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Role of cartridge-based nucleic acid amplification test in diagnosis of extrapulmonary tuberculosis; a cross-sectional study.

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Abstract

Background:
Tuberculosis (TB) is an infectious disease caused by an acid fast bacilli known as Mycobacterium tuberculosis. It primarily affects the lungs (Pulmonary TB) but can also affect other organs (EPTB) like lymph nodes, pleura, CNS, meninges, bones and joints, kidney, spine, etc, which remains a major public health problem.

Methodology:
Patients with presumptive extrapulmonary TB, presenting complaints, Comorbidities, HIV status, and any past & contact history of tuberculosis were noted. Investigations like CPB, ESR, HIV, and RBS were done in all cases. For patients with suspected TB lymphadenopathy, FNAC was done, and pus was obtained and sent for CBNAAT & culture. Patients with pleural TB & Other EPTB samples, like pleural fluid, CSF, and ascitic fluid aspirated and sent for ADA proteins, cytology, and CBNAAT & culture.

Results:
Out of 100 extra-pulmonary cases, 55% were males and 45% were females. The most common age group was 21-40 years. The most common EPTB found was lymph node TB (45%), followed by TB pleural effusion (30%) and abdominal TB (10%). Sensitivity and Specificity of CBNAAT were 62.2% and 74.50%, respectively, in comparison with Culture. Out of the 100 samples, CBNAAT was Positive in 42 samples, culture was positive in 45, of which 9(21.42%) were Rifampicin-resistant.

Conclusion:
The diagnostic yield of CBNAAT was 42% in the EPTB samples. Therefore, the present study concludes that CBNAAT definitely plays a crucial role in the diagnosis of EPTB because of its simplicity and rapid turnaround time of 2 hrs & detects Rifampicin Resistance simultaneously for early initiation of MDR TB treatment compared to culture, as it takes a longer duration.

Recommendation:
CBNAAT is recommended as the initial test for suspected extrapulmonary tuberculosis, providing rapid detection of TB and rifampicin resistance.

Keywords: *Tuberculosis, Extrapulmonary Tuberculosis, cartridge-based nucleic acid amplification test (CBNAAT). GeneXpert, Lymphnode TB, Pleural effusion,* Mycobacterium tuberculosis *(MTB), Tuberculosis culture*
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Introduction:
Mycobacterium tuberculosis (MTB) causes Tuberculosis (TB) disease, which is contagious in nature, predominantly involving the lungs.[1] Traditionally, it was believed that pulmonary TB constitutes around 85% of total TB cases, whereas the remaining 15% are extrapulmonary tuberculosis (EPTB) cases.[2] A huge variation is observed in the proportion of EPTB among all TB cases, ranging from 15% to 53% according to the current data from around the world.[3]

It primarily affects the lungs (Pulmonary TB) but can also affect other organs (EPTB) like lymph nodes, pleura, CNS, meninges, bones and joints, kidney, spine, etc. It remains a major public health problem. According to the WHO global report in 2025, TB is one of the top 10 causes of death

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globally and a leading cause from a single infectious agent (even above HIV/AIDS).In 2025, an estimated 10 million people were affected with tuberculosis worldwide, of whom 1.1 million died. Annually, India accounts for one-fourth of the global incident TB cases [4].

EPTB accounts for 15-20% of TB cases. It includes lymph node TB (cervical, axillary, hilar, inguinal), pleural effusion, Pott's spine, skeletal tb abdominal TB (usually with ascites), and genitourinary (renal) tuberculosis.[5] Disseminated/miliary tuberculosis often includes pulmonary and extrapulmonary sites. One of the important risk factors for the development of EPTB is weak cellular host immunity.HIV accounts for the highest number of EPTB cases, especially with low CD4 counts. In HIV negative EPTB, amounts to 20% compared to HIV-positive, in whom it is around 50-60% [6]. Other causes of Immunosuppression who are at increased risk of EPTB are Diabetes, smoking, low body weight, chronic kidney disease, malignancy, and use of immunosuppressive agents. Recently, low socioeconomic status has been identified as a risk factor for EPTB. EPTB poses a diagnostic challenge because of inaccessible sites for sampling and the paucibacillary nature of specimens. Hence, there is a need for a rapid and cost-efficient test to diagnose EPTB.[7]

