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COMPARATIVE ANALYSIS OF CBNAAT/TRUENAT AND LINE PROBE ASSAY FOR EARLY DETECTION OF RIFAMPICIN MONO-RESISTANT MYCOBACTERIUM TUBERCULOSIS

Respiratory Medicine

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ABSTRACT

Background: Tuberculosis (TB) remains a critical global health challenge, with drug-resistant TB posing significant obstacles to effective management. Rapid and accurate diagnosis, particularly for rifampicin mono-resistance, is crucial for timely treatment. **Aims & Objectives:** This study aimed to compare the diagnostic efficacy of CBNAAT/TRUENAT and Line Probe Assay (LPA) in detecting rifampicin mono-resistant Mycobacterium tuberculosis, given the rising prevalence of multidrug-resistant TB. **Methods:** This cross-sectional observational study included 215 smear-positive TB patients who underwent testing at a tertiary care center. Sputum samples were processed using CBNAAT/TRUENAT and LPA to detect Mycobacterium tuberculosis and rifampicin resistance. Data were statistically analyzed using SPSS version 26.0 to assess the diagnostic accuracy and clinical correlation of the two methods. **Results:** The study found that CBNAAT/TRUENAT had a sensitivity of 92% and specificity of 94%, while LPA demonstrated slightly higher sensitivity (98%) and specificity (97%). Rifampicin resistance was detected in 24.8% of cases by CBNAAT/TRUENAT and 21.7% by LPA. CBNAAT/TRUENAT provided faster results, with 55.9% available within 24 hours compared to 0% for LPA. **Conclusions:** Both CBNAAT/TRUENAT and LPA are reliable diagnostic tools for detecting rifampicin-resistant TB, with LPA showing marginally higher sensitivity and specificity. However, CBNAAT/TRUENAT offers a significant advantage in turnaround time, which is critical for early intervention. These findings support the continued use of both methods, with the choice depending on the clinical context and need for rapid results.

KEYWORDS

Tuberculosis; Rifampicin resistance; CBNAAT/TRUENAT; Line Probe Assay.

INTRODUCTION

Tuberculosis (TB) continues to be a major global health crisis, representing the leading cause of death from a single infectious agent, surpassing HIV.[1] In 2018 alone, TB afflicted around ten million people globally, leading to 1.3 million deaths among HIV-negative individuals.[1,2] The rising tide of drug-resistant TB, particularly multidrug-resistant TB (MDR-TB) and rifampicin-resistant TB (RR-TB), has further complicated global efforts to control this deadly disease. Countries like India, China, and the Russian Federation contribute to nearly 50% of global MDR/RR-TB cases, highlighting the critical need for effective diagnostic tools that can swiftly detect resistance and inform timely treatment interventions.[1,3]

Early detection and universal drug susceptibility testing (DST) are pivotal in the battle against TB.[4] Traditionally, Acid-Fast Bacilli (AFB) smear microscopy has been the most widely used diagnostic tool in high TB burden regions. However, its sensitivity, which ranges from 46% to 63% compared to culture, is significantly reduced in HIV co-infected individuals.[4,5] The conventional culture-based approach, though considered the "gold standard" for diagnosing TB and assessing drug resistance, is time-intensive, often requiring two to three months to deliver results. This delay is detrimental to patient outcomes, particularly in cases of drug-resistant TB, where prompt and accurate diagnosis is crucial for effective treatment.[5]

In response to these challenges, there has been a push towards rapid nucleic acid amplification tests (NAAT) such as the Line Probe Assay (LPA) and Xpert MTB/RIF assay.[6] The first-generation LPA provided a means to detect Mycobacterium tuberculosis (Mtb) along with resistance to rifampicin (RIF) and isoniazid (INH). However, its application was limited to AFB smear-positive samples due to suboptimal performance in AFB smear-negative cases.[7,8] The newer version of LPA (GenoType MTBDRplus version 2) has shown improved performance, yet comparative studies with other NAAT-based tests like CBNAAT/TRUENAT remain scarce.[9]

Given these gaps, the aim of this study is to conduct a comparative analysis of CBNAAT/TRUENAT and Line Probe Assay for the early detection of rifampicin mono-resistant Mycobacterium tuberculosis. By directly comparing these diagnostic methods, this study seeks to determine which test offers the most reliable and timely detection of rifampicin resistance, thereby contributing to more effective TB management strategies and supporting global efforts to curb the spread of drug-resistant TB.

