain a suspension containing approximately 3.0×10^8 CFU/ml. We performed 10-fold serial dilutions from the stock H37Rv strain to obtain inoculum sizes ranging from 3.0×10^8 to 3.0×10^{-2} CFU/ml.

Seven Bactec MGIT 960 culture tubes (Becton Dickinson and Company, Sparks, MD, USA) were inoculated with each suspension (1 ml/tube), and were incubated in a BD Bactec MGIT system (Becton Dickinson and Company, Taipei, Taiwan) at 37°C to evaluate the association between concentration and positive detection in the MGIT system. One of the seven tubes was left in the instrument until a positive result was obtained, to determine the time to positivity in MGIT broth cultures. The six other tubes were removed from the instrument for NAA tests (Cobas TaqMan MTB test, IS*6110* real time PCR, and *hsp65* PCR-restriction fragment length polymorphism [RFLP]) at days 1, 3, 5, 7, 10, and 14, and an aliquot of 300 μl was removed from each tube. The procedures were repeated thrice.

Clinical specimen processing, culture and identification

The study was approved by the Review Board Committee of Kaohsiung Medical University Hospital, Kaohsiung, Taiwan (IRB No. KMUH-IRB-970369). Two hundred clinical specimens, including 193 respiratory specimens, two cerebrospinal fluid, two pericardial effusion, two urine samples and one pleural effusion sample, were collected from patients with suspected TB at Kaohsiung Medical University Hospital, Kaohsiung, Taiwan, from October to December 2011. The specimens were subjected to subsequent mycobacterial tests, including AFB smear, microscopic examinations, MGIT culture, Cobas TaqMan MTB test, IS*6110*-based PCR, and *hsp65* PCR-RFLP analysis.

Clinical specimens were digested and decontaminated using the *N*-acetyl-L-cysteine-NaOH method, neutralized with phosphate buffer (67mM XX, pH 6.8), and centrifuged [9]. The auramine–rhodamine fluorochrome staining method was used for microscopic examination; positive microscopy results were identified by the Kinyoun acid-fast staining method [10].

Portions (0.5 ml) of the sediment from each specimen were used to inoculate a LJ tube, a 7H11 agar plate, and Bactec MGIT 960 tube (Becton Dickinson and Company) supplemented with oleic acid, albumin, dextrose, catalase (BBL MGIT OADC), and PANTA Plus (both products from Becton Dickinson and Company) [2].

The MGIT tubes were incubated at 35°C and monitored automatically every 1 h for fluorescence intensity. The tubes were incubated until they were positive or for 42 days. AFB smears from growth-positive MGIT tubes were produced. Colonies from AFB-positive tubes were subcultured onto a LJ tube and a 7H11 agar plate, which were incubated at 37°C for 8 weeks and examined weekly for positive cultures. Mycobacteria isolated from the cultures were identified by morphological and biochemical analyses and were further confirmed by BD MGIT TBc identification test (Becton Dickinson and Company) [11] and PCR-RFLP analysis as previously described [12].

DNA extraction from MGIT medium

An aliquot of 300 μl from each MGIT medium was mixed with 600 μL of cell lysis buffer (Geneaid, Taipei, Taiwan) using a vortex mixer and then incubated at 80°C for 5 min. Thereafter, this specimen was cooled at room temperature, and 300 μl of protein precipitation solution was added followed by centrifugation at 14,000 rpm for 10 min at 4°C. After the supernatant was removed, 800 μl of 100% isopropanol was added, and the solution was centrifuged at 14,000 rpm for 10 min after cooling at 4°C for 2 h. After the supernatant was carefully removed, 600 μl of 70% alcohol was added. Following centrifugation at 14,000 rpm for 10 min at 25°C,

the supernatant and residual alcohol were carefully removed. After desiccating the sample, 50 µl of water was added then incubated at 85°C for 10 min. The clarified lysate was stored at −20°C until further use.

