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Comparison of Xpert MTB/RIF (G4) and Xpert Ultra, including trace readouts, for the diagnosis of pulmonary tuberculosis in a TB and HIV endemic setting

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ABSTRACT

Background: There are limited data about Xpert-Ultra performance in different settings, in HIV-infected persons, in those with a history of previous TB, and with trace readouts.
Methods: We evaluated the relative accuracy of Xpert-MTB/RIF and Xpert-Ultra in 272 selected but well-characterized archived sputum samples. Of these, 168 were culture-positive (64/168 smear-positive and 104/168 smear-negative), and 104 were culture-negative (102/104 from patients with previous TB and 2/104 from patients without a TB history). Assay-specific limit-of-detection (LOD) experiments were conducted using serial dilutions of *Mycobacterium tuberculosis* H37Rv.
Results: Overall sensitivity (95%CI) in smear-negative culture-positive samples for Xpert-MTB/RIF and Xpert-Ultra were 71.2% (62.5–79.9) and 77% (68.9–85.1), respectively (and in HIV-infected persons: 63.5% (50–76.1) and 73.1% (61.1–85.2), respectively). The LOD for Xpert-Ultra was lower (9 versus 184 CFU/ml). There were a total of 9/272 (3.3%) Xpert Ultra trace readouts (6/104 [5.8%]) in smear-negative culture-positive persons, and 3/102 (3%) in culture-negative non-TB persons with a history of previous TB).
Conclusions: Xpert-Ultra had a lower LOD compared to Xpert-MTB/RIF. A small proportion of samples (<5%) from culture-negative patients but with a history of previous TB had a likely false-positive trace readout. These data inform the management of patients with suspected TB in endemic settings.

Introduction

The development of rapid and accurate diagnostic tests for tuberculosis (TB), which decreases the time of treatment initiation, is an important strategy to control the TB epidemic. The WHO recommended Xpert MTB/RIF assay (Cepheid, USA) is an automated cartridge-based, real-time polymerase chain reaction (PCR) test that has been proven to reduce time to treatment initiation in TB patients (Boehme et al., 2011; Calligaro et al., 2017) by detecting the presence of TB and drug resistance to rifampicin (RIF^R) in sputum samples within two hours (Theron et al., 2011). It has been shown to have better sensitivity (98%) at diagnosing TB over sputum smear microscopy (20%–60%; Walusimbi et al., 2013). However, it has suboptimal sensitivity in smear-negative sputum (67%; (Walusimbi et al., 2013)), which is often the case in HIV-infected patients (Theron et al., 2011). To improve the sensitivity, Xpert Ultra was introduced, which has an enhanced assay design and uses high-resolution melt analysis (Chakravorty et al., 2017).

The reported sensitivity for Xpert Ultra in smear-negative sputum reached 78.9%, which is higher than Xpert MTB/RIF (66.1%) (Chakravorty et al., 2017) and the assay performed better in sputum from HIV-infected individuals (87.5% versus 68.6% for Xpert Ultra versus Xpert MTB/RIF, respectively; Berhanu et al., 2018). The threshold for detection with Xpert Ultra was ~1-log CFU better over Xpert MTB/RIF (12 CFU/ml versus 130 CFU/ml) (Chakravorty et al., 2017). However, this improvement in sensitivity comes at the cost of decreased specificity (Garcia-Basteiro et al., 2017).

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However, there are several gaps in our knowledge about the utility of Xpert Ultra in HIV-infected persons, in those with a history of previous TB, and the epidemiology of trace readouts has been poorly studied. It has been suggested that the latter may represent true or false-positive results, and the optimal management of such patients is unclear. There are also limited data about the comparative limit of detection of the two Xpert assays. Therefore, in the current study, we sought to evaluate the diagnostic accuracy of Xpert MTB/RIF versus Xpert Ultra from selected patients in a TB and HIV endemic setting and explore the epidemiology of trace results.

Materials and methods

Case definitions

The following case definitions were used for the analysis:

(A) **Confirmed active TB** - patients fulfilled **all** of the following criteria:

(i) Presented at the TB clinic with at least one WHO defined symptom suggestive of TB.
(ii) **Positive** sputum culture for *Mycobacterium tuberculosis* (*M. tb*).
(iii) Initiated on TB treatment.
(iv) Had resolution of their TB symptom(s) at 8 weeks of follow-up.

