Rapid Molecular Detection of Mycobacterium Tuberculosis (MTB) in Pulmonary and Extrapulmonary Samples.

Dr. Sheeta Sharma[*1], Dr. Sumit Kumar[2], Dr. Sandeep Dogra[3]

[1]Fellowship in Clinical Microbiology Scholar, Ramnath Prasad Institute of Higher Education Foundation East Champaran (Bihar).
[2]Professor Microbiology, Ramnath Prasad Institute of Higher Education Foundation East Champaran (Bihar).
[3]Professor & HOD, Department of Microbiology, Govt. Medical College & Hospital Jammu (J&K).

Corresponding Author:
Dr. Sheetal Sharma
Fellowship in Clinical Microbiology Scholar, Ramnath Prasad Institute of Higher Education Foundation, East Champaran Bihar.Email: ssharmabyc@gmail.com

ABSTRACT

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading cause of death by an infectious disease. It remains a serious public health problem over the globe. Tuberculosis is a significant global health challenge, with an estimated 10 million people becoming sick with the disease and 1.5 million deaths due to TB in 2020 (1). The global public health response for TB has been complicated by challenges to diagnose and link people to care. In 2019, an estimated 2.9 million people with TB were undiagnosed and unreported (2), and in 2020 this estimate increased to more than 4 million people undiagnosed and unreported for TB during the COVID-19 pandemic (1). This was a retrospective study conducted by Department of Microbiology, Government Medical College, Rajouri in collaboration with District Tuberculosis Centre, Rajouri, J&K. A total of 248 samples of the patients with symptoms suggestive of pulmonary tuberculosis and Extra pulmonary samples were collected and tested over a period of ten month. Out of the 248 samples tested, 9 (3.6%) were positive for MTB by Truenat. Early morning, deep coughed sputum and EPTB samples was considered for the study. males and females among the study population with 126 males (50.8%) and 122 females (49.2%). Maximum number of suspected EPTB patients was in the age group of 50-59 and 60-69 age category (42.3%). In contrast, the present study aimed to assess the rate of inconclusive Truenat results in real-world situations and to determine their root causes. This knowledge is needed in order to optimize the testing performance of Truenat MTB-RIF, to ensure timely diagnosis, and thereby to reduce the magnitude of undiagnosed TB cases.

Keywords:*Mycobacterium tuberculosis (MTB), TrueNat, Pulmonary Tuberculosis (PTB), Extrapulmonary-tuberculosis (EPTB).*

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INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), is the leading cause of death by an infectious disease. It remains a serious public health problem over the globe. Tuberculosis is a significant global health challenge, with an estimated 10 million people becoming sick with the disease and 1.5 million deaths due to TB in 2020 (1). The global public health response for TB has been complicated by challenges to diagnose and link people to care. In 2019, an estimated 2.9 million people with TB were undiagnosed and

unreported (2), and in 2020 this estimate increased to more than 4 million people undiagnosed and unreported for TB during the COVID-19 pandemic (3). With approximately 2.8 million cases annually, India has the world's highest incidence of tuberculosis (4). Treating tuberculosis is extremely challenging if the bacteria that cause the disease are resistant to first-line drugs. If they are resistant to rifampicin, the disease is termed rifampicin-resistant tuberculosis (RR-TB), and if they are also resistant to isoniazid, the disease is termed multidrug-resistant tuberculosis (MDR-TB). Microbiological confirmation is recommended for diagnosing pulmonary tuberculosis (PTB) and extra-pulmonary tuberculosis (EPTB). The miniaturized forms of PCR tests have the advantages of a reduction in the cost of instruments and faster turnaround times in poor resource settings. The micro-PCR devices have the added advantages of better diagnostic sensitivity and portability. They are widely used in India and other South-East Asian countries (7). GeneXpert was endorsed by the World Health Organization (WHO) to be used in India as part of the National TB control programme (8). Cartridge Based Nucleic Acid Amplification Test (CBNAAT) is a test which detects TB bacilli and also screens for rifampicin drug resistance (9).

