Original Article

Simple and rapid identification of the mycobacterium tuberculosis complex by immunochromatographic assay using MPT 64 antigen detection test

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Abstract

Background: In developing countries like India, more than 90% of tuberculosis infections are still caused by *M. tuberculosis*. Phenotypic and genotypic methods used for identification of mycobacterial species are either cumbersome or expensive. The *Mycobacterium tuberculosis* protein 64 (MPT-64) antigen is an *M. tuberculosis* complex (MTBC) specific antigen and immunochromatographic test based on this Ag has been used to identify MTBC rapidly. The present study was undertaken to evaluate this commercial assay for characterization of Mycobacteria. **Material and Methods:** A total of100 isolates recovered from both pulmonary (50 isolates) as well as extra pulmonary specimens (50 isolates) were characterized as MTBC or NTM based on standard phenotypic characteristics and biochemical tests. These results were compared with commercial SD TB Ag MPT64 Rapid test. **Results:** Among 100 mycobacterial isolates, 98 isolates were identified as MTBC and the two isolates from extra pulmonary cases were identified as NTM on the basis of biochemical tests. There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test. **Conclusion:** The sensitivity and specificity of TB Antigen MPT64 rapid test was 100% as compared to biochemical methods. This commercial assay is rapid, simple, easy to perform, interpret and economical with high sensitivity and specificity to identify MTBC from culture isolates.

Keywords: Tuberculosis, *M. tuberculosis* complex, MPT64 Antigen, Immunochromatographic assay.

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INTRODUCTION

Tuberculosis (TB) is one of the major causes of morbidity and mortality worldwide. The *Mycobacterium tuberculosis* complex (MTBC) is a known agent for infectious pulmonary TB. It is caused by members of MTBC, which includes *M. tuberculosis, M. bovis, M. africanum, M. canetti and M. microti*[1]. In developing countries like India, more than 90% of tuberculosis infections are still caused by *M. tuberculosis*.Conventional phenotypic methods for identification of mycobacterial species are based on the results of rate of growth, pigmentation of colonies and various biochemical reactions. These methods are time-consuming, involve use of hazardous chemicals, and are prone to subjective error in interpretation of results[2]. Although, molecular methods are rapid, sensitive and specific, they are expensive and require trained personnel and special laboratory setup[3]. Hence, there is need for a rapid, accurate and simple test for identification of mycobacteria species. A variety of antigens have recently emerged for the immune diagnosis of TB. The *Mycobacterium tuberculosis* protein 64 (MPT-64) antigen

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is an *M. tuberculosis* complex (MTC) specific antigen secreted during bacterial growth[4]. This antigen is encoded by the RD2 region which is specific for MTBC and can be detected in culture isolates and biopsy samples[5]. Recently, Standard Diagnostics (SD, Korea) developed a simple and rapid assay, "SD BIOLINE TB Ag MPT 64 Rapid" (commercial assay) to identify and differentiate MTBC and nontuberculous mycobacteria (NTM) by immunochromatography (ICT). The present study was undertaken to evaluate this commercial assay for characterization of Mycobacteria already isolated on Lowenstein Jensen (L-J) medium from cases of pulmonary and extrapulmonary tuberculosis.

MATERIAL AND METHODS

In this prospective study, a total of 100 clinical specimens from adult patients with pulmonary (50 samples) and extra pulmonary (50 samples) infection were included. Samples were inoculated onto L-J medium and Ziehl-Neelsen (Z-N) staining was also done. The work was carried out in a Class II Biosafety Cabinet and level two bio safety practices were followed. After appearance of growth, the isolates were confirmed by Z-N staining. The positive cultures were screened to differentiate the growth of mycobacteria into MTBC and NTM by niacin accumulation test, nitrate reduction test, heat stable catalase at 68^0C/pH7 and Para- nitrobenzonic acid (PNB) susceptibility test[2]. The cultures which were not identified as MTBC and suspected to be NTM were further identified by rate of growth, pigment production, Urease test, Tween – 80 hydrolysis test, Arylsulfatase test, MacConkey agar test andSodium Chloride tolerance test[2]. All the cultures were also subjected to TB Antigen MPT64 rapid test for differentiation into MTBC and NTM. The test was performed as per the manufacturer's instructions[6]. The kit contains cassettes with nitrocellulose strip on which mouse monoclonal anti-MPT64 antibodies (test line) and goat anti-mouse antibody (control line) are immobilized. Another mouse monoclonal antibody recognizing a different epitope of MPT64 antigen and conjugated with colloidal gold particles is present in the sample well. If MPT64 antigen is added to the strip, it gets captured by both types of mouse monoclonal antibodies and gives a visible test band. The mouse monoclonal antibody conjugated with colloidal gold particles combines with goat anti-mouse antibody to give the control band. Briefly,[3-4] colonies from L-J were emulsified in 200 µl of extraction buffer. 100 µl of suspended solid culture in buffer was added into the sample well. The inoculated cassettes were kept undisturbed at room temperature and were examined at the end of15 minutes for presence of pink band in "Control" and "Test" region[6]. The appearance of control band confirmed the validity of the test. If the control band was not visible in 15 minutes, the result was considered invalid and the sample was retested. The presence of only control band in the absence of test band was considered a negative test. Presence of both control and test band indicated a positive test.

