

## Identification of TBc: Using MPT 64 protein and cord formation

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### Abstract

Tuberculosis (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis*. The genus *Mycobacterium* comprises over 150 species. Non-tuberculosis *Mycobacteria* are the cause of opportunistic infections and frequently presents with similar clinical features like tuberculosis so species identification is important for management. The current study was designed for presumptive diagnosis of MTB by detecting MPT 64 and Cord formation and the sensitivity and specificity of Cord Formation in MGIT positive samples. A cross sectional study consists of 100 MGIT positive samples. Ziehl-Neelsen Staining was performed to detect Cord Factor and TBc ID was undertaken to detect MPT 64 protein. Out of 100 MGIT positive samples 92 were positive for Cord factor and 08 were negative, whereas 89 samples were positive on MGIT TBc Identification Device and 11 were negative. The sensitivity for TBc ID was 94.6% and specificity was 100% with Positive Predictive Value and Negative Predictive Value of 98.9% and 81.8% respectively. TBc ID and Cord Factor seem to be highly sensitive and specific for rapid diagnosis and accurate differentiation *Mycobacterium tuberculosis* from Non-tuberculosis.

**Keyword:** MGIT, Tuberculosis, MPT 64, TBc ID.

### Introduction

Tuberculosis (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis*. It is a major cause of morbidity and mortality, particularly in developing countries. This bacterial infection is caused by various strains of *Mycobacteria*, usually *Mycobacterium tuberculosis* (MTB). This bacteria in most cases attacks the lungs (Pulmonary TB), but MTB can cause disease in any part of the body such as the spine, kidney and brain called Extra-pulmonary Tuberculosis (EPTB).<sup>1</sup> The *Mycobacterium tuberculosis* complex (MTC) consists of *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium canettii*, and

*Mycobacterium microti*. *M. tuberculosis*, the most common culprit behind TB is a small, aerobic, non motile bacillus.<sup>2</sup> Tuberculosis (TB) is one of the major public health problems in Pakistan. Pakistan ranks fifth amongst TB high-burden countries worldwide. It accounts for 61% of the TB burden in the WHO Eastern Mediterranean Region.

In 2007, the World Health Organization (WHO) adopted a policy that recommended the use of liquid culture method as a standard for tuberculosis diagnosis and case management.<sup>3</sup> Culture still represents the cornerstone on which a definitive diagnosis of tuberculosis and other mycobacteriosis relies. In recent years, the development of rapid and reliable methods for culture detection of acid fast bacilli has been regarded as worthy of absolute priority.<sup>4,5</sup>

The development and implementation of liquid culture system BACTEC MGIT 960 (Becton Dickinson's *Mycobacterium* growth Indicator Tube), is the most sensitive for recovery of mycobacteria from clinical samples.<sup>6</sup> The BACTEC MGIT 960 liquid culture system can shorten the time of recovery of mycobacteria to approximately 10 to 14 days.<sup>7,8</sup>

In liquid cultures, *Mycobacterium tuberculosis* displays characteristic serpentine cord formation. Cord effect is caused by major mycolic acid containing molecules such as trehalose-6, 6'-dimycolate (TDM), a component of the mycobacterium cell wall, implicated in major immunomodular mechanism that is responsible for rendering MTB virulent.<sup>8</sup> TDM is an abundant surface glycolipid in the mycomembranes which provide a potent biological barrier. TDM is also known as "cord factor" as it facilitates cord formation, as well as increasing impermeability and resistance to: (i) many antibiotics, thus improving survival inside macrophages.<sup>8,9</sup> MTB can be differentiated from Non-tuberculosis *Mycobacteria* (NTM) by the presence of cord factor because NTM is scattered or dispersed without any orientation. NTM does not form a serpentine cord.<sup>10</sup>

The TBc identification (TBc ID) is a device which is also used for the differentiation of NTM and MTB. TBc identification device is based on the detection of a protein (MPT64) secreted by *Mycobacterium tuberculosis* complex (MTC), can detect *Mycobacterium tuberculosis*

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in 15 min from acid-fast bacillus (AFB)-positive MGIT cultures.<sup>11</sup> MPT 64 is the protein produced only by Mycobacterium tuberculosis, not by Non-tuberculosis Mycobacteria. The combination of the two tests (Cord Factor and MGIT Tbc ID) is used for accurate and rapid identification of Mycobacterium tuberculosis from MGIT positive isolates. The current study was designed for rapid identification of MTB using MGIT 960 and a presumptive diagnosis of MTB by detecting MPT 64 and Cord formation.