(B) **Confirmed non-TB** - patients fulfilled **all** of the following criteria:

(i) Presented at the TB clinic with at least one WHO defined symptom suggestive of TB.
(ii) **Negative** sputum culture for *M. tb.*
(iii) **Not** Initiated on TB treatment.
(iv) Had resolution of their TB symptom(s) and/or who had a confirmed alternative diagnosis at 8 weeks of follow-up.

(C) **Previous TB** - patients fulfilled **all** of the following criteria:

(i) Presented at the TB clinic with at least one (new-onset) WHO defined symptom suggestive of TB.
(ii) Had a history of one or more episodes of culture or Xpert confirmed TB.
(iii) Had completed TB treatment at least 6 months before their current onset of symptoms.

Archived sputum samples

Sputum samples (n = 272) from symptomatic individuals suspected of having pulmonary TB were obtained between June 2013 and December 2015 from TB clinics in Cape Town, South Africa. The University of Cape Town Human Research Ethics Committee approved (approval # 068/2016) the current study, and all patients provided written informed consent for study participation and biobanking of clinical samples for downstream evaluation.

Xpert MTB/RIF and Xpert Ultra testing

The Xpert MTB/RIF and Xpert Ultra assays were performed as described previously (Helb et al., 2010). Briefly, sodium hydroxide and isopropanol-containing sample buffer (Cepheid, USA) was added to the sputum sample at a ratio of 2:1 and incubated for 15 min at room temperature with gentle intermittent agitation. Following incubation, 2 ml of sample was transferred to the Xpert MTB/RIF and/or Xpert Ultra cartridge and run on the Xpert system (Cepheid, Dx System Version 4.0c), depending on the outcome under investigation.

Limit-of-detection experiments

Sputum samples from culture and smear-negative patients with no history of previous TB were combined, diluted in sample buffer, and spiked with *M. tb* H37Rv at 1500, 750, 375, 188, 94, 47, 24, 12, 9, 5 and 0 CFUs/ml. Each dilution of spiked sputa was tested using the Xpert MTB/RIF, and Xpert Ultra assay in triplicate, and the results were compared at each concentration. Dilutions were plated onto Middlebrook 7H9 agar (Sigma, Germany) containing oleic albumin dextrose catalase (OADC) supplement (Becton Dickinson, USA), and colony forming units (CFUs) were counted to ensure that the spiked levels of CFU/ml were accurate.

The effect of serial freeze–thaw cycles on Xpert performance

Samples classified as smear-negative Xpert MTB/RIF-positive and culture-positive (n = 16) were randomly selected from biobanked samples to evaluate whether freeze–thawing the sample has an effect on test performance. The samples underwent three complete freeze–thaw cycles, after which Xpert MTB/RIF was performed as described previously. The cyclic threshold (Ct) values were compared to previously documented Xpert MTB/RIF results using a fresh sample at the same time of sample collection.

Statistical analysis

For demographic analysis, the chi-squared ($\chi 2$) test was employed for categorical variables; for continuous variables, the Mann–Whitney test was used for non-parametrically distributed data (GraphPad, Version 6). Diagnostic accuracy of Xpert MTB/RIF and Xpert Ultra including, sensitivity and specificity, with or without the trace call included for Xpert Ultra, was performed. The Fishers Exact Test was utilized for comparison of the diagnostic variables for Xpert MTB/RIF and Xpert Ultra. The diagnostic accuracy analysis was performed in Stata, Version 13. A p-value of <0.05 was considered significant for all statistical analyses.

Results

Patient clinical parameters and sputum sample characteristics

The demographic characteristics of the patients enrolled in the study are shown in Table 1. Patient subgroups were pre-selected to answer our research questions. Overall, the median age of the patients was 39, with ~60% (155/261) being males and ~40% being females (106/261).

The majority (~62% 104/168) of patients in the confirmed active-TB group were smear-negative (i.e., potentially with paucibacillary disease) with ~35% (58/168) of patients having had a previous history of TB. The confirmed non-TB group almost entirely [98% (102/104)] consisted of patients with a prior history of tuberculosis with an HIV prevalence of 33% (31/93) in this group. Xpert MTB/RIF and Xpert Ultra were positive for 82.1% and 82.7%, respectively, amongst the confirmed active-TB patients.

