Diagnostic Efficacy of New Xpert Ultra for Extrapulmonary Tuberculosis Using Culture and Composite Reference Standard

Abstract

Introduction: Xpert Ultra (Cepheid, USA) is recently introduced with an extra category of trace-positive results and higher sensitivity for tuberculosis (TB) diagnosis. **Objective:** The objective of the study was to assess the diagnostic accuracy of Xpert Ultra for extrapulmonary samples using culture and composite reference standard (CRS) as the gold standard. **Materials and Methods:** In a 1-year (March 2021–22) prospective observational study, samples of suspected extrapulmonary TB (EPTB) patients were subjected to Ziehl–Neelsen staining, culture, and Xpert Ultra (Cepheid, Sunnyvale, CA) tests. Relevant clinical and treatment information was noted. The diagnostic accuracy of Xpert Ultra compared with culture and CRS was calculated. **Results:** Out of 1720 suspected patients of EPTB, 223 (13%), predominantly males 135 (60%), with a mean age of 41.46 ± 19.81 years, were diagnosed as TB positive following CRS criteria. The maximum cases were of pleural TB (35.4%), followed by central nervous system TB (17.9%), gastrointestinal TB (17.5%), and lymph node TB (12.1%). Of all samples, 150 (8.7%) were microbiologically confirmed, including 141 detected by Xpert ultra, 67 culture positive, and only 16 smear positive. Among the Xpert Ultra-positive samples, 35 showed trace results, including six false-positive results. Considering culture and CRS as the gold standard, the sensitivity (86.57%, 59.64%) and specificity (94.98%, 99.47%) of Xpert Ultra were calculated, respectively. Rifampicin resistance was detected in 1 (0.70%) sample. **Conclusion:** Diagnosis of EPTB is a challenge and Xpert Ultra may detect TB at a very early stage. However, it is essential to rule out false-positive results. Additional studies are needed on Xpert Ultra to interpret trace results better.

Keywords: *Composite reference standard, culture, extrapulmonary tuberculosis, sensitivity, Xpert Ultra*

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Introduction

Extrapulmonary tuberculosis (EPTB) includes various clinical conditions caused by *Mycobacterium tuberculosis* (MTB) infecting organs other than the lungs and is a significant cause of morbidity and mortality worldwide. The sites commonly involved in EPTB are lymph nodes, meninges, genitourinary tract, pleura, peritoneum, pericardium, spine, bones, joints, skin, etc. Tubercular meningitis and pericarditis can be fatal, whereas others, such as tuberculosis (TB) spine and pleural TB, lead to disability. The burden of EPTB accounts for 15%–20% of all TB cases, almost double in HIV-positive patients.[1] Early diagnosis and timely treatment are the most critical steps to control the disease, but it is still a challenge due to its paucibacillary nature, vague clinical presentation, and varied site involvement; hence, sometimes, high clinical suspicion is the only guide to making a diagnosis. Furthermore, the emergence of multidrug-resistant MTB poses a more significant challenge for clinicians to diagnose and treat this disease. Other than radiology, raised adenosine deaminase (ADA), lymphocytosis, raised erythrocyte sedimentation rate (ESR), histopathology, microbiological methods, including acid-fast staining, culture, and various molecular tests confirm the diagnosis. Although culture is the gold standard, it has a prolonged turnaround (4–6 weeks). The smear microscopy has low sensitivity and cannot differentiate MTB from nontuberculous mycobacteria (NTM). For timely diagnosis of TB, nucleic acid amplification tests (NAATs) are increasingly being used worldwide, including Xpert Ultra, TRUNAT and line probe assay (LPA), which have revolutionized the diagnosis and treatment of EPTB. These polymerase

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chain reaction (PCR)-based tests provide results within a few hours with high sensitivity and specificity and simultaneously detect drug resistance.[2]

