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Performance of Xpert MTB/RIF, Xpert Ultra, and Abbott RealTime MTB for Diagnosis of Pulmonary Tuberculosis in a High-HIV-Burden Setting

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ABSTRACT More sensitive tests are needed for the diagnosis of smear-negative and HIV-associated tuberculosis. This study compares the sensitivities and specificities of three molecular tests, namely, the Xpert MTB/RIF test, the Xpert Ultra (Ultra), and RealTime MTB (RT-MTB), in a high HIV prevalence setting. Symptomatic adults were recruited from three outpatient sites, and each provided 4 sputum specimens. The diagnostic performance of Xpert MTB/RIF, Ultra, and RT-MTB was evaluated, with culture as a reference standard. HIV infection occurred in 62% of patients, with a median CD4 count of 220 cells/μl. The Ultra test had the highest sensitivity of 89.3% (95% confidence interval [CI], 78.1 to 96) compared to those of the Xpert MTB/RIF at 82.1% (95% CI, 69.6 to 91.1; $P = 0.12$) and RT-MTB at 78.6% (95% CI, 65.6 to 88.4; $P = 0.68$). The specificity was highest with the Xpert MTB/RIF at 100% (95% CI, 98 to 100), followed by RealTime MTB at 96.7% (95% CI, 92.9 to 98.8; $P = 0.03$) and the Ultra at 95.6% (95% CI, 91.5 to 98.1; $P = 0.08$). In patients with smear-negative disease, the Ultra was more sensitive than the Xpert MTB/RIF (64.7% [95% CI, 38.3 to 85.8] versus 41.2% [95% CI, 18.4 to 67.1], respectively; $P = 0.12$), and RT-MTB performed equally to Xpert MTB/RIF. In a comparison of the Ultra and RT-MTB on the same sputum specimen pellets, the Ultra was more sensitive than RT-MTB in the overall cohort (88.9% [95% CI, 77.4 to 95.8] versus 77.8% [95% CI, 64.4 to 88], respectively; $P = 0.03$) and among people with HIV (87.5% [95% CI, 71 to 96.5] versus 68.6% [95% CI, 50 to 83.9], respectively; $P = 0.03$). Although these results did not reach statistical significance, they suggest that the Ultra is more sensitive than the Xpert MTB/RIF and RT-MTB, most prominently in smear-negative disease. This was accompanied by a loss of specificity.

KEYWORDS Abbott RealTime MTB, South Africa, Xpert MTB/RIF, Xpert Ultra, molecular diagnostics, tuberculosis

The Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA), endorsed by the World Health Organization (WHO) in 2010, heralded a new era in rapid molecular diagnostics for tuberculosis (TB). The diagnosis of TB and rifampin resistance no longer required sophisticated laboratory infrastructure with mycobacterial culture capabilities and highly skilled laboratory technicians (1). Although the initial clinical trial of the Xpert MTB/RIF demonstrated sensitivity ranging between 98% in smear-positive TB and 72% in smear-negative cases (1), subsequent evaluations have demonstrated a tremendous vari-

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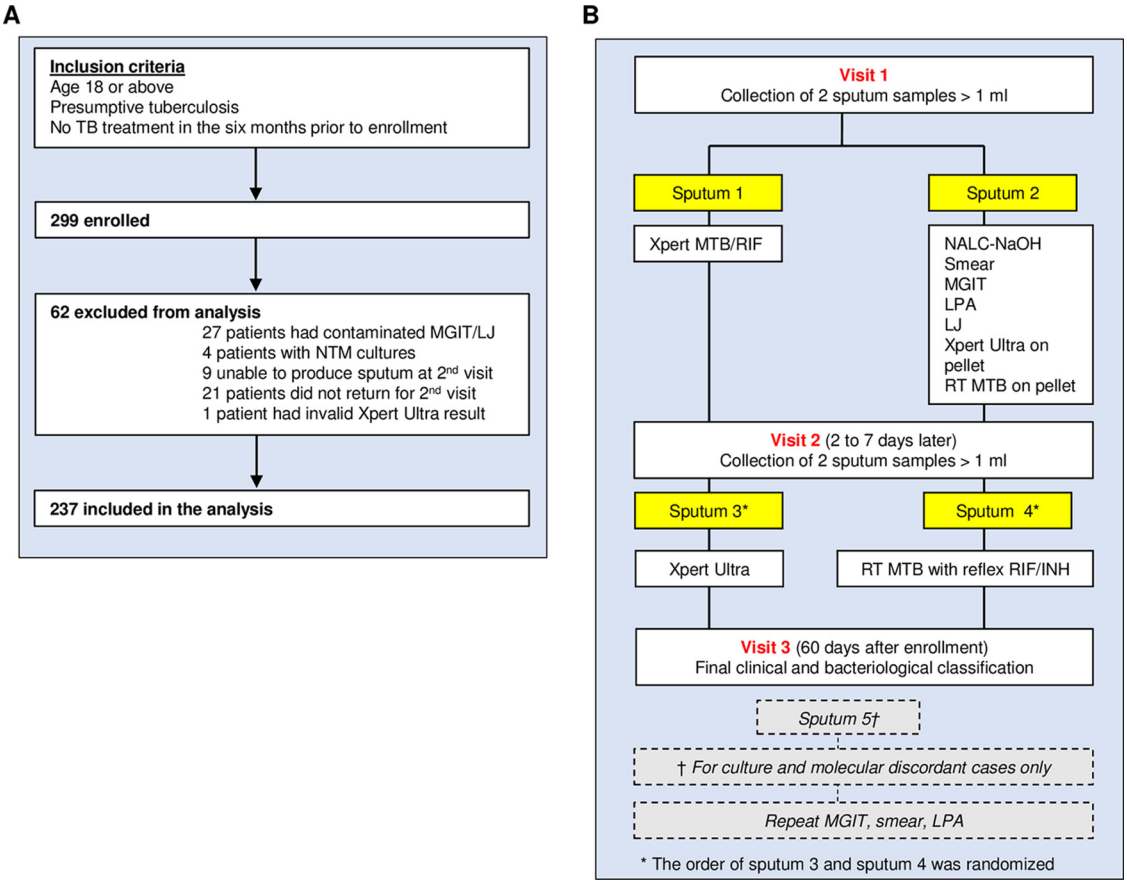

