International Journal of Research in Medical Sciences
Aich I et al. Int J Res Med Sci. 2026 Mar;14(3):1077-1084
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: https://dx.doi.org/10.18203/2320-6012.ijrms20260628

Original Research Article

Comparative evaluation of cartridge-based nucleic acid amplification test, Ziehl-Neelsen smear microscopy, and culture in Lowenstein Jensen media for diagnosis of pulmonary and extrapulmonary tuberculosis in a tertiary care hospital in West Bengal, India

Indranil Aich[1], Biyanka Sau[2], Somenath Acharya[1], Pushpen Ghosh[3], Sougata Ghosh[2], Manideepa Sengupta[2], Mitali Chatterjee[1], Subhranil Mal[4]*

[1]Department of Microbiology, PKG Medical College and Hospital, Kolkata, West Bengal, India
[2]Department of Microbiology, Medical College and Hospital, Kolkata, West Bengal, India
[3]Department of Community Medicine, PKG Medical College and Hospital, Kolkata, West Bengal, India
[4]Medical College and Hospital, Kolkata, West Bengal, India

Received: 30 December 2025
Revised: 06 February 2026
Accepted: 18 February 2026

***Correspondence:**
Subhranil Mal,
E-mail: subhranilmal@gmail.com

ABSTRACT

Background: Tuberculosis remains a major global health problem and continues to rank among the leading causes of mortality worldwide. Early and accurate diagnosis is crucial for infection control and appropriate clinical management. Cartridge-based nucleic acid amplification test (CBNAAT) enables rapid and accurate molecular diagnosis of *Mycobacterium tuberculosis*, with simultaneous rifampicin resistance detection capability.
Methods: This cross-sectional study was conducted in Department of Microbiology in collaboration with Department of Chest Medicine, Medical College and Hospital, Kolkata, from February 2018 to January 2019. Two hundred specimens from suspected pulmonary and extrapulmonary tuberculosis cases were evaluated by CBNAAT, Ziehl-Neelsen (ZN) microscopy and culture in Lowenstein-Jensen (LJ) media. After excluding 14 erroneous specimens, the remaining 186 specimens were analysed using appropriate statistical methods.
Results: Of the 186 specimens included, CBNAAT was able to detect 27 positive cases (14.5%), while ZN microscopy and LJ culture identified 6.5% and 11.3% positive cases, respectively. Among the 27 CBNAAT-positive specimens, 20 were culture-positive, and 11 were found to be ZN smear positive – revealing statistically significant associations ($p<0.001$). Rifampicin resistance was found in 2 cases (7.4%). CBNAAT positivity showed significant association with haemoptysis ($p=0.014$) and longer duration of fever ($p=0.006$)
Conclusions: CBNAAT demonstrated superior diagnostic performance to ZN smear microscopy and LJ culture for rapid and accurate diagnosis of tuberculosis. Its ability to detect smear-negative cases highlights its value for early diagnosis and effective clinical management, particularly in high-burden settings such as India.

Keywords: CBNAAT, Xpert MTB/RIF, Tuberculosis diagnosis, ZN smear microscopy, Lowenstein-Jensen culture, Rifampicin resistance, Molecular diagnostics

INTRODUCTION

Tuberculosis (TB) remains a major global health problem, especially affecting low and middle-income countries (LMICs).[1-3] According to the World Health Organization's Global (WHO) tuberculosis report 2021, an estimated 10.6 million cases and 1.6 million deaths occurred worldwide, with India contributing nearly 27% of the global burden –

a figure underscoring the endemicity of the disease in this subcontinent.[4] The causative organism, *Mycobacterium tuberculosis*, can affect the lungs, leading to pulmonary tuberculosis; or may involve other organs like skin, genital organs, lymph nodes, skeletal system and central nervous system, causing extrapulmonary tuberculosis.[5]

