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Narrative review

The rapid molecular test Xpert MTB/RIF ultra: towards improved tuberculosis diagnosis and rifampicin resistance detection

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ABSTRACT

Background: Tuberculosis diagnosis has dramatically improved since the introduction of the rapid molecular test Xpert MTB/RIF (Xpert) detecting *M. tuberculosis* and rifampicin resistance directly from clinical specimens, therefore shortening the turnaround time, reducing patient's isolation period and decreasing the time to start anti-TB drugs. The new version, Xpert MTB/RIF Ultra (Ultra), displays a higher sensitivity and an improved rifampicin resistance detection. Both tests have been endorsed by the World Health Organisation.

Aims: Xpert and Ultra rapidly became widespread and paved the way for new approaches and new paradigms as well as for the development of molecular point-of-care tests (POCTs). In this narrative review, we aimed to address their performance in the diagnosis of tuberculosis and to discuss the expectations of these tests as well as their limits and the unmet needs.

Sources: Peer-reviewed publications addressing the diagnostic performance of Ultra and Xpert.

Content: We focused on publications that evaluated the performance of Ultra and Xpert on the same group of patients or the same set of specimens in different tuberculosis-burden settings.

Implications: The studies published so far reported an increased sensitivity of Ultra when compared to Xpert, which represents a benefit for tuberculosis diagnosis. The fact that such a sensitive assay cannot distinguish between alive and dead bacilli emphasizes that caution should be exercised regarding indications and interpretation of results. Additional studies are needed to determine the true performance for the diagnosis of extrapulmonary tuberculosis because of the great diversity of the specimens.

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Introduction

Over the last few decades, the microbial diagnosis of tuberculosis has been improved by the direct detection of *Mycobacterium tuberculosis* DNA from clinical specimens using polymerase chain reaction (PCR). The molecular test Xpert MTB/RIF (Xpert) that detects *M. tuberculosis* DNA and resistance to rifampicin directly from clinical specimens within approximately 2 h, has dramatically improved the diagnosis of tuberculosis (TB) by reducing the time to results. This directly impacted the time a patient is required to wait in the emergency ward, shortening the patient's isolation period and decreasing the time to initiate anti-TB drugs [1]. Xpert, which is more sensitive and specific than smear microscopy is recommended by the World Health Organization (WHO) for the 'rapid' microbial diagnosis of tuberculosis [2]. The cost-effectiveness of Xpert applied to the microbial diagnosis of tuberculosis has been addressed in regions with both a high and low prevalence of tuberculosis [3–6].

Xpert MTB/RIF has been endorsed by the WHO for the diagnosis of tuberculosis and is now widespread, both in high- and low-tuberculosis-prevalence regions and in high- and low-income areas. Due to its easy format, this test has the capacity to be used both in laboratories and at the bedside, as a point-of-care test (POCT), provided that all biosecurity issues are appropriately tackled. Prior to this, the molecular diagnosis of tuberculosis was limited only to specialized laboratories. This advancement has

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increased knowledge regarding the diagnostic performance of PCR in the diagnosis of tuberculosis. In addition, the added value of Xpert and Ultra have opened the way for future molecular POCTs. Other real-time PCR-based assays for the diagnosis of tuberculosis exist but thus far remain less automated [7].

With a limit of detection (LOD) of ~116 colony forming units (cfu) per mL, Xpert remains less sensitive than culture (LOD ~1–10 cfu/mL). In addition, the rifampicin resistance detection approach with Xpert is exposed to false-positive results [8–11]. To circumvent these limitations, the Xpert MTB/RIF Ultra (Ultra), a new version of the Xpert has been introduced. Ultra displays an improved LOD for the detection of *M. tuberculosis* (~15.6 cfu/mL) by targeting multicopy sequences, namely IS6110 (~16 copies/cell) and IS1810 (~5 copies/cell) while Xpert targeted the single copy gene *rpoB* [1,12]. The LOD of Ultra, which is getting closer to the LOD of mycobacterial culture, is valid only for respiratory samples. The specificity of rifampicin resistance in Ultra has also been improved by relying on the interpretation of the melting curves of sloppy molecular probes to detect mutations in the active site of *rpoB* involved in drug resistance [12–14]. In contrast, Xpert relied on cycle threshold (Ct) values to detect mutations, which may cause false-positive results, mainly due to insufficient amounts of DNA [1,8,10,11,15].

Ultra is expected to improve the diagnosis of tuberculosis because of its increased sensitivity for *M. tuberculosis* DNA detection and its improved specificity for the detection of *rpoB* mutations. In this narrative review, we analysed the results of recent studies comparing the performance of Ultra and Xpert in different tuberculosis-burden settings (i.e. different prevalence) and we discuss the added value of the new 'Ultra' test. We focused on studies that compared the performance of Ultra and Xpert on the same set of patients or samples. Indeed, results of studies on tuberculosis (i.e. performance of diagnostic tests) can vary widely according to the disease prevalence and to the studied population.

