Original Article

Utility of a lateral flow assay for culture confirmation of Mycobacterium tuberculosis complex

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A B S T R A C T

Background: Therapy for the clinical management of patients with Mycobacterium tuberculosis complex (MTBC) and non tuberculous mycobacteria (NTM) is different. Prompt detection and discrimination is necessary for administration of suitable therapy.

Methods: The aim of the study was to evaluate the performance of an Immunochromatographic Test (ICT) in rapid differentiation of MTBC from NTM grown in Lowenstein-Jensen medium and MGIT broth in comparison to molecular methods.

Results: Of the 106 isolates in this study, 96 and 95 were identified as MTBC by 16S rRNA PCR and MPT64 respectively. The sensitivity and specificity of MPT64 was found to be 99% and 100% respectively.

Conclusion: The Lateral Flow Assay Test is a useful and specific tool in rapid differentiation of M. tuberculosis complex from culture. Therefore proper identification avoids unnecessary ATT to patients infected with NTM.

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Introduction

Non tuberculous mycobacterial (NTM) species have shown an increasing association with clinical infections. These NTM species are resistant to first-line anti-TB drugs and when erroneously identified as Mycobacterium tuberculosis, give rise to a mistaken diagnosis of multidrug-resistant TB (MDR-TB) when treated with ATT. Therefore, it is important to differentiate M. tuberculosis complex from NTM as soon as possible in both pulmonary as well as extrapulmonary clinical specimens.

The identification of M. tuberculosis using biochemical methods is a complex, labor-intensive, and time-consuming process. Nucleic acid amplification (NAA) methods, such as real time PCR, AccuProbe Mycobacterium tuberculosis complex culture identification test (Gen Probe Inc., CA), the GenoType Mycobacterium CM test (Hain Lifescience, GmbH, Germany) are both rapid and specific but are technically challenging and they require the use of sophisticated instruments. Antigen detection tests have not been widely used, although they are rapid, simple, and more affordable.

Amongst M. tuberculosis antigens, the most often studied have been LAM and MPT64. Lateral flow assays, also called immunochromatographic assays, have been developed for the discrimination between M. tuberculosis complex and non tuberculous mycobacteria. These include the SD Bioline Ag MPT64 Rapid assay (Standard Diagnostics, Kyonggi-do, Korea),

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Capilia TB (TAUNS, Numazu, Japan), and the MGIT TBc Identification Test (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD). These sandwich-type assays use a monoclonal antibody to detect the MPB64 protein (Rv1980c; also termed as MPT64), which is specifically secreted during growth by the M. tuberculosis complex. The MPB64 is a 24 kDa protein, highly specific for the M. tuberculosis complex and differentiates between MTBC and NTM. Rapid immunochromatographic tests (ICT) that detect the M. tuberculosis complex (MTBC) MPT64 protein are cheaper and simpler to use and thus may have an important role in resource poor settings and also early diagnosis.

**Material and Methods**

The study was carried out in a tertiary care teaching centre between January 2012 and May 2013. MGIT TBc identification test (TBc ID; Becton Dickinson, USA) was compared with Real time PCR targeted at 16S rRNA for identification of MTB complex after growth on liquid as well as solid media.

Assuming a sensitivity of 99% for the new test with 85% for the reference test and a power of 90% within confidence interval of 95% the estimated sample size required was 77. One hundred and thirty smear positive sputum specimens were processed by modified Petroff’s method and then cultured on LJ medium and MGIT broth (Middlebrook 7H9). Of these, 106 specimens showed growth of AFB in both solid and liquid culture media and were included in the study. Two LJ cultures showed contamination and were not included in the study. All the 106 isolates were subjected to the ICT Lateral Flow Assay and real time PCR targeting 16S rRNA.

The TBcID assay (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD) consists of a nitrocellulose membrane on a test device with immobilized anti-MPB64 mouse monoclonal antibodies conjugated with gold colloidal for the detection of the MPB64 protein. When samples are added to the test device, MPT64 antigen binds to anti-MPT64 antibodies conjugated to visualizing particles on the test strip. The antigen-conjugate complex migrates across the test strip to the reaction area and is captured by a second specific MPT64

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**Fig. 1 – MPT64 ICT – Identification of Mtb complex A:**
Negative for Mtb; B: Positive for Mtb complex.

**Fig. 2 – 16S rRNA Real Time PCR:** Panel A shows lack of Melting peak for NTM (Blue), Panel B Shows peak for Mtb.
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