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Multicenter Laboratory Evaluation of the MB/BacT *Mycobacterium* Detection System and the BACTEC MGIT 960 System in Comparison with the BACTEC 460TB System for Susceptibility Testing of *Mycobacterium tuberculosis*▽

Montserrat Garrigó,[1,2] Lina Marcela Aragón,[1] Fernando Alcaide,[3] Sonia Borrell,[4] Eugenia Cardeñosa,[3] Juan José Galán,[5] Julián Gonzalez-Martín,[4] Nuria Martin-Casabona,[5] Carmen Moreno,[1] Margarita Salvado,[6] and Pere Coll[1,2]*

Servei de Microbiología, Hospital de la Santa Creu i Sant Pau,[1] Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona,[2] Hospital Universitari de Bellvitge,[3] Departamento de Microbiología, Hospital Clínic-IDIBAPS,[4] Hospital Vall d'Hebron,[5] and Hospital del Mar,[6] Barcelona, Spain

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In this multicenter study, the reliability of two nonradiometric, fully automated systems, the MB/BacT and BACTEC MGIT 960 systems, for testing the susceptibilities of 82 *Mycobacterium tuberculosis* strains to isoniazid, rifampin, ethambutol, and streptomycin was evaluated in comparison with the radiometric BACTEC 460TB system. The arbitration of discrepant results was done by the reanalysis of the strain, the determination of the MIC, and the molecular characterization of some resistance determinants. The overall level of agreement with BACTEC 460TB results was 96% with the MB/BacT test and 97.2% with the BACTEC MGIT 960 system. With both methods, the level of agreement with BACTEC 460TB results was 96.3% for isoniazid, 98.8% for rifampin, and 98.8% for ethambutol. The level of agreement for streptomycin was 90.2% with MB/BacT and 97.5% with BACTEC MGIT 960. Overall, there were 11 very major errors and 2 major errors with the MB/BacT method and 5 very major errors and 2 major errors with the BACTEC MGIT 960 system. In general, the MB/BacT and BACTEC MGIT 960 systems showed good performance for susceptibility testing with first-line antituberculosis drugs.

Tuberculosis (TB) is one of the most prevalent infectious diseases in the world. According to a recent report of the World Health Organization, in 2003 there were 8.8 million new TB cases and around 1.7 million deaths attributable to the disease (25). In addition, multidrug-resistant TB is becoming increasingly common and is a major health concern in many regions of the world, particularly in developing nations (24). Rapid, accurate diagnosis and the determination of drug susceptibility are crucial to optimize treatment and prevent transmission. The most widely used method for *Mycobacterium tuberculosis* drug susceptibility testing is the proportion method, either on solid medium or on liquid broth. The BACTEC 460TB system (Becton Dickinson Biosciences, Sparks, MD) has been widely validated for approximately 20 years for the reliable and rapid testing of the susceptibilities of *M. tuberculosis* isolates (9, 16, 21). The radiometric BACTEC 460TB test requires fewer than 14 days of incubation before results are available, but it is semiautomated and entails the disposal of a radioactive substance (16).

New liquid medium-based systems have recently been introduced for the nonradiometric susceptibility testing of *M. tuberculosis* (4, 10, 17–19). These include the ESP Culture System II (AccuMed International, Westlake, OH), the MB Redox system (Biotest, Dreieich, Germany), the BACTEC MGIT 960 mycobacterial growth indicator tube system (Becton Dickinson Microbiology Systems, Sparks, MD), and the MB/BacT system (bioMérieux, Marcy l'Etoile, France).

In the present multicenter study, we evaluated the reliability of the MB/BacT and BACTEC MGIT 960 systems for testing *M. tuberculosis* susceptibility to streptomycin, isoniazid, rifampin, and ethambutol (a combination known as SIRE) and compared the results obtained with these methods to those obtained with the radiometric BACTEC 460TB system. The arbitration of discrepant results was done by the reanalysis of the strain, the determination of the MIC, and the molecular characterization of the most relevant determinants of resistance to isoniazid, rifampin, and ethambutol.