Performance of ultra for the diagnosis of pulmonary tuberculosis

Sensitivity

A first retrospective study compared the performance of Ultra and Xpert for the detection of *M. tuberculosis* on 277 respiratory specimens collected in five countries (Peru, Vietnam, South Africa, Georgia and India). Sensitivities of Ultra and Xpert were 88.7% and 81%, respectively, when considering all culture-positive specimens, 98.9% and 97.8% when considering smear-positive–culture-positive specimens, and 78.9% and 66.1% when considering smear-negative–culture-positive specimens [12]. In a prospective multicentre study, which included 2368 participants, sensitivities of Ultra and Xpert were 88% versus 83%, respectively, for all culture-positive specimens and 63% and 46% for smear-negative–culture-positive specimens [13]. These data did not show any difference in sensitivity between Ultra and Xpert for smear-positive specimens but a higher sensitivity of Ultra for smear-negative specimens. Similar results were obtained in a study conducted in a region of low tuberculosis prevalence; sensitivities of Ultra and Xpert were 95.7% and 82.9%, respectively, when considering all culture-positive specimens, 100% for both tests when considering smear-positive–culture-positive specimens, and 91.8% and 66.7% when considering smear-negative–culture-positive specimens [16]. This suggests an increased sensitivity for Ultra, especially for paucibacillary specimens. This may improve the diagnostic yield in paucibacillary infections such as miliary tuberculosis, tuberculous pleurisy, tuberculous meningitis or early infections.

Sensitivity in HIV positive patients

Tuberculosis is an important cause of death among people living with HIV [17,18]. Efficient diagnostic tools are crucial for prompt diagnosis and early adequate anti-TB treatment. Microbiological diagnostics based on direct detection of *M. tuberculosis* in respiratory specimens have limited sensitivity in HIV patients with miliary lung infiltrates, due in particular to paucibacillary specimens. When considering HIV-negative patients, Ultra and Xpert display similar sensitivity: 91% versus 90% [13]. When considering HIV-positive patients only, the overall sensitivity of Ultra and Xpert was 90% and 77%, respectively (Table 1, Fig. 1) [13]. Similar results have been obtained in a high-HIV-burden setting reporting a gain in sensitivity for Ultra of +11.7% in adult HIV patients whereas no gain was observed in HIV-negative patients [19].

Sensitivity in paediatric patients

Tuberculosis diagnosis in the paediatric population remains challenging. In a study conducted in Cape Town (South Africa), with 453 eligible children, the overall sensitivity and specificity of Ultra on one respiratory specimen was 75.3% and 96.9%, respectively [20]. Sensitivity of Ultra was similar in HIV-infected and HIV-uninfected children; in this study, HIV prevalence was 19.4%. Different reference standard gave different results. Ultra and Xpert sensitivity when determined on the first respiratory specimen and using culture of any specimen as reference were 65.8% and 64.4%. When using a composite reference including Xpert, Ultra, culture and clinical data, the sensitivity of Ultra (73.7%) was higher than Xpert (63.2%) (Table 1) [20]. Another study, conducted in two sites located in Tanzania, reported an increased sensitivity of Ultra when compared to Xpert performed on the first available sample: 64.3% versus 53.6%, respectively, when considering all children and all culture-positive specimens [21]. When considering all available specimens, the sensitivity of Ultra and Xpert were 75% and 60.7%, respectively, for a mean number of samples per patient of 2.46 and 2.11, respectively. For both tests, the specificity was 100% when considering only the first specimen and 98.1% for Ultra and 100% for Xpert when considering all tested specimens. Interestingly, when considering HIV-negative patients, no significant difference in sensitivity was observed between Ultra and Xpert (52.6% and 47.4%). In contrast, when considering HIV-positive patients, Ultra sensitivity (88.9%) appeared significantly higher than Xpert sensitivity (66.7%) (Table 1). This study suggested an increased sensitivity of Ultra when compared to Xpert, especially in HIV-positive paediatric patients, which represented 52% of all patients included in the study [21].

Specificity issue raised by a very sensitive nucleic-acids amplification test

Several studies conducted in medium- and high-tuberculosis-prevalence regions reported an increase in sensitivity of Ultra when compared to Xpert but a decreased specificity of Ultra (96%) as compared to Xpert (98.7%), using culture as the reference standard [12,13] (Table 1, Fig. 2). PCR-based tests are unable to discriminate between dead and alive bacilli as PCR can detect DNA from non-viable bacilli after the introduction of anti-TB drugs or from a previous history of tuberculosis, which can negatively affect the test's specificity [22–24] (Fig. 1). This is more likely to occur with ultrasensitive tests, explaining their slightly decreased specificity. In addition, decreased specificity due to a patient's previous history of tuberculosis is more likely to occur in the setting of medium to high tuberculosis prevalence when compared to region of low tuberculosis burden [16,25]. Ultra provides an additional

