Rapid Characterization of *Mycobacterium tuberculosis* Complex isolated from Clinical Samples by SD TB Ag MPT 64 kits

**Pratibha Sharma***, Deepthi Nair**, Monorama Deb***

**Abstract**

*Purpose:* The present study aimed at evaluation of SD TB Ag MPT 64 Rapid kit for confirmation of isolates of *Mycobacterium tuberculosis* complex (MTBC) grown from clinical samples.

*Material & Methodology:* The present study included a total of 105 mycobacterial growths recovered from various pulmonary and extra-pulmonary clinical specimens in a tertiary care center. Culture was performed using Lowenstein Jensen’s media and BacT ALERT 3D automation system. The growths were subjected to both Accuprobe culture identification (Genprobe, San Deigo, CA) and SD TB Ag MPT 64 rapid kit (Standard Diagnostics, INC, Korea). SD TB Ag MPT 64 rapid kit was evaluated in the present study using Accuprobe as gold standard.

*Results:* The sensitivity and specificity of SD TB Ag MPT 64 rapid kit was 92.3% and 100% respectively.

*Conclusion:* SD TB Ag MPT 64 rapid kit is easy to perform, rapid and cheap test which can be used as a screening tool for rapid confirmation of the MTBC isolates in a routine tertiary care setting.

**Keywords:** *Mycobacterium tuberculosis*, Rapid, identification, MPT 64 antigen assay.

**Introduction**

Definitive diagnosis of tuberculosis depends upon the isolation and identification of the etiological agent. In specific media, both *Mycobacterium tuberculosis* (MTB) and non tubercular mycobacterium (NTM) grow from clinical specimens. Rapid identification of culture isolates and detection of antitubercular drug resistance is essential for diagnosis and treatment purposes and hence appropriate management of the disease.

Disease caused by NTMs is on rise with parallel increase in HIV infection and other immune-compromised states. As liquid TB culture was recommended by WHO in 2007 for rapid detection and drug susceptibility test as standard method for TB diagnosis and case management, automated culture system like MGIT, BACTEC 460, MB Bact alert 3D automation system (Biomerieux, France) have significantly reduced the turnaround time for culture but do not help in differentiating MTB and NTMs. NTMs are clinically important as they trigger disease and true infection but are inappropriately managed with 1st line anti tubercular drugs because of lack of discrimination between MTB and NTMs in small hospital laboratories which further worsens the patient’s condition and hence raises the risk of drug resistance. Conventional methods are still used for confirmatory identification of MTB isolates which are laborious, time consuming and require elaborate safety precautions. Chemiluminescent DNA probes, nucleic acid amplification assays, high performance liquid chromatography and sequencing of 16S r RNA are more sophisticated, but are costly in terms of specialized equipment, labor and time. Hence, simple, sensitive, and rapid method for mycobacterial identification is necessary.

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SD BIOLINE TB Ag MPT 64 rapid kit, developed by Standard Diagnostics, Korea is based on confirmation of culture by antigen detection assay of tuberculosis. It is an immune-chromatography test for detection of Mycobacterium tuberculosis protein 64 (MPT64) antigen, also termed as RV 1980c, a 24 kDa protein which is secreted during bacterial growth and Mycobacterium tuberculosis complex except some strains of M. bovis BCG.\(^{12,13}\) Mouse monoclonal anti MPT 64 antibodies (test line) and goat anti mouse antibody (control line) are immobilized on a nitrocellulose strip. Another mouse monoclonal antibody recognizing a different epitope of MPT 64 antigen conjugated with colloidal gold particles is present in the sample well. On addition of MPT 64 antigen, it gets captured to both types of monoclonal antibodies and give a visible test band. The mouse monoclonal antibodies conjugated with colloidal gold particles combine with goat anti mouse antibody to give a control band. The present study evaluates the SD TB Ag MPT 64 Rapid kit for identification of Mycobacterium tuberculosis grown in Bact/ ALERT TB culture bottles from pulmonary and extra pulmonary samples.

