



EVALUATION OF DIAGNOSTIC UTILITY OF CBNAAT FOR DETECTING MYCOBACTERIUM TUBERCULOSIS IN DIVERSE EXTRAPULMONARY SPECIMENS USING LIQUID CULTURE AS "GOLD STANDARD"

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ABSTRACT

Background: Tuberculosis remains a formidable public health threat globally, with extrapulmonary tuberculosis constituting 20 % of cases in India. Extrapulmonary tuberculosis (EPTB) poses significant diagnostic challenges due to its paucibacillary nature. GeneXpert MTB/RIF assay has revolutionized tuberculosis diagnosis, but data on its performance across diverse extrapulmonary specimens remain limited. **Methodology:** This prospective observational study evaluated the diagnostic utility of GeneXpert MTB/RIF in 1997 extrapulmonary specimens. Samples were put for upfront GeneXpert MTB/RIF alongside liquid culture. Diagnostic performance metrics were calculated and statistical associations were evaluated. **Results:** The assay demonstrated variable sensitivity across specimen types, with the highest sensitivity observed in pus samples (90.4%) and synovial fluid samples (100%). Pleural fluid and lymph node samples showed moderate sensitivity (77.4% and 79.0%, respectively), while CSF and ascitic fluid samples exhibited lower sensitivity (63.3% and 55.6%, respectively). Specificity ranged from 79.4% to 100% across specimen types. A significant association was found between GeneXpert MTB/RIF and culture results ($p < 0.00001$). **Discussion:** GeneXpert MTB/RIF assay is a rapid, sensitive, and specific diagnostic tool for extrapulmonary tuberculosis, particularly valuable in resource-limited settings. The study reinforces the continued use of GeneXpert MTB/RIF within established diagnostic algorithms.

KEYWORDS : Extrapulmonary tuberculosis, GeneXpertMTB/RIF, Liquid culture, Mycobacterium tuberculosis, Molecular diagnosis tools

INTRODUCTION:

According to the Global Tuberculosis Report 2024, tuberculosis (TB) once again emerged as the foremost cause of death globally in 2023, after a three-year period during COVID-19 pandemic years. India contributes approximately one-quarter of the total global tuberculosis burden.¹ Extrapulmonary tuberculosis (EPTB), caused by infection with *Mycobacterium tuberculosis* complex, constitutes over one fifth of all tuberculosis cases reported in India.²

The diagnosis of EPTB remains challenging because of its typically paucibacillary nature and the frequent requirement for invasive procedures to obtain suitable clinical specimens.³ The disease exhibits a wide spectrum of clinical manifestations, ranging from non-specific constitutional symptoms to organ- and system-specific presentations depending on the site involved. Among extrapulmonary sites, lymph node tuberculosis is the most common form (33%), followed by pleural involvement as the second most frequent manifestation.² However, tuberculosis of the central nervous system (CNS) remains the most severe and potentially fatal form.⁴

In 2010, the World Health Organization (WHO) endorsed the Xpert MTB/RIF assay—a rapid, cartridge-based nucleic acid amplification test, fully automated molecular diagnostic test that can detect *Mycobacterium tuberculosis* complex and rifampicin resistance within less than two hours.⁵ Despite its proven utility in pulmonary specimens, there is a paucity of data evaluating the performance of the GeneXpert MTB/RIF assay across diverse extrapulmonary specimen types. Therefore, the present study was undertaken to evaluate the diagnostic utility of the CBNAAT for detection of *Mycobacterium tuberculosis* in extrapulmonary specimens obtained from clinically suspected cases of extrapulmonary tuberculosis.

Methodology:

A Prospective observational study was conducted in TB culture and DST laboratory of a tertiary care hospital from the period

of December 2022 to February 2024. A total of 1997 extrapulmonary specimens were put for upfront GeneXpert MTB/RIF as per the diagnostic algorithm under NTEP.

After decontamination and concentration methods, all extrapulmonary samples concentrates were put for liquid culture in MGIT 960 system. All data were entered into Microsoft Excel and analyzed. The statistical significance of association between test outcomes of GeneXpert MTB/RIF and liquid culture was evaluated using the Chi-square test or Fisher's exact test, as appropriate. A p-value of <0.05 was considered statistically significant.

