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Study of the Diagnostic Accuracy of Truenat Assays for Detecting Pulmonary Tuberculosis & Rifampicin Resistance

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ABSTRACT

Background: Tuberculosis (TB) remains a major global health problem, particularly in developing countries, where delayed diagnosis contributes to continued transmission and increased morbidity. Conventional diagnostic methods have limitations in sensitivity and turnaround time, while molecular assays like Truenat offer rapid, accurate, and decentralized testing for detection of Mycobacterium tuberculosis and rifampicin resistance. **Aim and Objectives**: The purpose of the study is to determine the diagnostic accuracy of Truenat assays for detecting pulmonary tuberculosis and to evaluate their performance in identifying rifampicin resistance. **Material and Methods**: A hospital-based cross-sectional observational study was conducted in Smt Jai Shri Sinha, RT-PCR BSL-2, Molecular Biology Diagnostic and Research Laboratory in Department of Microbiology at Muzaffarnagar Medical College & Hospital over 18 months. A total of 2000 patients with symptoms suggestive of tuberculosis were included. Sputum samples were collected and processed using the Truenat MTB real-time PCR assay, followed by Truenat MTB-RIF Dx for rifampicin resistance detection. Data were analysed using SPSS software. **Results**: The majority of participants belonged to the 31–45 years age group with slight male predominance. Pulmonary tuberculosis accounted for 84.2% of cases. The Truenat assay demonstrated 100% sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy. Rifampicin resistance was observed in a very small proportion of cases, with most being sensitive. **Conclusion**: Truenat is a highly sensitive, specific, and rapid diagnostic tool for tuberculosis and rifampicin resistance. Its portability and ease of use make it suitable for decentralized testing, thereby enhancing early diagnosis and contributing to effective TB control and elimination strategies.

Keywords: Tuberculosis, Truenat, Rifampicin resistance, Molecular diagnosis, Pulmonary TB, Diagnostic accuracy.

INTRODUCTION

Tuberculosis (TB) is an airborne communicable disease transmitted when a susceptible person inhales droplet nuclei released by an individual with active pulmonary TB during coughing, sneezing, or speaking. [1] It is mainly spread by airborne droplets from people who have active pulmonary illness and is caused by *Mycobacterium tuberculosis*. TB is nevertheless widespread, especially in low- and middle-income nations, due to its effective transmission and capacity to linger in a latent condition. [2]

A persistent cough lasting more than two weeks, fever, night sweats, weight loss, exhaustion, chest pain, and rarely hemoptysis are all common symptoms of pulmonary tuberculosis. Nevertheless, these symptoms are non-specific and can be confused with a number of other respiratory disorders, such as cancer, pneumonia, and chronic obstructive pulmonary disease. Therefore, if microbiological confirmation is not done, relying just on symptoms may result in a missed or delayed diagnosis. [2]

Depending on the organ affected, extra-pulmonary tuberculosis frequently manifests subtly with nebulous symptoms such persistent fever, weight loss, or localized pain. Classical symptoms may be absent or abnormal in immune-compromised people, such as those with diabetes mellitus or HIV infection. These difficulties draw attention to the shortcomings of symptom-based diagnosis and emphasize the necessity of sensitive laboratory-based diagnostic techniques for tuberculosis early and precise detection. [3][4]

For many years, conventional TB diagnostic techniques have been the mainstay of TB diagnosis and are still frequently employed, especially in areas with limited resources. Due to its simplicity, affordability, and convenience of use, Ziehl-Neelsen staining sputum smear microscopy is still the most often used technique. [9] Because of its great sensitivity and capacity to enable drug susceptibility testing, *Mycobacterium tuberculosis* culture on solid or liquid media is regarded as the gold standard for TB diagnosis. [2]

One of the first molecular tests to be widely used under national TB control programs was the Cartridge-Based Nucleic Acid Amplification Test (CBNAAT), also referred to as Xpert MTB/RIF. It enables quick turnaround times for the simultaneous detection of *M.tuberculosis* and rifampicin resistance. However, its application in outlying and resource-constrained health facilities has been constrained by its need for steady electricity, regulated environmental conditions, frequent calibration, and greater operating expenses. [2]

