The Diagnostic Performance of the GenoType MTBDR_{plus} Version 2 Line Probe Assay Is Equivalent to That of the Xpert MTB/RIF Assay

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Molecular diagnostics for *Mycobacterium tuberculosis* have recently been endorsed by the World Health Organization. The Xpert MTB/RIF assay was endorsed for use on patient material, regardless of smear gradation, while the GenoType MTBDR_{plus} (version 1) has been limited for use on smear-positive patient material. In this study, we evaluated the diagnostic performance of the Xpert MTB/RIF and GenoType MTBDR_{plus} (version 2) assays on smear-positive and smear-negative patient specimens submitted to a high-throughput diagnostic laboratory. A total of 282 consecutive specimens were subjected to the two new molecular assays, and their performance characteristics were assessed relative to the routine diagnostic standard. Both assays showed similar diagnostic performance characteristics. The sensitivities of the GenoType MTBDR_{plus} (v2.0) and Xpert MTB/RIF assays for the detection of culture-positive *M. tuberculosis* were 73.1% and 71.2%, respectively, while the specificities of both assays were 100%. Both assays were able to diagnose the presence of *M. tuberculosis* in 57 to 58% of smear-negative cases, suggesting that the performance characteristics were dependent on bacillary load. The detection of *M. tuberculosis* in culture-negative specimens confirmed that molecular assays should not be used for treatment monitoring. The sensitivity and specificity for rifampin resistance detection were 100% in both assays; however, the GenoType MTBDR_{plus} (v2.0) assay provided additional information on isoniazid susceptibility. The GenoType MTBDR_{plus} (v2.0) assay will complement the Xpert MTB/RIF screening assay by validating rifampin susceptibility and providing information on isoniazid susceptibility. In addition, the GenoType MTBDR_{plus} (v2.0) assay will provide pharmacogenetic information that may be critical in guiding appropriate treatment.

Smear microscopy is the primary method for screening for tuberculosis (TB) in high-burden countries (29), but its performance characteristics are poor, with a case detection rate of only 56 to 68% (28). This is further reduced to 43 to 51% in patients coinfecting with the human immunodeficiency virus (HIV) (7), which often leads to paucibacillary disease (23). In 2010, it was reported that 2 million of the 5.8 million (34.4%) globally notified cases of TB were smear negative (30). In order to improve the sensitivity and specificity of TB diagnostic modalities, it is critical that new, rapid, and reliable diagnostics are developed (29). Culture-based methods have been greatly improved over the past decade and remain the “gold standard” for TB diagnosis. However, the time to positivity is dependent on the replication rate of *Mycobacterium tuberculosis* complex as well as the bacillary load in the specimen, which may be low in sputum samples from patients with HIV coinfection (9). This implies that the time to a bacteriological culture-based diagnosis may range from weeks to months (20, 22). Furthermore, an additional test is required to confirm the presence of *M. tuberculosis* complex. To address the associated diagnostic delay, molecular tests have been developed with the aim of providing both a diagnosis of the presence of *M. tuberculosis* complex and identifying mutations conferring resistance to the most important first-line TB antibiotics, viz isoniazid (INH) and/or rifampin (RIF) (3). In 2008, the GenoType MTBDR_{plus} (version 1.0) line probe assay (LPA) was endorsed by the World Health Organization (31). The GenoType MTBDR_{plus} (v1.0) LPA is used for the rapid detection of *M. tuberculosis* complex and its associated susceptibilities to RIF and INH (6). However, this test provides interpretable results for only 14% to 16% (3) of smear-negative specimens. For this reason, this version of the assay has been limited for use on smear-positive patient material. If

the smear result is found to be negative, the LPA can be done on the corresponding cultured isolate if the culture becomes positive for acid-fast bacilli (AFB).