(These results were presented in part at the 12th Congress of the Spanish Society of Infectious Disease and Clinical Microbiology, Valencia, Spain, 2006.)

MATERIALS AND METHODS

Evaluation sites. Susceptibility testing was performed in the microbiology laboratories of six university hospitals in Barcelona, Spain. All these laboratories are enrolled in a World Health Organization external quality control program for *M. tuberculosis* susceptibility testing. All strains had been tested previously with the BACTEC 460TB system and were retested with the MB/BacT method in laboratories 1 to 3 and with the BACTEC MGIT 960 method in laboratories 4 to 6.

Strains. A total of 82 *M. tuberculosis* isolates retrieved from a culture collection of the Mycobacteria Study Group of Barcelona were tested. Forty-eight of these strains were considered to be resistant to isoniazid, 14 were resistant to rifampin, 12 were resistant to ethambutol, and 18 were resistant to streptomycin as determined by the BACTEC 460TB method.

* Corresponding author. Mailing address: Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau, Av. Sant Antoni Mª Claret, 167, 08025 Barcelona, Spain. Phone: 34 93 2919069. Fax: 3493 2919070. E-mail: pcoll@santpau.es.

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MB/BacT system. Susceptibility testing with the MB/BacT system was performed according to the manufacturer's recommendations. Each one of the lyophilized antibiotics was reconstituted with 5.0 ml of distilled water. Five hundred microliters of the reconstituted antibiotic (drug stock solution) was added to an MB/BacT bottle and supplemented with 0.5 ml of restoring fluid (RISE; bioMérieux). The final antibiotic concentrations were 0.1 μg/ml for isoniazid, 1 μg/ml for rifampin, 5 μg/ml for ethambutol, and 1 μg/ml for streptomycin. A bottle without antibiotic was used as a growth control.

Each of the bottles with antibiotic was inoculated with 0.5 ml of an *M. tuberculosis* test suspension adjusted to a McFarland standard of 2. A 10^{-2} dilution of the *M. tuberculosis* test suspension was added to the growth control. Drug susceptibility testing sets were entered into the MB/BacT instrument and continuously monitored. An organism was determined to be susceptible when no growth in the antibiotic-containing bottle was detected or when the time to detection was greater than that for the 10^{-2}-dilution control. An organism was determined to be resistant when the time to detection of growth in the antibiotic-containing bottle was equal to or less than that in the 10^{-2}-dilution control.

BACTEC MGIT 960 system. Susceptibility testing with the BACTEC MGIT 960 system was performed according to the manufacturer's recommendations. The lyophilized antibiotics were reconstituted with 5.0 ml of distilled water. One hundred microliters of the reconstituted antibiotic (drug stock solution) was added to M960 tubes supplemented with 0.8 ml of the provided enrichment solution (BACTEC MGIT SIRE supplement; Becton Dickinson). Susceptibility testing was performed with the following final drug concentrations: 0.1 μg/ml for isoniazid, 1 μg/ml for rifampin, 5 μg/ml for ethambutol, and 1 μg/ml for streptomycin. All the drug-containing tubes were inoculated with 0.5 ml of the positive broth culture. Mycobacterial suspensions were used undiluted from days 1 to 2 following the detection of growth, while the suspensions were diluted 1:5 with sterile saline from days 3 to 5. A SIRE drug-free control was inoculated with 0.5 ml of a 10^{-2} dilution of the positive culture broth in sterile saline. The tubes were then placed in an M960 set carrier and incubated in the M960 instrument. The tubes were continuously monitored until the results indicating susceptibility or resistance were automatically interpreted and reported using predefined algorithms that compared growth in the drug-containing tube to that in the control tube.