In contrast, the present study aimed to assess the rate of inconclusive Truenat results in real-world situations and to determine their root causes. This knowledge is needed in order to optimize the testing performance of Truenat MTB-RIF, to ensure timely diagnosis, and thereby to reduce the magnitude of undiagnosed TB cases, not only in India but also in other countries that have rolled out Truenat as a molecular point-of-care tool to strengthen the TB diagnostic care cascade in national TB programs. This study is also evaluate the diagnostic accuracy of Truenat MTB assay in both pulmonary (PTB) and extra-pulmonary (EPTB) tuberculosis cases in a tertiary care hospital of Rajouri District in Jammu & Kashmir India.

MATERIAL & METHODS

This study was conducted in the Department of Microbiology, Government Medical College and Associated Hospital, Rajouri Jammu and Kashmir in collaboration with District Tuberculosis Centre (DTO) Rajouri, J&K. Sputum and Extra Pulmonary samples were received from various blocks of Rajouri district. The study was carried over a period of 10 months i.e, from May 2024 to Feb 2025.

Inclusion Criteria

Patients with clinical suspicion of pulmonary tuberculosis based on symptoms (e.g., cough more than two weeks, hemoptysis, fever, loss of weight and night sweats) and Radiological features (e.g., nodule, consolidation, cavitation and other opacities) were included in the study.

Exclusion Criteria

Samples macroscopically resembling saliva and blood stained samples were excluded.

A total of 248 samples of the patients with symptoms suggestive of pulmonary tuberculosis and Extra pulmonary samples were collected and tested over a period of ten months. Early morning, deep coughed sputum and EPTB samples was considered for the study. All the details of the patients like Name, Address, Age, Sex, Treatment received and Name of the referring centre were noted down.

Fig 1: TrueNat PCR Work Station

Fig 2: TrueNat MTB Test kit

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Fig 3: TrueNat PCR Analyzer Result Screen

RESULT

A total of 248 suspected cases were studied over a period of ten months from May 2024 to February 2025. Samples were obtained from various departments like Surgery, Medicine, Orthopaedics, Paediatrics, , Pulmonary Medicine and Gynaecology. There was an almost equal distribution of males and females among the study population with 126 males (50.8%) and 122 females (49.2%). Maximum number of suspected EPTB patients was in the age group of 50-59 and 60-69 age category (12). Age wise distribution of samples is mentioned in table. Out of the 248 samples tested, 44 (17%) were positive for MTB by Truenat. Out of the different positive samples, three were Sputum (33.3%).

