

Performance evaluation of MPT64 Antigen detection for rapid confirmation of *Mycobacterium Tuberculosis* complex (MTBC) from solid culture isolates

Authors:

Reyaz Nasir¹, Maroof Peer², Muzafar Amin³, Bilal Tantry⁴

¹Associate Professor, Department of Microbiology Government Medical College, Srinagar.

²Associate Professor, Department of Microbiology Government Medical College, Srinagar

³Associate Professor, Department of Microbiology, Kashmir Medical College, Srinagar.

⁴Research Scientist, Vrdl Department of Microbiology, Government Medical College, Srinagar.

Corresponding Author:

Dr. Muzafar Amin, Associate Professor, Department of Microbiology, Kashmir Medical College, Srinagar.

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ABSTRACT

Background: Tuberculosis remains one of the most common infectious diseases in impoverished nations and increasing resistance to anti-tubercular drugs makes treatment difficult and early diagnosis imperative. Although molecular tests are increasingly being relied upon, culture remains the gold standard and a positive culture still needs to be confirmed as MTB Complex. The purpose of this study was to assess the MPT64 antigen detection assay's diagnostic performance in confirming MTB complex from solid culture isolates. **Objective:** To evaluate the MPT64 antigen detection assay's diagnostic performance for confirming *Mycobacterium tuberculosis* complex from positive LJ culture isolates. **Materials & Methods:** Clinical samples were obtained from patients suspected of having pulmonary or extra pulmonary tuberculosis. GeneXpert was conducted on all samples and GeneXpert positive samples were cultured on Lowenstein-Jensen (LJ) medium. Culture positive isolates were subjected to MPT64 antigen detection test. **Results:** On GeneXpert, 63 of the 1050 samples were positive for MTB complex. Out of the 63 GeneXpert positive samples that were subjected to culture on LJ medium, 42/63 (66.6%) yielded growth. Culture positivity was higher (39.6%) in pulmonary than extra pulmonary samples (26.9%). Contrary to this, culture negativity was higher in extra pulmonary (30.1%) than (3.1%) in pulmonary specimens. MPT64 antigen detected all 42/42 (100%) of culture positive isolates as MTBC that were confirmed by conventional biochemical testing. **Conclusion:** MPT64 antigen detection assay is a reliable, quick and economical test for verifying MTBC from positive cultures and easy to incorporate in the Mycobacteriology culture diagnostic workflow.

Keywords: *GeneXpert, Mycobacterium tuberculosis complex, MPT64 and Tuberculosis.*

INTRODUCTION

In 2023, TB returned to being the world's leading infectious disease killer, surpassing COVID-19. It was also the leading killer of people with HIV and a major cause of deaths related to antimicrobial resistance. In 2023, an estimated 10.8 million people fell ill with TB worldwide, of which 55% were men, 33% women and 12% children and young adolescents. Globally in 2023, TB caused an estimated 1.25 million deaths. Eight countries accounted for more than two thirds of the global burden: India, Indonesia, China, Philippines, Pakistan, Nigeria, Bangladesh and the Democratic Republic of Congo. Among the recognized pulmonary TB cases each year, 64,000 multidrug-resistant TB (MDR-TB) cases occur in India, out of an estimated 300,000 cases worldwide. ¹ For effectively managing

patients and preventing the spread of disease as well as anti-tubercular resistance, prompt and reliable diagnosis is essential. Many methods have been developed for the quick detection of MTB, including real-time polymerase chain reaction (PCR), the Gen-Probe MTB/RIF, GeneXpert MTB/RIF and Line probe Assay (LPA), however with their advantages, they have their shortcomings too.²⁻⁴ While Ziehl Neelsen (ZN) stain offers quick preliminary information, it has sensitivity especially so in extra pulmonary samples.⁵ However culture is still regarded as the gold standard.⁶ Usually two to six weeks are needed to grow MTB using the solid culture method. With modern automated liquid culture systems like BACTEC TB 460 and MIGIT, growth can be obtained as early as 2nd to 3rd week considerably lowering the

