ORIGINAL RESEARCH PAPER
Volume - 15 | Issue - 08 | August - 2026 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

A COMPARATIVE STUDY OF CONVENTIONAL, TIME INTENSIVE LOWENSTEIN-JENSEN (LJ) SOLID CULTURE MEDIUM VS NEW, RAPID MOLECULAR DIAGNOSTIC ASSAY GENEXPERT IN THE DETECTION OF EXTRAPULMONARY MYCOBACTERIUM TUBERCULOSIS

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ABSTRACT

Background: Extrapulmonary tuberculosis (EPTB) accounts for around 15% to 25% of all tuberculosis cases globally. It is very difficult to diagnose due to its non-specific symptoms and a low bacillary load which accounts for its high morbidity and mortality. Conventional, gold standard technique available for detection of EPTB is Lowenstein–Jensen (LJ) solid culture medium but its main disadvantage is time-consuming, while the newly available molecular assay GeneXpert MTB/RIF offers rapid molecular detection and gives results in two hours. **Materials and Methods:** This is a prospective, observational, comparative study which was conducted on 50 extrapulmonary specimens from clinically suspected EPTB patients in a tertiary care hospital. All the samples were analyzed using both GeneXpert assay and LJ solid culture medium. **Results:** GeneXpert detected 16 (32%) cases, whereas LJ culture identified 6 (12%). While GeneXpert showed 100% sensitivity rate and 100% negative predictive value, it has lower specificity rate (77.3%). The difference in detection rates between GeneXpert and LJ culture was statistically significant ($p < 0.05$). **Conclusion:** This study concludes that GeneXpert assay is a rapid and highly sensitive diagnostic tool for detecting EPTB in paucibacillary specimens. However, LJ culture medium remains the gold standard technique since it has 100% sensitivity and specificity rates.

KEYWORDS

EPTB - Extrapulmonary Tuberculosis; Genexpert, Lowenstein–jensen (LJ) Culture, Paucibacillary, Gold Standard

INTRODUCTION

The bacterium *Mycobacterium tuberculosis* is the primary pathogen responsible for the spread of tuberculosis, a highly infectious disease. It usually spreads through air or respiratory droplets. Tuberculosis affects the lungs primarily but it involves other organs such as lymph nodes, bones and joints, GIT, and CNS which is called as extrapulmonary involvement.

Extrapulmonary tuberculosis (EPTB) accounts for around 15% to 25% of all tuberculosis cases around the world (1). Unlike TB involving lungs, patients with extrapulmonary involvement do not always have any specific symptoms and also the bacterial load from such extra pulmonary specimens will be very low (2). These two challenges present a significant diagnostic dilemma and contribute significantly to mortality and morbidity.

Hence conclusive and fast diagnosis of EPTB is strenuous since solid culture methods like Lowenstein–Jensen (LJ) culture have its own limitations. But still it has been upheld as the gold standard technique, and can grow as few as 10 to 100 viable bacilli. Its diagnostic utility is severely hampered by a prolonged incubation period of three to eight weeks, which significantly delays life-saving treatment in life-threatening conditions like TB meningitis where in such delays are unacceptable and often necessitate empirical therapy, which may lead to mismanagement of multidrug-resistant tuberculosis and disease progression (3,4).

The usual method of diagnosis of TB involves growing the bacteria in a medium (Lowenstein–Jensen (LJ) culture medium) takes a long time usually around three to eight weeks. This prolonged incubation period significantly delays life-saving treatment in life-threatening conditions such as TB meningitis which may lead to disease progression (4).

The advent of new, accelerated molecular assays has transformed the diagnosis of tuberculosis landscape. By utilizing RT-PCR, the GeneXpert MTB / RIF assay provides results within two hours while simultaneously detecting rifampicin resistance. However, it lacks sensitivity depending on specimen type and it is very low in extrapulmonary samples. Various studies have reported variable sensitivity for GeneXpert MTB/RIF assay ranging from 52% - 89% in extrapulmonary specimens (5-8).

Therefore, comparative evaluation of rapid molecular diagnostic tools such as GeneXpert with conventional culture methods remains essential to establish an effective diagnostic algorithm for EPTB.

OBJECTIVE

To compare and evaluate the performance of the conventional Lowenstein-Jensen (LJ) solid culture method against Rapid molecular assay GeneXpert in the detection of *Mycobacterium tuberculosis* from various extra pulmonary specimens.

MATERIALS AND METHODS

This prospective, observational, and comparative study was conducted at the Department of TB and Chest Medicine in collaboration with the Department of Microbiology at a tertiary care hospital in Chennai. The study was approved by the Institutional Ethics Committee (IEC No: Inst/TN/2014/052). All participants were fully explained about the study procedures, and written informed consent was obtained before collecting samples. The confidentiality of the participants was strictly maintained.

