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# Evaluation of the Diagnostic Performance of GeneXpert MTB/RIF assay for Extra-pulmonary Tuberculosis: A Retrospective Study from Pakistan

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**Abstract** **Objective:** A retrospective cross sectional study was conducted to evaluate the diagnostic performance of Cepheid GeneXpert MTB/RIF assay (Xpert assay) for the Extra-pulmonary tuberculosis (EPTB). This study retrieved and analyzed the record of Provincial TB Reference Laboratory (PTRL), Hayatabad Medical Complex, Peshawar, Pakistan from January 2019 to December 2022. **Materials and Methods:** Different EPTB specimens received to the PTRL for routine diagnosis from EPTB suspected patients were recruited consecutively and anonymously. All the specimens were digested and decontaminated by NALC-NaOH method except CSF. Afterward, all the specimens were cultured on solid and liquid medium and tested by Xpert assay. The overall and for each individual sample, diagnostic performance of Xpert assay was measured by sensitivity, specificity and predictive values using culture as a gold standard. **Results:** A total of 2174 EPTB samples were recruited after meeting the inclusion criteria and the pooled sensitivity, specificity, positive predictive value and negative predictive value of Xpert assay was 67%, 94%, 52% and 96% respectively. Moreover, Xpert assay showed variable sensitivities for different EPTB specimens including Pus, Tissue biopsy, Urine, CSF, Pleural fluid, Pericardial fluid and Ascitic fluid i.e., 91, 83, 80, 67, 46, 44 and 36%, respectively. The specificity ranged from 85% in CSF to 99% in Urine. **Conclusion:** Xpert assay can early detect EPTB cases for prompt treatment but there is low certainty of negative result to rule out EPTB.

**Key Words** Tuberculosis, Extra-pulmonary tuberculosis, GeneXpert MTB/RIF assay

## INTRODUCTION

Tuberculosis (TB) has remained the deadliest public health problem across the globe for ages [1]. The global tuberculosis report 2023 documented 10.6 million incident cases of TB worldwide including 6.3% of people living with HIV, wherein, 1.3million people died of TB. The two thirds load of total incident cases of TB was constituted by eight countries including Pakistan with 0.6 million new cases of TB in 2022. Pakistan adds 6% to the world TB burden and rank 5$^{th}$ among countries with high burden of TB [2]. The typical manifestation of TB is pulmonary infection, but it can be manifested as extra-pulmonary TB (EPTB) in any other organ of the body. However, EPTB frequently involves sites encompassing lymph node, pleura, brain, bone, meninges, gastrointestinal tract, genitourinary tract, joints and skin etc. The proportion of EPTB account significant among different countries of the world, whilst it represents 20% of all the cases of TB in Pakistan [3]. EPTB is causing significant morbidity and mortality throughout the world, nevertheless, its diagnosis is a great obstacle because of its clinical non-specific presentation, resemblance with other clinical conditions, paucibacillary nature of the specimens and usually

require invasive procedure for obtaining the biological specimen for microbiological, cytological and histopathological diagnosis and lack of resources in the remote TB endemic areas [4].

There is no single efficient diagnostic modality for the diagnosis of EPTB; however, microbiological confirmation can only be made by the identification of Mycobacterium tuberculosis complex (MTBc) in the biological samples through microscopy, culture, or nucleic acid amplification assay. The conventional microscopic tools do not give promising results for the identification of MTBc in the EPTB samples because of the paucibacillary nature of these specimens; moreover, it cannot differentiate MTBc from Mycobacterium other than tuberculosis. Though culture is deemed reference standard for isolation of Tubercle bacilli, but it requires trained personnel, state of the art laboratory and several weeks for report, consequently , early diagnosis and initiation of immediate treatment to control the disease cannot be achieved [5].

Therefore, the World health organization (WHO) recommended Cepheid GeneXpert MTB/RIF assay (Xpert assay) for the diagnosis of EPTB in 2013. This is a fully automated semi-quantitative real time nucleic acid amplification test and can be run with minimal bio-safety requirement and insignificant training. Xpert assay operates in a closed chamber including automatic extraction of DNA with a turnaround time of 120 minutes. It offers efficient and promising results in the diagnosis of EPTB by targeting the *rpoB* gene for the detection of both MTBc and resistance against rifampicin simultaneously [6].

