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Original Article

Performance Assessment of AsiaGen MTB Detection Assay for Direct Detection of *Mycobacterium Tuberculosis* from Respiratory Specimens

Ya-Wen Cheng[1], Hsiu-Fang Lin[1], Chi- Sheng Chou[3], Chiou-Huey Wang[1], Tsun-Mei Lin[1,2]

Objective: Tuberculosis(TB) is a major public health threat worldwide. The incidence is still high in Southern Taiwan. Highly sensitive and reliable diagnostic methods for direct detection of Mycobacterium tuberculosis from respiratory specimens are necessary.

Methods: The performance of AsiaGen MTB detection assay was compared with that of Cobas Amplicor Mycobacterium system for M. tuberculosis rapid detection and Bactec MGIT 960 liquid culture system in clinical respiratory specimens at E-Da Hospital.

Results: The sensitivity and specificity of the AsiaGen MTB detection assay were 90.9% and 92.4%, and those of the Cobas Amplicor Mycobacterium system were 77.2% and 100%, respectively. Of the 121 cases with positive acid-fast stain, 98 (80%) were positive for TB by MGIT 960 TB culture, and 95 (80%) among them were positive by AsiaGen MTB detection assay. Of the 111 cases with stain-negative smears, 50 (45%) were positive for TB by MGIT 960 TB culture, but only 9 (18%) among them were positive by AsiaGen MTB detection assay. The sensitivity, specificity, and positive and negative prediction values in smear-positive cases were 96.9%, 25%, 96.9%, and 86.9%, respectively. However, the values in smear-negative cases were 18.0%, 84%, 52.9%, and 56.4%, respectively.

Conclusion: AsiaGen MTB detection assay has a similar diagnostic value compared with Cobas Amplicor Mycobacterium system for direct detection of M. tuberculosis from respiratory specimens. It might be helpful for clinical laboratory to detect tuberculosis infection immediately in smear-positive respiratory specimens; however, the performance is relatively low for smear-negative specimens.

Key words: Mycobacterium tuberculosis, AsiaGen MTB detection assay, Cobas Amplicor Mycobacterium system, MGIT 960 TB culture, respiratory specimens

From the [1]Department of Laboratory Medicine, and [2]Department of Medical Research, E-Da Hospital, I-Shou University, Kaohsiung, and [3]AsiaGen Corporation, Tainan, Taiwan.
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Address reprint request and correspondence to: Professor Tsun-Mei Lin, Department of Laboratory Medicine, E-Da Hospital, 1, Yida Road, Jiaosu Village, Yanchao District, Kaohsiung City 82445, Taiwan.
Tel: +886-7-6150011-2801, Fax: +886-7-6150925, E-mail: ed100744@edah.org.tw

Tuberculosis (TB) is an infectious disease of high prevalence worldwide. The World Health Organization reported an estimated 8.3 million new TB cases in the world.[1] *Mycobacterium tuberculosis* (MTB) is the principal TB etiological agent in humans; it is a weak Gram-positive rod-shaped bacterium that has no flagellum, does not form spores or produce toxins and has no capsule. The microbe's width and height vary from 0.3 to 0.6 and 1 to 4 μm, respectively, and it presents with a complex cellular envelope, considerably slow growth, and genetic homogeneity.[2,3] In 2008, the incidence and mortality of TB in Taiwan were 62 and 3.3 per 100,000, respectively.[4] The incidence of TB is still very high in Southern Taiwan. Generally, successful control of tuberculosis depends on a high index of suspicion for rapid detection of *M. tuberculosis* to allow early treatment and proper isolation of patients, thereby decreasing the likelihood of dissemination.[5] The conventional method for laboratory diagnosis of tuberculosis is based on acid-fast staining and culture by liquid and solid media. However, the staining lacks sensitivity (<50%), whereas culture results are usually not available in the first 2 to 3 weeks.[6-8] Various nucleic acid amplification assays have been developed for the direct detection and identification of *M. tuberculosis* in clinical samples, and the results are usually obtained within hours of sample collection.[9-11] The purpose of this study was to evaluate the performance of AsiaGen MTB detection assay (AsiaGen Corporation, Taiwan, ROC) compared with that of CobasAmplicor Mycobacterium system (Roche Diagnostic Systems, Inc., Branchburg, N.J.) for rapid detection of *M. tuberculosis* in clinical respiratory specimens.