RESULTS:

A total of 1997 extrapulmonary specimens were analyzed. The distribution of various sample types is shown in table 1. The most frequently received specimens were pleural fluid (443; 22.2 %), pus (401; 20.1%), and lymph node (346; 17.3 %) followed by CSF (352; 17.6 %) and ascitic fluid (186; 9.3 %). Less frequently received samples were synovial fluid (21; 1%), pericardial fluid (14, 0.7%), drain fluid (6; 0.3%), bone marrow (5; 0.25%), bone tissue (3; 0.15%), pleural nodule (1; 0.05%) and hydrocele fluid (1; 0.05 %).

Table 1. Distribution Of Various Extrapulmonary Specimens Analyzed

Type of Specimen	Number of Samples (n)
Pleural fluid	443
pus	401
Lymph node	346
Cerebrospinal fluid (CSF)	352
Ascitic fluid	186
Tissue biopsy (other than lymph node)	218
Synovial fluid	21
Pericardial fluid	14
Drain fluid	6
Bone marrow	5
Bone tissue	3

Pleural nodule	1
Hydrocele fluid	1
Total	1997

The total number of samples tested positive for Mycobacterium tuberculosis by GeneXpert MTB/ RIF and liquid culture respectively for various extrapulmonary specimen has been demonstrated in the table no 2.

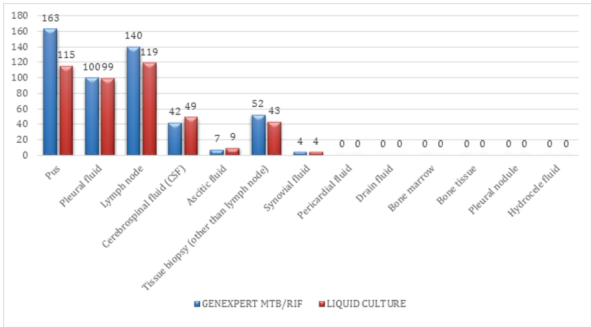


Figure 1: No. of samples with positive results for Mycobacterium tuberculosis detection in GeneXpert MTB/RIF and liquid culture

Table 2. Evaluation of Gene Xpert MTB/RIF With Liquid Culture (MGIT) For All Pus Samples (N = 401)

PUS SAMPLE (N= 401)	Liquid Culture Positive	Liquid Culture Negative	Total
GENEXPERT MTB/RIF POSITIVE	104	59	163
GENEXPERT MTB/RIF NEGATIVE	11	227	238
TOTAL	115	286	401

The assay showed sensitivity 90.4%, specificity 79.4%, PPV 63.8%, and NPV 95.4%. There was a significant association between GeneXpert and culture results (Chi-square = 165.9, df = 1, p < 0.00001), indicating reliable detection of Mycobacterium tuberculosis in pus.

Table 3. Evaluation of GeneXpert MTB/RIF With Liquid Culture (MGIT) For All Pleural Samples (N = 443)

Pleural fluid SAMPLE (N= 443)	LIQUID CULTURE POSITIVE	LIQUID CULTURE NEGATIVE	TOTAL
GENEXPERT MTB/RIF POSITIVE	77	23	100
GENEXPERT MTB/RIF NEGATIVE	22	321	343
TOTAL	99	344	443

The assay showed sensitivity 77.4%, specificity 93.3%, PPV 77.0%, and NPV 93.6%. There was a significant association between GeneXpert and culture results (Chi-square = 222.8, df = 1, p < 0.00001), indicating reliable detection of Mycobacterium tuberculosis in pleural fluid.

Table 4. Evaluation Of GeneXpert MTB/RIF With Liquid Culture (MGIT) For All Lymph Nodes Samples (N = 346)

Lymph node SAMPLE (N= 346)	LIQUID CULTURE POSITIVE	LIQUID CULTURE NEGATIVE	TOTAL
GENEXPERT MTB/RIF POSITIVE	94	46	140
GENEXPERT MTB/RIF NEGATIVE	25	181	206
TOTAL	119	227	346

The assay showed sensitivity 79.0%, specificity 79.8%, PPV 67.1%, and NPV 87.9%. There was a significant association between GeneXpert and culture results (Chi-square = 111.5, df = 1, p < 0.00001), indicating reliable detection of

Mycobacterium tuberculosis in lymph node samples.

