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ORIGINAL ARTICLE

Performance of Xpert MTB/RIF for the Diagnosis of Extrapulmonary Tuberculosis

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SUMMARY

Background: **Mycobacterial burden is low in extrapulmonary specimens, making diagnosis and treatment difficult. Xpert MTB/RIF is a real-time PCR assay for the detection of *Mycobacterium tuberculosis* and rifampin resistance. This study evaluated the performance of the Xpert MTB/RIF assay in extrapulmonary specimens.**
Methods: **Acid-Fast Bacilli (AFB) smear, culture, and Xpert MTB/RIF were performed on extrapulmonary specimens. Mycobacterial culture was performed on BACTEC MGIT liquid for 6 weeks and 2% Ogawa medium for 8 weeks. Overall sensitivity and specificity of Xpert MTB/RIF was estimated using culture as a gold standard. Xpert MTB/RIF sensitivity and cycle-threshold (Ct) values according to AFB smear grade were evaluated. The sensitivity, specificity, and concordance of rifampin resistance compared to the phenotypic drug sensitivity test were evaluated.**
Results: **A total of 1,289 specimens were included in the study. The overall sensitivity and specificity of the Xpert MTB/RIF assay were 59.4% (41/69, 95% CI 46.9 - 70.9%) and 99.3% (1,212/1,220, 95% CI 98.7 - 99.7), respectively. Positive predictive value of Xpert MTB/RIF was 83.7% (41/49, 95% CI 69.8 - 92.2) and negative predictive value was 97.7% (1,212/1,240, 95% CI 96.7 - 98.5%). Xpert MTB/RIF assay sensitivity significantly increased with increases in AFB smear grade ($p < 0.001$). AFB smear grades and Xpert MTB/RIF Ct values were negatively correlated. Rifampin resistance results of Xpert MTB/RIF and culture showed a concordance rate of 97.2%.**
Conclusions: **The Xpert MTB/RIF assay could be used to replace the AFB smear for the diagnosis of extrapulmonary tuberculosis, and has high specificity for the detection of rifampin resistance.**
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KEY WORDS

AFB smear, extrapulmonary tuberculosis, *Mycobacterium tuberculosis*, resistance, Xpert MTB/RIF

INTRODUCTION

Mycobacterium tuberculosis infection can occur in any organ. Globally, 6.3 million newly diagnosed tuberculosis (TB) cases were reported in 2016, and extrapulmonary TB comprised 15% of these cases [1]. The rate of extrapulmonary TB varies greatly from region to region, ranging from 8% in Western Pacific countries to 24% in Eastern Mediterranean countries. South Korea is an intermediate-TB burden country with annual incidence of

65.9 cases per 100,000 population in 2018 [2].

Diagnosing extrapulmonary TB is challenging since the bacterial burden is relatively low in specimens from tissues or fluids other than the lung. The infection may be deeply seated, making it difficult to access. In addition, extrapulmonary TB has complicated clinical features that require a high index of suspicion. Due to difficulties in diagnosis, treatment is often delayed, affecting morbidity and mortality.

The WHO has endorsed the Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) for the diagnosis of extrapulmonary TB in cerebrospinal fluid (CSF), lymph nodes, and tissue specimens. Xpert MTB/RIF is a cartridge-based nucleic amplification assay, which uses direct or processed specimens to detect *M. tuberculosis* complex and rifampin resistance. Xpert MTB/RIF includes five probes covering the rifampin resistance-determining region (RRDR) of the *rpoB* gene. The cycle-threshold (Ct) of the first positive probe determines the presence of *M. tuberculosis* and the delta Ct of each probe determines rifampin resistance. Xpert MTB/RIF offers results within 2 hours, which makes early diagnosis and timely treatment possible.

The purpose of this study was to compare the performance of the Xpert MTB/RIF assay for the detection of *M. tuberculosis* and rifampin resistance in extrapulmonary specimens with that of culture, acid-fast bacilli (AFB) smear, and phenotypic drug sensitivity tests (pDST).

MATERIALS AND METHODS

Study design

The medical records were reviewed retrospectively for patients who were tested with Xpert MTB/RIF at Asan Medical Center, a tertiary care center in Seoul, Korea from April 2015 and March 2018. Multiple specimens per patient were included in the study. The study was approved by the Institutional Review Board of Asan Medical Center.