Truenat assays were created as native, chip-based, real-time polymerase chain reaction (PCR) tests appropriate for near point-of-care application in order to get over these operational limitations. *M.tuberculosis* and rifampicin resistance can be directly detected from sputum samples using the Truenat platform, which consists of Truenat MTB, Truenat MTB Plus, and Truenat MTB-RIF Dx. The system is appropriate for decentralized testing because it is battery-operated, portable, and requires little laboratory infrastructure. [3]

The Truenat MTB assay detects *M.tuberculosis* DNA, while the MTB Plus assay offers improved sensitivity, particularly in smear-negative and paucibacillary cases. Samples positive for MTB can subsequently be tested using the Truenat MTB-RIF Dx assay, which identifies mutations in the *rpoB* gene associated with rifampicin resistance. This sequential approach facilitates early identification of drug-resistant tuberculosis and timely initiation of appropriate therapy. [5] Truenat has been approved by the World Health Organization and integrated into India's National TB Elimination Programme for use at district and sub-district levels because to the focus on early diagnosis, universal drug susceptibility testing, and decentralization of diagnostic services. [2][3] Therefore, evaluating the diagnostic precision of Truenat tests for rifampicin resistance and pulmonary tuberculosis is crucial to ascertaining their efficacy in programmatic and practical contexts.

Aim & Objectives: The purpose of this study is to determine the ddiagnostic accuracy of Truenat assays for detecting pulmonary tuberculosis and for detecting Rifampicin resistance.

MATERIAL AND METHODS:

A hospital-based cross-sectional observational study was conducted in Smt Jai Shri Sinha, RT-PCR BSL-2, Molecular Biology Diagnostic and Research Laboratory in Department of Microbiology at Muzaffarnagar Medical College & Hospital, a tertiary care center, over a period of 18 months (April 2024–March 2025). A total of 2000 OPD patients presenting with symptoms suggestive of tuberculosis (such as chronic cough >2 weeks, haemoptysis, fever, weight loss, and night sweats) were included using convenience sampling. Sputum samples were collected in sterile containers, transported promptly, and processed under biosafety level-2 conditions; samples with delay were stored at 4°C for up to 24 hours. Nucleic acid extraction was performed using the Trueprep AUTO system, followed by detection of Mycobacterium tuberculosis using the Truenat MTB real-time PCR assay based on TaqMan chemistry, where results were interpreted through amplification curves and Ct values. Positive samples were further tested for rifampicin resistance using Truenat MTB-RIF Dx. Quality control measures, proper biomedical waste disposal, and standard laboratory protocols were strictly maintained. Data were entered in MS Excel and analysed using SPSS software after obtaining institutional ethical clearance.

Inclusion criteria:

- Patient having symptoms like prolonged cough for >2weeks.
- Patients coughing up blood or mucus.
- Patients with chest pain or pain with breathing or coughing.
- Patients presenting with fever, chills, night sweats, weight loss, loss of appetite, tiredness and malaise.

Exclusion criteria:

- Patients who did not give consent.
- Patients with no history of Chronic cough.
- Follow up TB patients were also excluded from the study.

RESULTS:

The socio-demographic profile of the participants showed that the largest proportion belonged to the 31–45 years age group, comprising 440 (22.0%) participants. This was followed by the 46–60 years age group with 415 (20.8%), >60 years with 413 (20.7%), and 20–30 years with 383 (19.2%) participants. The 2–19 years age group accounted for 322 (16.1%), while very young children constituted only a small proportion, with 20 (1.0%) participants in the 1 month–1 year group and 7 (0.4%) in the <1 month group. Gender distribution showed a slight male predominance, with 1039 (52.0%) males and 961 (48.1%) females. **(Table 1)**

Table 2 showed that TRUENAT assay showed perfect agreement with the reference standard for pulmonary tuberculosis detection. Out of 1684 cases, 224 positive cases and 1460 negative cases were correctly identified, with no false positives or false negatives, indicating 100% sensitivity, specificity, and diagnostic accuracy.