FIG 1 (A) Study enrollment. (B) Study procedures. MGIT, mycobacterial growth indicator tube; LPA, line probe assay; LJ, Löwenstein-Jensen agar; RT-MTB, RealTi*m*e MTB; RIF, rifampin; INH, isoniazid; NTM, nontuberculous mycobacteria.

ability in test performance in smear-negative disease, with sensitivity ranging between 26% and 67% (1–6). This is particularly problematic in children and people living with HIV (particularly advanced HIV disease) who are more likely to have smear-negative disease and also experience high TB-associated mortality (7), highlighting the need for new tests with improved sensitivity for the detection of paucibacillary disease.

Despite the increased diagnostic yield compared to that of smear microscopy and the capacity for the rapid detection of rifampin resistance, the use of the Xpert MTB/RIF failed to translate into a decrease in mortality compared to that with smear microscopy in a variety of trial and observational study settings during the early implementation of the Xpert MTB/RIF in South Africa (7, 8). This lack of impact on mortality has been attributed to a number of causes, including the high rates of ongoing clinical diagnosis and empirical treatment, trial design limitations, and general health system weaknesses (8, 9). In settings where the Xpert MTB/RIF is widely used, much of the ongoing empirical treatment is driven by the limited sensitivity of the Xpert MTB/RIF in smear-negative disease.

Since the implementation of the Xpert MTB/RIF, two new rapid molecular assays for the detection of TB and rifampin resistance have become available, namely, the Xpert MTB/RIF Ultra (Ultra; Cepheid) and the RealTi*m*e MTB assay (RT-MTB; Abbott, Des Plaines, IL, USA), which offer the promise of increased sensitivity. The Ultra uses the same diagnostic platform as the Xpert MTB/RIF and incorporates several changes, which include two PCR amplification targets (*IS6110* and *IS1081*), a larger PCR chamber, and the use of a melt curve analysis to detect rifampin resistance (10). These improvements have resulted in a predicted limit of detection (LoD) of the Ultra of 15 CFU/ml of sputum, which approximates the LoD of TB in liquid culture and is a substantial

TABLE 1 Clinical and demographic characteristics of patients included in the analysis

Clinical and demographic characteristics[a]	Entire cohort ($n = 237$)	TB culture finding[b]	
		Positive ($n = 56$)	Negative ($n = 181$)
Age (yr) (median [range])	36 (18–77)	34 (20–63)	37 (18–77)
Female sex (no./total [%])	79/237 (33.3)	15/56 (26.6)	64/181 (35.4)
HIV infection (no./total [%])	147/237 (62)	34/56 (60.7)	113/181 (62.4)
HIV status unknown[c]	8/237 (3.4)	3/56 (5.4)	5/181 (2.8)
CD4 (cells/mm^3) (median [IQR])	220 (84–358)	125 (78–232)	278 (104–400)
On ART at enrollment	64/147 (43.5)	10/34 (29.4)	54/113 (47.8)
Prior history of tuberculosis (no./total [%])	43/237 (18.1)	10/56 (17.9)	33/181 (18.2)
BMI (kg/m^2) (median [IQR])	21 (18–24.5)	19.2 (18.1–21.6)	21.9 (18.5–25.6)
Prior antibiotic exposure (no./total [%])	61/237 (25.7)	14/56 (25)	47/181 (26)

[a]ART, antiretroviral therapy; IQR, interquartile range; BMI, body mass index.
[b]TB, tuberculosis. Findings based on positive mycobacterial growth indicator tube and/or Löwenstein-Jensen culture.
[c]Declined HIV testing.

improvement over the LoD of the Xpert MTB/RIF, estimated as 112 CFU/ml (10). In a multicountry clinical study conducted by the Foundation for Innovative Diagnostics (FIND), the Ultra demonstrated an improved sensitivity compared to that of the Xpert MTB/RIF in smear-negative disease (63% versus 46%, respectively), accompanied by a loss of specificity (98% versus 96%, respectively) (5). The Ultra shows tremendous promise for the diagnosis of TB in children (11) and TB meningitis (12), for which many TB tests, including the Xpert MTB/RIF, have traditionally underperformed (12).

The RT-MTB assay uses the *m*2000 automated platform, which is widely used for HIV load testing. Unlike the Xpert testing platform, which can be adapted to point-of-care use, the RT-MTB requires trained laboratory technicians and a centralized laboratory setting (13). The RT-MTB PCR assay targets *IS6110* and protein antigen B for *Mycobacterium tuberculosis* identification. The RT-MTB assay identifies both rifampin (RIF) and isoniazid (INH) resistance via the RealTime MTB RIF/INH reflex test (RT-MTB RIF/INH), which targets the rifampin resistance-determining region (RRDR) of *rpoB*, as well as the *katG* and upper *inhA* gene promoter regions. In analytical studies, RT-MTB has a LoD of 32 CFU/ml (13), and in clinical studies, the test sensitivity ranged from 74% in smear-negative specimens (6) to 100% in smear-positive pulmonary specimens (14–18). However, most of the studies of RT-MTB to date, aside from those by Scott and colleagues (6), did not include specimens from people living with HIV, which may impact the results.