time frame.⁷⁻⁹ But a positive acid fast growth on solid or liquid media does not definitively identify the isolate to be belonging to *Mycobacterium tuberculosis* complex (MTBC) as Non tuberculous mycobacteria (NTM) can easily grow on these media and stain vividly acid fast though they are largely considered as contaminants unless proved otherwise.⁶ Hence it is important to make a distinction between the two not only for determining clinical significance but also for tailoring treatment options. Besides growth rate and pigment production conventionally this differentiation between the two is made possible through a battery of tests like niacin accumulation, nitrate reduction, catalase production and Tween 80 hydrolysis.^{9,10} These tests nonetheless are not only cumbersome and time consuming but could be simultaneously dangerous for the bench worker especially so if utmost care to laboratory safety protocols is not adhered to. WHO advises against the use of serological testing for tuberculosis and their role is more often counterproductive.¹¹ But a common protein for quick confirmation of *Mycobacterium Tuberculosis complex* from solid and liquid cultures is MPT64, a 24-kDa protein of MTB complex that is found only in MTBC and not in NTMs.¹²⁻¹⁵ In this study our objective was to assess the performance of MPT64 for rapid confirmation of *Mycobacterium Tuberculosis complex* from solid cultures.

MATERIALS AND METHODS

Study Design

This observational cross-sectional laboratory based study was conducted in Department of Microbiology, Government Medical College Srinagar (GMCS), during March 2022 to March 2023. GMC Srinagar is a tertiary care, 2200 bedded hospital and caters to more than 7000 out patients in a day and is associated with hospitals like chest disease, bone and joint, paediatric and super speciality hospitals.

Sampling and processing

A total of 1050 patients aged 17-70 years, with clinical suspicion of tuberculosis (TB) were included in this study. BAL, sputum, CSF, Pus, endometrial curetting's, cervical lymph node aspirates and tissue biopsy samples from OPD and IPD were received in microbiology department along with the lab request form. Leakage, appropriate sample tube labelling, and form completion were all checked on these samples. All of the samples received in the lab were evaluated using GeneXpert in accordance with usual practice, without any sampling order, to prevent sampling bias. The samples that were found positive on GeneXpert were processed further for culture on Lowenstein-Jensen medium (LJ). Culture positive were subjected to MPT64 Ag detection test.

Culture of *Mycobacterium Tuberculosis*

N-Acetyl-L-Cysteine–Sodium Hydroxide (NALC-NaOH), was used for 15 minutes to decontaminate samples for *Mycobacterium Tuberculosis* culture, followed by dilution with phosphate-buffered saline (PBS), the final pH maintained at 6.8. Finally, the pellet was resuspended in 1.5 ml of PBS after the mixture was centrifuged for 15 minutes at 3000 g/min.¹⁶ The LJ medium is prepared in test tubes with a slanted surface for optimal bacterial growth. The tubes are sterilized in an inspissator before use. A sterile inoculating loop is used to transfer the sample onto the surface of the slant. The inoculum is streaked gently over the surface of the medium to allow the bacteria to grow, the inoculated LJ slants in duplicate were incubated at 37°C (the optimal growth temperature for *Mycobacterium tuberculosis*) for a maximum of eight weeks. The slants were observed weekly for growth and no growth after 8 weeks was regarded as negative culture.¹⁷ The standard operating procedures of the Mycobacteriology Laboratory (BSL-2) were followed.¹⁸ Any growth on LJ medium was subjected to AFB Stain and if positive was further processed for MPT64 antigen detection. Simultaneously conventional biochemical tests; Niacin accumulation, nitrate reduction, catalase production and Tween 80 hydrolysis were carried out to differentiate between MTB complex and NTMS.¹⁰

MPT64 antigen detection test (SD Bioline Abbott India)

Sample preparation: 3-4 colonies from LJ medium were suspended in 200ul extraction buffer before the test. 100 ul from this was added into the specimen well of the test kit. Interpretation of results was noted within 15 minutes. Positive results beyond 15 minutes were regarded as false positive. While as the presence of colour bands (CT and C) within the result window indicates a positive result, that of only C-band within the result window indicates a negative result.¹⁹ Known in house MTBC and NTM isolates were tested at the time of a fresh test.