NAA assays for analysis of specimens in MGIT media

We used three NAA tests to detect *M. tuberculosis* in MGIT medium. After the specimens were processed for incubation in the MGIT system, 300-µl aliquots from each sample were collected for detection by NAA assays at days 1, 3, 5, 7, 10, and 14.

a. Cobas TaqMan MTB test

The Cobas TaqMan MTB tests (Roche, Basel, Switzerland) were conducted according to the manufacturer's instructions, which included two major steps: preparation of specimen DNA and real-time PCR. The assay permits the detection of amplified TB amplicons and an internal control DNA, which are simultaneously amplified and detected. Briefly, 100-µl aliquots of liquefied, decontaminated specimens were mixed with 500 µl of specimen wash solution and centrifuged at 12,500 rpm for 10 minutes. The Cobas TaqMan 48 analyzer determined the cycle threshold value (C_T) for the *M. tuberculosis* DNA, and confirmed that the C_T values of the internal positive and negative control DNA were within the normal ranges. The internal control DNA was used to detect polymerase inhibitors that might be present in the specimens [7, 13].

b. IS*6110*-based PCR

IS*6110*-based PCR is a useful genotyping method for tuberculosis diagnostics and epidemiologic studies [14]. IS*6110*-based PCR was performed according to the method described by Thierry et al. [15]. The primers used for this assay were MTB1007 (5′-GTG CGG ATG GTC GCA GAG AT-3′) and MTB1008 (5′-CTC GAT GCC CTC ACG GTT CA-3′).

c. *hsp65* PCR-RFLP analysis

The *hsp65* gene is present in all species of *Mycobacteria*, and *hsp65* sequence variations are useful for phylogenetic analysis and species identification [16]. A 439-bp fragment of the *hsp65* gene was amplified by PCR using two specific primers Tb11 (5′- ACCAACGATGGTGTGTCCAT-3′) and Tb12 (5′-CTTGTCGAACCGCATACCCT-3′). The PCR products were digested with *BstE*II and the *Hae*III [17], and the species were identified by similarity of restriction patterns using the PRASITE database (http://app.chuv.ch/prasite/index.html).

Analysis of discrepant results

Conventional cultures were primarily considered the "gold standard" for performance calculations. When discrepant results between the three NAA tests and culture occurred, the test results from the adjunct BD MGIT TBc identification (Becton Dickinson and Company, Sparks, MD, USA) and GenoType *Mycobacterium* CM assays (Hain Lifescience GmbH, Nehren, Germany) and a review of the patient's clinical history were taken into consideration for resolving discrepancies. When a discrepancy concerning TB occurred, the medical data of a patient, including radiographic findings, chest symptoms, and history of antibiotics were also reviewed. Based on these data, patients were classified into five groups according to the recommendations of the American Thoracic Society; patients in groups 3, 4, or 5 were regarded as having TB [18]. For example, a NAA-positive but conventional culture-negative specimen was considered a true positive if the diagnosis of TB was supported by at least one other molecular assay and by the clinical data. In contrast, a NAA-positive but culture-negative specimen was considered a false positive if the positivity could not be confirmed by any other molecular assay, and diagnosis of TB could not be supported by the clinical data.

Performance analysis

Using the results of the conventional culture as the gold standard, the performance of these NAA assays was analyzed. To assess the performance of these assays, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated after discrepant analysis [19].

Results

Inoculum size and time to positive detection required for identification of *M. tuberculosis* reference strain by NAA tests

As shown in Table 1, the time to positivity (detected by the MGIT system) with MGIT broth cultures of inoculum sizes ranging from 3×10^8 to 3×10^{-2} CFU/ml was between 5 and 14 days. All three NAA tests were able to detect MTBC at day 1 of MGIT culture at inoculum sizes ranging from 3×10^8 to 3×10^2 CFU/ml. In addition, both IS*6110*-based PCR and Cobas TaqMan tests were able to detect *M. tuberculosis* after 1 day in culture with the lowest tested inoculum size (3×10^{-2} CFU/ml). After 5 days in culture in the MGIT instrument, *hsp65* PCR-RFLP analysis were also able to detect *M. tuberculosis* at an inoculum concentration of 3×10^{-2} CFU/ml (Table 1).