Table 1
Diagnostic performance of Xpert MTB/RIF Ultra and Xpert MTB/RIF

	Sensitivity ultra/sensitivity Xpert (difference ultra-Xpert)	Specificity ultra/specificity Xpert (difference ultra-Xpert)	References (number of patients or samples)
Respiratory specimen all specimen	87.5/81 (+6.5)	98.7/98.7 (0)	Chakravorty, Simmons et al., 2017 retrosp. ($n = 277$)
	88/83 (+5.4)	96/98 (-2)	Dorman, Schumacher et al., 2018 prosp. ($n = 1753$)
	89.3/82.1 (+7.2)	95.6/100 (-4.4)	Berhanu et al., 2018* ($n = 237$)
	95.7/82.9 (+12.8)	96.7/97.3 (-07)	Opota et al., 2019** ($n = 196$)
Respiratory smear positive specimen	98.9/97.8 (+1.1)	—	Chakravorty, Simmons et al., 2017 retrosp. ($n = 277$)
	100/100 (0)	—	Opota et al., 2019** ($n = 196$)
Respiratory smear negative specimen	78.9/66.1 (+12.8)	—	Chakravorty, Simmons et al., 2017 retrosp. ($n = 277$)
	63/46 (+17)	—	Dorman, Schumacher et al., 2018 prosp. ($n = 1753$)
	91.7/66.7 (+25)	—	Opota et al., 2019* ($n = 196$)
Adult HIV-positive only (all respiratory specimen)	90-77 (+13)	—	Dorman, Schumacher et al., 2018 prosp. ($n = 1753$)
	88.2/76.5 (+11.7)	94.7/100 (-5.3)	Berhanu et al., 2018 prosp. ($n = 237$)
Adult HIV-negative only (all respiratory specimen)	89.5/89.5 (0)	96.8/100 (-3.2)	Berhanu et al., 2018 prosp. ($n = 237$)
Extrapulmonary specimens	83.7/67.4 (+16.3)	92.0/96.0 (-4)	Wu et al., 2019 prosp. ($n = 200$)
Paediatric patient	64.3/53.6 (+10.7)	100/100 (0)	Sabi, Rachow et al., 2018 prosp. ($n = 215$)
	65.8/64.4 (+1.4)	96.6-99.6 (-3)	Nicol, Workman et al., 2018 ($n = 306$)[a]
	73.7/63.2 (+10.5)		Nicol, Workman et al., 2018 ($n = 76$)[b]
Paediatric patient (HIV-negative only)	52.6/47.4 (+5.2)	—	Sabi, Rachow et al., 2018 prosp. ($n = 215$)
Paediatric patient (HIV-positive only)	88.9/67.7 (+21.2)	—	Sabi, Rachow et al., 2018 prosp. ($n = 215$)
Rifampicin resistance detection	92.7/92.7 (0)	98/99 (-1)	Chakravorty, Simmons et al., 2017 retrosp. ($n = 277$)
	95/95 (0)	98/98 (0)	Dorman, Schumacher et al., 2018 prosp. ($n = 1753$)

*High number of HIV-infected patients (67%).
** Low tuberculosis prevalence.
[a] Sensitivity of one Xpert or Ultra compared to multiple cultures.
[b] Composite reference standard including medical history and all the microbiological results.

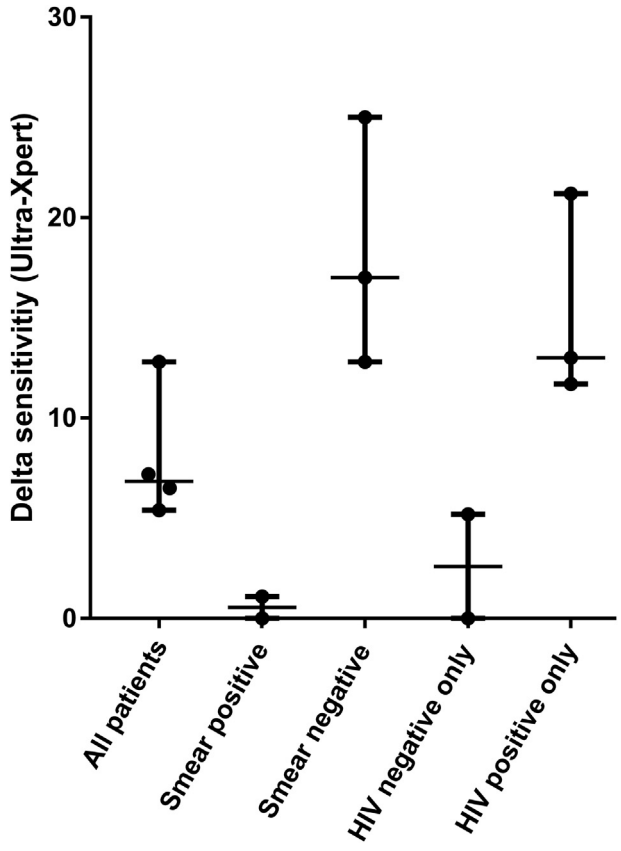

Fig. 1. Improved sensitivity of Xpert MTB/RIF Ultra as compared to Xpert MTB/RIF especially for paucibacillary samples. The data represent the difference in sensitivity (delta = Ultra − Xpert) for different patients and different clinical conditions on respiratory specimens with the exception of tuberculous meningitis which corresponds to CSF. Each dot represents data retrieved from publications comparing the performances of both tests used in the same study. The graph represents the Median with 95% confidence interval.

category called 'trace' that does not exist with Xpert, which corresponds to specimens positive for the PCR targeting the multicopy genes *IS6110* and *IS1810* and negative for the PCR targeting the single copy gene *rpoB*. The 'trace' category is the result of the improved LOD of the Ultra and probably contributes to the decreased specificity of the test. A 'trace' result represents the detection of a very low quantity of *M. tuberculosis* DNA. Clinical information of patients with Ultra 'trace' results helps to distinguish (a) ongoing active paucibacillary tuberculosis from (b) detection of dead bacilli from previous infections [16].

Tuberculosis diagnosis remains challenging and clinicians are often confronted with difficult decisions to make when tuberculosis remains culture negative. Similarly, PCR-positive–culture-negative tuberculosis needs to be analysed with caution, as demonstrated for paucibacillary specimens such as cerebrospinal fluid (CSF) that often represent true positives.