Figure 1A shows the study plan of the patients tested for Xpert MTB/RIF and Xpert Ultra (n = 272). Out of the 168 confirmed active-TB cases, 64 and 104 were smear-positive and smear-negative, respectively. For the confirmed active-TB, smear-negative sputum samples, Xpert Ultra could detect five and three more TB cases than Xpert MTB/RIF in the HIV-infected and HIV-uninfected patients, respectively. Within the confirmed non-TB group with a history of

Table 1
Demographic characteristics of the sub-groups (data are n (%) unless otherwise stated).

Demographic data	All samples (%) (n = 272)	Confirmed active-TB (%) (n = 168)	Non-TB (%) (n = 104)	p-Value
Age				
[a]Median years (range)	39 (19–68)	39 (19–65)	39 (26–68)	
Gender				0.1289
Male	164 (60.3)	94 (56)	70 (67.3)	
Female	108 (39.7)	74 (44)	34 (32.7)	
HIV-infected				<0.0001
Yes	148 (54.4)	113 (67.2)	35 (33.7)	
No	107 (39.3)	40 (23.8)	67 (64.4)	
Not determined	17 (6.5)	15 (8.9)	2 (1.9)	
CD4 count (cells/ml) (range)[b]	235 (6–788)[Ψ]	235 (6–788)[ω]	235 (25–681)[φ]	0.1494
Smear status				<0.0001
Smear-negative	191 (70.2)	104 (61.9)	87 (83.7)	
Smear-positive	68 (25)	64 (38.1)	4 (3.8)	
Unknown	13 (4.7)	–	13 (12.5)	
Previous TB				<0.0001
Yes	160 (58.8)	58 (34.5)	100 (98.0)	
<5 years ago	61	24	37	
5 ≤ 10 years ago	68	22	46	
>10 years	56	14	42	
No	111 (40.8)	109 (64.9)	2 (1.9)	
Unknown	1 (0.4)	1 (0.6)	–	
Xpert MTB/RIF				<0.0001
Positive	151 (55.5)	138 (82.1)	13 (12.5)	
Negative	121 (44.5)	30 (17.9)	91 (87.5)	
Xpert Ultra				<0.0001
Positive	151 (55.5)	139 (82.7)	12 (11.5)	
Negative	121 (44.5)	29 (17.3)	92 (88.5)	
Trace	9/272	6/168	2.9 (3/104)	

[a] Median (range).
[b] Performed if HIV-infected. There was no CD4 count data for 5[Ψ], 3[ω], and 2[φ] patients, respectfully.

