Rapid diagnosis of extrapulmonary tuberculosis with Xpert
Mycobacterium tuberculosis/rifampicin assay

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Abstract
Purpose. XpertMycobacterium tuberculosis/rifampicin (Xpert MTB/RIF) assay has been endorsed by the World Health Organization (WHO) for diagnosis of extrapulmonary tuberculosis (EPTB), while the sensitivity and specificity have not been fully evaluated. We aimed to evaluate the performance of Xpert MTB/RIF assay in the detection of different extrapulmonary specimens.

Methods. A total of 420 nonrespiratory specimens were detected with acid-fast bacilli (AFB) smear microscopy, solid culture, conventional drug susceptibility testing (DST) and Xpert MTB/RIF assay. Using solid culture and conventional DST as the gold standard, we assessed the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of the Xpert MTB/RIF assay for detecting MTB and rifampin resistance, respectively.

Results. When setting the solid culture results as the gold standard, the sensitivity, specificity, PPV, NPV and Kappa value of Xpert MTB/RIF assay and AFB smear results were 70.6 % (48/68), 91.96 % (318/346), 63.2 % (48/76), 94.1 % (318/338), 0.60, respectively. In addition, when compared with conventional DST results, the sensitivity, specificity, PPV, NPV and Kappa of the Xpert MTB/RIF assay for detecting rifampin resistance were 91.7 % (11/12), 100.0 % (47/47), 100.0 % (11/11), 97.9 % (47/48) and 0.95, respectively.

Conclusion. Compared with AFB smear and solid culture, Xpert MTB/RIF assay has high sensitivities and short detection time, so could be used as an alternative for the rapid diagnosis of EPTB and rifampin resistance in clinical practice.

INTRODUCTION
Tuberculosis (TB) is one of the most dangerous threats to public health. According to the World Health Organization (WHO’s) report in 2015 [1], 0.93 million TB patients emerged in China, accounting for 10 % of the global TB burden. In China, multidrug-resistant tuberculosis (MDR-TB) patients accounted for 12 000 of the new cases reported annually, and worldwide 24% were MDR-TB cases [2]. It was estimated that approximately 10–20% of all active TB was extrapulmonary tuberculosis (EPTB) in China [3]. Compared with pulmonary disease, diagnosis of EPTB is particularly difficult because of the low bacterial load in the nonrespiratory specimens and the challenging sample collection from deep-seated tissue [4]. Therefore, detecting EPTB in time, and effectively controlling the spread and prevalence of TB is an important issue.

Nowadays, conventional TB diagnostic tools, such as acid-fast bacilli (AFB) smear microscopy and AFB culture, no longer meet clinical demand. AFB smears have low sensitivity and whilst AFB culture is the gold standard for TB diagnosis, solid and liquid cultures are usually required 4–8 weeks and 4–10 days before the final results can be obtained, respectively. In addition, conventional drug susceptibility testing (DST) may take up to 7–13 weeks, which often results in a delay or deprivation of treatment in clinical practice. Thus, the application of new nucleic acid amplification diagnostic technologies provides a potential solution for making an early diagnosis to improve patient outcomes.

In recent years, molecular methods have been developed for the diagnosis of TB and rapid detection of drug resistance in clinical specimens, such as the Xpert Mycobacterium
**tuberculosis/rifampicin (MTB/RIF) assay.** In 2010, the WHO endorsed the Xpert MTB/RIF assay, which has widely been used in detecting pulmonary TB and has a high sensitivity in smear-negative pulmonary TB [5]. Recently, there has been increasing evidence supporting the use of this assay in nonrespiratory clinical samples from patients with EPTB [6–9]. In 2013, the Xpert MTB/RIF assay was recommended by the WHO for the diagnosis of EPTB [10]. Even though many research groups have diagnosed EPTB with the Xpert MTB/RIF assay, the sensitivity and specificity have not yet been fully evaluated because the number and type of clinical samples have been diverse. In China, most of the studies used sputum specimens to evaluate the TB value, while our study assessed the value of extrapulmonary diagnosis. More studies about these researches need to be provided. Therefore, the aim of this study is to evaluate the performance of the Xpert MTB/RIF assay in the detection of different extrapulmonary specimens in China.

**METHODS**

**Specimen collection**

From April 2013 to April 2015, a total of 420 extrapulmonary specimens, including 224 pleural fluid, 74 cerebrospinal fluid, 54 ascitic fluid, 35 joint cavity fluid and 33 urine, were obtained from Shaanxi Provincial Institute for Tuberculosis Control and Prevention and Shaanxi Tuberculosis Hospital. Consecutive specimens were obtained from patients who were diagnosed with suspected EPTB. Specimens of at least 5 ml were collected during operations before the start of anti-TB treatment.