Performance for the diagnosis of extrapulmonary tuberculosis

Many studies have addressed the performance of Xpert for the diagnosis of extrapulmonary tuberculosis showing that sensitivity may vary according to the specimen tested. A recent Cochrane study conducted on Xpert reported the following sensitivity and specificity: 71.1% and 98% for CSF, 50.9% and 99.2% for pleural fluid, 82.7% and 98.7% for urine samples [26]. So far only a few studies have addressed the performance of Ultra for the diagnosis of extrapulmonary tuberculosis. The study was conducted only on smear-negative specimens and reported an overall sensitivity and specificity of 75.9% and 100%, respectively [27] (Table 1). The sensitivity on pleural fluid, a paucibacillary specimen of poor sensitivity [28,29] was only 60.5%. When considering other sterile sites, the sensitivity was 100% (3/3) for CSF, 87.5% (7/8) for joint fluid, 33.3% (1/3) for ascitic fluid, and 66.6% (2/3) for pericardial fluid. The sensitivity was 100% (12/12) for urine samples. In this study, the specificity was 100% for all tested specimens. However, because of the small number of samples, the calculated performances require confirmation with larger studies. In a prospective

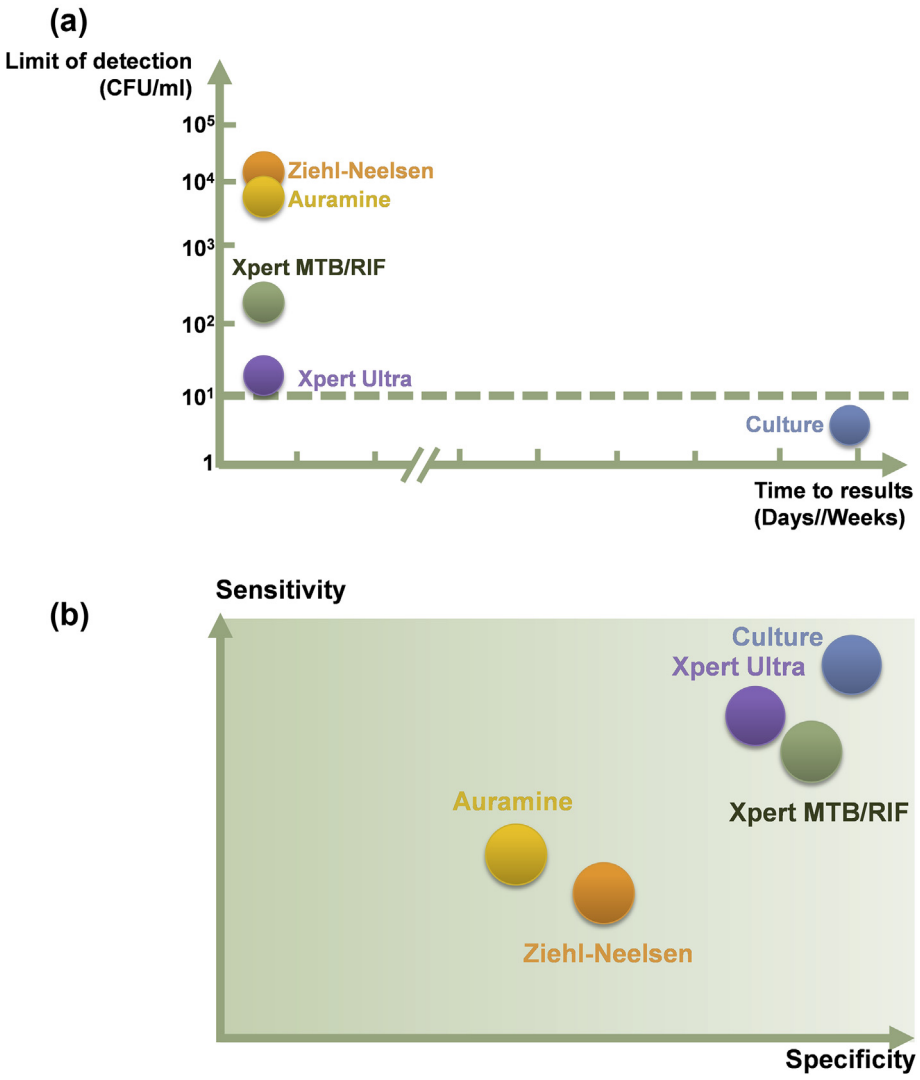

Fig. 2. Performance of Xpert MTB/RIF and Xpert MTB/RIF Ultra. Representation of the performance of some microbial diagnostic tests for tuberculosis.

study that included 200 cases of extrapulmonary tuberculosis the overall sensitivity of Ultra and Xpert was 83.7% and 67.4%, respectively, and the specificity was 92% and 96%, respectively [30]. The study included 103 pleural fluid samples for which the detection rate of Ultra and Xpert were 43.7% and 20.4%, respectively. The study also included 71 fine-needle aspirations of tissues for which the detection rate of Ultra and Xpert was 78.9% and 63.4%, respectively; this was slightly higher than that of pleural fluid. Among smear-negative specimens, the sensitivity of Ultra and Xpert on pleural fluid ($n = 108$) was 61.11% versus 34.26% [31].