India is now moving to TB elimination, and the national program has been rechristened as the National TB Elimination Program (NTEP); in the context of targets for drastic reduction of TB incidence and mortality, early diagnosis of EPTB becomes very important. Late diagnosis results in morbidity, increased mortality, and disease sequelae.[8] The laboratory diagnosis is an added hurdle, as a good number of specimens are paucibacillary or smear-negative, particularly for the detection of drug resistance, and it is not uncommon to miss the diagnosis of EPTB.[9]

This study aims to establish the diagnostic yield of CBNAAT in detecting mycobacterium tuberculosis bacilli in EPTB patients.

Materials and methods

Study design and setting

This hospital-based cross-sectional observational study used prospective data collection and was conducted in outpatient and inpatient departments of Government Chest and TB Hospital, Kakatiya Medical College, Hanumakonda, Telangana, India. The study was carried out over 24 months from May 2023 to April 2025.

Inclusion criteria

1. Patients of either sex, aged above 18 years with clinical history of presumptive Extrapulmonary Tuberculosis.
2. Hemodynamically stable EPTB cases

Exclusion criteria

1. Extrapulmonary Tuberculosis patients who were already on treatment
2. Scanty aspirate in whose case material could not be sent for CBNAAT

Variables and data sources

In the study, each patient was consecutively enrolled after fulfilling the inclusion and exclusion criteria for the study. Clinical assessment: Detailed history, physical examination, and symptom recording.Radiological assessment: Chest X-ray for confirmation of pleural effusion. Laboratory investigations: Blood tests: CBP, LFT, RFT, BT, CT, PT-INR, HIV.A detailed clinical history about fever, night sweats, anorexia, weight loss, and hemoptysis. History of diabetic status, hypertension, COPD, HIV/AIDS, and any history of tuberculosis or contact history were noted.

Under proper aseptic precautions, after obtaining informed consent for the procedure, samples from different sites like pleural fluid, peritoneal fluid, lymph node aspirate, pus, cerebrospinal fluid, synovial fluid, CSF and fine needle aspirate samples from suspected TB Lymphnode cases were collected in Falcon-tube and subjected to CBNAAT investigation (GeneXpert MTB/RIF assay) for detection of Mycobacterium tuberculosis and rifampicin resistance and mycobacterial culture. The growth of mycobacteria on culture medium was noted at the end of two weeks and every week thereafter. The final results were reported at the end of eight weeks in the Microbiology Department. Depending upon the site and type of lesion, all other standard investigations conventionally used for diagnosis of tubercular lymphadenitis and tubercular pleural effusion were also performed, such as smear by Ziehl–Neelsen Stain (ZN Stain), biochemical tests including pleural fluid adenosine deaminase (ADA), lactate dehydrogenase level (LDH), glucose and protein, cytology, and Mantoux test.

Sample size determination

Sample Size Calculation: Sample size was calculated using the formula:

$n= Z^2p(1-p)/d^2$

where: n = sample size , Z = confidence level (95%) , p = anticipated diagnostic yield of CBNAAT, d = desired level of precision.

Based on previous studies, the minimum required sample size was calculated as 100 patients.

Statistical analysis

Data were entered into a spreadsheet and analysed using standard statistical methods. Continuous variables were expressed as mean ± standard deviation, and categorical variables were expressed as frequency and percentage. The diagnostic accuracy, sensitivity, and specificity were also calculated.

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Measures to Minimize Bias

Several measures were adopted to minimize potential sources of bias. Consecutive patient recruitment was undertaken to reduce selection bias with predefined eligibility criteria. Standardized protocols were followed for sample collection, laboratory analysis, and clinical evaluation to minimize measurement bias. Use of a reference standard such as Mycobacterial culture was used as the microbiological reference standard. Regular equipment maintenance was performed for CBNAAT and culture processing to ensure accuracy and reproducibility of laboratory results. Uniform diagnostic criteria were applied for all participants to reduce classification bias.