Delayed diagnosis and under-detection, especially in smear-negative and extrapulmonary tuberculosis, continue to hamper effective disease control and transmission reduction. Early and accurate diagnosis of TB is therefore, of utmost importance for prompt pharmacotherapy. Conventional Ziehl-Neelsen (ZN) smear microscopy, though rapid and inexpensive, has low sensitivity as it requires a minimum concentration of 10^4-10^5 bacilli/ml to yield positive results.[6] Mycobacterial culture in Lowenstein-Jensen (LJ) media is considered to be the reference standard, due to its high sensitivity and ability to detect viable organisms. However, the process itself is time-consuming, often requiring upto 8 weeks – limiting its role in early decision-making.[7]

The advent of molecular diagnostics, particularly cartridge-based nucleic acid amplification test (CBNAAT) or Xpert MTB/RIF, has revolutionized TB diagnostics, owing to its ability to detect *Mycobacterium tuberculosis* in less than two hours and simultaneously identifying mutations in the rpoB gene.[8,9] Additionally, CBNAAT provides a semi-quantitative estimate of mycobacterial load, thereby reflecting the MTB burden, and also allowing correlation with smear microscopy grading and disease severity.[10]

Given the limitations of conventional diagnostic methods, the present study was undertaken in a tertiary care centre to assess the diagnostic yield of CBNAAT, examine its correlation with ZN smear microscopy and LJ medium culture, evaluate the association between MTB load and smear positivity, and analyse demographic and clinical factors influencing CBNAAT positivity.

METHODS

Study design and setting

This hospital-based, cross-sectional, observational study was undertaken in the Department of Microbiology in collaboration with the Department of Chest Medicine, Medical College and Hospital, Kolkata, over a period of 12 months (February 2018 – January 2019), after approval by the Institutional Ethics Committee.

Study population

Two hundred consecutive pulmonary and extrapulmonary specimens for the request of CBNAAT from indoor and outdoor patients from different departments of Medical College and Hospital, Kolkata, received by the Department of Microbiology, were included in the study.

Sample size

The initial sample size was 200 specimens. However, 14 specimens gave "ERROR" results in CBNAAT and were excluded, reducing the final sample size to 186 specimens.

Inclusion criteria

Patients with clinical suspicion of pulmonary tuberculosis, including symptoms of cough with or without expectoration, weight loss, fatigue and haemoptysis. Patients with clinical suspicion of extrapulmonary tuberculosis. Specimens from both indoor and outdoor patients were included.

Exclusion criteria

Specimens yielding "ERROR" results in CBNAAT and incomplete clinical information were excluded.

Specimen collection and processing

Specimens were collected under aseptic precautions. Pulmonary specimens included sputum, induced sputum, bronchoalveolar lavage (BAL) fluid, gastric lavage, and endotracheal tube suction. Extrapulmonary specimens included pus from various sites, tissue biopsies, lymph nodes, body fluids (pleural fluid, ascitic fluid, CSF), blood, and urine. Each specimen was divided into three portions: upper portion: used for ZN smear microscopy, middle portion: used for CBNAAT and lower portion: used for culture in LJ media.

ZN smear microscopy

Smears were prepared from the upper portion of specimens, heat-fixed, and stained using the ZN method. Slides were examined under oil immersion (100× objective) and graded according to RNTCP guidelines.

CBNAAT (Xpert MTB/RIF)

The middle portion of the specimens was processed according to the manufacturer's instructions using the GeneXpert system. The test provided results for *Mycobacterium tuberculosis* detection and rifampicin resistance status within 2 hours.

Culture in Lowenstein Jensen media

The lower portion of specimens underwent homogenization, decontamination using NALC-NaOH method, concentration by centrifugation, and inoculation into LJ media. Cultures were incubated at 37 °C and examined weekly for up to 8 weeks.

Clinical and demographic data collection

Detailed clinical history including age, sex, weight loss, past history of tuberculosis, family history of TB,

comorbidities (diabetes mellitus, COPD, and HIV), and symptoms (cough, fever, haemoptysis with duration) were recorded.