### Methodology

A cross sectional Study was conducted in the Department of Pathology, Allama Iqbal Medical College, Lahore and chest ward Jinnah Hospital Lahore, for a period of 6 months, Inclusion Criteria Single sputum sample taken from TB suspects having symptoms (fever, weight loss, and cough, hemoptysis, family history of TB) and radiographic evidence of TB from all age groups and both Genders. Prior to sample collection, patients were instructed in detail concerning the proper collection of adequate sputum specimen. Patients already on anti-tuberculosis treatment and MGIT culture negative cases were excluded. All collected samples were cultured on MGIT 960 following previously described method.<sup>12</sup> 100 MGIT positive samples were taken.

The isolates were stained with Ziehl-Neelsen staining technique for detecting acid-fast bacteria. Mycobacterium tuberculosis complex exhibited serpentine cording in the liquid medium. Smears were prepared from MGIT culture positive tubes and stained by ZN technique. Cording is the characteristic exhibited only by M. tuberculosis. Non-tuberculosis Mycobacteria are scattered or dispersed without any orientation as NTM does not form a serpentine cord.

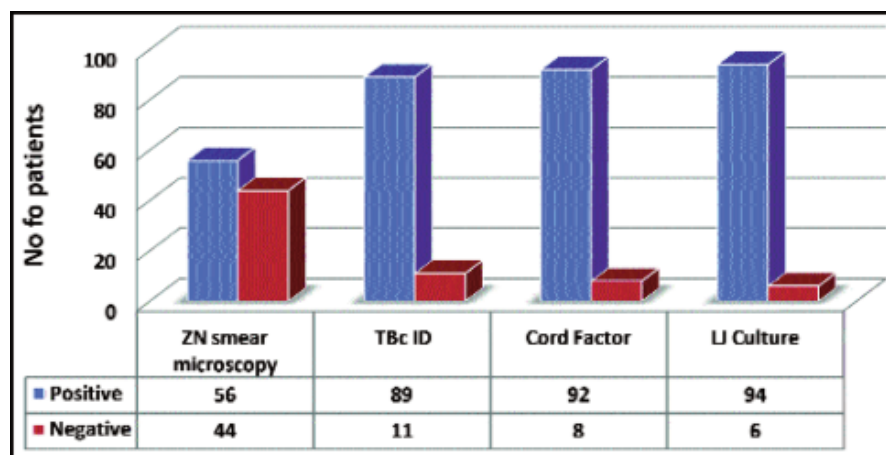
The BD MGIT TM Tbc ID (Becton,

Dickinson and Company) is a rapid immuno-chromatographic device which was used to determine clinical isolates to species level. Isolates from MGIT liquid culture were used for the qualitative detection of Mycobacterium tuberculosis complex from AFB positive MGIT tube, based on the detection of a protein (MPT64) secreted by Mycobacterium tuberculosis.

Each Tbc ID device was inoculated with 100µL of a positive MGIT culture medium from the bottom of the MGIT tube. The result was interpreted after 15 minutes of incubation at room temperature.

### Results

The results of our study, (Graph) showed Rate of positivity for tuberculosis and its comparison of ZN smear, LJ culture, Tbc ID and Cord Factor from all 100 MGIT positive samples. LJ diagnosed highest no of cases (94/100) followed by cord factor 92/100, and Tbc ID culture (89/100) respectively, and lowest number of cases were detected by ZN smear microscopy (56/100). The comparison between MGIT Culture and LJ Culture shows that out of 100 MGIT positive samples 94 were positive on LJ culture whereas 6 were negative. It concludes that



Graph showing the frequency of different diagnostic test for Tuberculosis as 56% using ZN smear microscopy, 89% TBcID, 92% Cord Factor and 94 LJ culture.

Graph: Frequency of different diagnostic tests for Tuberculosis.

Table-1: Diagnostic accuracy of ZN, cord factor, TBC ID.

Diagnostic accuracy	Sensitivity	Specificity	PPV	NPV
Zn staining	57.4% (54/94)	66.7% (4/6)	96.4%	9.1%
Cord factor	97.8% (92/94)	83.3% (5/6)	98.9%	75%
TBC Id	94.6% (89/94)	100.0% (6/6)	98.9%	81.8%

Showing the sensitivity, specificity, and Positive predictive value, Negative predictive value of ZN smear, Cord factor and Tbc ID. Positive predictive value (PPV), Negative Predictive Value (NPV).

MGIT culture (Liquid culture) is more sensitive than the conventional LJ culture technique. So, it can be used as a Gold Standard for Tuberculosis diagnosis.