Materials and Methods

Specimen collection and processing

A total of 232 sputum specimens including 121 smear-positive and 111 smear-negative on acid-fast stain from the patients with symptoms and/or signs suggesting pulmonary TB were collected from 2006 to Dec 2008 at E-Da hospital.

All specimens were processed and treated as previously described.[21] Briefly, each specimen was processed by adding an equal volume of NaOH-citrate-N-acetly-L-cysteine at room temperature for 15 min. Sterile phosphate buffer saline (PBS, pH 7.4) was added to the mixture to reach a final volume of 50 ml before being centrifuged for 15 min at 3,000 g. After centrifugation, the supernatant was discarded and the sediment was resuspended with PBS to achieve a final volume of 1-3 ml for preparing smears, inoculating culture media, and performing PCR assays.

Smear and culture

Smears of the processed specimens for acid-fast bacilli were stained with auramine-rhodamine fluorochrome and examined by standard procedures.[22] Fluorochrome stain-positive smears were confirmed by the Kinyoun stain method.[22] Cultures were performed by inoculating 0.5 ml portion of the sediment in the MGIT 960 system (Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD.). Vials were incubated in the appropriate incubator at 37°C for 8 weeks. O_2 consumption from growth of bacteria provokes a change in the color of the vial bottom, and alarms laboratory personnel with a sound signal.

AsiaGen MTB detection assay

This technique uses a nested PCR technique, which can improve the sensitivity of detection of MTB and decrease false-positive events. Extraction of DNA was started by resuspending pellet in 150 μl of lysis buffer I (KOH, pH 13.1) at room temperature for 10 min and at 10°C water bath for another 20 min. Then, 140 μl of lysis buffer II (HCl and acetic acid, pH 1.2) was added. After centri-

fuging at 10,000 g for 2 min, a 10 µl volume of supernatant was transferred to an amplification tube containing 50 µl of amplification reagent (Tris-HCl, $MgCl_2$, dATP, dGTP, dTTP, dCTP, external primer, and AmpliTaq DNA polymerase) for nested PCR. The primers for nested PCR were derived from the *M. tuberculosis* genome, encoding the insertion sequence IS*6110* with the sequence of the external primers: 5-GTGAGGGCATCGAGGTGG-3 and 5-CGTAGGCGTCGGTCACAAA-3 and internal primers: 5-GATGCACCGTCGAACG-3 and 5-biotin-CCACGGTAGGCGAACCCT-3. For each assay, one negative control containing the PCR mixture without the template DNA was prepared. In a hybridization tube, 10 µl of each amplified DNA sample and 290 µl of hybridization reagent, containing 15 µl of MagProbe (beads with probe; 5-amine-ACCTAACCGGCTGTGGGTAGCAGA) and 150 µl of hybridization buffer, was added, vortexed, and incubated at 95°C for 5 min and at 60°C for 20 min in a dry bath. Tubes were then transferred to magnetic wells for 5 min. The hybridization buffer was removed by aspiration without disturbing the Mag-Probe. After complete washing, blocking solution and streptavidin-horseradish peroxidase were added, vortexed, and kept from light at room temperature for 20 min. After washing twice and resuspending with PBS, the tube was placed in a luminometer to determine the number of relative light units (RLU) produced by the reaction. When the RLU of the corresponding control was less than 25,000, the sample was considered positive for *M. tuberculosis* complex if the sample's RLU was equal to or greater than 100,000; and negative if the sample's RLU was less than 25,000. If the sample's RLU was between these values, the sample was retested to verify the results. The sample was considered positive if the retest RLU value was equal to or greater than 25,000 and negative if it was less than 25,000.