TABLE 2 Final bacteriological and clinical classification

Classification	No./total no. (%)
Bacteriological ($n = 237$)	
Sputum culture positive pulmonary TB	56/237 (23.6)
Smear and culture positive for *M. tuberculosis*	39/237 (16.5)
Smear negative and culture positive *M. tuberculosis*	17/237 (7.2)
Smear negative and culture negative	181/237 (76.4)
Smear positive and culture negative	0
Rifampin resistance[a]	4/58 (6.9)
Clinical	
Definite TB[b]	59/237 (24.9)
Possible TB (i.e., clinical diagnosis)[c]	5/237 (2.1)
Death at 2 mo	6/237 (2.5)
LTFU[d] at 2 mo	10/237 (4.2)

[a]Detected by GenoType MTBDR*plus* line probe assay.
[b]Microbiologic evidence of TB on culture or Xpert MTB/RIF testing (mostly sputum, 1 patient diagnosed lymph node aspirate).
[c]Clinical diagnosis of TB by X ray or other method.
[d]LTFU, lost to follow-up.

TABLE 3 Diagnostic performance of Xpert MTB/RIF, Xpert Ultra, and RealTime MTB for identification of tuberculosis compared to MGIT culture

Assay	No./total no. (% [95% CI])			
	Full cohort (n = 237)			
	Sensitivity	Specificity	PPV[a]	NPV[b]
Sputum (direct)				
Xpert MTB/RIF	46/56 (82.1 [69.6–91.1])	181/181 (100 [98–100])	46/46 (100 [92.3–100])	181/191 (94.8 [90.6–97.5])
RealTime MTB	44/56 (78.6 [65.6–88.4])	175/181 (96.7 [92.9–98.8])[c]	44/50 (88 [75.7–95.5])	175/187 (93.6 [89.1–96.6])
Ultra	50/56 (89.3 [78.1–96])	173/181 (95.6 [91.5–98.1])[c]	50/58 (86.2 [74.6–93.9])	173/179 (96.6 [92.8–98.8])
Pellets[d]				
RealTime MTB	42/54 (77.8 [64.4–88])	172/180 (95.6 [91.4–98.1])	42/50 (84 [70.9–92.8])	172/184 (93.5 [88.9–96.6])
Ultra	48/54 (88.9 [77.4–95.8])[e]	169/180 (93.9 [89.3–96.9])	48/59 (81.4 [69.1–90.3])	169/175 (96.6 [92.7–98.7])

[a]PPV, positive predictive value.
[b]NPV, negative predictive value.
[c]P value of <0.05 versus Xpert MTB/RIF with culture as gold standard.
[d]Three participants with invalid Ultra test results were excluded from the pellet analyses involving the full, HIV-positive, and smear-negative cohorts.
[e]P value of <0.05 versus RT-MTB with culture as gold standard.

This early data for the Ultra and RT-MTB tests showed promise in their ability to detect TB in patients with paucibacillary disease for whom a highly sensitive rapid test for TB remains elusive. In addition, RT-MTB RIF/INH has the added advantage of identifying isoniazid resistance in addition to rifampin resistance. Given the variability in test performance in different HIV prevalence settings that was seen following the implementation of the Xpert MTB/RIF, there is an ongoing need for additional data obtained under pragmatic conditions. The primary objective of this study was to compare the sensitivity and specificity of the single Xpert MTB/RIF with those of the Ultra and RT-MTB for the detection of TB, with a specific focus on smear-negative and HIV-associated TB. Test performances were compared on both raw sputum samples and sputum pellets, and liquid culture was used as a gold standard. A secondary objective was to compare the performance of the Ultra and RT-MTB on a single specimen.

MATERIALS AND METHODS

Study setting and participants. Participants were recruited from three sites in Johannesburg, South Africa, between December 2016 and July 2017. The study sites included two community health care centers (Hillbrow Community Health Center and Witkoppen Health and Wellness Center) and the Helen Joseph Hospital outpatient TB clinic. Eligible study participants were adults (≥18 years old) who presented with at least one TB symptom, which included cough of any duration, fever, weight loss, and night sweats as per the WHO TB screening guidelines (19). The participants had to be willing to return for a second study visit within 7 days and a third study visit two months after enrollment in order to be eligible for the study. Patients who self-reported receiving TB treatment in the 6 months prior were ineligible, as this would impact test performance.

Study procedures. At the first study visit, the participants were offered HIV testing and counseling and underwent an interview during which demographic information and medical history were obtained, including a history of prior TB infection and antibiotic use in the preceding month. The patients were asked to provide 2 sputum specimens of at least 1 ml in volume on the first visit (sputum specimens 1 and sputum 2) (Fig. 1B). Patients returned within 2 to 7 days and provided a further 2 sputum samples of at least 1 ml in volume (sputum specimens 3 and 4). The order of the collection of sputum specimens 3 and 4 were randomized. Study staff, clinicians, and study participants were blinded to the Ultra and RT-MTB results, which were not used for clinical care. All patients with microbiologically confirmed or suspected TB infections were diagnosed and managed according to South African TB management guidelines (20). A third follow-up visit occurred between 42 and 70 days after study enrollment, and those who did not return for the third visit were contacted by phone and underwent a telephonic interview. The participants were asked about ongoing symptoms, whether a diagnosis of TB was made by their treating clinician(s) (e.g., by X ray, clinical diagnosis, lymph node aspirate, etc.), the date of TB treatment initiation (if applicable), and symptomatic response to TB therapy. In cases with molecular and phenotypic discordancy (i.e., positive Xpert MTB/RIF, RT-MTB, or Ultra result and lack of growth on MGIT and LJ culture), another sputum specimen was obtained for MGIT culture, smear, and line probe assay (LPA) at the third visit (sputum specimen 5). In these discordant cases, the baseline negative MGIT culture underwent extended incubation to see if extended incubation would result in growth.