Table 1 Comparison of MGIT broth culture and culture-enhanced NAA[a] tests for detecting *in vitro*-prepared *M. tuberculosis* suspensions

Inoculum size (CFU/ml)	Time to detection with MGIT culture (days)	NAA tests[b]	Culture-enhanced NAA test results according to the incubation period (days)					
			1	3	5	7	10	14
3×10^8	5	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
3×10^7	5	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
3×10^6	7	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
3×10^5	10	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
3×10^4	10	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
3×10^3	10	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
3×10^2	10	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	+	+	+	+	+	+
30	14	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	−	+	+	+	+	+
3	14	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	−	−	+	+	+	+
3×10^{-1}	14	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	−	−	+	+	+	+
3×10^{-2}	14	TaqMan	+	+	+	+	+	+
		IS*6110*	+	+	+	+	+	+
		hsp65	−	−	+	+	+	+

[a] NAA: nucleic acid amplification

[b] TaqMan: CobasTaqMan MTB test, IS*6110*: IS*6110*-based real time PCR, *hsp65*: *hsp65* PCR-RFLP analysis

### Detection of *M. tuberculosis* in clinical specimens using NAA tests

Clinical specimens, including 150 AFB smear-positive and 50 AFB smear-negative samples, were enrolled for evaluation. Of the 150 AFB smear-positive samples, 12 were AFB (±), 74 were 1+, 28 were 2+, 25 were 3+, and 11 were 4+. As shown in Fig. 1, 118 of 200 MGIT tubes (59.0%) yielded positive culture results detected by the MGIT system, of which 99 specimens (83.9%) were positive after 5 days inoculation.

Among these positive cultured isolates in the MGIT system, 83/118 (70.3%) were identified as *M. tuberculosis*, including 82 from AFB smear-positive specimens and one from an AFB smear-negative specimen.

Analysis of discrepant results

Of the 200 clinical samples analyzed, 84 were positive for TB, and 116 were negative for TB. The detailed analysis of the discrepant results is presented in Table 2. There were three

Fig. 1 Detection of 118 culture-positive reactions over the 14-day study period in MGIT system. Within the first 3 days, 19/118 positive reactions (16.1%) were detected. Among the specimens with positive reactions, 62/118 (52.5%) were confirmed as *Mycobacteria tuberculosis* and 31/118 (26.3%) were identified as nontuberculosis mycobacteria by conventional tests
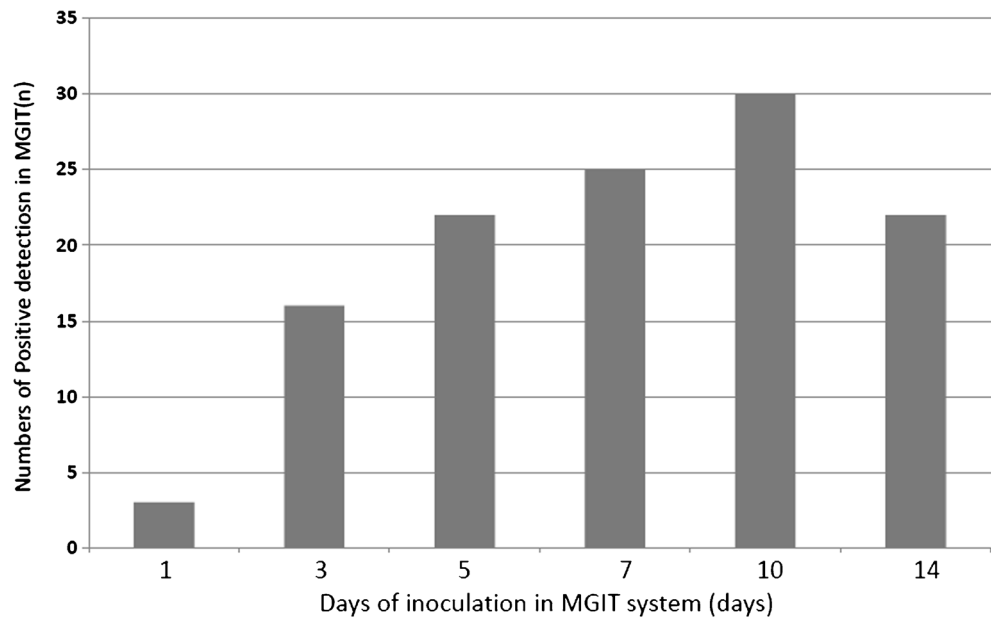
discrepancies between the NAA results and those from conventional culture and identification techniques. The Cobas TaqMan MTB assay yielded 199 concordant results (83 positive and 116 negative) with one false positive after the discrepancies were resolved. IS*6110*-based PCR yielded 197 concordant results (81 positive and 116 negative), and produced one false positive and two false negatives after the discrepancies were resolved. Finally, *hsp65* PCR-RFLP yielded 198 concordant results (81 positive and 117 negative) with two false negatives after the discrepancies were resolved.

