The Diagnostic Performance of the GenoType MTBDRplus 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 MTBDRplus (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 MTBDRplus (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 MTBDRplus (v2.0) assay provided additional information on isoniazid susceptibility. The GenoType MTBDRplus (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 MTBDRplus (v2.0) assay will provide pharmacogenetic information that may be critical in guiding appropriate treatment.

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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 specimen 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 PCR amplification and hybridization using the GenoType MTBDRplus 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 nontuberculous 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.
First-line DST results were available for 51 of the 52 M. tuberculosis complex-positive specimens using the GenoType MTBDRplus (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 MTBDRplus (v2.0) LPA diagnostic 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 MTBDRplus (v2.0) LPA and Xpert MTB/RIF, respectively, in this study setting relative to smear alone (3).
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 MTBDRplus system must not be ignored.

The most important advantage of the GenoType MTBDRplus (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 MTBDRplus (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 MTBDRplus (v2.0) LPA, after which the GenoType MTBDRplus 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 MTBDRplus (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).

**ACKNOWLEDGMENT**

We thank the hardworking staff of the Green Point Tuberculosis Reference Laboratory, without whom this study would not have been possible.

**REFERENCES**


**TABLE 3 Performance of the GenoType MTBDRplus (v2.0) LPA in combination with the GenoLyse kit relative to routine INH susceptibility testing**

<table>
<thead>
<tr>
<th>Assay result</th>
<th>No. of INH susceptibility results</th>
<th>Performance, % (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>GenoType MTBDRplus (v2.0) LPA and GenoLyse kit</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>34</td>
<td>0</td>
</tr>
<tr>
<td>R</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>MTB &lt;sup&gt;+&lt;/sup&gt;</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>6</td>
</tr>
</tbody>
</table>

*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 <sup>+</sup>, *Mycobacterium tuberculosis* complex absent.

† According to the GenoType MTBDRplus (v1.0) LPA.

<sup>+</sup> 95% CI, 95% confidence interval; PPV, positive predictive value; NPV, negative predictive value.