Chart 1: Distribution of Samples from various Clinical departments

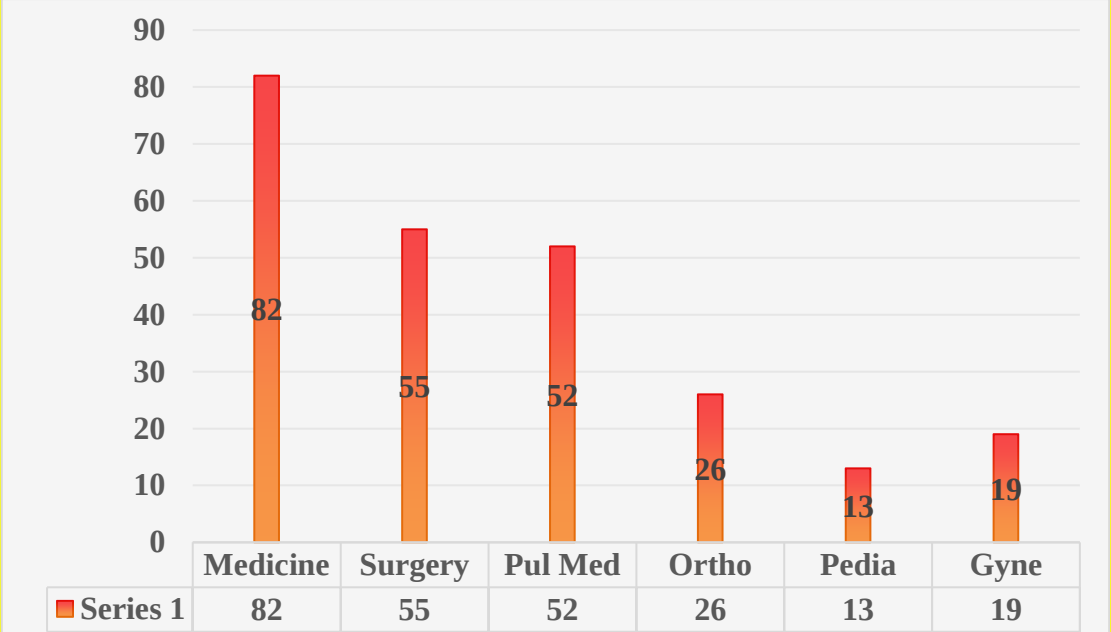


Chart 2: Age wise distribution of samples with MTB Positive result

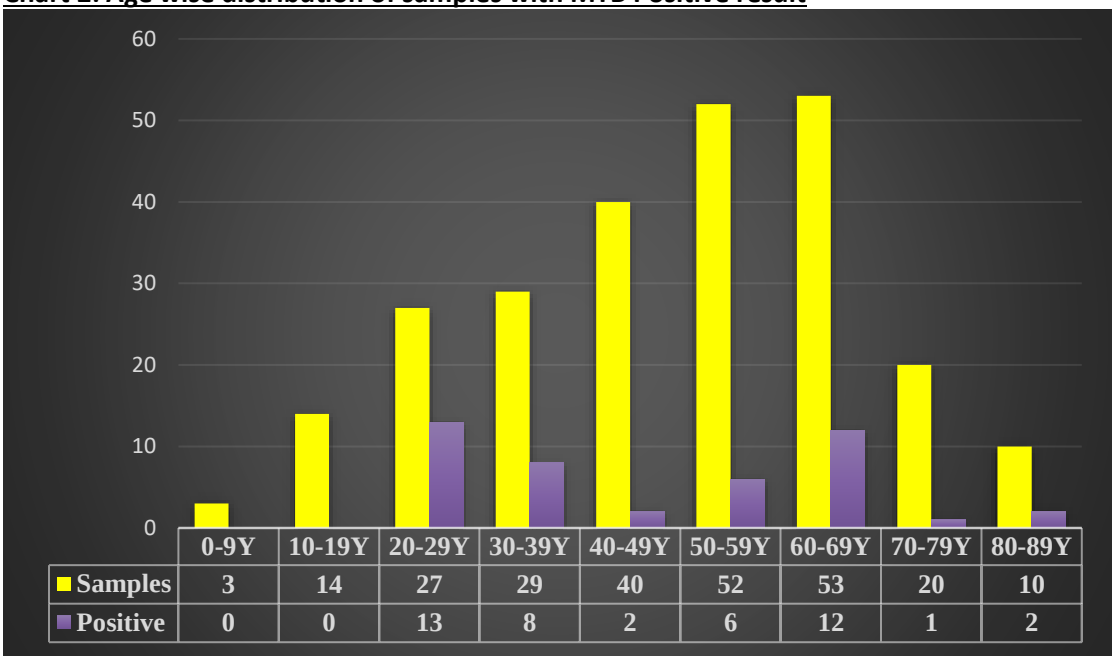


Chart 3: Symptology of study population

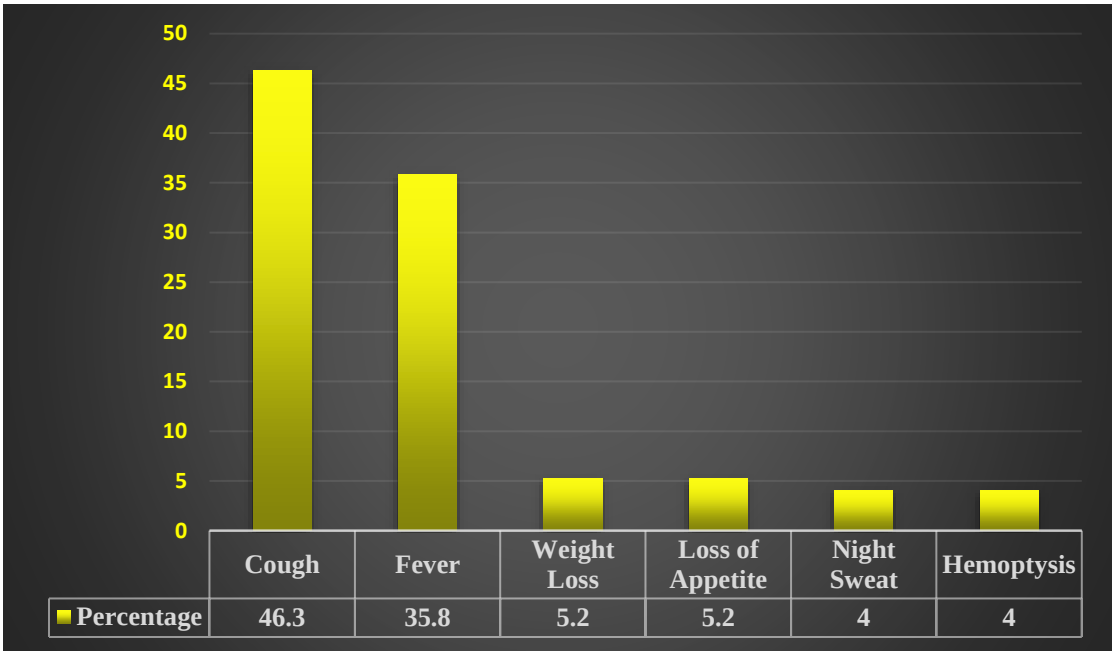
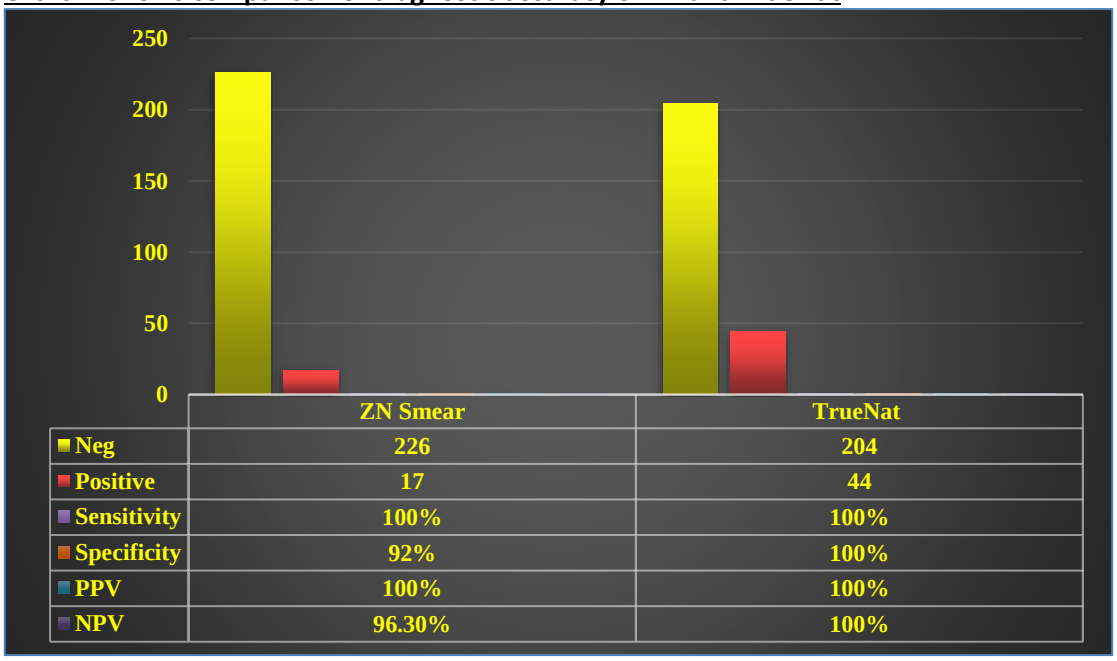


Chart 4: Shows comparison of diagnostic accuracy of ZN and TrueNat



DISCUSSION

A major WHO priority for TB diagnostics is to implement a rapid, sputum-based molecular test to replace smear microscopy at the peripheral level (i.e., microscopy centers and attached primary healthcare facilities) [7,8]. Our model-based analysis shows that in India, Truenat PCR system is a cartridge based test for the rapid diagnosis of tuberculosis along with the detection of rifampicin resistance which is developed in India. With the extensive availability of this economically viable diagnostic method in resource poor country like India will not only help in the early diagnosis but also in the appropriate management and infection control measures and controls the spread of this deadly disease.

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Truenat will improve linkage-to-care and increase life expectancy also.