Statistical analysis

Data were entered into Microsoft Excel and analysed using statistical package for the social sciences (SPSS) version 20. Categorical variables were expressed as numbers and percentages and compared using Pearson's Chi-square test for independence of attributes/Fisher's exact test as appropriate. Continuous variables were expressed as mean, median, and standard deviation and compared using unpaired t-test. An alpha level of 5% was taken; any $p<0.05$ was considered statistically significant.

RESULTS

Demographic characteristics

Among 186 specimens, males contributed 111 specimens (59.7%), while females contributed 75 specimens (40.3%), as depicted in Figure 1.

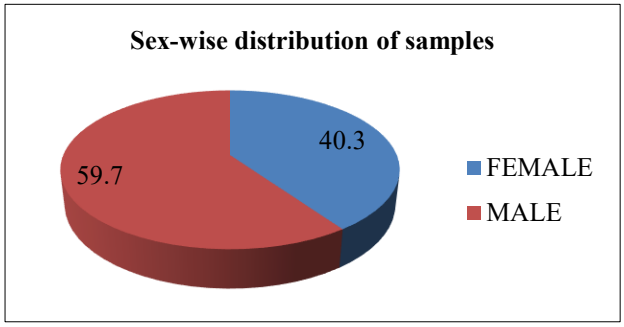

Figure 1: Distribution of samples received according to sex.

The majority of the specimens were found to be from the age group less than 10 years (42; 22.6%), followed by 41-50 years (29; 15.6%) and 21-30 years (28; 15.1%) – with the mean age being approximately 32.5 years. This is shown in Figure 2.

Analysis of income, family size and number of rooms indicated that patients from lower socioeconomic sections were more prone to tuberculosis, consistent with global epidemiological patterns.

Specimen distribution

Out of 186 specimens, 104 (55.9%) were pulmonary samples and 82 (44.1%) were extrapulmonary samples. The distribution of various specimens among pulmonary and extrapulmonary samples has been represented below in Table 1.

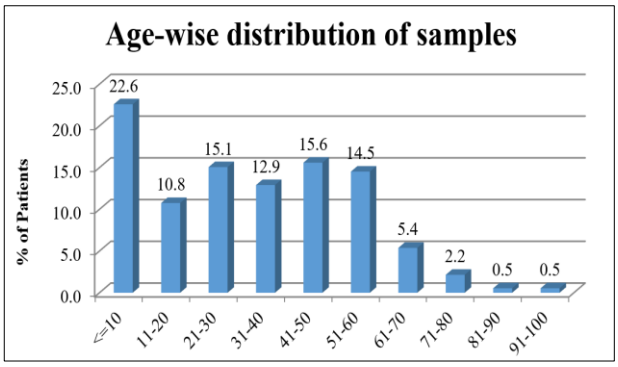

Figure 2: Distribution of samples received according to age.

Table 1: Distribution of pulmonary and extrapulmonary specimens included (n=186).

Specimen type	Number	Percent
Pulmonary	104	55.9
Sputum	59	31.7
Induced sputum	4	2.2
Endotracheal tube suction	2	1.1
BAL fluid	13	7.0
Gastric lavage	26	14.0
Extrapulmonary	82	44.1
Pus	23	12.4
Tissue	8	4.3
Lymph node	7	3.8
Ascitic fluid	6	3.2
Blood	3	1.6
Urine	1	0.5
CSF	14	7.5
Drain fluid	1	0.5
Pleural fluid	18	9.7
Fluid from fistula in ano	1	0.5
Total	186	100.0

Diagnostic test results

Out of the 186 specimens analysed, ZN smear microscopy was negative in 174 cases (93.5%) and positive in 12 cases (6.5%), with equal distribution of smear grades 1+ and 2+ (six cases each). CBNAAT detected Mycobacterium tuberculosis in 27 specimens, while 159 specimens were negative. Among the CBNAAT-positive cases, rifampicin resistance was identified in two specimens (7.4%).

