Comparison between TRCReady MTB and MTB ELITe MGB kit for the direct detection of *Mycobacterium tuberculosis complex* in respiratory and non-respiratory samples

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Summary

Background: A recent World Health Organization survey estimated about 10.4 million new tuberculosis (TB) cases per year with a high mortality rate. A rapid and accurate diagnosis of TB is very important for the optimal treatment and the prevention of spread. In the last few years rapid nucleic acid amplification tests for the detection of *Mycobacterium tuberculosis complex* (MTB) were developed.

Materials and methods: In this study we have compared TRCReady MTB, based on the Transcription Reverse Transcription Concerted Reaction technology (TRC) and MTB ELITe MGB Kit, a qualitative nucleic acid amplification assay, for the direct detection of MTB in 56 respiratory and 24 non-respiratory specimens, collected from September to November 2015.

Results: Both methods did not detect any MTB in 45 respiratory samples and in 22 non-respiratory specimens with negative cultures. MTB ELITe MGB Kit identified MTB in 8 respiratory samples with MTB positive cultures, 7 of which were detected by TRCReady MTB as well. Two non-respiratory MTB positive cultures were correctly identified by both methods. In two respiratory samples with *Mycobacterium Other Than Tuberculosis* positive cultures both methods provided negative results.

Conclusions: In conclusion, TRCReady MTB performance proved comparable to that of MTB ELITe MGB Kit in the diagnosis of pulmonary and extra-pulmonary TB, with a shorter analytical time (50 *vs* 110 min).

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Key words: Tuberculosis; Mycobacterium Tuberculosis Complex; TRC-80 Ready MTB.

Contributions: PAC, CA, PM, contributed equally to the study design and to the selection of cases under study; AG contributed to the preparation of the manuscript, performed the sensitivity and specificity experiments and the data review; PM and EV carried out the technical experiments.

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 15 May 2017.
Revision received: 7 June 2017.
Accepted for publication: 8 June 2017.

Microbiologia Medica 2017; 32:6794
doi:10.4081/mm.2017.6794

Introduction

Tuberculosis (TB) is currently a major global health problem. As published in a recent World Health Organization survey, in 2015 about 10.4 million new cases were estimated worldwide, with 1.4 million of TB-related deaths (11). Therefore a rapid and accurate diagnosis of TB is crucial in providing optimal treatment, in reducing transmission and in preventing hospital-acquired TB infections. Although culture method is considered the *gold standard* for the detection of *Mycobacterium Tuberculosis Complex* (MTB), it requires from 2 to 6 weeks to confirm an infection, since MTB are slow-growing organisms (1). On the other hand the fluorescent acid-fast bacillus staining, such as Auramine-O, can be carried out in a short time, but almost half of culture-positive TB cases are estimated to be smear negative (3).

In the last few years various molecular tests based on nucleic acid amplification were developed for the direct detection of MTB in clinical samples (2, 7). Recently a new method based on the Transcription Reverse Transcription Concerted Reaction technology (TRC) has been developed (6). As described elsewhere, TRC is an isothermal RNA amplification at 46°C with transcriptase and reverse transcriptase in the presence of intercalation activating fluorescence probes (INAF) (4). TRCReady MTB (TOSOH Corporation, Tokyo, Japan) is a totally automated system for a rapid extraction, amplification and detection of MTB 16 rRNA target, using TRC method (9). This study was performed to evaluate the performance of TRCReady MTB technology in comparison to the MTB ELITe MGB Kit (ELITech Group, Turin, Italy), a qualitative nucleic acid amplification assay currently in use at our laboratory, in respiratory and non respiratory clinical specimens. The results of both methods were compared to culture data.

Materials and Methods

Fifty-six respiratory specimens (26 sputum, 26 bronchoalveolar lavage-BAL, 4 bronchial aspirates) and 24 non-respiratory