TrueNat, when replacing smear microscopy and used at point-of-care, increases the number of TB cases correctly detected and linked to care by 590 per 10,000 individuals with presumptive TB. It also increases life expectancy by nearly 0.4 years and is cost-effective. While Truenat DMC was economically inefficient among the four strategies, it was cost-effective when compared directly to SSM. The cost-effectiveness of Truenat POC, compared to SSM, was consistent across a wide range of clinical and cost parameter values. The WHO's target product profile (TPP) of the "smear replacement test" includes a set of minimal and optimal requirements [7,8]. Truenat fits many minimal TPP standards, including battery-powered operation and less than 2 hours to result (22).

With culture as gold standard test, Truenat had a sensitivity of 100% and specificity of 95.1%. When compared with microscopy, Truenat test had 100% sensitivity and 96.6% specificity. In a similar study conducted by Nikam C et al., for evaluation of Truenat using sputum samples, they found a sensitivity value of 100% and a specificity value of 43.98% for Truenat over microscopy, whereas for Truenat over culture they obtained a sensitivity value of 94.70% and a specificity value of 52.85% (13).

Majority of the cases were in the age group of 60-69 years with male predominance. This was in agreement with studies conducted by Subbarao et.al (15) and Desai K et al. The most common symptoms in our study were cough (69.75%) and fever (56.27%). Similar findings were seen in study conducted by Avashia et al (16)as they found fever (69.4%) and cough (72.2%) as the main symptom.

Our analysis shows that scaling up molecular diagnostics will increase the required budget but the majority of the cost will be from MDR-TB treatment. A recent economic analysis for India similarly found that full replacement of smear microscopy with Xpert would substantially increase budget requirements but would result in lower cost per MDR-TB case initiated on treatment [21]. As the NTEP plans its NSP budget for 2020–2025, it should consider MDR-TB treatment costs as much as, if not more than, the prices of diagnostic tests.

Truenat MTB test has a higher sensitivity than other conventional diagnostic tests like smear microscopy or culture for MTB. The present study stresses the importance of this new tool, which is indigenous, economical and convenient to use in a low resource setting like India. This study paves and opens windows for larger studies for replacing other molecular diagnostic tests. The performance of Truenat has been evaluated extensively by various researchers and has been compared with conventional culture based as well as with other molecular diagnostic methods. Nikam et al., from Hinduja Hospital and Medical research centre, Mumbai evaluated the performance of Truenat RTPCR in comparison with GeneXpert on sputum samples from Pulmonary TB cases and found a high concordance (96%) with GeneXpert (9).

CONCLUSION

Truenat MTB-RIF assay (Truenat), a nucleic acid amplification test (NAAT), is a real-time polymerase chain reaction (RT-PCR) chip-based assay that can detect Mycobacterium tuberculosis (Mtb) and rifampicin (RIF) drug resistance using portable, battery-operated devices. The National TB Elimination Program (NTEP) in India introduced this novel tool at the district and subdistrict level in 2020. This study aimed to assess the level and causes of inconclusive results (invalid results, errors, and indeterminate results) in MTB and RIF

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testing at NTEP sites and the root causes of these in the programmatic setting.

This study was conducted in the Department of Microbiology, Government Medical College and Associated Hospital, Rajouri Jammu and Kashmir in collaboration with District Tuberculosis Centre (DTO) Rajouri, J&K. A total 248 clinical samples were collected and tested for mycobacterium tuberculosis infection and drug resistance by TrueNat a rapid PCR system from May 2024 to February 2025 about ten months.

The World Health Organisation (WHO) has endorsed the use of TrueNat PCR as a rapid diagnostic test for the diagnosis of TB and prioritized areas like drug-resistant TB, paediatric TB, TBHIV co-infection, extra-pulmonary TB, and sputum smear negative PTB for use of TrueNat (25). This study concludes that in less accessible and resource limited rural settings where establishing a sophisticated laboratory for culture to the prescribed biosafety levels is difficult, TrueNat PCR system provides a very good detection laboratory method. Extensive use of this assay thereby facilitates early treatment decisions and curbing transmission of Tuberculosis. Our study highlighted the usefulness of the TrueNat over and above the traditional smear microscopy for significantly higher positive results. It also has an added advantage of detection of multi-drug resistant cases, thus contributing as a milestone in 'End TB' strategy.

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