Ethical considerations

Institutional Ethics Committee approval was obtained from the **Kakatiya Institutional Ethics Committee**. The identity of the patient was kept confidential, and written informed consent was obtained from each enrolled patient. Only anonymized clinical data were used for analysis.

Results:

Participant flow:

A total of 153 presumptive TB cases were examined for eligibility. Of these, 110 were considered potentially eligible after initial screening. Following a detailed eligibility assessment, 100 were confirmed eligible, included in the study, and analysed. The reasons for exclusion are shown in Figure 1

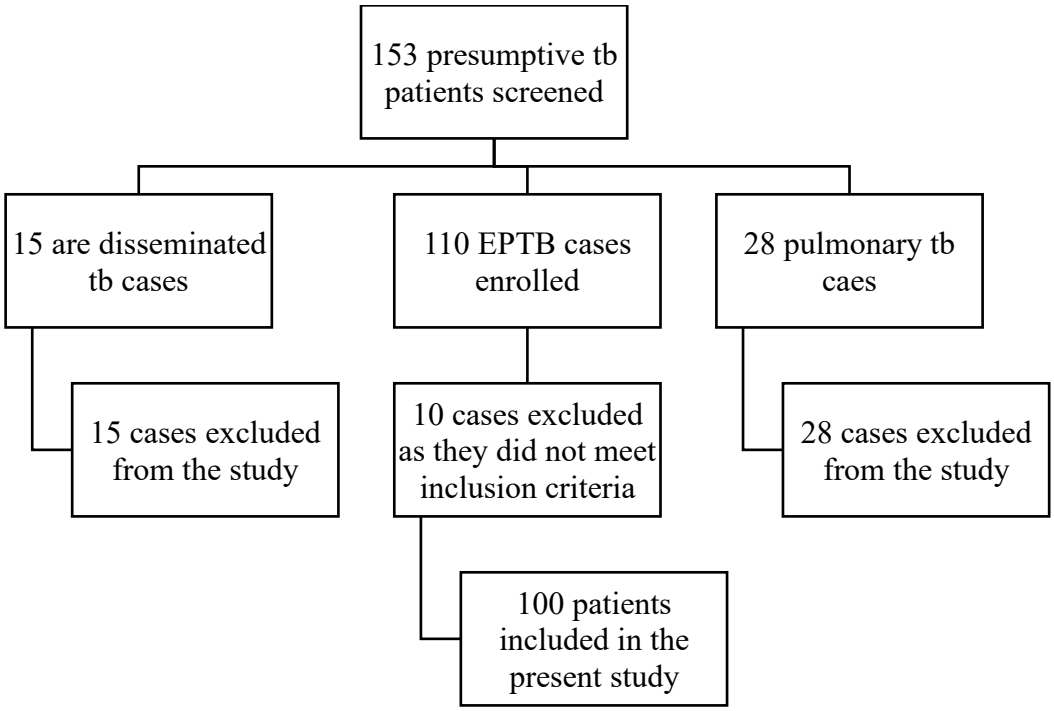

Figure 1. Participant flow diagram

100 patients were enrolled and analysed for this study: 55% were males and 45% were females. The mean age of the study population was 40.81 years, with age ranging from 18 to 79 years. The most common symptom was fever (94%). Comorbidity observed in the study population was diabetes mellitus (16%), 21% of the study population had a history of TB, and 11% had a history of contact with TB. [Table 1] The most common EPTB found in our study population was lymph node TB (45%), followed by TB pleural effusion (30%), abdominal TB (10%), bone and joint TB (7%), and urogenital TB(5%). The least common EPTB was central nervous system (CNS) TB (3%). [Table 2].

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Table 1: Descriptive data of the study population.

Variable	Total(n)	Percentage (%)
Gender		
Male	55	55%
Female	45	45%
Age in years		
0-20	20	20%
21-40	53	53%
41-60	24	24%
61-80	6	6%
SYMPTOMS		
Fever	94	94%
cough	29	29%
SOB	29	29%
Chest pain	20	20%
Lymph node swelling	45	45%
Constitutional symptoms	68	68%
Abdominal pain and distension	10	10%
Back pain	4	4%
headache	2	2%
Neck stiffness	1	1%
comorbidities		
Diabetes mellitus	16	16%
Hypertension	9	9%
HIV	12	12%
History of TB		
yes	21	21%
no	79	79%
TB contact		
yes	11	11%
no	89	89%

Note. *N = 100. Values are presented as frequency (n) and percentage (%). *Symptoms and comorbidities were not mutually exclusive; therefore, percentages may total more than 100%.*

Table 2: Distribution of study population based on type of EPTB disease.

