JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2006, p. 3025–3027 Vol. 44, No. 8
0095-1137/06/$08.00+0 doi:10.1128/JCM.00068-06
Copyright © 2006, American Society for Microbiology. All Rights Reserved.

Evaluation of the GenoType Mycobacteria Direct Assay for Detection of *Mycobacterium tuberculosis* Complex and Four Atypical Mycobacterial Species in Clinical Samples

F. Franco-Álvarez de Luna, P. Ruiz, J. Gutiérrez, and M. Casal*

Microbiology Service, Reina Sofia University Hospital, and Mycobacteria Reference Center, Faculty of Medicine, Cordoba University, Cordoba, Spain

Received 12 January 2006/Returned for modification 7 March 2006/Accepted 2 June 2006

We evaluated GenoType Mycobacteria Direct (GTMD), a novel commercial assay based on nucleic acid sequence-based amplification technology, for the detection of *Mycobacterium tuberculosis* complex, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. malmoense* directly from clinical specimens. A total of 134 respiratory and extrapulmonary samples from 65 patients were processed. Sensitivity, specificity, positive predictive, and negative predictive values for GTMD were 92, 100, 100, and 77%, respectively. The GMTD technique is useful, reliable, and rapid when used during the normal routine of a clinical laboratory.

Worldwide, tuberculosis (TB) is one of the major causes of death (7). The World Health Organization estimates that, annually, 9 million inhabitants of the world develop tuberculosis and that it is the cause of more than 2 million deaths (12). With the introduction of molecular techniques for the identification of mycobacteria from culture and the application of molecular techniques for direct detection in clinical specimens, technological development in TB diagnosis has experienced a considerable increase over the last few years (4, 9).

The aim of this study was to evaluate a recently commercialized novel genetic assay, namely, GenoType Mycobacteria Direct (GTMD) (Hain Lifescience GMBH, Nehren, Germany), that is capable of detecting *Mycobacterium tuberculosis* complex (MTBC) and four atypical mycobacterium species of clinical interest and to compare the results with those of the COBAS AMPLICOR MTB system (Roche Diagnostics).

The assay was performed by the Microbiology Service of Reina Sofia University Hospital (Cordoba, Spain), together with the Mycobacteria Reference Center (Faculty of Medicine, Cordoba University, Cordoba, Spain). We analyzed 134 samples (100 sputa, 1 bronchoalveolar lavage, 4 bronchial aspirate, 4 pleural fluid, 13 adenopathy biopsy, 2 purulent exudate, 2 abscess, 1 feces, 2 ascitic fluid, 2 cephalorhachidian fluid, and 3 urine samples) from 65 patients suspected of having TB or another type of mycobacteriosis. All samples, except those that came from sterile compartments, were decontaminated by treatment with *N*-acetylcysteine–4% NaOH and concentrated by centrifugation. For microscopic study, the fixed preparations were stained with auramine-rhodamine and visualized with a fluorescence microscope (magnification, ×40). All positive and doubtful samples were processed and examined for the detection of acid-fast bacilli with Ziehl-Neelsen staining. After digestion, decontamination, and concentration, the samples were seeded in MGIT and Lowenstein-Jensen media according to the manufacturer's instructions (Becton Dickinson, Sparks, MD). Two 500-μl aliquots were removed for preparation and processing following the protocols of the molecular techniques evaluated in our study, GenoType Mycobacteria Direct and COBAS AMPLICOR MTB. All isolates grown in different culture media were identified by the GenoType Mycobacterium CM assay (Hain Lifescience GMBH, Nehren, Germany) (11) or by AccuProbe hybridization probes (AccuProbe, Gen-Probe, Inc., San Diego, CA).

GenoType Mycobacteria Direct. This is a novel commercial assay based on the nucleic acid sequence-based amplification (NASBA) and DNA strip techniques, allowing the 23S rRNA amplification-based detection of MTBC, *M. avium*, *M. intracellulare*, *M. kansasii*, and *M. malmoense* directly from decontaminated clinical specimens. This assay is divided in three parts and was performed according to the manufacturer's instructions.