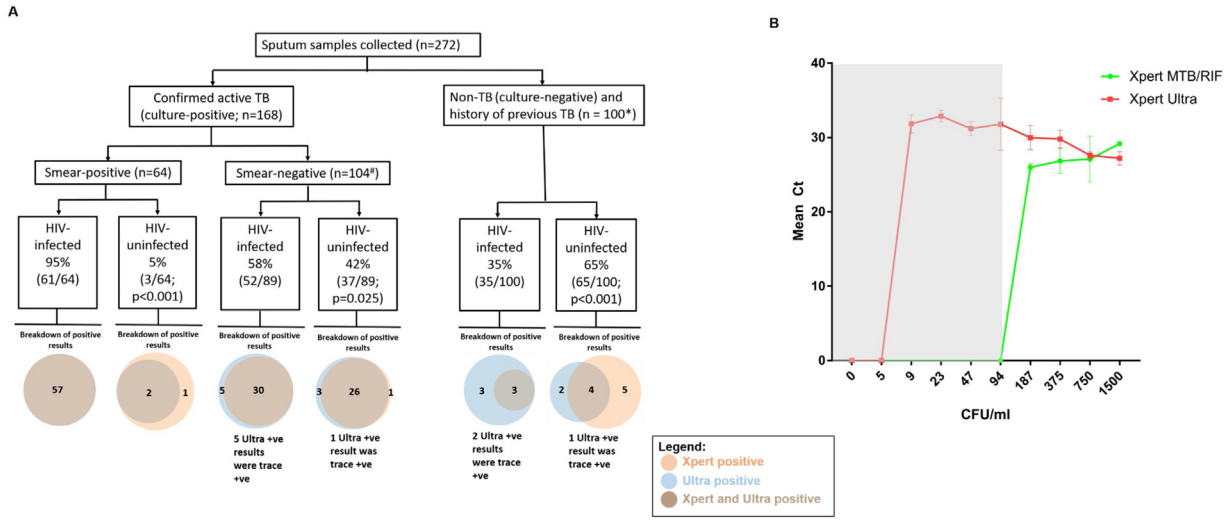

Figure 1. Study flow and LOD for Xpert MTB/RIF and Xpert Ultra. (A) Study flow showing the number of sputum samples analysed with Xpert MTB/RIF and Xpert Ultra. #Fifteen samples had an unknown HIV status. *Two patients had no history of previous TB and two HIV results were of unknown status. p-Values compare the HIV-infected versus uninfected status. (B) LOD for Xpert MTB/RIF and Xpert Ultra. Culture-negative, smear-negative pooled sputum samples were diluted 1:2 in lysis buffer. The diluted sputum was spiked with *M. tb* H37Rv at 1500, 750, 375, 188, 94, 47, 24, 9, 5 and 0 CFUs/ml. Each dilution was analysed by Xpert MTB/RIF and Xpert Ultra in triplicate. The shaded area indicates the relative CFU/ml where one or more of the replicates were either Xpert Ultra negative or trace. Xpert = Xpert MTB/RIF, Ultra = Xpert Ultra.

previous TB (n = 100), Xpert Ultra could detect an additional three TB cases above Xpert MTB/RIF in the HIV-infected patients and an additional two in the HIV-uninfected patients.

Limit-of-detection (LOD) of Xpert MTB/RIF versus Xpert Ultra

The LOD for Xpert MTB/RIF and Xpert Ultra are shown in Figure 1B. Culture-negative, Xpert MTB/RIF-negative sputum samples from individuals without a history of TB, and who did not receive TB treatment with resolution of their respiratory symptoms on follow-up, were pooled and diluted 2:1 (lysis buffer:sputum) in lysis buffer as indicated in the materials and methods. The sputum was spiked with 1500, 750, 375, 188, 94, 47, 24, 9, 5, and 0 CFUs/ml of *M. tb* H37Rv and analyzed by Xpert MTB/RIF and Xpert Ultra. The Xpert Ultra sputum samples spiked with 1500, and 750 CFU/ml had similar average *rpoB* Ct values of approximately 28. Average *rpoB* Ct values increased to approximately 29 when the Xpert Ultra sputum samples were spiked with 187 CFU/ml. Xpert MTB/RIF could not detect TB at a CFU/ml below 187, and Xpert Ultra was less reproducible below 94 CFU/ml (Figure 1B), where one or more of

the triplicates were negative for TB or trace. The LOD for Xpert Ultra was nine CFU/ml.

Sensitivity of Xpert MTB/RIF and Xpert Ultra in smear-negative sputum samples from HIV-infected and HIV-uninfected patients with definite TB

The sensitivity for Xpert MTB/RIF and Xpert Ultra for the HIV-infected and HIV-uninfected sputum samples is shown in Table 2. Overall the sensitivity for Xpert MTB/RIF and Xpert Ultra was 71.2% (95% CI; 62.5%–79.9%) and 77% (68.9%–85.1%), respectively. For the sputum samples from the HIV-infected individuals, the sensitivity for Xpert MTB/RIF was lower at 63.5% (50%–76.1%) compared to that of Xpert Ultra (73.1% [61.1%–85.2%]; Table 2); however, this was not significant. The sensitivity for Xpert MTB/RIF and Xpert Ultra was 73% (58.7%–87.3%) and 78.4% (65.1%–91.7%), respectively, for the sputum samples from HIV-uninfected individuals.