Type of EPTB	Number	percentage
Lymphnode	45	45%
Pleural effusion	30	30%
Abdominal	10	10%
Bone and joint	7	7%
Urogenital	5	5%
CNS	3	3%
Total	100	100%

Note. *EPTB = extrapulmonary tuberculosis; CNS = central nervous system. Percentages are based on the total study population (N = 100).*

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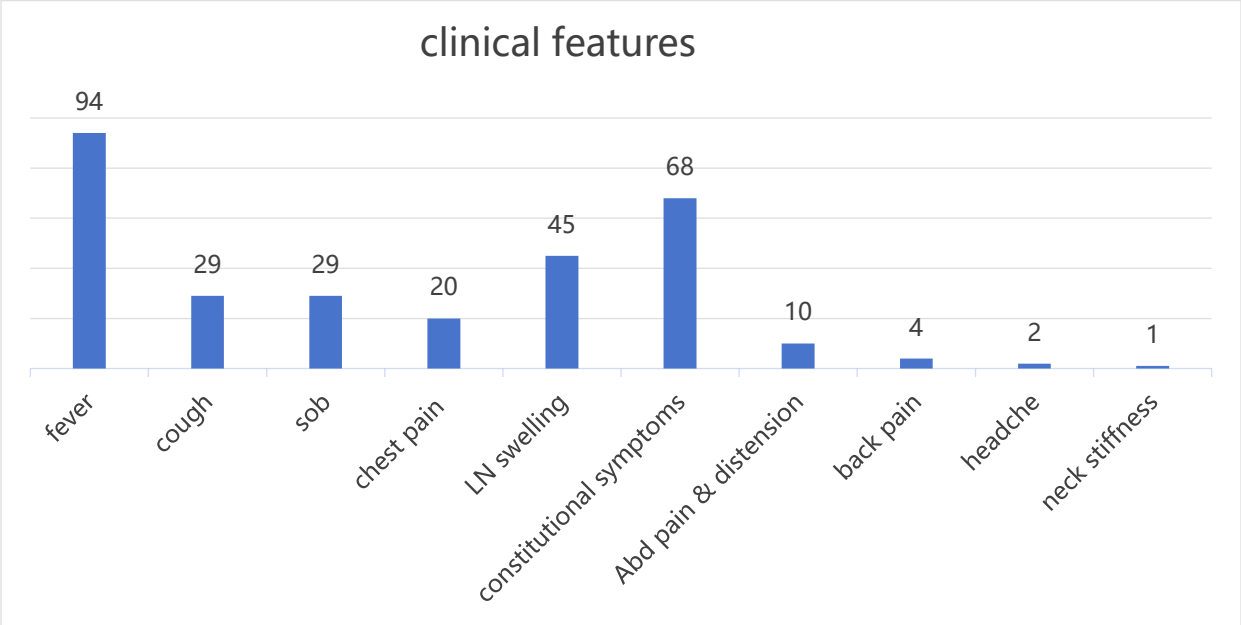
Graph 1: Figure showing the Clinical features in EPTB.



The clinical features among which the most common was fever (94%, followed by Constitutional symptoms 68%, LN swelling 45%, Cough 29%, sob 29%), chest pain 20%, Abdominal pain and distension 10%, Back pain 4%, headache (2%, Neck stiffness 1% [Graph 1].

Table 3: Distribution Of EPTB Samples with CBNAAT Positive and Rifampicin Sensitive or Resistant.