The study involved a sample of 50 extrapulmonary specimens collected from male and female patients aged 18 to 65 years. Patients who were clinically suspected of or confirmed to have extrapulmonary tuberculosis (EPTB) and gives written consent to participate in the study were included. Patients who were not willing to provide consent and those currently receiving anti-tubercular therapy or those diagnosed with any other co-morbid conditions such as HIV, syphilis, or diabetes were excluded from the study.

Around 5- 10 ml of extra pulmonary specimens were collected under aseptic precautions and transported promptly to the microbiology laboratory and processed without delay. All the specimens were subjected to digestion and decontamination by adding equal volumes of NALC (N-acetyl-L-cysteine) and NaOH (Sodium hydroxide) and allowed to stand for 15–20 minutes. Then a phosphate buffer with pH of 6.8 was added to all specimens and centrifuged at 3000 rpm for 20 minutes. The top layer, supernatant was then discarded, and the sediment was used for further analysis.

GeneXpert Assay:

For diagnosing *Mycobacterium tuberculosis* using GeneXpert assay, a portion of the processed sample from all the specimen were taken and the sample reagent was added in a 2:1 ratio according to the manufacturer's instructions. All the specimens were then thoroughly mixed and kept in room temperature. After 15 minutes, it was transferred into the test cartridge and loaded into GeneXpert system.

Conventional LJ (Lowenstein–Jensen) Culture Method:

For detection of *Mycobacterium tuberculosis* using conventional LJ culture method, another portion of the processed sample from the entire specimen was inoculated into the LJ solid culture medium. The inoculated media were then incubated at 37°C and observed weekly for up to 6 weeks for the growth of *Mycobacterium tuberculosis.*

Statistical analysis:

Statistical analysis was analyzed using appropriate statistical software. For all the categorical variables the results were expressed as percentages. The performance of GeneXpert was evaluated by calculating sensitivity, specificity, and predictive values, using LJ culture as the reference standard. Statistical significance was determined via chi-square analysis. Probability value < 0.05 was considered statistically significant.

RESULTS

A total of 50 extrapulmonary specimens were collected from clinically suspected EPTB patients. Among the 50 samples, majority 64% were pleural fluid aspirate. The details of the specimens collected and its frequency analysis were shown in table 1.

Table 1: Details of the specimen collected (n=50)

Specimen	Number of patients	Percentage (%)
Pleural fluid	32	64
Pus	11	22
Ascetic fluid	4	8
Lymph node aspirate	3	6

Among the study participants, 37 patients (74%) were males and 13 (26%) were females, showing a statistically significant male predominance. The most commonly affected age group was 41–60 years. The majority of patients belonged to the lower socioeconomic status, indicating a higher burden of disease in this group. The frequency analysis for various demographic details of the study participants were shown below in table 2.

Table 2: Demographic Details of Study Participants

Parameters	Frequency	Percentage (%)
Gender		
Male	37	74%
Female	13	26%
Age (in years)		
≤ 20	4	8%
21–40	15	30%
41–60	25	50%
> 60	6	12%
Socioeconomic Status		
Lower	32	64%
Middle	12	24%
Upper	6	12%

Table 3: Comparison of Diagnostic Methods

Diagnostic Methods	Positive Cases	Negative Cases	Chi square test	P value
LJ Culture method	6 (12%)	12%	-	-
GeneXpert Assay	16 (32%)	32%	4.72	0.03

The diagnostic performance of the GeneXpert and the LJ Culture method was compared using the Chi-Square test. The GeneXpert Assay yielded a significantly higher positivity rate of 32% (16/50) compared to the 12% (6/50) detected by LJ Culture method. The calculated Chi-Square value was 4.72 with a corresponding p-value of 0.03. Since the p-value is < 0.05, the null hypothesis is rejected, confirming that there is a statistically significant difference between the two diagnostic methods, with GeneXpert providing a superior diagnostic yield for extrapulmonary tuberculosis. The details of the positive cases from both the methods were summarized in table 3.

Evaluation of performance of GeneXpert assay:

Assuming LJ solid culture medium as the gold standard reference for detecting TB and all the 6 LJ-positive cases were also detected by GeneXpert, to evaluate its performance, parameters such as sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated below in table 4.

Table 4: Performance of GeneXpert assay

True Positives (TP) = 6	Sensitivity	100%
False Negatives (FN) = 0	Specificity	77.3%
False Positives (FP) = 10	Positive Predictive Value	37.5%
True Negatives (TN) = 34	Negative Predictive Value	100%

When compared against the standard solid LJ Culture method, the GeneXpert assay demonstrated a sensitivity rate of 100%, successfully identifying all 6 culture-positive cases. However, the specificity rate was 77.3%, as the assay detected 10 additional positive cases that were not recovered by the LJ method. This resulted in a Positive Predictive Value (PPV) of 37.5%. Notably, the Negative Predictive Value (NPV) was 100%, indicating that a negative GeneXpert result reliably ruled out the presence of Mycobacterium tuberculosis in the samples. These findings suggest that while GeneXpert is highly sensitive, its higher detection rate may reflect its ability to identify paucibacillary cases or dead bacilli that traditional culture methods fail to isolate.