The semi-quantitative MTB load reported by CBNAAT showed a predominance of low bacillary burden, with very low load in seven cases (25.9%), low load in fifteen cases (55.6%), medium load in four cases (14.8%) and high load in one case (3.7%). Culture on LJ medium yielded growth in 21 specimens (11.3%), whereas no growth was observed in the remaining 165 specimens (88.7%).

Interrelationship between diagnostic methods

CBNAAT versus ZN smear microscopy

Among 27 CBNAAT positive specimens, 16 (59.26%) were ZN smear negative, 6 (22.22%) were ZN positive 1+, and 5 (18.52%) were ZN positive 2+. The association was statistically significant (p=0.001) (Table 2).

CBNAAT versus LJ culture

Out of the 27 CBNAAT positive samples, 20 (74.07%) were found to be culture positive, while 7 (25.93%) were culture negative. On the other hand, 158 (99.37%) were culture negative and 1 (0.63%) was culture positive, among the 159 CBNAAT negative specimens – the association being statistically significant (p=0.001) (Table 3).

ZN smear microscopy versus LJ culture

Among 12 ZN smear positive specimens, 10 (83.33%) were culture positive. Among 174 ZN smear negative specimens, 164 (94.25%) were culture negative and 10 (5.75%) were culture positive. The association was statistically significant (p=0.001) (Table 4).

Table 2: Correlation between ZN smear microscopy grading and CBNAAT results.

ZN stain status	CBNAAT not detected (%)	CBNAAT detected (%)	Total (%)	P value
Negative	158 (99.37)	16 (59.26)	174 (93.55)	0.001
Positive 1+	0 (0)	6 (22.22)	6 (3.23)	
Positive 2+	1 (0.63)	5 (18.52)	6 (3.23)	
Total	159 (100)	27 (100)	186 (100)	

Table 3: Correlation between LJ medium culture and CBNAAT results.

Culture status	CBNAAT not detected (%)	CBNAAT detected (%)	Total (%)	P value
Negative	158 (99.37)	7 (25.93)	165 (88.71)	0.001
Positive	1 (0.63)	20 (74.07)	21 (11.29)	
Total	159 (100)	27 (100)	186 (100)	

Table 4: Correlation between ZN smear grading and LJ medium culture results.

Culture status	ZN negative (%)	ZN positive (%)	Total (%)	P value
Negative	164 (94.25)	1 (8.33)	165 (88.71)	0.001
Positive	10 (5.75)	10 (83.33)	20 (10.75)	
Total	174 (100)	12 (100)	186 (100)	

One specimen was CBNAAT negative but ZN smear and culture positive, with growth within 7 days, possibly representing rapid-growing atypical mycobacteria. Another specimen was CBNAAT and ZN positive but culture negative, likely due to non-viable bacilli.

Association between CBNAAT positivity and demographic characteristics

Sex and CBNAAT positivity

Among CBNAAT positive cases detected, majority were observed to be male (66.67%), while females contributed 33.33% of cases – reflecting a male predominance (Figure 3).

Age group and CBNAAT positivity

As depicted in Figure 4, CBNAAT positivity was higher among the 11-20- and 31-40-years age group, with 35.0% and 29.16% rates respectively. On the contrary, lower rates were reported in the dependent age group, with low positive cases among children and elderly.

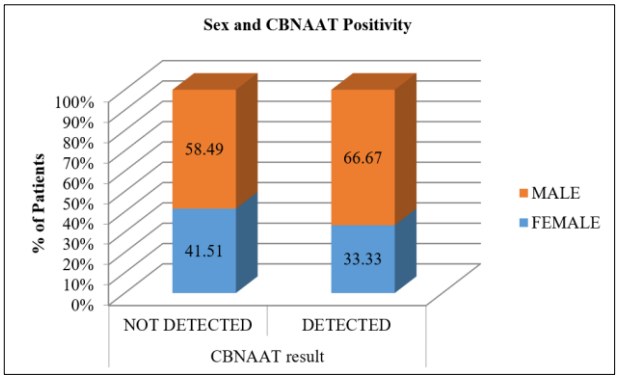

Figure 3: Component bar diagram showing distribution of CBNAAT results stratified by sex.

