Evaluation of the Xpert MTB/RIF for detection of *Mycobacterium tuberculosis* in cerebro-spinal fluid.

Pink F\(^1,2\), Brown TJ\(^2\), Kranzer K\(^2,3,4\), Drobniewski F\(^1,2,5\).

\(^1\)Department of Infection, Barts Health, 80, Newark St., London E1 2ES, UK
\(^2\)NMRL, Public Health England, 2, Newark St., London E1 2AT, UK
\(^3\)Department of Infectious Disease Epidemiology, London School of Hygiene and Tropical Medicine, UK
\(^4\)National Mycobacterium Reference Laboratory, Research Centre Borstel, Germany
\(^5\)Department of Infectious Diseases, Imperial College, Commonwealth Building, Hammersmith Campus, London, UK

**Abstract**

Studies investigating Xpert MTB/RIF diagnostic performance on cerebro-spinal fluid samples are lacking in resource rich settings. Xpert MTB/RIF results for 740 CSF samples from 698 patients across England were retrospectively compared with the results of culture of the same and contemporary samples. The overall sensitivity was calculated at 55%.

Central nervous system infection, particularly meningitis, caused by *Mycobacterium tuberculosis* occurs in around 1\% of all cases of tuberculosis and accounts for at least 5\% of extra-pulmonary tuberculosis diagnoses\(^1\). It is more common in children and patients with HIV and delayed diagnosis is associated with poor outcomes\(^2\). Laboratory diagnosis of tuberculous meningitis (TBM) is challenging due to paucibacillary disease. Examining large volumes of cerebrospinal fluid (CSF) improves the sensitivity of smear and culture\(^3\), but
volumes submitted for mycobacterial culture are often small. Thus in the majority of patients, TBM is diagnosed on the basis of clinical symptoms, cell count and protein concentration in the CSF rather than microbiological confirmation. The clinical value of culture remains limited to diagnostic confirmation and drug susceptibility testing; cultures may take several weeks to yield a positive result and negative results do not exclude TBM.

The Xpert MTB/RIF is an automated real-time molecular assay for rapid diagnosis of tuberculosis (TB) and detection of rifampin resistance. It was first endorsed by the World Health Organization (WHO) in December 2010. A recent systematic review reported the successful use of the Xpert MTB/RIF test on CSF samples, with a median sensitivity of 85%. The review included 10 studies representing a total of 126 positive CSF samples; one study from Vietnam contributed 103 positive samples. The second largest study was conducted in Italy including a total of 150 CSF samples of which 11 were TB culture positive. Despite its wide availability, studies investigating the diagnostic performance of Xpert MTB/RIF for TBM in resource rich settings are lacking. This study used routine retrospective data to determine the sensitivity and specificity of Xpert MTB/RIF compared to liquid Mycobacteria Growth Indicator Tube (MGIT)-based culture of CSF samples.

All CSF samples referred for Xpert MTB/RIF testing to the National Mycobacterium Reference Laboratory (NMRL), London between January 2011 and January 2015 were included in this analysis. The following data was extracted from the laboratory database: age, gender; Xpert MTB/RIF, culture and smear results. CSF samples for which Xpert/RIF testing was attempted were included in the analysis: a total of 741 samples with 740 valid Xpert MTB/RIF results and one test failure. Any CSF or central nervous system culture results from the same patient within three months of the index sample were reviewed. Only aliquots of >500µl underwent Xpert MTB/RIF testing to ensure sufficient sample volumes for culture. Microscopy was performed at the NMRL if microscopy results were not available from the referring laboratory, then up to 1.5ml of the primary sample was centrifuged, and any supernatant in excess of 600µl was removed and the centrifuged sample was mixed.
with Cepheid sample reagent. Following incubation for 15 minutes 2ml of the preparation were transferred into an Xpert MTB/RIF cartridge as per the manufacturer’s instructions. The remaining CSF was inoculated into a Bactec MGIT tube (Becton Dickinson, Oxford, UK) and Kirchner medium and incubated for 8 weeks.

Sensitivity and specificity and corresponding 95% confidence intervals (95%CI) of Xpert MTB/RIF were calculated using two gold standards: firstly comparison with the culture result on the same sample (table 1); secondly comparison with a composite of the culture result on the same sample and any sample within 7 days of the index sample (table 2). Samples with a positive Xpert MTB/RIF result and a negative culture result were excluded if the patient was receiving anti-tuberculous treatment at the time of sampling. A total of 740 samples were analysed from 698 patients: the median age was 46 (range 0 – 93 yrs), 59% were male. TBM was confirmed by culture of the primary specimen in 37 of 740 cases (4.5%). Including a further 8 samples with contemporary positive CSF or central nervous system cultures for *M. tuberculosis* increased the prevalence to 5.5% in this series. Seven of 740 specimens were smear positive, four of which were positive on both culture and Xpert MTB/RIF. Of the remaining 3 specimens all were culture and Xpert MTB/RIF negative for *M. tuberculosis*, a non-tubercular mycobacterium was cultured in one case. Based on the Xpert MTB/RIF and culture results on the same sample, the Xpert MTB/RIF sensitivity, specificity, positive predictive value and negative predictive values were calculated as: 54% (95% CI 38 - 70%); 98% (95% CI 97-99%); 60% (95%CI 44-77%) and 97% (95%CI 96-98%). Table 2 takes into account contemporary positive culture results for Xpert MTB/RIF positive samples (n=5), and Xpert MTB/RIF negative samples (n=3), improving sensitivity to 56% (95% CI 40-70.4%) and specificity to 99% (95%CI 98-99%)