The WHO recommended for the diagnosis of EPTB in 2020 that Xpert Ultra should replace smear microscopy/culture for primary diagnosis of EPTB except for blood and stool samples. Although culture is needed for follow-up of patients on the antituberculosis treatment (ATT). The Xpert MTB/rifampicin (RIF) system, developed by Cepheid in Sunnyvale, CA, USA, is a real-time PCR-based, rapid, automated cartridge-based NAAT that was endorsed by the WHO in 2010 for pulmonary TB and in 2015 for the initial diagnosis of EPTB. It has 45%–67% sensitivity for microbiologically proven EPTB samples, requires minimum biosafety measures, and detects RIF resistance simultaneously. Then, Xpert Ultra was introduced in 2017, which includes IS1081 and IS6110 insertion sequences and a larger amplification chamber that leads to better sensitivity and has 28 min shorter turnaround time (Xpert: 112 min, Xpert Ultra: 84 min). It detects additional trace categories. The studies have reported 70% to 90% sensitivity for Xpert Ultra in extrapulmonary samples.[3,4] LPA is a PCR-based molecular test that detects MTB and resistance genes by revere hybridization technology. MTB-positive samples are tested with the first-line LPA that detects isoniazid and RIF resistance. Second-line LPA test is recommended for susceptibility testing of second-line anti-tubercular drugs for the judicious use of ATT and better cure.[5] Most of the studies have been done on pulmonary samples, and the majority of studies on extrapulmonary samples have described the use of GeneXpert and TrueNat. There is a paucity of data on the performance of recently available Xpert Ultra in extrapulmonary samples. Hence, the present study aimed to evaluate the performance of the Xpert Ultra assay compared with the culture and composite reference standard (CRS) method in diagnosing EPTB.

Materials and Methods

The present study was conducted for 1 year in the department of microbiology, from March 2021 to March 2022. The ethical committee of the institute approved the study. All the clinically suspected patients of EPTB admitted to the hospital, whose requisition for Xpert Ultra was received, were included in the study. All the data enlisted in the pro forma, including patient details, clinical findings, history of TB, and laboratory tests such as ESR, hemoglobin, total leukocytes count, differential leukocyte count, ADA, radiological, histopathology findings, and ATT, were noted. All the extrapulmonary specimens such as body fluids (pleural fluid, pericardial fluid, and ascetic fluid), pus, tissue, lymph node aspirates, and cerebrospinal fluid (CSF), except blood and stool samples, were processed using standard methods. Decontamination of all samples (except body fluid samples) was done. The samples were subjected to microscopy, culture (Lowenstein–Jensen [LJ]/Mycobacterium growth indicator tube [MGIT]), and Xpert Ultra assay.[6]

Microscopy

A smear was made after concentration procedures from each sample. Ziehl–Neelsen (ZN) staining was used to see the presence of acid-fast bacilli.

Culture

Rapid culture (MGIT) was performed as per the requisition form. The rest of the samples was inoculated on conventional LJ medium.

Lowenstein–Jensen medium

LJ medium was inoculated with 0.1–0.25 mL of concentrated sample, incubated at 37°C and examined for 8 weeks for the detection of any growth. All culture bottles were examined daily for the first 7 days to rule out NTM *spp.* (rapid grower) and to check for bacterial contamination. After that, cultures were examined twice weekly till 8 weeks. As soon as any growth is evident on the culture media, preliminary identification of mycobacteria was made based on the growth rate, colony characteristics, and ZN staining.

Rapid culture: Mycobacterium growth indicator tube

MGIT Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, Azlocillin (PANTA) was reconstituted with 15.0 mL MGIT growth supplement and mixed until completely dissolved. 0.8 mL of MGIT growth supplement/PANTA was added aseptically to each MGIT tube, and 0.5 mL of a well-mixed-concentrated specimen was added to the MGIT tube. Immediately, the tube was recapped tightly and mixed by inverting it several times. The inoculated tubes were left at room temperature for 30 min. The inoculated MGIT media were incubated at 37°C ± 1°C for 6 weeks. Readings were recorded daily using the BACTEC Micro MGIT reader (Becton, Dickinson and Company, USA). Once the MGIT tube was found positive by fluorescence or visual observation, a smear was prepared and stained by the ZN method for acid-fast bacillus (AFB).

GeneXpert *Mycobacterium tuberculosis/*rifampicin ultra (Cepheid, Sunnyvale, CA) assay

Sample processing

The fluids (10–15 mL) were centrifuged, the pellet was suspended in 0.5 mL normal saline, and tissues (minimum ½inch) were washed if blood tinged and homogenized. Buffer (2 mL) was added to the processed samples and incubated at room temperature for 15 min with two vortex steps. Pipette 2 mL of it and load slowly into the S-chamber of the cartridge carefully without introducing any bubbles. The cartridge was loaded into the machine after adding the sample as per the manufacturer's guidelines.

Quality check

The system has inbuilt controls (sample processing control) and PCR reaction probe check control for quality check.

Yearly calibration was done by the company person. One positive or negative sample is sent monthly to the referral laboratory for quality check. Known positive and negative samples are tested on lot change of the kit.