DISCUSSION

India's focus towards TB Mukt Bharat Abhiyaan (Tuberculosis Free India Campaign) to eliminate Tuberculosis by 2025, ahead of the global 2030 Sustainable developmental goals target 3.3 aims to end the global Tuberculosis TB epidemic by 2030 (9). To achieve this initiative it is important to upgrade from the traditional solid culture methods to molecular detection. The current study focused on comparing the Nucleic Acid amplification assay GeneXpert with traditional solid LJ culture technique in extra pulmonary specimens.

Among the study participants, 37 patients (74%) were males and 13 (26%) were females, showing a statistically significant male predominance. This coincides with Mohamed et al (10) in which majority (78%) of their study population were males. Moreover males are having the habit of smoking & drinking alcohol, spending time in the highly crowded public places, being the developing country mens do work in poorly ventilated areas may contribute to the higher prevalence rate.

The most commonly affected age group was 41–60 years. The majority of patients belonged to the lower socioeconomic status, indicating a higher burden of disease in this group. This is in concordance with the research study conducted by Sriram selvaraju et al (11) where the commonest age group affected was more than 30 years.

In the present study, solid LJ culture medium detected tuberculosis in 6/50 (12%) which correlates with the study done by Nadeem et al (12) and AS Boinwad et al (13). The mean time for detection of Mycobacterium tuberculosis in this study is 29 days. The main disadvantage of solid LJ culture medium is its longer turnaround time. The current study found that the GeneXpert assay has detected tuberculosis in 16/50 (32%) of extrapulmonary specimens. This is higher when compared to the studies conducted by Nadeem et al (12), AS Boinwad et al (13), and Rangaiahagari Ashok et al (14) where they showed detection rate ranging from 6.8 % to 18%. This may be because our study includes more number of pus and lymph node aspirates in which the detection rate is higher for Gene X pert. Moreover suspecting the tuberculosis with clinical evidence implies a major challenge.

The current study found that the GeneXpert assay has detected tuberculosis in 32% of extrapulmonary specimens. This is significantly higher than the 12% detected by LJ solid culture medium. This difference in detection rates between GeneXpert assay and LJ solid culture medium was statistically significant (p = 0.03), indicating that the observed superiority of GeneXpert is unlikely to be due to chance. Similar findings have been reported in previous studies demonstrating the higher sensitivity rates of GeneXpert in extrapulmonary samples.

The increased detection rate or sensitivity with GeneXpert is because it can detect bacterial DNA even when the load is low particularly in extrapulmonary specimens (6). In contrast, the conventional LJ solid culture medium may fail to detect some EPTB cases because it requires presence of viable organisms and also it is time-consuming, leading to its lower detection rates. This suggests that the GeneXpert assay proved to be a rapid and highly sensitive diagnostic tool for detecting extrapulmonary tuberculosis, with significant advantages over conventional method.

The lower specificity (77.3%) observed with GeneXpert in the present

study can be attributed to the fact because of its detection of non-viable bacilli which is consistent with the findings from studies done by Amal H.G. et al (15) and Sunil Narute et al (16).

Additionally, the rapid turnaround time of GeneXpert provides an important clinical advantage, enabling early diagnosis and prompt initiation of anti-tubercular therapy, which may reduce disease progression and transmission. The ability of GeneXpert to simultaneously detect rifampicin resistance further enhances its clinical utility in managing drug-resistant tuberculosis.

However, despite its higher sensitivity, GeneXpert does not replace LJ culture entirely. Conventional LJ culture medium remains essential for confirming viable organisms and performing drug susceptibility testing, which is critical for guiding appropriate therapy in MDR & XDR drug resistant Tuberculosis. Hence Gene x pert helps diagnosis Tuberculosis early & culture testing may be useful for prognosis monitoring & follow up.

There are some limitations to this study. One limitation is that we did not compare the results of GeneXpert and LJ solid culture medium in a way that would have allowed us to use a statistical test. Another limitation is that our study had relatively small sample size. Future studies are recommended with larger sample sizes to compare different diagnostic tests.

CONCLUSION

This study highlights the clinical utility of the GeneXpert assay as a rapid and highly sensitive diagnostic tool for detecting extrapulmonary tuberculosis, particularly in paucibacillary specimens where bacterial loads are critically low. Transitioning from traditional, culture-based methods to molecular screening effectively bridges historical diagnostic gaps. Implementing this assay as a first-line diagnostic tool for high-risk cohorts specifically pediatric suspects, immunocompromised individuals, and patients living with HIV is essential. Ultimately, integrating molecular diagnostics into standard care pathways facilitates early case detection and the timely initiation of targeted therapy, leading to significantly optimized patient clinical outcomes.

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