MPT64 Antigen Simple and Rapid Test for Identification and Discrimination of *Mycobacterium tuberculosis* Complex from Nontuberculous Mycobacteria

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Abstract

Tuberculosis is a global pandemic and the incidence of nontuberculous mycobacteria (NTM) appears to be increasing worldwide. In this sense it is necessary that laboratories to use a rapid, accurate test, not laborious which does not require biosecurity measures. Molecular tests are accurate method, but is needs special equipments and lots labour, time and not economically. The aim for this study was to characterization the isolated of mycobacteria by immunochromatographic test, SD BIOLINE TB Ag MPT64 for differentiation into *Mycobacterium tuberculosis* (MTBC) complex and NTM. The immunochromatographic assay was evaluated with four reference strains of mycobacterial species and other clinical isolates of mycobacteria using the conventional methods as the gold standard. The results indicate that Ag MPT64 can be easily used for rapid discrimination between MTBC and NTM to confirm *Mycobacterium tuberculosis* specifically obtained from early stages of subculture without any technical complexity.

Keywords: Ag MPT64, *Mycobacterium tuberculosis* complex, Nontuberculous mycobacteria

INTRODUCTION

Tuberculosis is a global problem and the single most common cause of death from any bacterial agent (Laughon, 2007; Park *et al.*, 2009; WHO, 1996). Members of *Mycobacterium tuberculosis* complex (MTBC), which includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. canetti* and *M. microti* cause tuberculosis. In recent years, disease caused by nontuberculous mycobacteria (NTM) are increasing (Kanade *et al.*, 2011; Hankya *et al.*, 2011; van Ingen *et al.*, 2009).

Mycobacterium tuberculosis (MTB) and NTM are different clinically, therefore rapid detection, isolation, and discrimination are essential for appropriate management (ATS, 1997; Hasegawa *et al.*, 2002; Park *et al.*, 2009).

Detection of tuberculosis agent like NTM species by culture and microscopic methods is difficult and time consuming (Hatta *et al.*, 2010).

Therefore, the examination of cultured colonies should be able to discriminate between MTB and NTM to confirm MTB specifically (Tohir *et al.*, 2011).

Commercial diagnostic methods employ molecular biological tests to provide quick and specific methods for the identification of MTC, instead false positive results cannot be never excluded and these assays are still costly in terms of specialized equipment, labour and time (Tohir *et al.*, 2011; Wang *et al.*, 2006).

Considering all aspects and alternative methods that are inexpensive, rapid methods are vital for the identification of mycobacteria (Halima *et al.*, 2011).

Recently, MPT64 antigen assays were developed to discriminate between *Mycobacterium tuberculosis* complex (MTBC) from NTM by immunochromatography test (Halima *et al.*, 2011).

MPT64 is a mycobacterial protein secreted only by MTBC and has been proven to differentiate MTBC from NTM (Abe *et al.*, 1999; Halima *et al.*, 2011; Hasegawa *et al.*, 2002). The MPT64 gene is well characterized, and the antigen has been studied extensively (Halima *et al.*, 2011; Nakamura *et al.*, 1998; Oettinger *et al.*, 1994). MPT64, a 24 kDa secretory protein, is one of major antigens from tuberculosis (Tohir *et al.*, 2011).

Standard Diagnostics, Inc (SD) (Yongin, Korea) developed the SD BIOLINE TB Ag MPT64 RAPID® test, which is a simple and rapid test using a mouse monoclonal anti-MPT64 antibody that is capable to discriminate between MTC and NTM by immunochromatographic assay (Tohir *et al.*, 2011; Halima *et al.*, 2011).

MATERIALS AND METHODS

The commercial assay, “SD BIOLINE TB Ag MPT 64 Rapid®”, manufactured by Standard Diagnostics, Seoul, South Korea, based on the principle of immunochromatography test, was used as per the manufacturer’s instructions.

It was tested on the cultures identified at the Clinic of Pulmonary Disease and Faculty of Veterinary Medicine in Iasi.

For this study, were used 58 MTBC and 115 NTM strains from humans and 11 MTBC and 9 NTM from animals and birds (Tab. 1) which were compared with conventional culture identification methods as a gold standard.

The results obtained after the immunochromatographic assay with the strains isolated from hens and mammalian carnivores were compared

with PCR amplification by IS6110 sequence for detection of *Mycobacterium tuberculosis* complex and the insertion sequence IS 1245 for identified of *Mycobacterium avium*.

H₃₇Ra *Mycobacterium tuberculosis* strain and *Mycobacterium avium subsp. avium*, *Mycobacterium abscessus* and *Mycobacterium marinum* obtained from Spanish Type Culture Collection (CECT) were used as the standard positive and negative control for identification purpose (Tab. 1).

Strains that were to be tested by the commercial immunochromatographic assay were first subcultured on Lowenstein Jensen (LJ) medium and incubated for growth at 37°C for several weeks.

All cultures were examined using Ziehl-Neelsen (ZN) staining to confirm the presence of acid-fast bacilli (AFB). After cultivation in LJ medium were applied to the immunochromatographic assay slide directly.

