**Rapid detection of *Mycobacterium tuberculosis* by using**

**MPT64 immunochromatographic assay**

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**Abstract**

Tuberculosis (TB) is considered to be one of the major infectious diseases in the developing countries and is more prevalent in India. The confirmatory identification of *M. tuberculosis* (MTB) is still being done by the conventional, laborious and time consuming biochemical methods. Before this period of confirmation, an infected individual continues to spread the disease to many other susceptible individuals. Therefore, there is a need for a rapid and effective identification of Mycobacteria and a robust drug sensitivity testing for effective treatment. MPT64, one of the major culture filtrate protein, is a specific antigen that differentiates the M. tuberculosis complex from the mycobacteria other than tuberculosis (MOTT) species. The present study evaluated the identification of *Mycobacteria tuberculosis* from MOTT by using SD Bioline kit and the findings were correlated with a conventional biochemical test and the sensitivity, specificity, and predictive values of this kit were also assessed. 80(n=80) mycobacterial isolates from Sputum and BAL were tested by using MPT64 immunochromatographic assay and validated by standard microscopic and biochemical assays. It was observed there was no significant difference in identification of MTBC isolates between SD MPT64 TB Ag Kit® assay and Biochemical test. Hence, the sensitivity, specificity, positive predictive & negative predictive values of the SD Ag MPT64 kit was found to be 100%. Thus the simplicity, cost effectiveness, and the rapidity of the test make it appropriate for use in TB diagnostic laboratories.

**Keywords:** MPT64, MTB, Bioline

**Introduction:**

Tuberculosis (TB) is an infectious disease caused by the bacillus Mycobacterium tuberculosis (Mtb). It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB). It is spread by air and potentially fatal disease of human (16).

Developing TB disease is higher among people infected with HIV [16]. The 2013 World Health Organization (WHO) estimated and showed 9 million (range, 8.6 million - 9.4 million) cases globally. There were 1.5 million TB deaths [15]. In India, about 2.0 - 2.3 million incident cases of TB are reported annually, which account for a fifth of new cases in the world—a greater number than any other country [16]. The situation is now further worsened by the increasing number of drug-resistant cases of TB. Thus, there is a need for rapid and correct identification of Mycobacteria and rapid drug sensitivity testing for effective treatment of the disease [14, 15] .

Automated culture systems like Bactec 460, MBBacT (Bio Merieux, France), MGIT have significantly reduced the time for culture, but do not help in differentiating MTB & MOTT. Further Confirmatory Identification of *M. tuberculosis* (MTB) is still being done by conventional biochemical methods. These tests are laborious, time consuming and require elaborate safety precautions. During this long and laborious process, an infected individual continue to spread the disease to many other susceptible individuals. The recent objective of WHO is to reduce the time for culture, identification, and drug resistance detection to as short as 2 days by employing nucleic acid amplification assays. This method requires a specialized set up, trained personal & is not suitable for resource poor countries (5).

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). It is a lateral flow test has been reported to identify the M. tuberculosis complex from the MOTT using the mouse monoclonal anti-MPT64 antibody impregnated on nitrocellulose membrane. [1,7,10]

MPT64 is one of the major culture filtrate protein (24 kDa)[12,17] encoded by the region of RD2 genes[2] and it is a specific antigen that differentiates the M. tuberculosis complex from the mycobacteria other than tuberculosis (MOTT) species. The actively secreted proteins of M. tuberculosis are the first to interact with the host immune system and recognized by human Th1 cells. Therefore it could be useful for TB diagnosis or as part of a novel candidate vaccine against TB (8,9). A large amount of variability in the diagnostic accuracy of MPT64 has been reported, depending on the recombinant antigen used in assays [12,17]

The present study was conducted to evaluate the identification of *Mycobacteria tuberculosis* from MOTT by using SD Bioline kit for and findings were correlated with a conventional biochemical test and the sensitivity, specificity, and predictive values of this kit were also assessed.

**Materials and Methods:**

80(n=80) mycobacterial isolates from Sputum and BAL, one control strain H3R7V, 15(n=15) Gram positive and gram negative bacterial isolates from Urine, Sputum and BAL were tested by using MPT64 immunochromatographic assay and validated by standard microscopic and biochemical assays.

