Xpert MTB/RIF, a new pillar in the diagnosis of extrapulmonary tuberculosis?

Viral Vadwai, M.Tech.,1 Catharina Boehme, M.D.,2 Pamela Nabeta, M.D.,2 Anjali Shetty, M.R.C.P., F.R.1 David Alland, M.D.3 Camilla Rodrigues, M.D.1,*

1P. D. Hinduja National Hospital and Medical Research Centre, Mahim, Mumbai.
2Foundation for Innovative New Diagnostics, Geneva, Switzerland.
3Department of Medicine, New Jersey Medical School, University of Medicine and Dentistry, New Jersey, Newark, New Jersey.

*Corresponding Author:
Dr. Camilla Rodrigues,
Consultant Microbiologist.
P.D. Hinduja National Hospital and Medical Research Centre.
Email: dr_crodrigues@hindujahospital.com
Tel. No. +91-22-24447795.

Running Title: Diagnosis of extrapulmonary TB using Xpert MTB/RIF
Abstract

10-15% of tuberculosis (TB) cases in India are estimated to have extrapulmonary disease and due to lack of diagnostic means, often remain untreated. Early detection of *Mycobacterium tuberculosis* (MTB) and multi-drug resistance is a priority in TB diagnosis to improve the successful treatment rate of TB and reduce transmission. Xpert MTB/RIF test (Xpert) - recently endorsed by World Health Organization for the detection of pulmonary TB - was evaluated to test its utility in 546 patients suspected of extrapulmonary tuberculosis. 546 extrapulmonary specimens were split and processed simultaneously for both culture (solid and liquid) and Xpert. For culture, the sensitivity was low - 53% (150/283). Results of Xpert sensitivity and specificity were assessed in comparison to a composite reference standard made up of smear and culture results, clinical, radiological and histological findings. The sensitivity of Xpert was 81% (228/283), (64% [89/138] for smear negative cases and 96% [139/145] for smear positive cases), with a specificity of 99.6%. The sensitivity was found to be high for the majority of specimen types (63-100%) except for cerebrospinal fluid, which was 29% (2/7). Xpert correctly identified 98% of phenotypic Rifampin (RIF) resistant cases and 94% of phenotypic RIF susceptible cases. Sequencing of the 6 discrepant samples resolved 3 of them, resulting in an increased specificity of 98%. In conclusion, the results of this study suggest that Xpert also shows good potential in the diagnosis of extrapulmonary TB and that its ease of use makes it applicable for TB endemic countries.
Introduction

India has the world’s largest burden of tuberculosis (TB), accounting for one fifth of the global TB incidence. The global annual incidence estimate is 9.4 million cases, of which 1.98 million cases are from India.\textsuperscript{10} TB remains the largest infectious killer disease affecting adults in developing countries.\textsuperscript{1} In India, TB disproportionately involve the young. Almost 50% of multi-drug resistant TB (MDR-TB, resistant to at least rifampin [RIF] and isoniazid) cases worldwide are estimated to occur in China and India.\textsuperscript{21} TB manifests clinically as pulmonary or extrapulmonary tuberculosis (EPTB), the former being more common. In India, 10 to 15% of TB cases are estimated to be EPTB (condition, which mainly affects the lymph nodes, meninges, kidney, spine and growing ends of the bones), with 25-50% case mortality within months. In this situation, not only rapid TB case detection but also early determination of MDR status is important. The major challenge in the diagnosis of EPTB is the frequently atypical clinical presentation simulating other inflammatory and neoplastic conditions, and which frequently results in delay or deprivation of treatment. Therefore, a high index of suspicion is necessary to make an early diagnosis and, quite often, more than one procedure is necessary for the confirmation of diagnosis. In lower-income countries, the lack of diagnostic infrastructure substantially aggravates the problem.\textsuperscript{5} Reports on biological tests such as enzyme linked immune sorbent assay, slide agglutination techniques and polymerase chain reaction (PCR) are available in EPTB, however, the specificity and sensitivity of these tests are variable.\textsuperscript{5} Also, these tests require a number of manual steps, and some have a relatively long turn-around-time.