All specimens were detected by AFB smear microscopy, solid Lowenstein–Jensen (L–J) culture and Xpert MTB/RIF assay (Fig. 1).

**AFB smear and solid culture**

Clinical specimens were processed with N-acetyl-L-cysteine and sodium hydroxide (NALC-NaOH) to a final concentration of 1% [11]. Then samples were centrifuged for 20 min at 3000 g. The sediment was resuspended in 1.0 ml of phosphate buffer (pH 6.8), then divided into two parts: one part was used for microscopy with Ziehl–Neelsen (Z–N) staining, the other part was subjected to cultivation on solid medium L–J following WHO guidelines [12]. Each specimen was inoculated onto two L–J slants and each slant was inoculated with 0.1 ml.

**Identification of MTB and DST**

Identification of species was performed with the GenoType MTBC and CM/AS assays (Hain Lifescience GmbH, Nehren, Germany) according to the manufacturer’s protocol [13]. Conventional DST for rifampin resistance was determined with the proportional method, and the concentration of RIF in the solid medium was 40 µg ml⁻¹, as recommended by WHO guidelines.

**Xpert MTB/RIF assay**

The Xpert MTB/RIF assay was performed following the manufacturer’s instructions (Cepheid, Sunnyvale, CA, USA). All specimens were added to an Xpert MTB/RIF assay sample reagent (SR) at a 1:2 ratio in a closed container [6–8]. The mixture was vortexed twice at room temperature for 15 min. In total, 2 ml of the mixture was transferred into the test cartridge and then integrated into the GeneXpert instrument. Xpert MTB/RIF assay results were reported automatically.

**Statistical analysis**

All statistical analyses were performed using the SPSS software (version 17.0). The positive rate of AFB smear, solid culture and Xpert MTB/RIF assay were compared using the χ² test. P<0.05 was considered significant. Using solid culture on L–J medium and drug sensitivity test results as the gold standard, consistency tests for Xpert MTB/RIF, AFB smear detection and the gold standard test (Kappa test), kappa value >0.8, showed excellent agreement.

**RESULTS**

**Study population**

A total of 420 extrapulmonary specimens were obtained from 420 suspected patients, including 221 pleural TB, 74 meningeal TB, 54 peritoneal TB, 35 joint cavity TB and 30 urinary tract TB. Of the 420 specimens from suspected EPTB, three solid L–J culture results were contaminated and three Xpert MTB/RIF detections failed. In total, 414 patients (median age, 48.5±10.2 years; 251 males and 163 females) were included in our study. The demographic characteristics of patients are shown in Table 1.

**Comparison of the Xpert assay with AFB smear, solid culture method results for TB detection**

Through the examination of all specimens, the positive rate of the Xpert MTB/RIF assay was 18.4% (76/414), higher than that of solid culture (16.4%, 68/414) and AFB smear (9.2%, 38/414). Comparison of the Xpert MTB/RIF assay with the AFB staining method showed that the difference was statistically significant (χ²=14.69, P<0.01). No significant difference was identified between the Xpert MTB/RIF assay and solid culture (χ²=0.54, P>0.05). Compared with the AFB smear method and solid culture, the difference was statistically significant (χ²=9.74, P<0.05) (Table 2).

**Comparison of the Xpert MTB/RIF assay with AFB smear method results for TB detection**

Using solid culture results as the gold standard, overall sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the Xpert MTB/RIF assay were 70.6% (48/68), 91.9% (318/346), 63.2% (48/76), 94.1% (318/338), respectively. The Kappa test was chosen to assess the Xpert MTB/RIF assay and solid culture methods (κ=0.60); the two methods have a better degree of consistency (Table 3).
Rifampin resistance analysed by the Xpert assay and DST

For culture-positive isolates, the average turnaround time was 24±6 days (range 18–42 days) in solid culture. The 68 positive culture specimens from each isolate underwent rifampin susceptibility testing and identification by the GenoType MTBC and CM/AS assays. Among the 68 positive isolates, nine were nontuberculous mycobacterium (NTM) and 59 were MTB strains. All specimens of the nine NTM strains in culture were negative in the Xpert assay. Thus, 59 isolates were analysed finally. There were 11 (100.0 %) and 12 (91.7 %) isolates identified as RIF resistant by the Xpert assay and DST, respectively. No significant difference was identified between the Xpert MTB/RIF assay and DST ($\chi^2=2.05$, $P>0.05$). Setting the conventional drug susceptibility testing as the gold standard, the sensitivity, specificity, PPV and NPV in detecting rifampin resistance was 91.7 % (11/12; 95 % CI:61.5–99.8), 100.0 % (47/47; 95 % CI:92.5–100.0), 100.0 % (11/11; 95 % CI:71.5–100) and 97.9 % (47/48; 95 % CI:88.9–99.9), respectively. The Xpert MTB/RIF assay and conventional DST had a good consistency ($\kappa=0.95$, Table 4).