Association between CBNAAT positivity and clinical parameters

As elaborated in Table 5, CBNAAT positivity showed a statistically significant association with duration of fever (mean 61.24±79.99 days versus 26.59±31.03 days;

p=0.006) and presence of haemoptysis (p=0.014). However, no significant statistical association was noted with age, weight loss, duration of cough, previous history of tuberculosis or presence of comorbidities.

Correlation between MTB Load and ZN smear positivity

Smear positivity increased progressively with higher MTB load, with 100% smear positivity observed in medium and high MTB load categories, thus demonstrating a strong association between mycobacterial load and ZN smear positivity.

Rifampicin resistance

Out of 27 CBNAAT positive samples, 2 (7.4%) were found to be resistant to rifampicin. This is in agreement with the India TB report 2024, published by NTEP, which identified roughly 7.1% resistance to rifampicin, of the 746,607 MTB-positive cases under the purview of the study.[11]

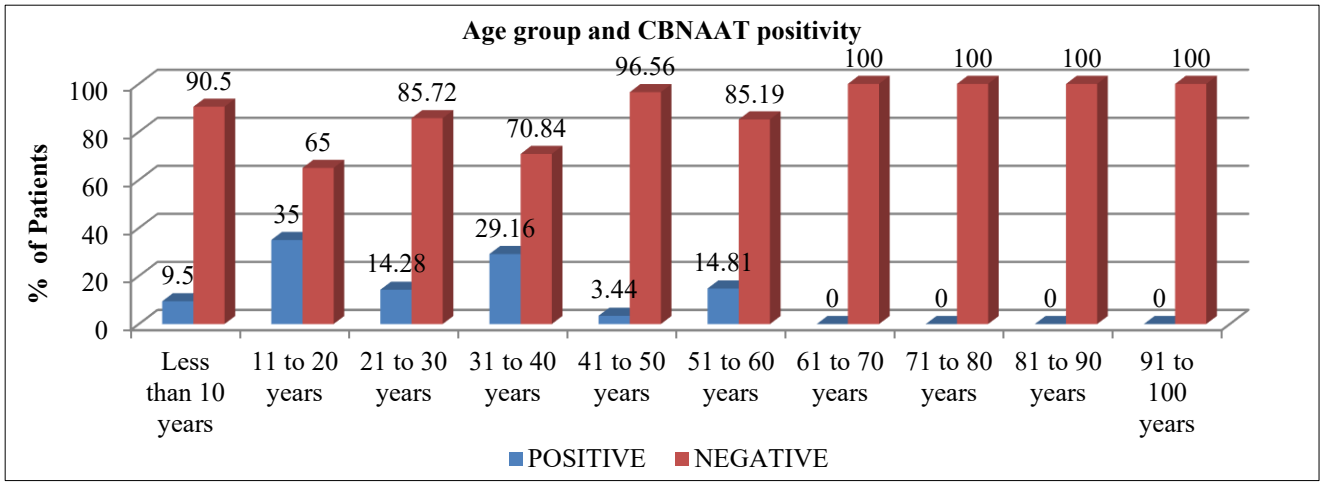

Figure 4: Compound bar diagram showing age-wise distribution of CBNAAT positivity and negativity.

Table 5: Comparison of clinical and demographic parameters between CBNAAT-positive and CBNAAT-negative patients.

