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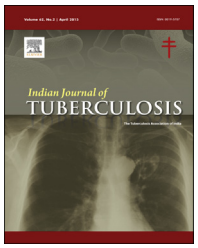

Original Article

Rapid identification of *Mycobacterium tuberculosis* complex in clinical isolates by combining presumptive cord formation and MPT64 Antigen Immunochromatographic Assay

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ABSTRACT

Purpose: Combining the results of presumptive cord formation in smear and MPT64 Antigen Immunochromatographic Assay has been suggested to reduce the false negative and positive rates for identification of *Mycobacterium tuberculosis* (MTB) complex in liquid culture. This study was done to evaluate the clinical utility of combining the results of the two tests for rapid identification MTB complex in mycobacterial isolates.

Methods: 484 isolates of mycobacteria obtained in MGIT culture were identified using presumptive cord formation in smear and further by MPT64 Antigen ICT assay. Result obtained were analyzed taking PNB inhibition test as the reference standard.

Results: Combining the results of the two tests, 464 (95.9%) isolates were correctly identified while discrepant results were obtained in 20 (4.1%) isolates. When the results of the two tests were intersected, the specificity and PPV was 100%, but the sensitivity decreased to 96.4% and the NPV to 68.6%. On the other hand, when the results of the two methods were combined, the sensitivity and NPV was 100%, but the specificity decreased to 88.6% and the PPV to 99.1%.

Conclusion: Presumptive cord formation and MPT64 antigen ICT assay can be used in combination for identification of MTB complex. When both the test are positive, the culture can be reported to contain MTB complex. If both the tests are negative, the culture should be reported to contain NTM. Only when discrepant results are obtained by the two tests, further evaluation is necessary to ensure an accurate diagnosis.

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1. Introduction

The global burden of tuberculosis (TB) remains enormous. In 2012, there were an estimated 8.6 million estimated cases of TB, out of which 2.3 million were from India. The emergence and spread of drug-resistant *Mycobacterium tuberculosis* (MTB) strains poses significant challenges to disease control. As per the WHO Global Report on Tuberculosis 2013, India accounts for 64,000 multidrug-resistant tuberculosis (MDR-TB) cases out of 300,000 cases estimated globally to occur among the notified pulmonary TB cases annually.[1] Rapid and accurate diagnosis of these cases is crucial for patient management and control of disease transmission.

Culture remains the gold standard for the definitive diagnosis of tuberculosis. Introduction of liquid culture technology have aided in the rapid isolation of mycobacterial species. Various commercially available liquid culture systems use Middlebrook 7H9 broth for better recovery and faster growth of mycobacteria. The media is very sensitive and as a result is prone to contamination not only by non mycobacterial organisms but also by Non tuberculous Mycobacteria (NTM) originating from patients flora or laboratory reagents. These NTM isolates have to be differentiated from MTB complex before proceeding to drug susceptibility testing as clinical significance of NTM, especially in high prevalence TB setting is still under debate.[2]

Several methods are available to identify MTB and differentiate it from NTM. Molecular methods and high performance liquid chromatography have been described as accurate and rapid, but require procedures that are technically complex, laborious and costly. Para-Nitrobenzoic Acid (PNB) inhibition test is recommended for differentiation of MTB from NTM in commercial liquid culture systems but it requires additional time of 4–11 days from the detection of a positive culture to the identification of an isolate.[3]

Presumptive identification of MTB complex by its ability to grow as serpentine cords in liquid culture medium has been previously reported.[4,5] Visualisation of cord formation by Ziehl–Neelsen (ZN) stain provides rapid preliminary information before the results of other identification methods are available. However few NTMs also form true cords or pseudocords, that are loose aggregate of bacilli, in liquid media leading to false results.[5] MPT64, a 24 kDa protein, is one of the major antigens secreted by members of MTB complex in culture medium. Detection of antigen by immunochromatography (ICT) using monoclonal antibodies against MPT64 antigen has been suggested to be a rapid and cost effective method of identification of MTB complex isolates.[6] The specificity and sensitivity of >92% has been reported in various studies.[6–8] Reported false-negatives have likely resulted from low numbers of bacteria in the cultures or mutations in the MPT64 gene of the bacteria.[7,9]

This study was undertaken to evaluate the clinical utility of combining presumptive cord formation and MPT64 Antigen ICT Assay to rapidly and accurately identify MTB complex in positive liquid culture isolates.

2. Methods

This prospective study was conducted at a large tertiary care hospital of India. Specimens submitted for routine analysis in mycobacteriology laboratory were studied. All procedures requiring biosafety precautions including processing of specimens, inoculation of media, and identification were performed in a Class II Biosafety Cabinets dedicated for mycobacterial work.

A total of 484 mycobacterial isolates obtained in Mycobacterium Growth Indicator Tube (MGIT) liquid culture (Becton Dickinson and Company, USA) from 2286 consecutive clinical specimens over a period of one year were included in the study. Smears made from positive MGIT tube were stained with ZN stain to confirm the isolate as Acid Fast Bacilli (AFB) and serpentine cord morphology was recorded. Serpentine cording was defined as tight, rope-like aggregates of acid-fast bacilli in which the long axes of the bacteria paralleled the long axis of the cord. Microscopic morphology and organism orientation that did not meet the above criteria were considered negative for cording (Fig. 1).

All AFB positive isolates were further subjected to identification of MTB complex by MPT64 Antigen ICT assay (SD Bioline TB Ag MPT64 rapid kit) as per manufacturer's instructions. Briefly 100 μl of sample obtained from positive MGIT tube was applied directly to the sample well without preparation. Tests were interpreted 15 min after sample application. The presence of a control band alone indicates a negative result, whereas the presence of two color bands (control and test bands), no matter which band appears first, indicates a positive result for MTB complex. A color band of any intensity was read as a positive reaction. The absence of control band after the test was considered invalid (Fig. 2).

Identification of MTB complex by PNB inhibition test was taken as reference standard. PNB was added to get a final concentration of 500 μg/ml in the MGIT tube. 0.5 ml of positive culture was inoculated into two MGIT tubes with and without PNB and incubated at 37 °C. MGIT tubes were read daily using Micro MGIT fluorescence reader (Becton Dickinson and Company, USA). A strain was considered susceptible when the tube containing the PNB did not show fluorescence 2 days after positive results were observed in the control tube, whereas if fluorescence occurred the strain was considered resistant. Discordant results were resolved by means of results of biochemical tests and molecular test – Line probe assay. The result obtained were subjected to statistical analysis.

The reference strain H37Rv was used as positive control for MGIT, MPT64 antigen ICT assay and PNB inhibition test.

3. Results

Of the 484 mycobacterial isolates obtained in MGIT liquid culture, 449 were identified as MTB complex and 35 as NTM. Result of identification by presumptive cord formation and MPT64 Antigen ICT assay in comparison to reference PNB inhibition test is shown in Table 1. Four hundred and thirty eight MTB complex isolates and three NTM isolates demonstrated