Cobas Amplicor MTB test

This system is capable of semi-automatically performing rapid detection of MTB which uses an internal amplification control designed to detect the presence of inhibiting substances. The procedure was performed according to the manufacturer's instructions, starting from a 100 µl sediment sample portion. It is a two-step preparation process, combining fully automated amplification and detection. The internal control DNA sequence contained primer-binding regions identical to those of the MTB target sequence. A unique probe-binding region differentiated the internal control from the target amplicon. A colorimetric reading exhibiting absorbance values greater than 0.35 optical density units was considered positive. Since no equivocal optical density range was defined, all readings of ≤ 0.35 were considered to be negative results, irrespective of observed inhibition. The test results were considered to be correct if the A450 values of the positive and negative controls were ≥ 2.0 and ≤ 0.25, respectively. If these criteria were not met, the entire amplification was repeated.

Results

Comparison of AsiaGen MTB detection assay and Cobas Amplicor Mycobacterium system

A total of 110 specimens including 44 culture-positive and 66 culture-negative for *M. tuberculosis* were included in this portion of comparison study. Of the 44 culture-positive specimens, only 23 were smear-positive on acid-fast stain. As shown in Table 1, the cumulative difference for all MTB-positive specimens (40 by AsiaGen MTB detection assay and 34 by Cobas Amplicor Mycobacterium system) was not significant (P=0.08). All 23 samples that were smear-positive and culture-positive for tuberculosis complex were AsiaGen MTB detection assay-positive but only 19 were Cobas Amplicor Mycobacterium system-posi-

Table 1. Correlation between two amplification assays for detection of M. tuberculosis and culture results among 110 respiratory specimens

Assay	AFS	No. of samples Culture(+) n=44 PCR(+)	PCR(-)	Culture(-) n=66 PCR(+)	PCR(-)	Sensitivity(%)	Sepcificity(%)	Predictive Value(%) Positive	Negative
Asia Gene	Positive(25)*	23	0	0	2#	100	100	100	100
	Negative(85)*	17	4	5	59	80.9	92.1	77.3	93.7
	Overall(110)*	40	4	5	61	90.9	92.4	88.6	93.8
Cobas	Positive(25)*	19	4	0	2#	82.6	100	100	33.3
Amplicor	Negative(85)*	15	6	0	64	71.4	100	100	91.4
	Overall(110)*	34	10	0	66	77.2	100	100	85.7

*No of samples #Non-Tuberculosis mycobacterium

tive. Of the 21 samples that were smear-negative but culture-positive for MTB, 17 (80.9%) were AsiaGen MTB detection assay-positive and 15 (71.4%) were Cobas Amplicor Mycobacterium system-positive. However, among 66 samples with negative acid fast stain and culture, five of these samples were detected to be positive by AsiaGen MTB detection assay. All these 5 cases were previously diagnosed to have pulmonary tuberculosis and were receiving anti-TB treatment.

Table 1 shows comparison of these two rapid amplification assays for detection of *M. tuberculosis* in respiratory specimens in reference to the culture results. The AsiaGen MTB detection assay detected 40 of the 44 samples that were culture-positive for *M. tuberculosis* (sensitivity, 90.9%). Among the 66 culture-negative samples, five was positive by AsiaGen MTB detection assay (specificity, 92.4%). Positive and negative predictive values were 88.6% and 93.8%, respectively. The Cobas Amplicor Mycobacterium system detected 34 of the 44 samples that were culture-positive for MTB (sensitivity, 77.2%), and it identified none of the 66 culture-negative samples as positive (specificity, 100%). Positive and negative predictive values for the Cobas Amplicor Mycobacterium system were 100% and 85.7%, respectively.

Table 2 shows the correlation between the results obtained with the AsiaGen MTB detection assay and the Cobas Amplicor Mycobacterium system for the detection of MTB. Agreement was 77.3% (34 of 44 samples) and 92.3% (65 of 66 samples) in MTB-positive and MTB-negative samples, respectively. There were 4 specimens of MTB culture-positive specimens not detected by both commercial kits, 6 specimens were detected by AsiaGen MTB detection assay, but not by Cobas Amplicor Mycobacterium system. Five specimens among the 66 MTB culture-negative specimens were demonstrated to be positive by AsiaGen MTB detection assay. Sixty-one specimens were shown to be negative by both assay kits.

