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Comparison of the Real-Time PCR Method and the Gen-Probe Amplified *Mycobacterium tuberculosis* Direct Test for Detection of *Mycobacterium tuberculosis* in Pulmonary and Nonpulmonary Specimens

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Real-time PCR was compared to Amplified *Mycobacterium tuberculosis* Direct Test (AMTDII) for 100 clinical specimens. The overall sensitivities of the real-time PCR method and AMTDII were similar for respiratory and nonrespiratory specimens. However, real-time PCR seemed to be less susceptible to amplification inhibitors than AMTDII.

During the last decade, a number of nucleic acid amplification (NAA) methods have been developed for rapid detection and identification of *Mycobacterium tuberculosis* complex (MTB) in clinical specimens (3, 5, 9, 10). These techniques are attractive due to the direct detection of few copies of genomic sequence of MTB in clinical specimens. However, all NAA methods are not as sensitive as culture, especially for the diagnosis of smear-negative tuberculosis (TB), and false-positive results consecutive to cross-contamination have been reported (11). Recently, real-time PCR methods have been proposed for the detection of a variety of microorganisms, including mycobacteria (1, 2). The present study evaluated real-time PCR in comparison to the Amplified *Mycobacterium tuberculosis* Direct Test (AMTDII) (Gen-Probe, San Diego, Calif.) in the setting of a routine university hospital laboratory. For this purpose, real-time PCR (ABI Prism SDS 7000; Applied Biosystems, Foster City, Calif.) and the AMTDII, as well as conventional identification methods, were performed on 100 clinical specimens collected from 93 patients hospitalized at the Lille University Medical Center from January to December of 2003.

After decontamination with *N*-acetyl-L-cysteine–2% NaOH (7) and centrifugation (3,000 × *g* for 20 min at 4°C), the sediments of the pulmonary specimens were stained with auramine-rhodamine fluorochrome for fluorescent microscopy and inoculated into both a Löwenstein-Jensen medium and an antibiotic-supplemented BacT/Alert MP culture bottle (bioMérieux, Marcy l'Etoile, France). Nonpulmonary specimens from normally sterile sites were cultured without prior decontamination. After inoculation, the remainder of the specimen was aliquoted into two portions (one for AMTDII and the other for the real-time PCR) and frozen until the amplification. NAA tests were applied in parallel, on the frozen aliquots from the same specimen, the same day, in a blind manner by one laboratory technician, independently of the smear and the culture results.

The AMTDII was carried out according to the manufacturer's instructions. Briefly, 450 μl of specimens was placed in lysing solution, and then 25 μl of lysate was used for amplification. All specimens were tested in duplicate. One of them was spiked with a known quantity of *M. tuberculosis* to detect inhibiting substances. All AMTDII series included one negative and one positive amplification control. The run was validated when the negative and positive controls were <20,000 and >1,000,000 relative light units (RLUs), respectively. For real-time PCR assay, whenever possible, approximately 500 μl of sample (minimal volume of 100 μl) was used for DNA extraction with the MagNA Pure LC DNA isolation kit III (Roche Molecular Biochemicals, Mannheim, Germany) as recommended by the manufacturer. The primers and the fluorogenic probe for the IS*6110* sequence (GenBank accession no. X52471) were designed with the Primer Express software, version 2.0 (Applied Biosystems), and were obtained from Applied Biosystems (Warrington, United Kingdom). The nucleotide sequences of the forward and the reverse primers were 5′-CCGAGGCAGGCATCCA-3′ (positions 1062 to 1077) and 5′-GATCGTCTCGGCTAGTGCATT-3′ (positions 1112 to 1132). The sequence of the minor groove binder probe was 6-carboxyfluorescein–5′-TCGGAAGCTCCTATGAC-3′ (positions 1095 to 1111). PCR amplifications were performed in 25-μl reaction volumes including 1× TaqMan Universal Master Mix (Applied Biosystems) containing dUTP and uracil-*N*-glycosilase, each primer at a final concentration of 100 nM, the fluorescent-labeled probe at a final concentration of 300 nM, and 5 μl of extracted DNA. Each run included positive and negative controls, the former being prepared from 10^{-2}, 10^{-4}, and 10^{-6} dilutions of 45-μg/ml DNA from *M. tuberculosis* strain H37Rv, and the latter consisted of sterile water. Quality control was acceptable when the negative control had an undetectable cycle threshold (CT) and the 10^{-2}, 10^{-4}, and 10^{-6} dilutions of H37Rv DNA had CT values between 17 and 19, 25