Performance analysis after discrepancies were resolved

As shown in Table 3, after a 1-day inoculation in the MGIT system, the sensitivity, specificity, PPV, and NPV for the Cobas TaqMan MTB assay were 70.2%, 100%, 100%, and 82.3% respectively. For IS*6110*-based PCR, these results were 63.1%, 100%, 100%, and 78.9% respectively, while they were 88.1%, 100%, 100%, and 92.1% respectively for *hsp65* PCR-RFLP (Table 3).

After a 3-day incubation in the MGIT system, both the specificity and PPV were 100% for all three NAA tests in AFB smear-positive specimens, and the specificity was 100% for all of three NAA tests in smear-negative specimens. With the exception of one false negative result by the three NAA tests, the other 49 specimens revealed good agreement between the NAA results and conventional culture results. Only the Cobas TaqMan test detected this one sample as TB-positive after a 14-day inoculation in the MGIT system. As shown in Table 4, the overall specificity and PPV were 100% for all three NAA tests, with the Cobas TaqMan MTB assay having the best sensitivity (97.6%).

For the liquid culture after a 14-day incubation, the sensitivity, specificity, PPV, and NPV for the Cobas TaqMan MTB assay were 100%, 99.1%, 98.8%, and 100% respectively; these values were 98.8%, 100%, 100%, and 99.1% respectively for IS*6110*-based PCR and 97.6%, 100%, 100%, and 98.3% for *hsp65* PCR-RFLP (Table 3). In comparison, the sensitivity, specificity, PPV and NPV for the conventional culture method were 98.8%, 100%, 100%, and 99.1% respectively.

Table 2 Analysis of the discrepancies in detecting *M. tuberculosis* from three clinical specimens

Specimen no. (AFB smear score)	Conventional culture results	Detection of *M. tuberculosis* complex (other mycobacterium detected)			Geno type CM test	Anti-TB therapy[a]	Final interpretation of TB growth results
		CobasTaqMan MTB test	IS*6110* based PCR	*hsp65*PCR-RFLP			
T16620 (1+)	−	+	−	−	+	Yes	Pulmonary TB
T17274 (1+)	−	+	+	−	−	No	NTM[b]
T18852 (−)	+	+	−	−	+	Yes	Pulmonary TB

[a] TB, tuberculosis [b] NTM, nontuberculous mycobacteria

Table 3 Results of nucleic acid amplification (NAA) tests for clinical samples after different incubation periods in the MGIT system

Time of incubation in MGIT (days)	Cobas TaqMan MTB		IS6110-based PCR		hsp65 PCR-RFLP	
	Positive[a]	Negative[b]	Positive[a]	Negative[b]	Positive[a]	Negative[b]
Day 1[c]	59	141	53	147	74	126
Day 3	82	118	61	139	81	119
Day 5	84	116	76	124	82	118
Day 7	84	116	83	117	82	118
Day 14[d]	85	115	83	117	82	118

[a] NAA tests detected the growth of *M. tuberculosis*

[b] The results of NAA tests were negative for *M. tuberculosis*

[c] The final interpretation of the *M. tuberculosis* results showed 84 isolates were positive for *M. tuberculosis*, and 116 were negative. After a 1-day inoculation in the MGIT system, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for the Cobas TaqMan MTB assay were 70.2%, 100%, 100%, and 82.3% respectively. For IS6110-based PCR, these results were 63.1%, 100%, 100%, and 78.9% respectively, while they were 88.1%, 100%, 100%, and 92.1% respectively for hsp65 PCR-RFLP.