Though, this assay is endorsed by WHO for the EPTB diagnosis, however, its sensitivity in different non-respiratory specimens is significantly vary among various geographical zones because of distinct factors encompassing low number of bacilli in the specimens and variable endemicity of TB [7]. Therefore, it is utmost important to evaluate the diagnostic performance of Xpert assay for EPTB diagnosis in each TB endemic region. The aim of the current study was to evaluate the diagnostic performance of Xpert assay for the most common specimens of EPTB received to the provincial TB reference laboratory (PTRL), Hayatabad Medical Complex (HMC), Peshawar, Pakistan.

## METHODS

This retrospective study retrieved and analyzed the record of PTRL, HMC, Peshawar from January 2019 to December 2022. All the EPTB specimens received to the PTRL from EPTB suspected patients, belonging to the different geographical areas of the province Khyber Pakhtunkhwa of Pakistan were consecutively and anonymously recruited in the study. The specimens were collected at the health care facility of the respective clinicians who referred them for the diagnosis of EPTB. The specimens collected from follow up patients and/or who empirically started anti-tuberculosis treatment was excluded from the study.

### Specimens work flow

All the EPTB specimens recruited in the study excluding Pus and Bone marrow aspirate were concentrated before digestion and decontamination. The liquid specimens including Synovial fluid, Pericardial fluid, Pleural fluid, Ascitic fluid, Cerebrospinal fluid (CSF), bronchoalveolar lavage, Gastric aspirate and urine were concentrated at 3000g for 15 minutes and the sediment was re-suspended in 2-5ml of Phosphate buffer saline (PBS). While the solid specimens encompassing Bone, Lung biopsy and Lymph node were mashed in PBS with sterile scalpel and the resultant saline were then centrifuged for concentration as mentioned above.

For digestion and decontamination of the specimens and/or sediment of the specimens except CSF were treated with N-acetyl-L-cysteine-sodium hydroxide (NALC-NaOH). In brief, NALC-NaOH solution was equally added to the sediment and/or specimens and left at the bench of laboratory with intermittent shaking for 15 minutes in the Falcon tube. Afterward, PBS was added to the Falcon tube up to the mark of 50ml and was centrifuged for 15 minutes at 4000g, the sediment was re-suspended in 2ml of PBS after discarding the supernatant [8]. The sediment was then inoculated on the slope of Lowenstein Jensen (LJ) medium and in tube of BD, Bactec Mycobacterium growth indicator tube (MGIT) 960 for TB culture and also tested by Xpert assay for detection of TB genome, while the sediment of CSF was used without decontamination for culture and Xpert assay.

### TB culture

Each of the two LJ slopes were inoculated with two drops of the re-suspended sediment with the help of a sterile dropper and incubated at 37°C. The LJ slopes were observed every day for the first 7 days for any growth and later on twice a week till appearance of growth or declared negative after 8 weeks of incubation. The growth of TB bacilli was confirmed by growth rate, colony morphology and ZN staining. While MGIT 960 tube supplement with 0.8 ml of PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) and OADC (oleic acid-albumin-dextrose-catalase complex) was inoculated with 0.5 ml of the re-suspended sediment. The tube was incubated in the MGIT 960 system at 37°C which monitors the growth automatically. Any tube with growth was confirmed as TB bacilli by TBc identification test and the tube failed to grow for 6 weeks was recorded negative [9].

### Xpert Assay

In line with manufacturer instructions 1 ml of the re-suspended sediment was added with 2ml of sample reagents in 50 ml Falcon tube and left for 15 minutes at room

temperature with interval mixing. Afterward, 2 ml of the suspension was poured into the cartridge and the cartridge was placed in the chamber for processing the assay. The Xpert assay reports the results as MTB not detected or MTB detected semi quantitatively measuring the bacilli load (very low, low, medium and high) [10].

### Statistical Analysis

Data was entered into the spread sheet of Excel and the sensitivity, specificity, Positive Predictive Value (PPV) and negative predictive value (NPV) were calculated by 2x2 table method using TB culture as a reference standard.