Table 2. Agreement between the results obtained with AsiaGen MTB detection assay and Cobas Amplicor Mycobacterium system in attempts to detect MTB in respiratory samples

		AsiaGen Nested-PCR Positive	Negative
Cobas Amplicor-PCR	Positive	34(19)[a]	0(0)
	Negative	11(4)	65(2)

[a] Number in parentheses indicates the number of smear-positive samples

Correlation between AsiaGen MTB detection assay and culture results

Of the 121 cases with positive acid fast staining (AFS), 117 (96.7%) were positive for culture by MGIT 960 system, 98 (81.0%) were confirmed to be MTB by molecular method, and only 95 (96.9%) were positive by AsiaGen

Table 3. Correlation of AsiaGen MTB assay for MTB detection and culture results among 232 respiratory specimens

AsiaGen	Culture (+); n=178				Culture(-) n=54		Sensitivity (%)	Specificity (%)	Predictive value (%)	
	MTB; n=148		NTM; n=30						Positive	Negative
	+	−	+	−	+	−				
AFS(Positive;n=121)	95	3	0	19	3	1	96.9	25	96.9	86.9
AFS(Negative; n=111)	9	41	0	11	8	42	18	84	52.9	56.4
Overall; n=232	104	44	0	30	11	43	70.2	79.6	90.4	62.4

MTB: *Mycobacteruium tuberculosis complex* ; NTM: Non-*Tuberculosis mycobacterium*

MTB detection assay. Of the 111 cases with negative AFS smears, 50 (45.0%) showed positive culture for MTB, and only 9 (18.0%) were positive by AsiaGen MTB detection assay.

As shown in Table 3, the cumulative difference for all MTB-positive specimens (98 positive by AFS methods and 104 positive by AsiaGen MTB detection assay) was not significant (P=0.454). Based on culture and molecular methods for detecting MTB, the overall sensitivity, specificity, and positive and negative prediction values of AsiaGen MTB detection assay were 70.2%, 79.6%, 90.4%, and 62.4%, respectively. However, the values in AFS positive detection were 96.5%, 25.0%, 96.9%, and 86.9%, respectively. The AsiaGen MTB detection assay was significantly more sensitive for AFS-positive than for AFS-negative specimens (96.9% vs. 18.0%; $p<0.001$). The AsiaGen MTB detection assay was highly specific for MTB detection, since all non-Tuberculosis mycobacterium (NTM) specimens were negative (Table 3).

Discussion

It is important to diagnose TB as early as possible due to high incidence of spreading of *M. tuberculosis* via person-to-person contact. Since conventional methods, including acid-fast staining and culture, are insensitive and time-consuming, new developments through application of molecular biology techniques[12,13] for rapid diagnosis of *M. tuberculosis* are of great interest in laboratory medicine.[14-16] Recently, the AsiaGen Corporation in Taiwan developed a new assay for detection of *M. tuberculosis* complex, AsiaGen MTB detection assay. This technique uses a nested PCR technique, which can improve the sensitivity of detection of *M. tuberculosis* complex.[17,18] With this technique, the false-positive events can be decreased since the probability of a wrong locus being mistakenly amplified twice is very low. In addition, the overall time for processing this assay requires only 5 hours. The assay significantly reduces manipulation time as compared with conventional culture method.

In the first part of our study, the two commercial kits for MTB detection in respiratory specimens were compared. The AsiaGen MTB detection assay is entirely manual, whereas the Cobas Amplicor Mycobacterium system is semi-automated. Our results demonstrated that MTB in clinical samples can be rapidly detected by both of these amplification assays within a few hours. Both kits including all of the reagents needed for sample amplification and detection are easy to prepare and the assays are easy to perform. The differences of the results from cutoff values, values in controls, and values in samples were broad enough to allow easy discrimination in both assays. We demonstrated that the AsiaGen MTB detection assay has a diagnostic value similar to that of the Cobas Amplicor Mycobacterium system for direct detection of MTB in clinical specimens. Since it is critical to identify all cases of active TB to interrupt the dissemination and transmission of the organ-