Accuracy of Xpert MTB/RIF and Xpert Ultra in sputum samples from non-TB patients with previous history of TB (false positivity rate)

The diagnostic accuracy of Xpert MTB/RIF and Xpert Ultra for non-TB patients with previous history of TB is shown in Table 3. The sensitivity for Xpert MTB/RIF and Xpert Ultra were 12.7% [7%–20.8%; p = 0.470] and 11.8% [6.2%–19.6%], respectively. In the HIV-infected individuals versus the HIV-uninfected individuals the sensitivity of Xpert MTB/RIF was 11.4% [3.2%–26.7%] versus 13.8% [6.5%–24.7%]; p = 0.732), respectively and Xpert Ultra was 17.1% [6.6%–33.6%] versus 9.2% [3.5%–19%; p = 0.246], respectively.

The significance of Xpert Ultra trace readouts

In the smear-negative active TB group (Table 2)

When the trace results for Xpert Ultra were excluded from the analysis, the sensitivity did not change for both the HIV-infected group (63.5% [49%–76.4%] and the HIV-uninfected groups (75.7% [58.8%–88.2%]) (Table 2). The Xpert Ultra trace readouts appeared higher in the HIV-infected (9.6% [3.2%–21%]; p = 0.199) versus the HIV-uninfected individuals (2.7% [0%–14.2%]), but this was not significant.

In the non-TB group with a previous history of TB (Table 3)

When the trace results were excluded for Xpert Ultra, the sensitivity in the HIV-infected versus the HIV-uninfected individuals was similar at 11.4% (3.2%–26.7%; p = 0.534) and 7.7% (2.5%–17%), respectively.

Positive predictive values (PPV) and negative predictive values (NPV) of Xpert MTB/RIF and Xpert Ultra overall and when stratified to HIV-infected and smear-negative samples

The PPV and NPV values for the study cohort overall and when stratified according to HIV-infected and smear-negative sputum samples is shown in Figure S1 (Supplementary material). Overall the PPV and NPV did not change for Xpert MTB/RIF versus Xpert Ultra (91.4% [55.9%–89.3%] vs. 92.1% [77.4%–100%] and 75.2% [60.6%–92.3%] vs. 76% [61.3%–93.3%], respectively). When stratified to HIV-infected sputum samples, the NPV appeared to decrease for both Xpert MTB/RIF (75.2% [60.6%–92.3%] vs. 62% [42.1%–88%]) and Xpert Ultra (76% [61.3%–93.3%] vs. 61.7% [41.3%–88.6%]), but this was not significant. The PPV and NPV did not change for both tests overall compared to smear-negative sputum samples.

The effect of freeze–thaw cycles on the performance of Xpert MTB/RIF

The effect of freeze/thawing sputum samples on Xpert MTB/RIF performance is shown in Figure 2. Eight pairs of culture-positive, Xpert MTB-RIF-positive sputum samples were randomized and either remained fresh or were subjected to three freeze/thaw cycles before performing Xpert MTB/RIF. On average, the Ct values for the freeze/thawed sputum samples (Ct = 23; p = 0.078) were similar to the fresh sputum samples (Ct = 26), indicating that freeze/thawing sputum samples does not affect Xpert MTB/RIF performance (Figure 2).

Distribution of test-positive results for culture, Xpert MTB/RIF, and Xpert Ultra

The relationship between test positivity for culture, Xpert MTB/RIF, and Xpert Ultra is shown in Figure 3. Culture could detect TB in an additional 19 sputum samples above Xpert MTB/RIF and Xpert

Table 2
Sensitivity of Xpert MTB/RIF and Xpert Ultra in smear-negative culture-positive samples stratified according to HIV status.

	Confirmed smear-negative TB (n = 104[a])		
	Overall (n = 104) Positive (%, 95%CI, n/N, p-value)	HIV-infected (n = 52) Positive (%, 95%CI, n/N, p-value)	HIV-uninfected (n = 37) Positive (%, 95%CI, n/N, p-value)
Xpert MTB/RIF	71.2%, 62.5%–79.9%, 74/104	63.5%, 50%–76.1%, 33/52	73.1%, 58.7%–87.3%, 27/37 **p = 0.345
Xpert Ultra (with trace)	77%, 68.9%–85.1%, 80/104, p = 0.343	73.1%, 61.1%–85.2%, 38/52, *p = 0.292	78.4%, 65.1%–91.7%, 29/37, *p = 0.588 **p = 0.568
Xpert Ultra (without trace)	71.2%, 61.4%–79.6%, 74/104, p = 0.484	63.5%, 49%–76.4%, 33/52,	75.7%, 58.8%–88.2%, 28/37, *p = 0.790 **p = 0.221
Xpert Ultra (trace readout)	5.8% 2.1%–12.1% 6/104	9.6% 3.2%–21% 5/52	2.7% 0%–14.2% 1/37 **p = 0.199

[a] Fifteen patients had an unknow HIV status.
* P values are for comparison between Xpert MTB/RIF and Xpert Ultra.
** P values are for comparison between HIV-infected and uninfected.