Table 5. Evaluation of GeneXpert MTB/RIF With Liquid Culture (MGIT) For All CSF Samples (N = 352)

CSF SAMPLE (N= 352)	LIQUID CULTURE POSITIVE	LIQUID CULTURE NEGATIVE	TOTAL
GENEXPERT MTB/RIF POSITIVE	31	11	42
GENEXPERT MTB/RIF NEGATIVE	18	292	310
TOTAL	49	303	352

The assay demonstrated sensitivity 63.3%, specificity 96.4%, PPV 73.8%, and NPV 94.2%. There was a significant association between GeneXpert and culture results (Chi-square = 142.6, df = 1, p < 0.00001), indicating reliable detection of Mycobacterium tuberculosis in CSF.

Table 6. Evaluation Of GeneXpert MTB/RIF With Liquid Culture (MGIT) For All Ascitic Fluid Samples (N= 186)

ASCITIC FLUID SAMPLE (N=186)	LIQUID CULTURE POSITIVE	LIQUID CULTURE NEGATIVE	TOTAL
GENEXPERT MTB/RIF POSITIVE	05	2	7
GENEXPERT MTB/RIF NEGATIVE	04	175	179
TOTAL	09	177	186

The assay showed sensitivity 55.6%, specificity 98.9%, PPV 71.4%, and NPV 97.8%. There was a significant association between GeneXpert and culture results (Fisher's exact p = 0.004), indicating reliable detection of Mycobacterium tuberculosis in ascitic fluid.

Table 7. Evaluation of GeneXpert MTB/RIF with Liquid Culture (MGIT) For All Tissue Samples (N = 218)

TISSUE SAMPLE (N=218)	LIQUID CULTURE POSITIVE	LIQUID CULTURE NEGATIVE	TOTAL
GENEXPERT MTB/RIF POSITIVE	29	23	52
GENEXPERT MTB/RIF NEGATIVE	14	152	166
TOTAL	43	175	218

The assay showed sensitivity 67.4%, specificity 86.8%, PPV 55.8%, and NPV 91.6%. There was a significant association between GeneXpert and culture results (Chi-square = 55.9, df = 1, p < 0.00001), indicating reliable detection of Mycobacterium tuberculosis in tissue samples.

Table 8. Evaluation of GeneXpert MTB/RIF with Liquid Culture (MGIT) For All Synovial Fluid Samples (N = 21)

SYNOVIAL FLUID SAMPLE (N=21)	LIQUID CULTURE POSITIVE	LIQUID CULTURE NEGATIVE	TOTAL
GENEXPERT MTB/RIF POSITIVE	04	00	04
GENEXPERT MTB/RIF NEGATIVE	00	17	17
TOTAL	04	17	21

The assay showed sensitivity 100 %, specificity 100 %, PPV 100 %, and NPV 100 %. There was a significant association between GeneXpert and culture results (Fisher's extract test, p = 0.000167), indicating reliable detection of Mycobacterium tuberculosis in synovial fluid samples.

Rifampicin Resistance:

Of 1997 samples put for GeneXpert MTB/RIF, a total of 508 samples showed positive results for Mycobacterium

tuberculosis in GeneXpert MTB/RIF, of which 25% samples showed Rifampicin resistance.

RIFAMPICIN RESISTANCE DETECTED

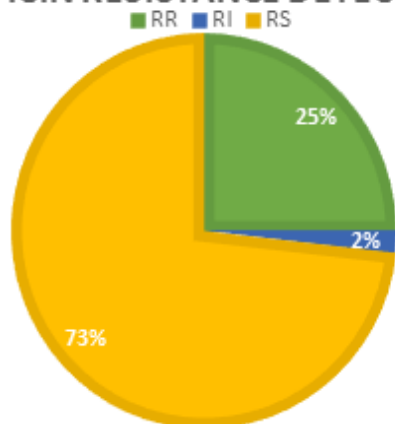


Figure 2:

DISCUSSION:

In the present study, a total of 1997 extrapulmonary specimens were analyzed for the detection of *Mycobacterium tuberculosis* (MTB) using GeneXpert MTB/RIF assay and liquid culture serving as the 'gold standard'. The majority of specimens comprised pleural fluid (22.2%), pus (20.1%), lymph node (17.3%), and cerebrospinal fluid (17.6%), which are among the most frequently encountered extrapulmonary tuberculosis (EPTB) samples in clinical practice. Similar distribution has been documented by Jain et al who reported lymph node, pus and CSF as predominant extrapulmonary specimens.⁶