The entire test procedure was carried out inside a biological safety cabinet class II. The solid culture isolates were scrapped from the solid medium and suspended in 200 µl of extraction buffer (SD) provided in the kit and the suspension was added to the sample well.

When it were condensation fluid in the slant tubes, it was taken direct 100 µL of condensation fluid and was applied directly to the sample well without using an extraction buffer that was much faster.

Tests were interpreted after 15 minutes from sample application at room temperature.

The presence of both control and test pink colored bands, indicates the test is positive and

Tab.1. Different Mycobacterial isolates tested with SD BIOLINE TB Ag MPT 64 Rapid®

Species	Isolates (n)
<i>Mycobacterium tuberculosis</i> complex	
<i>Mycobacterium tuberculosis</i> H ₃₇ Ra	1
<i>Mycobacterium tuberculosis</i> complex (humans)	58
<i>Mycobacterium tuberculosis</i> complex (mammalian carnivores)	11
Nontuberculous <i>Mycobacteria</i>	
<i>Mycobacterium avium subsp. avium</i> 7407	1
<i>Mycobacterium abscessus</i> 8517	1
<i>Mycobacterium marinum</i> 7091	1
<i>Mycobacterium avium subsp. avium</i> (hens)	9
Nontuberculous <i>Mycobacteria</i> unidentified (humans)	115
Total	197

the presence of only the control band indicates negative result for MTBC. The absence of control band after the test was considered invalid.

The negative results after the commercial immunochromatographic test (ITC) at hens were compared by IS 1245 PCR assay for *Mycobacterium avium*. The positive results at mammalian carnivores after the ITC were compared with IS6110 PCR for *Mycobacterium tuberculosis* complex (Miller J. *et al.*, 1999).

RESULTS AND DISCUSSIONS

Sensitivity and specificity of the commercial assay was calculated using the results of conventio-

nal phenotypic as a golden standard from the MTBC and NTM isolates.

The reference strain ($H_{37}Ra$) showed the presence of MPT64 antigen in the test band (Fig. 1). Similar positive bands were obtained in MTBC isolates from humans and animals (n=69) and negative bands were detected for any NTM isolates (n=124) with rapidly growing from humans and hens and for the NTM type strain (n=3) which indicates a test sensitivity and specificity of 100% compared with conventional methods (Tab. 2).

The PCR assay was performed on 20 samples, isolated from animals and birds.

Fig. 1. Identification of the *M. tuberculosis* complex by the TB Ag MPT 64 kit; strong positive – $H_{37}Ra$ *M. tuberculosis*; negative – *M. avium subsp. avium*

Tab. 2. Sensitivity and Specifity of SD BIOLINE Ag MPT 64

Culture method		LJ	
Test method		MTBC	NTM
SD BIOLINE TB Ag MPT 64	Positive	70	0
	Negative	0	127
	Total	70	127
Sensitivity & Specifity (%)		100 (197/197)	
Test method		PCR (IS6110/IS1245)	
		MTBC	NTM
SD BIOLINE TB Ag MPT 64	Positive	11	0
	Negative	0	9
	Total	11	9
Sensitivity & Specifity (%)		100 (20/20)	

After the immunochromatographic test, were obtained 9 isolates negatives and 11 isolates positives, this results were confirmed by PCR assay (Tab. 2).

Tests were interpreted in only 15 min after the culture application.

The presence of a control band indicates a negative result, and the presence of two color bands (control and test bands), no matter which band appears first, indicates a positive result. A color band of any intensity was read as a positive result (Fig. 1.).

These results are similar to those observed in other studies in order to assess the sensitivity and specificity of the test (Abe *et al.*, 1999; Fabre *et al.*, 2010; Gaillard *et al.*, 2011; Tohir *et al.*, 2011; Kumar *et al.*, 2011). Ismail *et al.* (2009), used the same test to demonstrate sensitivity, specificity and positive and negative predictive values of 97%, 100%, 100% and 92%, respectively. Park *et al.*, (2009) also reported excellent sensitivity (99%) and specificity (100%) of the test, with a detection limit by (10^5 CFU/mL).

The detection limit of this assay is 10^5 CFU/ml, mentioned and in manufacturer's instructions, which can be prepared with colonies from a solid medium, obtained from early stages of subculture. Therefore, the tuberculosis confirmation can be available with only three colonies from early stage of culture. This shortens the overall time of identification for the clinical isolate by three-four weeks. Both the culture suspension as well as the culture filtrate gave equally satisfactory results.

Instead, the molecular tests requires special equipment and are more expensive for an isolate identification compared with immunochromatographic test and is not able to identify or differentiate the members of MTB complex (Kanade *et al.*, 2012).

This rapid method for confirming MTB culture isolates is reliable, rapid and inexpensive.

CONCLUSION

In both humans and animals, the immunochromatographic assay it could be a good alternative for a rapid identify MTC from NTM.

Because the optimal management and treatment of a patient with tuberculosis is different from that of patients infected with mycobacteria other than tuberculosis, is necessary to use this rapid and extremely simple test.

"SD BIOLINE TB Ag MPT 64®" assay is a simple, rapid, without any special equipment for protection, economical and accurate test for characterization of clinical mycobacterial isolates suitable for laboratories with poor resource.

Therefore, the workload in order to confirm TB can be reduced remarkably.

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