Evaluation of Methods for Rapid Detection of Resistance to Isoniazid and Rifampin in *Mycobacterium tuberculosis* Isolates Collected in the Caribbean

Patrick Eberechi Akpaka,1* Shirematee Baboolal,1,2 Denise Clarke,2 Lorraine Francis,2 and Nalin Rastogi3

Department of Para-Clinical Sciences, Faculty of Medical Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago; Caribbean Epidemiology Centre, Jamaica Boulevard, Port of Spain, Trinidad and Tobago; and Institut Pasteur de la Guadeloupe, Abymes, Guadeloupe

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The rapid identification of drug-resistant strains of *Mycobacterium tuberculosis* is crucial for the timely initiation of appropriate antituberculosis therapy. The performance of the Genotype MTBDRplus assay was compared with that of the Bactec 460 TB system, a “gold standard” culture-based method. The Genotype MTBDRplus assay was quicker and more cost-effective for the detection of rifampin resistance, but it was not as good for the detection of isoniazid-resistant strains in our setting.

The major serious challenges associated with the management of tuberculosis in Caribbean countries are diagnosis of the infection, drug resistance, and the paucity of reports on the prevalence of drug resistance. Although laboratories in many of these countries have the ability to perform smear microscopy, there is still a shortage of a laboratory capability for the performance of accurate, rapid culture and drug susceptibility tests (DSTs). As a result, many cases with low bacillary loads are misdiagnosed, underdiagnosed, or poorly treated.

The rapid detection of drug-resistant *Mycobacterium tuberculosis* strains facilitates early access to the appropriate therapy, reduces rates of transmission, and improves treatment outcomes (12). *M. tuberculosis* resistance to isoniazid (INH) and rifampin (RIF), which are two of the most important antituberculosis drugs, often results in treatment failure and death (6); hence, the detection of resistance to these agents is crucial.

The phenotypic DST method is routinely performed only after a pure, viable culture is obtained, and this usually takes 4 to 6 weeks, resulting in long diagnostic delays (7). The administration of inappropriate therapy during the period of delay may lead to the acquisition of further drug resistance as well as the dissemination of drug-resistant strains through person-to-person transmission. To improve treatment and prevent the transmission of drug-resistant *M. tuberculosis* strains, effective alternative diagnostic tests that will enable the rapid detection of drug resistance after the collection of specimens from infected patients are required. This study was carried out to determine the turnaround time, cost, and reliability of the Genotype MTBDRplus assay, a relatively new molecular test, in comparison with those of the Bactec 460 TB system, a “gold standard” conventional method, for the identification and the detection of susceptibility to INH and RIF of *M. tuberculosis* isolates from the Caribbean.

### MATERIALS AND METHODS

Eighty-one samples (26 sputum specimens and 55 culture material specimens on Lowenstein-Jensen medium) received at the Caribbean Epidemiology Centre of Trinidad and Tobago from several Caribbean islands for further confirmatory tests were used for this study. These were processed by standard microbiological methods (9, 11) to identify and confirm the presence of *M. tuberculosis* as well as to carry out the DSTs (3, 14). Informed consent was not required since no detailed patient information was known and the results were not linked to a patient identifier at the center.

The Genotype MTBDRplus assay (Hain LifeScience, Nehren, Germany), which is based on a DNA strip technology that permits the molecular identification of isolates of the *M. tuberculosis* complex and their susceptibilities to RIF and INH, were performed either with the cultured sample on Lowenstein-Jensen material or directly from the sputum specimen, according to the manufacturer’s instruction and as reported elsewhere (8; product insert, Hain LifeScience).

The turnaround time, which is time required to process, inoculate, identify, and perform the DST with the *M. tuberculosis* isolate, was measured. The costs of the reagents needed to perform either the MTBDRplus or the Bactec procedure were determined. These costs did not include the cost for labor (the technologists’ hands-on time) or for the purchase of any major equipment, such as the Bactec 460 system, microscopes, power supplies, and other infrastructural elements. Quality control of the procedure was performed by using aliquots of DNA extracts of pansusceptible *Mycobacterium tuberculosis* ATCC 27294 isolates. The data were analyzed by using Epi Info software (version 3.4.3) (5). Comparisons were done by chi-square analysis and Fisher’s exact test. A P value of ≤0.05 was considered significant.

### RESULTS AND DISCUSSION

In this study, evaluation of the performance characteristics of a molecular test and a conventional test that is an efficient, cost-effective, and timely method for use in determining whether *M. tuberculosis* isolates are resistant to INH and RIF revealed that the MTBDRplus assay performed very well only for the detection of RIF resistance, while the Bactec 460 system performed well for the detection of both RIF and INH resistance. Resistance to RIF, which is conferred by mutations in the *rpoB* gene, was correctly identified in 23 of 24 isolates (95.8%) by use of the Genotype MTBDRplus assay. This good...
TABLE 1. Genes mutations and corresponding wild-type and mutation probes in the MTBDRplus test for detection of RIF and INH resistance in Mycobacterium tuberculosis isolates from the Caribbean