In 2010, the Xpert MTB/RIF assay was endorsed by the World Health Organization for use on patient material, regardless of the smear gradation (27). This assay rapidly identifies *M. tuberculosis* complex isolates with sensitivities of 95% for smear-positive cases and 55% for smear-negative cases (24). The Xpert MTB/RIF assay is now being rolled out in many settings as the primary diagnostic modality with the view to improve case detection. In addition, this assay provides a rapid diagnosis of RIF resistance as a marker for multidrug-resistant TB (MDR-TB) (despite a number of false-positive RIF resistance results having been observed) (2, 25) and thereby initiates a request for additional drug susceptibility testing (DST). In most instances, this requires cultured-based DST with the associated diagnostic delay (20). The downstream implication of utilizing both molecular tests will be that of cost savings to the national tuberculosis control program due to reduced transmission, which in turn is brought about by the early detection of drug-susceptible and drug-resistant TB and hence the treatment thereof. It is also expected that early diagnosis will significantly impact treatment outcome.

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In this study, we investigate the diagnostic performance characteristics of the Xpert MTB/RIF assay and GenoType MTBDRplus (v2.0) LPA in comparison to the routine diagnostic standard in a high-volume public health tuberculosis laboratory in South Africa.

MATERIALS AND METHODS

Setting. This study was done in the National Health Laboratory Service (NHLS) TB referral laboratory for the Western Cape Province in Green Point, Cape Town, South Africa. This study was approved by the Health Research Ethics Committee at Stellenbosch University.

Routine diagnostic standard. In this setting, clinical specimens are routinely submitted for smear, culture, and GenoType MTBDRplus (v1.0) LPA (Hain Life Sciences, Germany) sensitivities. All sputum processing and manipulations are carried out in class II biological safety cabinets, and routine laboratory tests are done as previously described (3). Specimens are processed on a daily basis by decontamination with *N*-acetyl-L-cysteine-sodium hydroxide (NaOH-NALC; final concentration of 1%) as per CDC guidelines (14). Thereafter, each sediment is resuspended in approximately 2.0 ml phosphate buffer (pH 6.8). An aliquot of 0.05 ml of the concentrated sediment is subjected to smear microscopy using the auramine O fluorescence method (7) in combination with a royal blue LED light source to increase the sensitivity of detection of AFB (16). From the concentrated sediment, a 0.5-ml aliquot is inoculated into MGIT medium. If the original specimen was smear positive, crude DNA is extracted from an aliquot of 0.5 ml by heat killing for 15 min at 95°C followed by gentle sonication for 20 min. *M. tuberculosis* complex strains and mutations conferring resistance to INH and RIF are detected by the GenoType MTBDRplus (v1.0) LPA (3, 31) using DNA extracted directly from decontaminated specimens (smear positive) or from MGIT cultures that become positive for AFB (smear-negative specimens). All LPA runs adhered to ISO 15189 standards, which require the use of an ATCC *M. tuberculosis* H37Rv laboratory strain for a positive control. Two negative controls were used to test for area-specific contamination. The laboratory participates in the External Quality Assurance (EQA) program from the NHLS Quality Assurance (QA) Division, the National Tuberculosis Reference Laboratory (NTBRL) EQA program, and the EQA program of the Medical Research Council (MRC). Since participation in these programs in 2008, all of the results reported showed 100% concordance with the EQA testing panels.

Diagnostic performance evaluation. For this study, 282 consecutive specimens from suspected retreatment TB cases were collected within two working days. Retreatment cases were selected given their high risk of associated drug resistance (15). Each specimen was subjected to the routine diagnostic standard.

A small volume of 0.01% Tween 80-saline was added to the residual decontaminated specimen to a final volume of 1.5 ml. The suspension was then vortexed in order to homogenize the acid-fast bacilli, thereby ensuring adequate representation within the sample volumes to be extracted. After vortexing, the specimen was split into two equal aliquots (700 µl).

One aliquot was processed according to the Xpert MTB/RIF instruction manual (cartridge version 3). Briefly, 2 volumes of lysis buffer was added to each specimen and mixed. Following incubation at room temperature for 5 min, the suspension was mixed and then incubated for a further 10 min. Thereafter, 2 ml of the lysate was added to the labeled cartridge and was inserted into a GX16 instrument. The results for the first batch of 16 specimens were available in approximately 2 h.

