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Research Article

MPT64 Antigen Detection for Rapid Confirmation of *Mycobacterium tuberculosis* Isolates

Ashraf Ullah Stanikzai*

Chancellor, University of Moraa, Kabul, Afghanistan

***Corresponding Author:** Ashraf Ullah Stanikzai, Chancellor, University of Moraa, Kabul, Afghanistan.

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Abstract

Background: The SD MPT64TB Ag Kit is a newly developed, rapid immunochromatographic test that utilizes a mouse monoclonal antibody against the MPT64 antigen to detect *Mycobacterium tuberculosis* (MTB) isolates. This enables the rapid identification of MTB in cultured samples. Developed by SD Bioline in South Korea, the kit demonstrates varying predictive values, types, and specificities. It provides both positive and negative detection results, along with specificity and range parameters.

Methods: In laboratories with limited resources, this rapid method for confirming *Mycobacterium tuberculosis* (*M. tuberculosis*) culture isolates is reliable, efficient, and cost-effective. The broth consisted of LJ (Löwenstein-Jensen) broth, twelve non-mycobacterial isolates, twelve nontuberculous mycobacteria (NTM) isolates, and forty-four culture isolates of *Mycobacterium tuberculosis*. The SD Bioline immunochromatography (ICT) assay kit was used to detect the presence of *Mycobacterium tuberculosis* in five sputum and pus samples using the MPT64 antigen. The MPT64 antigen band was detected in the H37Rv strain as a positive reference control.

Results: All 54 *Mycobacterium tuberculosis* (MTB) isolates tested also exhibited a similar band, indicating 100% sensitivity. The test kit demonstrated 100% specificity, as no MPT64 band was observed in any of the non-MTB test isolates. In terms of both positive and negative predictive values, the PPV and NPV are 100%.

Conclusion: The global epidemic of tuberculosis persists. For precise drug susceptibility testing, *Mycobacterium tuberculosis* (*M. tuberculosis*) culture isolates must be identified quickly. The MPT64 TB Ag detection ICT kit provides a quick and accurate substitute for molecular identification techniques.

***Keywords:** MPT64 Antigen; Mycobacterium tuberculosis; Rapid Diagnosis; Immunochromatographic Test (ICT)*

Introduction

Pakistan has the highest prevalence of tuberculosis (TB) and bears a significant share of the global TB burden. The World Health Organization (WHO) states that the TB-HIV/AIDS epidemic and the rise in multidrug-resistant (MDR) *Mycobacterium tuberculosis* (MTB) cases have created significant treatment challenges in India, hampering efforts to control this devastating disease.

Timely antituberculosis testing has long been hindered by the slow growth rate of *Mycobacterium tuberculosis* (*M. tuberculosis*) and the labor-intensive, hazardous, and technically demanding nature of its diagnosis. In Pakistan, the increase in TB cases is due to the long time required for accurate identification.

Although automated culture systems, such as Bactec 460, MB/BacT (BioMérieux, France), and MGIT, can speed up culture turnaround times, they cannot differentiate between *Mycobacterium tuberculosis* (*M. tuberculosis*) and non-tuberculous mycobacteria (NTM). While traditional biochemical methods are still used to confirm MTB, they are time-consuming, require considerable effort, and demand stringent biosafety measures. During this lengthy diagnostic process, infected individuals may continue to transmit the disease to others.

The WHO has introduced the use of line probe tests to address this issue. This can reduce the time required to detect and identify drug resistance by up to two days. However, this method demands specialized infrastructure, trained personnel, and is not feasible for resource-limited settings.

In search of an alternative, faster, and more reliable method for identifying *Mycobacterium tuberculosis* (*M. tuberculosis*) culture isolates, researchers discovered MPT64, a 28 kDa antigen specific to *M. tuberculosis* and absent in BCG strains [1]. Both native and recombinant forms of MPT64 have been shown to effectively distinguish individuals vaccinated with BCG from those infected with MTB [2]. Consistent screening of *Mycobacterium tuberculosis* isolates for antigen detection in culture provides a rapid, sensitive, and accurate approach.

The SD TB Ag MPT64 Rapid immunochromatographic (ICT) test, developed in Seoul by Standard Diagnostics, utilizes a monoclonal antibody against the MPT64 antigen to confirm *Mycobacterium tuberculosis* isolates [3,4]. The test kit has been evaluated for its clinical utility, specificity, selectivity, and ability to produce accurate positive and negative results. Notably, the MPT64 antigen is present only in the *Mycobacterium tuberculosis* (*M. tuberculosis*) complex.