Table 3
False-positive rates (specificity) of Xpert MTB/RIF and Xpert Ultra for the detection of TB in sputum samples from non-TB patients with a previous history of TB.

	Non-TB with history of previous TB (n = 102[a])		
	Overall (n = 102) Positive (%, 95%CI, n/N, p-value)	HIV infected (n = 35) Positive (%, 95%CI, n/N, p-value)	HIV uninfected (n = 65) Positive (%, 95%CI, n/N, p-value)
Xpert MTB/RIF	12.7%, 7%–20.8%, 13/102	11.4% 3.2%–26.7% 4/35	13.8% 6.5%–24.7% 9/65 **p = 0.732
Xpert Ultra (with trace)	11.8%, 6.2%–19.6%, 12/102, p = 0.470	17.1% 6.6%–33.6% 6/35 *p = 0.495	9.2% 3.5%–19% 6/65 *p = 0.410 **p = 0.246
Xpert Ultra (without trace)	8.8%, 4.1%–16.1%, 9/102, p = 0.70	11.4% 3.2%–26.7% 4/35	7.7% 2.5%–17% 5/65 *p = 0.258 **p = 0.534

[a] Two patients had an unknow HIV status.
* P values are for comparison between Xpert MTB/RIF and Xpert Ultra.
** P values are for comparison between HIV status.

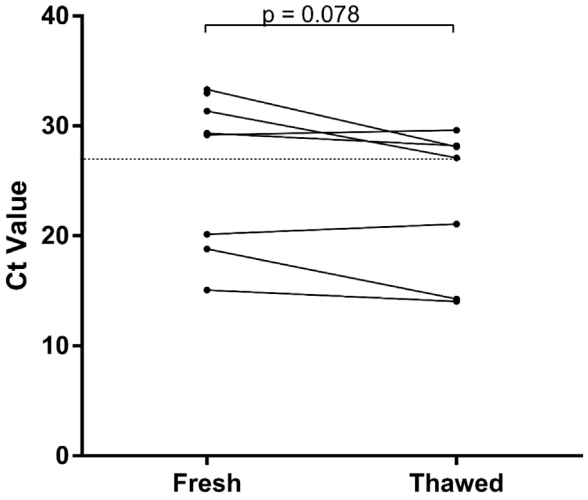

Figure 2. Ct values of fresh or freeze–thawed (3 cycles) sputum samples (16 sputum samples; 8 pairs of samples). Smear-negative culture-positive Xpert MTB/RIF-positive sputum samples were subjected to three rounds of freeze/thaw prior to repeat Xpert MTB/RIF. The dotted line represents the median Ct value = 27.

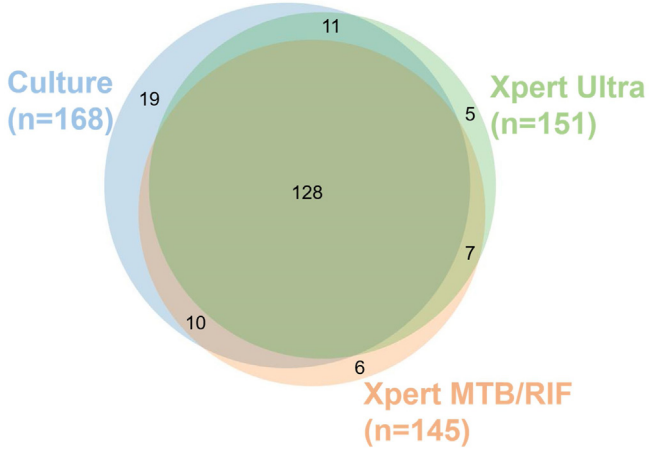

Figure 3. Venn diagram showing the relationship between test positivity for Xpert MTB/RIF (n = 145), Xpert Ultra (n = 151), and culture (n = 168).