Overall Xpert MTB/RIF’s sensitivity for detecting MTB in CSF was 55%. This is similar to the 59% determined by Nhu et al ‘s study of 379 patients with probable TBM and Patel at al’s figure of 62% in their South African study of over 200 predominantly HIV positive patients. However it is significantly lower than the 85% sensitivity reported in an Italian
study including only 11 MTB cases\textsuperscript{7}. These studies utilised clinical diagnosis as well as culture as the reference standard reflecting the difficulties in diagnosing MTB microbiologically. Unfortunately clinical and laboratory information such as cell count are rarely provided to the NMRL by the referring hospital, making it impossible to use a composite reference standard such as that proposed by Marais et al\textsuperscript{8}. Differences in reference standards, make head-to-head comparison with the other studies difficult.

The Vietnamese study was conducted in a tertiary specialist hospital. An MTB case series from the same hospital reported mortality in excess of 30\% possibly reflecting advanced stages of MTB disease. Bacillary burden might be higher in advanced cases of MTB resulting in a high sensitivity of laboratory diagnosis. Patel et al noted lower Xpert sensitivities in HIV negative patients with TBM (42\%). In contrast our study included samples from routine clinical practice, drawn from general and specialist hospitals across England. Both prevalence of MTB and the bacillary burden of patients presenting with MTB are highly context specific. Centrifugation and sample volumes might explain differences in sensitivity of Xpert MTB/RIF in different settings: Bahr et al describe a sensitivity of 72\% when comparing Xpert analysis of centrifuged CSF directly with Xpert on 2ml unprocessed CSF (sensitivity 28\%), and against microscopy and culture\textsuperscript{9}. Median CSF samples in this Ugandan study were large (6ml), whereas the NMRL is typically sent 0.5-1ml.

Culture sensitivity is also a function of sample volume. To address the issue of false negative cultures due to low CSF volumes this study took account of other culture results within 7 days of the sample received for Xpert MTB/RIF testing. It is still possible that small culture volumes and undisclosed TB treatment account for a proportion of Xpert MTB/RIF ‘false positive’ results. If samples were submitted with fuller clinical details, including HIV status, imaging findings and cell counts we would have been able to employ a more robust reference standard.
A sensitivity of 55% compared to a suboptimal reference standard (culture) is unlikely to impact heavily on clinical decision-making. At best a positive test result will confirm clinical suspicion and enable a clinician to stop broad-spectrum antibiotics. In settings with a high prevalence of rifampicin resistance, Xpert MTB/RIF results may confer some value regarding rpoB mutations. Bahr et al (9) conclude a positive culture or Xpert should be the laboratory diagnostic standard and a negative Xpert MTB/RIF result should not outweigh a high level of clinical suspicion10.

Table 1: Xpert MTB/RIF compared to culture for identification of Mycobacterium tuberculosis complex

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<th>Culture positive</th>
<th>Culture negative</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Xpert MTB/RIF positive</td>
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<td>13*</td>
<td>33</td>
</tr>
<tr>
<td>Xpert MTB/RIF negative</td>
<td>17</td>
<td>690**</td>
<td>707</td>
</tr>
<tr>
<td>Total</td>
<td>37</td>
<td>703</td>
<td>740</td>
</tr>
</tbody>
</table>

* Includes 5 instances where specimen was not culture positive but a contemporary CSF specimen was culture positive

**Includes 3 instances where other contemporary CSF/CNS cultures were positive for M. tuberculosis
Table 2: Xpert MTB/RIF compared to culture, including contemporary (within 7 days)

CSF samples for identification of Mycobacterium tuberculosis complex

<table>
<thead>
<tr>
<th></th>
<th>Culture positive</th>
<th>Culture negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xpert MTB/RIF</td>
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<tr>
<td>positive</td>
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<td>Xpert MTB/RIF</td>
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</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>690</td>
<td>735</td>
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*5 Xpert MTB/RIF positive and MGIT culture negative results were excluded as request forms stated the patients were on TB treatment

PLEASE ENSURE THAT THE REFERENCES ARE ALL IN THE SAME FORMAT. THANKS