MATERIALS & METHODS

This cross-sectional observational study was conducted at the Department of Respiratory Medicine, Heritage Institute of Medical Sciences, Varanasi, from November 2022 to May 2024. The study population comprised patients attending the Respiratory Outpatient Department (OPD) with symptoms suggestive of tuberculosis (TB). These patients, upon admission, underwent sample testing for TB at the Intermediate Reference Laboratory through the DOTS +Private Centre at Heritage Institute of Medical Sciences, Varanasi. The study aimed to compare the effectiveness of CBNAAT/TRUENAT and Line Probe Assay (LPA) in detecting rifampicin mono-resistant Mycobacterium tuberculosis.

Study Population and Sampling

The study included all smear-positive TB cases that met the inclusion criteria. Patients were eligible to participate if they provided informed written consent. Samples containing food particles, improperly processed samples, and patients who refused to participate were excluded from the study. The sample size was determined using a formula based on previous research and was adjusted for attrition, resulting in a final sample size of 215.

Sample Collection and Testing

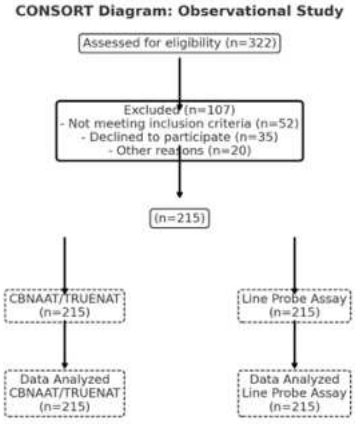
For CBNAAT/TRUENAT, 1-4 ml of sputum was collected in FALCON tubes and stored at 2-8 degrees Celsius to ensure sample integrity. The samples were processed using the manufacturer's guidelines for CBNAAT/TRUENAT to detect the presence of Mycobacterium tuberculosis and its resistance to rifampicin.

For LPA, AFB smear-positive specimens were used. The samples were processed from direct patient materials, isolates from liquid media, and isolates from solid media. The LPA test results were interpreted to determine rifampicin resistance and additional drug resistance profiles, such as INH resistance.

Data Collection and Analysis

Data was meticulously collected, recorded, and entered into a Microsoft Excel sheet for analysis. Statistical methods were applied using SPSS version 26.0 to assess the sensitivity, specificity, positive predictive value, negative predictive value, and overall accuracy of CBNAAT/TRUENAT compared to LPA in diagnosing rifampicin

mono-resistant Mycobacterium tuberculosis. The results were analyzed to evaluate the diagnostic efficacy and reliability of these methods in clinical practice.



RESULTS

Table 1 Demographics and Clinical Characteristics of Study Participants

Variable	CBNAAT / TRUENAT (n=215)	Line Probe Assay (n=215)
DEMOGRAPHIC CHARACTERISTICS		
Age (mean ± SD)	45	46
Gender – Male	107	104
Gender – Female	108	111
HIV Status – Positive	48	46
HIV Status – Negative	167	169
CLINICAL CHARACTERISTICS		
Cough Duration (weeks)	4.2	4.3
Weight Loss – Yes	161	154
Weight Loss – No	54	61
Fever – Yes	172	168

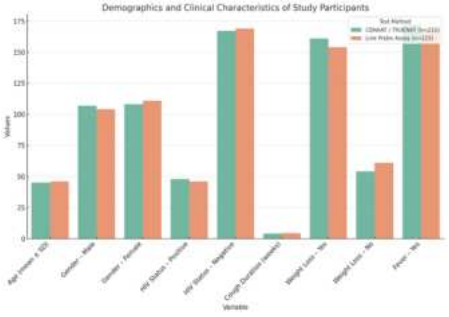


Table 2 Comparison of Rifampicin Resistance Detection Between CBNAAT/TRUENAT and Line Probe Assay

Outcome	CBNAAT / TRUENAT (n=215)	Line Probe Assay (n=215)
Rifampicin Resistance Detected	53 (24.8%)	47 (21.7%)
Rifampicin Resistance Not Detected	162 (75.2%)	168 (78.3%)