Laboratory procedures. All testing was conducted at the Clinical Laboratory Services (CLS) in Johannesburg, South Africa. The CLS is a quality-assured clinical trial laboratory and a division the University of Witwatersrand's School of Pathology. Sputum 1 was used for Xpert MTB/RIF testing (standard of care) using a 2:1 sample reagent buffer-to-specimen ratio (Fig. 1B). Sputum 2 was decontaminated with *N*-acetyl-L-cysteine and sodium hydroxide and concentrated; the resultant pellet was

TABLE 3 (Continued)

HIV-positive cohort only (n =147)			
Sensitivity	**Specificity**	**PPV**	**NPV**
26/34 (76.5 [58.8- 89.3])	113/113 (100 [96.8–100])	26/26 (100 [86.8–100])	113/121 (93.4 [87.4–97.1])
24/34 (70.6 [52.5–84.9])	109/113 (96.5 [91.2–99])	24/28 (85.7 [67.3–96])	109/119 (91.6 [85.1–95.9])
30/34 (88.2 [72.5–96.7])	107/113 (94.7 [88.8–98])[c]	30/36 (83.3 [67.2–93.6])	107/111 (96.4 [91–99])
22/32 (68.8 [50–83.9])	107/112 (95.5 [89.9–98.5])	22/27 (81.5 [84.8–93.7])	107/117 (91.5 [84.8–95.8])
28/32 (87.5 [71–96.5])[e]	104/112 (92.9 [86.4–96.9])	28/36 (77.8 [60.8–89.9])	104/108 (96.3 [90.8–99])

resuspended and used for auramine O staining, direct GenoType MTBDR*plus* line probe assay (Hain Lifesciences, Germany) on smear-positive specimens (0.5 ml), inoculation in liquid culture using MGIT with incubation in a Bactec 960 instrument (BD Microbiology Systems, USA) (0.5 ml), and inoculation on LJ solid medium (0.2 ml). MTBDR*plus* and phenotypic drug susceptibility testing were performed on *M. tuberculosis* culture-positive specimens. The remainder of the concentrated pellet was reserved for Ultra (0.5 ml) and RT-MTB testing using the automated *m*2000 platform (0.5 ml). At the second visit, patients provided a further 2 sputum samples of at least 1 ml in volume. The order of specimens was randomized and the full volume was used for Ultra (Sputum 3) and RT-MTB with reflex RIF/INH (sputum 4). These specimens were tested raw (unprocessed) on the Ultra and RT-MTB assays. For the Ultra, a 2:1 sample reagent buffer-to-specimen ratio was used. The specimen for RT-MTB underwent preparation with inactivation reagent buffer in a 3:1 buffer-to-specimen ratio. Specimens that were positive for *M. tuberculosis* with RT-MTB underwent reflex RT-MTB RIF/INH testing. The final visit occurred approximately 2 months after enrollment (between 42 and 70 days), and the subset of patients with positive molecular (Xpert MTB/RIF, Ultra, or RT-MTB) and negative phenotypic (MGIT or LJ) culture results had a repeat sputum sent for MGIT culture, smear, and LPA (sputum 5). The baseline negative MGIT for these discordant cases was reincubated for a further 42 days.

Statistical analysis. Patients were excluded from the primary analysis if culture contamination occurred, if the culture was positive for nontuberculous mycobacteria, and if they did not return for the