The first part consists of the stabilization and RNA isolation of decontaminated specimens using a capture method (magnetic bead capture probe). The process began with cellular rupture: 300 μl of inhibitory removing buffer was pipetted into a 2-ml tube containing 500 μl of decontaminated specimen. After homogenizing, the mixture was centrifuged for 3 min at 12,000 × *g*. The supernatant was removed, and the pellet was resuspended with 100 μl of RNA capture buffer. The sample was then incubated at 95°C for 30 min, followed by immersion in a preheated 60°C ultrasonic bath for 15 min. Subsequently, 10 μl of magnetic beads was added to a mixture of 90 μl of internal control and the capture probe and 100 μl of the previously obtained cellular lysate in a 0.5-ml tube. After vortexing, a binding reaction was performed in a TwinCubator (Hain Lifescience GMBH, Nehren, Germany) by using a program with two steps of different incubation temperatures. In the first step, the samples were incubated at 65° for 10 min; this was followed by a second incubation step of 17 min at 25°C combined with shaking at a frequency of 1,100 rpm. For washing, the samples were transferred to a magnetic separator. After a dark-colored precipitate formed (approximately 3 min), the supernatant was removed, the magnetic bar of the magnetic

* Corresponding author. Mailing address: Mycobacteria Reference Center, Faculty of Medicine, Cordoba University, 14004 Cordoba, Spain. Phone: 34 957 218284. Fax: 34 957 218229. E-mail: mi1carom@uco.es.

TABLE 1. Results for sensitivity, specificity, PPV, and NPV of the genetic assays used to detect MTBC[a]

Technique and result	No. of samples from[a]:		Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
	Tuberculosis patients (n = 101)	Nontuberculosis patients (n = 27)				
GenoType Mycobacteria Direct						
Positive	93	0	92	100	100	77.1
Negative	8	27				
COBAS AMPLICOR MTB						
Positive	92	0	91	100	100	64.3
Negative	9	27				

[a] The total number of samples was 128.

separator was withdrawn, and the pellet was resuspended in 200 μl of washing buffer. This washing process was performed twice. After the second wash, 20 μl of magnetic beads buffer was added to the pellet, resulting in "loaded magnetic beads."

The second part of the procedure comprised an isothermic amplification of RNA by the NASBA method, in which there is a mixture of primers/nucleotides labeled with biotin and a mixture of enzymes: for each reaction, 30 μl of primers/nucleotides labeled with biotin was added to a 0.5-ml tube and mixed with 10 μl of loaded magnetic beads. The sample was covered with 20 to 30 μl of liquid paraffin oil, and the tubes were transferred to the TwinCubator. The process began with a preliminary denaturation step, in which the temperature of the samples was raised to 65°C for 8 min. Subsequently, the samples were cooled to 41°C and the NASBA reaction was started by adding 10 μl of the previously reconstituted enzyme mixture. The amplification process then commenced for a period of 60 min.

Finally, in the third part of the process, reverse hybridization of the amplified products was performed using an automated system (Profiblot; Tecan, Maennedorf, Switzerland) (2, 11). This machine allows the shaking, heating, and dispensing of the reagents required for the hybridization reaction on membrane strips. Once the strips were completely dry, they were pasted on an evaluation sheet and interpreted using a reading card, both provided by the manufacturer.

PCR COBAS AMPLICOR MTB. PCR COBAS AMPLICOR MTB is a qualitative test for the detection of *M. tuberculosis* complex microorganisms. The test technique was performed following the manufacturer's instructions (3).

A total of 134 samples from 65 patients were processed. Positive mycobacterium cultures were found in 97 samples. The growth of *M. tuberculosis* complex was detected in 91 samples, whereas the other samples were positive for *M. intracellulare* (1 sample), *M. malmoense* (1 sample), *M. kansasii* (2 samples), and *M. avium* (2 samples). The GenoType Mycobacteria Direct assay detected a total of 99 positive responses from different types of samples. The GTMD assay detected 87 specimens with a positive culture and 12 specimens with a negative culture. Compared to results from culture, this indicates a sensitivity and specificity of the GenoType Mycobacteria Direct assay of 89 and 67%, respectively. The COBAS AMPLICOR MTB system that amplifies DNA of only *M. tuberculosis* complex detected 92 positive samples, 11 of which had been negative in mycobacterium cultures. Compared to those from culture, these results indicate a sensitivity and specificity of the COBAS AMPLICOR MTB of 83 and 70%, respectively. These values increased to 89 and 70%, respectively, when samples with only MTBC isolation were considered.

