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

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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 The MB/Bact system (bioMérieux) has also been withdrawn from DST.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).

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TABLE 1 Drug susceptibilities for strains of *M. tuberculosis* determined by the MGIT 960 system compared to results with the VersaTREK system[a]

Phenotype determined by VersaTREK	No. of strains with drug susceptibility phenotype in Bactec MGIT 960									
	Isoniazid		Rifampin		Streptomycin		Ethambutol		Pyrazinamide	
	S	R	S	R	S	R	S	R	S	R
S	14		38		39	**3**	50		40	**2**
R		53		29		25		17		25
Total	14	53	38	29	39	28	50	17	40	27

[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 resistance 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; 0.968 (95% CI, 0.907 to 1.028) and 0.938 (95% CI, 0.853 to 1.022) for STR, and 0.904 (95% CI, 0.798 to 1.009) and 0.905 (95% CI, 0.801 to 1.008) for PZA by VersaTREK and MGIT, respectively.

The correlation between the genotype and the VersaTREK results (Table 3) for low (LC) and high (HC) drug concentrations of INH, STR, and EMB showed that the presence of mutations in *katG* (58.9%) was predictive for HC resistance ($P \leq 0.001$) and mutations in *inhA* (71.4%) for LC resistance among the INH^r strains ($P = 0.001$). The presence of mutations in *rpsL* (64.3%) among the STR^r strains was predictive for HC resistance ($P = 0.04$). However, the mutations in *embB* among EMB^r strains was not correlated with HC or LC resistance ($P = 0.4$).

Both systems achieved greater than 95% agreement for all drugs. Few discrepancies between the two systems were observed for PZA and STR. The VersaTREK and MGIT 960 systems detected six discrepancies with results of the original B460TB DST. Nonetheless, the kappa index agreement was above 0.9.

INH and RIF test results showed perfect agreement, similar to findings in previous studies (6, 7, 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 resolution[a]

Strain	Drug	Phenotype determined by DST method			Molecular characterization[b]	Final resolution
		MGIT 960	VersaTREK	B460TB		
056/R	Pyrazinamide	R	S	R	*pncA*	Resistant
076/R	Pyrazinamide	R	S	S	WT	Indeterminate
036/R	Streptomycin	R	S	S	*rpsL*	Resistant
106/R	Streptomycin	R	S	S	WT	Indeterminate
261/R	Streptomycin	R	S	R	*rpsL*	Resistant
005/R	Rifampin	S	S	R	*rpoB*	Resistant
107/R	Ethambutol	S	S	R	WT	Indeterminate
305/R	Ethambutol	S	S	R	WT	Indeterminate
12492[c]	Ethambutol	S	S	R	*embB*	Resistant
042/R	Pyrazinamide	R	R	S	WT	Indeterminate
250/R	Pyrazinamide	R	R	S	WT	Indeterminate

[a] DST, drug susceptibility testing; S, sensitive; R, resistant; WT, wild type.
[b] Mutated gene or wild type (WT).
[c] WHO reference strain.

TABLE 3 Genotypes of drug resistance determinants according to the drug concentration observed in DST by the VersaTREK system[a]

	No. of strains for drug and genotype											
	Isoniazid			Rifampin		Streptomycin			Ethambutol		Pyrazinamide[b]	
Phenotype	*inhA*	*katG*	WT	*rpoB* c516	*rpoB* c531	*rpsL*	*rrs*	WT	*embB*	WT	*pncA*	WT
S				1		2		1	1	2	1	1
R				9	20						17	4
LCR	10		4			3	4	7	8	4		
HCR	5	23	11			9	1	1	4	1		
Total	15	23	15	10	20	14	5	9	13	7	18	5

[a] DST, drug susceptibility testing; S, sensitive; R, resistant; WT, wild type; LCR, low-concentration resistance; HCR, high-concentration resistance.
[b] Four strains were not genotyped.

in obtaining interpretative 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.

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