Methodology

The MPT64 antigen was tested for in 54 confirmed *Mycobacterium tuberculosis* (MTB) isolates, one H37Rv culture, 10 MTB isolates from pus, and 12 gram-positive and gram-negative bacterial isolates from sputum and urine using the immunochromatographic assay method (Table 1). The manufacturer specifies this procedure. A Class II Biosafety Cabinet (BSC-II) is where all testing is performed.

Nature of Sample	Number	MPT64 Negative	Total Number
H37Rv	1	0	01
MTB culture isolates	54	0	54
MOTT	10	10	10
NMB isolates	12	12	12
Total	77	22	77

Table 1: *Sample distribution.*

Three to four colonies of H37Rv broth isolate and Löwenstein-Jensen (LJ) medium were emulsified in 200 µL of extraction buffer. For the ICT sample cassette, 100 milliliters of the mixture were added to the sample well. For the BacT/Alert MP bottle, 100 milliliters of the culture medium of positive cultures were directly added to the test cassette. In addition, 100 milliliters of non-mycobacterial isolate cultures (*Acinetobacter baumannii, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli*) were emulsified in buffer and then added to the well.

Following initial homogenization, five positive swab samples were processed in various ways. A single sample was centrifuged at 3000 rpm for 10 minutes. Next, the sample well was filled with 100 milliliters of supernatant. For the remaining sputum samples, buffer was added in volumes of 100 µL, 200 µL, and 100 µL, respectively, followed by direct addition of the samples into the cassette wells.

After use, the test cassette is left for twenty minutes at room temperature. If only the “C” region displays a pink band and no pink band is present in the “T” region, the test is considered harmful to MPT64. If no pink band is present in the “C” region, the test is considered invalid.

Results

The presence of a control band (C band) in all 77 samples indicated the validation of the test. The H37Rv control showed MPT64 antigen in the test region (T band), which showed a strong pink band. 53 isolates of *M. tuberculosis* also had a noticeable light red band in the test area. 54 out of 54 isolates displayed a slight pink band. A mutation in the isolate or a decreased antigen concentration in the test sample could be the cause of this. The test kit demonstrated 100% sensitivity. Isolates with less intense bands were sent for genetic characterization.

No pink bands were found in the *Mycobacterium* Other Than Tuberculosis (MOTT) isolate or the other 22 test samples. This shows that the MPT64 antigen was not present, and the ICT kit's specificity was 100% (Table 2). Additionally, this study showed that the ICT MPT64 Ag Rapid kit could detect antigen in sputum smear samples that yielded positive results. None of the five sputum samples showed a test band after 20 minutes; however, a band appeared following overnight incubation. This suggests that, for practical application of the rapid test to clinical samples, the antigen extraction procedure may need to be optimized to increase antigen concentration. Upon re-examination of the test cassettes after overnight incubation at room temperature, none of the negative samples displayed a test band.

Nature of Sample	Number	MPT 64 Negative	MPT 64 Positive
H37Rv	1	0	01
MTB culture isolates	54	0	54
MOTT	10	10	0
NMB isolates	12	12	0
Total	77	22	55

Table 2: *MPT64 test results.*

Discussion

An endemic illness that has existed for a very long time is tuberculosis. Not a single race or belief has been spared in its conquest of the whole planet. Pakistan has treated most of the world's tuberculosis cases, making it a serious public health problem. Approximately 0.87 million of the roughly 2 million TB cases that occur in Pakistan each year are infectious. An estimated 330,000 Pakistanis are thought to lose their lives to tuberculosis each year. One of the main causes of death in Pakistan is tuberculosis (TB), which claims the lives of two people every three minutes, or around 1,000 per day.

The treatment of TB patients depends on early diagnosis, which is supported by a Mycobacteriology laboratory capable of performing drug susceptibility testing, culture, and identification of *Mycobacterium tuberculosis* (MTB) from clinical specimens. Although automated technologies have accelerated the isolation process, rapid identification of MTB isolates remains essential. In TB research, as well as for diagnosis and treatment, developing new methods for the quick detection of culture isolates and isolates resistant to anti-tubercular drugs has become a significant priority.

Modern molecular techniques are not cost-effective for laboratories with limited resources. In low-resource countries, a simple, rapid and accurate identification method would be highly beneficial. Recent findings suggest that new rapid immunochromatographic techniques are among the best diagnostic tools for TB control programs.

The primary goal of this study was to develop a readily accessible and rapid test that can identify isolates of *M. tuberculosis* cultured in media [5]. Differentiating *Mycobacterium tuberculosis* from *Mycobacterium* other than Tuberculosis is of clinical and therapeutic importance. Most mycobacteriology laboratories rely on standard biochemical assays to detect MTB, which are labor-intensive and require specialized biosafety equipment.