samples, including 12 pleural effusions, 2 urines, 3 cerebrospinal fluid (CSF), 3 lymph node aspirates, 2 abscess fluids, 1 gastric aspirate and 1 pulmonary biopsy were collected from September to November 2015 and were processed for MTB detection. All specimens, except CSF, were decontaminated by NaCl-NaOH procedure, as previously described (5). Fixed smears were automatically stained with Truant aramine-rhodamine stain (Areospray® TB). The Mycobacterial cultures were performed in liquid (BACTEC MGIT 960; Becton Dickinson, Milan, Italy) and in solid (Lowenstein-Jensen; Becton Dickinson) media at 37°C for up to 8 weeks. Isolates of mycobacteria from positive cultures were identified by Genotype Mycobacterium CM (Arnika). An automated DNA extraction instrument (Easy MAG; bioMérieux, Mercy-L'Etoile, France) was utilized. About 70 minutes to obtain the DNA extracts were usually needed. The DNA extraction products were used for MTB ELITe MGB Kit (ELITech Group, Turin, Italy), a qualitative real-time amplification reaction in microplate for the detection of MTB DNA (including *M. tuberculosis*, M. *africanum*, *M. bovis*, *M. bovis BCG*, *M. microti* and *M. canettii*). A programmable thermostat provided with an optical system for fluorescence emission (7500 Fast Dx, Applied Biosystems) was used for the MTB detection. In each well 20 µL of MTBQ-PCR Mix, constituted by specific primers and probes for the region of the IS6110 gene of MTB and a for the 5' UTR region of the human beta Globin gene (internal control), were added to 20 µL of DNA extract. The MTB-specific probes, labelled with FAM fluorophore, were activated when a hybridization took place with the specific products of the MTB amplification reaction. This process, including 45 cycles of amplification and detection, required 49 minutes. If a positive signal was detected between the 35th and the 37th cycle, a careful check of the reaction curves should be undertaken, to verify the correctness of curve shape. In case a positive signal was detected over the 38th cycle, the test was repeated to confirm or exclude the positivity. For TRCReady MTB the manufacturer's instructions were followed. Briefly 200 mL of MB-Lysis reagent and 500 mL of decontaminated sample were dispensed into a screw cap tube. After a rapid spinning, the tube was incubated at 80°C in the dry heat block for 10 minutes. 400 mL of the lysed sample were added to the denaturant reagent tube that was placed into the P-cartridge. The P-cartridge was inserted in the TRCReady that automatically extracted, amplificated and detected MTB 16 rRNA target. MTB 16 rRNA amplification was detected by measuring the fluorescence emitted at 520 nm, whereas that of the nucleic acid internal control was detected by measuring the fluorescence emitted at 610 nm. Samples with any positive signal at 520 nm were considered positive, while samples with a positive signal at 610 nm and no positive signal at 520 nm were considered negative. For TRCReady MTB 50 minutes of processing time were necessary (10 minutes for manual lysis and 40 minutes for automated extraction, amplification and detection) and positive or negative judgements have been automatically reported.

Diagnostic sensitivity and specificity of TRCReady MTB were verified using our 30 frozen cell cultures of MTB and *Mycobacterium Other Than Tuberculosis* (MOTT). Statistical comparison was performed using Fisher's exact test. A P-value <0.05 was considered significant.

This study was approved by the Technical and Scientific Advisory Committee of the Western Milan Area Hospital Consortium, Italy.

Results

All 16 frozen cell cultures of MTB were correctly detected by TRCReady MTB, that did not show any positive signal in the 14 frozen cell MOTT cultures (2 *Mycobacterium avium*, 2 *M. avium complex*, 2 *M. xenopi*, 2 *M. gordonae*, 1 *M. fortuitum*, 3 *M. kansasii*, 1 *M. intracellulare*, 1 *M. chelonae,* 1 *M. kumamotonense*). Eleven respiratory specimens (19.6%) and 2 non-respiratory samples (8%) showed positive cultures for mycobacteria. MTB was identified in 9 respiratory specimens with positive cultures and in both non-respiratory samples (pleural effusion and lymph-node aspirate). In 2 respiratory specimens with positive cultures *M. kansasii* and *M. intracellulare* were identified. Forty-five respiratory specimens and 22 non-respiratory samples showed negative cultures.

TRCReady MTB and MTB ELITe MGB Kit did not detect any MTB in 45 respiratory samples and in 22 non- respiratory specimens with negative cultures, with a specificity of 100% for both methods. MTB ELITe MGB Kit identified MTB in 8 respiratory samples with MTB positive cultures. Three samples with negative smears gave a MTB signal between the 35th and 36th cycle of reaction. Only in one specimen with positive culture and negative smear, MTB was not detected by MTB ELITe MGB Kit (Table 1). MTB ELITe MGB Kit detected MTB in 2 non-respiratory samples, however in a pleural effusion mycobacteria were detected at the 37th cycle of amplification. Both samples showed negative smears (Table 2). TRCReady MTB detected MTB in 2 non-respiratory

Table 1. Comparison of TRCReady MTB with MTB ELITe MGB Kit in respiratory specimens *versus* cultures.

Cultures	TRCReady MTB, n (%)		MTB ELITe MGB Kit, n (%)		Smear, n (%)	
	Positive	Negative	Positive	Negative	Positive	Negative
MTB positive, 9 (16%)	7 (78)[a,b]	2 (22)	8 (89)[a,b]	1 (11)	5 (56)	4 (44)
MOTT positive, 2 (4%)	0	2 (100)	0	2 (100)	1 (50)	1 (50)
Negative, 45 (80%)	0	45 (100)[c]	0	45 (100)[c]	0	45 (100)

a, sensitivity; b, P>0.5; c, specificity.

