Utility of MPT64 Antigen Detection for Rapid Confirmation of *Mycobacterium tuberculosis* Complex

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ABSTRACT

Background: Rapid differentiation of the *Mycobacterium tuberculosis* complex (MTBC) and mycobacteria other than tuberculosis (MOTT) is crucial to facilitate early and effective treatment of the patients. Clinical presentation of MTBC and MOTT is not always very clear and routine conventional methods are time consuming. **Materials and Methods:** In the present study, the MPT64 protein detection-based immunochomatographic test (SD Bioline Kit, Standard Diagnostics, Inc., Korea) was compared with the conventional biochemical method. **Results:** The sensitivity, specificity, positive predictive, and negative predictive values of the SD AgMPT64 kit were found to be 100, 96.4, 98.72, and 100%, respectively. **Conclusions:** Our results have demonstrated that the SD bioline kit is a rapid, reliable method and it can be used in the Revised National Tuberculosis Control Program (RNTCP) of India, for the appropriate management of tuberculosis.

Key words: Biochemical identification, MPT64 antigen, *M. tuberculosis* complex

INTRODUCTION

The *Mycobacterium tuberculosis* complex (MTBC) is a known agent for infectious pulmonary tuberculosis (TB). On the other hand mycobacteria other than tuberculosis (MOTT), can also cause a similar disease. The sign and symptoms of pulmonary infection due to MTBC or MOTT often resemble, and their differentiation through acid fast stain is incomprehensible. Identification and speciation of the mycobacterium becomes essential for the appropriate management and treatment of the affected individuals.[1,2]

The Revised National Tuberculosis Control Program (RNTCP) recommends the use of liquid culture for rapid diagnosis and drug sensitivity testing (DST) for all Intermediate Reference Laboratories (IRLs) and National Reference Laboratories (NRLs), as it significantly reduces the turnaround time for culture and drug susceptibility testing.[3,4] Rapid confirmatory identification of *M. tuberculosis* is important, as the conventional biochemical methods are laborious, time-consuming, and require elaborate safety precautions. During this long and tedious process, an infected patient may spread the disease to many other individuals. Nucleic acid amplification-based methods are rapid and specific, but require a person who is technically skilled and also require expensive equipment.[5] Thus a simple, rapid, and sensitive discriminatory test for rapid identification of the *M. tuberculosis* complex is necessary for accurate diagnosis and treatment of the disease.

MPT64 is one of the major culture filtrate protein (24 kDa)[6,7] encoded by the RD2 region genes[8] and has been shown to be a specific antigen that differentiates the *M. tuberculosis* complex from the mycobacteria other than tuberculosis (MOTT) species.[5,9] An MPT64-based, simple and rapid immunochromatographic assay was developed by the Standard Diagnostics, Inc. (SD) (Yongin, Korea), known as the SD Bioline TB Ag MPT64 RAPID® test (SD bioline kit). This lateral flow test has been reported to identify the *M. tuberculosis* complex from the MOTT using the mouse monoclonal anti-MPT64 antibody.[10-12]

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The present study was conducted to check the utility of the SD Bioline kit for the identification of mycobacteria in the liquid media. These findings were correlated with a conventional biochemical test and the sensitivity, specificity, and predictive values of this kit were also assessed.

MATERIALS AND METHODS

Study subjects

The Department of Microbiology, National Institute of TB and Respiratory Diseases is a National Reference Laboratory (NRL) under the RNTCP, India. The smear-positive diagnostic samples are subjected directly to Line probe assay for rapid diagnosis of multidrug-resistant tuberculosis (MDR-TB), whereas, the smear-negative and critical month follow up samples of MDR patients (third, fourth, fifth, sixth, eighteenth, twenty-first, and twenty-fourth months) are put into an MGIT 960 (Mycobacteria Growth Indicator Tube, Becton Dickinson, Franklin Lakes, NJ; USA) liquid culture system. The study was performed directly on 105 random samples flagged as positive by the MGIT 960 instrument, for a period of four months (December 2012-March 2013).