**Material & Methods**

The present study was conducted over a period of nine months (March 2012-Dec 2012) in Department of Microbiology, Vardhman Mahavir Medical College and Safdarjung Hospital, 1600 bed, multi specialty tertiary care center, New Delhi, India. Clinical samples received by mycobacteriology laboratory on routine basis were processed for culture by Bact Alert 3D automation (Biomerieux, France) as per the manufacturer’s guidelines. The mycobacterial growths so obtained were confirmed to be Mycobacterium tuberculosis complex by AccuProbe culture identification kit and SD TB MPT 64 Ag rapid kit. AccuProbe culture identification kit was used as the gold standard and SD MPT 64 Ag rapid kit was evaluated in statistical terms. AccuProbe culture identification kit (Gen Probe, San Diego, CA) was performed with the growths in Middle brook 7H9 broth with turbidity >1 McFarland 1 Nephelometer standard as per the manufacturer’s recommendations. Samples were prepared by pipetting 100ul of lysis reagent, 100ul of hybridization buffer with 100ul of culture of mixed culture broth, sonicated for 15 min after degassing the sonicator. Sample lysis was performed by placing the lysing tubes at 95\(^\circ\)C for 10 min. Probes were hybridized (DNA:RNA hybrid) by placing 100ul of lysed specimen in probe tubes and incubating for 15min at 59.5\(^\circ\)C-61\(^\circ\)C heating block. Hybridized and non hybridized probes were selected by adding 300ul selection reagent and reading in chemiluminometer using appropriate protocol. Samples producing signals >30,000RLU were considered positive and below 20,000 RLU were negative. Signals between 20,000 and 29,999 RLU range were repeated. SD TB Ag MPT 64 Rapid kit was performed by directly applying 100ul of liquid culture broth on the sample well (S) without use of sample preparation procedure. From solid cultures, 3-4 colonies were suspended in 200ul of extraction buffer provided in kit prior to test. 100ul of this suspension was placed on the sample well. The test results were interpreted in 15 min. The presence of only control band (‘C’ band) within the result window indicates a negative. The presence of two control bands (‘C’ & ’T’ band) within the test window indicates positive result.

**Results**

A total of 105 Mycobacterium isolates were recovered from sputum (42), pus (28), cerebrospinal fluid (22), aspirates (5) and urine (8) over a period of ten months. Of these isolates, 74.2% (78/105) was confirmed to be Mycobacterium tuberculosis complex by AccuProbe culture identification kit. Of these 105 isolates, 68.5% (72/105) could be detected by MPT 64 TB Ag Rapid kit. All the 25.7% (27/105) of the isolates which were negative by AccuProbe identification also gave negative result with MPT 64 TB antigen kit. Table 1 shows the sample distribution and MTB confirmed by both the techniques. The sensitivity, specificity, positive predictive value and negative predictive value for MPT 64 Ag rapid kit is shown in Table 2.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of total MTBC isolates recovered (n)</th>
<th>AccuProbe positive MTBC (n)</th>
<th>AccuProbe negative isolates (n)</th>
<th>AccuProbe MTBC Positives (%)</th>
<th>MPT 64 TB Ag positive MTBC (n)</th>
<th>MPT 64 TB Ag negative isolates (n)</th>
<th>MPT 64 TB Ag Pos MTBC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>42</td>
<td>30</td>
<td>12</td>
<td>71.4</td>
<td>29</td>
<td>13</td>
<td>69.0</td>
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<tr>
<td>Pus</td>
<td>28</td>
<td>22</td>
<td>6</td>
<td>78.5</td>
<td>19</td>
<td>9</td>
<td>67.8</td>
</tr>
<tr>
<td>CSF</td>
<td>22</td>
<td>17</td>
<td>5</td>
<td>77.3</td>
<td>16</td>
<td>6</td>
<td>72.7</td>
</tr>
<tr>
<td>Aspirate</td>
<td>5</td>
<td>5</td>
<td>0</td>
<td>100</td>
<td>5</td>
<td>0</td>
<td>100</td>
</tr>
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<td>Urine</td>
<td>8</td>
<td>4</td>
<td>4</td>
<td>50.0</td>
<td>3</td>
<td>5</td>
<td>37.5</td>
</tr>
<tr>
<td>Total</td>
<td>105</td>
<td>78</td>
<td>27</td>
<td>74.2</td>
<td>72</td>
<td>33</td>
<td>68.5</td>
</tr>
</tbody>
</table>

