

Xpert MTB/RIF Ultra: Optimal procedures for the detection of *Mycobacterium tuberculosis* in cerebrospinal fluid



ARTICLE INFO

Keywords:

Tuberculosis
Meningitis
Cerebrospinal fluid
Xpert
PCR

ABSTRACT

Tuberculosis is the leading infectious cause of death globally and extra-pulmonary disease occurs in 15% of incident cases annually. Tuberculous meningitis (TBM) is arguably the most lethal form of tuberculosis and requires prompt diagnosis and initiation of treatment to prevent death and serious neurological disability. The development of rapid diagnostic tests using polymerase chain reaction (PCR) technology for the detection of *Mycobacterium tuberculosis* (MTB), including the World Health Organization (WHO) – endorsed Xpert MTB/RIF Ultra assay, has allowed earlier definite diagnosis of TBM than conventional culture methods which usually take two weeks or longer for positive identification of MTB. Detection of MTB in cerebrospinal fluid (CSF) using PCR assays requires special attention to the collection, handling, and processing of CSF. Herein we present best practices guidance to maximize the detection rate of MTB in CSF using Xpert MTB/RIF Ultra.

1. Introduction

Xpert MTB/RIF (Xpert) is a cartridge-based fully automated nucleic acid amplification technology used worldwide since 2010 for the rapid detection of MTB and to determine resistance of MTB to rifampicin (RIF) in pulmonary and extra-pulmonary samples [1]. Xpert uses a single amplification target, the MTB *mpoB* gene. Xpert MTB/RIF Ultra (Xpert Ultra) is a second-generation test recently developed to boost the sensitivity for detection of MTB to be comparable to standard MTB culture [1,2]. Xpert Ultra includes two additional amplification targets, the IS6110 and the IS1081 genes, and has a larger PCR chamber volume (50 μ l versus 25 μ l). In analytical studies of sputum samples spiked with a test strain of MTB, the calculated limits of detection for Xpert Ultra and Xpert were 15.6 and 112.6 colony forming units/ml respectively [2]. A semi-quantitative category of “trace” was introduced with Xpert Ultra to report samples positive for IS6110 and/or IS1081 but negative for *mpoB*. Trace positivity does not provide information on RIF resistance.

In March 2017, the WHO recommended Xpert Ultra as an alternative to Xpert as the initial diagnostic test for adults and children with suspected tuberculosis including TBM. Xpert Ultra is currently being rolled out globally to replace Xpert. Both Xpert and Xpert Ultra are included on the WHO Model List of Essential In Vitro Diagnostics for healthcare facilities with clinical laboratories [3]. However, testing of specimens other than sputum is considered an off-label use of both assays. The manufacturer Cepheid does not provide technical instructions for extra-pulmonary specimens. In this paper, we provide our recommendations for the safe and optimal collection, handling, and processing of CSF specimens to maximize the detection rate of MTB using Xpert Ultra.

2. How should CSF be collected during lumbar puncture?

CSF should be collected during lumbar puncture into sterile plain plastic tubes with leak-proof screw caps. Screw caps allow the gentle opening of tubes, reducing the chances of spilling or splashing of the contents. In many low-resource settings, however, blood collection tubes are routinely used to collect CSF, a practice which may lead to false-negative results on PCR-based tests. A large number of substances can inhibit PCR including additives to blood collection tubes; EDTA (purple/lavender top tubes) and heparin (green top tubes) [4,5]. Blue top blood collection tubes contain sodium citrate which, like EDTA, can bind Mg^{2+} , a co-factor for PCR [4]. Red top blood collection tubes can be used if sterile plain tubes are not available. However, they contain clot activators (e.g. silica or glass particles) and it is not known if these particles can adversely affect the Xpert Ultra assay [6]. A reasonable option is the slow aspiration of a small volume of CSF from the lumbar puncture needle into a sterile syringe which can be delivered directly to the laboratory, although this technique carries an increased risk of post-lumbar puncture headache [7]. We are not aware of any studies that have compared the performance of Xpert or Xpert Ultra on CSF collected in blood collection tubes versus plain tubes. The risk of obtaining false-negative results with Xpert and Xpert Ultra if CSF is collected in blood collection tubes is therefore theoretical.