The recently developed CE-marked Xpert MTB/RIF (Xpert) test (Cepheid Inc.), based on nested real-time PCR and molecular beacon technology, has been shown to be rapid with a result for TB and RIF resistance in under 2 hours;\textsuperscript{12} is not prone to cross-contamination; requires
minimal biosafety facilities;\textsuperscript{4} can be performed by technicians with little training; and has a high
sensitivity in smear-negative pulmonary TB (the last factor being particularly relevant in patients
with HIV infection). These characteristics make it a potentially attractive tool also for
extrapulmonary specimens. A series of meta-analyses have shown that nucleic acid amplification
tests (NAATs) have high specificity and positive predictive value with highly variable
sensitivity, especially in EPTB.\textsuperscript{11,16-18} In these studies, NAAT has usually been compared to
culture, which is known to be a very suboptimal reference standard for EPTB. Therefore, we
have also compared it against a composite reference standard (CRS) to evaluate the true
diagnostic potential of Xpert in EPTB.\textsuperscript{3,6,14} The CRS for this study was composed of smear
microscopy, culture (both liquid and solid), clinical findings, histology/cytology, site-specific
computerized tomography scan/magnetic resonance imaging and follow-up (FU) after 3 months
from the date of enrollment. This study was carried out in accordance with recommendations on
design and conduct of diagnostic accuracy assessments.\textsuperscript{9}
Materials and Methods

Study population and samples

This study was conducted in a private tertiary care hospital, Mumbai, from January to August 2010. After screening of 630 consecutively presenting patients with symptoms suggestive of EPTB, 547 patients met all inclusion criteria and were enrolled at the point of presentation to the consulting physician. Consenting patients were enrolled only if they could provide detailed clinical history, radiological and histology/cytology reports, along with an adequate amount of specimen material. The collected specimen types included 284 biopsies (from tissues [n=147], lymph nodes [n=82], fine needle aspirates [n=55]), 147 specimens of pus, 93 specimens of body fluids (synovial [n=11], pericardial [n=3], pleural fluid [n=66], peritoneal [n=13]) and 23 cerebrospinal fluid (CSF) specimens were included in the study. The minimum volume of sample required was: 3 ml for any kind of body fluid including pus; 2.5 ml for CSF; 1 cm × 1 cm for biopsies. Patients were excluded if they were initiated on anti-tubercular treatment (ATT) within past 60 days. This study was approved by the Institutional Review Board of our hospital and informed consent was obtained from each patient. The sample was divided equally into 3 parts, each part was uniquely coded. Two parts were assigned to 2 different technologists, one in Mycobacteriology laboratory where they read smears, inoculated cultures and performed drug susceptibility testing (DST), and the other in the Research laboratory where they performed Xpert assay; thus blinding the technologists to the results of other tests. The third part was stored at -80°C.

Methods

The sample was divided equally into 3 parts: One part was used for the Xpert test, the second was stored at -80°C and the third was tested for direct and concentrated AFB microscopy
(Ziehl–Neelsen [ZN] staining) followed by processing with N-acetyl L-cysteine and sodium hydroxide (NALC–NaOH), and centrifugation. The resuspended pellet was subjected to cultivation on both solid medium (egg-based Löwenstein– Jensen [LJ]) and liquid medium (BACTEC MGIT [mycobacteria growth indicator tube] 960 culture; BD Microbiology Systems). Culture positives were confirmed for *Mycobacterium tuberculosis* (MTB) species by p-nitrobenzoic acid assay and subjected to indirect drug-susceptibility testing with MGIT SIRE.

Xpert

Xpert was performed as described previously. A 2:1 volume of sample reagent buffer (SR) was added to biopsies after they had been chopped into very small pieces with a sterile blade in a sterile Petri dish. Care was taken to ensure that at least one piece entered the cartridge. Fluids were processed directly by addition of 2:1 volume of SR, except for CSF (usually <1 ml), which was raised to 2 ml adding SR. The results obtained were in a simple text format which could be read easily. In case of reporting as ‘Invalid’, ‘No Result’ or ‘Error’, the sample was reprocessed and rerun if sufficient material was available.