Laboratory diagnostic process

All specimens were simultaneously subjected to AFB smear microscopy, culture, and the Xpert MTB/RIF assay. Biopsy specimens including lymph nodes were disrupted and homogenized using a bead beater (FastPrep-24, MP Biomedicals, LLC, Irvine, CA, USA). AFB smear was performed using auramine-rhodamine staining, followed by Ziehl-Neelsen staining. Smear grade was assigned as per CLSI M48-A guidelines [3]. After treatment with N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) and centrifugation, the resuspended pellet was used for culture. Mycobacterial culture was performed on BACTEC MGIT liquid medium (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) for 6 weeks and 2% Ogawa medium (Korean Institute of Tuberculosis, South Korea) for 8 weeks. Culture positive specimens were tested with Seeplex TB detection (Seegene, Seoul, Korea) or Advansure TB/NTM real-time PCR (LG Chemistry, Seoul, Korea) to differentiate *M. tuberculosis* complex from nontuberculous mycobacteria. If *M. tuberculosis* complex was confirmed on culture, antimicrobial susceptibility test was performed by both pDST and genotypic drug sensitivity test. pDST was performed at the Korean National Tuberculosis Association. The absolute concentration method on Löwenstein-Jensen (L-J) agar containing 40 μg/mL rifampin and 20 μg/mL isoniazid was used as previously described [4]. The genotypic drug sensitivity test was performed with GenoType MTBDR*plus* line probe assay (Hain Lifescience, Nehren, Germany). *rpoB* gene sequencing was performed as previously described to confirm discrepant cases [5].

Xpert MTB/RIF assay

Xpert MTB/RIF cartridges and the GeneXpert Dx system (Cepheid, CA, USA) were used in this study. Sterile samples were directly used without the decontamination process. Non-sterile samples such as colonoscopic biopsy were decontaminated with NALC-NaOH. The assay was performed according to the manufacturer's instructtions and previously described methods [6].

Extrapulmonary tuberculosis diagnosis

Cases showing discrepancies between the results of the culture test and those of Xpert MTB/RIF were categorized according to composite reference standards as previously described, and were classified into the following groups: (1) confirmed TB (culture was positive regardless of AFB smear results), (2) probable TB (culture was negative but clinical symptoms, radiologic findings, histology/cytology results were compatible with TB, or improvement was observed after TB medication), and (3) not TB (culture and all other TB tests were negative) [7].

Statistical analysis

The results of the Xpert MTB/RIF assay were analyzed for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). One-way ANOVA was performed to compare Ct values between different AFB smear groups. Excel 2013 (Microsoft Corporation, Redmond, WA, USA), MedCalc (Medcalc, Mariakerke, Belgium), and SPSS software version 19.0 (SPSS, Chicago, IL, USA) were used for statistical analysis.

RESULTS

Specimen characteristics

A total of 1,289 specimens were included in the study, including 797 biological fluid samples (549 CSF, 111 pleural fluids, 67 ascites, 32 urine, 23 pericardial fluids, and 15 joint fluids), 372 biopsy or aspirate samples (268 tissue biopsy samples other than lymph nodes, 103 lymph node biopsies, and 1 bone marrow aspirate), and

Table 1. Comparison of Xpert MTB/RIF and the culture test.

		Culture		Total
		Positive	Negative	
Xpert MTB/RIF	Positive	41	8	49
	Negative	28	1,212	1,240
Total		69	1,220	1,289

Table 2. Discrepant cases with positive Xpert MTB/RIF assay results and negative culture test results.

No.	Xpert grade	Smear grade	Culture	Specimen	Clinical information	EPTB interpretation
1	Medium	-	no growth	LN	suggestive of tuberculosis on histology	probable TB
2	Low	-	no growth	LN	TB PCR (+) on another sample	probable TB
3	Very low	-	no growth	LN	TB PCR (+) and chronic inflammation with fibrosis on histology	probable TB
4	Medium	1+	no growth	Biopsy	TB PCR (+) on another sample	probable TB
5	Low	-	no growth	Pus	improvement after TB medication	probable TB
6	Medium	1+	no growth	LN	improvement after TB medication	probable TB
7	Very low	-	no growth	CSF	improvement after TB medication	probable TB
8	Medium	1+	no growth	Urine	diagnosed as disseminated TB, culture (+) with CSF and sputum specimen	probable TB

Abbreviation: EPTB - extrapulmonary tuberculosis, LN - lymph node, TB - tuberculosis.

Table 3. Performance of Xpert MTB/RIF assay compared with that of culture in different types of specimens.

