Identification *Mycobacterium tuberculosis* complex using an immunochromatographic test

**Running title: A useful test for *M. tuberculosis* identification**

Received for publication, February 11, 2011  
Accepted, January 25, 2012

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**Abstract**

**Purpose of the study:** In order to identify *Mycobacterium tuberculosis* complex from liquid and solid colonies, was used both phenotypic methods and SD Bioline Ag MPT64 Rapid test.

**Methods:** We have tested 47 cultures identified in the Microbiology Laboratory between November and December 2009. All cases were selected from Microbiology Laboratory, of the Clinic of Pulmonary Disease, Iasi, Romania. The tests were performed according to the producer’s recommendation.

**Results:** The test sensitivity and specificity was determined using 47 strains. The mean of the ages of the study group was 51 years. The Immunochromatographic Rapid Test was positive in 57.4% of cultures tested. All positive tests were identified *M. tuberculosis* based on the phenotypic features. From negative tests, 70% were *M. non-tuberculosis*, since 30% were classified as *M. tuberculosis*.

**Conclusion:** The test showed good qualities and advantages compared with classical methods for identification of the *Mycobacterium tuberculosis* complex. For lack of some performance and expensive systems, this rapid and inexpensive method for mycobacteria identification could be a real useful tool.

**Keywords:** immunochromatographic, *Mycobacterium tuberculosis*, identification

**Introduction**

One-third of the European tuberculosis (TB) patients live in Romania. Besides, a relative important number of TB cases are non-reported.(1) The National Health System is facing with a high number of TB patients, so it needs more and more diagnostic tools and treatment. Nowadays, the gold standard for TB diagnosis is the bacteriological exam. The modern methods for TB diagnosis are not largely available in Romania, and they surpass the financial capacity of the TB Program. Due to increasing complexity of TB cases, part of the drugs resistant mycobacteria, and on the other hand due to non-TB infections, the responsibilities of the bacteriological labs are increasing.

For lack of some performance and expensive systems, the implementation of the other rapid and inexpensive methods for mycobacteria identification could be a real useful tool. In this way, an immunochromatographic test to differentiate TB from non-TB mycobacteria was used in the Bacteriological Laboratory from the Clinic of Pulmonary Disease was used.
Material and methods

Study population, study year and case definition

The current study has been performed in the Bacteriological Laboratory from the Clinic of Pulmonary Disease from Iasi. The Lab tests in microscopy and culture all specimens from patients who live in Iasi City, and the surrounded area. The antibiograms are performed on specimens from Pascani, Harlau, and the Counties of Suceava, Botosani, Neamt, Bacau, Vaslui. The technical endowment of the lab allows microscopical examinations (optical, fluorescence) and culture on Lowenstein-Jensen medium, and on liquid medium (MB/BacT) respectively. Mycobacteria identification has been performed using fenotipical methods: medium colony aspects, biochemical reactions (niacin, reductasis, cathalasis), para-amino salicilic acid (PAS) susceptibility. About 38,737 microscopical examinations and cultures and 878 antibiograms respectively are processed every year.

In this study we included 47 culture positive specimens, from patients with TB suspicions. All tests were performed between November and December 2009, respecting the producer’s standards and requirements.

The Ethic Committee approved the current study, but the patient’s informed consent wasn’t necessary because an additional test for an already collected product was performed, in a similar purpose with a traditional implemented test.

For all cases, the clinical and chest X-ray data sugest TB diagnosis. All kind of specimens were used: sputum, sputum induction, bronchial lavage etc. The specimens were decontaminated with sodium hydroxide (NaOH) 4%, 15-20 min, before cultivation. For neutralization was used HCl 8%, and blue-bromtimol like pH marker. After neutralization the product was centrifuged and the sediment was used for cultivation on Lowenstein Jensen medium, and/or on liquid medium. On solid medium, the *Mycobacterium tuberculosis* (*M. tuberculosis*) colonies are „R” type (rough, dry, with irregular edges), non-pigmented, slow-growing. Identification is based on the macroscopic and microscopic morphology, biochemical tests.

Differential characteristics of *M. tuberculosis* used in laboratory are niacin positive, susceptibility to pyrazinamide, absence of catalase production at 68°C, and nitrase activity. In addition, for the studied cultures, there has been performed an immunochromatographic test, available on the market, produced by SD Bioline, Standard Diagnostics Inc, Korea. The test is based on immunological and molecular characters of the *M. tuberculosis*, and consists in protein identification, MPT64, secreted by those bacteria, in grown phase. The protein is a major antigen (Ag) of the *M. tuberculosis* and is recognized by CD4+ lymphocytes.

Procedure of the test:

- 3 - 4 colonies should be suspended in 200 μL of extraction buffer prior to test;
- from condensation fluid, 100 μL of sample is applied directly to the well;
- test results are ready in 15 minutes after sample application;
- if a control band appears at the left section of the result window it shows that the test is working properly;
- in positive tests, another colored band appears at the right section of the result window (fig 1).
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**Results**

From 47 cultures, on the phenotypic tests there have been identified 27 cases of *M. tuberculosis*, and 20 non-tuberculosis respectively. The results of the rapid immunochromatographic test are illustrated in table 1. Comparing the results of the immunochromatographic TB Ag MPT64 Rapid test and the results of the phenotypic tests we have noticed a perfect correlation for all positive tests for *M. tuberculosis* (table 1). The sensibility of the test was 100%.

**Table 1.** Results of the phenotypic tests versus immunochromatographic

<table>
<thead>
<tr>
<th>TB Ag MPT64</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>27</td>
<td>0</td>
<td>27/27</td>
</tr>
<tr>
<td>Negative</td>
<td>6</td>
<td>14</td>
<td>14/20</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>14</td>
<td></td>
</tr>
</tbody>
</table>

From non-tuberculosis cultures on phenotypic tests only 70% have been confirmed by immunochromatographic TB Ag MPT64 Rapid test, which means a lower percentage comparing with the results from the other studies.(2)
Discussions

The immunochromatographic TB Ag MPT64 Rapid test, recently introduced on the market, is a simple and rapid differentiation test. The purpose of the current study was to determine the utility and the performance of the test in a bacteriological Romanian lab. Due to the high number of TB patients, the labs are confronting with a high number of the positive cultures which require identification tests. Without modern identification tests, the labs used traditional phenotypic tests, and they were time consuming and they needed specialized people. The results of the immunochromatographic TB Ag MPT64 Rapid test suggest a good sensibility and specificity, a cheaper and shorter time for work. Similar results are quoted in the speciality literature.(2,3)

Also there are not necessary new precautions for probes manipulation. The test is easy to use, it can be preserved at room temperature, and seems to be very useful for countries with a high incidence of TB. It can also be performed using both solid and liquid medium, and it is inexpensive. The disadvantage of this test is that it cannot differentiate \textit{M. tuberculosis} and \textit{M. bovis}.(4)

Conclusions

The immunochromatographic TB Ag MPT64 Rapid test has excellent results to differentiate tuberculosis versus non-tuberculosis mycobacteria. In countries with a high TB incidence it could be an efficient alternative test for traditional phenotypic tests.

Abbreviations

TB – tuberculosis
\textit{M. tuberculosis} – \textit{Mycobacterium tuberculosis}
Ag – antigen

References