Patient categories

Based on the CRS, patients were categorized into 4 groups: confirmed TB cases (culture positive or smear negative/culture positive or smear positive/culture positive); probable TB cases (culture negative but showing clinical symptoms, radiological findings and/or histology/cytology suggestive of TB); possible TB cases (negative culture, other tests and only clinical symptoms/signs suggestive of TB, in this group the patient follow-up indicated response to empirical ATT after 3 months); and not TB (culture and all other tests for TB were negative, and
patient improved without receiving TB treatment). In case of smear-positive, culture-negative patients, their LJ were checked for 10 weeks before discarding and all culture negative patients were followed-up after 3 months. All those patients whose culture grew non-tuberculous mycobacteria (NTM), were lost to follow up or died before follow up were excluded from the study (see patient flow in figure 1). Based on clinical history, smear microscopy and culture reports, radiological reports and/or histology/cytology results and follow-up, two experts in this field who were blinded to the Xpert test results categorized the patients into the four diagnostic groups. Table 1a. shows the symptoms and signs taken into consideration according to site of infection from where the specimen was obtained. Table 1b. represents a detailed algorithm used for categorization of patients into different categories of the composite reference standard.

Analysis of RIF-discordant strains: Bi-directional sequencing was carried out on RIF resistance determining region of the \( rpoB \) gene in all the RIF-discordant strains using forward 
\[
\text{CGTTGATCAACATCCGGCCGGTG}
\]
and reverse 
\[
\text{CCACCTTGCACGTACGCGTT}
\]
primers, and analyzed using Chromas version 2.33 software.

Statistical Analysis: Sensitivity and specificity of smear microscopy, culture and Xpert were calculated against CRS based on the single direct test run. Forest plots displaying sensitivity and specificity estimates and their 95% confidence intervals (CI) for each specimen were created using the Meta-Disc software ver 1.4.\textsuperscript{22} Wilson’s binomial method was used to calculate 95% CI.\textsuperscript{2} The indeterminate rate was the number of tests classified as “invalid”, “error” or “no result” divided by the total number performed. When results were indeterminate and
sufficient sample remained, the assay was repeated once, and the second result was used for analysis.
Results

Patients: 547 patients were enrolled in the study (fig. 1). A total of 14 (3%) patients were excluded from the study since 5 (9%) were NTM positive, 7 (1%) were lost to follow up and 2 (0.5%) died; thus, 533 patients was the final sample size for the analysis. Of these, 150 (27%) were culture positive ‘confirmed TB’ cases (58 [11%] being smear negative and 92 [17%] being smear positive); 129 (24%) were clinically, radiologically and/or histologically/cytologically positive suggestive of ‘probable TB’ cases; 4 (1%) were only clinically positive and responding to ATT suggestive of ‘possible TB’ cases and 250 (46%) patients had no evidence of TB and were ‘not TB’ cases. Of the total culture positive cases, 50 (33%) patients were found to have MDR-TB on phenotypic DST. Out of 547, 16 patients (3%) were found to be HIV positive. The median age of the patients was 37 years (8 months – 94 yrs). The male to female sex ratio was 0.85.

Sensitivity and Specificity

Case Detection: The sensitivity of smear microscopy was found to be 61% (91/150) among patients with positive culture and 51% (145/283) among patients with positive CRS. On comparison with composite reference standard, the pooled sensitivity of culture was found to be 53% (150/283), with 42% (59/138) for smear negative CRS positive (S-CRS+) and 63% (91/145) for smear positive CRS positive (S+CRS+) cases. The sensitivity of Xpert test against CRS was found to be 81% (228/283), 64% (89/138) for S-CRS+ and 96% (139/145) for S+CRS+ cases with a specificity of 99.6% (249/250). Xpert in comparison against culture showed a pooled sensitivity of 83% (125/150), being 66% (38/58) for smear negative culture positive and 95% (87/92) for smear positive culture positive cases; with a specificity of 73% (277/382). Cultures had an average time to positivity (TTP) of 25 days, on liquid culture and 5
weeks on solid media. Table 2a describes in detail the sensitivity and specificity of culture and
Xpert with respect to different specimen groups in comparison with the CRS. Table 2b describes
in detail the sensitivity and specificity of Xpert against culture among different specimen groups.
Figure 2 gives the details of sensitivity of Xpert against CRS for each kind of specimen.