Tuberculous meningitis

The diagnosis of tuberculous meningitis, a life-threatening manifestation of extrapulmonary tuberculosis, remains challenging and is often delayed and associated with a high mortality rate (>60% in HIV-infected patients) [32,33]. In particular, the sensitivity of the microbial diagnostics in CSF is not satisfying because tuberculous meningitis is a paucibacillary infection. Ultra and Xpert were not initially considered for CSF but have been tested in several studies. Wang and colleagues observed an increased sensitivity of Ultra (44.19%) when compared with Xpert (18.60%) on CSF of HIV-negative patients ($n = 43$ patients with tuberculous meningitis) [31]. In another study performed in HIV-positive patients (n = 23 patients with probable or definite tuberculous meningitis), the sensitivity of Ultra and Xpert for the diagnosis of tuberculous meningitis in HIV positive patients was 70% and 43%, respectively (Table 1, Fig. 1) [34]. When using a composite definition of tuberculous meningitis, as any CSF that tested positive by microscopy, Xpert, Ultra or Culture, sensitivity of Ultra and Xpert and culture was 95%, 45% and 45%, respectively. Tuberculous meningitis is challenging because CSF volume is often limited. In this study, 1 mL of centrifuged CSF was analysed with Xpert and Ultra [32]. Microbiological diagnosis positively correlates with the volume of specimen; however, a large initial volume of CSF is not always available in routine diagnostics. This study also underlines the high rate of culture-negative tuberculous meningitis [34].

Altogether, these data suggest an increased sensitivity of Ultra when compared to Xpert for the diagnosis of extrapulmonary tuberculosis. Nevertheless, care must be taken because of the limited number of studies and the limited number of samples. Noteworthy, the performance of these rapid molecular tests may largely vary depending on the sample.

Rifampicin resistance detection

A major limitation of Xpert was the occurrence of false-positive results for rifampicin detection. The positive predictive value of

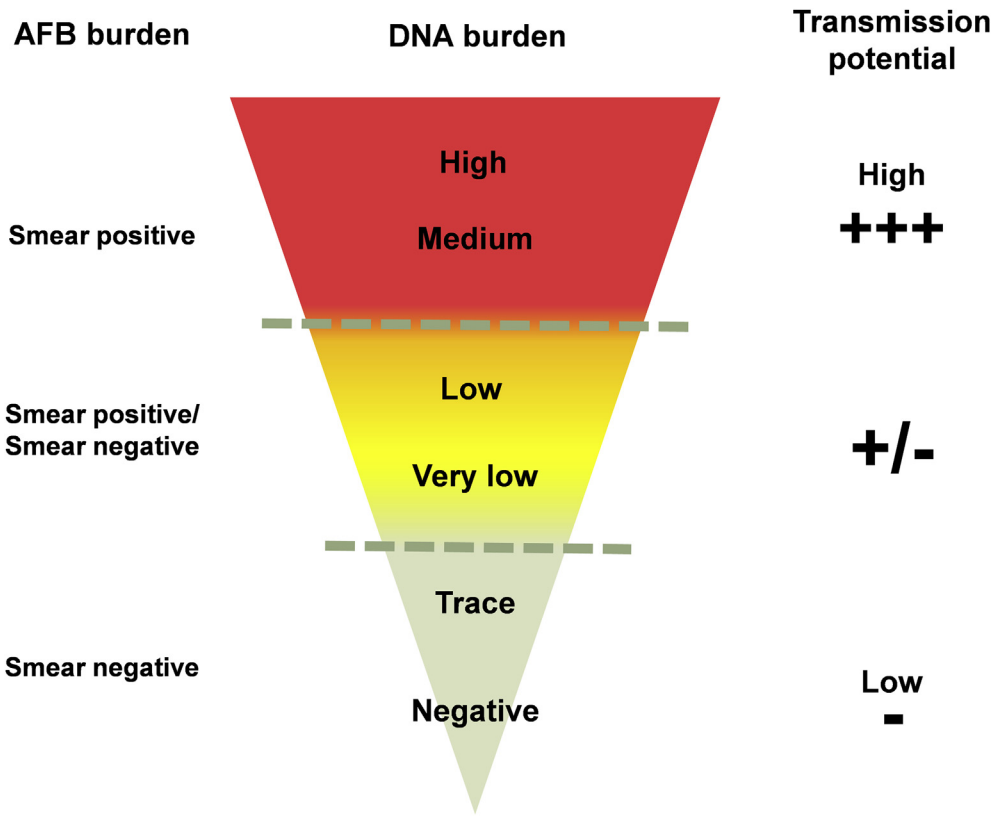

Fig. 3. Correlation between DNA burden and smear microscopy results. The semi-quantitative results of Xpert and Ultra high, medium, low, very low (trace for Ultra only) and negative were found to positively correlate with acid-fast bacilli detection (smear microscopy) and could be used to evaluate patient's transmission potential and guide isolation decision in smear-independent algorithms. Patients with negative Xpert could be considered poorly infectious, whereas patients with positive results high and medium should be considered as the most infectious; patients with Xpert low, very low or trace should be considered as potentially infectious and other clinical information should be considered to guide isolation measures. This algorithm may apply for any quantitative or semi-quantitative nucleic acid amplification test.

rifampicin resistance detection ranged from 70% to 90% for setting of MDR-TB<5% and MDR-TB>15% [8–11,15]. This was due to the fact that the detection of rifampicin resistance relies, with Xpert, on the absence of binding of specific DNA probes on the 81-bp region of the *rpoB* genes in which mutations occur and were more likely to occur in specimens with low bacterial load. In the new Ultra cartridge, the resistance to rifampicin is detected through a new resistance detection algorithm analysing molecular probes melting curves [13]. Thus, the Ultra is unlikely to produce false-positive results. Ultra and Xpert display similar sensitivity for the detection of rifampicin resistance (92.7–95%), this is due to the fact that both tests target the single-copy gene *rpoB* [12,13]. Interestingly, no significant difference in specificity for rifampicin resistance detection was observed between Ultra (98%) and Xpert (99%) [12,13] (Table 1). Larger studies including a higher number of paucibacillary tuberculosis, corresponding to specimens with very low quantities of *M. tuberculosis* DNA, might be necessary to address the benefit of the new resistance-detection algorithm.