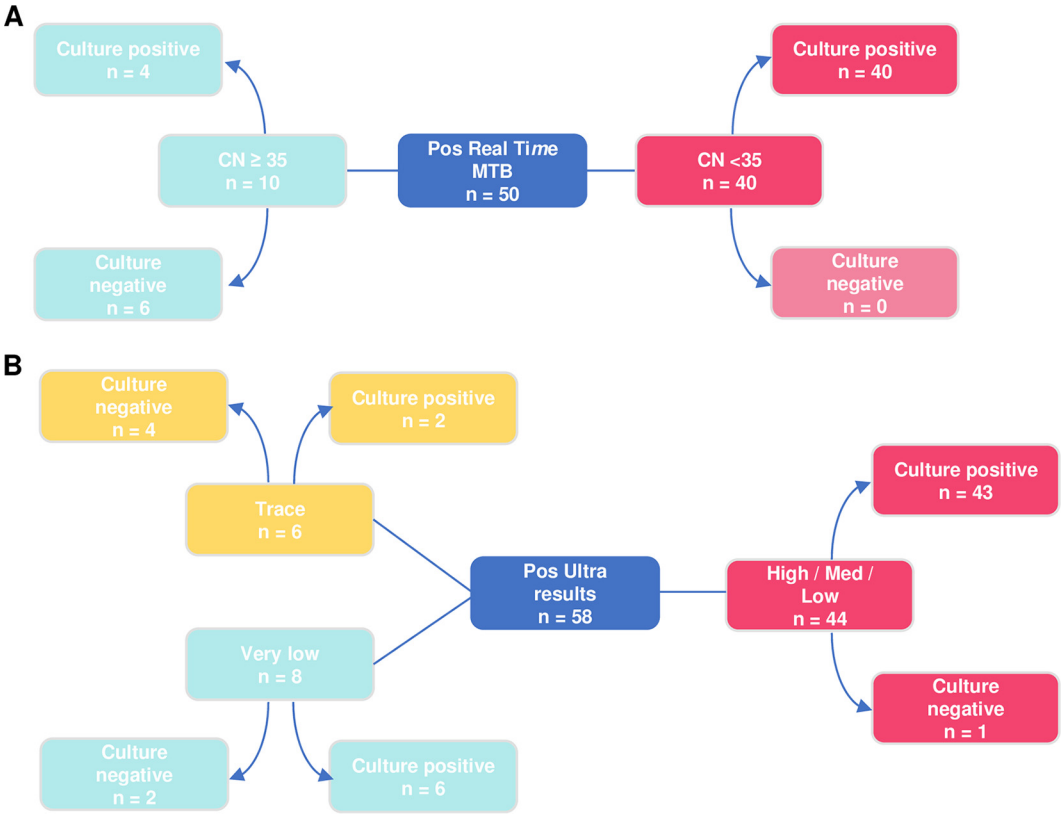

FIG 2 (A) RealTime MTB molecular and culture discordant cases. (B) Ultra molecular and culture discordant cases. CN, cycle number (C_N) in the RT-MTB assay; Pos, positive.

TABLE 3 (Continued)

HIV-negative cohort only (n = 82)				Smear-negative cohort only (n = 198)	
Sensitivity	**Specificity**	**PPV**	**NPV**	**Sensitivity**	**Specificity**
17/19 (89.5 [66.9–98.7])	63/63 (100 [94.3–100])	17/17 (100 [80.5–100])	63/65 (96.9 [89.3–99.6])	7/17 (41.2 [18.4–67.1])	181/181 (100) [98–100])
17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	7/17 (41.2 [18.4–67.1])	175/181 (96.7) [92.9–98.8])[c]
17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	11/17 (64.7 [38.3–85.8])	173/181 (95.6) [91.5–98.1])[c]
17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	4/16 (25% [7.27–52.4])	172/180 (95.6) [91.4–98.1])
17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	10/16 (62.5 [35.4–84.8])[e]	169/180 (93.9) [89.3–96.9])

second visit or were unable to produce sputum at the second visit. The culture endpoint was an aggregate of MGIT and/or LJ culture. In instances where MGIT was contaminated and LJ positive, the culture was considered positive. If MGIT was contaminated and LJ was negative, the culture was considered contaminated and the patient was excluded from the analytic cohort, as LJ was not considered to be sufficiently sensitive alone. The Xpert MTB/RIF and the Ultra cycle threshold (C_T) values were used as a measure of mycobacterial concentration, as was the cycle number (C_N) value in the RT-MTB assay. The Xpert MTB/RIF and the Ultra report semiquantitative results according to the C_T value, including high, medium, low, and very low. The Ultra has an additional semiquantitative category termed trace. Statistical analysis was performed using Stata version 14 (StataCorp, College Station, TX, USA). The sensitivity and specificity for each of the molecular diagnostic tests were estimated on the basis of a single test conducted on raw sputum or pellet and compared to those of culture. The sensitivity and specificity were analyzed overall and according to smear and HIV status and reported with 95% CIs. The PPV and NPV for each test were also calculated and presented with corresponding 95% CIs. The sensitivity, specificity, PPV, and NPV of the Ultra and RT-MTB performed on the concentrated sputum pellets were also assessed. Two-sided McNemar's test with exact binomial tests and significance at a P value of <0.05 were used to compare the sensitivities and specificities of the Ultra and RT-MTB to those of the Xpert MTB/RIF, using culture as a gold standard. The McNemar's test was used to compare the sensitivities and specificities of the Ultra and RT-MTB performed on sputum pellets, with culture as a gold standard. The Ultra has an additional lower threshold of detection, designated trace. Due to the poor specificity of Ultra trace results found in the FIND trial (5), an additional analysis was performed to assess the sensitivity, specificity, PPV, and NPV of the Ultra if trace results were reclassified as negative in the overall cohort and by HIV status and smear status. Discordant cases were defined as a patient with a positive molecular test (i.e., Xpert MTB/RIF, Ultra, or RT-MTB) on raw specimen or concentrated pellet accompanied by a negative MGIT and LJ culture. RRs for discordance are reported with 95% CIs and Fisher exact test results.

Ethics approval. Ethics approval for this study was obtained from the University of the Witwatersrand Human Ethics Review Committee (M1511110).

RESULTS

Cohort characteristics. A total of 299 patients that met the initial eligibility criteria across the three sites were enrolled in the study (Fig. 1A). Sixty-two patients were excluded from the primary analysis: 27 patients with contaminated mycobacterial growth indicator tube (MGIT)/Löwenstein-Jensen (LJ) culture, 4 patients with cultures positive for nontuberculous mycobacteria, 9 patients who were unable to produce sputum at the second visit, 21 patients who did not return for the second visit within 7 days (including 4 patients who were hospitalized prior to the second visit), and 1 patient with an invalid Ultra result. Patients with contaminated MGIT and negative LJ results were excluded from the analysis. Two patients with contaminated MGIT and positive LJ results were included. The primary analysis was performed on data from 237 patients.