BACTEC 460TB system. Susceptibility testing with the BACTEC 460TB system was performed according to the manufacturer's recommendations. After the lyophilized drugs were rehydrated, 100 μl of each antibiotic solution was added to the 12B vial. The test was performed with the following final drug concentrations: 0.1 μg/ml for isoniazid, 2 μg/ml for rifampin, 7.5 μg/ml for ethambutol, and 6 μg/ml for streptomycin. A 12B vial with a growth index ranging from 500 to 800 was used for the direct inoculation of SIRE drug-containing BACTEC 460TB 12B vials and, after a 10^{-2} dilution of the contents, also the SIRE drug-free control. All vials were incubated at 37°C and tested daily in the BACTEC 460TB instrument. Readings were evaluated according to the established criteria for calculating susceptible, resistant, and borderline results (13, 20).

Resolution of discrepant results. When the MB/BacT and/or BACTEC MGIT 960 system and the BACTEC 460TB system showed discrepant results for a strain, the strain was reanalyzed by the three systems. The results for a strain were finally considered discrepant when the second test gave the same result. The MIC of the antibiotic for the strain in question was determined by the BACTEC 460TB method. The mechanisms of resistance to isoniazid, rifampin, and ethambutol were characterized molecularly according to previously published methodology (2, 7, 15). The targets were codon 315 in the *katG* gene and the *mabA-inhA* regulatory region for isoniazid, 81 bp of the core region of the *rpoB* gene for rifampin, and codon 306 of the *embB* gene for ethambutol.

Concordance was defined as a coefficient of agreement between the results of the MB/BacT or BACTEC MGIT 960 method and the BACTEC 460TB method. False resistance results were defined as major errors (ME), and false susceptibility results were defined as very major errors (VME).

Quality control. A reference strain of *M. tuberculosis* H37Rv (ATCC 27294) was tested by all methods used in this study as a quality control.

Analysis. Data were analyzed using SPSS statistical software. Agreement between the qualitative test results was assessed by using the kappa statistic.

RESULTS

MB/BacT results in comparison with those of BACTEC 460TB. Table 1 summarizes the results obtained with the MB/BacT and BACTEC 460TB methods. In total, these systems gave discrepant results for 13 strains. Ten strains had initial false susceptibility results, six for streptomycin, three for ethambutol, and one for isoniazid. Three strains showed initial false resistance results, two for isoniazid and one for rifampin.

TABLE 1. Initial drug susceptibility results for 82 clinical strains of *M. tuberculosis* as determined by the MB/BacT and BACTEC MGIT 960 methods in comparison with the BACTEC 460TB system[a]

System and phenotype	No. of strains with the indicated phenotype as determined by the BACTEC 460TB method							
	INH		RIF		EMB		STR	
	R	S	R	S	R	S	R	S
MB/BacT								
R	47	2	14	1	9	0	12	0
S	1	32	0	67	3	70	6	64
BACTEC MGIT 960								
R	45	0	14	1	11	0	17	3
S	3	34	0	67	1	70	1	61

[a] Drug concentrations used for MB/BacT and MGIT 960 testing were 0.1 μg/ml for isoniazid, 1 μg/ml for rifampin, 5 μg/ml for ethambutol, and 1 μg/ml for streptomycin. Drug concentrations used for BACTEC 460TB testing were 0.1 μg/ml for isoniazid, 2 μg/ml for rifampin, 7.5 μg/ml for ethambutol, and 6 μg/ml for streptomycin. INH, isoniazid; RIF, rifampin; EMB, ethambutol; STR, streptomycin; S, susceptible; R, resistant.

BACTEC MGIT 960 results in comparison with those of BACTEC 460TB. Table 1 summarizes the results obtained with the BACTEC MGIT 960 and BACTEC 460TB methods. In total, these systems yielded discrepant results for nine strains. Five strains had initial false susceptibility results, three for isoniazid, one for ethambutol, and one for streptomycin. Four strains had initial false resistance results, three for streptomycin and one for rifampin.

The SIRE susceptibility test results with the MB/BacT system were obtained in 8.2 days on average, whereas with the BACTEC MGIT 960 system they were obtained in 13.3 days and with the BACTEC 460TB system the results were available in 10.6 days.

