

## Research Article

# Utility of Xpert MTB/RIF Assay for Diagnosis of Pediatric Tuberculosis Under Programmatic Conditions in India

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## ABSTRACT

Tuberculosis (TB) diagnosis in children still remains a challenge in developing countries. We analyze the performance of Xpert MTB/RIF assay for the diagnosis of pediatric TB under programmatic conditions. We retrospectively analyzed the performance of Xpert MTB/RIF assay from February 2016 to March 2018. A total 2678 samples from TB suspects below 14 years were received in the laboratory and were frontline tested by Xpert MTB/RIF assay according to the manufacturer's instructions. If sample was sufficient, the smear microscopy and culture were performed as per standard World Health Organization's guidelines. The smears and cultures were performed in 2178 and 588 samples, respectively. Among 2678 samples, 68 were rejected, Xpert MTB/RIF assay was positive in 357/2610 (13.6%) cases, while the smear was positive in 81/2178 (3.3%) cases. The sensitivity of smear and Xpert MTB/RIF when compared with culture was 24.6% (14.1–37.8%) and 81% (68.6–90.1%), respectively. The diagnostic accuracy of Xpert MTB/RIF and smear was 97.1% and 92.2%, respectively. Thirty samples (8.5%) were detected as rifampicin resistance by Xpert MTB/RIF assay. The Xpert MTB/RIF increased the detection rate up to fourfold when compared with smear microscopy. Xpert MTB/RIF assay is the most rapid, sensitive, and specific method for microbiological confirmation and rifampicin resistance detection in pediatric tuberculosis.

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## 1. INTRODUCTION

Childhood tuberculosis (TB) constitutes a major but underappreciated burden of disease in endemic countries as the national TB programs mainly focus on adult TB [1,2]. According to the global TB report, 1.04 million children were diagnosed with TB and 0.2 million deaths were estimated in 2016 [3]. In India, pediatric TB accounts for 6% of the total TB burden [4]. For better treatment outcome, timely treatment initiation is required with the help of rapid diagnostics. However, especially in children, the diagnosis of pediatric TB has become more complex because of limited World Health Organization (WHO) endorsed tests. Each test has its own limitations, for example, smear microscopy has a low sensitivity due to paucibacillary nature of TB in children and lacks reproducibility [5]. It is unable to differentiate the disease caused by other mycobacterial species. The gold standard for TB diagnosis is *Mycobacterium tuberculosis* culture, which is laborious and time consuming [6]. The sensitivity of culture for the diagnosis of pediatric TB, as comparison to clinical standard, ranges from 25 to 75% depending upon the specimen's type, quality, and also the severity of disease [6].

Nucleic acid amplification test (NAAT) for TB diagnosis, especially in adults, has a very high sensitivity and specificity [7–9]. However, for the diagnosis of pediatric TB, the sensitivity and specificity of different NAATs are lower than in adults, taking culture as a gold standard. For diagnosis of adult TB and rifampicin resistance, Xpert MTB/RIF assay (a hemi-nested real-time polymerase chain reaction) showed very high sensitivity and specificity in smear-positive TB. The assay provides results in 90 min and also offers a promising solution in addressing the challenges in the diagnosis of pediatric pulmonary tuberculosis (PTB). Several studies have been conducted for determination of accuracy of Xpert MTB/RIF assay in pediatric TB. Among smear-positive and smear-negative samples, the sensitivity of Xpert MTB/RIF assay was 95–96% and 55–62%, respectively, using culture as a reference standard [10]. After a meta-analysis, WHO recommended that the Xpert MTB/RIF assay can be used as frontline test rather than conventional microscopy and culture in all children suspected of TB. In India, under Revised National Tuberculosis Control Program (RNTCP), the Xpert MTB RIF assay was also recommended to be used as a first choice for the diagnosis of pediatric TB at different levels. This study was thus conducted to retrospectively analyze the utility of Xpert MTB/RIF assay under routine programmatic conditions in a tertiary care center.

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## 2. MATERIALS AND METHODS

### 2.1. Study Design

A total of 2678 samples were received from pediatric population under RNTCP from February 2016 to March 2018. All the samples, respiratory and nonrespiratory, were received from presumptive TB patients as defined by RNTCP, India. These samples were received in the RNTCP center, Mycobacteriology laboratory of the Department of Medical Microbiology, Post Graduate Institute of Medical Education & Research (PGIMER), Chandigarh, for routine diagnosis of pediatric TB. The Mycobacteriology laboratory is certified by the International Organization for Standardization 15189:2007 and also a DST-approved center by India's RNTCP. The data were analyzed retrospectively and patient information including age, sex, type of sample, HIV status, previous history, and contact history were collected from the RNTCP request form received in the laboratory. These samples were first processed for Xpert MTB/RIF assay, and if the sample was sufficient, then the other methods like smear microscopy and culture were performed. As this is a retrospective analysis of laboratory data, approval was taken from Intramural Institutional Ethics Committee, PGIMER, Chandigarh.