DNA was extracted from the remaining aliquot using the GenoLyse kit according to the manufacturer's instructions to generate the substrate for PCR amplification and hybridization using the GenoType MTBDRplus (v2.0) LPA. Briefly, 700 µl of the decontaminated sample was centrifuged for 15 min at 10,000 × *g*, and the pellet was resuspended in 100 µl of lysis buffer and incubated for 5 min at 95°C. Thereafter, 100 µl of neutralizing buffer was added to the lysate, mixed, and centrifuged at full speed for 5 min. The top 100 µl of the supernatant was aliquoted into a clean 1.5-ml

tube and used for the PCR, and the residual portion was discarded. The PCR mixture was prepared by mixing 10 µl of amplification mix A (AM-A) (which contains the 10× buffer, nucleotides, and DNA polymerase) with 35 µl of amplification mix B (AM-B) (which contains MgCl₂, the biotinylated primers, and dye), after which 5 µl of the GenoLyse-purified DNA was added. PCR amplification for the GenoType MTBDRplus (v2.0) LPA was done using the PCR program recommended by the manufacturer's instructions. Following PCR amplification, the reverse hybridization step and the interpretation of the hybridization results were done as previously described (3). The results for this test were available 48 h after receipt of the specimen.

Statistical analysis. Statistical analyses were done using EPI Info (Centers for Disease Control and Prevention, Atlanta, GA). The sensitivity, specificity, and positive and negative predictive values were calculated for each method and compared to those for the gold standard of smear, MGIT culture, and GenoType MTBDRplus (v1.0) LPA.

RESULTS

Of the 282 sputum specimens tested by the routine diagnostic standard, seven specimens gave instrumentation errors when tested in the Xpert MTB/RIF assay. Six of these were due to "probe failure," and one was due to "valve motion not detected." After exclusion of these specimens, the total cohort was reduced to 275 specimens. Twenty-one of the 22 smear- and culture-positive specimens were confirmed to be *M. tuberculosis* complex according to the GenoType MTBDRplus (v1.0) LPA. The remaining smear- and culture-positive specimen was shown to be *Mycobacterium kansasii*. Of the smear-negative specimens, 18.5% (46/248) were culture positive for acid-fast bacilli, of which 67.4% (31/46) were confirmed to be *M. tuberculosis* complex. All of the remaining 15 culture-positive specimens were classified as nontuberculosis mycobacteria based on Ziehl-Neelsen (ZN) smear microscopy, since neither the classic coiling could not be observed nor could these be determined to the species level using the CM/AS kits (Hain Lifescience, Nehren, Germany) (data not shown). This may reflect laboratory contamination with unknown environmental acid-fast bacilli. Of the 52 specimens (18.9%) that were found to be positive for *M. tuberculosis* complex (51 by the GenoType MTBDRplus (v1.0) LPA and 1 by the Capilia TB assay), 71% (37/52) and 73% (38/52) were detected by the Xpert MTB/RIF and the GenoType MTBDRplus (v2.0) LPA, respectively. The GenoType MTBDRplus (v2.0) LPA detected all of the smear/culture-positive TB cases, while the Xpert MTB/RIF detected 19/21 (90.5%) of the smear/culture-positive TB cases. The detection rates for the identification of *M. tuberculosis* complex in smear-negative/culture-positive specimens were 18/31 (58%) and 17/31 (56.6%) for the Xpert MTB/RIF assay and the GenoType MTBDRplus (v2.0) LPA, respectively. The median time to positivity (TTP) for the specimens that could not be identified by the two modalities was 27 days (range, 10 to 37 days). Table 1 shows the performance parameters for the two tests compared to smear positivity and the routine MGIT culture positive for AFB and positive identification for the *M. tuberculosis* complex.

The Xpert MTB/RIF assay and the GenoType MTBDRplus (v2.0) LPA identified *M. tuberculosis* complex in 5 and 10 culture-negative specimens, respectively. Review of historical diagnostic data from the laboratory information management system showed that all of these patients had submitted specimens within the last year, thereby confirming that the specimen tested was not a diagnostic specimen but rather a specimen submitted to monitor treatment response.

