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MPT 64 antigen detection for Rapid confirmation of Mycobacterium tuberculosis complex

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ABSTRACT
The Mycobacterium tuberculosis complex (MTBC) is a known agent for infectious pulmonary 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. The SD MPT 64 TB Ag Rapid ICT kit is a simple, reliable, rapid, low-tech identification kit which can be used as a diagnostic tool in resource poor diagnostic centers for accurate identification of MTB isolates.

Keywords: Mycobacterium tuberculosis, NTM, MPT 64

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]

Characterization of mycobacteria can be done phenotypically or genotypically. 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 (growth on PNBA medium), involve use of hazardous chemicals, some of which are carcinogenic (niacin test) and are prone to subjective error in interpretation of results (nitrate reduction). (3) On the other hand, molecular methods that identify specific nucleic acid sequences are rapid, sensitive and specific, but are expensive and require trained personnel and special laboratory setup.(4) Hence there is need for a rapid, accurate and simple test for characterization of mycobacteria.

The Mycobacterium tuberculosis protein 64 (MPT-64) antigen is one of the major culture filtrate protein (24 kDa) encoded by the RD2 region genes and has been shown to be a specific antigen that differentiates the MOTT species (5). An MPT64-based, simple and rapid immunochromatographic assay known as the SD Bioline TB Ag MPT64 RAPID test (SD Bioline kit) has been reported to identify the *M. tuberculosis* complex from the NTM using the mouse monoclonal anti-MPT64 antibody (6). The present study was conducted to check the utility of the SD Bioline kit for the identification of mycobacteria. These findings were correlated with a conventional biochemical test.

MATERIALS AND METHODS:

In this study, a total of 100 mycobacterial isolates on Lowenstein-Jensen (L-J) medium recovered from both pulmonary (50 isolates) as well as extra pulmonary specimens(50 isolates)

were included. The positive cultures were screened to differentiate the growth of mycobacteria into MTBC and NTM by niacin accumulation test, catalase 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, Mac Conkey agar test and Sodium Chloride tolerance test. All the cultures were also subjected to TB Antigen MPT64 rapid test for differentiation into MTBC and NTM. Manufacturer's instructions were followed during the test. The entire test procedure was carried out inside a biosafety cabinet category -II (BSC-II).

3- 4 colonies from L J Medium isolates & H37 Rv strain were emulsified in 200µl 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 of 15 minutes for presence of pink band in "Control" and "Test" region (7) . 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. (7)

RESULTS:

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, 47 were positive for niacin test, negative for catalase and PNB test. The three isolates from extrapulmonary cases were positive for PNB test. Therefore, all the 97 isolates were identified as MTBC and the three isolates from extrapulmonary cases identified as NTM on the basis of biochemical tests. These three NTM isolates from extrapulmonary specimens were further identified as *Mycobacterium fortuitum(2)* and *Mycobacterium chelonae(1)* on the basis of biochemical tests. These isolates belong to Group IV (Rapid Growers) of Runyon's Classification.

H37 Rv control showed the appearance of pink band in the test region (T band) confirming the presence of MPT 64 antigen. When subjected to TB Ag MPT64 Rapid test, the control band was seen in all the tested cultures (n=100), validating the test. All the 50 isolates from pulmonary cases and 47 isolates from extrapulmonary cases showed positive results (visible band for MPT64 antigen) and the three 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 (Table 1).The sensitivity and specificity of TB Antigen MPT64 rapid test was found to be 100% as compared to biochemical methods.

Table 1: comparison of MPT 64 and Biochemical test

MPT 64 Antigen test result	**Biochemical test result**		**Total**
	MTBC	**NTM**	
Positive	97	0	97
Negative	0	3	3
Total	97	3	100

DISCUSSION:

Tuberculosis is an endemic disease of great antiquity. Tuberculosis (TB) is a major public health problem

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in India. India accounts for one-fifth of the global TB incident cases. Each year nearly 2 million people in India develop TB, of which around 0.87 million are infectious cases. It is estimated that annually around 330,000 Indians die due to TB. TB is one of the leading causes of mortality in India killing 2 persons every three minutes, nearly 1,000 every day. Early diagnosis well supported by a Mycobacteriology laboratory equipped to perform culture, identification and drug susceptibility testing of *Mycobacterium tuberculosis* (MTB) from clinical specimens is vital in the management of tuberculosis patients. The automated systems have improved the speed of isolation but there is a need for rapid identification of MTB isolates. Novel technologies for rapid identification of the culture isolates and anti-tubercular drug resistant isolates has become a top priority not only in TB research but also for diagnosis & treatment purposes.

The modern molecular methods are not economically suitable for resource poor laboratories. A cheap, rapid & reliable identification test method will be of enormous help in resource poor countries. The new rapid Immunochromatographic methods have been found to be such ideal diagnostic aid in TB control programme.

Mycobacterium tuberculosis protein 64 (MPT-64) antigen is found to be specific for MTBC which is secreted during mycobacterial growth. Cost-effective analytical studies of MPT64 TB Ag test, other rapid molecular methods and culture combined with conventional biochemical tests have shown MPT64 TB Ag test as more economical than the other two methods. Further, this test requires only 15 min of analysis as compared to other methods. Kumar et al(India) (8) , Toihir et al(Madagascar) (9) and Abe et al (Japan) (6) showed 100% sensitivity and specificity as seen in the present study. The MPT-64 TB antigen is a *M.tuberculosis* complex specific antigen secreted during bacterial growth, thus making it an excellent antigen for the identification of MTBC.

CONCLUSION:

The SD MPT 64 TB Ag Rapid ICT kit is a simple, reliable, rapid, low-tech identification kit which can be used as a diagnostic tool in resource poor diagnostic centres for accurate identification of MTB isolates. The SD MPT64 assay showed excellent performance as a tool for the rapid identification of MTBC. The rapidity of this test will markedly reduce the turnaround time in MTB culture & Drug susceptibility testing. The low cost, rapidity, High specificity, Sensitivity of the MPT 64 antigen detection makes that it is a very useful diagnostic tool.

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