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TABLE 1. Comparison of AMTDII and real-time PCR for detection of TB in clinical specimens

Direct microscopy and culture findings (*n*)[a]	Number of specimens positive with:		Number of specimens inconclusive with[c]:	
	AMTDII	Real-time PCR	AMTDII	Real-time PCR
Respiratory specimens				
AFB+/C+ (13)	13	13	0	0
AFB−/C+ (8)	3	6	1	0
AFB−/C− (21)	1[b]	0	0	0
Nonrespiratory specimens				
AFB+/C+ (7)	6	7	1	0
AFB+/C− (4)	2	4	1	0
AFB−/C+ (13)	6	8	2	0
AFB−/C− (34)	2[b]	0	4	0

[a] AFB, acid-fast bacilli; C, culture. Positive and negative findings are indicated.
[b] Specimens yielding RLUs of >200,000 and ≤500,000 in the equivocal zone.
[c] Inconclusive due to inhibitors.

and 27, and 33 and 35, respectively. During the test period, the 10^{-2}, 10^{-4}, and 10^{-6} dilutions of H37Rv DNA had CT values of 18.6 ± 0.6, 25.7 ± 0.25, and 34.3 ± 0.21, respectively. Each sample was tested in duplicate, and an exogenous internal positive control (Applied Biosystems) was added to the reaction chamber in each duplicate and coamplified for detecting endogenous PCR inhibitors. Standard amplification parameters were as follows: 50°C for 2 min and 95°C for 10 min, followed by 45 cycles of 95°C for 15 s and 60°C for 1 min. Real-time data were analyzed with SDS software, version 1.0.

Clinical and radiological data were collected from 93 patients by review of their clinical records. A case of TB was defined as a patient with either specimens positive for MTB in culture or specimens negative for MTB in culture if the following criteria were present: (i) the specimen was isolated from a patient treated by antituberculous drugs, (ii) the specimen was isolated from a patient whose other specimens were culture positive, and (iii) the patient's clinical history provided evidence of TB sufficient to initiate antituberculous therapy.