Table 1. Demographic characteristics of patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>48.5±10.2</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>251 (60.6)</td>
</tr>
<tr>
<td>Female</td>
<td>163 (39.4)</td>
</tr>
<tr>
<td>Clinical diagnosis</td>
<td></td>
</tr>
<tr>
<td>Pleural TB</td>
<td>221 (53.4)</td>
</tr>
<tr>
<td>Meningeal TB</td>
<td>74 (17.9)</td>
</tr>
<tr>
<td>Peritoneal TB</td>
<td>54 (13.0)</td>
</tr>
<tr>
<td>Joint cavity TB</td>
<td>35 (8.5)</td>
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<tr>
<td>Urinary tract TB</td>
<td>30 (7.2)</td>
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</tbody>
</table>

DISCUSSION

With the development of molecular techniques, the MTB drug resistance mechanism becomes increasingly clarified. Numerous studies have investigated whether more than 95 % of rifampin-resistant MTB strains have a mutation within the 81 bp hot spot region of the *rpoB* gene [14, 15]. The Xpert MTB/RIF assay is a fully automated molecular diagnostic platform that used hemi-nested real-time PCR to
Previous study highlighted that the Xpert MTB/RIF assay’s impressive value in diagnosing pulmonary TB has been outlined in numerous publications. But the various reports showed significant differences in this assay’s performance for the diagnosis of EPTB. In this study, we found that the overall sensitivity and specificity of the Xpert MTB/RIF assay were 70.6 and 91.9%, values which were lower than those from Vadwai et al. (80.6 and 99.6%, respectively) [7]. Previous studies of the Xpert MTB/RIF assay have reported that test sensitivities on pleural fluid varied from 0 to 100% [16]. In this paper, the detection sensitivity in pleural fluid specimens was 40.0%, which is similar to that reported by Tortoli et al. [17], whereas the sensitivity for cerebrospinal and urine specimens in our study (75.0, 75.0%, respectively) was lower (84.6, 84.6%, respectively). The sensitivity of the Xpert MTB/RIF assay in pleural fluid specimens was lower than other specimens in our study, which could be explained by the low bacterial load in these specimens [18–20]. In our study, the sensitivity of the Xpert MTB/RIF assay was found to be similar to that previously reported in all these specimens; however, the specificity was lower than reported by other studies [6, 8, 9, 21]. This might be related to the solid culture used as the gold standard in this study, which has a lower sensitivity than the liquid culture [22, 23]. The sensitivity of solid culture (16.4%) being lower than GeneXpert methods (18.4%) can be explained as follows: (1) the distribution of bacteria in the extrapulmonary specimens was uneven; (2) the decontamination procedure was too drastic and lost the viable bacteria. The heterogeneity between studies may be associated with the differences in the quality of the samples, the number of samples and the diagnostic gold standard used. The limitation of this study was the small number of joint cavity fluid and urine specimens. Therefore, we will collect more samples in future work. The other limitation of our study was that there were not enough specimen types. In our study, we collected only fluid samples and there were no tissue specimens; however, we will start to collect tissue samples for future work.

A few studies have shown that the detection limit of AFB smear microscopy is 5000 c.f.u. ml\(^{-1}\), while the Xpert MTB/RIF assay was able to detect as few as 131 c.f.u. ml\(^{-1}\) in sputum specimens [24, 25]. In our study, we compared the sensitivity and specificity of the Xpert MTB/RIF assay with AFB smear microscopy for the diagnosis of MTB in extrapulmonary clinical specimens. The results showed that the positive rate of specimens with Xpert MTB/RIF assay was the highest, compared with culture and AFB smear methods. Taking the DST result as the gold standard, the Xpert MTB/RIF assay and conventional DST had very good consistency. The observed sensitivity and specificity of the Xpert MTB/RIF assay of 91.7% (11/12) and 100.0% (47/47), respectively, for detecting rifampin resistance were similar to those in previously reported studies [4]. One of these samples was rifampicin resistant by phenotypic DST but rifampicin sensitive by GeneXpert. For these cases, we detected the specimen which had a wild-type sequence by GenoType MTBC assays. This might be because resistant bacteria account for only a small proportion of the total number of bacteria. In total, compared with AFB smear microscopy and solid culture, the Xpert MTB/RIF assay can both increase the sensitivity and reduce the detection time greatly, and so could play an important role in the detection of EPTB.