MICs for and molecular resistance mechanisms of the strains with persistent discrepant results. The final categorization of 13 strains with discrepant results from the MB/BacT system and 9 strains with discrepant results from the BACTEC MGIT 960 system confirmed the BACTEC 460TB results in 14 cases, the MB/BacT results in 3 cases, and the BACTEC MGIT 960 results in 4 cases (Table 2).

There were no changes in the initial isoniazid sensitivity categorization by the BACTEC 460TB method after discrepancy resolution. Therefore, there were three false susceptibility results, or VME, by the BACTEC MGIT 960 system, whereas the MB/BacT test had one VME and two false resistance results, or ME. However, the MIC of isoniazid for the strain corresponding to one of the three BACTEC MGIT 960 VME was 0.125 μg/ml, and the strain had no mutations at the studied targets. The MIC for another BACTEC MGIT 960 VME strain was 0.25 μg/ml, and this strain had a Trp728Tyr mutation. Both MICs were very close to the critical concentration used. This was not the case for the MIC for the remaining BACTEC MGIT 960 VME strain (059R), also associated with the MB/BacT VME, which was 1 μg/ml. MICs for all the ME strains were <0.05 μg/ml.

TABLE 2. Resolution of discrepant results from the MB/BacT and BACTEC MGIT 960 systems in comparison with results from the BACTEC 460TB method[a]

Drug	Strain	Result from:			MIC (μg/ml)	Molecular characterization	Final categorization
		BACTEC 460TB system	MB/BacT system	BACTEC MGIT 960 system			
INH	058R	R	R	S	0.125	*katG*, wild-type; *mabA-inhA*, wild-type	R
	009R	R	R	S	0.25	*katG*, Trp728Tyr; *mabA-inhA*, wild-type	R
	059R	R	S	S	1	*katG*, wild-type; *mabA-inhA*, wild-type	R
	04/017	S	R	S	<0.05	*katG*, wild-type; *mabA-inhA*, wild-type	S
	03/025	S	R	S	<0.05	*katG*, wild-type; *mabA-inhA*, wild-type	S
RIF	078R	S	S	R	1	*rpoB*, Leu533Pro	R
	VH10241	S	R	S	1	*rpoB*, Asp516Tyr	R
ETB	VH10649	R	S	S	4	*embB*, wild-type	S
	VH8552	R	S	R	4	*embB*, wild-type	S
	VH9511	R	S	R	8	*embB*, wild-type	R
STR	058R	S	S	R	0.5		S
	006R	R	R	S	8		R
	019R	R	S	R	8		R
	062R	R	S	R	8		R
	VH10241	R	S	R	8		R
	03/033	R	S	R	8		R
	080R	R	S	R	8		R
	082R	R	S	R	>8		R
	017R	S	S	R	8		R
	018R	S	S	R	>8		R

[a] INH, isoniazid; RIF, rifampin; ETB, ethambutol; STR, streptomycin; R, resistant; S, sensitive.

The MIC of rifampin for two strains (078R and VH10241) was 1 μg/ml. Strain 078R had a Leu533Pro mutation, and strain VH10241 had an Asp516Tyr mutation. As rifampin concentrations of 0.5 μg/ml are bactericidal for wild-type isolates of *M. tuberculosis* (11), both strains were finally categorized as resistant. The BACTEC 460TB system considered these strains to be sensitive, and the MGIT 960 system classified strain 078R as resistant and VH10241 as sensitive, whereas the MB/BacT system considered strain VH10241 to be resistant and strain 078R to be sensitive. There was therefore one BACTEC MGIT 960 VME and another MB/BacT VME.