**Table 1. Sample Distribution and MTBC Confirmed by both the methods (Accuprobe identification and MPT 64 TB antigen kit)**
Discussion

Rapid & early diagnosis, culture identification and drug susceptibility testing of *Mycobacterium tuberculosis* complex isolates recovered from clinical specimen is vital in management of tuberculosis patients. Researchers were continuously in search of an alternative, rapid and reliable method for identifying mycobacterium species isolated in culture. A rapid immuno chromatographic (ICT) test has been recently developed for confirmation of the MTBC isolates. The purpose of our study was to evaluate an ICT kit based on detection of MPT 64 TB antigen, commercialized in name of SD Bioline MPT 64 TB Ag Rapid kit against Accuprobe as gold standard, which is a hybridization assay and has been used as a molecular test for identification of MTBC isolates grown in culture.  

In the present study, MPT 64 TB Ag rapid kit could identify 92.3% of the *Mycobacterium tuberculosis* isolates as compared to AccuProbe culture identification assay which is a probe based signal assay. However, it was 100% specific. The positive predictive value was 100% and the negative predictive value was 81.8%. An excellent sensitivity of 99%-100% and specificity of 100% have been reported in other studies.  

Another ICT assay, Tbc ID test (Becton Dickinson, USA) had a sensitivity, specificity, PPV and NPV of 96.9%, 100%, 100% and 88.9% when used in MGIT culture positive growth which is very much similar to the present study. In yet another study, the values for SD MPT 64 TB Ag were 97%, 100%, 100% and 92% respectively.  

In our study, 6 isolates were not picked up by MPT 64 TB Ag kits which were confirmed *Mycobacterium tuberculosis* isolates by probe based hybridization assay, the reference method of our study. The false negativity may be attributed to the lower concentration of MPT 64 protein on the 1st culture positive day which has also been observed before. False negativity could also be explained by the deletion and mutation in the mpt64 gene leading to the production of incomplete protein. As our strains were not analyzed by sequence analysis, we may not be able to say this surely. Previously confirmed three isolates of MTBC when tested again by both the methods remained negative in case of Accuprobe but were MPT 64 TB Ag positive which may be explained with the loss of viability on prolonged storage, so could not be picked by nucleic acid hybridization assay which is a RNA based detection assay. Since MPT 64 antigen is stable in culture medium, hence it gave positive results even in prolonged cultures.

Molecular methods for diagnosis and identification of MTBC isolates are not economically suitable for resource poor countries like India, which is an endemic nation for tuberculosis. An accuprobe culture identification kit costs Rs ~20,000/- per 20 tests costing Rs 1000/- per test, whereas MPT 64 TB Ag kit costs ~ Rs 4250/- per 20 tests costing Rs 170/- per test. Accuprobe culture identification is labor intensive, time taking (~1.5 hour test), requires equipments, technical skill and biosafety as compared to MPT 64 TB Ag based detection which is simple, labor sensitive, requiring no lab infrastructure and technical skill and is a 15 minute detection test. In view of this, rapid immunochromatography detection of MTBC specific antigen for rapid identification of isolates which is sensitive and specific has proved an ideal diagnostic tool in TB control program. It has been observed that a negative test result does not always rule out the possibility of isolating mycobacterium belonging to *Mycobacterium tuberculosis* complex. Other techniques must be used if a negative ICT slide test result is obtained.

The present study concludes that the MPT 64 TB Ag rapid kit is an easy to perform, rapid, sensitive and a specific method for characterization of MTB isolates recovered from pulmonary and extra pulmonary samples. However, this rapid test could be used as a screening tool and in TB control program but the negative results must be further confirmed using other methods.

**Conflict of Interest:** Nil

**References**


3. World Health Organization. Use of liquid TB culture


