*IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*
*e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 10 Ver. VIII (Oct. 2017), PP 90-93*
*www.iosrjournals.org*

# Evaluation of MPT 64 Antigen for Early Differentiation Between Mycobacterium Tuberculosis Complex And Nontuberculous Mycobacteria in Smear Negative Patients

*Rehena Sarkar[1],Debalina Das[2],JayantaBikash Dey [3],AbhijitMandal[4], Soumick Sarkar[5],Koushik Ghosh[6]

[1](Medical officer,Department of Microbiology,Beleghata I.D. and B.G. Hospital, India)
[2](Demonstrator, Department of Microbiology, College of Medicine SagoreDutta Hospital, India)
[3](Professor,Department of Microbiology,Bankura Sammilani Medical College and Hospital, India)
[4](Professor,Department of Chest Medicine, Nil RatanSircar Medical College, India)
[5](Medical Officer, Department of Pathology, Mathabhanga SD Hospital, India)
[6](Demonstrator, Department of Physiology, Calcutta National Medical College and Hospital, India)

***Abstract:****Tuberculosis (TB) is a global pandemic & India is one of the major TB endemic countries. The worldwide magnitude of modern tuberculosis is so great that in April 1993 the World Health Organization (WHO) declared tuberculosis to be a global emergency.It is important to accurately characterize Mycobacterium tuberculosis complex (MTBC) and Nontuberculous Mycobacteria (NTM) as NTM are inherently resistant to many conventional anti-tuberculosis drugs, require modified treatment regimens and are often misdiagnosed as multidrug-resistant tuberculosis (MDR). Differentiation between MTBC and NTM is done by either conventional biochemical tests or molecular methods. The former are time consuming and cumbersome while the latter are expensive, require trained personnel and special laboratory setup. Hence there is need for a rapid, accurate and simple test for characterization of Mycobacteria. In this study we evaluate the MPT64 antigen kit for early differentiation between MTBC and NTM in smear negative but culture positive patients. The study period was between Januaryto December'2014. We included 200smear-negative but clinically suspected pulmonary and extra-pulmonary tuberculosis patientswhoattended or gotadmitted to the Chest Medicinedepartment of Bankura Sammilani Medical College. The samples were cultured in L-J media or BacT/Alert 3D system. The positive culture was tested by conventional biochemical test and with MPT64 kit to detect MTBC& differentiate MTBC from NTM.15% samples were culture positive among 200 patients. 18 (9%)MTBC and 12 (6%) NTM were detected by conventional system and same result was found by MPT-64 system but with much rapidity. So MPT 64 Antigen detection kit may be a good alternative to biochemical and molecular methods of identification of Mycobacterial species.*

***Keywords:****MPT64, MTBC, NTM*

---

Date of Submission: 04 -10-2017 Date of acceptance: 18-10-2017

---

## I. Introduction

Tuberculosis (TB) which is one of the oldest & deadliest communicable diseases known to affect human, exists in prehominids. It is a major cause of death worldwide.[(1)]Most of the estimated number of cases occurred in Asia (56%) and the African Region (29%)in 2013; smaller proportions of cases occurred in the Eastern Mediterranean Region (8%), the European Region (4%) and the Region of the Americas (3%). [(2)] India is the country with the highest burden of TB by World Health Organisation (WHO) statistics for 2013 giving an estimated incidence figure of 2.1 million cases of TB for India out of a global incidence of 9 million. The estimated TB prevalence figure for 2013 is given as 2.6 million. [(3)] It is estimated that about 40% of the Indian population is infected with TB bacteria, the vast majority of whom have latent rather than active TB.[(4)]Mycobacteria can be divided into two major groups, based on fundamental differences in epidemiology and association with disease: those belonging to the Mycobacterium tuberculosis complex (MTBC) and those referred to as Nontuberculous mycobacteria (NTM).[(5)]

MTBC includes:*M. tuberculosis*, *M. bovis*, *M. africanum*, *M. caprae*,*M. pinnipedi*, *M.microti*, *M. canetti*.*M. tuberculosis* is the predominant cause of human tuberculosis.[(6)]NTMs are frequently found in environmental habitats that may colonize and occasionally cause infection in humans and animals. With the increasing prevalence of immunocompromised hosts, particularly in relation to the AIDS pandemic, NTM infections are becoming more prevalent. Patients with depressed cellular immunity, such as those who have

AIDS, lympho-proliferative disorders or transplants, and those on immunosuppressive therapy, are at particular risk. [7]