The TRUENAT assay showed that all rifampicin resistance-related results (indeterminate, resistant, and sensitive) were detected only in TRUENAT-positive cases, while TRUENAT-negative cases were mostly not applicable (86.6%). The association was statistically highly significant ($p < 0.001$), indicating a strong correlation between TRUENAT positivity and rifampicin sensitivity results. **(Table 3)**

The TRUENAT assay demonstrated excellent diagnostic performance, with 100% sensitivity, specificity, PPV, NPV, and overall accuracy, indicating it is a highly reliable test for detection of pulmonary tuberculosis. **(Table 4)**

Figure 1 shows that the majority of participants had pulmonary tuberculosis, accounting for 1684 (84.2%) cases, while extra-pulmonary tuberculosis constituted a smaller proportion with 316 (15.8%) cases, indicating pulmonary TB as the predominant form in the study population.

Table 1- Socio-demographic profile of participants:

Variables	Frequency	Percentage (%)
Age Group		
<1 month	7	0.4%
1 month-1 year	20	1.0%
2 year-19 years	322	16.1%
20-30 years	383	19.2%
31-45 years	440	22.0%
46-60 years	415	20.8%
>60 years	413	20.7%
Gender		
Female	961	48.1%
Male	1039	52.0%

Table 2: Comparison of TRUENAT Assay with Reference Standard for detection of Pulmonary Tuberculosis:

TRUENAT Result	Reference Test Result		
	Negative	Positive	Total
Negative	1460	0	1460
Positive	0	224	224
Total	1460	224	1684

Table 3: Distribution of Rifampicin Sensitivity Results by TRUENAT Assay:

Rifampicin Sensitivity	TRUENAT					
	Negative		Positive		Total	
	N	%	N	%	N	%
Indeterminate	0	0%	19	.95%	19	1.0%
Not applicable	1732	86.6%	0	0.0%	1732	86.6%
Resistant	0	0.0%	10	.5%	10	.5%
Sensitive	0	0.0%	239	12.0%	239	12.0%
p-value	**<0.001**					

Table 4: Diagnostic Accuracy of TRUENAT assay for detection of Pulmonary Tuberculosis:

Parameter	Value (%)
Sensitivity	100
Specificity	100
Positive Predictive Value (PPV)	100
Negative Predictive Value (NPV)	100
Overall Diagnostic Accuracy	100

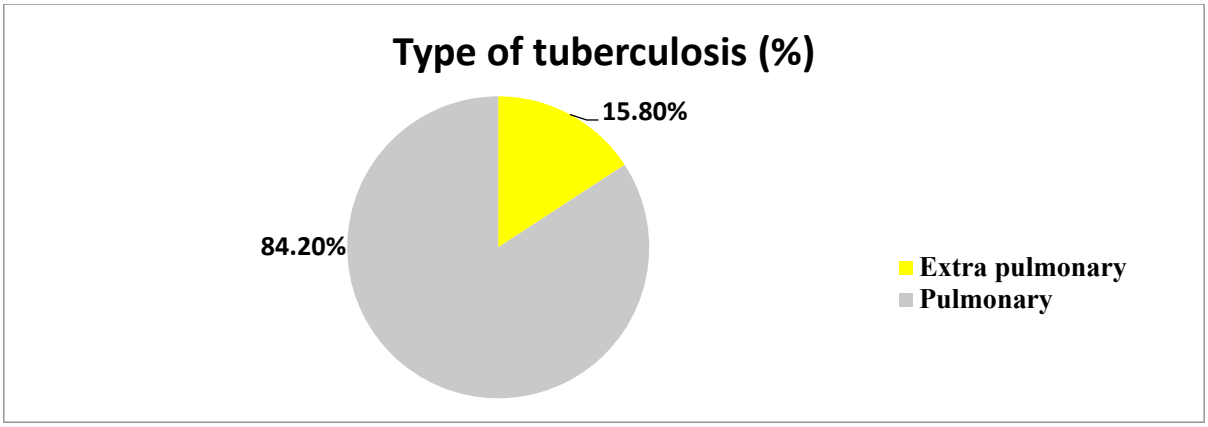

Figure 1: Distribution of study participants according to type of tuberculosis:

DISCUSSION:

In present study, largest cohort was the 31–45 years age group, representing 22.0% (N=440) of the total population. Kumar et al. [6] reported a similar peak in the 30–45 years category, accounting for 24.5% of their study population, which aligns closely with our finding of 22.0%. In contrast, a 2021 study by Singh et al. [7] observed a slightly higher prevalence in the older demographic, with the >60 years group constituting 26.8%, compared to our study's 20.7%. The gender distribution showed a marginal male predominance, with males accounting for 52.0% (N=1039) and females accounting for 48.1% (N=961). The observed male-to-female ratio in our study is notably more balanced than that reported by Onifade et al., [8] who found a significantly higher male prevalence of 64.2% in their tuberculosis survey. In a 2022 study by Narasimhan et al., [9] the male proportion was recorded at 58.0%, which is slightly higher than your finding of 52.0%.

The diagnostic evaluation for Pulmonary Tuberculosis (PTB) using the reference standard yielded a positivity rate of 13.3% (n = 224) among the 1,684 participants tested. This detection rate highlights a significant burden of confirmed disease within the symptomatic cohort, yet it also underscores that a vast majority (86.7%) of those presenting with respiratory complaints like cough were not suffering from active PTB. This finding is consistent with recent data from Saha et al. (2025), where the prevalence of microbiologically confirmed TB in symptomatic outpatient populations was found to range between 10% and 15%, reflecting the high overlap of symptoms between TB and other chronic respiratory conditions. [10] Furthermore, the gap between clinical suspicion and laboratory confirmation observed here mirrors findings by Satia et al. (2025), who noted that in specialized clinics, the diagnostic yield for TB typically plateaus around 12–18% when utilizing molecular or culture-based reference standards. [11]

The implementation of the Truenat assay for the detection of Pulmonary Tuberculosis (PTB) in this study yielded a positivity rate of 13.3% (n = 224). This observation is reinforced by the work of Rao et al. (2025), whose evaluation of chip-based real-time PCR in decentralized settings found that Truenat displayed high sensitivity and a similar diagnostic yield in adult symptomatic cohorts, making it a reliable pillar for active case finding. [12] The high proportion of negative results (86.7%, n = 1,460) highlights the assay's utility in rapid rule-out protocols within high-burden settings. This high negativity rate is consistent with research by Maclean et al. (2020), who emphasized that molecular diagnostic tools are essential for the rapid exclusion of tuberculosis in symptomatic individuals, where approximately 85% to 90% of presumptive cases in primary care settings are often found to be TB-negative [13].

The prevalence of rifampicin-sensitive cases (12.1%) in our cohort aligns with the diagnostic yield observed in other high-burden settings. For instance, Mohanty et al. (2025) reported that while overall TB positivity was high (44.17%), the proportion of sensitive cases significantly outweighed resistant ones in tertiary care screening. [14] As emphasized by Inbaraj et al. (2026), the assay's sensitivity for detecting drug-resistant mutations (80–86%) makes it a vital tool for decentralized resistance surveillance. [15] Our study observed an indeterminate rate of 2.2% (n = 7) for rifampicin sensitivity. This is significantly lower than the 26.4% indeterminate rate reported in larger regional evaluations of the Truenat platform by Rajendran et al. (2025). [16] Inbaraj et al. (2026) highlighted the operational superiority of the Truenat platform, noting that

its portable, battery-operated design allows for high-quality molecular testing in decentralized settings where traditional laboratory infrastructure—such as that required for MGIT culture—is lacking. [17]

