Comparison of Two Commercially Available DNA Line Probe Assays for Detection of Multidrug-Resistant *Mycobacterium tuberculosis*

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Two commercially available DNA line probe assays, Genotype MTBDR and INNO-LiPA Rif. TB, were evaluated for their abilities to detect resistance to isoniazid (INH) and rifampin (RIF) in 52 *Mycobacterium tuberculosis* isolates. The test results were compared to those obtained by phenotypic drug susceptibility testing and sequencing. Compared to the results of phenotypic drug susceptibility testing, the Genotype MTBDR test results were concordant for INH for 47 of the 52 (90.4%) isolates, and both the Genotype MTBDR and the INNO-LiPA Rif. TB test results were concordant for RIF for 51 of the 52 (98.1%) isolates. The Genotype MTBDR test results correlated with the sequencing results for 48 of the 52 (92.3%) isolates and the INNO-LiPA Rif. TB results for 50 of the 52 (96.2%) isolates. Both assays are useful for the rapid screening of *M. tuberculosis* isolates obtained from patients suspected of having multidrug-resistant tuberculosis, but the Genotype MTBDR assay has the advantage of being able to detect resistance to both INH and RIF simultaneously.

Multidrug-resistant (MDR) *Mycobacterium tuberculosis* isolates are resistant to at least isoniazid (INH) and rifampin (RIF), the two most important components of effective anti-tuberculosis therapy. Incorrect use of the drugs, treatment failures, and the transmission of MDR isolates have caused multidrug-resistant tuberculosis (MDR-TB) to become a rapidly increasing health problem in both developed and developing countries (3, 5). Drug susceptibility testing by conventional methods takes several weeks, while early diagnosis of the disease and the rapid identification of resistant strains are essential for efficient treatment and control of the MDR strains.

In the last few years, there has been considerable progress in our understanding of the mechanisms of action and of the basis of resistance to the antituberculous drugs, especially resistance to INH and RIF. Resistance to INH has been associated with alterations in at least four genes, but extensive studies have demonstrated that INH resistance is most frequently associated with a specific mutation in codon 315 of *M. tuberculosis* catalase peroxidase (*katG*) gene (13). The prevalence of *M. tuberculosis katG* mutations varies geographically, but the *katG* codon 315 mutation is found in between 60 and 90% of the INH-resistant strains (1, 2, 4, 9, 10, 15). The mechanism of resistance to RIF involves missense mutations in a well-characterized region of the beta subunit of DNA-dependent RNA polymerase (which is encoded by the *rpoB* gene). Several studies have shown that more than 95% of RIF-resistant strains harbor a mutation within an 81-bp region of the *rpoB* gene (8, 16, 17).

Generally, DNA sequencing-based approaches are considered the reference assays for the detection of mutations, but often, they have been found to be too cumbersome for routine use. The commercial strip assay INNO-LiPA Rif. TB (Innogenetics, Ghent, Belgium) has previously been evaluated for the detection of mutations conferring resistance to RIF in *M. tuberculosis* (7, 11, 14). The Genotype MTBDR assay (Hain Lifescience GmbH, Nehren, Germany) is a novel kit-based method for the detection of the most common mutations in *M. tuberculosis katG* and *rpoB*.

The Genotype MTBDR test is based on the same general principle as the INNO-LiPA Rif. TB test, but it has the advantage of being able to detect the presence of mutations in both *katG* and *rpoB* simultaneously and thus predict resistance to both INH and RIF (6).

Both strip tests are based on reverse hybridization of amplicons (*katG* in the Genotype MTBDR test and *rpoB* in both tests) to immobilized, membrane-bound probes covering the wild-type (WT) sequences; the *katG* 315 mutation (the Genotype MTBDR test); and the *rpoB* mutations Asp516Val, His526Tyr, His526Asp, and Ser531Leu. The aim of this study was to compare the performances of the Genotype MTBDR test and the INNO-LiPA Rif TB test for the rapid detection of MDR among Finnish and Russian *M. tuberculosis* isolates. The line probe assay results were compared to the results obtained by conventional sequencing and phenotypic drug susceptibility testing.