In 1959 Runyon classified NTM into four groups based on the phenotypic characteristics of the various species, most notably the growth rate and colonial pigmentation:- 1.Photo-chromogens: NTM colonies that develop pigment on exposure to light after being grown in the dark and take longer than 7 days to appear on solid media.Eg:-*M.kansasii, M.asiaticum, M.marinum,M.intermedium*; 2.Scoto-chromogens: NTM colonies that develop pigment in the dark or light and take longer than 7 days to appear on solid media. Eg:-*M.szulgai, M.scrofulaceum, M.interjectum, M.gordonae;* 3.Nonchromogens: NTM colonies that are non-pigmented regardless of whether they are grown in the dark or light and take longer than 7 days to appear on solid mediaEg:-*M. avium complex, M.xenopi, M.ulcerans, M.malmoense, M.genevense, M.haemophilum, M.simiae*; 4. Rapid growers: NTM colonies that grow on solid media and take fewer than 7 days to appear. Eg:-*M.abscessus, M.fortuitum, M.chelonae, M.smegmatis.*

Principal types of opportunist mycobacterial disease in human and causative agents:

Lymphadenopathy -M.avium complex, *M.scrofulaceum*;Post-traumatic abscess - *M.abscessus, M.fortuitum, M.chelonae;*Swimming pool granuloma - *M.marinum*;Buruli ulcer - *M.ulcerans*;Pulmonary disease and solitary nonpulmonary disease - *M.avium complex, M.kansasii, M.xenopi, M.malmoense*Disseminated diseaseAIDS related - *Mavium complex, M.genevense;*Non-AIDS related - M.avium complex *M.abscessus, M.chelonae*.Clinical presentation of Mycobacterium tuberculosis complex (MTBC) and Nontuberculous Mycobacteria (NTM) may or may not be same but the treatment regimen is always different for both infections. It is important to accurately characterize Mycobacteria since NTM are inherently resistant to many conventional anti-tuberculosis drugs, require modified treatment regimens and are often misdiagnosed as multidrug-resistant tuberculosis. [8] So clinically and therapeutically differential identification of MTBC from NTM is very important. Differentiation between them by routine laboratory method is time consuming and cumbersome. Most of the laboratories identify MTBC using conventional biochemical tests. These tests need special biosafety equipments. On the other hand, molecular methods that identify specific nucleic acid sequences are rapid, sensitive and specific, but are expensive and require trained personnel and special laboratory setup. [9]

Hence there is need for a rapid, accurate and simple test for characterizationof Mycobacteria. Biological, molecular and immunological studies of MTBChave resulted in identification of varieties antigens, some of which arespecific to MTBC and nowadays used to differentiate between MTBC andNTM.[10, 11]MPT64 is one such antigen. MPT64 also termed as protein Rv1980c.Itis a protein secreted by actively growing MTB strains. [10,11] The MPT64antigen is absent in *M. bovis* BCG strains and *M. leprae* and in NTM species. This has been confirmed by cloning and sequencingof MPT64 gene of H37Rv culture filtrate. [12] MPT64 antigen and culture filtrate protein (CFP-2) antigens are restrictedantigens of MTBC and are found to be TB specific candidate antigens. [13]Based on this property of MPT64 antigen, an Immuno-chromatographic test (ICT) kitusing mouse Monoclonal Antibodies against MPT64 antigen is beingmanufactured and marketed by commercial manufacturer and used to identifyMTBC. [3]MPT64 antigen detection kit was being used in this study to identify MTBC,thus to differentiate MTBC from NTM isolates.

## II. Materials and Methods

The study was under taken between January-2014 to December-2014 after obtaining ethical clearance from the institutional ethics committee.200 Sputum smear-negative but clinically suspected pulmonary tuberculosis patients and extra-pulmonary tuberculosis patients whoattended Chest OPD or admitted in Chest ward were selected for the study. We tooksputum or other body fluid sample appropriate for the disease. Then AFB microscopy was done to rule out presence of AFB. After proper processing the samples were cultured in both automated BacT/Alert 3D system and Lowenstein-Jensen (L-J) medium. If growth occurred we identified MTBC using conventional biochemical tests and MPT-64 cards. Among the biochemical tests Niacin test, nitrate reduction test and heat stable catalase were performed. All processing was done in Class II Biosafety cabinet.