Parameter	CBNAAT negative	CBNAAT positive	P value
Mean fever duration (days)	26.59±31.03	61.24±79.99	0.006*
Haemoptysis present (%)	17 (10.69)	8 (29.63)	0.014*
Mean cough duration (days)	84.95±210.72	136.58±255.27	0.150
Age (years)	33.20±22.56	27.19±17.54	0.051
Weight loss (kg)	3.25±2.71	5.89±11.14	0.301
Past history of TB (%)	21 (13.21)	5 (18.52)	0.546
Comorbidities (%)	38 (23.9)	7 (25.93)	0.820
Type II DM (%)	27 (16.98)	7 (25.93)	0.284

Values are expressed as mean±standard deviation or number (percentage), *represents statistically significant association, i.e., p<0.05

DISCUSSION

This cross-sectional, comparative study of CBNAAT, ZN smear microscopy and LJ culture conducted at a tertiary care centre in Kolkata elucidates their relative diagnostic utility, particularly highlighting the superiority of CBNAAT in detecting both pulmonary and extrapulmonary tuberculosis.

In the current study, CBNAAT-positive cases were predominantly clustered in the 11-20- and 31-40-year age groups, indicating a higher preponderance towards the economically productive age bracket. This concurs with the Global Burden of Disease (GBD) analysis done in 204 countries, with incidence and prevalence of TB being highest between ages 15-44 years and frequently peaking around 30–34 years.[12] According to WHO surveillance, over 80% of TB cases affect adults in their most productive years of life, while Ogbo et al, in a research conducted in Nigeria, recorded highest mortality rates among people aged 15-49 years of age.[13,14] This age predilection ratifies the substantial socioeconomic impact of TB in endemic regions. A clear male predominance was seen in our study, with an approximate male-to-female ratio (M: F) 2:1. This corroborates with studies done by Schaberg et al and Humayun et al, with M:F ratios 2.08 and 1.53 respectively.[15,16]

Pulmonary specimens constituted 55.9% of total samples, while extrapulmonary samples accounted for an

appreciable 44.1%. This distribution shows a relatively higher representation of extrapulmonary tuberculosis compared to many articles reviewed, including those by Shrivastava et al and Eddabra et al, while being comparable to the findings of Sunnetcioglu et al, who reported nearly equal number of pulmonary and extrapulmonary TB cases (50.6% versus 50.3%).[17-19] Among extrapulmonary specimens, pus contributed the maximum with 23 samples, followed closely by pleural fluid (n=18) and CSF (n=14). The presence of varied specimens enhances the generalisability of the present findings and strengthens the applicability of CBNAAT across pulmonary and extrapulmonary TB cases alike. However, the higher proportion of extrapulmonary samples in our setting might be reflective of referral bias typical of tertiary care centres.

With respect to diagnostic utility, CBNAAT was observed to be superior to conventional methods of microbial detection, i.e., ZN smear and culture in LJ medium. Of the 186 samples tested, CBNAAT detected *Mycobacterium tuberculosis* in 14.5% cases, while ZN smear microscopy identified only 12 cases (6.5%) and LJ culture yielded 21 cases (11.3%). Notably, 59.26% of CBNAAT positive cases were ZN smear negative, thus underscoring the limited sensitivity of microscopy in detecting paucibacillary disease and reinforcing the use of molecular assays. The strong statistical association between CBNAAT and LJ culture positivity (p=0.001) supports the high diagnostic accuracy of CBNAAT.

This result echoes with multiple studies done in India and abroad.[20-23] For instance, research conducted by Gupta et al in Central India (2024) showed that among 319 clinical samples, ZN smear positivity was 18.2%, while GeneXpert/CBNAAT was positive in 21.6% of cases. Also, higher sensitivity and specificity values were noted with GeneXpert than with ZN smear, using culture as the reference standard.[20] In another study instituted in 2025, GeneXpert showed 93.6% sensitivity compared to the 71.3% for smear microscopy, and had a stronger alignment with culture results than the latter.[21] These demonstrate that CBNAAT outperforms conventional diagnostics – particularly ZN smear microscopy – in detecting *Mycobacterium tuberculosis*. Such advantages support the use of CBNAAT as a frontline diagnostic test in high-burden setups.