Detection of RIF resistance: The sensitivity and specificity of the Xpert test compared to
phenotypic DST was found to be 97.5% (39/40), correctly determining RIF resistance, and 94%
(80/85), correctly determining RIF susceptibility. However, there were 6 patients whose
phenotypic DST results for RIF were in discordance with the Xpert result. Five of these samples
were RIF sensitive by phenotypic DST but RIF resistant by Xpert; and 1 sample was RIF
resistant by phenotypic DST and RIF sensitive by Xpert. This discrepancy in results was
resolved by bidirectional sequencing. Of the 5 phenotypically proven RIF sensitive strains, 2
were found to have a wild-type sequence while the other 3 showed the same point mutation at
codon 533 (CTG to CCG). The correlation between 533 codon mutation and RIF resistance is
controversial. The remaining 1 sample showed the presence of a mutation at codon 531 (TCG
to TTG) on sequencing. Additionally, results of 4 randomly sequenced samples were in
concordance with Xpert. Considering the phenotypic DST and sequencing results together,
sensitivity using Xpert was found to be 98% (42/43) and specificity 98% (80/82) (Table 3).

Indeterminate Rate.
The indeterminate rate was the number of tests classified as “invalid”, “error” or “no
result” divided by the total number performed. Xpert was indeterminate in 0.7% (4/547) tests
performed, a rate lower than overall contamination rate (2.1%) in 11/547 cultures, both liquid
and solid. Allowing for one repeat test, the indeterminate result rate dropped to 0% (0/547), with 100% (4/4) valid results.
Discussion

A recent study by Boehme et al.\textsuperscript{8} has successfully shown the use of Xpert for point-of-treatment in low-income countries for detecting RIF resistance in pulmonary TB cases. Along with high specificity, the study showed a sensitivity of 90\% in smear negative pulmonary TB cases. Since we were one of the sites evaluating the test, we decided to also evaluate its utility in paucibacillary extrapulmonary specimens. The test identified 83\% (125/150) of all ‘confirmed TB’ cases, including 64\% (38/59) of smear negative TB cases. It was also observed that Xpert diagnosed TB in 80\% (103/129) of the ‘probable TB’ cases, whose culture was negative but had positive radiological tests and/or positive histology/cytology reports, while some of them were already on anti-tubercular treatment at the point of enrollment in the study.

The specificity of Xpert test (99.6\%) was found to be similar to that reported by Boehme et al.\textsuperscript{8} In the case of extrapulmonary specimens, sensitivity of smear (51\%) and culture (53\%), though comparable, were found to be low in comparison with Xpert (81\%); and the average TTP of culture is 25 days for MGIT only. Culture is seen to have low sensitivity in case of smear positive patients (n=57, smear positive culture negative) because 80\% (45/57) of patients were on ATT for varying periods of time ranging from 4 to 6 months when enrolled in the study and 16\% (9/57) of patients had completed their treatment regimen. Thus, both groups of patients were expected to turn culture negative.

The low of sensitivity of culture (53\%) in comparison with Xpert (81\%), against CRS can be explained as follows: 1) 78\% (104/133) of culture negative, Xpert positive patients were on anti-tubercular treatment for varying periods of time when enrolled in the study; 2) paucibacillary nature of extrapulmonary specimens with tendency of \textit{M. tuberculosis} to form clumps leading to uneven distribution of the bacilli; 3) loss of viable bacilli during NALC-NaOH processing (due to decanting supernatant steps) unlike Xpert processing wherein the entire
volume of the processed specimen is used; 4) better homogenization and liquefaction efficiency of Xpert sample reagent compared to NALC-NaOH processing.

The study shows that Xpert has true diagnostic potential with good sensitivity (86-100%) for specimens like synovial, pericardial, peritoneal fluids, pus, and fine needle aspirates; moderate sensitivity (63-73%) for tissues, lymph nodes, pleural fluid but poor sensitivity (29%) in the case of CSF, at least in this small number of samples. A pre-processing step (concentrating the specimen by centrifuging it a high speed and then using the pellet for processing) might be required to increase sensitivity in paucibacillary specimen types such as CSF. There is a need to evaluate and confirm the utility of this tool on a large sample size with specimens like CSF, other body fluids; and urine which are easier to obtain.

Finally, not only MTB detection, but also rapidly determining the patient’s MDR status is of prime importance in bringing to an end the spread of MDR-TB and decreasing mortality. Conventional DST results take at least 2 months from the time when the culture is inoculated. Faster methods that allow starting MDR regimens early are urgently needed. Conventional procedures are laborious, require high infrastructure laboratories and trained personnel, a luxury that is only available in a few reference centers and not in resource-limited settings or decentralized laboratory settings where they are most required.