Interpretation

The results were interpreted as MTB detected (high/medium/low/very low/trace) or MTB not detected. RIF resistance detected (mutation detected in the *rpoB* gene)/not detected (no mutation in the *rpoB* gene)/indeterminate (could not determine RIF-R due to insufficient signal detection). The tests showed invalid results/errors were repeated.

Composite reference standard

The CRS criteria included patients positive for culture or Xpert Ultra positive (high/medium) or Xpert Ultra positive (low/very low/trace) with clinical features, cytology/radiology/other laboratory tests, suggestive of TB and response to ATT and Xpert Ultra negative with clinical features/cytology/radiology/other laboratory tests, suggestive of TB and response to ATT.[7]

Statistical analysis

The data were entered in Microsoft Excel and analyzed as frequencies, relative frequencies, range, and mean ± standard deviation. Statistical analysis was performed using the SPSS 20.0 version (SPSS Inc., Chicago, IL, USA) of Microsoft Windows, and the sensitivity, specificity, and predictive values were calculated as appropriate.

Results

Of 1720 suspected patients of EPTB, the majority were males, 1064 (61.9%) and belonged to more than 60 years (33%) of age. Of these, 223 (13%) patients were considered TB-positive following CRS criteria, including males predominantly 135 (60.5%) and had a mean age of 41.46 ± 19.81 years. The maximum cases were of pleural TB 79 (35.4%), followed by central nervous system TB 40 (17.9%), gastrointestinal TB 39 (17.5%), lymph node TB 27 (12.1%), musculoskeletal TB 23 (10.3%), genitourinary TB 12(5.4%), cutaneous TB 2 (0.9%), and pericardial 1 (0.45%) TB. The Monteux test was performed on 203 patients, and 20.2% showed positive reactions. Out of them, eight were detected positive for active TB.

Out of the total, microbiological methods confirmed 150 (8.7%) samples as TB positive. The distribution of samples and test results of various microbiology methods is shown in Table 1. Out of these, 141 were detected by Xpert Ultra, 67 showed growth in culture, and only 16 smears showed the presence of AFB. Therefore, 82 (54.6%) samples were detected positive for MTB only by Xpert Ultra, which were missed in culture and smear. In nine samples, MTB was caught only by the culture that was found negative in Xpert Ultra and smear [Figure 1].
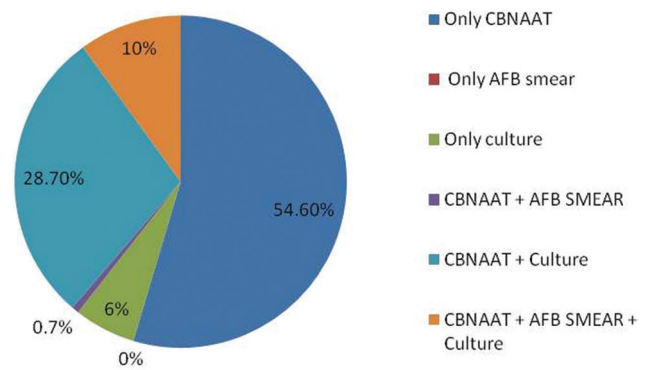

Figure 1: Distribution of positive tests (*n* = 150). CBNAAT: Cartridge-based nucleic acid amplification tests; AFB: Acid-fast bacilli

Among Xpert Ultra-positive samples, high 5 (3.5%), medium 17 (12.1%), low 59 (41.9%), very low 25 (17.7%), and trace 35 (24.8%) results were observed. Out of the total Xpert Ultra-positive samples, RIF resistance was seen in 1 (0.70%) case, and 35 (24.8%) samples showed indeterminate results due to very low copy numbers in the trace-detected samples.

Among the 223 TB-positive patients, 90 (40.4%) patients were detected negative by Xpert Ultra. Eight patients detected MTB positive by Xpert Ultra were not treated for TB and were considered false positive. Among them, six were detected as trace and two were detected as low by Xpert Ultra.

Considering culture and CRS as the gold standard, the sample-wise sensitivity (86.57%, 59.64%), specificity (94.98%, 99.47%), positive predictive value (41.13%, 94.33%), negative predictive value (99.43%, 94.30%), and accuracy (94.65%, 94.30%) of Xpert Ultra were calculated, respectively [Table 2]. AFB smear showed a sensitivity of 22.39% and a specificity of 99.94% compared with culture. Using CRS as the reference standard, the sensitivity and specificity of smear, Xpert Ultra, and culture were calculated [Table 3].