**MATERIALS AND METHODS**

**Bacterial isolates.** A total of 52 *M. tuberculosis* isolates obtained from patients in Finland and Russia were included in the study. The isolates were cultured on Lowenstein-Jensen medium, and drug susceptibility testing was performed by the agar proportion method according to an international standard (18). For PCR amplification, a loopful of bacteria was suspended into 1 ml of 1× TE (Tris-EDTA) buffer and inactivated at 95°C for 15 min. The suspension was mixed by vortexing and centrifuged at 12,000 rpm for 5 min in a 5415 D centrifuge (Eppendorf AG, Hamburg, Germany), and the supernatant was used for PCR. PCR amplification and conventional sequencing of the *katG* and *rpoB* loci were performed as described previously (10, 12).

**Genotype MTBDR and INNO-LiPA Rif. TB assays.** The Genotype MTBDR and the INNO-LiPA Rif. TB assays were carried out according to the manufac-
turers' instructions with the reagents provided in the kits. Both assays consist of PCR amplification, hybridization of the PCR products to the probe-containing strips, and detection and interpretation of the results.

Both the Genotype MTBDR and the INNO-LiPA Rif. TB test strips have a conjugate control line to ensure that reactive conjugate and substrate have been added and an M. tuberculosis complex-specific probe to confirm that the bacterial isolate being tested belongs to the M. tuberculosis complex. In addition to this, the Genotype MTBDR test has a universal control probe that detects mycobacteria and other gram-positive bacteria with a high genomic G+C contents and positive controls for the katG and rpoB genes. Only bands with intensities as strong as or stronger than that of the universal control are considered in the interpretation of the Genotype MTBDR test results.

Although these strip tests do not contain probes for all the possible rpoB mutations, five WT rpoB probes cover the whole S1 bp resistance-determining region of the gene. Both the GenoType MTBDR and INNO-LiPA Rif. TB test instructions state that the absence of a signal with any of the WT probes indicates resistance to RIF. In addition to this, the GenoType MTBDR test strip has four katG-specific probes: the katG Uni probe, which detects a katG-specific gene region (and which should be positive for all M. tuberculosis isolates); a katG WT probe; a katG MUT1/T1 probe, which detects the AGC—ACC (S315T) mutation; and a katG MUT2/T2 probe, which detects the AGC—ACA (S315T) mutation.

RESULTS AND DISCUSSION

According to the results of conventional drug susceptibility testing, 28 of the 52 isolates were MDR, 4 were resistant to INH only, and 20 were fully susceptible. Of the 32 isolates that were phenotypically resistant to INH, 27 (84.4%) had a Ser315Thr mutation in katG codon 315. The remaining five (15.6%) INH-resistant isolates did not contain a mutation in this region. None of the 20 phenotypically INH-susceptible isolates harbored an H526D mutation. Twenty-four of the 28 (85.7%) isolates were correctly identified as MDR by the Genotype MTBDR test.

Comparison of the test results is shown in Tables 1 and 2.

### TABLE 1. Comparison of agar proportion method, katG sequencing, and Genotype MTBDR test for detection of INH susceptibility among the 52 M. tuberculosis isolates

<table>
<thead>
<tr>
<th>INH susceptibility</th>
<th>Agar proportion method</th>
<th>katG sequencing</th>
<th>Genotype MTBDR test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>32</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Susceptible</td>
<td>20</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

TB tests. A fourth MDR isolate harboring an rpoB S531L mutation failed to hybridize with the GenoType MTBDR test MUT3 (S531L) probe, although the test with the WT 5 probe was negative.

Of the 28 isolates that were phenotypically MDR, all 28 contained a mutation in rpoB and 24 contained a mutation in katG codon 315. Twenty-four of the 28 (85.7%) isolates were correctly identified as MDR by the Genotype MTBDR test. Comparison of the test results is shown in Tables 1 and 2.