Table 2. Comparison of TRCReady MTB with MTB ELITe MGB Kit in non-respiratory specimens *versus* cultures.

Cultures	TRCReady MTB, n (%)		MTB ELITe MGB Kit, n (%)		Smear, n	
	Positive	Negative	Positive	Negative	Positive	Negative
MTB positive, 2 (8%)	2 (100)	0	2 (100)	0	0	2
Negative, 22 (92%)	0	22 (100)	0	22 (100)	0	22

samples with MTB positive cultures and in 7 respiratory samples. TRCReady MTB did not identify MTB in 2 respiratory samples with positive cultures and negative smears, one of which resulted MTB positive by MTB ELITe MGB Kit as well. The sensitivity of MTB ELITe MGB Kit was 89% and the sensitivity of TRCReady MTB was 78%. However, this difference was not statistically significant (P>0.5). In 2 respiratory samples with MOTT positive cultures, both methods did not detect any MTB.

Discussion and Conclusions

In the last decade various studies reported the usefulness of TRC in the detection of MTB in clinical specimens (2, 8). Takakura evaluated the performance of TRC method for the detection of MTB in respiratory samples in comparison to that of COBAS AMPLICOR PCR (8). In that study the author did not find significant differences in sensitivity between COBAS PCR assay and TRC method (95% *vs* 90.7%) with 100% of specificity for both techniques. In a recent study Tsuyuguchi compared the performance of Xpert ® MTB/RIF assay, which is the most rapid and sensitive molecular test recommended by WHO for the diagnosis of TB, with other Nucleic Acid Technologies such as TaqMan MTB and TRC MTB assay (10, 11). The authors did not find any difference in terms of performance between the two molecular technologies. In an European multicentre study, Drouillon analysed 633 respiratory and non-respiratory samples with TRC assay and the results were compared to culture data. In that study the sensitivity and the specificity of TRC method in respiratory samples were respectively 88.2% and 97.4% and in non-respiratory specimens were 83.3% and 95.8% (2).

Our study was performed to evaluate the performance of TRCReady MTB assay, based on TRC technology, in comparison to the MTB ELITe MGB Kit currently in use at our laboratory for the direct detection of MTB in respiratory and non-respiratory clinical specimens. TRCReady MTB assay showed several advantages such as a shorter analytical time (50 minutes of processing time *vs* 110 minutes with MTB ELITe MGB Kit), a total automation that does not require a high level of expertise, a reduced use of consumables (for example the reagents used for nucleic acid extraction) and provided clearcut results (*i.e.* positive or negative judgment) without the need to review the amplification curve. Since a MTB positive culture is still considered the *gold standard* for the diagnosis of pulmonary and extra-pulmonary TB, TRCReady MTB assay and MTB ELITe MGB Kit were compared to culture data. A total of 80 samples (56 respiratory samples and 24 non-respiratory samples) were included. 30 frozen cell cultures of Mycobacteria (16 MTB and 14 MOTT) were also tested by TRCReady MTB assay. TCR Ready MTB assay correctly detected MTB in the 16 MTB frozen cell cultures whereas no positive MTB detection was detected in 14 MOTT frozen cell cultures. In none of 45 respiratory samples and in none of 22 non-respiratory specimens with negative cultures, false positive results were recorded with either methods (100% of specificity). In 11 respiratory samples and 2 non-respiratory samples the cultures resulted positive in 15-20 days (data not shown). In 9/11 respiratory samples and in both non-respiratory specimens with positive cultures MTB was detected.

Our data showed that fluorescent acid–fast bacilli were not detected by microscopy analysis (negative smear) in almost half of positive mycobacteria cultures, as also reported by Gavin (3). In 2 respiratory specimens with positive cultures and negative smears, MTB was not detected by TRCReady MTB. In one of these samples, MTB was revealed by MTB ELITe MGB kit. In 4 specimens (3 respiratory samples and in 1 pleural effusion) MTB ELITe MGB kit detected MTB over the 35th amplification cycle. In all these cases we have rechecked the PCR curves and have assigned a positive judgment.

The sensitivity of MTB ELITe MGB Kit was 89% and the sensitivity of TRCReady MTB was 78%, without a statistically significant difference (P>0.5). We have found a sensitivity level for TRC method lower than that was reported in other studies (2), but in our study the number of specimens was relatively small. The specificity for both methods was 100%. No false positive samples have been found.

In conclusion, our data showed that TRCReady MTB performance has been comparable to that of MTB ELITe MGB Kit. TRCReady MTB showed high specificity and good sensitivity for the direct detection of MTB in respiratory and non-respiratory specimens. Since TRCReady is a rapid and user-friendly method, it may be used in the diagnosis of pulmonary and extra-pulmonary TB.

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