The demographic and clinical characteristics of all enrolled patients are shown in Table 1. The median age was 36 years, with women making up one-third (33.3%) of the participants. HIV testing and counseling was offered to all patients; 3.4% declined testing, and there was an overall HIV prevalence of 62% with a median CD4 count of 220 cells/μl (interquartile range [IQR], 84 to 358). Fewer than half (43.5%) of the patients with HIV were on antiretroviral therapy (ART) at study enrollment. The rates of HIV infection in patients with and without culture-positive TB were similar (60.7% versus 62.4%, respectively). Among the participants with HIV, those with culture-positive TB had lower rates for preceding ART treatment (29.4% versus 47.8%) and lower median CD4 values (125 cells/mm^3 versus 278 cells/mm^3) at study enrollment than those who were not culture positive. A prior history of TB was reported in 18.1%, and antibiotic

TABLE 4 Diagnostic performance of Ultra based on reclassification of trace results as negative

Classification	No./total no. (% [95% CI])			
	Full cohort ($n = 237$)			
	Sensitivity	Specificity	PPV[a]	NPV[b]
Ultra trace included	50/56 (89.3 [78.1–96])	173/181 (95.6 [91.5–98.1])	50/58 (86.2 [74.6–93.9])	173/181 (96.6 [92.8–98.8])
Ultra trace reclassified as negative	49/56 (87.5 [75.9- 94.8])	178/181 (98.3 [95.2–99.7])	49/52 (94.2 [84.1–98.8])	178/185 (96.2 [92.4–98.5])

[a]PPV, positive predictive value.
[b]NPV, negative predictive value.

exposure during the month prior to study enrollment was reported in one-quarter (25.7%) of the patients enrolled in the study.

The final bacteriological and clinical classifications (definite TB versus possible TB versus no TB) took place at the final 2-month study visit (Table 2). Culture-positive pulmonary TB occurred in 58/237 (24.5%) cases. Fifty-six patients had positive baseline cultures, 1 patient had a positive culture upon extended incubation of MGIT, and 1 patient had a negative baseline culture and positive culture upon repeat MGIT testing (sputum 5) following discordant molecular and phenotypic results. Smear- and culture-positive disease occurred in 39/237 (16.5%) patients. Rifampin resistance was detected by GenoType MTBDR*plus* in 4/58 (6.9%) patients with culture-positive disease. A definite diagnosis of TB, defined as microbiologic confirmation of TB (by culture or the Xpert MTB/RIF) in sputum or an extrapulmonary specimen, occurred in 59/237 (24.9%) patients, which includes 58 patients with pulmonary TB and a 1 patient who was diagnosed with TB on the basis of a lymph node aspirate. A further 2.1% ($n = 5$) had possible TB, defined as a clinical diagnosis of TB made by X ray or other means with subsequent empirical treatment.

Diagnostic performance of Ultra and RT-MTB for identification of *M. tuberculosis*. The sensitivities and specificities of the three assays (Xpert MTB/RIF, RT-MTB, and Ultra) on raw sputum samples are described in Table 3. The test performance of the Ultra and RT-MTB were compared to that of the Xpert MTB/RIF. The sensitivity of the Ultra of 89.3% (95% confidence interval [CI], 78.1 to 96) was higher than that of the Xpert MTB/RIF (82.1% [95% CI, 69.6 to 91.1]; $P = 0.13$). The Ultra performed better in HIV-positive individuals, with a sensitivity of 88.2% (95% CI, 72.5 to 96.7) compared to 76.5% (95% CI, 58.8 to 89.3) with the Xpert MTB/RIF ($P = 0.13$). The Ultra also performed better than the Xpert MTB/RIF in smear-negative disease (64.7% [95% CI, 38.3 to 85.8] versus 41.2% [95% CI, 18.4 to 67.1], respectively; $P = 0.12$). The differences in sensitivity between RT-MTB and the Xpert MTB/RIF were smaller in all the cohorts. The overall RT-MTB sensitivity was 78.6% (95% CI, 65.5 to 88.4) compared to 82.1% (95% CI, 69.6 to 91.1) with the Xpert MTB/RIF ($P = 0.68$) and 70.6% (95% CI, 52.5 to 84.9) versus 76.5% (95% CI, 58.8 to 89.3) ($P = 0.68$), respectively, among people with HIV. There was no difference in sensitivity when testing patients with smear-negative disease.

Overall, the specificity of the Xpert MTB/RIF (100% [95% CI, 98 to 100]) was higher than that of RT-MTB (95.5% [95% CI, 91.4 to 98.1]; $P = 0.03$) and the Ultra (96.7% [95% CI, 92.9 to 98.8]; $P = 0.007$). Among people with HIV, the Xpert MTB/RIF specificity (100% [95% CI, 96.8 to 100]) was higher than that of the Ultra (94.7% [95% CI, 88.8 to 98]; $P = 0.03$) and RT-MTB (96.5% [95% CI, 91.2 to 99]; $P = 0.13$).

Performance of Ultra and RT-MTB on concentrated sputum pellets. Concentrated pellets were made from the second sputum specimen (sputum 2) on which MGIT, Ultra, and RT-MTB testing were conducted. The testing of the same specimens with all three tests provided the opportunity to compare the performance of the Ultra with that of the RT-MTB. In the overall cohort, the Ultra was more sensitive than RT-MTB (88.9% [95% CI, 77.4 to 95.8] versus 77.8% [95% CI, 64.4 to 88], respectively; $P = 0.03$) (Table 3). Although the Ultra was more sensitive (87.5% [95% CI, 71 to 96.5]) than RT-MTB (68.8% [95% CI, 50 to 83.9]) in patients with HIV ($P = 0.03$), the difference seen in the smear-negative cohort (62.5% [95% CI, 35.4 to 84.8] versus 25% [95% CI, 7.27 to 52.4], respectively) was not statistically significant.