Decontamination of specimens and culture procedure

The sputum samples were processed in a BSL III Laboratory using the standard N-Acetyl L-cysteine (NALC)/Sodium Hydroxide (NaOH) method.[13] The processed sample (0.5 ml) was inoculated and incubated in the MGIT 960 system till the tubes were flagged positive by the machine or till it was negative (42 days). Ziehl Neelsen (ZN) smears were performed on the positive cultures, to determine the presence of acid fast bacilli and serpentine cords. The sterility of MGIT positive cultures was performed on blood agar plates. One hundred microliters of culture were inoculated into the Lowenstein Jensen (LJ) slants in duplicate for biochemical identification.

SD bioline tuberculosis assay

The SD bioline assay was performed on acid fast bacilli — positive MGIT cultures, as described by the manufacturer. Liquid culture (0.1 ml) was put onto the sample well. The test cassettes strips were incubated for 15-30 minutes at room temperature (RT). The pink band in the 'C' region confirmed the test validity. An additional pink band in the 'T' region was interpreted as positive for the MPT64 Ag. Only the pink band in the 'C' region and no band in the 'T' region were considered negative for the MPT 64 antigen. No band in 'C' region was interpreted as an invalid test. H37Rv was taken as a positive control for each new kit.

Biochemical identification

The LJ cultures were observed for colony morphology and pigmentation, and stained by using the Ziehl Neelsen (ZN) method. They were subjected to conventional biochemical identification using niacin, nitrate, and the heat-resistant catalase test (HRCT). The technician performing the test was blinded against the SD Bioline results.

RESULTS

A total of 105 acid fast bacilli–positive liquid cultures were selected for the present study. The control band was seen in all tested cultures, validating the test. The H37 Rv control showed the appearance of a pink band in the test region (T band), confirming the presence of the MPT 64 antigen. Seventy-seven isolates were identified as *M. tuberculosis* and 27 were identified as MOTT by both biochemical identification and the SD Bioline assay.

Discrepant results were found among two isolates. One culture, positive for the SD Bioline test, was reported as MOTT by the conventional biochemical test (false positive), whereas, another was negative by the SD Bioline test and reported *M. tuberculosis* by biochemical identification (false negative). The SD Bioline test was repeated for both the isolates. A positive band was observed in one of the isolates reported negative earlier by the SD Bioline test, whereas, for the second isolate the results were the same. The sensitivity, specificity, positive predictive and negative predictive values of the SD AgMPT64 kit were found to be 100, 96.4, 98.72, and 100%, respectively [Table 1].

Turnaround time

The average turnaround time from sample collection to identification of the *M. tuberculosis* complex by the SD Bioline assay was 18 days for smear negative samples, 14 days for samples with a 1+ smear and 7 days for samples with 2+ and 3+ smears. The biochemical identification of the clinical isolates required a time range of 30 to 70 days.

Presence/absence of cords

The SD Bioline test results were correlated with the presence or absence of cords in liquid culture smears. Twenty-two liquid cultures identified as MOTT showed

Table 1: Comparative results of the biochemical test and the SD bioline assay for identification of mycobacteria

SD bioline assay	Biochemical identification		Sensitivity (%)	Specificity (%)	Predictive value (%)	
	M. tuberculosis	MOTT			Positive	Negative
Positive	77	1	100	96.4	98.72	100
Negative	0	27				
Total	77	28				

atypical morphology/lack of cords/loose clusters in ZN smears [Figure 1]. The remaining five SD bioline-negative clinical isolates showed cords ($n = 5$) and all these isolates were also reported as MOTT by the biochemical test.

DISCUSSION

Tuberculosis remains the major public health problem in India and accounts for one-fifth of the global TB incident cases. The capacity of the laboratories to perform a rapid culture, identification and DST of *M. tuberculosis* from clinical specimens is vital in the management of tuberculosis patients. RNTCP has recommended the automated culture system for rapid isolation of the mycobacteria. Although most of the mycobacterial cultures belong to the *M. tuberculosis* complex, clinically and therapeutically the differential identification of *M. tuberculosis* from mycobacteria other than tuberculosis (MOTT) is very important. Therefore, a simple and rapid method for the identification of mycobacterial culture isolates will be of enormous help in resource-poor countries.