### 2.2. Xpert MTB/RIF Assay

The Xpert MTB/RIF assay was used as frontline test for diagnosis of TB. About 1 ml sample was mixed with double the amount of reagent buffer and incubated and transferred into the cartridge. The cartridge was then placed into the instrument. The Xpert MTB/RIF were interpreted as *M. tuberculosis* complex detected or not detected and rifampicin resistance detected and not detected [11].

### 2.3. Routine Microbiological Assays

The direct smear was prepared and reported as per the RNTCP guideline, India. The remaining sample was processed/decontaminated by NALC–NaOH method [12]. A total of 500 µl of the processed sample was transferred in MGIT tube (Becton Dickinson, USA). The MGIT tube was incubated at 37°C for 42 days in the MGIT 960 instrument as per the manufacturer's instructions. The positive tubes given by MGIT 960 instrument were confirmed by using SD BIOLINE TB Ag MPT64 Rapid (Standard Diagnostics, Inc., Republic of Korea) for *M. tuberculosis* complex.

### 2.4. Statistical Analysis

All the statistical parameters were calculated using *M. tuberculosis* culture as a reference standard. The parameters like sensitivity and specificity were calculated using online calculator (<https://www.medcalc.org/calc/diagnostictest.php>). The positive predictive value (PPV), negative predictive value (NPV), and concordance were also calculated.

## 3. RESULTS

### 3.1. Study Population

A total of 2678 samples were received from presumptive pediatric TB patients from February 2016 to March 2018. Sixty-eight samples

were rejected due to unavailability of complete information ( $n = 66$ ) and invalid test results ( $n = 2$ ) by Xpert MTB/RIF assay. Therefore, 2610 samples were analyzed for this study, of which 1551 (59.4%) were male and 1059 (40.6%) were females, with age ranging from 1 month to 14 years with median age of 7 years. Among the 2610 samples, 1626 (62.1%) were respiratory samples including induced sputum and sputum (584, 22.4%), gastric aspirate/gastric lavage (740, 28.4%), bronchoalveolar lavage (252, 9.6%), endotracheal (ET) aspirate (50, 1.9%). The nonrespiratory samples were 984 (37.9%) including ascitic fluid (33, 1.3%), bone marrow aspirates (24, 0.9%), tissue biopsies (24, 0.9%), cerebrospinal fluid (CSF) (384, 14.7%), endobronchial ultrasound-guided transbronchial needle aspirate (EBUS-TBNA) (3, 0.1%), fine needle aspiration cytology (FNAC) (142, 5.6%), lymph node aspirate (50, 1.9%), pleural fluid (183, 7%), pus (120, 4.6%), synovial fluid (8, 0.3%), and other extra-pulmonary tuberculosis (EPTB) samples (13, 0.5%) (Table 1). Among 2610 samples, the smear and culture were performed for 2178 and 588 samples, respectively. The smear was positive in 81/2178 (3.3%) cases and the culture for *M. tuberculosis* complex was positive for 58/588 (9.9%) cases (Figure 1). The smear and culture positive were found in 14/567 (9.9%) cases, smear negative and culture positive were 43/567 (7.6%), and there was 1 (0.2%) smear positive and culture negative case.

A total of 357 (13.6%) samples were diagnosed as TB by Xpert MTB/RIF assay, including 250 (70.02%) respiratory samples and 107 (29.9%) nonrespiratory samples. Among positive respiratory samples, 24/252 (9.5%) were bronchoalveolar lavage (BAL), 10/45 (22.2%) ET aspirates, 56/740 (7.6%) gastric aspirate (GA)/gastric lavage (GL), and 160/584 (27.4%) were induced sputum or sputum. Among positive nonrespiratory samples, there were 3/33 (9.1%) ascitic fluid, 2/24 (8.3%) aspirates, 29/384 (7.6%) CSF, 2/3 (66.7%) EBUS-TBNA, 28/142 (19.7%) FNAC, 9/50 (18%) lymph node aspirate, 7/183 (3.8%) pleural fluid, 24/120 (20%) pus, 1/8 (12.5%) synovial fluid, and 2/13 (15.4%) other EPTB samples. Thirty (8.5%) samples were found rifampicin resistant, of which there were 20 (2 BAL, 2 ET aspirate, 5 GL, and 11 sputum) respiratory samples and 10 (3 CSF, 2 FNAC, 1 LN aspirate, 1 pleural fluid, and 3 pus) were nonrespiratory samples.