### Table 2. Comparison of the positive results obtained using three different methods for MTB detection

<table>
<thead>
<tr>
<th>Method</th>
<th>Pleural fluid (n=221) (%)</th>
<th>Cerebrospinal fluid (n=74) (%)</th>
<th>Ascitic fluid (n=54) (%)</th>
<th>Joint cavity fluid (n=35) (%)</th>
<th>Urine (n=30) (%)</th>
<th>Total (n=414) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFB smear</td>
<td>11 (5.0%)</td>
<td>0 (0.0%)</td>
<td>1 (1.9%)</td>
<td>20 (57.1%)</td>
<td>6 (20.0%)</td>
<td>38 (9.2%)</td>
</tr>
<tr>
<td>Solid culture</td>
<td>25 (11.3%)</td>
<td>4 (5.4%)</td>
<td>4 (7.4%)</td>
<td>27 (77.1%)</td>
<td>8 (26.7%)</td>
<td>68 (16.4%)</td>
</tr>
<tr>
<td>Xpert MTB/RIF assay</td>
<td>28 (12.7%)</td>
<td>6 (8.1%)</td>
<td>5 (9.3%)</td>
<td>28 (80.0%)</td>
<td>9 (30.0%)</td>
<td>76 (18.4%)</td>
</tr>
</tbody>
</table>

### Table 3. Diagnostic performance of the Xpert MTB/RIF assay with the solid culture method as the gold standard

<table>
<thead>
<tr>
<th>Specimen type</th>
<th>No. of positive tests/no. of positive samples (% sensitivity, 95% CI)*</th>
<th>No. of negative tests/no. of negative samples (% specificity, 95% CI)</th>
<th>No. of diagnoses of positive samples/no. of total positive tests (% PPV, 95% CI)</th>
<th>No. of diagnoses of negative samples/no. of total negative tests (% NPV, 95% CI)</th>
<th>Kappa value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pleural fluid</td>
<td>10/25 (40.0%, 21.1–61.3)</td>
<td>178/196 (90.8%, 86.8–94.9)</td>
<td>10/28 (35.7%, 18.6–55.9)</td>
<td>178/193 (92.2%, 88.5–96.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>3/4 (75.0%, 19.4–99.4)</td>
<td>67/70 (95.7%, 91.0–100.0)</td>
<td>3/6 (50.0%, 11.8–88.2)</td>
<td>67/68 (98.5%, 95.7–100.0)</td>
<td>0.57</td>
</tr>
<tr>
<td>Ascitic fluid</td>
<td>3/4 (75.0%, 19.4–99.4)</td>
<td>48/50 (96.0%, 86.3–99.5)</td>
<td>3/5 (60.0%, 14.7–94.7)</td>
<td>48/49 (98.0%, 89.1–99.9)</td>
<td>0.64</td>
</tr>
<tr>
<td>Joint cavity fluid</td>
<td>26/27 (96.3%, 81.0–99.9)</td>
<td>6/8 (75.0%, 34.9–96.8)</td>
<td>26/28 (92.9%, 76.5–99.1)</td>
<td>6/7 (85.7%, 42.1–99.6)</td>
<td>0.75</td>
</tr>
<tr>
<td>Urine</td>
<td>6/8 (75.0%, 34.9–96.8)</td>
<td>19/22 (86.4%, 65.1–97.1)</td>
<td>6/9 (66.7%, 29.9–92.5)</td>
<td>19/21 (90.5%, 69.6–98.8)</td>
<td>0.59</td>
</tr>
<tr>
<td>Total</td>
<td>48/68 (70.6%, 59.8–81.4)</td>
<td>318/346 (91.9%, 89.0–94.8)</td>
<td>48/76 (63.2%, 52.3–74.0)</td>
<td>318/338 (94.1%, 91.6–96.6)</td>
<td>0.60</td>
</tr>
</tbody>
</table>

*CI, confidence interval.
Table 4. Xpert MTB/RIF assay for the detection of rifampin resistance in comparison with DST as the gold standard

<table>
<thead>
<tr>
<th>Xpert MTB/RIF assay</th>
<th>DST</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resistant</td>
<td>Susceptible</td>
</tr>
<tr>
<td>Resistant</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>Susceptible</td>
<td>1</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>47</td>
</tr>
</tbody>
</table>

In conclusion, the Xpert MTB/RIF assay not only has better sensitivity and specificity for the diagnosis of TB and detection of rifampin resistance in EPTB in under 2 h, but also requires minimal biosafety facilities. Furthermore, it is less dependent on the techniques of the user. However, the new molecular technique is considerably more expensive, because of the high price of reagents and instrument maintenance, than conventional methods. Overall, the Xpert MTB/RIF assay has a high clinical application value in earlier diagnosis of EPTB, which can be applied to areas of the country as resources allow.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
This study was approved by the Medical Ethics Committee of Shaanxi Provincial Institute for Tuberculosis Control and Prevention.

References