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Xpert MTB/RIF Ultra in the auxiliary diagnosis of tuberculosis among people living with human immunodeficiency virus

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SUMMARY Clinical diagnosis of tuberculosis (TB) in people living with the human immunodeficiency virus (HIV) poses a challenge. The Xpert MTB/RIF Ultra (Ultra) has displayed greater sensitivity at diagnosing tuberculosis and rifampicin resistance compared to the Xpert MTB/RIF (Xpert). However, whether Ultra is able to facilitate an auxiliary diagnosis of TB in patients with an HIV-TB co-infection remains unclear. Accordingly, the current study evaluated the use of Ultra in patients with an HIV-TB co-infection by summarizing relevant studies. The sensitivity and specificity of Ultra and Xpert at diagnosing patients with an HIV-TB co-infection have been summarized and compared. The performance of Ultra in diagnosing extrapulmonary tuberculosis was also summarized. Although a large-cohort, multi-center study needs to be conducted to assess Ultra's ability to detect TB in AIDS patients in the future, the current evidence supports the use of Ultra for the assessment of patients with an HIV-TB co-infection.

Keywords Xpert MTB/RIF Ultra, HIV, tuberculosis, diagnosis

People living with human immunodeficiency virus (PLHIV) are susceptible to tuberculosis (TB) due to their deficient immunity, and this leads to accelerated disease progression (*1*). However, cases of an HIV-TB co-infection are difficult to diagnose due to their subtle clinical manifestations and low rate of detection by conventional diagnostic assays (*2*). Therefore, a rapid and precise method of auxiliary diagnosis of an HIV-TB co-infection is urgently required. The Xpert MTB/RIF (Xpert) assay is a classic rapid nucleic acid amplification test (NAAT) that rapidly measures *Mycobacterium tuberculosis* and rifampicin resistance and that has a high level of sensitivity. However, Xpert has been reported to have inferior performance in diagnosing an HIV-TB co-infection compared to TB alone (*3*). To solve this problem, the Xpert MTB/RIF Ultra (Ultra) was updated by Cepheid (Sunnyvale, USA). As a new-generation rapid molecular test, Ultra has a larger chamber for DNA amplification and 2 multicopy amplification targets for TB (IS6110 and IS1081, for a lower limit of detection of 16 CFU/mL), which renders it more sensitive than Xpert (*4*). Thus, Ultra may improve the detection of TB among PLHIV. However, the ability of Ultra to detect TB among PLHIV has not yet been adequately investigated. Summarized here is the diagnostic performance of Ultra in patients with an HIV-TB co-infection.

To summarize the sensitivity and specificity of Ultra's performance, there are both advantages and disadvantages in Ultra's diagnosis of TB among AIDS patients. On the one hand, Ultra is more sensitive at diagnosing TB among AIDS patients. Berhanu *et al*. reported that Ultra's sensitivity was 11.7% higher (88.2% *vs*. 76.5%) in adult HIV-positive patients compared to Xpert (*5*). Ultra's sensitivity has been found to be superior to that of Xpert only in patients with an HIV-TB co-infection. Similar results have been reported by Dorman *et al*., who found that Ultra was 13% more sensitive than Xpert (90% *vs*. 77%) (*6*). Moreover, Ultra has also displayed higher sensitivity in children with HIV. A comparative study reported that Ultra's sensitivity was 22.2% higher (88.9% *vs*. 66.7%) in children with an HIV-TB co-infection than Xpert (*7*). In 2020, a two-cohort study was conducted by Mishra *et al*. in South Africa (*8*). They reported positive results only in HIV-TB cases in cohort A, where Ultra's sensitivity increased by 14% (81% *vs*. 67%), but there was little difference in sensitivity in HIV-negative patients (87% *vs*. 89%). They reported in cohort B that, unlike in cohort A, Ultra's sensitivity decreased in HIV-positive cases (87% *vs*. 92%), and Ultra's sensitivity decreased by 13% (94% *vs*. 81%) in HIV-negative patients.

On the other hand, Ultra's specificity in diagnosing

Table 1. Sensitivity and specificity: Xpert MTB/RIF Ultra *vs*. Xpert MTB/RIF

Cohorts	Samples (*n*)	Xpert MTB/RIF Ultra		Xpert MTB/RIF		*Ref.*
		Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	
HIV-positive adults	48	(81; 59-95)	(78; 58-92)	(67; 44-86)	(100; 88-100)	(*8*)
	121	(87; 47-99)	(71; 55-84)	(92; 64-99)	(86; 75-94)	(*8*)
	147	(88.2; 72.5-96.7)	(94.7; 88.8-98)	(76.5; 58.8-89.3)	(100; 96.8-100)	(*5*)
	115	(77; 68-84)	/	(90; 83-95)	/	(*6*)
HIV-positive children	9	(88.9; 51.8- 99.7)	/	(66.7; 29.9-92.5)	/	(*7*)

This table shows the performance of Xpert MTB/RIF Ultra and Xpert MTB/RIF in diagnosing HIV according to the original papers listed. The tests were compared in the same studies.