Among the 100 specimens analyzed, 45 and 55 were isolated from 40 TB and 53 non-TB patients, respectively. Out of the 45 clinical specimens isolated from TB patients, 21 and 24 were pulmonary and nonpulmonary specimens, respectively. The pulmonary specimens consisted of sputum ($n = 17$), bronchial aspirate ($n = 3$), and bronchoalveolar lavage fluid ($n = 1$). The nonpulmonary specimens included lymph node ($n = 8$), tissues ($n = 4$), urine ($n = 3$), synovial fluid ($n = 3$), abscess ($n = 3$), and pleural fluid ($n = 3$). Amplification results with smears and cultures data are summarized in Table 1. Among the 21 pulmonary specimens isolated from TB patients, 16 (76%) and 19 (90%) were AMTDII and real-time PCR positive, respectively. The number of positive results was lower among the 24 nonrespiratory specimens with both methods. Indeed, 14 (58%) were found positive with AMTDII and 19 (79%) with real-time PCR. Nine (9%) specimens (four lymph nodes, three sterile fluids, one abscess, and one bronchial aspirate), of which five were from four different TB patients, could not be interpreted with AMTDII due to the presence of inhibitors of amplification. Complete inhibition of the AMTDII with inhibiting substances was previously reported, particularly in nonpulmonary specimens (3). Therefore, it was suggested that all specimens be decontaminated, even those from sterile sites, to reduce proteins and enzymes which likely inhibit the amplification reactions. However, the sample manipulations during decontamination may cause sampling errors such as (i) inappropriate specimen dilution, (ii) accidental aspiration of the pellet when removing supernatant, and (iii) cross-contamination. In addition, decontamination methods have been reported to cause a large reduction (80%) of CFU recovered in cultures (13). This may increase the likelihood of culture-negative results among paucibacillary specimens. In contrast, no inhibitor was detected with the real-time PCR method. Likely, the method of DNA extraction used for real-time PCR as well as the smaller volume amplified (5 versus 25 μl) may reduce inhibitors better than AMTDII. Finally, out the 40 TB patients, 29 (73%) and 37 (93%) were conclusively detected as positive with AMTDII and real-time PCR, respectively. Among the 55 non-MTB specimens, two cerebrospinal fluids and one bronchial aspirate yielded equivocal results (RLUs ranging from 200,000 to 500,000) with AMTDII. Some authors have stated that these samples should be retested (6, 12). Unfortunately, no residual specimens were available for retesting, and AMTDII could not be done on additional specimens because patients were discharged from the hospital with favorable clinical outcome in the absence of antituberculous treatment. In contrast, no equivocal results were observed with the real-time PCR method. Although the specimen processing for smear decontamination and culture was done in a separated area with distinct equipment for NAA testing, it is possible that the equivocal results were due to cross-contamination from a positive specimen in an adjacent test position. Indeed, the AMTDII required multiple user-dependent steps for manipulations that have the potential for cross-contamination. In contrast, the sample preparation for real-time PCR is automatically performed on the MagNA Pure and the SDS 7000 systems, thus limiting the carryover contamination.

Finally, in excluding noninterpretable specimens (false-negative inhibitory and false-positive equivocal specimens), the sensitivity, specificity, and positive and negative predictive values of real-time PCR obtained with respiratory and nonrespiratory specimens were nearly similar to those of AMTDII (Table 2). Indeed, the sensitivity, specificity, and negative and positive predictive values for AMTDII were 80, 100, 92, and 100% for respiratory specimens and 70, 100, 89, and 100% for nonpulmonary specimens, respectively. The corresponding values for real-time PCR were 90, 100, 96, and 100% for respiratory specimens and 80, 100, 92, and 100% for nonrespiratory specimens, respectively. The slight differences observed between these two methods were not statistically significant (paired χ^2 test). As expected, a reduced sensitivity was seen among smear-negative specimens with both methods. However, the reduced sensitivity appeared to be less marked with real-time PCR (67% with real-time PCR versus 50% with AMTDII), but it was also not statistically significant. A low sensitivity of NAA testing on the smear-negative specimens is common, especially because few bacilli are present in these type of specimens (4, 5, 8, 9).

In conclusion, real-time PCR appeared to be as sensitive and specific as the AMTDII and less susceptible to amplification inhibitors in the absence of a systematic decontamination pro-

TABLE 2. Sensitivity, specificity, and negative and positive predictive values of AMTDII and real-time PCR[a]

Specimen	Sensitivity (%)		Specificity (%)		NPV (%)		PPV (%)	
	AMTDII	Real-time PCR	AMTDII	Real-time PCR	AMTDII	Real-time PCR	AMTDII	Real-time PCR
Respiratory	80	90	100	100	92	96	100	100
Nonrespiratory	70	80	100	100	89	92	100	100
AFB positive	95	100	100	100	98	100	100	100
AFB negative	50	67	100	100	84	89	100	100
All	75	85	100	100	83	89	100	100

[a] AFB, acid-fast bacilli; NPV, negative predictive value; PPV, positive predictive value.

cedure of specimens from sterile sites. However, a further study including more specimens is required to determine if significant differences in sensitivity and specificity might be observed between these two methods.