ism in the case of respiratory tract disease, the second part of our study aimed at evaluating the sensitivity and specificity of the AsiaGen MTB detection assay based on the results of acid-fast stain and MGIT liquid culture methods. In comparison with culture results of MGIT 960, three false-positive and three false-negative results were observed in AFS-positive samples with AsiaGen MTB detection assays (Table 3). These 3 samples of false-positivity were demonstrated in patients with a previous diagnosis of pulmonary tuberculosis receiving anti-TB treatment. The dead mycobacteria in the specimens would have contributed to the positive results from the AsiaGen MTB assay. The false negative PCR results from 3 specimens that were both culture- and staining-positive might be explained by the presence of inhibitors of enzymatic amplification and/or an unequal distribution in the test samples. For respiratory specimens, the sensitivity and specificity values of AsiaGen MTB assay were 70.2 and 79.6%, respectively, and were lower compared to those reported by Wang et al.,[19] 96.7 and 95.2%. In our study, for the smear-positive specimens, the sensitivity was 96.9% and the specificity was only 25.0%. For the smear-negative respiratory specimens, the sensitivity was 18.0%; only 17 specimen from 111 smear-negative specimens, including 50 culture-positive for MTB and 50 culture-negative for MTB, showed positive AsiaGen MTB results, the data indicate that the sensitivity of AsiaGen MTB detection assay is not high enough for smear negative specimens. The problem of smear-negative pulmonary tuberculosis is worth particular attention, because these patients have been reported to be responsible for about 17% of tuberculosis transmission.[20,21] Other reasons for a loss of sensitivity, besides PCR inhibitor, could be technical errors (e.g., accidental aspiration of pellet when removing supernatant after centrifugation), the presence of a low number of mycobacteria, especially in specimens from patients undergoing treatment, and/or an unequal distribution of bacilli in the test suspension.[22-26] It is well known that MTB have a tendency to form clumps and cords. Moreover, it must be mentioned that all 41 false-negative PCR, culture-positive specimens were recovered from the MGIT after a period of more than 20 days of incubation, which indicates the presence of a small number of mycobacteria in the clinical specimen examined. However, with the implementation of nucleic acid-based test systems, the ability to detect MTB in most samples within a few hours rather than in 2 to 6 weeks seems to be a considerable advance from the practical point of view. Samples that are negative by the direct test may still prove positive by culture, and a negative test result should not be interpreted as ruling out TB. Therefore, increased sensitivity for AsiaGen MTB would be desirable.

In summary, the resurgence of tuberculosis has highlighted the urgent need for sensitive, correct, and fast methods for the laboratory detection of MTB. Our results indicate that the diagnostic value of AsiaGen MTB detection assay is comparable to that of Cobas Amplicor Mycobacterium system for respiratory specimens. We conclude that the AsiaGen MTB detection assay is a rapid and easy-to-perform method for clinical laboratories to detect MTB infection immediately in AFS-positive as well as AFS-negative respiratory specimens and is a useful tool for improving patient management.