The high cost of this sophisticated technology is offset to an extent by the rapid turn-around-time almost similar to smear microscopy (<2 hrs), with less biohazard and only minimal training. In conclusion, the GeneXpert MTB/RIF test not only has good sensitivity and specificity for diagnosis of TB and detection of RIF resistance in EPTB but also perfectly fits the requirements of the Indian health care setting.
Acknowledgements

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Detection of Mycobacterium tuberculosis and Rifampin Resistance by Use of On-

MTBDRplus Assay for Rifampin and Isoniazid Susceptibility Testing of Mycobacterium


Fig 1. This flowchart explains the patient flow in this study.

630 patients were screened.
Inclusion criteria:
- Suspected EPTB.
- Patient should be able to give detailed clinical history.
- Should be able to provide CT/MRI reports of the site of body from where the specimen is removed and/or histology/cytology reports of the specimen.
- The specimen material should be 1 cm × 1 cm for biopsies, 3 ml for body fluids, pus and 2.5 ml in case of CSF.

83 patients excluded.
- 14 patients provided an insufficient specimen volume.
- 20 patients could not give entire history.
- 13 patients died within days after enrolment.
- 36 didn’t have contact nos. to keep their follow up.

547 patients were eligible and included in the study.
- 284 Biopsies.
- 147 Pus.
- 93 Body fluids.
- 23 CSF.

Specimen

MTB/RIF test

Direct and concentrated AFB smear microscopy, BACTEC MGIT 960 and LJ.

Store specimen at -80 °C

Preliminary data analysis

14 patients excluded.
- 5 cultures grew NTM.
- 2 patients died before follow up.
- 7 were lost on follow up.

533 patients (specimens were included in the main analysis)

283 being CRS positive were 'TB' cases.
- 138 were smear negative.
- 145 were smear positive.

250 being CRS negative were 'No TB' cases.
- 247 were smear negative.
Fig 2. The forest plot gives the details of sensitivity of Xpert against CRS for each kind of specimen.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Sensitivity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissues</td>
<td>0.73 (0.59 - 0.84)</td>
</tr>
<tr>
<td>Lymph Nodes</td>
<td>0.73 (0.59 - 0.84)</td>
</tr>
<tr>
<td>Fine needle aspirate</td>
<td>0.86 (0.68 - 0.96)</td>
</tr>
<tr>
<td>Pus</td>
<td>0.95 (0.89 - 0.98)</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>1.00 (0.16 - 1.00)</td>
</tr>
<tr>
<td>Pericardial fluid</td>
<td>1.00 (0.16 - 1.00)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>0.63 (0.42 - 0.81)</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>1.00 (0.29 - 1.00)</td>
</tr>
<tr>
<td>CSF</td>
<td>0.29 (0.04 - 0.71)</td>
</tr>
</tbody>
</table>

Pooled Sensitivity = 0.81 (0.76 to 0.85)
Chi-square = 39.71; df = 8 (p = 0.0000)
Inconsistency (I-square) = 79.9%
Table 1a. Symptoms and signs taken into consideration based on the site of infection.

<table>
<thead>
<tr>
<th>Site of Infection</th>
<th>Symptoms/Signs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Irritability, restlessness, stiff neck, headache persistent for 2-3 weeks, vomiting, seizures, changes in mental condition or behavior.</td>
</tr>
<tr>
<td>Intestinal tract, abdomen</td>
<td>Abdominal pain, diarrhea.</td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>Enlargement of lymph nodes, mass formation in the neck.</td>
</tr>
<tr>
<td>Cardio-respiratory</td>
<td>Shortness of breath, low blood pressure, chest pain, dyspnea.</td>
</tr>
<tr>
<td>Endometrium</td>
<td>Pelvic pain, pelvic mass, irregular periods, infertility.</td>
</tr>
<tr>
<td>Skin (cutaneous)</td>
<td>Visible presence of ulcers or lesions, tender nodules.</td>
</tr>
</tbody>
</table>

Weight loss, persistent cough and fever for 2-3 weeks were also considered for all kinds of specimens.

Table 1b. An algorithm for patient categorization into different categories of composite reference standard (CRS).

<table>
<thead>
<tr>
<th>CRS category</th>
<th>AFB Smear</th>
<th>Culture</th>
<th>Symptoms/Signs</th>
<th>Radiology</th>
<th>Histology/Cytology</th>
<th>Follow-up at 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Confirmed TB</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>Probable TB</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Possible TB</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Not TB</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

As described in Table 1a.