The sensitivity, specificity, positive predictive and negative predictive values of the SD AgMPT64 kit was found to be 100, 96.4, 98.72, and 100%, respectively.

There was a strong intensity of the test band in the SD bioline assay against all *M. tuberculosis* isolates except one culture, which showed a weak reaction. The band intensity was most likely proportional to the available antigen concentration in the liquid culture and it could be related to less production of protein due to a low bacillary load in the culture.

It has been shown that the MPT64 protein is not produced by some *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) substrains.[14] Few studies have evaluated the SD MPT64 TB antigen rapid ICT kits,[11,15,16] with sensitivity ranging from 97 to 100% and a specificity of 100%. False negative results due to unique mutations in the *mpb64* gene have been reported,[17] which necessitate validation in diverse settings.

One isolate was reported as MOTT by biochemical identification and *M. tuberculosis* by SD Bioline. The reason

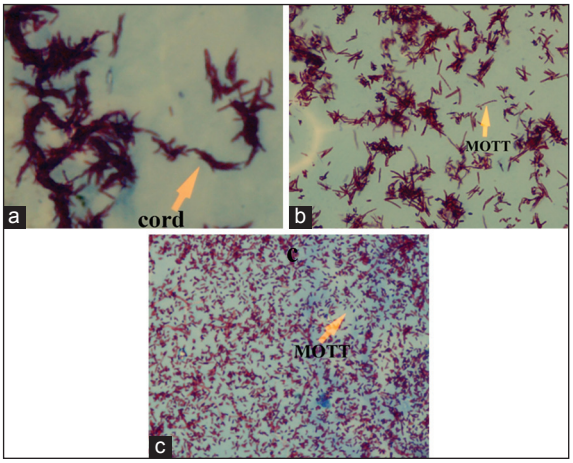

Figure 1: Structure of the *M. tuberculosis* complex (a) and MOTT (b and c) in liquid culture, (a) serpentine cords, (b) loose cords, and (c) individual scattered acid fast bacilli

could be a mixed infection by both the *M. tuberculosis* complex and MOTT, which led to a positive test result for SD Bioline as well as niacin, nitrate. One isolate that was initially negative by the SD Bioline assay turned out to be positive after repeating the test from the subculture. For cultures with SD bioline negative results, ZN smear should be checked for the presence/absence of serpentine cords, repeating test from the subcultures and correlation with biochemical tests/molecular methods should be considered before reporting the results.

Serpentine cord formation is a differential characteristic of the *M. tuberculosis* complex, as MOTT could be found in the form of clusters or remained single.[4] in the liquid media. ZN staining was is a very simple and rapid technique, but does not accurately discriminate *M. tuberculosis* and MOTT. In an Indian study, 46% of the MOTT cultures were noted for cord formation. In our study, out of 28 MOTT isolates, six showed either true cords or unusual cords (21.4%). Studies conducted to observe cord formation for the *M. tuberculosis* complex showed specificity ranging from 92.9 to 99.6%.[18,19] Therefore, only a ZN smear examination of the liquid culture is not accurate for the identification of *M. tuberculosis* complex. The number of MOTT included in the present study does not reflect the prevalence of MOTT in the community, as a majority of SD-negative MGIT cultures were subcultured into the LJ medium for

biochemical identification, to include a representative number of such isolates.

The SD Bioline TB Ag MPT64 kit is currently available at a negotiated cost of Rs. 4250/ per box of 25 tests or Rs. 170/ per specimen. The cost of consumables for differentiation by biochemical tests will be approximately the same. The rapidity of the test markedly reduces the turnaround time in the MTB culture and DST and can contribute significantly to the TB control program.

Our results demonstate the utility of the SD Bioline test for identification of the mycobacteria. Immunochromatograpic assays detecting the MPT64 antigen can be used as replacement for the conventional identification tests. The simplicity of the method, low cost, and the rapidity of the test make it appropriate for use in TB diagnostic laboratories.

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