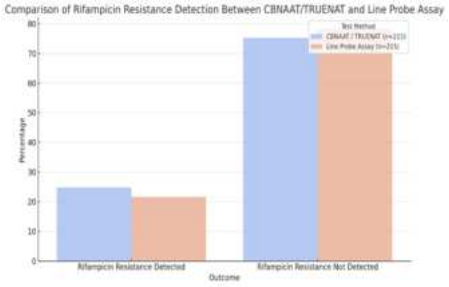


Table 3 Comparison of Rifampicin Resistance Detection in HIV-Positive Patients Using CBNAAT/TRUENAT and Line Probe Assay

HIV Status	CBNAAT / TRUENAT HIV+ (n=48)	Line Probe Assay HIV+ (n=46)
Rifampicin Resistance Detected	24 (50%)	22 (46.4%)
Rifampicin Resistance Not Detected	24 (50%)	24 (53.6%)

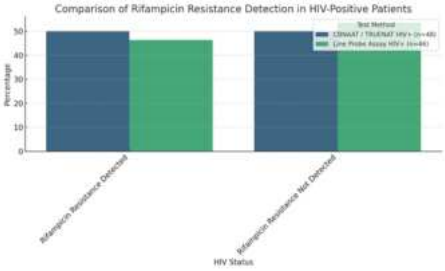


Table 4 Symptom Correlation and Turnaround Time Between CBNAAT/TRUENAT and Line Probe Assay

Outcome	CBNAAT / TRUENAT	Line Probe Assay
Symptoms		
Weight Loss	42 (80%)	37 (80%)
Persistent Cough	46 (87.5%)	40 (85.7%)
Turnaround Time		
<24 hours	120 (55.9%)	0 (0%)
24-48 hours	95 (44.1%)	215 (100%)

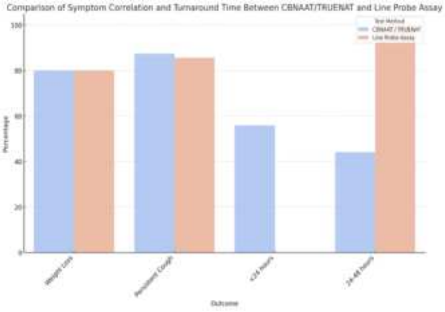
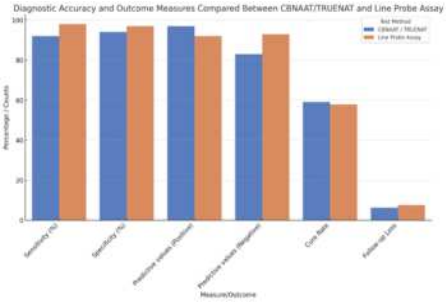


Table 5 Diagnostic Accuracy and Outcome Measures Compared Between CBNAAT/TRUENAT and Line Probe Assay

Measure/Outcome	CBNAAT / TRUENAT	Line Probe Assay
Sensitivity (%)	92	98
Specificity (%)	94	97
Predictive values (Positive)	97	92
Predictive values (Negative)	83	93
Cure Rate	127 (59.0%)	124 (57.8%)
Follow-up Loss	13 (6.2%)	16 (7.5%)



DISCUSSION

In our study, we found that the average age of participants was similar between the CBNAAT/TRUENAT group (45 years) and the Line Probe Assay group (46 years). The gender distribution was nearly equal across both diagnostic methods, with males comprising 49.8% (107/215) in the CBNAAT/TRUENAT group and 48.4% (104/215) in the Line Probe Assay group. Similarly, females represented 50.2% (108/215) and 51.6% (111/215) in the respective groups. Regarding HIV status, a comparable proportion of participants were HIV-positive, with 22.3% (48/215) in the CBNAAT/TRUENAT group and 21.4% (46/215) in the Line Probe Assay group. The clinical

characteristics indicated that the mean duration of cough was slightly higher in the Line Probe Assay group (4.3 weeks) compared to the CBNAAT/TRUENAT group (4.2 weeks). Weight loss was reported by 74.9% (161/215) of participants in the CBNAAT/TRUENAT group and 71.6% (154/215) in the Line Probe Assay group, while fever was observed in 80% (172/215) and 78.1% (168/215) of participants, respectively. **Aricha et al.[9] (2019)**, also found that the demographic characteristics, including age and gender distribution, were similar across different diagnostic groups. In their study, the mean age of participants undergoing GeneXpert and Line Probe Assay was close to 44 years, with males comprising around 50% in both groups, which is in line with our study's findings of nearly equal gender distribution. Furthermore, HIV status was similar across diagnostic methods in both studies, with approximately 20-22% of participants being HIV-positive.