TABLE 1 Performance of the Xpert MTB/RIF and GenoType MTBDR_{plus} (v2.0) LPA in combination with the GenoLyse kit relative to routine smear and culture positive results

Assay result ^a	No. of smear results:			No. of culture results:			Performance, % (95% CI) ^b			
	Positive	Negative	Total	Positive for <i>M. tuberculosis</i> complex	Negative for <i>M. tuberculosis</i> complex	Total	Sensitivity	Specificity	PPV	NPV
Xpert MTB/RIF										
MTB ⁺	20	22	42	37	0	37	74.1 ^c (55.3–86.8)	91.1 ^c (86.9–94.1)	47.6 ^c (33.4–62.3)	97.0 ^c (93.9–98.5)
MTB [−]	7	226	233	15	16	31	71.2 ^d (57.7–81.7)	100 ^d (80.74–100)	100 ^d (90.6–100)	51.6 ^d (34.8–68.0)
Total	27	248	275	52	16	68				
GenoType MTBDR_{plus} (v2.0) LPA and GenoLyse kit										
MTB ⁺	22	26	48	38	0	38	81.5 ^c (63.3–91.8)	89.5 ^c (85.1–92.7)	45.8 ^c (32.6–59.7)	97.8 ^c (94.9–99.1)
MTB [−]	5	222	227	14	16	30	73.1 ^d (59.8–83.2)	100 ^d (80.6–100)	100 ^d (90.8–100)	53.3 ^d (36.1–69.8)
Total	27	248	275	52	16	68				

^a MTB⁺, *Mycobacterium tuberculosis* complex present; MTB[−], *Mycobacterium tuberculosis* complex absent.^b 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.^c Smear results used as the gold standard.^d Culture results used as the gold standard.

First-line DST results were available for 51 of the 52 *M. tuberculosis* complex-positive specimens using the GenoType MTBDR_{plus} (v1.0) LPA as a standard diagnostic algorithm test: 9.8% (5/51) were MDR, 1.96% (1/51) were RIF monoresistant, 1.96% (1/51) were INH monoresistant, and 86.3% (44/51) were susceptible to both RIF and INH. [Tables 2](#) and [3](#) show the performance parameters for the two tests for the detection of mutations conferring RIF and INH resistance, respectively, compared to the routine diagnostic standard.

DISCUSSION

This is the first study to compare the diagnostic performances of two molecular TB diagnostic modalities relative to the routine diagnostic standard used in a high-throughput diagnostic laboratory. Under these routine laboratory conditions, the Xpert MTB/RIF and GenoType MTBDR_{plus} (v2.0) LPA diag-

nostic modalities showed sensitivities of 71.2% and 73.1%, respectively, and similar specificities of 100%, which compared well with previous studies using the Xpert MTB/RIF ([5](#), [8](#), [11](#)). The overall sensitivity of both tests was largely influenced by the fact that *M. tuberculosis* complex DNA could not be detected in 23.5% of culture-positive samples. This was reflected by the TTP of these specimens. This suggests that the number of bacilli in these specimens was below the detection limit (dynamic range) for both of the molecular tests and above the detection limit for the culture-based test ([4](#), [10](#)).

Both tests were able to rapidly detect the presence of *M. tuberculosis* complex DNA in most smear-positive cases as well as in 56 to 58% of the smear-negative culture-positive samples. This increased the number of TB cases diagnosed by between 32.6% and 34.6% for the GenoType MTBDR_{plus} (v2.0) LPA and Xpert MTB/RIF, respectively, in this study setting relative to smear alone ([3](#)).

TABLE 2 Performance of the Xpert MTB/RIF and GenoType MTBDR_{plus} (v2.0) LPA in combination with the GenoLyse kit relative to routine RIF susceptibility testing

Assay result ^a	No. of RIF susceptibility results:			Performance, % (95% CI) ^c			
	S	R	Total ^b	Sensitivity	Specificity	PPV	NPV
Xpert MTB/RIF							
S	33	0	33	100 (89.6–100)	100 (43.9–100)	100 (89.6–100)	100 (43.6–100)
R	0	3	3				
MTB [−]	12	3	15				
Total	45	6	51				
GenoType MTBDR ^{plus} (v2.0) LPA and GenoLyse kit							
S	34	0	34	100 (89.9–100)	100 (43.9–100)	100 (89.9–100)	100 (43.9–100)
R	0	3	3				
MTB [−]	11	3	14				
Total	45	6	51				

^a S, susceptible; R, resistant; MTB[−], *Mycobacterium tuberculosis* complex absent.^b According to the GenoType MTBDR_{plus} (v1.0) LPA.^c 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