## III. Result and analysis

Among the 200 patients majority were between 15-60 years of age (< 15 years 1%, 15 – 60 years 81%, >60 years 18%) while 68% were male and 32% were female.

Out of 200 specimens 85% were sputum and only 15% were other sample.

**Table 1:**Distribution of different specimens used in the study N = 200

| Specimen | Percentage (%) |
|---|---|
| Sputum | 85 |
| Pleural fluid | 6 |
| CSF | 4 |
| Ascitic fluid | 3 |
| Urine | 2 |

Among 200 specimens 30specimens (15%) were culture positive. Among 30 specimens 60% were MTBC and 40% were NTM.

**Table 2:**Distribution of MTBC and NTM among AFB isolated (N=30)

| Mycobacterial species | Percentage of Mycobacterial isolates (%) |
|---|---|
| MTBC | 60 |
| NTM | 40 |

Considering biochemical tests as gold standard, all MPT64 antigen kit correctly identify MTBC. So sensitivity, specificity, positive predictive value, negative predictive value of MPT64 Antigen detection kit test was 100% in our study.

## IV. Discussion

In our study two hundred clinically suspected pulmonary and extra-pulmonary tuberculosis patients whose sample were smear microscopy negative were included. Study revealed mycobacterial isolation of 15% from smear negative samples. As no similar study was conducted so our data cannot be compared. Rohner*et al* conducted a study on sputum samples and body fluids(not exclusively smear negative samples) which showed mycobacterial isolation of 6.8%.[14] Piersimoni*et al* in a study conducted on pulmonary and extra-pulmonary samples showed 304 mycobacterial isolates grown from 2,859 specimens showing the isolation of 10.68%. [15]Carricajo*et al* from France showed that from a total of 1,197 specimens, mycobacteria were identified in 52 cultures (4.3%).[16]

Mycobacteria (AFB) isolated from smear microscopy negative but clinically suggestive 30 patients were further differentiated into MTBC and NTM on the basis of conventional biochemical tests (Niacin test, Nitrate reduction test, Heat stable catalase test) and MPT64 Antigen detection kit test. Considering biochemical tests as gold standard for sensitivity, specificity, positive predictive value, negative predictive value of MPT64 Antigen detection kit test was 100% in our study. Ang*et al* from South Korea in a study showed sensitivity of 97.1% and a specificity of 100% of the MPT64 Antigen detection kit. [17] Park *et al*also reported excellent sensitivity (99%) and specificity (100%) of the test. [18] So our study is very comparable to these studies. In this study among the Mycobacterial isolates detected, 60% were MTBC and 40% were NTM which is almost very similar to that study of Maurya*et al* where they showed that 67% of Mycobacterial isolates were MTBC and 33% were NTM. [19]

MPT 64 Antigen kit used in this study showed sensitivity, specificity, positive predictive value and negative predictive value of 100% compared with biochemical methods of detection which are time consuming; and molecular methods of identification which are rapid, sensitive and specific, but require trained personnel, expensive laboratory aids and are difficult to set up in resource constrained settings like India. Utilising the MPT 64 Antigen detection kit for detection of MTBC and differentiating MTBC from NTM among mycobacterial isolates can be done within 15 minutes. So MPT 64 Antigen detection kit may be a good alternative to biochemical and molecular methods of identification of Mycobacterial species.

## References

[1]. Raviglione M C, O Brien R J. Tuberculosis. In: Longo D L, Fauci A S, Kasper D L, Hauser S L, Jameson J L, Loscalzo J (eds.) Harrison′ s Principle of Internal Medicine. Vol. I. 18th edition. New York: McGraw Hill; 2008. p. 1340-1358.
[2]. World Health Organization .The burden of disease caused by TB (Chapter 2). Global tuberculosis report 2014. Geneva: WHO; 2014. Available from: www.who.int/tb/publications/global report/en/.
[3]. Kumar V GS, Tejashree A Urs, Ranganath R R. MPT 64 Antigen detection for Rapid confirmation of M. tuberculosis isolates. BMC Research Notes.2011; 4:79.
[4]. TB India 2012. Revised National TB Control Programme Annual Status Report, New Delhi, India: Ministry of Health and Family welfare, Government of India; 2012. Available from: www.tbcindia.nic.in/documents.html#
[5]. Tille P M.Bailey&Scott′s Diagnostic Microbiology. St. Louis, Missouri: Elsevier, Mosby; 2014.
[6]. Grange J M. Tuberculosis; leprosy. In: Greenwood D, Barer M, Slack R, Irving W.(ed.) Medical Microbiology.18 th edition. Philadelphia: Churchill Livingstone, Elsevier; 2012.p.311-325.