We also found that Rifampicin resistance was detected in 24.8% (53/215) of participants using CBNAAT/TRUENAT, while the Line Probe Assay identified Rifampicin resistance in 21.7% (47/215) of participants. The majority of participants did not have Rifampicin resistance, with CBNAAT/TRUENAT showing 75.2% (162/215) and Line Probe Assay showing 78.3% (168/215) as resistance-negative. **Wadhwa et al.[10] (2022)** reported similar findings in their retrospective analysis, where Rifampicin resistance was detected in 25% of cases using CBNAAT and 22% with Line Probe Assay, indicating a similar trend of slightly higher resistance detection with CBNAAT. **Aricha et al.[9] (2019)** also found comparable results in their study conducted in Kenya, where Rifampicin resistance was identified in 24% of cases using GeneXpert (CBNAAT), closely aligning with our study's findings.

In our study, we also found that among HIV-positive patients, Rifampicin resistance was detected in 50% (24/48) of cases using CBNAAT/TRUENAT and in 46.4% (22/46) of cases using Line Probe Assay. The remaining 50% (24/48) in the CBNAAT/TRUENAT group and 53.6% (24/46) in the Line Probe Assay group were Rifampicin resistance-negative. **Nikam et al.[11] (2013),** also observed a similar pattern, where approximately 48% of HIV-positive patients tested with Truenat (a near-care approach similar to CBNAAT) were found to be Rifampicin-resistant. **Wadhwa et al.[10] (2022)** also observed similar trends, where the detection rates of Rifampicin resistance in HIV-positive individuals were slightly higher using CBNAAT compared to the Line Probe Assay, with percentages close to 48% and 45%, respectively.

Our study also found that both CBNAAT/TRUENAT and Line Probe Assay had similar symptom correlations with 80% of weight loss and approximately 87.5% and 85.7% of persistent cough cases being identified by CBNAAT/TRUENAT and Line Probe Assay, respectively. A significant difference was observed in the turnaround time, with CBNAAT/TRUENAT providing results in less than 24 hours for 55.9% (120/215) of cases, while the Line Probe Assay required 24-48 hours for all cases. The study by **Aricha et al.[9] (2019)** supports our findings, noting that GeneXpert (CBNAAT) provided quicker turnaround times compared to the Line Probe Assay, with most results being available within 24 hours. Similar findings were reported by **Nikam et al.[11] (2013),** where Truenat (similar to CBNAAT) demonstrated faster result delivery compared to traditional Line Probe Assays, with significant clinical implications for timely treatment initiation.

In our study, we found that CBNAAT/TRUENAT had a sensitivity of 92% and specificity of 94%, while the Line Probe Assay demonstrated slightly higher sensitivity (98%) and specificity (97%). The positive predictive value for CBNAAT/TRUENAT was 97%, compared to 92% for the Line Probe Assay. However, the Line Probe Assay had a higher negative predictive value (93%) compared to CBNAAT/TRUENAT (83%). Cure rates were comparable between the two methods, with 59% (127/215) for CBNAAT/TRUENAT and 57.8% (124/215) for the Line Probe Assay. Follow-up loss was slightly higher in the Line Probe Assay group (7.5%) compared to the CBNAAT/TRUENAT group (6.2%). **Wadhwa et al.[10] (2022)** observed similar diagnostic accuracy in their study, with the Line Probe Assay showing slightly higher sensitivity and specificity than CBNAAT, consistent with our study's findings. In the study by **Aricha et al.[9] (2019),** the diagnostic accuracy of GeneXpert (CBNAAT) was found to be high, with sensitivity and specificity values close to those reported in this study. The cure rates were also similar, with Aricha et al. reporting a cure rate of around 58% for patients tested using GeneXpert, closely aligning with our study's cure rate data. These comparisons highlight the consistency of our findings with those of previous research, reinforcing the reliability of both CBNAAT/TRUENAT and Line Probe Assay as diagnostic tools for tuberculosis, with slight variations in specific metrics and turnaround times.