RESULTS

Out of 1050 patients, 63 were positive for MTBC by the GeneXpert system, while 987 patients tested negative. Pulmonary were 27/63(42.8%) and extra pulmonary 36/63(57.1%). All the 63 positive samples were rifampicin sensitive. Among these, 31 (49.2%) were male, and 32 (50.7%) were females. The mean age of the participants was 25.16 years, with ages ranging from a minimum of 17 years to a maximum of 75 years. In terms of geographic location, 08 (12.6%) patients were from urban areas, while 55(87.3%) were from rural areas. The sample distribution for diagnostic testing was as follows: BAL (10); Sputum (17); CSF (08); lymph node aspirate (07); pleural fluid (05); hip joint fluid (02); Tubo- ovarian abscess (TOA) (03); abdominal peritoneal deposits (01); ascitic fluid

(02); omental biopsy (03); ear infection (01); pus (01); knee aspirate (03) (Table 1).

Table 1: Baseline characteristics of patients who tested positive on GeneXpert (n=63)

Characteristics	N=63	Frequency %
Gender		
Male	31	49.2
Female	32	50.7
Sample		
BAL	10	15.8
SPUTUM	17	26.9
CSF	08	12.6
Lymph Node Aspirate	07	11.1
Pleural Fluid	05	7.9
Hip Joint Fluid	02	3.1
Tubo ovarian abscess	03	4.7
Abdominal Peritoneal Deposit	01	1.6
Ascitic Fluid	02	3.1
Omental Biopsy	03	4.7
Ear Infection	01	1.6
Pus	01	1.6
Knee Aspirate	03	4.7
Urban	08	12.6
Rural	55	87.3

The samples (n= 63) that were positive on GeneXpert were cultured on Lowenstein-Jensen (LJ) medium. Of these, 42 samples exhibited visible growth that was acid fast on ZN staining. Subsequently, all 42 culture-positive samples were subjected to the MPT64 antigen detection test. The test returned positive results for all 42 samples, confirming the presence of Mycobacterium tuberculosis complex in 42/42 (100 %) of culture positive samples. The culture positivity of GeneXpert positive samples on LJ medium as such was 42/63 (66.6%). (Table 2).

Table 2: Results of various diagnostic methods for Mycobacterium Tuberculosis Complex

Diagnostic Methods			
	GeneXpert MTB/RIF assay (n=1050)	Mycobacterial culture on LJ Medium (n=63)	MPT64 (n=42)
Positive	63	42	42
Negative	987	21	0

While the distribution of culture positive TB types revealed that 25/63 (39.6%) patients had pulmonary TB, while 17/63 (26.9%) had extra pulmonary TB. Twenty-one (21) out of 63 GeneXpert positive samples were negative on L J medium culture (33.3%). Among the culture negatives 2/63 (3.1%) were pulmonary samples while as 19/63 (30.1%) were extra pulmonary. (Table 3).

Table 3. Sample distribution of GeneXpert & culture positive isolates

Sample type	GeneXpert positive	Culture positive
BAL	10	10
Sputum	17	15
CSF	8	3
Lymph node aspirate	7	3
Pleural fluid	5	2
Hip joint fluid	2	2
Tubo ovarian abscess	3	2
Abdominal peritoneal deposit	1	0
Ascitic fluid	2	0
Omental biopsy	3	1
Ear infections	1	1
Pus	1	1
Knee aspirate	3	2
Total	63	42