Ultra. Both Xpert MTB/RIF and Xpert Ultra performed equally by detecting TB in an additional six and five sputum samples, respectively.

Discussion

We evaluated the diagnostic accuracy of Xpert MTB/RIF and Xpert Ultra using selected archived sputum samples from patients with suspected TB in a high TB and HIV prevalence setting. The three key findings in our study were: (1) Xpert Ultra had lower LOD for *M. tb* compared to Xpert MTB/RIF, (2) trace results with Xpert Ultra were relatively infrequent even in the group of patients who have a predisposition for trace false positive readouts (i.e., non-TB with a previous history of TB who turned out to be culture-negative and remained well on follow-up), Xpert Ultra positivity was ~3%, (3) Xpert Ultra sensitivity was consistently lower in HIV-infected persons and trace readouts were higher in this group, and (4) overall, Xpert Ultra was 6% more sensitive than Xpert MTB/RIF in smear-negative samples, although this did not reach statistical significance.

Overall, a minority of samples (3%) constituted trace readouts. This was less than the ~10% overall trace readout found by Berhanu et al. in South Africa (Berhanu et al., 2018) . In that study, reclassification of trace results resulted in a loss of sensitivity of 5.6% in the smear-negative group (Berhanu et al., 2018). In the study by Dorman et al., the reduction in sensitivity from excluding the trace readout was almost 9% in smear-negative culture-positive persons (Dorman et al., 2018). In our study, this figure was less than 6% in the smear-negative group, though this increased to almost 10% in HIV-infected persons. This may be due to several factors, including using a convenience sample set, patient classification based on follow-up, differential disease burden, and different TB strains. The interpretation of trace readouts on the Xpert Ultra semi-quantitative scale is controversial.

On the one hand, it may represent a true positive, i.e., detection of *M. tb* DNA, where the culture result is falsely negative. This could be due to a variety of factors including sub-clinical TB, differentially culturable mycobacteria that do not optimally grow on conventional culture media (Motyl et al., 1990), sampling error (sequential paired samples collected in the field are known to be discordant

due to random sampling error), or alternatively samples may be falsely culture-negative due to sample preparation, death of mycobacteria during transport to the laboratory, or overgrowth of mouth flora. Indeed, in the Dorman study, longer-term follow-up uncovered Xpert Ultra-positive culture-negative patients who subsequently turned out to be culture-positive (Dorman et al., 2018). In the Berhanu report, some samples became culture-positive well beyond the 42-day culture threshold limit (Berhanu et al., 2018). On the other hand, trace readouts may be falsely positive due to technical factors including detection of DNA artifacts (e.g., primer dimers) and inherent noise at the limit of detection of the fluorescence signal (false positive signal at very low-level fluorescence). A drawback of our study was that we did not sequence the amplicons from the Xpert Ultra cartridges, which could inform on the issue of technical artifacts. Dorman et al. sequenced amplicons obtained from 14 cartridges (samples from 14 participants that were Xpert Ultra-positive but culture-negative); in 12 of the 14 participants detection of *M. tb* DNA was confirmed (Dorman et al., 2018).

How trace readouts should be handled in clinical practice remains unclear, and there are implications for missing a TB diagnosis versus erroneously prescribing potentially toxic treatment. The current WHO guidelines suggest that trace readouts should signal TB treatment in paucibacillary disease (e.g., HIV co-infection, extra-pulmonary TB, etc.), while in other contexts, repeat testing should be performed. However, this may not be feasible in an endemic setting where almost ~10% of readouts are trace (Dorman et al., 2018). A prior modeling study has shown that Xpert Ultra, despite lower specificity (but higher sensitivity), could have a mortality benefit in TB and HIV hyper-endemic settings. At the same time, over-treatment of false-positive cases will likely occur in low prevalence regions (Kendall et al., 2017).

