In a previous publication (1), we evaluated the Genotype MTBDR (GT-MTBDR; Hain Lifescience, Nehren, Germany) assay as a rapid diagnostic tool for multidrug resistance (MDR) detection in Mycobacterium tuberculosis isolates and clinical specimens. The main limitation of the test was the low sensitivity for isoniazid resistance detection (70%), due to the targeting of only one (katG) of the genes involved (2). Furthermore, the test performance on clinical specimens was suboptimal because of the lack of a clear hybridization signal (unpublished data).

Recently, a new version of the test, the Genotype MTBDRplus (GT-MTBDRplus) assay, has been developed in order to improve the assay’s sensitivity for isoniazid resistance and direct detection of drug resistance in clinical specimens. It targets all the genes included in the previous test with the addition of codons 505 to 510 in the hot-spot region of rpoB, to improve the detection of rifampin resistance, and the promoter region of the inhA gene (nucleotides −8, −15, and −16) for isoniazid resistance.

We tested with the GT-MTBDRplus assay 173 isoniazid-resistant isolates (Table 1) and 78 clinical specimens (Fig. 1) that had been previously analyzed with the GT-MTBDR version and compared the results with those of sequence analysis. DNA extraction from isolates and clinical specimens was performed as previously described (1).

The use of the GT-MTBDRplus assay allowed the correct identification of an additional 11.6% (20/173) of isoniazid-resistant strains compared to the results with the GT-MTBDR, with a specificity of 100%. In one sample, none of the tests allowed the detection of the katG gene due to the presence of several mutations in the same gene, as confirmed by the sequence analysis. Both the assays were interpreted as indeterminate for the katG analysis.

The two tests were also evaluated side by side (Fig. 1) on a panel of 78 respiratory samples that were positive by M. tuberculosis Direct test (MTD; bioMerieux, Marcy l’Etoile, France). Among the 78 clinical specimens analyzed, 31 were smear negative (25 fully susceptible, 4 isoniazid resistant, and 2 MDR) and 47 smear positive (41 fully susceptible, 5 isoniazid resistant, and 1 MDR). With the GT-MTBDR assay, we obtained a valid test for 18/31 (58.1%) smear-negative and 45/47 (95.7%) smear-positive samples and an indeterminate result defined as absence of positive amplification control in 15 cases (13 smear negative, 2 smear positive). In 12 cases (6 smear negative, 6 smear positive), despite the presence of a positive amplification control, the interpretation of the test was difficult due to the faint staining of some hybridization bands. The number of valid tests obtained by the new assay was 100% (47/47) for smear-positive and 70.9% (22/31) for smear-negative samples, with no ambiguity in the hybridization pattern. With smear-negative samples, the GT-MTBDRplus assay allowed the reduction of indeterminate results from 41.9% to 29.0% and improved the concordance rate with culture-based DST, drug susceptibility testing; INH-R, isoniazid resistance.

**TABLE 1. Detection of isoniazid resistance in clinical isolates by the G-MTBDR and the G-MTBDRplus assays compared with sequencing data**

<table>
<thead>
<tr>
<th>Assay</th>
<th>Gene</th>
<th>No. (%) of isolates carrying:</th>
<th>Wild-type gene</th>
<th>Isoniazid-resistant gene</th>
<th>Indeterminate gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT-MTBDR</td>
<td>katG</td>
<td>55 (31.8)</td>
<td>117 (67.6)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>GT-MTBDRplus</td>
<td>katG</td>
<td>55 (31.8)</td>
<td>117 (67.6)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>inhA</td>
<td>153 (88.4)</td>
<td>20 (11.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
<tr>
<td>Sequence</td>
<td>katG</td>
<td>55 (31.8)</td>
<td>117 (67.6)</td>
<td>1 (0.6)</td>
<td></td>
</tr>
<tr>
<td>analysis</td>
<td>inhA</td>
<td>153 (88.4)</td>
<td>20 (11.6)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

* The isolates tested (n = 173) were previously determined to be isoniazid resistant by drug susceptibility testing.
* The GT-MTBDR assay did not target the inhA gene.
* The isolates tested (n = 173) were previously determined to be isoniazid resistant by drug susceptibility testing.

**FIG. 1.** Comparison of the performance of the Genotype MTBDR assay with the Genotype MTBDRplus assay on clinical specimens. “Double pattern” refers to samples showing mutated and wild-type patterns simultaneously. DST, drug susceptibility testing; INH-R, isoniazid resistance.
The increased sensitivity of GT-MTBDRplus is due to the introduction of new targeted genes for isoniazid resistance coupled with better primer and probe design, allowing improved sensitivity with direct clinical specimens to provide rapid direct detection of drug resistance, essential to improve case management and, consequently, tuberculosis control.

REFERENCES


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* Published ahead of print on 31 October 2007.