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REFERENCES

1. **Cleary, T. J., G. Roudel, O. Casillas, and N. Miller.** 2003. Rapid and specific detection of *Mycobacterium tuberculosis* by using the Smart Cycler instrument and a specific fluorogenic probe. J. Clin. Microbiol. **41:**4783–4786.
2. **Desjardin, L. E., Y. Chen, M. D. Perkins, L. Teixeira, M. D. Cave, and K. D. Eisenach.** 1998. Comparison of the ABI 7700 system (TaqMan) and competitive PCR for quantification of IS*6110* DNA in sputum during treatment of tuberculosis. J. Clin. Microbiol. **36:**1964–1968.
3. **Gamboa, F., G. Fernandez, E. Padilla, J. M. Manterola, J. Lonca, P. J. Cardona, L. Matas, and V. Ausina.** 1998. Comparative evaluation of initial and new versions of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens. J. Clin. Microbiol. **36:**684–689.
4. **Johansen, I. S., V. O. Thomsen, A. Johansen, P. Andersen, and B. Lundgren.** 2002. Evaluation of a new commercial assay for diagnosis of pulmonary and nonpulmonary tuberculosis. Eur. J. Clin. Microbiol. Infect. Dis. **21:**455–460.
5. **Jouveshomme, S., E. Cambau, D. Trystram, M. Szpytma, W. Sougakoff, J. P. Derenne, and J. Grosset.** 1998. Clinical utility of an amplification test based on ligase chain reaction in pulmonary tuberculosis. Am. J. Respir. Crit. Care Med. **158:**1096–1101.
6. **Kerleguer, A., J. L. Koeck, M. Fabre, P. Gerome, R. Teyssou, and V. Herve.** 2003. Use of equivocal zone in interpretation of results of the Amplified Mycobacterium Tuberculosis Direct test for diagnosis of tuberculosis. J. Clin. Microbiol. **41:**1783–1784.
7. **Metchock, B. G., F. S. Nolte, and R. J. Wallace, Jr.** 1999. *Mycobacterium*, p. 399–437. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 7th ed. ASM Press, Washington, D.C.
8. **O'Sullivan, C. E., D. R. Miller, P. S. Schneider, and G. D. Roberts.** 2002. Evaluation of Gen-Probe Amplified Mycobacterium Tuberculosis Direct test by using respiratory and nonrespiratory specimens in a tertiary care center laboratory. J. Clin. Microbiol. **40:**1723–1727.
9. **Piersimoni, C., A. Callegaro, C. Scarparo, V. Penati, D. Nista, S. Bornigia, C. Lacchini, M. Scagnelli, G. Santini, and G. De Sio.** 1998. Comparative evaluation of the new Gen-Probe *Mycobacterium tuberculosis* Amplified Direct Test and the semiautomated Abbott LCx *Mycobacterium tuberculosis* assay for direct detection of *Mycobacterium tuberculosis* complex in respiratory and extrapulmonary specimens. J. Clin. Microbiol. **36:**3601–3604.
10. **Piersimoni, C., C. Scarparo, P. Piccoli, A. Rigon, G. Ruggiero, D. Nista, and S. Bornigia.** 2002. Performance assessment of two commercial amplification assays for direct detection of *Mycobacterium tuberculosis* complex from respiratory and extrapulmonary specimens. J. Clin. Microbiol. **40:**4138–4142.
11. **Sarmiento, O. L., K. A. Weigle, J. Alexander, D. J. Weber, and W. C. Miller.** 2003. Assessment by meta-analysis of PCR for diagnosis of smear-negative pulmonary tuberculosis. J. Clin. Microbiol. **41:**3233–3240.
12. **Woods, G. L., J. S. Bergmann, and N. Williams-Bouyer.** 2001. Clinical evaluation of the Gen-Probe Amplified Mycobacterium Tuberculosis Direct Test for rapid detection of *Mycobacterium tuberculosis* in select nonrespiratory specimens. J. Clin. Microbiol. **39:**747–749.
13. **Yajko, D. M., C. Wagner, V. J. Tevere, T. Kocagoz, W. K. Hadley, and H. F. Chambers.** 1995. Quantitative culture of *Mycobacterium tuberculosis* from clinical sputum specimens and dilution endpoint of its detection by the Amplicor PCR assay. J. Clin. Microbiol. **33:**1944–1947.