In concentrated AFB microscopy results versus LJ media results, LJ culture was taken as Gold Standard. Out of 94 culture positive cases, 54 were positive on ZN microscopy and 40 were negative on microscopy. While out of 6 culture negative samples 4 were also negative on ZN, but 2 cases were positive on ZN but negative on LJ. These two samples were being taken as false positive.

Therefore, sensitivity, specificity, PPV and NPV of ZN smear as followed 57.4%, 66.7%, 96.4% and 9.1%.

Out of 94 LJ culture positive cases, 92 were positive for cord formation on microscopy and 02 were negative on microscopy. Therefore, sensitivity, specificity, PPV and NPV of ZN smear were 97.8%, 83.3%, 98.9% and 75.0% respectively Table 01.

TBc Identification device results with comparison to LJ media out of 94 LJ culture positive cases, 88 were positive on TBc ID device and 06 were negative. Therefore, sensitivity, specificity, PPV and NPV of ZN smear were 94.6%, 100%, 98.9% and 81.8% respectively Table-1.

## Discussion

Tuberculosis (TB) is a major cause of morbidity and mortality, particularly in developing countries. For the diagnosis of TB ZN smear microscopy has limitation due to low sensitivity and LJ culture due to the intrinsic slow growth of MTB (4-8 weeks). In 2007, the World Health Organization (WHO) recommended the use of liquid culture method as a standard for tuberculosis (TB) diagnosis which markedly reduces the time of detection of active TB.<sup>13</sup>

MTB can be differentiated from Non-tuberculosis Mycobacteria (NTM) by the presence of cord factor which gives characteristic serpentine cord like appearance to MTB while NTM are scattered or dispersed without any orientation as NTM does not form a serpentine cord. The TBc Identification Device (TBc ID) is used for the differentiation of NTM and MTB, which is based on detection of MPT 64 gene.<sup>14</sup>

In the present study, TBc Identification Device and Cord Factor were used for the rapid species identification of MTB. This study demonstrated that the combination of Cord Factor and TBc ID can rapidly (within an hour) and accurately differentiate *M. tuberculosis* from NTM. Results of our study show 89 samples to be Positive on TBc ID and 92 were Positive on Cord Factor detection out of total 100 MGIT positive samples. The sensitivity for TBc ID was

94.6% and specificity was 100% with Positive Predictive Value and Negative Predictive Value of 98.9% and 81.8% respectively. Whereas the Cord Factor sensitivity was 97.85% and specificity was 83.3% with Positive Predictive Value and Negative Predictive Value of 98.9% and 75% respectively.

Machado Det al.,<sup>11</sup> studied the Assessment of the BD MGIT TBc. This corresponds to a sensitivity of 90.14%, specificity of 100%, and positive and negative predictive values of 100% and 80.55%, respectively. Barouni A et al.,<sup>15</sup> studied the Evaluation of the BD MGIT TBc identification test for rapid identification of Mycobacterium tuberculosis Complex from positive BACTEC MGIT 960 cultures. The sensitivity, specificity, and positive/negative predictive values of TBc ID test for identifying *M. tuberculosis* complex were 96.9%, 100%, 100% and 88.9% respectively. Yu M-Cet al.,<sup>16</sup> studied Evaluation of the Rapid MGIT TBc Identification Test for Culture Confirmation of Mycobacterium tuberculosis Complex Strain Detection. The sensitivity, specificity, positive predictive values, and negative predictive values of the TBc ID test were 98.8%, 100%, 100%, and 95.1%, respectively.

Arora J et al.,<sup>17</sup> studied the Presumptive identification of Mycobacterium tuberculosis complex based on cord formation in BACTEC MGIT 960 medium. The sensitivity and specificity of cord formation were found to be 99.7% and 89.9%, respectively. Another study, the Cord Formation in BACTEC Medium Is a Reliable, Rapid Method for Presumptive Identification of Mycobacterium tuberculosis Complex.<sup>18</sup> Cord formation had a sensitivity, specificity, positive predictive value, and negative predictive value of 89.2%, 99.2%, 98.5%, and 94.2%, respectively. Kadam M. et al.,<sup>19</sup> studied "Can cord formation in BACTEC MGIT 960 medium be used as a presumptive method for identification of *M. tuberculosis* complex? The sensitivity, specificity, positive and negative predictive values are found to be 99.6%, 54%, 96% and 91% respectively.

## Conclusion

The results of our study showed that the combination of MGIT TBc ID and Cord Factor seems to be highly sensitive and specific to rapidly and accurately differentiate Mycobacterium tuberculosis from Non-tuberculosis Mycobacteria from MGIT (Liquid Culture) positive isolates as compared to traditional sub culturing techniques.

**Disclaimer:** None to declare.

**Conflict of Interest:** None to declare.

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