Discrepant results (positive results obtained with GenoType Mycobacteria Direct and COBAS AMPLICOR MTB, but a negative culture) were considered true positive when the patients suspected of having active pulmonary tuberculosis or another type of mycobacteriosis had, after a review of their clinical histories, responded to the antituberculous treatment or exhibited positive mycobacterium cultures in specimens obtained during the study period (1, 8). In this study, 12 samples from 7 patients with negative cultures were positive by at least one of the amplification techniques for MTBC and 11 were positive by both methods. Five of the seven patients in the discrepant panel had other positive cultures for mycobacteria in the study period as well as clinical signs of disease and responded to antituberculous treatment. The other two patients had clear signs of tuberculosis and responded to the antituberculous treatment. Consequently, these 12 samples were considered true positives, increasing the sensitivity and specificity of the GTMD technique to 92 and 100%, respectively, and that of COBAS AMPLICOR MTB to 91 and 100%, respectively, for the samples of patients with MTBC isolations (Table 1). Results from the GenoType Mycobacteria Direct assay were negative for eight specimens with positive culture. Positive microscopy was found in only one of these specimens. These samples were taken from seven patients who were not receiving antituberculous treatment when the specimens were taken. The growing time of these samples in liquid medium was between 6 and 25 days, with a median of 14 days. Both amplification assays were made from the same specimen and at the same time. GenoType Mycobacteria Direct detected all six atypical pathogen mycobacterium isolations collected during this study, corresponding to one *M. intracellulare*, one *M. malmoense*, and 2 isolations each of *M. avium* and *M. kansasii* (Table 2).

The new genetic GenoType Mycobacteria Direct assay is based on NASBA technology described for the first time at the beginning of the 1990s by Kievits et al. (10). Since then, several advances and improvements of this process have been reported (5, 6) that have been used in a multitude of assays for the amplification of viral, bacterial, or parasitic RNA. A characteristic of this technology is its capacity for detecting and amplifying RNA. In our study, GTMD detected samples with a count of less than 10 bacilli per 100 fields in the auramine-rhodamine staining. This new technique includes a laborious

TABLE 2. Mycobacteria detected by GTMD in different clinical samples

Sample type (no. of samples)	GTMD result (no. of samples)
Sputum (91)	*M. tuberculosis* complex (86)
	M. kansasii (2)
	M. malmoense (1)
	M. avium (2)
Biopsy (4)	*M. tuberculosis* complex (3)
	M. intracellulare (1)
Bronchial aspirate (2)	*M. tuberculosis* complex (2)
Abscess (1)	*M. tuberculosis* complex (1)
Feces (1)	*M. tuberculosis* complex (1)

extraction process and requires more manipulation than does the COBAS AMPLICOR MTB system, which is more automated. Both assays take about 5 h to perform. The direct cost of the GenoType Mycobacteria Direct assay is approximately 50 Euros, whereas that of COBAS AMPLICOR MTB is about 30 Euros. The detection of the amplified product with the COBAS AMPLICOR MTB system is performed with spectrophotometry reading, whereas GenoType Mycobacteria Direct uses reverse hybridization technology of the amplified product on membrane strips (2). The visualization of the bands on the strips is more objective and interpretation is quick and easy.