## RESULTS

This four years retrospective study recruited 2174 EPTB specimens which were comprised of 14 different EPTB types encompassing Pleural fluid 670 (30.9%), CSF 401 (18.4%), Tissue biopsy 268 (13.2%), Ascitic fluid 246 (11.3%), Pus 233 (10.8%), Urine 147 (6.8%), Pericardial fluid 98 (4.6%), Synovia fluid 29 (1.3%), Gastric aspirate 26 (1.1%), Bronchoalveolar lavage 25 (1.1%), Lymph node 24 (1.1%), Bone 03 (0.1%), Bone marrow 03 (0.1%) and Lung Biopsy 01 (0.04%).

Out of 2174 specimens, culture declared 200 (9.1%) samples positive for MTBc growth while 1974 (90.9%) samples were recorded negative because no growth appeared during required incubation period. Xpert assay detected MTBc in 255 (11.7%) specimens while failed to detect TB genome in the remaining 1919 (88.3%) samples.

In the study population, the overall sensitivity, specificity, PPV and NPV of Xpert assay were 67, 94, 52 and 96%, respectively, as shown in Table 1. Out of the 14 different types of EPTB specimens 07 types of the specimens including Synovial fluid (29), Gastric aspirate (26), Bronchoalveolar lavage (25), Lymph node (24), Bone (1), Bone marrow (1) and Lung Biopsy (1) were excluded from the evaluation of Xpert assay for its diagnostic performance for these samples because their sample size was very small, thus, it can create inconclusive results. The diagnostic performance of Xpert assay for the other 07 EPTB specimens were evaluated based on sensitivity, specificity, PPV and NPV. Wherein, the highest sensitivity was recorded for Pus and lowest sensitivity was calculated for Ascitic fluid, whereas the highest specificity was measured for both Urine and Ascitic fluid and lowest specificity was measured for CSF as detailed in Table 2.

## DISCUSSION

EPTB is multifaceted complex disease and constitute a substantial portion of the global TB with increasing death rate particularly in the areas with limited resources. The early diagnosis and prompt institution of the treatment is utmost important to curb its morbidity, mortality and disability. Xpert assay has a profound effect on the diagnosis of EPTB and holds promises for the early and prompt diagnosis of it with minimum biosafety requirements, infrastructure and trained personnel. Nevertheless, the sensitivity of this assay varies for the detection of TB bacilli in different EPTB samples because of the extent of the endemicity of TB [7]. Therefore, we endeavored to evaluate the efficiency of the Xpert assay for the diagnosis of different forms of EPTB. The overall sensitivity (67%), specificity (94%), PPV (52%) and NPV (96%) of the Xpert assay were measured and it was in concordance with the results of Zahid *et al.* [11] from Indus hospital, Karachi, Pakistan, for sensitivity (69.4%), specificity (94.3%), PPV (61.2%) and NPV (95.9%) for EPTB.

Table 1: Overall sensitivity, specificity, PPV and NPV of Xpert assay

| | Xpert assay | | | | | |
|---|---|---|---|---|---|---|
| Culture | Positive | Negative | Sensitivity | Specificity | PPV | NPV |
| Positive | 134 | 66 | 67% | 94% | 52% | 96% |
| Negative | 121 | 1853 | | | | |

Table 2: Sensitivity, specificity, PPV and NPV of Xpert assay for individual specimen

| | | Xpert assay | | | | | |
|---|---|---|---|---|---|---|---|
| Specimens | Culture | Positive | Negative | Sensitivity | Specificity | PPV | NPV |
| Pleural fluid (670) | Positive | 23 | 27 | 46% | 96% | 47% | 96% |
| | Negative | 26 | 594 | | | | |
| CSF (401) | Positive | 22 | 11 | 67% | 85% | 40% | 94% |
| | Negative | 21 | 347 | | | | |
| Tissue Biopsy (268) | Positive | 20 | 4 | 83% | 86% | 38% | 98% |
| | Negative | 33 | 211 | | | | |
| Ascitic fluid (246) | Positive | 5 | 9 | 36% | 99% | 71% | 96% |
| | Negative | 2 | 230 | | | | |
| Pus (233) | Positive | 49 | 5 | 91% | 87% | 67% | 97% |
| | Negative | 24 | 155 | | | | |
| Urine (147) | Positive | 4 | 1 | 80% | 99% | 67% | 99% |
| | Negative | 2 | 140 | | | | |
| Pericardial Fluid (98) | Positive | 4 | 5 | 44% | 97% | 57% | 95% |
| | Negative | 3 | 86 | | | | |

Moreover, our results also extend the findings published previously [12-14] regarding the variation in the sensitivity of this assay for different EPTB samples.