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References

1. Shi W, Feng J, Zhang M, et al: Biosynthesis of isoprenoids: characterization of a functionally active recombinant 2-C-methyl-D-erythritol 4-phosphate cytidyltransferase (IspD) from Mycobacterium tuberculosis H37Rv. J Biochem Mol Biol 2007;40:911-20.
2. Ducati RG, Ruffino-Netto A, Basso LA, ea at: The resumption of consumption -A review on tuberculosis. Mem Inst Oswaldo Cruz 2006;101:697-714.
3. Cole ST, Brosch R, Parkhill J, et al: Deciphering the biology of Mycobacterium tuberculosis from the complete genome sequence. Nature 1998;393:537-44.
4. Center for Disease Control, Department of Health, Executive Yuan, Taiwan. Tuberculosis annual report (Taiwan);2009. Available at: http://www.cdc.gov.tw.
5. Wang JY, Lee LN, Lai HC, et al: Performance assessment of the Capilia TB assay and the BD ProbeTec ET system for rapid culture confirmation of Mycobacterium tuberculosis. Diagn Microbiol Infect Dis 2007;59:395-9.
6. Boyd JC, Marr JJ: Decreasing reliability of acid-fast smear techniques for detection of tuberculosis. Ann Intern Med 1975;82:489-92.
7. Murray PR, Elmore C, Krogstad DJ: The acid-fast stain: a specific and predictive test for mycobacterial disease. Ann Intern Med 1980;92:512-3.
8. Pfyffer GE, Brown-Elliott BA, Wallace RJJ: Mycobacterium In Manual of Clinical Microbiology. 6th ed. Washington, DC: American Society for Microbiology, 2005;532-559.
9. Piersimoni C, Scarparo C, Piccoli P, et al: Performance assessment of two commercial amplification assays for direct detection of Mycobacterium tuberculosis complex from respiratory and extrapulmonary specimens. J Clin Microbiol 2002;40:4138-42.
10. Huang TS, Huang WK, Lee SS, et al: Rapid detection of pulmonary tuberculosis using the BDProbeTEC ET Mycobacterium tuberculosis Complex Direct Detection Assay (DTB). Diagn Microbiol Infect Dis 2003;46:29-33.
11. Piersimoni C, Scarparo C: Relevance of commercial amplification methods for direct detection of Mycobacterium tuberculosis complex in clinical samples. J Clin Microbiol 2003;41:5355-65.
12. Hale YM, Pfyffer GE, Salfinger M: Laboratory diagnosis of mycobacterial infections: new tools and lessons learned. Clin Infect Dis 2001;33:834-46.
13. Soini H, Musser JM: Molecular diagnosis of mycobacteria. Clin Chem 2001;47:809-14.
14. Brown TJ, Power EG, French GL: Evaluation of three commercial detection systems for Mycobacterium tuberculosis where clinical diagnosis is difficult. J Clin Pathol 1999;52:193-7.
15. Dalovisio JR, Montenegro-James S, Kemmerly SA, et al: Comparison of the amplified Mycobacterium tuberculosis (MTB) direct test, Amplicor MTB PCR, and IS6110-PCR for detection of MTB in respiratory specimens. Clin Infect Dis 1996;23:1099-106; discussion 1107-8.
16. Reischl U, Lehn N, Wolf H, et al: Clinical evaluation of the automated COBAS AMPLICOR MTB assay for testing respiratory and nonrespiratory specimens. J Clin Microbiol 1998;36:2853-60.
17. Yuen KY, Yam WC, Wong LP, et al: Comparison of two automated DNA amplification systems with a manual one-tube nested PCR assay for diagnosis of pulmonary tuberculosis. J Clin Microbiol 1997;35:1385-9.
18. Montenegro SH, Gilman RH, Sheen P, et al: Improved detection of Mycobacterium tuberculosis in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. Clin Infect Dis 2003;36:16-23.
19. Wang JY, Lee LN, Chou CS, et al: Performance assessment of a nested-PCR assay (the RAPID BAP-MTB) and the BD ProbeTec ET system for detection of Mycobacterium tuberculosis in clinical specimens. J Clin Microbiol 2004;42:4599-603.
20. Behr MA, Warren SA, Salamon H, et al: Transmission of Mycobacterium tuberculosis from patients smear-negative for acid-fast bacilli. Lancet 1999;353:444-9.
21. Hernandez-Garduno E, Cook V, Kunimoto D, et al: Transmission of tuberculosis from smear negative patients: a molecular epidemiology study. Thorax 2004;59:286-90.
22. Centers for Disease Control and Prevention. Update: Nucleic acid amplification tests for tuberculosis. MMWR Morb Mortal Wkly Rep 2000;49:593-4.
23. Bogard M, Vincelette J, Antinozzi R, et al: Multicenter study of a commercial, automated polymerase chain reaction system for the rapid detection of Mycobacterium tuberculosis in respiratory specimens in routine clinical practice. Eur J Clin Microbiol Infect Dis 2001;20:724-31.
24. Clarridge JE, 3rd, Shawar RM, Shinnick TM, et al: Large-scale use of polymerase chain reaction for detection of Mycobacterium tuberculosis in a routine mycobacteriology laboratory. J Clin Microbiol 1993;31:2049-56.
25. Levidiotou S, Vrioni G, Galanakis E, et al: Four-year experience of use of the Cobas Amplicor system for rapid detection of Mycobacterium tuberculosis complex in respiratory and nonrespiratory specimens in Greece. Eur J Clin Microbiol Infect Dis 2003;22:349-56.
26. Oh EJ, Park YJ, Chang CL, et al: Improved detection and differentiation of mycobacteria with combination of Mycobacterium Growth Indicator Tube and Roche COBAS AMPLICOR System in conjunction with Duplex PCR. J Microbiol Methods 2001;46:29-36.