DISCUSSION

Tuberculosis the disease of antiquity still remains a public health concern in vast majority of underdeveloped and developing countries owing to the share number of infections and deaths it causes annually. With the evolving HIV pandemic, rising HIV and TB confections and mounting resistance to both antitubercular and HIV drugs tuberculosis poses a formidable challenge vis a vis diagnosis and treatment.²⁰ While rapid molecular diagnostics like GeneXpert have transformed the tubercular diagnostic landscape,²¹ unfortunately their availability is still limited in many resource poor countries where AFB staining and culture are still mainstay of diagnosis. However, any acid fast growth on culture needs further confirmation by a battery of biochemical tests that are not only time consuming but could be hazardous for the laboratory personnel. The introduction of MPT64 Antigen detection test that is an immunochromatographic lateral flow assay has provided a viable alternative that is not only quick but relatively safe as well. MPT64 antigen is a culture filtrate protein that is specifically produced by Mycobacteria belonging to MTB complex and not by the rest of Non tuberculous mycobacteria.¹²⁻¹⁵ The present study was aimed to determine the diagnostic performance of MPT64 antigen in confirming mycobacterium tuberculosis complex from growths on solid LJ medium cultures.

In the current study out of 1050 samples that were sent for GeneXpert testing, 63 were positive for MTB complex accounting for 6% positivity which is slightly lower than 9.5% reported by Jagadevi et al.²² While most other Indian studies like by Gupta et al report higher GeneXpert positivity (16.9%).²³ This lower positivity in our study could be attributed to more paucibacillary extrapulmonary samples, poor patient selection criteria and suboptimal quality and quantity of specimens. Males and females represented 49.2% and 50.7% respectively. Majority of the GeneXpert positive cases 55 (87.3%) belonged to rural areas. This disproportionately major contribution from rural areas could be possibly attributed to poverty, overcrowding, lack of access to healthcare facilities and health education in rural areas compared to urban. There were more extra pulmonary 36/63 (57.1%) than pulmonary 27/63 (42.8%) GeneXpert positive cases. The high proportion of extra pulmonary cases could be because of the fact that our hospital is one of the only two tertiary care hospitals in the valley that caters to cases of extra pulmonary tuberculosis like meningeal, abdominal, synovial and musculoskeletal that are often difficult to diagnose in peripheral healthcare setups. Moreover, a large chunk of patients with pulmonary tuberculosis preferentially attend a dedicated chest diseases hospital (Govt Chest Disease Hospital Dalgate Srinagar).

Out of 63 GeneXpert positive samples 42/63 (66.6%) samples were positive on L J medium culture while as 21/63 (33.3%) samples were negative after 8 weeks of incubation. Among culture positive isolates 25/63(39.6%) were pulmonary while as 17/63(26.9%) were extra pulmonary tuberculosis. In the GeneXpert positive culture negative 21/63 (33.3%) samples, 2/63 (3.1%) were pulmonary and 19/63 (30.1%) were extra pulmonary tuberculosis. A relatively lower culture positivity overall (66.6%) in the current study could be explained by the fact that a good majority of the GeneXpert positive samples (57.1%) were from extra pulmonary sites that are known to be paucibacillary and have low yield on culture. This is evident in the current study from the fact that among the culture positives 39.6% were pulmonary and 26.9% extra pulmonary while as in the culture negatives, pulmonary represented only 3.1% while as extra pulmonary represented 30.1% samples. Moreover some of the patients were already receiving ATT and in such cases it is well known that even though while GeneXpert may amplify the DNA of dead bacilli, cultures will fail to grow such non viable bacteria.²⁴ There have been studies with results similar to and differing from our findings as the diagnostic algorithm and the study patient population differ across the studies.^{25, 26}

All 42 culture positive samples were subjected to MPT64 antigen detection test. The test was positive in all 42/42(100%) of culture positive isolates. Subsequently all the 42 isolates were confirmed to be belonging to MTB Complex by conventional biochemical tests. As such in the present study the MPT64 antigen successfully confirmed all culture positive isolates as MTBC enabling earlier rapid confirmation compared to conventional biochemical tests that are labour intensive and time consuming. Similar findings have been reported by numerous other studies.^{27,28} However since the study population was restricted to GeneXpert positive samples only, the data was enriched for MTBC and lacked an adequate representation of NTM. Therefore the ability of MPT64 test to discriminate MTBC from NTM could not be reliably assessed owing to the study design limitation.