Discussion

In our study, 13% (223) TB positivity, the most common being pleural TB, was observed, comparable to the study conducted by Gupta *et al*., who had also reported 13% EPTB positivity.[8] The male predominance of TB-positive patients was concordant with the national data and other studies.[1,5] The mean age of the TB-positive patients was 41.46 ± 19.81 years, which is in line with other studies,[9] whereas Alwani *et al*. reported that most EPTB patients of <20 years of age group.[10] Although the clinical symptoms were observed according to the site involved, like others, we also observed fever (97.3%) as the most common symptom, followed by loss of appetite (78.9%) and weight loss (69.1%).[11,12] In the present study, the maximum samples were pleural fluid (30%), followed by CSF (24.7%), ascitic fluid (16.5%), lymph node aspirate/

Table 1: Tuberculosis positivity of extrapulmonary samples by various methods (*n*=1720)

Sample	Xpert-Ultra, *n* (%)	AFB smear, *n* (%)	Culture (LJ/MGIT), *n* (%)	TB positive by any method, *n* (%)
Pleural fluid (*n*=516)	47 (9.1)	5 (1)	24 (4.7)	53 (10.3)
Cerebrospinal fluid (*n*=425)	16 (3.8)	1 (0.2)	5 (1.2)	17 (4)
Ascitic fluid (*n*=283)	7 (2.5)	0	1 (0.4)	7 (2.5)
Lymph node/tissue (*n*=252)	33 (13.1)	5 (2)	20 (7.9)	34 (13.5)
Pus (*n*=165)	35 (21.2)	5 (3)	17 (10.3)	36 (21.8)
Urine (*n*=52)	2 (3.8)	0	0	2 (3.8)
Pericardial fluid (*n*=27)	1 (3.7)	0	0	1 (3.7)
Total (*n*=1720)	141 (8.2)	16 (0.9)	67 (3.9)	150 (8.7)

TB: Tuberculosis; LJ: Lowenstein–Jensen; MGIT: Mycobacterial growth indicator tube; AFB: Acid-fast bacilli

Table 2: Sample-wise sensitivity, specificity, positive predictive value, and negative predictive value of Xpert Ultra compared with culture and composite reference standard

Sample	Gold standard	Sensitivity	Specificity	PPV	NPV	Accuracy
Pleural fluid	Culture	75	94.11	38.31	98.72	93.22
	CRS	62.32	99.11	91.49	94.46	94.19
Cerebrospinal fluid	Culture	80	97.14	25	99.76	96.94
	CRS	40.54	99.74	93.75	94.62	94.59
Ascitic fluid	Culture	100	97.87	14.29	100	97.88
	CRS	46.15	99.63	85.71	97.46	97.17
Lymph node/tissue	Culture	95	93.97	57.58	99.54	94.05
	CRS	70.45	99.04	93.94	94.06	94.05
Pus	Culture	94.12	87.16	45.71	99.23	87.88
	CRS	63.64	100	100	84.62	87.88
Total	Culture	86.57	94.98	41.13	99.43	94.65
	CRS	59.64	99.47	94.33	94.30	94.30

CRS: Composite reference standard; PPV: Positive predictive value; NPV: Negative predictive value

Table 3: Sensitivity, specificity, positive predictive value, and negative predictive value of various microbiological methods compared with composite reference standard

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)
Xpert Ultra	59.64	99.47	94.33	94.30	94.30
Ziehl–Neelsen Staining	07.17	100	100	87.85	87.97
Culture	30.04	100	100	90.56	90.93

PPV: Positive predictive value; NPV: Negative predictive value

tissue (14.7%), and pus (9.6%). Similarly, various other studies have reported pleural fluid as the most common sample received.[8,9,13,14] However, lymph node aspirate was obtained as the most common sample (32.5%), followed by pleural fluid (29.3%) in another study.[10]

A total of 150 (8.7%) samples were confirmed TB positive by microbiology tests. Yadhav and Veena have reported 23.8% positivity among extrapulmonary samples, with the highest positivity in lymph nodes.[9] The maximum positivity was seen in pus, 21.8%, followed by lymph node/tissue (13.5%) in our study. Avashia *et al.* also reported maximum MTB positivity in pus samples (56.7%), followed by lymph node samples (54.2%), CSF samples (33.3%), pleural fluid samples (23.3%), and ascitic fluid samples (20%).[15] Another study reported the highest positivity in biopsies or fine-needle aspirates (35%), followed by gastric aspirates (23%), pus (21%), urine (6%), and CSF (5%).[16]