RESULTS

One hundred clinical specimens were collected from adult patients and demographic details of these patients were recorded. Maximum number of patients belonged to 16-45 years (83%) of age group followed by 46-60 years (16%) of age group in both pulmonary and extrapulmonary cases. There were 53 males and 47 females with male predominance in extra pulmonary cases (M:F=1.27:1). Most common symptom in pulmonary cases was cough (92%) followed by fever (74%) and weight loss (64%) while in extrapulmonary cases most common symptom was fever (44%) followed by lymphyadenopathy (36%). The pus (60%) was most common specimen followed by pleural fluid (14%) and lymph node aspirate (12%) amongst extrapulmonary specimens. Pleural involvement (32%; pleural effusion and empyema thoracis) followed by lymph node involvement (18%) was most commonly seen in extrapulmonary cases. Other sites (16%) included axillary, mastoid and submandibular abscesses. Out of 50 culture positive pulmonary specimens, only 23 (46%) were positive on primary ZN smear. Whereas, among 50 culture positive extrapulmonary specimens, only 18 (36%) were primary ZN smear positive. All the isolates from pulmonary cases (n=50) were positive for niacin and nitrate reduction test while negative for catalase and PNB test. In extrapulmonary isolates, 48 were positive for niacin test, negative for catalase and PNB test while 49 were positive for nitrate reduction test. The two isolates from extrapulmonary cases were positive for PNB test. Therefore, all the 98 isolates were identified as MTBC and the two isolates from extrapulmonary cases identified as NTM on the basis of biochemical tests. These two NTM isolates from extrapulmonary specimens were further identified as *Mycobacterium fortuitum* and *Mycobacterium chelonae* on the basis of biochemical tests. These isolates belong to Group IV (Rapid Growers) of Runyon's Classification. The control band was seen in all the tested cultures (n=100), validating the ICT test. All the 50 isolates from pulmonary cases and 48 isolates from extrapulmonary cases showed positive results (visible band for MPT64 antigen) and the two isolates from extrapulmonary cases gave negative results. There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test. The sensitivity and specificity of TB Antigen MPT64

rapid test was found to be 100% as compared to biochemical methods.

DISCUSSION

In the present study, majority of patients (83%) belonged to 16 – 45 years of age group in both pulmonary and extra pulmonary cases with slight male predominance (M:F = 1.27:1) seen in extra pulmonary cases. Similar demographic profile of patients was found in a study by Sivasankari *et al*[7]. Pus (60%) was most common specimen and lymph node aspirate (12%) amongst extra-pulmonary specimens in the present study. Previous studies also found pus as the most common specimen followed by lymph node[8-12]. In the present study, most common symptom in pulmonary cases was cough (92%) followed by fever (74%) and weight loss (64%) while extrapulmonary cases showed fever (44%) as most common symptom followed by lymphyadenopathy (36%).Sharma *et al*[13] also reported fever (98%) and cough (95%) as most common symptom in pulmonary tuberculosis. Whereas Koshti *et al*[14] documented cough (66.6%) as most common symptom in pulmonary cases while fever (86.8%) followed by weight loss (58.1%) in extra-pulmonary cases. In the present study, 46% and 36% of Z-N smear positivity was seen in pulmonary and extrapulmonary cases respectively. A study published by Selvakumar *et al*, reported ZN smear sensitivity of 47% from liquefied sputum samples[15]. In the present study, 98% isolates were found *M. tuberculosis* complex while only 2% were NTM. Both the NTM were isolated from extrapulmonary tuberculosis cases. There was no NTM isolated from pulmonary tuberculosis cases. MTBC was found to be the most common isolate than NTM in previous studies. In developing countries like India, more than 90% of tuberculosis infections are still caused by *M. tuberculosis*. The major attraction for using MPT64 TB Ag test was its claim to characterize mycobacterial isolates accurately in 15 minutes with a low cost (Rs. 125/- per test). This assay was evaluated for rapid characterization of 100 cultureisolates and whether it could replace the tedious conventional phenotypic methods.

Table 1: Comparison of differentiation of MTBC and NTM by conventional biochemical methods and TB Antigen MPT64 Rapid test

MPT64 Ag Test Result	Biochemical Test Result		Total
	MTBC	NTM	
Positive	98	0	98
Negative	0	2	2
Total	**98**	**2**	**100**

There was no discrepancy found in differentiation of these isolates between biochemical tests and TB Antigen MPT64 rapid test (Table 1). The sensitivity and specificity of TB Antigen MPT64 rapid test was found to be 100% as compared to biochemical methods. Other studies have also demonstrated specificity of 100% and sensitivity ranging from 96.5% to 100%[16-18]. To conclude, this commercial assay is rapid, simple, easy to perform, interpret and economical with high sensitivity and specificity to identify MTBC from culture isolates.

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