[d] After a 14-day incubation, the sensitivity, specificity, PPV and NPV for the Cobas TaqMan MTB assay were 100%, 99.1%, 98.8%, and 100% respectively; these values were 98.8%, 100%, 100%, and 99.1% respectively for IS6110-based PCR and 97.6%, 100%, 100%, and 98.3% for hsp65 PCR-RFLP

Discussion

In the first part of this study, we found that both IS6110-based PCR and the Cobas TaqMan tests were able to detect specimens with a low inoculum concentration (3×10^{-2} CFU/ml) of TB after only 1 day in the MGIT system. After a 5-day incubation in the MGIT system, all three NAA assays had positive detection regardless of the inoculum sizes. This preliminary experiment revealed the possibility of detecting TB after only a 1-day incubation in a broth culture with a bacterial load of $\geq 3 \times 10^{-2}$ CFU/ml by both IS6110-based PCR and Cobas

TaqMan tests. Using the MGIT culture system alone required 5–14 days to detect culture-positive reactions for the inoculation sizes tested. Thus, use of both procedures (NAA methods for incubated MGIT cultures) decreased the time to detect TB as compared to the MGIT system alone

It has been reported that a specimen with a bacterial load of $\geq 10^4$ CFU/ml is required to yield a positive smear [20]. It is presumed that the 150 AFB smear-positive specimens used in this study had bacterial loads of at least 10^4 CFU/ml. Given that all three NAA tests could detect the MGIT-incubated specimens with an initial bacterial load of 3×10^2 CFU/ml

Table 4 Performance analysis in three NAA[a] assays at day 3 after discrepancy analysis

Specimen group (no. of specimens)	NAA results		Final interpretation of TB results[b]		% (95% confidence interval) for the NAA assay			
			Positive	Negative	Sensitivity	Specificity	PPV	NPV
AFB smear-positive (150)	CobasTaqMan MTB test	Positive	82	0	99.0	100	100	98.5
		Negative	1	67	(0.93–0.99)	(0.93–1.00)	(0.94–1.00)	(0.91–0.99)
	IS6110-based PCR	Positive	61	0	73.5	100	100	75.3
		Negative	22	67	(0.63–0.82)	(0.93–1.00)	(0.93–1.00)	(0.65–0.84)
	hsp65 PCR- RFLP	Positive	81	0	97.6	100	100	97.1
		Negative	2	67	(0.91–0.99)	(0.93–1.00)	(0.94–1.00)	(0.89–0.99)
All (200)	CobasTaqMan MTB test	Positive	82	0	97.6	100	100	98.3
		Negative	2	116	(0.91–0.99)	(0.96–1.00)	(0.94–1.00)	(0.94–1.00)
	IS6110-based PCR	Positive	61	0	72.6	100	100	83.5
		Negative	23	116	(0.62–0.82)	(0.96–1.00)	(0.93–1.00)	(0.76–0.89)
	hsp65 PCR- RFLP	Positive	81	0	96.4	100	100	97.5
		Negative	3	116	(0.89–0.99)	(0.96–1.00)	(0.94–1.00)	(0.92–0.99)

[a] NAA, nucleic acid amplification

[b] For AFB smear-positive isolated ($n = 150$), final interpretation of *M. tuberculosis* growth results showed 83 isolates were positive for *M. tuberculosis*, and 67 were negative. For all clinical isolates ($n = 200$), final interpretation of *M. tuberculosis* results showed 84 isolates were positive for *M. tuberculosis*, and 116 were negative

after only 1 day in culture, we hypothesize that the turnaround time for recovery of TB could be shortened to 1 day with this procedure for AFB smear-positive specimens. However, the sensitivity, specificity, PPV, and NPV for the NAA tests were less satisfactory for clinical specimens after a 1-day MGIT culture (Table 3). Moreover, although hsp65 PCR-RFLP revealed relatively less sensitivity for 1-day culture specimens of the laboratory strain, it had better sensitivity and NPV for 1-day cultures of clinical specimens. The negative results obtained from the culture-positive specimens using the NAA tests after a 1-day incubation in the MGIT system, and the inconsistent performance results between the laboratory strain and clinical specimens, may be related to a low bacterial load, the presence of PCR inhibitors, an unequal distribution of bacteria in the clinical specimens, or a sample volume effect [7, 21].

