Evaluation of the VersaTREK System Compared to the Bactec MGIT 960 System for First-Line Drug Susceptibility Testing of Mycobacterium tuberculosis

M. Espasa,⁎ M. Salvadó,⁎ E. Vicente,⁎ G. Tudó,⁎ F. Alcaide,⁎ P. Coll,⁎ N. Martin-Casabona, † M. Torra,⁎ D. Fontanals,⁎ and J. González-Martín⁎

Laboratori de Microbiologia, UDIAT-CD, Corporació Sanitària Parc Taulí, Sabadell, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; Laboratory of Microbiology, Universitat de Barcelona (UB), Barcelona, Spain; Laboratory of Microbiology, Hospital Universitari Vall d’Hebron, Barcelona, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona (UB), Barcelona, Spain; Laboratori de Microbiologia, Laboratori de Referència de Catalunya, Barcelona, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; Servei de Microbiologia-CDB, Hospital Clínic de Barcelona, Departament d’Anatomia Patològica, Farmacologia i Microbiologia, Universitat de Barcelona (UB), and Barcelona Centre for International Health Research (CRESIB, Hospital Clinic-Universitat de Barcelona), Barcelona, Spain; Servei de Microbiologia, Hospital Universitari de Bellvitge, Hospitalet de Llobregat. Departament de Patologia y Terapéutica Experimental, Universitat de Barcelona (UB), Barcelona, Spain; Servei de Microbiologia, Hospital Universitari Santa Creu i Sant Pau, Barcelona, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain; and Servei de Microbiologia, Hospital Universitari Vall d’Hebrón, Barcelona, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona (UB), Barcelona, Spain.

The aim of this study was to evaluate the reliability of the VersaTREK system for Mycobacterium tuberculosis drug susceptibility testing compared with results obtained with the Bactec MGIT 960 system. A total of 67 strains were evaluated. Overall agreement was at 98.5%. Kappa indexes were 1.0 for isoniazid, rifampin, and ethambutol, 0.937 for pyrazinamide, and 0.907 for streptomycin. The VersaTREK system is validated for M. tuberculosis drug susceptibility testing.

Tuberculosis (TB) is one of the most prevalent infectious diseases worldwide (25). Moreover, Mycobacterium tuberculosis multidrug-resistant (MDR) strains are a serious problem (2, 24, 26). Rapid drug susceptibility testing (DST) is essential to prevent MDR transmission.

The most widely used method for M. tuberculosis DST has been the radiometric Bactec 460TB system (B460TB; Becton Dickinson) (9, 14, 19), considered the reference method (14). However, the use of radioactivity recently led to its discontinuation. The MB/Bact system (bioMérieux) has also been withdrawn from DST. Despite the introduction of molecular resistance rapid detection systems, DST must still be performed (8). Only two automated systems for M. tuberculosis DST currently have FDA approval: the Bactec 960 mycobacterial growth indicator tube system (MGIT 960; Becton Dickinson) (13, 17, 18) and the VersaTREK culture system (TREK Diagnostics), formerly the ESP culture system II. The MGIT 960 system has been evaluated widely (1, 5, 10, 16, 20), while only four studies have evaluated the VersaTREK system for M. tuberculosis DST (6, 7, 11, 15) and none have compared VersaTREK with MGIT 960.

The objective of this multicenter study was to evaluate the reliability of the VersaTREK system for M. tuberculosis DST against isoniazid, rifampin, streptomycin, ethambutol, and pyrazinamide, comparing the results with those obtained by MGIT 960 using a collection of strains.

A total of 57 M. tuberculosis strains retrieved from clinical isolates were tested at three hospitals in Barcelona, Spain. All strains had been previously studied by DST using the B460TB system and genotyped by DNA sequencing for mutations in the codon 315 region in the katG gene and the mabA–inhA regulatory region for isoniazid (INH), the 81-bp region of the rpoB gene for rifampin (RIF), the entire embB gene for ethambutol (EMB), rrs (530 loop, 238 bp, and 912 region, 240 bp), and the entire rpsL gene for streptomycin (STR) and entire pncA gene for pyrazinamide (PZA). Seven strains were susceptible to all drugs. Among the 50 resistant strains, 48 were resistant to INH, 26 to RIF, 20 to STR, 16 to EMB, and 20 to PZA. Twenty-six strains were MDR. For the resistance genotypes of these strains, see Table 3. Additionally, the study included 10 WHO reference strains (12), with validated phenotypic and genotypic results provided by the Supranational Reference Laboratory of Vall d’Hebron (Spain).

DST with the VersaTREK system was performed according to the manufacturer’s instructions (21–23), using drug concentrations of 0.1 and 0.4 μg/ml for INH, 1 μg/ml for RIF, 5 and 8 μg/ml for EMB, 2 and 8 μg/ml for STR, and 300 μg/ml for PZA. DST with the MGIT 960 system was performed following the manufacturer’s instructions (3, 4), using drug concentrations of 0.1 μg/ml for INH, 1 μg/ml for RIF, 5 μg/ml for EMB, 1 μg/ml for STR, and 100 μg/ml for PZA.

For discrepant results, tests were repeated once with both methods. If the discrepancy persisted, the presence of mutations in the determinants of resistance was analyzed. The DST discrepancies with wild genotype results were considered an indeterminate result. Data were analyzed using SPSS statistical software (v18.0). Agreement of results was assessed using the kappa statistic and the coefficient of agreement. The two systems were considered equal in performance if the concordance was above 97% and 99% for INH and RIF, respectively, if the agreement for EMB, STR, and PZA was above 92%, and finally if the kappa value was above 0.7 (12).