Radiology, positive if presence of infiltrates or cavities or hilar lymph nodes or pleural effusions or tuberculomas were noted.

Histology/cytology, positive if presence of caseation necrosis with epitheloid granulomas was reported irrespective of the visual presence or absence of acid fast bacilli.

Follow-up at 3 months, positive if the patient was on anti-tubercular treatment (ATT) and negative if the patient has responded to non ATT.
Table 2a The Sensitivity and Specificity of Culture and Xpert with respect to different specimen group on comparison with composite reference standard (CRS).

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Culture Sensitivity</th>
<th>Xpert Sensitivity</th>
<th>Xpert Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All CRS positive</td>
<td>S-CRS+</td>
<td>S+CRS+</td>
</tr>
<tr>
<td>Biopsies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total</td>
<td>50 (70/139)</td>
<td>75 (105/139)</td>
<td>62 (48/78)</td>
</tr>
<tr>
<td>no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>42-59</td>
<td>68-82</td>
<td>50-72</td>
</tr>
<tr>
<td>Pus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total</td>
<td>64 (56/103)</td>
<td>95 (98/103)</td>
<td>90 (26/29)</td>
</tr>
<tr>
<td>no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>45-64</td>
<td>89-98</td>
<td>73-97</td>
</tr>
<tr>
<td>Body Fluids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total</td>
<td>62 (21/34)</td>
<td>71 (24/34)</td>
<td>57 (13/23)</td>
</tr>
<tr>
<td>no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>45-76</td>
<td>54-83</td>
<td>37-74</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total</td>
<td>43 (3/7)</td>
<td>29 (2/7)</td>
<td>29 (2/7)</td>
</tr>
<tr>
<td>no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>16-75</td>
<td>8-65</td>
<td>8-65</td>
</tr>
<tr>
<td>Total (Pooled)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total</td>
<td>53 (150/283)</td>
<td>81 (228/283)</td>
<td>64 (89/138)</td>
</tr>
<tr>
<td>no.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>47-59</td>
<td>76-85</td>
<td>56-72</td>
</tr>
</tbody>
</table>

\(^a\)S-CRS+, Smear negative CRS positive; \(^b\)S+CRS+, Smear positive CRS positive.
Table. 2b The Sensitivity and Specificity of Xpert with respect to different specimen group on comparison with culture.

<table>
<thead>
<tr>
<th>Specimen Type</th>
<th>Xpert Sensitivity</th>
<th>Xpert Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All Culture positive</td>
<td>Smear negative Culture positive</td>
</tr>
<tr>
<td>Biopsies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total no.)</td>
<td>77 (54/70)</td>
<td>62 (21/34)</td>
</tr>
<tr>
<td>95% CI</td>
<td>66-86</td>
<td>45-76</td>
</tr>
<tr>
<td>Pus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total no.)</td>
<td>96 (54/56)</td>
<td>89 (8/9)</td>
</tr>
<tr>
<td>95% CI</td>
<td>87-100</td>
<td>54-100</td>
</tr>
<tr>
<td>Body Fluids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total no.)</td>
<td>76 (16/21)</td>
<td>62 (8/13)</td>
</tr>
<tr>
<td>95% CI</td>
<td>55-90</td>
<td>35-82</td>
</tr>
<tr>
<td>CSF</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total no.)</td>
<td>33 (1/3)</td>
<td>33 (1/3)</td>
</tr>
<tr>
<td>95% CI</td>
<td>6-80</td>
<td>6-80</td>
</tr>
<tr>
<td>Total (Pooled)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% (no./total no.)</td>
<td>83 (125/150)</td>
<td>66 (38/58)</td>
</tr>
<tr>
<td>95% CI</td>
<td>77-89</td>
<td>53-76</td>
</tr>
</tbody>
</table>
Table 3. Sensitivity and Specificity of the Xpert for the Detection of Rifampin Resistance, as Compared with Phenotypic DST Alone and in Combination with Sequencing for Discrepant Samples.

<table>
<thead>
<tr>
<th></th>
<th>Phenotypic DST*</th>
<th>Phenotypic DST + Sequencing for discrepant samples*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>% (no./total no.)</td>
<td>98 (39/40)</td>
<td>94 (80/85)</td>
</tr>
<tr>
<td>95% CI</td>
<td>86-100</td>
<td>87-98</td>
</tr>
</tbody>
</table>

*This is the reference standard for Xpert.