### 3.2. Sensitivity and Specificity of Xpert MTB/RIF

Among the 588 TB suspects, whose request was also received for culture and processed for liquid culture, smear was positive in 15 cases

**Table 1** | Xpert MTB/RIF positivity among the respiratory and nonrespiratory samples

Type of sample	Total no	Xpert MTB/RIF positive (%)
Bronchoalveolar lavage	252	24 (9.5)
ET aspirate	50	10 (20)
Gastric aspirate/gastric lavage	740	56 (7.6)
Induced sputum/sputum	584	160 (27.4)
Ascitic fluid	33	3 (9.1)
Aspirate	24	2 (8.3)
Biopsy	24	0
CSF	384	29 (7.5)
EBUS–TBNA	3	2 (66.7)
Fine-needle aspiration	142	28 (19.7)
Lymph node aspirate	50	9 (18)
Pleural fluid	183	7 (3.8)
Pus	120	24 (20)
Synovial fluid	8	1 (12.5)
Other EPTB	13	2 (15.4)

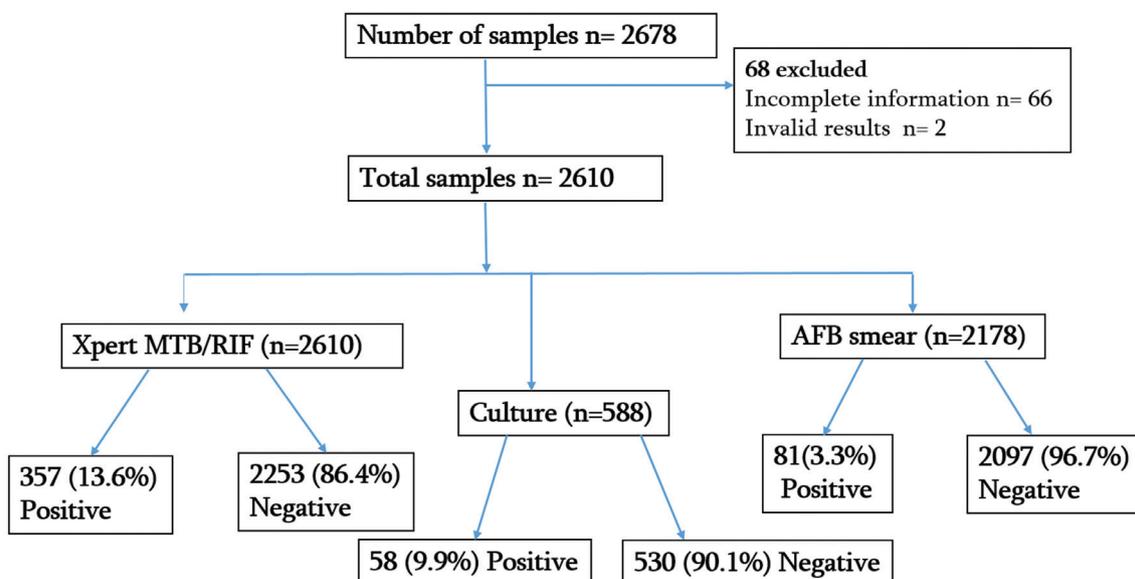


Figure 1 | Participant flowchart in the study. AFP, acid-fast Bacilli.

Table 2 | Performance of diagnostic tests for detection of *M. tuberculosis*

Type of diagnostic test	Sensitivity in % (95% CI)	Specificity in % (95% CI)	PPV (95% CI)	NPV (95% CI)
Smear	24.6 (14.1–37.7)	99.8 (98.9–100)	93.3 (65.2–99.1)	92.2 (91–93.2)
Xpert MTB/RIF—overall	81 (68.6–90.1)	95.5 (93.3–97.1)	66.2 (56.5–74.7)	97.8 (96.4–98.7)
Xpert MTB/RIF—respiratory samples	81.2 (67.4–91.5)	96.6 (94.3–98.1)	73.6 (62.1–82.6)	97.7 (96–98.7)
Xpert MTB/RIF—nonrespiratory samples	80 (44.4–97.5)	94.6 (90.2–97.4)	44.4 (28.9–61.2)	98.8 (96.2–99.7)