Type of EPTB sample	Number(n)	CBNAAT positive	Rifampicin sensitive	Rifampicin resistance
Lymph node pus	45	23	18	5
Pleural fluid	30	13	10	3
Ascitic fluid	10	3	2	1
Synovial fluid	7	2	2	0
Genitourinary fluids	5	0	0	0
CSF	3	1	1	0
Total	100	42	33	9

Note. *CBNAAT = Cartridge-Based Nucleic Acid Amplification Test; EPTB = extra pulmonary tuberculosis; CSF = cerebrospinal fluid. Rifampicin sensitivity and resistance are reported only among CBNAAT-positive samples.*

Lymph node pus contributes to the majority of the extra pulmonary samples, contributing to 45, followed by pleural fluid 30 of the total 100 extra pulmonary samples. Out of the 45 lymph node pus samples 23, pleural fluid samples 13 were found CBNAAT positive, of which 5 and 3 were found to be Rifampicin resistant, respectively [Table 3].

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Table 4: Distribution of CBNAAT-positive and negative samples Vs Liquid Culture Positive and Negative Samples

CBNAAT Result	Culture Positive, *n*	Culture Negative, *n*	Total, *n*
Positive	28	14	42
Negative	17	41	58
Total	**45**	**55**	**100**

Note. *CBNAAT = Cartridge-Based Nucleic Acid Amplification Test. True positive (TP) = 28; true negative (TN) = 41; false positive (FP) = 14; false negative (FN) = 17.*

Table 5: Diagnostic Accuracy Measures of CBNAAT Using Culture as the Reference Standard

Diagnostic Parameter	Value
Sensitivity (%)	62.2
Specificity (%)	74.5
Positive Predictive Value (PPV, %)	66.7
Negative Predictive Value (NPV, %)	70.7
Overall Accuracy (%)	69.0
Cohen's Kappa (κ)	0.37

Note. *Culture was used as the reference standard for evaluating the diagnostic performance of CBNAAT. A Cohen's kappa (κ) value of* ***0.37*** *indicates* ***fair agreement*** *between CBNAAT and culture.*

Out of a total of 100 samples, CBNAAT was positive in 42 samples, culture was positive in 45, and 28 samples were positive for both CBNAAT & culture. True positive-28, True negative-41, False positive-14, False negative-17 Sensitivity-62.2% Specificity-74.5% PPV-66.7% NPV-70.7% Accuracy-69.0%, Kappa statistics-0.37 [Table 4&5].

Discussion

The present hospital-based cross-sectional study with prospective data collection evaluated Diagnostic Accuracy Measures of CBNAAT Using Culture as the reference standard in 100 EPTB patients. The sensitivity and specificity of CBNAAT in the present study were 62.2% and 74.5%, respectively. The diagnostic yield of CBNAAT was 42% in the EPTB samples. The sensitivity in this study also falls within the wide range (25%–96%) reported in a systematic review by Lawn et al., which shows that specimen type and bacterial load affect test results. [10]. In the present study, the sensitivity and specificity of CBNAAT compared to culture were 62.2% and 74.5%, respectively, which is nearly consistent with Suresh et al [11]. The sensitivity in this study was lower than that reported by Tortoli et al., who found a sensitivity of 81.3% and specificity of 99%. [12] This difference may be due to differences in sample types, lab techniques, bacterial load, and the population studied. The inclusion of more pleural and abdominal fluid samples, which usually have fewer bacteria, may have contributed to the lower sensitivity.

The demographic profile showed a predominance of adults in the economically productive age group, with a mean age of 41.8 years and more males (55%) than females (45%) in the present study. Similar demographic characteristics were reported by Chakravorty et al., who observed that EPTB commonly affects adults in the working age group (13). The higher number of males in this study also matches the typical pattern of tuberculosis in India.

Fever was the most common symptom (94%) most common comorbidity observed in the study population was diabetes mellitus (16%). 21% of the study population had a history of TB, and 11% had a history of contact with TB. These results show the importance of identifying underlying risk factors and carefully evaluating patients with presumptive TB symptoms.