Correlation between the semi-quantitative result of ultra and smear microscopy

Several publications have reported on the correlation between the semi-quantitative results of Xpert and smear microscopy [35]. This positive correlation made Xpert semi-quantitative results useful to estimate patients' infectious potential and to guide airborne isolation strategies when integrated with other clinical features in smear independent algorithms [36–38] (Fig. 3). Similarly, a correlation is also observed between the semi-quantitative result of Ultra and smear microscopy. Thus, Ultra might also help to rapidly identify the most infectious patients (those with Ultra-positive medium and high) as well as the less infectious patients, those with ultra-negative [12,16]. Studies making a direct link between the DNA load and the transmission potential are still missing.

Conclusions

The molecular test Xpert and more recently the Ultra rapidly became widespread. It is important to understand in detail their advantages and their limits in order to achieve optimal use but also because they have opened the way for future molecular POCTs for the diagnosis of tuberculosis. We conducted a narrative review to address the performance of Ultra and Xpert with particular interest to articles that compared the performance of both tests on the same sets of samples. Ultra displays an overall higher sensitivity than Xpert. A significant improvement is observed for HIV-positive patients for which pulmonary tuberculosis often presents as a paucibacillary infection, especially for miliary tuberculosis [39]. Increased sensitivity is observed for other paucibacillary infections such as pleural tuberculosis and smear-negative tuberculosis. Increased sensitivity of Ultra is the result of an increased reaction volume and the use of multicopy genes *IS6110* and *IS1810* as PCR targets. The copy number of *IS6110* may vary widely amongst mycobacteria from the complex tuberculosis in a lineage-specific manner and hence could affect molecular test sensitivity [40]. Animal-adapted mycobacteria have fewer copies of *IS6110*; it is generally accepted that *Mycobacterium bovis* contains only one

copy of this gene. The East African Indian Lineage 1 strain also contains a limited number of IS6110. In contrast isolates from modern lineage 2 (Beijing/W), 3 (CAS) and 4 (Haarlem, T4, S, X) can contain more than 20 copies of IS6110. Therefore, comparative studies achieved in various regions, and in various populations are still necessary.

So far, the LOD of Ultra has been evaluated only on respiratory specimens. In addition, very few publications have addressed the performance of Ultra for the diagnosis of extrapulmonary tuberculosis. Caution should be taken when using Ultra as well as Xpert for the diagnosis of extrapulmonary specimens, in particular pleural fluids.

As a PCR-based test, Ultra is able to detect DNA from dead bacilli in patients with a previous history of tuberculosis [22]. Careful anamnesis and clinical data addressing the pre-test probability are paramount for both requesting the test and the interpretation of any result, in particular trace results. Decreased specificity is more likely to occur in regions of medium and high tuberculosis prevalence. However, low-prevalence regions can encounter patients coming from high-prevalence countries for which the risk of a false positive due to a previous history of tuberculosis exists.

Studies conducted so far do not reveal an increased sensitivity or specificity of Ultra for rifampicin resistance detection. Regarding sensitivity, no gain was expected, as the target gene for PCR is still the single-copy gene *rpoB*. In contrast, a gain was expected for the specificity due to the introduction of melting curve analysis. The absence of significant differences between Ultra and Xpert could be explained by the fact that the impact on specificity is more likely to occur in regions of low prevalence of MDR-TB while thus far most studies have been conducted in regions of medium and high prevalence of MDR-TB.

An increased sensitivity of the Xpert MTB/RIF Ultra assay has been reported, especially in the setting of paucibacillary infections such as pulmonary TB in HIV-positive patients, tuberculous meningitis or tuberculous pleurisy. Therefore, the data suggests that an overall improvement for the diagnosis of tuberculosis seems assured with the Ultra assay. Nevertheless, negative Ultra tests cannot rule out tuberculosis. Culture remains one of the reference methods of choice for the diagnosis of tuberculosis despite the fact that culture-negative tuberculosis exists. Therefore, studies using composite references or case definitions of tuberculosis based on microbiological findings and clinical data would make the evaluation of the performance of new tests more reliable. More studies should also address the impact of molecular tests on the number of diagnosed cases.

Increased specificity for rifampicin resistance detection is expected to occur in the setting of low prevalence of MDR-TB and on paucibacillary specimens. Recent studies have essentially addressed the sensitivity and specificity of Ultra. It will be important for future studies to look at the positive and negative predictive values of these tests, knowing that such characteristics are dependent on the tuberculosis prevalence. Future studies should also define the optimal number of samples to be tested with regard to the diagnosis of pulmonary tuberculosis. The insertion sequences, at least IS6110, are also used in many other nucleic acid amplification tests for tuberculosis [41,42]. For instance, Berhanu and colleagues compared the performance of Ultra with the Real-Time MTB assay (RT-MTB) (Abbott, Des Plaines, USA) [43]. Sensitivity of Ultra and RT-MTB on the same specimens were 88.9% and 77.8%, respectively [19]; in contrast, Xpert and RT-MTB displayed similar performances [44]. It would be beneficial to have more studies that compare the performance of Xpert and Ultra with the other tests that target the same DNA sequences, in particular for the diagnosis of extrapulmonary tuberculosis. Finally, because of the increasing number of MDR and XDR strains, rapid molecular tests giving information regarding other resistance markers such as resistance to isoniazid, quinolones or aminoglycosides would be useful. The next generation of NAATs might also be able to discriminate between dead and living bacteria.