The MIC for two strains (VH10649 and VH8552) initially considered to be ethambutol resistant according to the results of the BACTEC 460TB system was 4 μg/ml, and these strains were therefore sensitive. Thus, two out of three initial ethambutol VME by the MB/BacT system and a VME by the BACTEC MGIT 960 system were considered true results. Moreover, the MIC for one strain (VH9511) initially considered to be resistant by the BACTEC MGIT 960 and BACTEC 460TB methods and sensitive by the MB/BacT method was 8 μg/ml, and this strain was finally considered to be resistant. Therefore, finally, there was one VME by the MB/BacT system and one ME by the BACTEC MGIT 960 system.

The MIC for two strains (017R and 018R) with discrepant results initially considered to be streptomycin sensitive by the BACTEC 460TB method was ≥8 μg/ml; therefore, these strains were resistant. As a result, there were one VME and one ME by the MGIT 960 system and eight VME by the MB/BacT method.

MB/BacT accuracy after discrepancy resolution. The overall level of agreement between the MB/BacT results and those of the BACTEC 460TB method was 96%. The concordance values were 96.3% for isoniazid (kappa statistic, 0.924), 98.8% for rifampin (kappa statistic, 0.960), 98.8% for ethambutol (kappa statistic, 0.940), and 90.2% for streptomycin (kappa statistic, 0.694). In total, the MB/BacT system had 11 VME (1 each for isoniazid, rifampin, and ethambutol and 8 for streptomycin) and 2 ME for isoniazid.

BACTEC MGIT 960 accuracy after discrepancy resolution. The overall level of agreement between the BACTEC MGIT 960 results and those of the BACTEC 460TB method was 97.2%. The concordance values were 96.3% for isoniazid (kappa statistic, 0.926), 98.8% for rifampin (kappa statistic, 0.960), 98.8% for ethambutol (kappa statistic, 0.945), and 97.5% for streptomycin (kappa statistic, 0.934). In total, the MGIT 960 system had five VME (three for isoniazid and one each for rifampin and streptomycin) and two ME (for ethambutol and streptomycin).

DISCUSSION

Delayed diagnosis, inadequate treatment regimens, and mortality characterize drug-resistant and multidrug-resistant TB. The importance of rapidly available results of *M. tuberculosis* susceptibility testing is universally acknowledged. Traditional drug susceptibility testing procedures, such as the proportions methods on Löwenstein-Jensen or agar medium, are time-consuming. Liquid systems have been introduced as an alternative method for mycobacterium susceptibility determinations. The major disadvantages of the BACTEC 460TB system are that it is semiautomated and that it uses radioactive material, with the need for the disposal of the radioactive waste. A number of automated, nonradiometric detection systems able to perform susceptibility testing are presently com-

mercially available to clinical laboratories. In this study, we independently compared the MB/BacT mycobacterium detection system and the BACTEC MGIT 960 system with the BACTEC 460TB method.

Methodological differences may explain some of the discrepant results. The BACTEC MGIT 960 method, for example, uses a pipette rather than a fine-needle syringe to seed the tubes. A pipette may collect large mycobacterial clumps and make inoculum standardization difficult (14). Such methodological differences would be expected to affect susceptibility testing for all drugs in the same way, but this was not the case in the present study. On the other hand, the concentrations used to test each antibiotic in the MB/BacT and the MGIT 960 systems were identical. However, the concentrations of rifampin, ethambutol, and streptomycin used in the BACTEC 460TB system were different from those used in the other two systems. The three critical concentrations were higher in the BACTEC 460TB system. This difference may account for some of the ME found, as will be discussed later on.

In studies described in previously published papers, discrepancies between results from the evaluated methods were resolved by means of reanalysis with the two methods (12) or with the proportion method on Löwenstein-Jensen slants (3–5, 22) or in Middlebrook 7H11 agar (19). However, our study is unique in the arbitration of discrepant results; we reanalyzed the strain using the three systems, determined the MIC, and characterized some of the molecular resistance determinants.