and Xpert MTB/RIF was positive in 71 samples. Among 71 Xpert MTB/RIF-positive samples, 47 and 24 were culture positive and culture negative, respectively. The sensitivity and specificity of smear were 24.6% (14.1–37.7%) and 99.8% (98.9–100%), respectively. The overall sensitivity of Xpert MTB/RIF was 81.03% (68.6–90.1%) and specificity was 95.5% (93.3–97.1%) (Table 2). The PPV and NPV of Xpert MTB/RIF was 66.2% (56.5–74.7) and 97.8% (96.4–98.8%), respectively. The diagnostic accuracy of smear and Xpert MTB/RIF assay was 92.2% (89.7–94.3%) and 94% (91.8–95.8%), respectively. In respiratory samples, the Xpert MTB/RIF was positive in 53 cases including 49 in culture-positive samples. The overall sensitivity of Xpert MTB/RIF in respiratory samples was 81.2% (67.3–91.5%), while the specificity of Xpert MTB/RIF was 96.6% (94.3–98.1%). The PPV and NPV of Xpert MTB/RIF were 73.6% (62.1–82.6%) and 97.8% (96–98.7%), respectively. In nonrespiratory samples, the Xpert MTB/RIF was positive in 12 cases including 9 in culture-positive samples. The overall sensitivity of Xpert MTB/RIF in nonrespiratory samples was 80% (44.4–97.5%), while the specificity of Xpert MTB/RIF was 94.6% (90.2–97.4%). The PPV and NPV of Xpert MTB/RIF were 44.4% (28.9–61.2%) and 98.8% (96.2–99.7%), respectively. A  $\kappa$ -value and proportion of agreement between two diagnostic tests, that is, smear microscopy and Xpert MTB/RIF, were detected as 0.35 and 0.89, respectively (Table 3).

## 4. DISCUSSION

The accurate diagnosis of pediatric TB is still a difficult task. In this study, we retrospectively analyzed the data of routinely used Xpert MTB/RIF from February 2016 to March 2018 in a tertiary care

Table 3 | Concordance between smear and Xpert MTB/RIF assay

	Smear positive	Smear negative	<i>k</i> -value	Proportion of agreement
Xpert MTB/RIF—positive	77	233	0.3555	0.89
Xpert MTB/RIF—negative	5	1923		(0.88–0.90)

hospital of North India under programmatic conditions. Overall, the bacteriological confirmation by Xpert MTB/RIF was demonstrated in 13.6% cases, which was much higher than the smear microscopy that had 3.3%. The previous Indian studies have also shown two- to threefold increase in positivity rate by using Xpert MTB/RIF as a frontline test in pediatric TB suspects [13]. Other studies from South Africa and Uganda have also shown the proportion of Xpert MTB/RIF-positive results ranging from 13 to 14% in pediatric TB [14,15]. Overall, the smears were positive in 3.3% cases and the culture was positive in 9.9% cases. There were low positivity of smear and culture in our study because both types of samples, respiratory and nonrespiratory, were included. In a meta-analysis by Detjen et al. [16], the sensitivity of smear in respiratory samples ranges from 0 to 60%. In our study also, the Xpert MTB/RIF had fourfold higher positivity than the microscopy. In the nonrespiratory samples, the microbiological confirmation was 10.9%, which was substantially higher than that of Gupta et al. [17] who reported 4% positivity.

The sensitivity of Xpert MTB/RIF when culture was taken as a reference standard was 79.5%. One study from Uganda has also shown similar sensitivity of 81.3% in pediatric TB while another from Germany has shown a pooled sensitivity of 54.7% [15]. The specificity was 95.5% and comparable to the meta-analysis done by

Detjen et al. [16], in which the specificity ranged from 86 to 100% in respiratory samples. In respiratory samples, Xpert MTB/RIF's sensitivity was 81.2% in our study while other studies have shown a range of 25–100%. In nonrespiratory samples, Xpert MTB/RIF's sensitivity was 80%, which was comparable to the respiratory samples.

Only a very few studies report resistance in pediatric TB. In our study, Xpert MTB/RIF assay detected 8.5% (30) cases of rifampicin resistance in respiratory and nonrespiratory samples. In India, Raizada et al. [13] also have shown 17.4% rifampicin resistance cases among the bacteriological confirmed cases. There is one limitation of this study that culture was not performed on all samples due to less amount of the samples.

## 5. CONCLUSION

The Xpert MTB/RIF assay was found to have a pooled sensitivity of 81.2% and a specificity of 95.5% for rifampicin resistance detection, which was found in line with WHO's recommendation for the use of Xpert MTB/RIF assay as a frontline test for the diagnosis of pediatric TB.

## CONFLICTS OF INTEREST

The authors declare they have no conflicts of interest.

## AUTHORS' CONTRIBUTION

RY and SS designed the study; RY wrote the manuscript; JM, PV, MS, SV, PK, and RK helped in sample collection; RY and SS did the statistics; JM, PV, MS, SS, and PA modified the manuscript; and SS supervised the project.

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