Transparency declaration

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References

[1] Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010;363:1005–15.
[2] World Health Organization. Automated real-time nucleic acid amplification technology for rapid and simultaneous detection of tuberculosis and rifampicin resistance: Xpert MTB/RIF assay for the diagnosis of pulmonary and extrapulmonary tb in adults and children. 2013. Policy update.
[3] Abimbola TO, Marston BJ, Date AA, Blandford JM, Sangrujee N, Wiktor SZ. Cost-effectiveness of tuberculosis diagnostic strategies to reduce early mortality among persons with advanced HIV infection initiating antiretroviral therapy. J Acquir Immune Defic Syndr 2012;60:e1–7.
[4] Andrews JR, Lawn SD, Rusu C, Wood R, Noubary F, Bender MA, et al. The cost-effectiveness of routine tuberculosis screening with Xpert MTB/RIF prior to initiation of antiretroviral therapy: a model-based analysis. AIDS 2012;26:987–95.
[5] Menzies NA, Cohen T, Lin HH, Murray M, Salomon JA. Population health impact and cost-effectiveness of tuberculosis diagnosis with Xpert MTB/RIF : a dynamic simulation and economic evaluation. PLoS Med 2012;9:e1001347.
[6] Vassall A, van Kampen S, Sohn H, Michael JS, John KR, den Boon S, et al. Rapid diagnosis of tuberculosis with the xpert mtb/rif assay in high burden countries: a cost-effectiveness analysis. PLoS Med 2011;8:e1001120.
[7] Garcia-Basteiro AL, DiNardo A, Saavedra B, Silva DR, Palmero D, Gegia M, et al. Point of care diagnostics for tuberculosis. Pulmonology 2018;24:73–85.
[8] Claessens J, Mathys V, Derdelinckx I, Saegeman V. Case report of a false positive result of the Xpert MTB/RIF assay for rifampicin resistance in *Mycobacterium tuberculosis* complex. Acta Clin Belg 2017;72:195–7.
[9] Cayci YT, Bilgin K, Coban AY, Birinci A, Durupinar B. An evaluation of false-positive rifampicin resistance on the Xpert MTB/RIF. Mem Inst Oswaldo Cruz 2017;112:756–9.
[10] Ocheretina O, Byrt E, Mabou MM, Royal-Mardi G, Merveille YM, Rouzier V, et al. False-positive rifampin resistant results with Xpert MTB/RIF version 4 assay in clinical samples with a low bacterial load. Diagn Microbiol Infect Dis 2016;85:53–5.
[11] Van Rie A, Mellet K, John MA, Scott L, Page-Shipp L, Dansey H, et al. False-positive rifampicin resistance on Xpert MTB/RIF: case report and clinical implications. Int J Tuberc Lung Dis 2012;16:206–8.
[12] Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, 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.
[13] Dorman SE, Schumacher SG, Alland D, Nabeta P, Armstrong DT, King B, et al. Xpert MTB/RIF Ultra for detection of mycobacterium tuberculosis and rifampicin resistance: a prospective multicentre diagnostic accuracy study. Lancet Infect Dis 2018;18:76–84.
[14] Ng KCS, van Deun A, Meehan CJ, Torrea G, Driesen M, Gabriels S, et al. Xpert Ultra can unambiguously identify specific rifampin resistance-conferring mutations. J Clin Microbiol 2018;56.
[15] Williamson DA, Basu I, Bower J, Freeman JT, Henderson G, Roberts SA. An evaluation of the xpert mtb/rif assay and detection of false-positive rifampicin resistance in *Mycobacterium tuberculosis*. Diagn Microbiol Infect Dis 2012;74:207–9.
[16] Opota O, Zakham F, Mazza-Stalder J, Nicod L, Greub G, Jaton K. Added value of Xpert MTB/RIF Ultra for diagnosis of pulmonary tuberculosis in a low-prevalence setting. J Clin Microbiol 2019;57.
[17] Luetkemeyer AF. Current issues in the diagnosis and management of tuberculosis and HIV coinfection in the United States. Top HIV Med 2010;18:143–8.
[18] Rockwood N, Wilkinson RJ. Understanding and intervening in HIV-associated tuberculosis. Clin Med (Lond) 2015;15:s43–9.
[19] Berhanu RH, David A, da Silva P, Shearer K, Sanne I, Stevens W, et al. Performance of xpert MTB/RIF, xpert ultra, and abbott real time MTB for the