TABLE 4 (Continued)

HIV-positive cohort (n = 147)			
Sensitivity	**Specificity**	**PPV**	**NPV**
30/34 (88.2 [72.5–96.7])	107/113 (94.7 [88.8–98])	30/36 (83.3 [67.2–93.6])	107/111 (96.4 [91–99])
29/34 (85.3 [68.9–95])	111/113 (98.2 [93.8–99.8])	29/31 (93.5 [78.6–99.2])	111/116 (95.7 [90.2–98.6])

Performance of molecular assays for the identification of rifampin and isoniazid drug resistance. The study was not designed to evaluate the sensitivity and specificity of the Ultra and RT-MTB for the detection of rifampin and isoniazid resistance. The susceptibility testing results can be found in Tables S1 and S2 in the supplemental material.

Challenges in interpretation: molecular and culture discordant cases. Among the culture-negative cases (n = 181), no false-positive results were observed from the Xpert MTB/RIF. There were 26 patients with positive molecular testing on raw and/or pellet specimens and negative MGIT and LJ cultures (see Table S3). Of these MGIT specimens, 25/26 underwent extended incubation for a further 42 days, and 1 was positive for *M. tuberculosis* after a total 51 days of incubation. Eight of twenty-six patients provided a specimen for repeat culture at the third study visit (sputum 5), and 1 patient had a positive MGIT. Four of twenty-six patients were started on TB therapy. There was no association between a history of TB (risk ratio [RR], 1.10; 95% CI, 0.44 to 2.77), HIV status (RR, 0.90; 95% CI, 0.40 to 2.00), prior antibiotic use (RR, 1.21; 95% CI, 0.54 to 2.73), and discordancy.

The majority of molecular and phenotypic discordancy occurred in specimens with low bacterial burdens. Of the 10 positive RT-MTB tests (on raw specimens) with a cycle number (C_N) of >35, 6 were culture negative and 4 were culture positive (Fig. 2A). All positive RT-MTB raw specimens with a C_N of <35 were culture positive. In addition, 4/6 with Ultra "trace" results (on raw specimens) were MGIT negative; 2/8 with "very low" results were MGIT negative, and 1/44 of the high/med/low results were culture negative (Fig. 2B).

Ultra assay performance based on reclassifying Ultra trace as negative. An additional analysis was conducted to assess the effect of reclassifying trace results as negative on Ultra test performance (Table 4). This resulted in a modest 1.8% loss of sensitivity (from 89.7% to 87.9%) accompanied by a 2.7% gain in specificity (95.6% to 98.3%). The positive predictive value (PPV) for the test improved from 86.7% (95% CI, 75.4 to 94.1) to 94.4% (95% CI, 84.6 to 98.8) without any appreciable change in the negative predictive value (NPV). However, there was a larger loss in sensitivity in the smear-negative cohort where sensitivity dropped by 5.6% (from 61.1% to 66.7%), with a 2.7% increase in specificity (95.6% to 98.3%).

DISCUSSION

In this clinical study, we compared two new molecular assays designed for the rapid detection of pulmonary tuberculosis and rifampin resistance, namely, the Ultra and RT-MTB, to the Xpert MTB/RIF in a cohort with a high rate of HIV coinfection. Although the results did not reach statistical significance, we found that the Ultra was 7% more sensitive than the Xpert MTB/RIF overall and 23% more sensitive among patients with smear-negative tuberculosis. RT-MTB was 3% less sensitive than the Xpert MTB/RIF overall, and performed equally to the Xpert MTB/RIF in patients with smear-negative disease. Both the Ultra and RT-MTB had decreased specificity compared to the Xpert MTB/RIF. The PPVs of 86.7% for the Ultra and 88.2% for RT-MTB suggest that approximately 1 in 6 positive tests would be a false-positive result with these two assays compared to that of culture. The specificity of the Ultra and RT-MTB did not vary by HIV or smear status. In a direct comparison of the Ultra to RT-MTB, the Ultra was 11% more sensitive overall (P = 0.03) and 38% more sensitive for smear-negative TB (P = 0.03).

TABLE 4 (Continued)

HIV-negative cohort ($n = 82$)				Smear-negative cohort ($n = 198$)	
Sensitivity	Specificity	PPV	NPV	Sensitivity	Specificity
17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	17/19 (89.5 [66.9–98.7])	61/63 (96.8 [89–99.6])	11/17 (64.7 [38.3–85.8])	173/181 (95.6 [91.5–98.1])
17/19 (89.5 [66.9–98.7])	62/63 (98.4 [91.5–100])	17/18 (94.4 [72.7–99.9])	62/64 (96.9 [89.2–99.6])	10/17 (58.8 [32.9–81.6])	178/181 (98.3 [95.2–99.7])

Our results mirror the test performance of the Ultra in a multicountry trial comparing the Ultra to the Xpert MTB/RIF conducted by FIND (5). Similar to the results of the FIND trial, most of the false-positive Ultra tests were trace, with trace representing the lowest semiquantitative value reported by the Ultra. We found that 10% of all positive Ultra results in our cohort were trace, and that 5 of 6 patients with trace-positive results had negative cultures. The reclassification of the Ultra trace results as negative resulted in a modest 1.8% loss of sensitivity accompanied by a 2.7% gain in specificity and a 7.7% increase in the PPV of the test. This suggests that the false-positive rate would drop from 1 in 6 positive tests to 1 in 20 positive tests if the Ultra trace results were reclassified as negative, without an appreciable loss of sensitivity in the overall cohort but with the caveat that the largest loss of sensitivity was in the smear-negative cohort, where sensitivity dropped by 5.6%.