False resistance results were major errors (ME), and false susceptibility results were very major errors (VME).
TABLE 1 Drug susceptibilities for strains of *M. tuberculosis* determined by the MGIT 960 system compared to results with the VersaTREK system

<table>
<thead>
<tr>
<th>Phenotype determined by VersaTREK</th>
<th>Isoniazid</th>
<th>Rifampin</th>
<th>Streptomycin</th>
<th>Ethambutol</th>
<th>Pyrazinamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>S</td>
<td>14</td>
<td>38</td>
<td>39</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>R</td>
<td>53</td>
<td>29</td>
<td>25</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>53</td>
<td>38</td>
<td>29</td>
<td>50</td>
</tr>
</tbody>
</table>

a S, sensitive; R, resistant. Boldface indicates discrepant results.

Table 1 summarizes the comparative DST results. The two methods gave discrepant results for 5 strains (Table 2): STR results for two strains were considered VME, and one VME was found in testing of PZA for VersaTREK. Results for the two remaining strains were considered indeterminate (one for PZA and another for STR).

The VersaTREK system showed an overall agreement of 98.5% with results obtained with the MGIT 960 system. The kappa index was 1.0 for INH, RIF, and EMB (100% concordance), 0.937 (95% confidence interval [CI], 0.850 to 1.023) for PZA (97% concordance), and 0.907 (95% CI, 0.805 to 1.008) for STR (95.5% concordance).

Additionally, the results of the MGIT 960 and VersaTREK tests agreed for 6 strains, being discrepant from the previous B460TB DST results (Table 2): one VME was found in RIF testing, 1 VME was found in EMB testing, and two ME were found for PZA. The remaining four results were considered indeterminate (two for EMB and two for PZA). Comparing the results of the two methods with the previous B460TB results, the overall agreement was 97.6% and 97.3% for VersaTREK and MGIT, respectively. The kappa index for each drug was as follows: 1 for INH; 0.97 (95% CI, 0.911 to 1.028) for RIF and 0.888 (95% CI, 0.764 to 1.011) for EMB by both methods; 0.907 (95% CI, 0.805 to 1.008) for STR (95.5% concordance).

The correlation between the genotype and theVersaTREK results differed slightly from those in the study by LaBombardi (11), which showed no discrepancy, while the results for STR were similar to those in previous studies (6, 15). The results with EMB were also at 100% agreement despite a previous study for which lower agreement was reported (6). This could be explained by the fact that Bergmann et al. did not study the low concentration for EMB, which allowed detection of 12 resistant strains with low levels of resistance in the present study. The results with PZA in our study differed slightly from those in the study by LaBombardi (11), which showed no discrepancy, while the results for STR were similar to those in previous studies (6, 15).

The major weakness of the study was the use of retrospective phenotype results since the B460TB test could not be repeated. The use of genotype results was useful for validating discrepant results (n = 5), as in the study by Garrigo et al. (10).

The determination of resistance at high and low concentrations for INH and STR showed a high correlation with the genotypes for *katG, inhA*, and *rpsL*, respectively, which may aid

### Table 2 Discrepant results

<table>
<thead>
<tr>
<th>Strain</th>
<th>Drug</th>
<th>Phenotype determined by DST method</th>
<th>Molecular characterization</th>
<th>Final resolution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MGIT 960</td>
<td>VersaTREK</td>
<td>B460TB</td>
</tr>
<tr>
<td>056/R</td>
<td>Pyrazinamide</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>076/R</td>
<td>Pyrazinamide</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>036/R</td>
<td>Streptomycin</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>106/R</td>
<td>Streptomycin</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>261/R</td>
<td>Streptomycin</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>005/R</td>
<td>Rifampin</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>107/R</td>
<td>Ethambutol</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>305/R</td>
<td>Ethambutol</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>12492*</td>
<td>Ethambutol</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>042/R</td>
<td>Pyrazinamide</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>250/R</td>
<td>Pyrazinamide</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

a DST, drug susceptibility testing; S, sensitive; R, resistant; WT, wild type.
b Mutated gene or wild type (WT).
c WHO reference strain.
in obtaining interpretable phenotype results for genotype detection systems and clinical decision making for TB treatment. Although a difference in the time to response between both systems was not found, it was not included in the analysis. Both systems require trained personnel to manipulate M. tuberculosis strains: needles are used in the VersaTREK system to inoculate the samples, which decreases the possibility of contamination but increases the risk of occupational transmission; in the MGIT 960 system, contamination can take place when the tubes are opened. Both systems can be connected to laboratory information system (LIS), and data analysis is facilitated by growth curve information.

Overall, our results indicate that the VersaTREK system is a validated methodology for drug susceptibility testing of M. tuberculosis and did not show results inferior to those of the MGIT 960 system, the currently most validated and broadly used system.

ACKNOWLEDGMENTS

We belong to the Study Group of Mycobacterial Infections (GEIM) of the Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC).

This work was supported financially by Spanish Network for Research in Infectious Diseases (REIPI, RD06/0008) from the Ministry of Health, Spain. bioMérieux supported the cost of culture media, drugs, and reagents for drug susceptibility testing.

We have no conflicts of interest of declare.

REFERENCES