The sensitivity of the tested assay in individual sample was 46% for Pleural fluid, while Sharif *et al.* [15] reported 57.14% sensitivity for Pleural fluid from Agha Khan hospital, Pakistan, however, they have tested only 58 samples which could be the reason of much higher sensitivity. A systematic review [16] summarized 49.5% sensitivity of Xpert for Pleural fluid from 25 eligible articles in 2022, which is nearly comparable to our results. The sensitivity of Xpert assay for CSF, Ascitic fluid and Pericardial fluid was 67, 36 and 44%, respectively, a comparable sensitivity has been reported by researchers in their studies in 2024, for CSF from Pakistan (75%) [11] and China (71.1%) [17]. Whereas, a study [18] from Iran documented 40 and 100% sensitivity of Xpert assay for Pericardial fluid and Ascitic fluid respectively, wherein, sensitivity regarding Pericardial fluid is in concordance with our finding, however the sensitivity for Ascitic fluid is maximum which is also supported by a study [19] from Pakistan. The maximum yield of Xpert assay in Ascitic fluid in the above mentioned two studies may be explained by difference in sample size because the Iranian study included merely 38 samples whilst a study from Pakistan includes 58 specimens, hence, their results could be by chance. The lower sensitivity of Xpert assay in case of body fluid is also supported by other previously published studies [20].

The current study measured 91% sensitivity for Pus, which shows the similar trend of more than 90% sensitivity for Pus as already reported by other findings [20, 21], while, 83 and 80%, sensitivity of Xpert assay for Tissue biopsy and Urine respectively, also extend the previous knowledge pertinent to the sensitivity of the Xpert assay for Urine [22] and Tissue biopsy [18, 21]. The specificity of the Xpert assay for individual sample was high i.e., ranged from 85% in CSF to 99% in Urine which is almost in agreement with other studies, furthermore, specificity is not markedly varies among the different specimens [23, 24].

Contrary to the sputum, the composition of different EPTB specimens is distinct from each other, therefore, the detection limit of Xpert assay in various EPTB specimens is also varied, which subsequently affect the efficiency of Xpert assay in the diagnosis of different EPTB sample [25].

The current study indicated 66 specimens which were declared positive by culture, but this assay failed to detect MTBc in these specimens, hence they were considered false negative and it is supported by the difference in the limit of detection of both techniques, because culture can detect almost 10 times lower number of TB bacilli in specimen than Xpert assay [26]. Therefore, clinicians should take caution while interpreting the negative result of the Xpert assay because it alone does not rule out EPTB.

Interestingly, 121 EPTB specimens which were reported negative by culture, nevertheless, MTBc was detected in these specimens by Xpert assay. According to our reference standard, the results were false positive, however, there are other studies with similar findings and they considered these results as true positive using composite reference standard instead of culture as a gold standard, which further enhance the specificity of Xpert assay [27,28]. Hence, this high specificity put sufficient confidence in positive result of Xpert assay to rule in EPTB.

In addition to this, when we compare our number of false positive cases in pooled and individual specimens with other studies, the number is higher in our work, which could be due to the lack of standardization in collection of specimens because we received specimens collected at other places. Moreover, the specimens were transported to the laboratory by the attendant of the patient, hence the condition during transportation and time between collection and processing of sample was also not standardized which can affect the viability of tubercle bacilli and its subsequent growth in culture.

Limitations of our study are the unavailability of any other standard and/or technique to evaluate the false positive results of Xpert assay. The recruitment of unequal number of different specimens limited the measurement of the diagnostic performance of the tested assay for all individual specimens. Conclusively, Xpert assay showed higher specificity for EPTB detection, however the sensitivity is relatively lower, therefore, Xpert assay can early detect EPTB cases for prompt treatment but there is low certainty of negative result to rule out EPTB.

## Conflict of Interests

Authors declare no conflict of interests.

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