Table 2. Diagnostic performance of Xpert MTB/RIF Ultra in detecting extrapulmonary tuberculosis

Specimens	HIV-positive patients	Sensitivity (%)	Specificity (%)	*Ref.*
CSF	41 (98%)	(76.5; 62.5-87.2)	(100; 97.6-100)	(*11*)
CSF	129 (100%)	(70; 47-87)	/	(*12*)
CSF	103 (17%)	(64.3; 38.8-83.7)	(100; 43.9-100)	(*10*)
Lymphadenitis	77 (58%)	(91; 76-98)	(67; 50-81)	(*13*)
Pleural fluid	9 (6.5%)	(37.5; 23.8-51.2)	(98.8; 96.5-100)	(*14*)

This table shows: (i).The performance of Xpert MTB/RIF Ultra with respect to various specimens from people living with HIV according to the original papers listed; (ii).Xpert MTB/RIF Ultra has a potential advantage at diagnosing tuberculous meningitis in patients with HIV.

patients with an HIV-TB co-infection was reported to be lower than that of Xpert. Berhanu *et al*. reported that Ultra's specificity was 94.7% compared to 100% for Xpert (*5*). Although Ultra's sensitivity yielded conflicting results in a two-cohort study, Ultra's specificity was 22% (78% *vs*. 100%) and 15% (71% *vs*. 86%) lower in patients with an HIV-TB co-infection than that of Xpert (*8*). In short, Ultra has a higher sensitivity than Xpert at detecting TB in HIV-positive populations, but Ultra has a lower specificity than Xpert (Table 1). The discrepancy may be due to an insufficient sample size and may be resolved by another study with a larger sample.

M. tuberculosis is known to usually infect the lungs, but it can also infect other organs. However, due to the difficulty in obtaining extra pulmonary samples and the paucibacillary nature of the disease, the diagnosis of extrapulmonary TB (EPTB) is challenging (*19*). Many other studies have concentrated on Xpert's performance in diagnosing EPTB. A recent Cochrane study reported that Ultra had better performance, including a higher sensitivity and lower specificity, than Xpert in diagnosing EPTB (*9*). However, limited studies have thus far focused on Ultra's effectiveness in diagnosing patients with an HIV-EPTB co-infection. A search of PubMed yielded only 5 studies on Ultra's effectiveness in diagnosing patients with an HIV-EPTB co-infection (Table 2). Three of those studies on Ultra involved cerebrospinal fluid (CSF). One reported that Ultra had a sensitivity 76.5% and specificity of 100% in diagnosing patients with an HIV-EPTB co-infection, another reported that Ultra had a sensitivity of 64.3% and a specificity of 100%, and yet another reported that Ultra had a sensitivity of 70% (specificity was not reported) (*10-12*). Minnies *et al*. reported that Ultra had a sensitivity of 91% and specificity of 67% in diagnosing lymphadenitis in patients with an HIV-EPTB co-infection (*13*). Another prospective study reported that Ultra had a sensitivity of 37.5% and specificity of 98.8% when testing pleural fluid from patients with an HIV-EPTB co-infection (*14*). Recently, Boloko *et al*. reported that the Ultra was able to test pre-processed blood from suspected cases of HIV-associated TB (*15*). They provided additional prognostic information that may include other available markers. A retrospective cohort study reported Ultra's accuracy in diagnosing TB in various samples from adult patients (n = 183 (4.8%)). Kaswala *et al*. reported that Ultra's sensitivity when testing pus was 92.0%. However, Ultra had the lowest sensitivity when testing CSF (38.5%) (*16*). In a nutshell, Ultra can detect pathogens in various types of specimens, but studies with larger cohorts and at multiple centers should be conducted, and especially in the HIV-TB population.

Management and control of resistant TB relies on timely and correct diagnosis. Unlike the previous generation of the assay (Xpert), Ultra can detect rifampicin resistance by using melting temperature analysis and 4 probes that target the rpoB gene (*4*). However, Ultra and Xpert have similar performance in terms of sensitivity and specificity in detecting rifampicin resistance. Dorman *et al*. reported that Ultra and Xpert had a similar sensitivity (95% *vs*. 95%) and specificity (98% *vs*. 98%) in people living with HIV. In that study, most patients were from Belarus, China, India, and Georgia. The Ile491Phe mutation that Ultra

was able to detect was not found in patients originally from these regions, resulting in similar findings for both assays (*8*). As such, a multi-center, large-cohort study of patients with an HIV-TB co-infection may need to soon be conducted to confirm the detection of resistance.

Ultra has been reported to have an increased sensitivity and decreased specificity in diagnosing patients with an HIV-TB co-infection. However, few studies reported on testing for rifampicin resistance with Ultra in patients with an HIV-TB co-infection. Recently, Martyn *et al*. reported that Ultra's Ct values could also indicate patients with tuberculous meningitis at increased risk of death (*17*). Shapiro *et al*. reported that tongue swab testing may complement non-sputum samples for diagnosis of TB in PLHIV (*18*). However, studies at more centers and with larger cohorts need to be conducted to determine Ultra's accuracy when testing various specimens.

In summary, Ultra has a higher sensitivity at detecting an HIV-TB co-infection than Xpert. Moreover, Ultra could potentially be used to detect an HIV-EPTB co-infection in the future, and especially one involving tuberculous meningitis. However, Ultra had a lower specificity at detecting an HIV-TB co-infection than Xpert. In addition, Ultra offers no benefits over Xpert in detecting rifampicin resistance among PLHIV, but further studies are needed.

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