This same pattern of false-positive molecular testing in the setting of very low mycobacterial quantities and negative culture was also seen with the RT-MTB assay but to a lesser extent than with the Ultra. Six of ten patients with positive RT-MTB results and C_N values of >35 (i.e., a very low bacterial burden) had negative cultures. We did not find an association between false-positive Ultra and RT/MTB results and a history of HIV status, prior antibiotic use, or, as was seen in the FIND Ultra trial, a history of tuberculosis (5). However, these conclusions are limited by the small number of Ultra trace and RT-MTB results with C_Ns of >35.

Although we considered molecular test-positive culture-negative cases to be false positives in our analysis, this is not entirely certain. The possible mechanisms explaining the molecular and phenotypic discrepancies include the limitations of MGIT culture methods as a gold standard for the detection of very low levels of MTB, the impact of preceding antibiotic use, and as was seen in the FIND trial but not in our study, the persistence of nonviable mycobacterial DNA in patients with a history of treated TB. In our study, we extended the liquid culture incubation period for patients who had a positive molecular test (Xpert MTB/RIF, Ultra, or RT-MTB) and a negative MGIT culture 42 days beyond the standard 42-day MGIT incubation period. We found a single patient that was Ultra trace positive who had growth of *M. tuberculosis* detected at 59 days of MGIT incubation. In addition, there was 1 patient with a positive repeat MGIT culture 2 months after the enrollment visit. These two cases provide limited evidence that molecular tests can detect very low burdens of viable mycobacteria not identified by current liquid culture standards.

Given the small number of patients with molecular and phenotypic test discordance in our study, we must be cautious in the interpretation of the significance and wider applicability of these results. Performance studies of these new assays in a variety of clinical settings, with different levels of background HIV infection, are needed to better understand the clinical significance of the discrepancies. In particular, longitudinal studies monitoring patients with discordant results over time are needed, especially in people who have very low levels of *M. tuberculosis* detected by molecular tests, such as an Ultra trace result and an RT-MTB with a C_N of >35.

Laboratories and national TB programs implementing these new diagnostic tests will have to determine how best to interpret Ultra trace and high C_N RT-MTB results while awaiting further research on the impact of these new assays on mortality and empirical treatment rates. Modeling studies suggest that the impact of switching from the Xpert MTB/RIF to the Ultra will be highly dependent on the clinical setting: countries with hyperendemic TB and high rates of HIV coinfection are predicted to see

a mortality benefit from the increased sensitivity of the Ultra assay, whereas the loss of specificity will result in overtreatment of false-positive cases in low TB prevalence regions (21). In its endorsement of the Xpert Ultra, the WHO recommended that a trace result should be considered a true positive in people with HIV, in children, and in extrapulmonary specimens due to the paucibacillary nature of the disease (22). However, in persons not at risk for HIV, the WHO recommended that an initial trace result be followed up with a repeat Ultra test: a second trace result should be considered positive, and a second negative result would require further clinical and radiologic follow-up prior to making a treatment decision.

The limitations of this study include the small sample size, which contributed to the lack of statistical significance in the comparison between the Ultra, RT-MTB, and the Xpert MTB/RIF. Larger cohorts are needed to confirm the findings of this study. Despite this limitation, we believe the trend of increased sensitivity of the Ultra in critical populations (smear negative and HIV-associated disease) is important to report. Given the variability in the performance of the Xpert MTB/RIF that was reported after the initial clinical trials, specifically the lower sensitivity in smear-negative disease (3, 4), we believe that this type of pragmatic study in a region with high HIV prevalence is of value.

Another important limitation is that the Xpert MTB/RIF, the Ultra, and RT-MTB assays performed on raw sputum were conducted on different specimens collected on days different from the one that was cultured. This may have resulted in variable sampling that might contribute to some of the molecular and phenotypic discordance seen in the study. This approach was chosen due to the logistic difficulty of collecting large volumes of sputum for testing on a single day. We aimed to design our study to evaluate the performance of the assays under realistic conditions, where often small volumes of sputum are collected. Limiting the study to patients who can provide large volumes of sputum can introduce bias toward more heavily symptomatic people with a greater burden of disease.

Conclusion. The expansion of molecular test options for the rapid diagnosis of TB and drug resistance to isoniazid and rifampin in both point-of-care and centralized laboratory settings is exciting following a long era of slow progress in diagnostic tests for TB. Although the results did not reach statistical significance, we found a trend of increased sensitivity of the Ultra assay compared to that of the Xpert MTB/RIF, which was more pronounced in patients with smear-negative TB. In a direct comparison between the Ultra and RT-MTB, the Ultra was 11% more sensitive overall and 38% more sensitive in smear-negative TB. These results suggest that the Ultra may improve diagnostic test performance in patients with paucibacillary disease for whom better diagnostics are critically important: children and people with advanced HIV. This increased sensitivity is accompanied by a loss of specificity, resulting in an increased number of false-positive tests, specifically associated with Ultra trace results. National TB programs will have to make individual determinations regarding the interpretation of Ultra trace results on the basis of their local TB epidemic and rates of HIV infection.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.00560-18.

SUPPLEMENTAL FILE 1, PDF file, 0.04 MB.

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