Comparison of the GenoType® MTBC Molecular Genetic Assay with culture methods in the diagnosis of tuberculosis

Muhammet Guzel Kurtoglu[1], Mehmet Ozdemir[2], Recep Kesli[1], Bulent Baysal[2]

[1]Department of Microbiology and Clinical Microbiology, Konya Education and Research Hospital, Konya, Turkey
[2]Department of Microbiology and Clinical Microbiology, Necmettin Erbakan University Meram Faculty of Medicine, Konya, Turkey

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Corresponding author:
Dr. Muhammet Guzel Kurtoglu
Konya Egitim
ve Arastirma Hastanesi
Meram Yeniyol
Meram/Konya, Turkey
Phone: 90 332 3236709
Fax: +90 332 323 67 23
E-mail: kurtoglumg@hotmail.com

Abstract

Introduction: Clinical samples from 433 patients pre-diagnosed with tuberculosis in Konya, Turkey, were investigated prospectively to compare the GenoType® MTBC test (GenoType® MTBC) with conventional "gold standard" culture methods.
Material and methods: Lowenstein Jensen (LJ) and Mycobacteria Growth Indicator Tube (MGIT)-960 culture methods and GenoType® MTBC were performed together.
Results: *Mycobacterium tuberculosis* (*M. tuberculosis*) detection rates were 16.2% by culture methods, 15.4% by GenoType® MTBC, and 6% by acid-fast bacilli microscopy. The LJ or MGIT-960 with GenoType® MTBC detected *M. tuberculosis* in 12 samples each that were negative according to the other culture method alone. Among 70 *M. tuberculosis*-positive samples, detection rates were 37% (26/70) by microscopy and 82.8% (58/70) by LJ and MGIT-960, but 95.7% (67/70) by GenoType® MTBC.
Conclusions: GenoType® MTBC may be used as a beneficial adjunct test to culture methods for the detection of M. tuberculosis.

Key words: *Mycobacterium tuberculosis*, genotype, culture.

Introduction

The tuberculosis agent Mycobacterium tuberculosis complex (*Mtc*) presents a significant health challenge worldwide [1]. Acid-fast bacilli (AFB) microscopy using Ziehl-Neelsen (ZN) staining has been evaluated as the simplest and fastest diagnostic method for the laboratory diagnosis of *Mtc*, but it has low sensitivity and specificity [2], and requires 5,000-10,000 bacilli per milliliter [3]. Diagnostic methods for mycobacteria identification involve reproduction in culture media, considered the "gold standard" method, which can be 500× more sensitive than ZN staining but is time-consuming and difficult, and needs additional biochemical tests. The need for methods that are faster, simpler and have a high rate of sensitivity and specificity has therefore led to the development of molecular and immunochromatographic test methods [4, 5].

Molecular methods can detect as few as 10 bacilli per milliliter, and can also be used for description, subtyping, determination of drug resistance, and

Table I. Detection of *M. tuberculosis* complex by traditional methods in GenoType® MTBC-positive (67/433) and GenoType® MTBC-negative (366/433) samples

	GenoType® MTBC-positive (*n* = 67)	GenoType® MTBC-negative (*n* = 366)
AFB positive	25	1
AFB negative	42	365
MGIT-960 positive	55	3
MGIT-960 negative	12	363
LJ positive	55	3
LJ negative	12	363

AFB – acid-fast bacilli, MGIT – Mycobacteria Growth Indicator Tube, LJ – Lowenstein Jensen

rapid laboratory diagnosis of tuberculosis [6]. The GenoType® MTBC Assay, V-4.0 (Hain Lifescience GmbH, Nehren, Germany), based on nucleic-acid sequence-based amplification (NASBA), can be directly performed on blood-free samples of patients taking anti-tuberculosis drugs for up to seven days, or from suspected treatment-naive tuberculosis cases.

In this study GenoType® MTBC was compared with two culture methods, Lowenstein Jensen (LJ) medium and the BACTEC Mycobacteria Growth Indicator Tube 960 system (MGIT) (Becton Dickinson and Company-BD, Sparks, MD, USA) in order to determine existence of *Mtc* from various clinical samples.

Material and methods

Preparation of samples and culture method

The study was carried out at Konya Education and Research Hospital, Microbiology Laboratory, Molecular Diagnostic Unit, Turkey, between January 2008 and December 2009. Inoculation and ZN staining of sputum, bronchoalveolar lavage, fasting gastric juice, urine, and abscess samples (after homogenization/ decontamination using *N*-acetyl-L-cysteine-sodium hydroxide [NALC-NaOH]), and of cerebrospinal, peritoneal, and pleural fluids (without homogenization/ decontamination) were performed. NALC-NaOH solution was mixed with 5–10 ml of each sample, vortexed (≤ 30 s), and incubated at room temperature for 15 min with periodic rotation. Phosphate buffer (0.067 M, pH = 6.8) was then added for a total volume of 50 ml, followed by 15 min centrifugation (3000 × g). The pellet was then resuspended in 1-2 ml phosphate buffer (pH = 6.8), 0.5 ml of which was inoculated into MGIT (BD) and LJ (BD), and incubated at 37°C for 6–8 weeks. The culture was considered negative if no growth was observed after this period.

MGIT, BACTEC-NAP Test

MGIT tubes have a silicon rubber base containing an oxygen-sensitive fluorescent indicator, and dissolved oxygen suppresses fluorescence. Reproduced microorganisms consume the oxygen, leading to a fluorescence signal that is detected by the MGIT device. In the study, MGIT cultures with a positive signal were subcultured in Columbia agar containing 5% sheep blood (BD) in order to check whether contamination existed. Additionally, ZN-stained slides from growth-positive tubes were examined for existence of AFB and cord-forming mycobacteria under a light microscope. The BACTEC NAP Tuberculosis Differentiation test was used for differentiating *Mtc* from mycobacteria other than tuberculosis.