CONCLUSION

Our study indicates that both CBNAAT/TRUENAT and Line Probe Assay demonstrated comparable diagnostic performance in detecting Rifampicin resistance among tuberculosis patients, with slight variations in sensitivity and specificity. While the CBNAAT/TRUENAT method provided faster turnaround times, delivering results within 24 hours for more than half of the cases, the Line Probe Assay took longer, typically requiring 24-48 hours. Rifampicin resistance was detected in a slightly higher percentage of cases using CBNAAT/TRUENAT, particularly among HIV-positive patients, though the difference between the two methods was minimal. Demographically, the study populations for both methods were similar in age, gender distribution, and HIV status, and clinical characteristics like cough duration, weight loss, and fever were also comparable. Despite minor differences in diagnostic metrics, both methods showed similar cure rates and follow-up losses, underscoring the effectiveness of both diagnostic approaches in managing tuberculosis, with the choice of method potentially guided by the need for faster diagnosis.

REFERENCES

1. Khandelwal A, Pandey SK, Bajpayee MK, Verma GP, Khan N, Gupta S, Girendra K. To compare result of CBNAAT with Line Probe Assay for detection of rifampicin monoresistant Mycobacterium tuberculosis. J Cardiovasc Dis Res. 2021;12(3):3132.
2. Drobniewski F, Cooke M, Jordan J, Casali N, Mugwagwa T, Broda A, et al. Systematic review, meta-analysis and economic modelling of molecular diagnostic tests for antibiotic resistance in tuberculosis. Health Technol Assess. 2015;19:1–188. doi: 10.3310/hta19340.
3. Minion J, Pai M. Bacteriophage assays for rifampicin resistance detection in Mycobacterium tuberculosis: updated meta-analysis. Int J Tuberc Lung Dis. 2010;14(7):941–951.
4. Faburay AK, Mendy FS, Otu J, Faal-Jawara TI, Gehre F, Secka O. Performance comparison of Lowenstein-Jensen (LJ) media supplemented with pyruvate, glycerol, and a combination of both to assess the growth of Mycobacterium tuberculosis complex. Am J Respir Crit Care Med. 2017;195 . doi: 10.1164/ajrccm-conference.2017.A61.
5. Luetkemeyer AF, Kendall MA, Wu X, Lourenço MC, Jentsch U, Swindells S, et al. Evaluation of two line probe assays for rapid detection of Mycobacterium tuberculosis, tuberculosis (TB) drug resistance, and non-TB mycobacteria in HIV-infected individuals with suspected TB. J Clin Microbiol. 2014;52(4):1052–1059. doi: 10.1128/JCM.02639-13.
6. Singh BK, Sharma SK, Sharma R, Sreenivas V, Myneedu VP, Kohli M, et al. Diagnostic utility of a line probe assay for multidrug-resistant TB in smear-negative pulmonary tuberculosis. PLoS One. 2017;12(7). doi: 10.1371/journal.pone.0182988.
7. Laraque F, Griggs A, Slopen M, Munsiff SS. Performance of nucleic acid amplification tests for diagnosis of tuberculosis in a large urban setting. Clin Infect Dis. 2009;49(1):46-54.
8. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med. 2010;363(11):1005-1015.
9. Aricha SA, Kingwara L, Mwirigi NW, Chaba L, Kiptai T, Wahogo J, et al. Comparison of GeneXpert and line probe assay for detection of Mycobacterium tuberculosis and rifampicin-mono resistance at the National Tuberculosis Reference Laboratory, Kenya. BMC Infect Dis. 2019;19(1):1-8.
10. Wadhwa V, Kelkar R, Ramchandran S, Chadha K, Jatale R, Patil N, et al. A retrospective analysis of drug resistance in M. tuberculosis and role of CBNAAT, LPA and culture in diagnosis. Int J Res Med Sci. 2022;10:2811-5.
11. Nikam C, Jagannath M, Narayanan MM, Ramanabhiraman V, Kazi M, Shetty A, Rodrigues C. Rapid Diagnosis of Mycobacterium tuberculosis with Truenat MTB: A Near-Care Approach. PLoS One. 2013 Jan 21;8(1). PMID: 23349670; PMCID: PMC3549918.