diagnosis of pulmonary tuberculosis in a high HIV burden setting. J Clin Microbiol 2018.
[20] Nicol MP, Workman L, Prins M, Bateman L, Ghebrekristos Y, Mbhele S, et al. Accuracy of Xpert MTB/RIF Ultra for the diagnosis of pulmonary tuberculosis in children. Pediatr Infect Dis J 2018;37:e261–3.
[21] Sabi I, Rachow A, Mapamba D, Clowes P, Ntinginya NE, Sasamalo M, et al. Xpert MTB/RIF Ultra assay for the diagnosis of pulmonary tuberculosis in children: a multicentre comparative accuracy study. J Infect 2018;77:321–7.
[22] Arend SM, van Soolingen D. Performance of Xpert MTB/RIF Ultra: a matter of dead or alive. Lancet Infect Dis 2018;18:8–10.
[23] Theron G, Venter R, Smith L, Esmail A, Randall P, Sood V, et al. False-positive Xpert MTB/RIF results in retested patients with previous tuberculosis: frequency, profile, and prospective clinical outcomes. J Clin Microbiol 2018;56.
[24] Theron G, Venter R, Calligaro G, Smith L, Limberis J, Meldau R, et al. Xpert MTB/RIF results in patients with previous tuberculosis: can we distinguish true from false positive results? Clin Infect Dis 2016;62:995–1001.
[25] Kendall EA, Schumacher SG, Denkinger CM, Dowdy DW. Estimated clinical impact of the Xpert MTB/RIF Ultra cartridge for diagnosis of pulmonary tuberculosis: a modeling study. PLoS Med 2017;14:e1002472.
[26] Kohli M, Schiller I, Dendukuri N, Dheda K, Denkinger CM, Schumacher SG, et al. Xpert MTB/RIF assay for extrapulmonary tuberculosis and rifampicin resistance. Cochrane Database Syst Rev 2018;8:CD012768.
[27] Perez-Risco D, Rodriguez-Temporal D, Valledor-Sanchez I, Alcaide F. Evaluation of the Xpert MTB/RIF Ultra assay for direct detection of *Mycobacterium tuberculosis* complex in smear-negative extrapulmonary samples. J Clin Microbiol 2018;56.
[28] Du J, Huang Z, Luo Q, Xiong G, Xu X, Li W, et al. Rapid diagnosis of pleural tuberculosis by Xpert MTB/RIF assay using pleural biopsy and pleural fluid specimens. J Res Med Sci 2015;20:26–31.
[29] Walmsley S. Acp journal club: review: Xpert MTB/RIF assay detects extrapulmonary TB in lymph nodes and CSF, but not pleural fluid. Ann Intern Med 2015;162:JC11.
[30] Wu X, Tan G, Gao R, Yao L, Bi D, Guo Y, et al. Assessment of the Xpert MTB/RIF Ultra assay on rapid diagnosis of extrapulmonary tuberculosis. Int J Infect Dis 2019.
[31] Wang G, Wang S, Jiang G, Yang X, Huang M, Huo F, et al. Xpert MTB/RIF Ultra improved the diagnosis of paucibacillary tuberculosis: a prospective cohort study. J Infect 2019.
[32] Khonga M, Nicol MP. Xpert MTB/RIF Ultra: a gamechanger for tuberculous meningitis? Lancet Infect Dis 2018;18:6–8.
[33] Thwaites GE. Advances in the diagnosis and treatment of tuberculous meningitis. Curr Opin Neurol 2013;26:295–300.
[34] 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.
[35] Lange B, Khan P, Kalmambetova G, Al-Darraji HA, Alland D, Antonenka U, et al. Diagnostic accuracy of the Xpert MTB/RIF cycle threshold level to predict smear positivity: a meta-analysis. Int J Tuberc Lung Dis 2017;21:493–502.
[36] Opota O, Senn L, Prod'hom G, Mazza-Stalder J, Tissot F, Greub G, et al. Added value of molecular assay Xpert MTB/RIF compared to sputum smear microscopy to assess the risk of tuberculosis transmission in a low-prevalence country. Clin Microbiol Infect 2016;22:613–9.
[37] Poonawala H, Leekha S, Medina-Moreno S, Filippell M, Johnson JK, Redfield RR, et al. Use of a single Xpert MTB/RIF assay to determine the duration of airborne isolation in hospitalized patients with suspected pulmonary tuberculosis. Infect Control Hosp Epidemiol 2018;39:590–5.
[38] Lippincott CK, Miller MB, Popowitch EB, Hanrahan CF, Van Rie A. Xpert MTB/RIF assay shortens airborne isolation for hospitalized patients with presumptive tuberculosis in the United States. Clin Infect Dis 2014;59:186–92.
[39] Theron G, Peter J, Dheda K. Characteristics of Xpert MTB/RIF-negative patients with pulmonary tuberculosis. Clin Infect Dis 2012;55:472–3. author reply 3-4.
[40] Gonzalo-Asensio J, Perez I, Aguilo N, Uranga S, Pico A, Lampreave C, et al. New insights into the transposition mechanisms of is6110 and its dynamic distribution between *Mycobacterium tuberculosis* complex lineages. PLoS Genet 2018;14:e1007282.
[41] Wang HY, Lu JJ, Chang CY, Chou WP, Hsieh JC, Lin CR, et al. Development of a high sensitivity taqman-based pcr assay for the specific detection of *Mycobacterium tuberculosis* complex in both pulmonary and extrapulmonary specimens. Sci Rep 2019;9:113.
[42] Joon D, Nimesh M, Varma-Basil M, Saluja D. Evaluation of improved is6110 lamp assay for diagnosis of pulmonary and extra pulmonary tuberculosis. J Microbiol Meth 2017;139:87–91.
[43] Chen JH, She KK, Kwong TC, Wong OY, Siu GK, Leung CC, et al. Performance of the new automated abbott realtime mtb assay for rapid detection of *Mycobacterium tuberculosis* complex in respiratory specimens. Eur J Clin Microbiol Infect Dis 2015;34:1827–32.
[44] Wang SF, Ou XC, Li Q, Zheng HW, Wang YF, Zhao YL. The Abbott Realtime MTB assay and the Cepheid GeneXpert assay show comparable performance for the detection of *Mycobacterium tuberculosis* in sputum specimens. Int J Infect Dis 2016;45:78–80.