In the case of a traumatic lumbar puncture, three to four tubes of CSF should be collected and the last tube with the least amount of blood should be tested. Hemoglobin and other blood components are known inhibitors of PCR [8]. Xanthochromic CSF, due to subarachnoid hemorrhage or jaundice, may cause a false negative result since bilirubin is a reported PCR inhibitor [8]. Finally, the required test sample volume for Xpert Ultra cartridges is 2 ml. For direct testing of undiluted CSF with Xpert Ultra, a minimum of 2 ml should be collected in addition to the necessary CSF volume for routine analyses and other microbiological studies.

<https://doi.org/10.1016/j.jctube.2019.01.002>

Received 9 August 2018; Received in revised form 2 January 2019; Accepted 3 January 2019

2405-5794/ © 2019 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

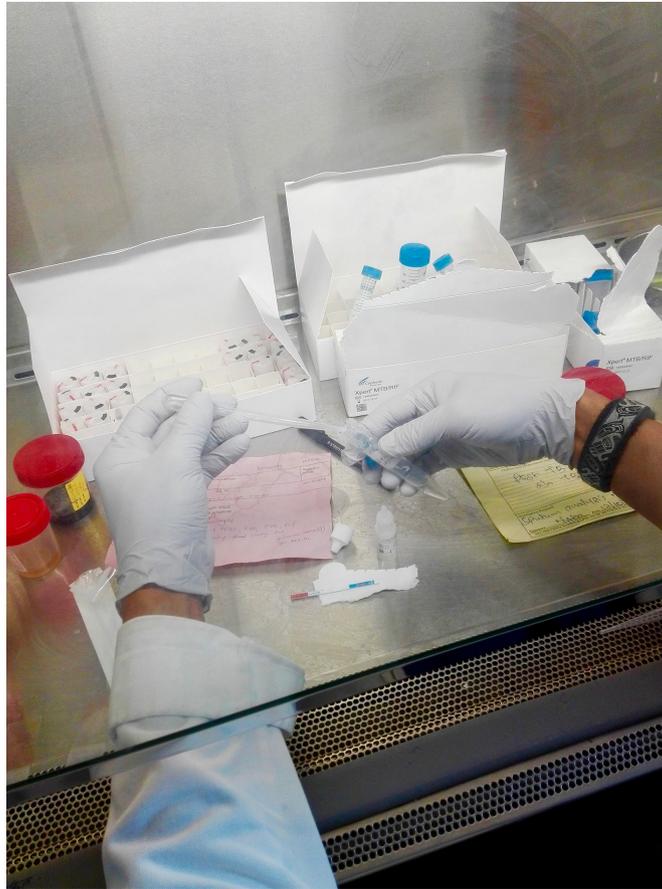


Fig. 1. Safe handling of a cerebrospinal fluid specimen in a biological safety cabinet (photo taken in Kampala, Uganda).

3. Does centrifugation of CSF improve the detection rate of Xpert Ultra?

Published studies of Xpert and Xpert Ultra for the diagnosis of TBM have used widely different protocols for processing CSF before testing. Comparing CSF samples from the same patients, Bahr et al. [9] reported improved sensitivity of Xpert with centrifugation (5/18 positive for uncentrifuged CSF versus 13/18 positive for centrifuged CSF). Xpert testing was performed without the addition of sample reagent (2 ml of CSF test sample) and the median volume of CSF centrifuged was 6 ml. Patel et al. [10] reported a higher sensitivity of Xpert on centrifuged CSF versus uncentrifuged CSF. However, this finding was based on a comparison of the results of testing centrifuged CSF samples ($N = 34$) with the results of testing uncentrifuged CSF samples ($N = 84$) obtained from mostly different patients (only 12 samples were paired samples from the same patients). The investigators separately reported the results of Xpert testing of the available paired CSF samples from the same patients and found no difference in Xpert sensitivities between centrifuged and uncentrifuged samples (58% versus 67%, $p = 0.6$). Xpert testing was performed using 1.0 ml of CSF test sample diluted with 1.0 ml of sample reagent and the volume of CSF centrifuged was 3 ml. Luo et al. [11] tested CSF spiked with MTB using nine different sample processing protocols, e.g. varying the amount of sample and sample reagent and centrifugation or no centrifugation, and found that differences in Xpert cycle threshold values may be due to intra-assay variations and not related to the protocol used.