Specimen	Sensitivity % (n/N) (95% CI)	Specificity % (n/N) (95% CI)	PPV % (n/N) (95% CI)	NPV % (n/N) (95% CI)
Tissue biopsy and aspirate (n = 372)	57.6 (19/33) (39.4 - 74.0)	98.5 (334/339) (96.4 - 99.5)	79.2 (19/24) (57.3 - 92.1)	96.0 (334/348) (93.2 - 97.7)
Lymph node (n = 103)	43.8 (7/16) (20.8 - 69.4)	95.4 (83/87) (88.0 - 98.5)	63.6 (7/11) (31.6 - 87.6)	90.2 (83/92) (81.8 - 95.2)
Biopsy and BM aspirate (n = 269)	70.6 (12/17) (44.0 - 88.6)	99.6 (251/252) (97.4 - 100)	92.3 (12/13) (62.1 - 99.6)	98.0 (251/256) (95.2 - 99.3)
Biological fluid (n = 797)	53.6 (15/28) (34.2 - 72.0)	99.7 (767/769) (99.0 - 100)	88.2 (15/17) (62.3 - 97.9)	98.3 (767/780) (97.1 - 99.1)
CSF (n = 549)	25.0 (1/4) (1.3 - 78.1)	99.8 (544/545) (98.8 - 100)	50.0 (1/2) (2.6 - 97.3)	99.5 (544/547) (98.3 - 99.9)
Pleural fluid (n = 111)	54.5 (6/11) (24.6 - 81.9)	100.0 (100/100) (95.4 - 100)	100.0 (6/6) (51.7 - 100)	95.2 (100/105) (88.7 - 98.2)
Ascites (n = 67)	20.0 (1/5) (1.1 - 70.1)	100.0 (62/62) (92.7 - 100)	100.0 (1/1) (5.4 - 100)	93.9 (62/66) (84.4 - 98.0)
Urine (n = 32)	100.0 (5/5) (46.3 - 100)	96.3 (26/27) (79.1 - 99.8)	83.3 (5/6) (36.5 - 99.1)	100.0 (26/26) (84.0 - 100)
Pericardial fluid (n = 23)	66.7 (2/3) (12.5 - 98.2)	100.0 (20/20) (80.0 - 100)	100.0 (2/2) (19.8 - 100)	95.2 (20/21) (74.1 - 99.8)
Joint fluid (n = 15)	NA	100.0 (15/15) (74.7 - 100)	NA	100.0 (15/15) (74.7 - 100)
Pus (n = 120)	87.5 (7/8) (46.7 - 99.3)	99.1 (111/112) (94.4 - 100)	87.5 (7/8) (46.7 - 99.3)	99.1 (111/112) (94.4 - 100)
Pooled (n = 1,289)	59.4 (41/69) (46.9 - 70.9)	99.3 (1,212/1,220) (98.7 - 99.7)	83.7 (41/49) (69.8 - 92.2)	97.7 (1,212/1,240) (96.7 - 98.5)

Sensitivity and positive predictive values of Xpert MTB/RIF assay for joint fluid could not be calculated, since there was no culture or Xpert MTB/RIF positive case.
Abbreviation: BM - bone marrow, CI - confidence interval, CSF - cerebrospinal fluid, NA - not available, NPV - negative predictive value, PPV - positive predictive value.

Table 4. Distribution of AFB smear grade among culture confirmed cases, and sensitivity and mean Ct value of Xpert MTB/RIF according to AFB smear grade.

AFB smear	Xpert MTB/RIF sensitivity % (95% CI)	Mean Ct value (range)
Negative (n = 53)	49.1 (26/53) (35.3 - 63.0)	25.3 (18.2 - 27.1)
Trace (n = 7)	85.7 (6/7) (42.0 - 99.2)	23.6 (16.9 - 26.9)
1+ (n = 2)	100 (2/2) (19.8 - 100)	21.2 (19.7 - 25.2)
2+ (n = 3)	100 (3/3) (31.0 - 100)	21.4 (20.6 - 21.9)
3+ (n = 3)	100 (3/3) (31.0 - 100)	14.3 (10.7 - 18.1)
4+ (n = 1)	100 (1/1) (5.5 - 100)	20.6

Abbreviation: AFB - acid-fast bacilli, CI - confidence interval.

120 pus samples (111 closed pus and 9 open pus). Among the 1,289 extrapulmonary specimens, AFB smears of 20 samples (1.6%) were positive, and *M. tuberculosis* complex was cultured from 69 specimens (5.4%). Forty-nine samples (3.8%) were Xpert MTB/RIF positive.