Our finding of 100% specificity aligns with the high-performance benchmarks set by the World Health Organization (2016) during the initial endorsement of rapid nucleic acid amplification tests (NAATs). [18] The data established a highly significant association between Truenat positivity and rifampicin sensitivity profiling ($p < 0.001$). Among confirmed TB cases, 12.0% (n = 239) were rifampicin-sensitive, while only 0.5% (n = 10) showed resistance. This 0.5% resistance prevalence is notably lower than the 3.39% reported by Maity et al. (2025) in a recent West Bengal tertiary care study. [19] The Truenat assay demonstrated a perfect performance profile for pulmonary tuberculosis detection, with 100% sensitivity, specificity, PPV, NPV, and overall diagnostic accuracy. Steingart et al. (2015) reported a pooled sensitivity of 88% and specificity of 98% in their landmark systematic review, highlighting the significant technological maturation of nucleic acid amplification tests (NAATs) over the past decade. [20]

Our results align with recent high-performance evaluations conducted within the National TB Elimination Programme. A 2023 study by Gopalaswamy et al. confirmed that under optimized laboratory conditions, Truenat's specificity remains exceptionally high, though they noted that programmatic challenges elsewhere often lead to lower sensitivities than the 100% achieved in our controlled study environment. [21]

In our study, Pulmonary TB (PTB) was diagnosed in 84.2% (n = 1684) of the participants. A comprehensive real-world study by Saha et al. (2025) indicates that in high-burden regions, pulmonary involvement typically accounts for 75% to 85% of all TB cases reported in clinical registries, closely mirroring the 84.2% observed. [10]

Our study showed that sputum samples constituted 84.2% (n = 1684) of all collected specimens. According to Saha et al. (2025), sputum remains the "gold standard" and most accessible diagnostic specimen in respiratory health programs, typically representing over 80% of the workload in tertiary diagnostic centers. [10] Extra-pulmonary specimens were diverse, with the "Others" category comprising 12.6% (n = 252) of the total. Among specific non-respiratory samples, Pus was the most frequent at 1.85% (n = 37). This finding is consistent with Jindal et al. (2025), who noted that suppurative lesions—particularly from cold abscesses or lymph node liquefaction—are common sources of diagnostic material in suspected extra pulmonary TB. [22] Notably, Gastric Aspirates (totaling 0.45%) and Endometrial Tissue (0.1%) represent niche but vital diagnostic pathways. This is supported by Satia et al. (2025), who highlighted that while gastric lavage is highly specific, it accounts for a minor fraction of the total specimen volume in adult-dominated studies due to the discomfort of the procedure. [11]

CONCLUSION:

The present study concludes that the Truenat assay is a highly sensitive, specific, and accurate diagnostic tool for pulmonary tuberculosis, demonstrating 100% diagnostic accuracy. Pulmonary tuberculosis was the predominant form observed, while rifampicin resistance was minimal in the study population. A strong correlation between Truenat positivity and rifampicin sensitivity highlights its reliability in detecting drug resistance. The assay's rapid turnaround time and ease of use make it suitable for routine clinical application. Thus, Truenat can be effectively utilized for early diagnosis and decentralized TB testing. Its implementation can significantly strengthen tuberculosis control and elimination efforts.

Limitations of the study:

The study was conducted at a single center with convenience sampling, limiting generalizability. Only symptomatic patients were included, possibly missing early or asymptomatic cases. Long-term follow-up and comparison with other diagnostic methods were not performed.

Relevance of the study:

The present study highlights the significant clinical and public health relevance of the Truenat assay as a rapid, accurate, and point-of-care diagnostic tool for tuberculosis. Its high diagnostic performance facilitates early detection and prompt initiation of treatment, thereby reducing disease transmission and improving patient outcomes. Additionally, the ability to detect rifampicin resistance enables timely identification and management of drug-resistant tuberculosis. The portability and minimal infrastructure requirements of the Truenat system make it particularly suitable for use in decentralized and resource-limited settings. Overall, the findings of this study support the wider implementation of Truenat in routine clinical practice and reinforce its role in strengthening tuberculosis control programs and achieving national TB elimination targets.

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