The mutations that the INNO-LiPA Rif. TB and/or GenoType MTBDR test missed should have been detected by the line probe assays, since a specific probe is included in the test strip (katG S315T and rpoB A516G, H526D, and S531L). In two cases both a WT genotype and a mutated genotype were present in the PCR amplificons, which would have compromised the DNA-based analysis. However, the sequencing results for these two isolates did not indicate a mixed population. The fact that the same two isolates that displayed the WT and the mutated genotype gave discrepant results in both the line probe tests could indicate that the strip tests are better able than sequencing to detect mixed bacterial populations. Another possibility is that the line probe assays displayed nonspecific hybridization. The rpoB D516G mutation that both the assays missed should have been detected indirectly by omission of a signal from the corresponding WT-specific probe. However, it is possible that if the WT DNA is present in large excess, the sensitivity of the molecular assay may not be high enough to detect a small amount of mutated DNA.

Both the Genotype MTBDR and the INNO-LiPA Rif. TB assays were straightforward and easy to perform, with mass production of the test strips. There is no expensive instrumentation needed, and the reading of the strips is easy. In the INNO-LiPA Rif. TB assay, the hybridization band intensities were always approximately the same and the bands were of

### TABLE 2. Comparison of agar proportion method, rpoB sequencing, Genotype MTBDR test, and INNO-LiPA Rif. TB test for detection of RIF susceptibility among the 52 M. tuberculosis isolates

<table>
<thead>
<tr>
<th>RIF susceptibility</th>
<th>Agar proportion method</th>
<th>rpoB sequencing</th>
<th>Genotype MTBDR test</th>
<th>INNO-LiPA Rif. TB assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistant</td>
<td>28</td>
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</tr>
<tr>
<td>Susceptible</td>
<td>24</td>
<td>24</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>
equal size, whereas in the Genotype MTBDR assay, the band intensity as well as the band size varied. Thus, the interpretation of the Genotype MTBDR test results was not as clear as that of the INNO-LI PA Rif. TB test results. Also, we found the labeling of the probes to be somewhat confusing in both assays. On the evaluation sheet, the probes specific for the katG mutation were labeled T1 and T2 and the probes specific for the rpoB mutation were labeled MUT1, MUT2A, MUT2B, and MUT3. In the INNO-LiPA Rif. TB assay, the rpoB mutations are labeled R2, R4a, R4b, and R5. The corresponding mutations can be found in the instruction manuals and in the Genotype MTBDR reading chart. However, the interpretation of the results would be clarified if the same terminology, preferably, the amino acid codon of each mutation, were used in all parts of the assay.

One disadvantage that the line probe tests share with other probe-based assays is that they can identify only the most frequent mutations and otherwise rely on the negative hybridization result with the WT-specific probe as a marker for additional mutations. Since no sequence data are provided and not every mutation confers drug resistance, the significance of the mutation detected can be difficult to interpret. Moreover, the Genotype MTBDR test cannot identify those INH-resistant isolates that have resistance-conferring mutations other than S315T in katG. Hence, the clinical utility of the test varies depending on the prevalence of the particular mutation at this locus. As only resistance to INH and RIF can be predicted, the molecular assays do not give any information about the most appropriate therapy for the patient. The primary use for the molecular tests is the rapid identification and isolation of the patients infected with MDR isolates and, thus, minimization of the transmission of MDR-TB. Since the assays are PCR based, they could even be used for the direct detection of M. tuberculosis, at least for smear-positive sputum specimens. However, neither assay is currently designed for that purpose. Unfortunately, the costs of the molecular assays often prohibit their use in the settings where they would be urgently needed.

In conclusion, the Genotype MTBDR assay was found to be a useful tool for the rapid screening of M. tuberculosis isolates obtained from patients suspected of having MDR-TB. Compared to the previously available INNO-LiPA Rif. TB test, it has the advantage of being able to detect resistance to both INH and RIF. However, the molecular drug susceptibility testing results should always be confirmed by phenotypic methods.

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REFERENCES

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