Strengths of the Study

The present study has several strengths. First, it evaluated MPT64 antigen detection in a real-world tertiary-care microbiology laboratory setting using clinically relevant pulmonary and extrapulmonary specimens. Second, the study included a relatively diverse range of specimen types, including sputum, BAL, CSF, lymph node aspirates, pleural fluid, synovial fluid, tissue and other extrapulmonary samples. Third, all culture-positive isolates were

evaluated by MPT64 antigen detection and subsequently confirmed using conventional biochemical methods, providing an additional reference for identification of MTBC. Fourth, the study assessed MPT64 detection using a commercially available immunochromatographic assay that can potentially be incorporated into routine mycobacteriology laboratory workflows without requiring sophisticated instrumentation. Finally, the study highlights the potential utility of MPT64 antigen detection for reducing the time and laboratory workload associated with conventional biochemical identification of MTBC from culture isolates.

Limitations of the Study

The study has several limitations that should be considered when interpreting the findings. First, the study was conducted at a single tertiary-care centre, which may limit the generalizability of the findings to other healthcare settings and geographical regions. Second, only GeneXpert-positive specimens were taken forward for culture, resulting in a study population enriched for MTBC and limiting assessment of the diagnostic performance of MPT64 across a broader range of mycobacterial isolates. Third, the number of culture-positive isolates was relatively small (n=42). Fourth, an adequate number of non-tuberculous mycobacterial (NTM) isolates was not included; therefore, the ability of the MPT64 assay to discriminate MTBC from NTM and its specificity could not be reliably estimated. Fifth, the study evaluated isolates obtained from solid Lowenstein-Jensen medium and therefore the findings may not be directly applicable to liquid culture systems. Finally, sensitivity, specificity, positive predictive value and negative predictive value could not be comprehensively calculated because the study design did not include an appropriate panel of confirmed MTBC-negative and NTM-positive culture isolates.

Future Directions

Future multicentric studies involving larger numbers of culture-positive isolates are warranted to further validate the diagnostic utility of MPT64 antigen detection. Such studies should include adequate numbers of confirmed NTM isolates and MTBC-negative cultures to allow robust estimation of sensitivity, specificity, positive predictive value and negative predictive value. Comparative evaluation of MPT64 antigen detection with conventional biochemical identification, molecular identification and automated liquid culture systems such as MGIT may also provide more comprehensive evidence regarding its diagnostic accuracy and workflow advantages. Cost-effectiveness, turnaround time and laboratory biosafety considerations should additionally be evaluated in different healthcare settings, particularly in resource-limited laboratories. Further studies comparing MPT64 assays across different

commercial platforms and specimen/culture conditions may help establish their broader applicability in routine tuberculosis diagnostic laboratories.

CONCLUSION

The MPT64 antigen detection test is a rapid, user friendly and reliable method for confirmation of Mycobacterium tuberculosis complex from solid culture isolates demonstrating a high identification rate in the current study. It can be readily incorporated into the routine Mycobacterial culture diagnostic workflows without need for extensive training.

Declarations

Ethics Approval and Consent to Participate

The study was conducted in accordance with applicable institutional and ethical standards. Ethical approval and/or waiver of informed consent was obtained from the appropriate institutional ethics committee, as applicable to this laboratory-based observational study. All procedures were performed in accordance with institutional guidelines and relevant standards for the handling and processing of mycobacterial specimens.

Consent for Publication

Not applicable. The study does not contain individually identifiable personal information or images requiring consent for publication.

Availability of Data and Materials

The data generated and/or analyzed during the present study are available from the corresponding author on reasonable request, subject to institutional policies and applicable ethical and confidentiality requirements.

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Competing Interests

The authors declare that they have no competing interests or conflicts of interest related to this study.

Authors' Contributions

RN and MP contributed to the study conception, laboratory investigation and data collection. MA contributed to study supervision, interpretation of results and manuscript development. BT contributed to laboratory investigation, data analysis and manuscript preparation. All authors reviewed and approved the final manuscript and agree to be accountable for all aspects of the work.

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AI Use Disclosure

The authors declare that no generative artificial intelligence tools were used to generate or substantially modify the scientific content of this manuscript.

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