Comparison of Xpert MTB/RIF and the culture test

Using culture as the gold standard, the overall sensitivity of Xpert MTB/RIF was 59.4% (41/69) and specificity was 99.3% (1,212/1,220) (Table 1). Eight samples were Xpert MTB/RIF positive but did not grow on culture (Table 2). These samples had low bacterial load showing smear grade negative to 1+. These cases were all diagnosed as probable TB according to composite reference standards. The positive predictive value was 83.7% (41/49) and the negative predictive value was 97.7% (1,212/1,240) (Table 3). The sensitivity of Xpert MTB/RIF for the detection of *M. tuberculosis* in lymph nodes was 43.8%, whereas it was 70.6% for other biopsies and bone marrow aspirates. Among biological fluids, the sensitivity of the Xpert MTB/RIF assay for the detection of *M. tuberculosis* in urine was 100%, 66.7% in pericardial fluid, 54.5% in pleural fluid, 25.0% in CSF, and 20.0% in ascites. In pus specimens, Xpert MTB/RIF showed a sensitivity of 87.5%.

Comparison of Xpert MTB/RIF and AFB smear

Among 69 culture-positive samples, only 16 (23.2%) samples were AFB smear-positive (Table 4). The sensitivity of Xpert MTB/RIF assay increased significantly as AFB smear grade increased from negative, to trace, to positive, from 49.1% to 85.7% to 100%, respectively ($p < 0.001$).

Xpert MTB/RIF Ct values tended to decrease as smear grade increased. The mean Ct value of Xpert MTB/RIF positive samples was 23.7 ± 4.4. AFB smear negative, trace, 1+, 2+, 3+, and 4+ groups showed Ct values of 25.3 ± 3.5 (n = 31), 23.6 ± 4.3 (n = 6), 21.2 ± 3.1 (n = 5), 21.4 ± 0.7 (n = 3), 14.3 ± 3.7 (n = 3), and 20.6 (n = 1). Since only one case was AFB smear grade 4+, smear grade 3+ and grade 4+ were analyzed together. AFB smear negative group and smear trace groups had significantly higher Ct values than the AFB smear 3+ and 4+ group ($p < 0.001$ and $p = 0.013$).

Comparison of Xpert MTB/RIF and pDST rifampin resistance detection

Of 49 samples with positive Xpert MTB/RIF, all samples showed susceptibility to rifampin with Xpert MTB/RIF. Of these, 36 were subjected to pDST. Thirty-five (35/36, 97.2%) showed concordance between Xpert MTB/RIF and pDST results. Only one sample (1/36, 2.8%) was discordant, showing rifampin sensitivity in the Xpert MTB/RIF assay and resistance in pDST. This case had *rpoB* WT8 loss with GenoType MTBDR*plus*. *rpoB* gene sequencing confirmed a CTG533CCG (L533P) mutation.

DISCUSSION

The most commonly affected sites of extrapulmonary TB are the lymph nodes, pleura, and the osteoarticular system [8,9]. Diagnosis of *M. tuberculosis* infection from extrapulmonary samples is challenging because these samples yield very few bacilli. AFB smears require 5,000 - 10,000 bacilli/mL to obtain a positive result, and the sensitivity of the AFB smear is only 10 - 20% [10,11]. The Xpert MTB/RIF assay can measure mycobacterial burden in samples with 100 bacilli/mL within 2 hours [12]. Microbiological culture remains the gold standard for extrapulmonary TB diagnosis, and bacterial loads as low as 10 - 100 bacilli/mL can be detected using this method, but long incubation periods are required [9].

Using culture as the gold standard, the specificity of Xpert MTB/RIF was over 95%. Overall sensitivity was 59.4%. This low sensitivity of the Xpert MTB/RIF assay is probably due to the paucity of mycobacteria in extrapulmonary samples and the presence of PCR inhibitors [13]. Xpert MTB/RIF sensitivity ranged from 20%

to 100% according to specimen type, indicating considerable heterogeneity. Previous studies have shown that lymph node, biopsy, and pus specimens have higher sensitivity (around 60 - 80%) than body cavity fluids such as pleural fluid and ascites, which show sensitivities of 30 - 50% [7,13-15]. In this study, urine, pus, and biopsy specimens other than lymph nodes showed a sensitivity of over 70%. Biological fluids other than urine showed lower sensitivity. CSF and ascites showed the lowest sensitivity (< 30%). Therefore, a negative Xpert MTB/RIF result in a fluid specimen cannot be used to exclude a diagnosis of extrapulmonary TB. In comparison with previous reports, the sensitivity of Xpert MTB/RIF for the detection of tuberculosis in lymph node specimens in the current study was only 43.8% [16]. This may be because Xpert MTB/RIF was tested with a small volume of lymph node specimens. In our hospital, the anatomic pathology laboratory and the clinical pathology laboratory is separated. Therefore, samples are divided into two and delivered to each laboratory. Another possible reason is that sample processing, such as decontamination and homogenization is different in various studies [15]. For sterile samples, we used direct specimens without decontamination, and mechanical homogenization is applied if needed.