The combination of NAA with liquid culture methods has been evaluated in two studies [22, 23]. Aono et al. [22] used a procedure that combined the BACTEC MGIT 960 system with the COBAS Amplicor assay from Roche Diagnostics on day 7, and reported that the sensitivity, specificity, PPV, and NPV were 95.4 %, 100%, 100%, and 90.9% respectively using respiratory specimens. In addition, Noussair et al. [23] used a BacT/Alert instrument from bioMerieux and the GenoType Mycobacteria Direct (GTMD) assay from HainLifescience on day 15, which resulted in a sensitivity, specificity, PPV, and NPV of 88.6 %, 100%, 100%, and 97.9% respectively using nonrespiratory specimens. Both studies reported that the combination of liquid cultures with NAA tests was a highly sensitive and specific method for the detection of TB in different specimens. Our study is based on combining the MGIT 960 system with three NAA tests, including the in-house IS6110-based PCR, hsp65 PCR-RFLP analysis, and Cobas TaqMan MTB assay, which were able to detect M. tuberculosis after a 3-day incubation.

Both the IS6110 insertion element and the hsp65 gene have been widely investigated as target DNA fragments for the diagnosis of TB, with appropriate sensitivities and specificities for clinical specimens [24, 25]. Identification of TB using an IS6110-based PCR assay in positive MGIT cultures led to excellent sensitivity (100%) and specificity (100%) within 7–14 days [25, 26]. The hsp65 PCR-RFLP analysis is often used to identify M. tuberculosis and differentiate other nontuberculous mycobacteria species in positive cultures, and it could be directly applied on clinical samples with acceptable sensitivity (84.5%) and specificity (100%) [27]. Our study confirmed that the inexpensive in-house NAA methods could be applied to MGIT culture specimens with acceptable sensitivity and excellent specificity (Table 4). The in-house tests cost less, and may be suitable for resource-limited conditions. The Cobas TaqMan MTB test is a quantitative assay that amplifies a segment of the 16S rRNA gene with the use of a TaqMan probe for the detection of M. tuberculosis DNA in clinical specimens [28]. The specificity of the Cobas TaqMan MTB assay is better than its predecessor, the Cobas Amplicor MTB system [29]. Furthermore, our results indicate that combining the Cobas TaqMan MTB assay with the MGIT system could shorten the turnaround time of identifying TB as compared to the combination assay used by Aono et al. [22].

For AFB smear-positive specimens after 3-day MGIT culture, the sensitivity, specificity, PPV, and NPV for the COBAS TaqMan tests were 99.0 %, 100%, 100%, and 99.0% respectively. There was one specimen that required a 14-day inoculation in the MGIT system to be identified by the COBAS TaqMan test. Thus, our results indicate that a negative Cobas TaqMan test result at day 14 can be sufficient to exclude the presence of TB. With AFB smear-positive and negative specimens taken together, the overall sensitivity, specificity, PPV, and NPV of the Cobas TaqMan test were 97.6%, 100%, 100%, and 98.3% respectively at day 3. The reported sensitivity, specificity, PPV, and NPV of the same test used directly for respiratory specimens (without inoculation in the MGIT system) were 91.5%, 98%, 91.5%, and 98.7% respectively [7]. Thus, detecting TB in MGIT culture specimens by NAA can lead to better sensitivity, specificity, and PPV.

In conclusion, the combination of MGIT and NAA tests is a highly sensitive and specific method for the early detection of M. tuberculosis in clinical specimens. The Cobas Taqman MTB test led to an excellent detection outcome at day 3. In addition, the in-house NAA methods, such as IS6110-based PCR and hsp65 PCR-RFLP, also can be useful for incubated broth culture specimens when cost is a concern.

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