Numerous helpful antigens have been found through biological, molecular, and immunological investigations of the *Mycobacterium tuberculosis* complex. The MPT64 TB antigen is also known as the Rv1980c protein [6,7]. Growing strains of *Mycobacterium tuberculosis* secrete it [8]. No strains of *M. bovis* or *M. leprae* contain the MPT64 antigen. The MPT64 gene from the H37Rv culture filtrate was cloned and sequenced to confirm this [9]. Recent studies have shown that MPT64 influences the immune system [10,11]. Restricted antigens of the *Mycobacterium tuberculosis* complex and candidate tuberculosis (TB)-specific antigens are considered to be the MPT64 antigen and culture filtrate protein 2 (CFP-2) [12]. In addition, it has been shown that the MPT64 antigen is only found in proliferating *Mycobacterium tuberculosis* (*M. tuberculosis*) cells [13].

The accuracy of a commercial ICT test kit (Capilia TB) in rapidly identifying 784 culture isolates was evaluated using the AccuProbe-MTB reference method. The rapid test kit demonstrated a sensitivity of 99.2% (381/384). One of the key findings of the study was the absence of false-positive results. Consequently, researchers concluded that Capilia TB ICT test kits are a suitable option for the routine and rapid identification of *Mycobacterium tuberculosis* isolates [14,15]. Rapid ICT tests have demonstrated 100% accuracy.

Compared to traditional biochemical tests and AccuProbe sequencing, rapid ICT kits have shown 100% specificity and 97% sensitivity. These rapid ICTs offer lower costs and significantly shorter turnaround times for accurately identifying MTB [16]. The SD MPT64 TB Ag rapid ICT kit has 100% specificity, 97% sensitivity, 92% positive predictive value (PVV), and 100% negative predictive value (NPR) because it combines encryption with high specificity and sensitivity, thus producing no false positive results when compared with molecular techniques [17]. This study did not produce false positive or negative results. Traditional recognition tests can be replaced by ICT which can detect MPT64 antigen in one day [18].

Rapid ICTs are a practical option for TB diagnostic laboratories due to their affordability and ease of use compared to molecular techniques for confirming MTB isolates. This study demonstrated the complete sensitivity of the SD Ag MPT64 kit. The bands produced by all 54 MTB isolates were comparable in intensity to those of the H37Rv culture. None of the mycobacterial and nontuberculous species under investigation showed any bands for the MPT64 antigen. The MPT64 antigen's specificity for *Mycobacterium tuberculosis* was further supported by the fact that it was absent from non-TB isolates.

The positive and negative predictive values in this study were both 100%. In one study showing a PPV of 100%, the NPV was lower (92%) [19], possibly due to false-negative results. However, another study showed an NPV of 100% (260/260) [20]. The ICT technique occasionally produces false-negative results in a small number of genetically confirmed MTB strains, this has been attributed to treatment of specific genes in the isolates. For example, in a study using the Capilia TB ICT kit, six MTB isolates produced false-negative results. Genomic analysis confirmed that all six strains had mutations, a limitation observed across commercial kits.

The predictive value, prevalence, and specificity of isolates in LJ medium and liquid culture were comparable; however, the band intensity in liquid culture was more pronounced. In this study, ICT successfully detected the MPT64 antigen in smear-positive serum samples. Antigen detection in clinical samples using the SD MPT64 TB Ag rapid kit could be enhanced with improved antigen extraction methods. Similar outcomes were reported in other studies. Compared to traditional biochemical and culture-based methods, analytical evaluations of the SD MPT64 TB Ag ICT and other rapid ICT kits have proven to be more cost-effective. This investigation did not require highly trained personnel or advanced laboratory equipment [21].

Conclusion

Mycobacterium tuberculosis isolates can be accurately identified in diagnostic centres with limited resources by using the SD MPT 64 TB Ag Rapid ICT Kit, a quick, simple, low-tech identification test. The turnaround time for MTB culture and drug susceptibility testing will be greatly reduced with a faster test. Therefore, this test is an important part of tuberculosis control efforts. Due to its low cost, speed, specificity, sensitivity, high NPP, and NNP, ICT is an invaluable diagnostic tool for detecting MPT 64 antigen. Since Pakistan is the primary endemic country for tuberculosis, it is more important than any other nation in the world to implement quick diagnostic techniques.

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Availability of Data and Materials

The manuscript contains complete data supporting our findings and results.

Consent for Publish

There is no individual persons' data in this manuscript.

Declaration of Competing Interest and Ethics

There is no conflict of interest. This research study complies with research publishing ethics. The scientific and legal responsibility for manuscripts published in OPS Journal belongs to the authors.

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