The most common EPTB found in our study population was lymph node TB (45%), followed by TB pleural effusion (30%) and abdominal TB (10%), which is similar to Sharma and Mohan's findings, who noted that lymph node tuberculosis is the most common form of EPTB [14]. Armand et al. and Hillemann et al also found that lymph

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node samples make up a large part of extrapulmonary tests using Xpert MTB/RIF. [15,16]

Among those with lymph node tuberculosis, CBNAAT detected Mycobacterium tuberculosis in 51.1% of samples, and 5 cases showed resistance to rifampicin. This rate is close to what Armand et al. reported and is similar to findings from other studies. This suggests that CBNAAT is effective in diagnosing lymph node tuberculosis and also helps detect drug resistance quickly. In cases of pleural tuberculosis, CBNAAT was positive in 43.3% of samples, with three 3 showing rifampicin resistance. This lower result is expected because pleural fluid usually has very few bacteria. Study by Nishal et al studies have shown that CBNAAT is less sensitive in pleural tuberculosis compared to tissue samples.[17]

CBNAAT was positive in 30% of cases, even though all pleural and ascitic fluid samples showed signs of tuberculosis, such as high protein levels, elevated adenosine deaminase (ADA), and lymphocyte dominance. These results show that while biochemical tests can suggest tuberculosis, a microbiological test is still needed, especially to detect drug resistance. CBNAAT identified tuberculosis in two patients with osteoarticular tuberculosis and one with central nervous system tuberculosis. Similar results were reported by Komanapalli et al., who said CBNAAT improves the diagnosis of EPTB when used with traditional methods. [18]

The main advantage of CBNAAT is its quick turnaround time—about two hours—which helps in early diagnosis and quick treatment. It also quickly detects rifampicin resistance, allowing for early treatment of drug-resistant tuberculosis. Yadhav et al, Veena et al and Kasat et al.have also highlighted similar benefits, stating that CBNAAT significantly speeds up diagnosis compared to traditional methods. Despite these benefits, CBNAAT has some limitations [19,20]. It is less sensitive in samples with low bacterial content, such as pleural fluid, cerebrospinal fluid, and ascitic fluid. It only detects rifampicin resistance and cannot replace full drug susceptibility testing. Additionally, the test requires special equipment and a steady electricity supply, which may limit its use in areas with fewer resources. In conclusion, this study supports the use of CBNAAT as a quick and reliable first test for EPTB. Even though its sensitivity depends on the type of sample, its high accuracy, fast results, and ability to detect rifampicin resistance make it an important tool in diagnosis. However, CBNAAT should be used with clinical findings, imaging, histopathology, and culture to best diagnose and manage EPTB.

Conclusion

The present study was conducted to study the role of CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) in the diagnosis of presumptive extrapulmonary Tuberculosis patients in the Chest & TB Hospital. A total of 100 cases were taken into the study. The study concluded: Diagnostic yield of CBNAAT was 42% in the EPTB samples. In our study, CBNAAT had a sensitivity of 62.2% and specificity of 74.5% in Extra Pulmonary Samples. Therefore, the present study concludes that CBNAAT/gene xpert definitely is a very useful, rapid, and economical tool for diagnosing EPTB and detecting rifampicin resistance simultaneously for early treatment initiation and decreasing morbidity, mortality, and TB sequelae.

Limitations

Limited sample size, and this study focuses on a single tertiary centre.

All the study samples were not correlated with biopsy or clinic-radiological reports.

Source of funding

The study had no funding.

Conflict of interest

The authors declare no conflict of interest.

Data availability

Data Available on request

Author contributions

Dr. Divya Sree Lavudya contributed to the conception and design of the study, clinical data supervision, interpretation of findings, manuscript drafting, and final approval of the manuscript.

Dr. Naresh Mamidi contributed to study planning, data collection, literature review, analysis support, manuscript revision, and critical review of the manuscript.

Dr. Sindhuja Vemula contributed to clinical coordination, methodological support, data verification, interpretation of relevant clinical findings, and final approval of the manuscript.

Author biography

Dr. Divya Sree Lavudya is a qualified medical professional with an MBBS degree from Mamata Medical College (2008–2013) and an MD in Respiratory Medicine from Osmania Medical College (2017–2020). She has over 7 years of teaching experience in the field of Respiratory Medicine, contributing actively to the academic training of undergraduate and postgraduate students. Her areas of expertise include the diagnosis and management of respiratory diseases, tuberculosis, pulmonary function

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