In our study, Xpert Ultra was found to be positive in 141 (8.2%) samples, culture in 67 (3.9%) samples, and AFB was seen in 16 (0.9%) samples. Another study has reported an MTB positivity of 14.4% by Gene Xpert Ultra.[7] Some authors have shown higher positivity of Xpert Ultra (37%) and ZN smear (13.3%).[15] A low-culture positivity (45%) was seen in our study, which may be due to low bacterial load and a high percentage of lymph node tissues that may remain sterile in culture. Most patients were on antibiotics; also, the quality of samples, transport conditions, and over-decontamination could be possible reasons.[17,18]

Previous studies have shown varied sensitivities and specificities of Xpert Ultra for various extrapulmonary samples using different reference standards. Although culture remains the gold standard, due to its limitations, CRS has been used as the reference standard. In our study, 40% of patients treated for TB were not detected by Xpert Ultra. This could be due to samples contaminated

with blood, and other PCR inhibitors possibly present in the samples, and inadequate sampling from inaccessible sites. However, eight (5.7%) patients detected by Xpert Ultra were not treated for TB and were considered false positives, which was low in our study as compared to other studies reported 11.3% Xpert Ultra-positive results as TB negative.[7]

The sensitivity and specificity of AFB smear in diagnosing EPTB were observed as 22.39% and 99.94%, respectively, using culture as a gold standard in our study. However, Mechal *et al*. have reported a higher sensitivity of 47.3% and a lower specificity of 96.8%.[19] In our study, using culture and CRS as the gold standard, Xpert Ultra assay has shown sensitivity (86.57% and 59.64%) and specificities (94.98% and 99.47%) in EP samples, respectively. We observed the lowest sensitivity in pleural fluids using culture, and in CSF using CRS, while the best sensitivity was seen in lymph node samples which are in line with other recent studies. A review article has compiled many previous studies on the performance of Xpert Ultra using culture and CRS as the gold standard and has reported highly varied sensitivities as 50%–84% and 37%–61% in pleural fluid, 50%–100% and 27%–70% in lymph node tissue/aspirates, 71%–96% and 33%–95% in CSF samples, and 100% and 18%–34% in urine samples, respectively.[20]

Mekkaoui *et al*. have reported sensitivity (90%) and specificity (91.9%) with culture as the reference standard, including low performance in pleural fluid (66.7% and 98.5%) and good results with lymph node (95.8% and 93.3%) samples.[17] Likely, in their study, Srivastava and Srivastava reported very low sensitivity (25.2%) and specificity (61.9%) of Xpert Ultra in pleural fluid samples. They reported the highest sensitivity with pus samples (92% and 100%) and poor performance with body fluids (48.1% and 98.9%) and CSF (38.5% and 99.4%).[14] Kaswala *et al*. have shown sensitivity and specificity using CRS as a reference method, including trace at 87.8% and 98.1% and excluding trace at 72.1% and 100%.[7]

A meta-analysis study has shown around a 6% increase in sensitivity over Xpert MTB/RIF, and this increase is mainly due to trace results of Xpert Ultra. Although interpreting trace results are challenging for clinicians, a repeat test is hardly logistically possible. Previous studies have demonstrated 3%–30% of trace results.[21] We observed 24.8% of trace results, and 82.9% of these were treated as TB positive.

In our study, RIF resistance was detected in only 1 (0.7%) pleural fluid sample. At the same time, 24.8% of the tests showed indeterminate RIF resistance results due to trace detection of MTB. Adhikary *et al*. have reported higher resistance (10.75%). The difference could be due to variations in sample size, varied patient selection criteria, and geographic area variation.[22]

The strength of our study is a good sample size, and it will be helpful for the clinicians to better interpret the Xpert Ultra results and value addition to the literature available on EPTB diagnosis. However, a few limitations were low culture positivity, and the culture method was used as per the patient's request. Most of the patients followed up telephonically to see the treatment response.

Conclusion

Xpert Ultra is a recent advanced molecular diagnostic tool helpful in reducing morbidity and mortality by confirming difficult-to-diagnose fatal EPTB cases within a few hours, leading to timely management. It detects smear-negative, paucibacillary samples with very low copy numbers as trace results; however, it is essential to rule out false-positive results. The sensitivity and specificity of the test varies with different samples and gold standards. More literature on Xpert Ultra trace results, clinical association, and treatment response are needed to guide clinicians.

Ethical statement

The study has been approved by the ethics committee of the Dayanand Medical College and Hospital (reference number - DMCH/P/2021/141-7).

Conflicts of interest

There are no conflicts of interest.

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