Xpert(®) MTB/RIF assay sensitivity with different methods of CSF processing for the diagnosis of TB meningitis

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patients and our epidemiological context with high co-infection rates, efforts are needed to attain maximum HIV testing coverage. Risk perception and access to health care may be barriers to be addressed in Northern Portugal.

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References


Xpert® MTB/RIF assay sensitivity with different methods of CSF processing for the diagnosis of TB meningitis

A 64-year-old woman born in India was admitted to our hospital with headache and fever of 3 weeks, associated with transient diplopia and dizziness. On examination, the patient was alert with mild neck stiffness and left fourth cranial nerve palsy. Laboratory tests were within normal values (including leucocyte count and C-reactive protein, human immunodeficiency virus negative), except for moderate hyponatraemia. Magnetic resonance imaging of the brain showed a 2 mm cerebellar lesion.

Lumbar puncture showed mononuclear pleocytosis with low glucose concentrations and elevated proteins. No acid-fast direct stain could be performed due to the scarcity of cerebrospinal fluid (CSF). The Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) was initially negative for Mycobacterium tuberculosis complex using 0.5 ml vortexed CSF. A repeated spinal fluid sample was Xpert-positive, with no rifampicin resistance.

An intensified regimen containing high-dose rifampicin, moxifloxacin, isoniazid and pyrazinamide was started, together with dexamethasone. The following day, the patient developed comatose mental status related to hydrocephalus, requiring placement of a lumbar drainage device. A large volume of CSF (30 ml) was sent for repeated investigations, as detailed below. After the drainage and initiation of treatment, progressive improvement in neurological status was observed. The patient’s outcome was good, with no neurological sequelae. Cultures were positive for susceptible M. tuberculosis.

The performance of the Xpert assay was assessed by evaluating four aliquots of CSF treated differently before polymerase chain reaction (PCR), as follows: aliquot 1, 0.5 ml CSF without vortexing and without centrifugation; aliquot 2, 0.5 ml CSF with vortexing and without centrifugation; aliquot 3, a 0.5 ml pellet obtained by centrifugation of 1 ml CSF for 10 min at 3500 × g followed by vortexing; and aliquot 4, 0.5 ml supernatant obtained by centrifugation at the above conditions. The PCR results are summarised in the Table.

The quantitative PCR data showed that vortexing CSF before PCR seemed not to affect the sensitivity of the assay. However, when PCR was performed on the CSF pellet (aliquot 3) rather than on the supernatant (aliquot 4), a difference was observed in that only the pellet sample was positive, and the threshold cycles were lower than for the non-centrifuged CSF aliquots (1 and 2), thus further improving the sensitivity of the assay.

TB meningitis remains a difficult diagnosis, and improvements in the sensitivity of diagnostic tools, allowing rapid confirmation of the diagnosis, are still needed. Sole reliance on a false-negative test result may delay treatment initiation. Several studies have examined the accuracy of the Xpert assay on CSF, showing that while specificity is consistently very high, sensitivity values are heterogeneous and depend on sample size, tuberculosis (TB) prevalence and the test used as gold standard.1–4 As a consequence, the World Health Organization (WHO) has endorsed...
using Xpert in preference to conventional microscopy as the initial diagnostic test for patients presumed to have TB meningitis, given the urgency of obtaining a rapid diagnosis.

The effect of CSF sample preparation on test sensitivity may also be of key importance. Although the WHO Xpert implementation manual recommends centrifugation for samples of \( \geq 5 \) ml, CSF preparation is not standardised among studies, some of which use a concentrated\(^4\) and others a native specimen.\(^5\) A growing body of evidence suggests, however, that centrifugation of CSF is recommended, if a sufficient amount of fluid is available, to improve sensitivity. Our results confirm that testing concentrated rather than native CSF lowers the cycle threshold for detection, and may thereby increase the sensitivity of the PCR assay.

In conclusion, we recommend performing the Xpert assay on concentrated CSF whenever possible. At present, Xpert can neither exclude the diagnosis of TB meningitis nor obviate the need for urgent empirical therapy when clinical suspicion is high, despite its improved sensitivity.

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**Table**

<table>
<thead>
<tr>
<th>Aliquot*</th>
<th>Probe A†</th>
<th>Probe B†</th>
<th>Probe C†</th>
<th>Probe D†</th>
<th>Probe E†</th>
<th>SPC‡</th>
<th>Reported result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>31.4</td>
<td>30.5</td>
<td>31.1</td>
<td>31.3</td>
<td>33.0</td>
<td>24.5</td>
<td><em>M. tuberculosis detected; very low</em></td>
</tr>
<tr>
<td>2</td>
<td>31.2</td>
<td>31.0</td>
<td>31.1</td>
<td>31.4</td>
<td>33.4</td>
<td>26.5</td>
<td><em>M. tuberculosis detected; very low</em></td>
</tr>
<tr>
<td>3</td>
<td>29.7</td>
<td>28.5</td>
<td>29.0</td>
<td>29.6</td>
<td>30.9</td>
<td>23.1</td>
<td><em>M. tuberculosis detected; very low</em></td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>25.5</td>
<td><em>M. tuberculosis not detected</em></td>
</tr>
</tbody>
</table>

* See text for description.
† Probes A to E targeting various \( rpoB \) gene mutations associated with resistance to rifampicin.
‡ Internal extraction-amplification control; this control is included in the assay and co-processed with every individual specimen.
§ Quantity of target detected.

PCR = polymerase chain reaction; SPC = sample processing control.

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**References**


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Can resuscitation-promoting factors be used to improve culture rates of extra-pulmonary tuberculosis?

Mycobacterial culture is the gold standard for tuberculosis (TB) diagnosis, and it is essential for ascertaining drug susceptibility and strain typing. In the United Kingdom nearly half of all TB cases are extra-pulmonary, most commonly lymph node TB (35.6% of all cases). Culture rates for extra-pulmonary TB are significantly lower than for pulmonary disease, leading to greater diagnostic and management uncertainty in this group.

Possible reasons for lower culture rates include existence of fewer mycobacteria at extra-pulmonary sites and/or reduced growth potential of these organisms. The host immune system’s ability to stimulate the transition of *Mycobacterium tuberculosis* to a non-replicating state that requires external stimulation to reinitiate growth is well recognised. Previous studies of *M. tuberculosis* in spumt reveal a subpopulation of bacteria that do not grow in standard conditions but require an exogenous source of resuscitation-promoting factor (Rpf) for growth. Rpf is a family of enzymes secreted by growing mycobacteria that have been implicated in the resuscitation and growth stimulation of dormant...