Eight cases were positive according to the Xpert MTB/RIF assay but negative according to culture and were categorized as probable TB based on clinical information. Three had received TB treatment, which could explain the discrepancy between the Xpert MTB/RIF and culture results since the PCR-based Xpert MTB/RIF assay will yield a positive result even when the bacteria are not viable. Another possible explanation for negative culture might be due to loss of bacilli during NALC-NaOH processing, which decants supernatants, whereas the Xpert MTB/RIF assay uses the entire volume of the sample [7]. In this context, the Xpert MTB/RIF assay could be used to complement the culture method for the diagnosis of extrapulmonary TB.

In this study, AFB smear sensitivity was 23.2%, when culture test results were used as the standard. This indicates that AFB smears are likely to lead to the misdiagnosis or mistreatment of a significant proportion of extrapulmonary TB cases. The sensitivity of Xpert MTB/RIF among AFB smear-negative, culture-positive cases was 49.1%, suggesting that it could be used to replace AFB smears for initial diagnosis.

As previously reported, there was a correlation between AFB smear grade and Xpert MTB/RIF assay sensitivity [17,18]. The sensitivity of the Xpert MTB/RIF assay significantly increased with increases in AFB smear grade. In addition, we observed that AFB smear grade and Xpert MTB/RIF Ct values were negatively correlated. Xpert MTB/RIF is a qualitative test, but some reports have used Xpert MTB/RIF Ct values to predict bacterial burden [19]. To our knowledge, this is the first study to evaluate and compare the AFB smear grades and Xpert MTB/RIF Ct values of extrapulmonary specimens. We suggest that Xpert MTB/RIF Ct values could be used to measure disease severity or therapeutic responses.

The concordance between Xpert MTB/RIF assay and pDST results was 97.2% (35/36) for rifampicin resistance. Only one case was discordant, showing rifampin-sensitivity in the Xpert MTB/RIF assay and rifampin-resistance in the pDST and GenoType MTBDR*plus.* This was caused by the CTG533CCG (L533P) mutation. L533P is considered a disputed mutation which is known to be associated with variable susceptibility results in growth-based assays. Many cases of L533P mutation show Xpert MTB/RIF resistance with pDST rifampin resistance and rifabutin susceptible pattern. However, some cases with L533P mutation were reported to show Xpert MTB/RIF susceptible, GenoType MTBDR*plus* resistant, and pDST rifampin monoresistant pattern [20]. It seems that L533P mutation is suboptimally identified in Xpert MTB/RIF [21].

pDST of 60 specimens revealed a rifampin resistance rate of 3.3% (2/60). A previous study reported rifampin resistance rates of 1.8% in Korean extrapulmonary TB patients [20]. Drug resistance of extrapulmonary TB may be lower than that of pulmonary TB, since drug-resistant bacteria have lower virulence, making them less likely to spread from the lung. Also, extrapulmonary TB is often the result of reactivation of *M. tuberculosis* after many years or decades of latency and its drug-resistance pattern tends to resemble that of the previous infection [22]. In this study, the rifampin resistance rate of extrapulmonary TB was similar to that of pulmonary TB in Korea, which is estimated to be 3.5% [23]. However, drug resistance data for extrapulmonary TB patients in Korea is limited and further study is needed.

Limitations of this study include the fact that multiple specimens were derived from each patient. Sensitivity and specificity were analyzed based on culture results only, and composite reference standards were not applied. Heterogeneity of results may be partly due to the small number of positive results.

In conclusion, considering the characteristics and low numbers of bacilli in extrapulmonary specimens, the Xpert MTB/RIF assay should be considered a workable replacement for AFB smears. In addition, Xpert MTB/RIF Ct values could be used to estimate bacterial burden, which would allow assessments of disease severity and therapeutic responses. Although the sensitivity of the Xpert MTB/RIF assay for the detection of *M. tuberculosis* in biological fluid samples is low, specificity and rifampin resistance detection showed excellent performance.

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Declaration of Interest:
The authors declare that there is no conflict of interest regarding the publication of this paper.

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