GenoType® MTBC Test

Decontaminated, blood-free pulmonary or extrapulmonary samples from suspected (untreated) tuberculosis patients, or those treated with anti-

Table II. Detection rates of *M. tuberculosis* complex in 70 culture-positive clinical samples by traditional and molecular methods

Samples	Total, *n* (%)	AFB (+)	AFB (–)	LJ (+)	LJ (–)	MGIT (+)	MGIT (–)	MTBC (+)	MTBC (–)
Sputum	43 (61.4)	19	24	38	5	36	7	41	2
Urine	6 (8.6)	2	4	5	1	4	2	6	0
CSF	1 (1.4)	0	1	1	0	1	0	1	0
Pleural fluid	3 (4.3)	1	2	3	0	3	0	3	0
Bronchoalveolar lavage	7 (10)	3	4	6	1	5	2	7	0
Fasting gastric juice	5 (7.2)	0	5	2	3	5	0	5	0
Abscess	2 (2.8)	1	1	1	1	2	0	1	1
Peritoneal fluid	3 (4.3)	0	3	2	1	2	1	3	0
Total (*n*)	70	26	44	58	12	58	12	67	3
(%)	100	37.1	62.9	82.8	17.2	82.8	17.2	95.7	4.3

AFB – acid-fast bacilli, LJ – Lowenstein Jensen, MGIT – Mycobacteria Growth Indicator Tube, CSF – cerebrospinal fluid

tuberculosis drugs for seven days at most, were tested directly by using GenoType® MTBC for detecting *M. avium*, *M. intracellulare*, *M. kansasii*, *M. malmoense*, and *M. tuberculosis*. Three-stage procedures were used:

1. RNA isolation using magnetic beads (Magnetic Separator-V.3, Hain Lifescience GmbH, Nehren, Germany).
2. RNA amplification through NASBA with a thermal Cycler (Bioer XP Cycler, Bioer Technology Co., Ltd., Japan).
3. Reverse hybridization of amplification products using GenoType® MTBC test strips and an Auto-LIPA device (Tecan ProfiBlot T48, Austria).

This study was approved by the local research ethics committee, and written informed consent was obtained from all participants.

Statistical analysis

χ^2 and McNemar tests were performed by using Statistical Package for the Social Sciences (SPSS) for Windows (version 15.0; SPSS Inc., Chicago, IL, USA).

Results

GenoType® MTBC, ZN, and culture methods were used together for identifying mycobacteria in 433 patients pre-diagnosed with tuberculosis. The AFB were detected in 26 samples (6%); of the remaining 407 AFB-negative samples, 44 were culture-positive for *Mtc*; bacterial reproduction was detected in 8 by LJ alone, 9 by MGIT alone, and 27 when both LJ and MGIT were used. LJ combined with GenoType® MTBC, however, detected reproduction in 12 samples that were negative by MGIT alone, and MGIT combined with GenoType® MTBC detected reproduction in 12 samples that were negative by LJ alone. In total, 67 (15.4%) and 70 (16.2%) *Mtc* strains were identified using GenoType® MTBC and culture methods, respectively. Of the three culture-positive samples, one from an abscess and two from sputum tested negative by GenoType® MTBC, giving a false-negative rate of 4.2% for GenoType® MTBC. Of the 70 culture-positive samples, 42 (60%) were obtained from males and 28 (40%) were from females, 1 patient being a child; the mean age of the patients was 52.3 ±18.09 years (range: 6–85 years).

Table I shows microscopy-, culture-, and GenoType® MTBC-based results from all 433 samples (Table I). Table II shows results from the 70 culture-positive samples, subclassified by sample type and method (Table II). There were significant differences between AFB microscopy and culture positivity; GenoType® MTBC and culture positivity; and AFB microscopy and GenoType® MTBC positivity (p = 0.01). Sensitivity, specificity, and positive and negative predictive values of GenoType® MTBC were found to be 95.7%, 100%, 100%, and 99.1%, respectively.

Discussion

The main goal of tuberculosis control programs is prevention of transmission through rapid diagnosis and treatment of sputum-positive patients. While clinical and radiological findings were evaluated as valuable, accurate and definitive diagnosis of the tuberculosis must be done by using microbiological methods. Hence, the diagnostic "gold standard" is reproduction of *Mtc* isolated from clinical samples in culture medium, followed by microscopic examination. The time-consuming nature of the culture methods, however, can lead to delay in the diagnostic period of the tuberculosis up to 6 to 8 weeks; molecular methods can be used in order to shorten the duration of the detection of mycobacteria [2–4]. The sensitivity of molecular methods depends on different conditions such as clinical sample type, homogenization-decontamination procedure, and nature of the molecular method used. The 4.2% false-negativity rate obtained from the present study is consistent with those reported as 1-19% in the different studies [3, 7–10], suggesting that diagnosis should not be made solely by using GenoType® MTBC.

In conclusion, according to the results of this study, aimed at comparing the GenoType Mycobacteria Direct Test with traditional culture methods in the laboratory diagnosis of tuberculosis, the sensitivity, specificity, and positive and negative predictive values of GenoType® MTBC were 95.7%, 100%, 100%, and 99.1%, respectively, with regards to the culture methods. It was concluded that GenoType® MTBC may be used as a beneficial adjunct to culture methods for the detection of *M. tuberculosis*.

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