MB/BacT mycobacterium detection system. The overall level of agreement between results from the MB/BacT system and those from the BACTEC 460TB method was 96%. Concordance values for isoniazid (96.3%), rifampin (98.8%), and ethambutol (98.8%) were good. The level of agreement for streptomycin (90.2%) was considerably lower than the range in previously published results of 95.3 to 100% (4, 6). All testing and even retesting were performed with the same production lot. One may speculate that the lot had an antibiotic concentration error.

The MB/BacT system presented one VME (false susceptibility) for isoniazid. The MIC for this strain was 1 μg/ml, and the molecular characterization of codon 315 in the *katG* gene and the *mabA-inhA* regulatory region showed wild-type sequences. False susceptibility results represent a serious drawback, as they can lead to the failure of anti-TB chemotherapy (14). False susceptibility to isoniazid as determined using the MB/BacT system in comparison with the BACTEC 460TB method has been reported previously (6, 23). However, this finding is infrequent in the literature. The two ME (false resistance) observed label as ineffective a very important drug that could be successfully used. This fact, although considered a less serious problem than false susceptibility results (14), considerably complicates the tuberculosis treatment.

There were three VME for ethambutol. However, the MIC for two of the three corresponding strains was 4 μg/ml, thus confirming the initial MB/BacT results. The final critical concentration of ethambutol in the MB/BacT system has been modified several times by the manufacturer. For a series of 115 strains, Brunello and Fontana (6) reported five ME with 2.5 μg/ml as the critical concentration. Díaz-Infantes et al. (8) described three VME and five ME among results for 36 strains with 2 μg/ml as the critical concentration. The critical concentration was later modified to 3.5 μg/ml. Using this concentration, Bemer et al. found no false resistance results and no increase in the number of false susceptibility results (one VME among results for 166 strains) (4). In spite of this, the manufacturer modified this concentration to 5 μg/ml, which was the concentration used in this study. As mentioned above, with this concentration we observed no false resistance results and one false susceptibility result among results for 82 strains. The increase in the ethambutol critical concentration therefore seems adequate.

Although the published levels of agreement for streptomycin range between 95 and 100% (4, 6), in our study the level of agreement was only 90.2%; that is, 8 of 18 resistant strains were categorized as sensitive (a VME). On the other hand, we did not observe false resistance with this drug. These results seem to favor an unacceptably high antibiotic concentration. Nevertheless, 1 μg/ml, the concentration used in this study, is the same as that used in the cited studies. As we performed all our testing with the same production lot, this discrepancy may reflect a specific problem of this lot not detected by the control strain that is sensitive to streptomycin.

BACTEC MGIT 960 system. The overall level of agreement between results from the BACTEC MGIT 960 and BACTEC 460TB systems was 97.2%. Concordance values were 96.3% for isoniazid, 98.8% for rifampin and ethambutol, and 97.5% for streptomycin. Previous studies that compared the automated BACTEC MGIT 960 and BACTEC 460TB methods obtained highly similar results (1, 3, 5, 19, 22).

The BACTEC MGIT 960 system gave three VME for isoniazid. The MICs for the corresponding strains were 0.125 μg/ml, 0.25 μg/ml, and 1 μg/ml. The molecular targets characterized showed wild-type regions. The MICs associated with two of the three VME were so close to the critical concentration used (0.1 μg/ml) that they could easily give discrepant results when tested in two different systems.

Although ethambutol is considered to have the least concordant results among the first-line drugs (1, 5, 22), in our study the concordance value for ethambutol with the BACTEC MGIT 960 system was good.

Regarding false sensitivity to streptomycin (VME), the majority of discrepant results in the literature are due to false resistance (3, 5, 19, 22), as in our study.

In conclusion, the MB/BacT and BACTEC MGIT 960 systems showed good performance for susceptibility testing with first-line antituberculosis drugs. The main advantages of these methods are the automated, continuous monitoring and the nonradiometric detection. In addition, both systems are less labor-intensive than the BACTEC 460TB system because tubes are placed in the respective instrument only once, whereas BACTEC 460TB vials are incubated offline and manually loaded and unloaded every day during the total incubation period.

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