Among different sample categories, the assay exhibited the highest sensitivity of 90.4% in pus specimens, suggesting effective detection in tissue-rich, high bacillary-load samples. Comparable studies by Set et al, Mechai et al reported lower sensitivities of 76.19 % and 64.3 %, underscoring the comparative stronger performance in the present study.^{7,8}

Pleural fluid samples showed moderate sensitivity (77.4%) alongside high specificity (93.3%), findings consistent with the inherently low bacillary yield of pleural tuberculosis. Similar findings were noted by Elbroisy et al who reported GeneXpert sensitivity of 77.8 % in pleural fluid, whereas, Patil et al's reported marginally higher diagnostic indices with 83.3 % sensitivity and specificity of 98.8 %. Despite moderate sensitivity, the strong co-relation between GeneXpert and culture results ($p < 0.00001$) in the present study underscores its utility as a rapid screening tool in clinically suspected cases.^{9,10}

Lymph node specimens also showed good diagnostic performance, with sensitivity and specificity of 79.0% and 79.8%, respectively. Observations from study of Patil et al demonstrated a higher diagnostic performance than current study with sensitivity of 100 % and specificity of 87.5 %. M.Hueda-Zavaleta et al also reported higher sensitivity of 100 % than our study but a lower specificity of 69.2 % as compared to our study.^{10,11} These results re-enforce GeneXpert MTB/RIF as a highly valuable diagnostic aid in tuberculous lymphadenitis.

Cerebrospinal fluid (CSF) samples yielded a sensitivity of 63.3% and specificity of 96.4%. Studies by Mechai et al established sensitivity of 85.7 % and specificity of 98%, which are superior values than current study. Similar study by Patil et al shows sensitivity of 100 % which is significantly higher than our study and specificity of 89.3 %.^{8,10}

Similarly, in ascitic fluid, GeneXpert showed a lower

sensitivity (55.6%) but retained excellent specificity (98.9%), reflecting inherent diagnostic limitations associated with peritoneal tuberculosis. The assay nonetheless demonstrated significant concordance with culture (Fisher's exact $p = 0.004$), supporting its adjunctive role in early diagnosis. Similar study by Bera et al shows a sensitivity of mere 19% and specificity of 100 %, the higher specificity is consistent with our findings.¹²

For tissue samples, the assay demonstrated sensitivity of 67.4% and specificity of 86.8%, suggesting moderate accuracy likely influenced by sample heterogeneity and presence of necrotic material. Hilman et al demonstrated sensitivity of 69% and specificity of 98.4 % aligning with present study findings.¹³

The 100% sensitivity and 100 % specificity observed in synovial fluid, though this observation is limited by small sample size, it reinforces the potential of GeneXpert MTB/RIF as a reliable diagnostic tool even in rare extrapulmonary presentations. Studies evaluating the performance of GeneXpert MTB/RIF on synovial fluid remain scarce, warranting further investigation.

CONCLUSION:

The present findings reaffirm CBNAAT based GeneXpert MTB/RIF as a rapid, sensitive and specific diagnostic tool for extrapulmonary tuberculosis, particularly valuable in resource-constrained, high-burden settings. The simultaneous detection of rifampicin resistance, allowing for timely initiation of appropriate therapy and improved patient management. The significant statistical associations between GeneXpert MTB/RIF and culture results across all specimen types ($p < 0.00001$ in most) confirm the reliability of the assay in detecting MTB in extrapulmonary samples. However, variable sensitivity across specimen types highlights the need for cautious interpretation and co-relation with culture in extrapulmonary samples. The high specificity of GeneXpert MTB/RIF in all extrapulmonary samples indicates its excellent ability to discriminate *Mycobacterium tuberculosis* complex from other non-tuberculous mycobacteria or contaminants. The molecular amplification of *rpoB* gene region, which is unique to the *Mycobacterium tuberculosis* complex, contributes to this high specificity making GeneXpert MTB/RIF a superior rapid diagnostic tool and reducing diagnostic ambiguity. The high negative predictive value further supports its role in ruling out tuberculosis in clinically suspected cases. Taken together, these findings underscore the pivotal role of GeneXpert MTB/RIF as a cornerstone in the rapid diagnosis of extrapulmonary tuberculosis and reinforce its continued use within established diagnostic algorithms, particularly in high-burden endemic settings.

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