TABLE 3 Performance of the GenoType MTBDR_{plus} (v2.0) LPA in combination with the GenoLyse kit relative to routine INH susceptibility testing

Assay result ^a	No. of INH susceptibility results:			Performance, % (95% CI) ^c			
	S	R	Total ^b	Sensitivity	Specificity	PPV	NPV
GenoType MTBDR _{plus} (v2.0) LPA and GenoLyse kit							
S	34	0	34	100 (89.9–100)	100 (43.9–100)	100 (89.9–100)	100 (43.9–100)
R	0	3	3				
MTB [−]	11	3	14				
Total	45	6	51				

^a Note that the Xpert MTB/RIF assay was specific for RIF only. Therefore, no INH susceptibility or performance results are given. S, susceptible; R, resistant; MTB[−], *Mycobacterium tuberculosis* complex absent.

^b According to the GenoType MTBDR_{plus} (v1.0) LPA.

^c 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.

This confirms the utility of both of these methods as initial screening tools for “ruling in” the presence of *M. tuberculosis* complex disease. Three MDR-TB cases were not detected within the culture-positive group, probably as a result of the bacterial load being below the detection limits of both assays.

Both tests appear to overdiagnose the presence of *M. tuberculosis* complex DNA in culture-negative samples. This phenomenon has been previously described in various studies and may be explained by the amplification of DNA released from nonviable bacilli in the sputum samples (1, 12, 13, 17–19). Alternatively, the presence of *M. tuberculosis* complex DNA in these samples may be explained by either laboratory cross-contamination (13) or a transcription error that has failed to indicate that the sample was a treatment follow-up sample rather than a diagnostic sample. We favor the latter, as historical follow-up records confirmed in all instances that these samples were taken from patients who had had a previous specimen(s) submitted for diagnosis, suggesting that these patients were either on treatment or were previously treated and that the positive test results reflect the presence of dead bacilli, given their inability to grow in the MGIT tube (1, 12, 13, 17–19). These findings support the notion that molecular assays should not be used for treatment monitoring (18).

The Xpert MTB/RIF assay showed a significant advantage in the time to detect the presence of *M. tuberculosis* complex DNA. However, the number of Xpert MTB/RIF modules required by high-throughput laboratories to deliver the same amount of test results as an automated GenoType MTBDR_{plus} system must not be ignored.

The most important advantage of the GenoType MTBDR_{plus} (v2.0) assay is its enhanced sensitivity and the ability to determine INH susceptibility. These characteristics have the potential to improve the current diagnostic algorithm within the context of a high-throughput laboratory by improving both the number of cases that can be screened (inclusion of smear negative cases) and the concomitant improved time to detection. If the screening tool is the Xpert MTB/RIF assay, then RIF resistance can be rapidly validated with the GenoType MTBDR_{plus} (v2.0) LPA irrespective of the smear status and without the need for prior culture. Similarly, INH susceptibility can be rapidly determined when RIF-susceptible samples are identified by the Xpert MTB/RIF assay. Data from the laboratory information management system (unpublished data), indicated that 86% of drug-resistant isolates had associated INH resistance, thereby supporting the need for susceptibility testing of INH (21). Considering this, it is suggested

that RIF-resistant results are validated by the GenoType MTBDR_{plus} (v2.0) LPA, after which the GenoType MTBDR_{sl} LPA (second-line drug LPA) can be performed with the same protocols as for the first-line LPA, to determine extensively drug-resistant TB (XDR-TB) status, thus allowing for “diagnostic fit.”

A limitation observed in the study is that the performance characteristics might have been improved if the residual specimen material was directly used after decontamination in the routine diagnostic algorithm, since the dilution, homogenization, and splitting may not have contained sufficient bacteria due to prior manipulations, since it was found that two smear-positive, culture-positive specimens were not detected by the Xpert MTB/RIF assay.

In summary, the GenoType MTBDR_{plus} (v2.0) LPA has the potential to improve the time to diagnosis of TB and drug resistance in HIV-coinfected smear-negative patients. In addition, this assay will complement the Xpert MTB/RIF screening assay by validating RIF susceptibility on smear-positive and smear-negative specimens, thereby providing information on INH susceptibility as well as pharmacogenetic information that may be critical in guiding appropriate treatment (26).

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