Our study highlights that trace-positive results in those with a previous history of TB should be carefully considered before TB treatment is commenced. Prospective studies in patients with trace readouts will be required to provide more guidance on how to optimally manage such patients in different clinical settings. However, we also quantified the magnitude of trace readouts in those who were culture-negative but with a prior history of TB. Our cohort was particularly well-characterized, and, in such patients, we had a follow-up of at least two months with a resolution of symptoms suggesting that Xpert Ultra in this context was detecting 'old' residual DNA from a prior episode. Indeed, detection of DNA in patients with previous TB is well-described, and Xpert Ultra cannot distinguish between the DNA from viable organisms and those that have demised (Theron et al., 2016; Theron et al., 2018). We also saw the well-described phenomenon of improved sensitivity with a trace readout but reduced specificity as outlined by others (Berhanu et al., 2018; Dorman et al., 2018). In our study, trace readouts occurred mostly in patients with a history of TB treatment within two years of their most recent TB episode. In this group of patients, analyzing the data by re-classifying trace calls as "negative" improves the specificity from 62% to 77%.

There is little data about Xpert Ultra's performance in HIV-infected persons, including those with a previous history of TB. Our results suggest that Xpert Ultra sensitivity (irrespective of the version) was lower in HIV-infected participants than HIV-uninfected participants, probably related to the more paucibacillary nature of the disease (at least in sputum samples) in such patients. The contribution of trace readouts to improving sensitivity was higher in HIV-infected than uninfected persons. Given the low burden of mycobacteria in the sputum of HIV-infected patients, the higher mortality seen in such patients, and inherent difficulties in diagnosis, the WHO has recommended that trace readouts in such patients should signal initiation of TB treatment. Our data showed that a history of previous TB had minimal impact on HIV-infected compared to uninfected persons.

There are limited data comparing performance between Xpert Ultra and Xpert MTB/RIF (Berhanu et al., 2018; Dorman et al., 2018). Overall, though our limited sample sizes did not reach significance, we did confirm the higher sensitivity of Xpert Ultra compared to Xpert MTB/RIF. Also, we provide laboratory evidence that Xpert Ultra performed better in *in vitro* studies using serial dilutions of *M. tb*. This is concordant with the findings of Chakravorty et al. (LOD for Xpert Ultra was 15.6 CFU/ml versus 112.6 CFU/ml for Xpert MTB/RIF) (Chakravorty et al., 2017). An interesting finding is that there were also individuals that were Xpert MTB/RIF cartridge positive but Xpert Ultra negative. This probably represents sampling error, and discordance between sequentially obtained samples is well recognized (Chakravorty et al., 2017).

Our study has several limitations. First, the small sample sizes limited our power to make intergroup sensitivity and specificity comparisons. However, we were limited by the size of our biobank, and our principal aim was to perform a preliminary interrogation of trace readouts and to gain more information about performance in HIV-infected persons. Second, we used biobanked rather than fresh samples, which may have impacted our results. However, similar trends and results were shown in the Berhanu, and Dorman reports. We also undertook experiments showing that freeze–thaw probably had a negligible effect on the study findings (though again sample sizes were limited and, if anything, freeze–thaw improved performance). Third, we did not perform sequencing of the cartridge amplicons. However, we were limited by resource constraints; this would have enabled us to consolidate technical false-positives but would have not corrected misclassification bias in those with previous TB. Fourth, the lower than predicted proportion of trace readouts compared to previous reports (Berhanu et al., 2018; Dorman et al., 2018) may have been due to suboptimal sample volume in some of the samples (not strictly recorded before running the Ultra assay) (Ho et al., 2015; Zimba et al., 2019) through our biobanking protocols stipulated collection of at least a one ml volume of sputum.

In conclusion, Xpert Ultra had a lower limit of detection compared to Xpert MTB/RIF. Moreover, we confirmed that a significant minority of samples (<5%) comprised trace readouts, and this may represent a false-positive signal in those with previous TB. Prospective studies are required on how to optimally manage such patients.

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Ethical approval

This study was reviewed and approved by The University of Cape Town Human Research Ethics Committee (approval # 068/2016).

Conflict of interest statement

None.

Author's contributions

AE, MT, EM, RM, and KD had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. AE, MT, EM, and KD conceived the

study, interpreted the data and results, and drafted the manuscript. MT and RM were involved in the biobanking aspects of the research and performing laboratory-related procedures. AE, MT, EM, and KD analyzed and interpreted the data. All authors critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ijid.2020.03.025.

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