The only published investigation of Xpert Ultra for the diagnosis of TBM reported a higher sensitivity of Xpert Ultra (70%) compared to Xpert (43%) for the detection of MTB in CSF obtained from 23 HIV-infected individuals with probable or definite TBM [12]. In this study, CSF (median 8 ml) was centrifuged and all but 2 ml of supernatant was

removed. For Xpert testing, 1.0 ml of the remaining 2 ml was mixed with 1.0 ml of sample reagent. For Xpert Ultra testing, 0.5 ml of the remaining 2 ml was mixed with 1.5 ml of sample reagent. This 1:4 dilution essentially returned the concentration of MTB bacilli in the test sample to near that of the original CSF specimen prior to centrifugation. It is likely that a similarly high sensitivity of Xpert Ultra would have been obtained by testing 2 ml of uncentrifuged and undiluted CSF.

Based on the evidence summarized above and the very high analytical sensitivity of Xpert Ultra [2], we believe that centrifugation of CSF offers a minimal advantage if any for the detection of MTB by Xpert Ultra. If centrifugation is performed, the safety of laboratory personnel needs to be ensured. Handling and processing specimens obtained from TB-infected individuals carries a risk of infection due to the potential for aerosolization of samples and subsequent inhalation of droplets [13]. The likelihood of generating infectious aerosols is lowest for direct sputum-smear microscopy, intermediate for processing and concentration of specimens, and highest for manipulation of cultures. Centrifuging, vortexing, and shaking of liquid samples such as CSF must be performed in biological safety cabinets (Fig. 1) which are not available in many laboratories in high-burden resource-limited regions.

4. Is dilution of CSF with sample reagent necessary?

The manufacturer-supplied sample reagent, which contains sodium hydroxide and isopropyl alcohol, is designed for the liquefaction and decontamination of sputum and tissue specimens. Since CSF is a liquid specimen, addition of sample reagent is not necessary and doing so will result in the dilution of the MTB bacillary concentration in the test sample, potentially reducing the likelihood of a positive detection result. To our knowledge, the report of Bahr et al. [9] is the only study of Xpert that tested CSF without dilution with sample reagent (see details

in section above). Our laboratory protocol is to test uncentrifuged and undiluted CSF directly with Xpert and Xpert Ultra. We started using Xpert Ultra in October 2017 for the routine testing of clinical samples and have since tested CSF obtained from 11 patients with probable or definite TBM defined by uniform case definition [14]. Xpert Ultra results were positive for 7 of the CSF samples, Xpert results were positive for 4 of the CSF samples, and both Xpert Ultra and Xpert were negative for 4 of the CSF samples (unpublished observations).

5. Summary recommendations for the collection, handling, and processing of CSF for testing with Xpert Ultra

- (1) CSF should be collected in sterile plain plastic tubes, preferably with screw caps. A minimum of 2 ml should be collected for Xpert Ultra testing. Additional CSF should be obtained for routine analyses and other studies as needed.
- (2) CSF (2 ml) should be slowly pipetted directly into the Xpert Ultra cartridge. CSF should only be diluted with the manufacturer-supplied sample reagent if less than 2 ml of CSF are available for Xpert Ultra testing.
- (3) Centrifugation of CSF can be performed, if desired, when more than 2 ml of CSF are available for Xpert Ultra testing and biological safety cabinets are employed for the safe handling of centrifuged specimens. After centrifugation, supernatant should be removed to leave 2 ml of CSF sample for direct application into the Xpert Ultra cartridge.
- (4) If the Xpert Ultra result is negative or trace detected, a second assay should be performed with a CSF sample from the same specimen if available and/or a new specimen if possible.

6. Conclusions and future directions

The scale-up of Xpert Ultra access worldwide holds the potential to improve the diagnosis, treatment, and outcomes of persons with TBM. Careful attention to the collection, handling, and processing of CSF as recommended here will maximize the rate of detection of MTB using Xpert Ultra. In contrast to pulmonary tuberculosis, Xpert has been reported to have substantially reduced sensitivities for diagnosing TBM in patients without HIV infection compared to HIV-infected patients [10,15], suggesting that MTB bacillary loads in CSF are lower in individuals without HIV infection. Prospective studies are needed to compare the performances of Xpert Ultra for detecting TBM in patients with and without HIV infection, including children, using identical CSF testing protocols. In practice, the diagnosis of TBM should be made using experienced clinical judgement after integrating clinical information, radiologic findings, and CSF results, including Xpert and/or Xpert Ultra tests if available [14,16].