The study demonstrated a strong correlation between CBNAAT-detected MTB load and ZN smear positivity. Only 14.28% of very low MTB load specimens were ZN positive, compared to 100% of medium and high MTB load specimens. This graded relationship is consistent with findings from previous studies, as explored in previously reported evidences.[24-27] In a cohort consisting of 204 sputum samples, Martin-Higuera et al demonstrated that Xpert Ultra cycle threshold values correlated strongly with smear grade ($r \approx 0.78$; $p<0.005$), while Fradejas et al found a 90.5% sensitivity in predicting smear positivity, further quantifying this association.[24,25] This finding furthers the superior sensitivity of CBNAAT, especially in paucibacillary disease.

CBNAAT-positivity exhibited statistically significant association with prolonged fever duration and presence of haemoptysis ($p < 0.05$ in each). These point to the features of active TB infection and collate with previously conducted studies (Pai et al and Flynn et al).[28,29] CBNAAT positive patients had significantly longer duration of fever (mean 61.24 days versus 26.59 days), suggesting a higher mycobacterial burden; whereas the association with haemoptysis, in 29.63% of CBNAAT positive patients, is reflective of more extensive pulmonary involvement. Though weight loss was observed in 66.67% of CBNAAT-positive patients (mean 5.89 kg), this association did not achieve statistical significance (p=0.301). Type II diabetes mellitus was present in 25.93% of CBNAAT positive patients, reflecting the well-established bidirectional relationship between diabetes and tuberculosis.[30-33] However, no significant associations were found with other comorbidities including HIV, COPD, hypertension, or cardiovascular diseases.

The superior performance of CBNAAT, particularly in detecting smear-negative cases and providing rapid rifampicin resistance information, has valuable implications for TB control in India. Given India's TB burden, widespread implementation of CBNAAT could significantly improve case detection, reduce diagnostic delays and enable early MDR-TB detection – especially for extrapulmonary tuberculosis cases.

Limitations

The relatively small sample size of CBNAAT positive cases (27 out of 186) limits the generalisability of some findings. Additionally, the specimen processing method (using different portions for different tests) may have introduced bias, as mycobacterial concentration could vary within specimens. The study also did not compare CBNAAT with liquid culture systems like MGIT or BACTEC, which are more sensitive than LJ culture. Furthermore, rifampicin resistance detected by CBNAAT was not confirmed by phenotypic or other genotypic methods.

CONCLUSION

The present cross-sectional study therefore illuminates upon the superiority of CBNAAT in TB diagnostics, over ZN smear microscopy and LJ medium culture – for both pulmonary and extrapulmonary cases. The higher detection rate, strong concordance with culture and ability to identify smear-negative cases highlight the value of CBNAAT in paucibacillary disease. The significant correlation between CBNAAT-detected mycobacterial load and smear positivity also supports its diagnostic reliability. By providing rapid and accurate results, CBNAAT reduces the interval between clinical suspicion and confirmed diagnosis, thereby leading to faster

initiation of pharmacotherapy. Overall, CBNAAT offers a reliable option as a frontline diagnostic modality in high-burden, resource-limited setups – as in this subcontinent. When used in conjunction with smear microscopy and culture, CBNAAT may serve as a fulcrum in achieving India's goal of eliminating tuberculosis-related morbidity and mortality.

ACKNOWLEDGEMENTS

Authors would like to thank the Heads of the Departments of Microbiology and Chest Medicine of Medical College and Hospital, Kolkata, for their valuable support and guidance during the course of this study. They also acknowledge the support of all medical and technical staff who contributed to sample collection and data acquisition. Lastly, they extend appreciation to the patients and their families for their cooperation, without whom this study would not have been possible.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Aich I, Sau B, Acharya S, Ghosh P, Ghosh S, Sengupta M, et al. Comparative evaluation of cartridge-based nucleic acid amplification test, Ziehl-Neelsen smear microscopy, and culture in Lowenstein Jensen media for diagnosis of pulmonary and extrapulmonary tuberculosis in a tertiary care hospital in West Bengal, India. Int J Res Med Sci 2026;14:1077-84.