<table>
<thead>
<tr>
<th>Gene</th>
<th>Missing wild-type probe</th>
<th>Codon analyzed</th>
<th>Mutation probe</th>
<th>Nucleotide, amino acid changes</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>rpoB</td>
<td>WT a 3</td>
<td>513–517</td>
<td>Mut 1</td>
<td>GAC516→GTC, Asp→Val</td>
<td>7 (20)</td>
</tr>
<tr>
<td></td>
<td>WT 4</td>
<td>516–520</td>
<td>Mut 1</td>
<td>GAC516→GTC, Asp→Val</td>
<td>4 (11.4)</td>
</tr>
<tr>
<td></td>
<td>WT 7</td>
<td>525–530</td>
<td>Mut 2A</td>
<td>CAC526→TAC, His→Tyr</td>
<td>3 (8.6)</td>
</tr>
<tr>
<td></td>
<td>WT 8</td>
<td>530–533</td>
<td>Mut 2B</td>
<td>CAC526→GAC, His→Asp</td>
<td>1 (2.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mut 3</td>
<td>TCG531→TGT, Ser→Leu</td>
<td>20 (57.1)</td>
</tr>
<tr>
<td>katG</td>
<td>WT</td>
<td>315</td>
<td>Mut 1</td>
<td>AGC315→ACC, Ser→Thr</td>
<td>7 (77.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mut 2</td>
<td>AGC315→ACA, Ser→Thr</td>
<td>2 (22.2)</td>
</tr>
</tbody>
</table>

a Analyzed on the basis of information provided by the manufacturer (product insert, Hain Lifescience).

b WT, wild-type pattern.
c Mut, mutation.

performance of the MTBDRplus assay for the detection of RIF resistance agrees with the findings of previous reports (8, 13, 16). The MTBDRplus assay allowed the rapid and specific detection of most mutations that confer resistance to RIF and, to a lesser extent, INH. Collective observations have indicated that mutations in the rpoB gene may account for >96% of the resistance to RIF (2, 8, 15), and a similar observation was also made for the Caribbean strains tested in this study.

The rate of INH resistance detection by the MTBDRplus method was a very disappointing 34.6%, and for multidrug-resistant isolates the rate of resistance detection rate was even less (29.4%). This observation is not unique, since the molecular mechanisms required for INH resistance are not fully understood and about 25 to 30% of the phenotypic INH resistance-associated mutations are still unaccounted for (10). It has been reported that the MTBDRplus assay was unable to detect low levels of INH resistance (1, 15); the assay also detects M. tuberculosis isolates in which resistance originates only in the rpoB, katG, and inhA regions (product insert, Hain Lifescience).

The low rate of detection of INH resistance by the MTBDRplus method in the present study may be due to the fact that only a single concentration of INH was used (0.1 μg/ml) or that the Caribbean strains harbor resistance mutations at unidentified gene regions. The changes in the M. tuberculosis rpoB and katG genes that denote susceptibility or resistance in the isolates detected by Genotype MTBDRplus test are shown in Table 1. The rpoB gene codons most frequently involved in mutations were S531L (57.1%) and S516L (20%) in isolates resistant to RIF. Twenty isolates carried the most common mutation, Ser531→Leu. A similar result was reported by Cavusoglu et al. (4), but our result was not in agreement with the findings of Barnard et al., who reported that most mutations occur at several other codons (2). As for INH resistance, of the nine resistant isolates that the Genotype MTBDRplus detected, 78% of them carried a mutation at the S315T codon of the katG gene and showed an AGC→ACC mutation, and 22% showed a AGC→ACA mutation (Table 1).

In two of the M. tuberculosis isolates from among the clinical specimens, the assay detected no missing band for the wild type but detected bands for mutations at Mut 2A and Mut 3 of the rpoB gene, signifying RIF resistance.

The high proportion of mutational changes detected in the katG gene S315T codon in INH- and RIF-monoresistant isolates in the present study is not in agreement with results reported elsewhere (2). This most frequent change seen in the S315T codon reported elsewhere was in a setting with a high TB burden, but this was not the case among the M. tuberculosis isolates encountered in the Caribbean region.

The turnaround time required to complete the test with the Bactec 460 culture system was 32 days, while it was 1 day for the MTBDRplus assay (P = 0.0001). The cost of the reagents required to identify the M. tuberculosis isolates and perform DST with the Bactec 460 assay was $78, while it was $23 for the MTBDRplus assay (P = 0.0001); the costs did not include technologist time or infrastructural requirements. The MTBDRplus assay required less time than the Bactec 460 TB system to detect resistance in M. tuberculosis isolates. This is in agreement with the time reported elsewhere (Caribbean Epidemiology Centre laboratory data, 2006 and 2007).

Although the MTBDRplus assay has limitations, as is the case with any DNA-based assay that screens the nucleic acid sequence and not the amino acid sequence, it is possible that mutations that do not cause an amino acid exchange (silent mutations) will still result in the absence of one of the wild-type probes. Also, the assay detects resistance only when the origin of resistance is in the M. tuberculosis rpoB, katG, and inhA regions.

In conclusion, the high sensitivity of RIF resistance detection is a merit point for the MTBDRplus test, as it can be used for the detection of RIF resistance, which is a surrogate marker for multiple-drug resistance in M. tuberculosis isolates. In our study, the results for the detection of INH resistance were not as good as those for the detection of RIF resistance and may be a setback of this assay as a method for the detection of INH resistance in M. tuberculosis isolates; but the assay is an excellent test for use with selected clinical samples, because results can be obtained within 24 h after receipt of the specimen. Culture of the specimen is not required, and contaminated as well as nonviable cultures can be used. Finally, use of the test method could be cost-effective in resource-poor countries like those in the Caribbean region.

ACKNOWLEDGMENTS

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