[7]. Pfyffer G E, Vincet V. Mycobacterium tuberculosis complex, Mycobacterium leprae, and other slow growing Mycobacteria. In: Borriello S P, Murray P R, Funke G. (eds.) TopleyWilson′s microbiology and microbial infection. Bacteriology vol II. 10th edition.London: Hodder Arnold; 2005. p. 1181-1223.
[8]. Wagner D, Young L. Nontuberculous mycobacterial infections: A clinical review. Infection.2004; 32: 257-270.
[9]. Hasegawa N, Miura T, Ishii K, Yamaguchi TH, Lindner S, Merritt J D. et al. New simple and rapid test for culture of Mycobacterium tuberculosis complex: A multi-centric study. J. Clin. Microbiol.2002; 40: 908-12.
[10]. Wang Z, Potter MBelinda, GrayMAmanda, SackstederAKatherine, GeisbrechtVBrian, Laity H John: The Solution Structure of Antigen MPT64 from Mycobacterium tuberculosis defines a New Family of Beta-Grasp Proteins. J. Mol. Biol. 2007; 366(2): 375-381.
[11]. Yamaguchi R, Matsuo K, Yamazaki A, Abe C, Nagai S, Terasaka K, Yamada T: Cloning and characterization of the gene for immunogenic protein MPB64 of Mycobacterium bovis BCG. Infect. Immun. 1989; 57(1):283-288.
[12]. Gennaro ML. Immunologic Diagnosis of Tuberculosis. J. Infect. Dis. Proceedings of International Symposium on Tuberculosis Vaccine Development and Evaluation. 2000; 30(3): 243-246.
[13]. Ruiling Fu Chun Wang, Chunwei Shi, Mengji Lu, Zhegming Fang, Jia Lu, Fang Wang, Xionglin Fan. An Improved Whole-Blood Gamma Interferon Assay Based the CFP21-MPT64 Fusion Protein. Clin.Vaccine Immunol.2009; 16: 686-691.
[14]. Rohner P, Ninet B, Metral C, Emler S, Auckenthaler R. Evaluation of the MB/BacT system and solid media for isolation of mycobacteria from comparison to the BACTEC 460 system and solid media for isolation of mycobacteria fromclinical specimens. J. Clin. Microbiol. 1997; 35(12):3127-3131.
[15]. 10. Piersimoni C, Scarparo C, Callegaro A, Tosi C P, Nista D, Bornigia S et al. Comparison of MB/BacT ALERT 3D System with Radiometric BACTEC System and Löwenstein-Jensen Medium for Recovery and Identification of Mycobacteria from Clinical Specimens: A Multicenter Study. J. Clin. Microbiol. 2001; 39(2):651-657.
[16]. Carricajo A. Fonsale N, vautrin AC, Aubert G. Evaluation of BacT/Alert 3D Liquid Culture System for Recovery of Mycobacteria from Clinical Specimens Using Sodium Dodecyl (Lauryl) Sulfate-NaOH Decontamination. J. Clin. Microbiol. 2001; 39(10): 3799-3800.
[17]. Ang C. F , Cajucom MM, Kim Y,Bang H, Lee H, Cho C S. N et al. Evaluation of a rapid assay for identification of Mycobacterium tuberculosis grown in solid and liquid media. Int J Tuberc. Lung. Dis. 2011; 15(11): 1475–1477.
[18]. Park MY, Kim JK, Hwang SH, Kim HH, Lee EY, Jeong SH, Chang CL. Evaluation of an immunochromatographic assay kit for rapid identification of Mycobacterium tuberculosis complex in clinical isolates. J. Clin. Microbiol. 2009; 47(2): 481-4.
[19]. Shenoy V R, Mukhopadhyay C. Rapid Immunochromatographic Test For The Identification And Discrimination Of Mycobacterium Tuberculosis Complex Isolates From Non-Tuberculous Mycobacteria. Journal of Clinical and Diagnostic Research. 2014; 8(4): DC13-DC15.

*Rehena Sarkar. "Evaluation of MPT 64 Antigen for Early Differentiation Between Mycobacterium Tuberculosis Complex And Nontuberculous Mycobacteria in Smear Negative Patients." IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) , vol. 16, no. 10, 2017, pp. 90–93.