**Immunochromatographic assay:**

TB Antigen MPT64 rapid ICT kit, manufactured by SD Bioline, Seoul, South Korea, was used as per the manufacturer’s instructions [13].Test kit immobilized with Mouse monoclonal antibodies, against MPT64 antigen, on a nitrocellulose membrane for confirmation of MTB isolates. The entire test procedure was carried out inside a biosafety class II cabinet.

2 - 4 isolated colonies were scraped from the solid LJ medium and suspended in 200 μl of extraction buffer provided in the kit. The emulsified solution was applied in the sample well as per manufacture instruction. After 15 minutes of sample application the test was interpreted at room temperature. The absence of band in the control region was considered invalid. All the ICT results were validated by biochemical test (Niacin test and Nitrate Reduction test).

**Results:**

80(n=80) mycobacterial isolates were cultured in LJ media, after 8 weeks of incubation it was further confirmed by smear by using Ziel Neelson method. Validation of mycobacterial spp was done by niacin test and nitrate reduction test of these 80 isolates 75 were (n=75) MTB and 5(n=5) isolates were MOTT shown in figure

All 80 isolates showed pink color band in control line and One H37Rv control strain shows pink colored band in both control and test line for confirming the presence of Tb MPT64 ag. 73 isolates of *M.tuberculosis* showed dark pink band in test line, 2 isolates showed faint band on test region were subjected to a repeat test with a longer incubation period of 48 hours as well as by Biochemical test (niacin and nitrate reduction test) and found to be MTBC.15 Gram positive and gram negative isolates shows negative band seen in control line, not seen in test line.

No significant difference was shown in identification of MTBC isolates between SD MPT64 TB Ag Kit® assay and Biochemical test. Hence, the sensitivity, specificity, positive predictive & negative predictive values of the SD Ag MPT64 kit was found to be 100%

**Discussion:**

Tuberculosis is one among the major public health issues in developing countries and stands for one-fifth of the total world TB incident cases and it is leading issues of mortality in India killing 2 persons every three minutes, nearly 1,000 every day (16).

Microscopy detection of AFB is rapid but less sensitivity and cannot discriminate between mycobacterium spp. Early diagnosis of TB is too difficult and well-equipped biosafety laboratory need to perform culture, identification and drug susceptibility testing of *Mycobacterium tuberculosis* and it is crucial thing for the management of tuberculosisincidence (16).

RNTCP has recommended the automated culture system for rapid detection of the mycobacterial isolates (5), but still need rapid method for detection of MTB isolates. Newer rapid identification method of the culture and Anti tubercular drug resistant isolates has become a high priority for diagnosis & patient management of tuberculosis (16).The new rapid Immunochromatographic methods have been found to be such ideal diagnostic tool in TB control programme (6)

The main aim of the study is to evaluate rapid and economically cheaper test which detect accuracy of mycobacterial isolates. Differentiation of MTB from MOTT is very important for clinically and therapeutically. In most of the laboratories using conventional biochemical and smear microscopy for differentiation. It requires skilled labor and time consuming process for identification. But these new immunochromatographic tests doesn’t not require skilled labor and require biosafety cabinet to perform, to get accuracy of the results within 20 minutes to confirm.

A commercial available ICT test kit was evaluated by 784 culture isolates for Rapid Identification of MTB, by using Accu probe -MTB as the reference method for MTB identification & comparative evaluation of the rapid kit. The sensitivity of the rapid test kit was found to be 99.2%. (381/384) and there is no false positive results were detected in this study. Thus the studies showed the specificity as 100% and concluded that the ICT test is an useful method for rapid & routine identification of MTB isolate (11).

Few studies have evaluated by using SD MPT64 TB antigen rapid ICT kits,[1,3,4] with sensitivity ranging from 97 to 100% and a specificity of 100% and false negative results occurs due to unique mutations in the mpb64 gene have been reported,[4]. This is a common feature encountered with both commercial kits.

In this study, there was no significant difference was found in the predictive values, sensitivity & specificity for isolates. The band intensity was more prominent in the liquid cultures. During the study was made to attempt and evaluate the ICT for identification of MPT 64 antigen in smear positive sputum samples. The cost of consumables for differentiation to biochemical tests will be approximately the same. The rapidity of the test will reduce markedly at the time of the MTB culture and DST and can contribute significantly to the TB control program. Our results demonstrate and found 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.

**Conclusions:**

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