The main difference in favor of the new GenoType Mycobacteria Direct assay is its ability to simultaneously detect five mycobacterium species of clinical interest directly from clinical specimens, thus eliminating the necessity of waiting for the culture results. COBAS AMPLICOR also offers diagnostic assays for the detection of *M. avium* and *M. intracellulare* but not for *M. kansasii* and *M. malmoense*. Both assays harbor an internal amplification control that allows the detection of the presence of inhibitors in the sample. For the GenoType Mycobacteria Direct assay, the absence of the so-called amplification control band on the membrane strip is indicative of the presence of inhibitors in case none of the species-specific bands is visible. In our study, we detected only two samples with inhibitors for the GenoType Mycobacteria Direct assay, whereas we detected three inhibited samples with the COBAS AMPLICOR. In the first test of this novel assay, conducted with patients who were not treated or who received antituberculous treatment for fewer than 7 days, the sensitivity and specificity were 87.5 and 100%, respectively (M. Weizenegger, personal communication). These results are slightly inferior to ours and were presented at the 25th Annual Congress of the European Society of Mycobacteriology in 2004.

In conclusion, the GenoType Mycobacteria Direct is an easy interpretable assay, which is relatively fast, as results can be obtained within one working day. It is very reliable, as we obtained values for sensitivity and specificity of 92 and 100, respectively, and a positive predictive value (PPV) and a negative predictive value (NPV) of 100 and 77%, respectively. Moreover, GTMD has the advantage of being able to detect various species of mycobacterial pathogens in one assay and from the same clinical sample.

REFERENCES

1. **American Thoracic Society.** 2000. Diagnostic standards and classification of tuberculosis in adults and children. Am. J. Respir. Crit. Care Med. **161:**1376–1395.
2. **Boden, D., M. Weizenegger, K. Benz, W. Ponstingl, M. Hengstler, S. Ruschgerdes, A. Fahr, and J. Sartel.** 1998. Reverse hybridization assay for rapid identification of Mycobacteria from culture samples. Clin. Lab. **44:**687–692.
3. **Bodmer, T., A. Gurtner, M. Scholkmann, and L. Matter.** 1997. Evaluation of the COBAS AMPLICOR MTB system. J. Clin. Microbiol. **35:**1604–1605.
4. **Centers for Disease Control and Prevention.** 2000. Nucleic acid amplification tests for tuberculosis. Morb. Mortal. Wkly. Rep. **49:**593–594.
5. **Chan, A. B., and J. D. Fox.** 1999. NASBA and other transcription-based amplification methods for research and diagnostic microbiology. Rev. Med. Microbiol. **10:**185–196.
6. **Deiman, B., P. van Aarle, and P. Sillekens.** 2002. Characteristics and applications of nucleic acid sequence-based amplification (NASBA). Mol. Biotechnol. **20:**163–179.
7. **Dye, C., C. J. Watt, D. M. Bleed, S. M. Hosseini, and M. C. Raviglione.** 2005. Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. Special communication: W.H.O. Stop TB department. JAMA **282:**677–686.
8. **Goessens, W. H. F., P. De Man, J. G. M. Koeleman, A. Luijendijk, R. te Witt, H. P. Endtz, and A. van Belkum.** 2005. Comparison of the COBAS AMPLICOR MTB and BDProbeTec ET assays for detection of mycobacterium tuberculosis in respiratory specimens. J. Clin. Microbiol. **43:**2563–2566.
9. **Ieven, M., and H. Goossens.** 1997. Relevance of nucleic acid amplification techniques for diagnosis of respiratory tract infections in the clinical laboratory. J. Clin. Microbiol. **10:**242–256.
10. **Kievits, T., B. Van Gemen, D. Van Strijp, R. Schukkink, M. Dirks, H. Adriaanse, L. Malek, R. Sooknanan, and P. Lens.** 1991. NASBA isothermal enzymatic in vitro nucleic acid amplification optimized for the diagnosis of HIV-1 infection. J. Virol. Methods **35:**273–286.
11. **Ruiz, P., J. Gutierrez, F. J. Zerolo, and M. Casal.** 2002. Genotype mycobacterium assay for identification of mycobacterial species isolated from human clinical samples by using liquid medium. J. Clin. Microbiol. **40:**3076–3078.
12. **World Health Organization.** 2005. Global tuberculosis control: surveillance, planning, financing. [Online.] http://www.who.int/tb/publications/global_report/2005/en/.