Acknowledgments

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. All authors report no conflicts of interest.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jctube.2019.01.002.

References

- [1] WHO. WHO Meeting Report of a Technical Expert Consultation, Non-Inferiority Analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF, <http://www.who.int/tb/publications/2017/XpertUltra/en/>, Date last accessed: January 2, 2019.
- [2] Chakravorty S, Simmons AM, Rowneki M, Cao Y, Ryan J, Banada PP, et al. The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. *mBio* 2017;8:e00812–7. <https://doi.org/10.1128/mBio.00812-17>.
- [3] WHO. World Health Organization model list of essential in vitro diagnostics. <http://apps.who.int/medicinedocs/documents/s23461en/s23461en.pdf>. Date last accessed: January 2, 2019.
- [4] Schrader C, Schielke A, Ellerbroek L, John R. PCR inhibitors – occurrence, properties and removal. *J Appl Microbiol* 2012;113:1014–26.
- [5] Huggett JF, Novak T, Garson JA, Green C, Morris-Jones SD, Miller RF, et al. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon. *BMC Res Notes* 2008;1:70. <https://doi.org/10.1186/1756-0500-1-70>.
- [6] Bowen RAR, Remaley AT. Interferences from blood collection tube components on clinical chemistry assays. *Biochemia Medica* 2014;24:31–44.
- [7] Engelborghs S, Niemantsverdrieta E, Struyfs H, Blennow K, Brouns R, Comabella M, et al. Consensus guidelines for lumbar puncture in patients with neurological diseases. *Alzheimer's Dement* 2017;8:111–26.
- [8] Al-Soud WA, Radstrom P. Purification and characterization of PCR-inhibitory components in blood cells. *J Clin Microbiol* 2001;39:485–93.
- [9] Bahr NC, Tugume L, Rajasingham R, Kiggundu R, Williams DA, Morawski B, et al. Improved diagnostic sensitivity for tuberculous meningitis with Xpert MTB/RIF of centrifuged CSF. *Int J Tuberc Lung Dis* 2015;19:1209–15.
- [10] Patel VB, Theron G, Lenders L, Matinyena B, Connolly C, Singh R, et al. Diagnostic accuracy of quantitative PCR (Xpert MTB/RIF) for tuberculous meningitis in a high burden setting: a prospective study. *PLOS Med* 2013;10(10):e1001536 <https://doi.org/10.1371/journal.pmed.1001536>.
- [11] Luo RF, Gaur RL, Banaei N. Using cerebrospinal fluid for the diagnosis of tuberculous meningitis with GeneXpert. *Eur Respir J* 2014;44:1094–5.
- [12] Bahr NC, Nuwagira E, Evans EE, Cresswell FV, Bystrom PV, Byamukama A, et al. Diagnostic accuracy of Xpert MTB/RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. *Lancet Infect Dis* 2018;18:68–75.
- [13] WHO. Tuberculosis Laboratory Biosafety Manual, http://www.who.int/tb/publications/2012/tb_biosafety/en/, Date last accessed: January 2, 2019.
- [14] Marais S, Thwaites G, Schoeman JF, Török ME, Misra UK, Prasad K, et al. Tuberculous meningitis: a uniform case definition for use in clinical research. *Lancet Infect Dis* 2010;10:803–12.
- [15] Nhu NT, Heemskerck D, Thu do DA, Chau TT, Mai NT, Nghia HD, et al. Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2014;52:226–33.
- [16] Chin JH. Xpert MTB/RIF Ultra: the long-awaited game changer for tuberculous meningitis? *Eur Resp J* 2017;50(4):1701201 <https://doi.org/10.1183/13993003.01201-2017>.

Jerome H. Chin^{a,*}, Willy Ssengooba^b, Scott Grossman^a, Jacob Pellinen^a, Vincent Wadda^c

^a Department of Neurology, NYU Langone Health, New York, NY, USA

^b Department of Medical Microbiology, Makerere University, Kampala, Uganda

^c Department of Medicine, Makerere University, Kampala, Uganda

E-mail address: jerome.chin@nyulangone.org (J.H. Chin).

* Corresponding author at: Department of Neurology, NYU Langone Health, New York, NY 10016, USA.