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Evaluation of the MB/BacT Mycobacterium Detection System for Susceptibility Testing of *Mycobacterium tuberculosis*

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The MB/BacT mycobacterium detection system was evaluated for its performance in the susceptibility testing of *Mycobacterium tuberculosis*. Eighty-three *M. tuberculosis* isolates were processed. Results for all isoniazid- and rifampin- and streptomycin-susceptible, isoniazid-resistant, and rifampin-resistant *M. tuberculosis* isolates with the MB/BacT system agreed 100% with those obtained by the agar proportion method. The agreements between the two methods for streptomycin- and ethambutol-resistant isolates were 96.4 and 90.4%, respectively. The susceptibility test results were obtained in 7 days, on average. These data demonstrate that the MB/BacT system is an accurate, nonradiometric method for rapid susceptibility testing of *M. tuberculosis*.

At least one-third of the world’s population is infected with *Mycobacterium tuberculosis*, and there are about 9 million new tuberculosis cases every year (6, 7). In addition, multidrug-resistant *M. tuberculosis* strains have been emerging in both developed and underdeveloped countries. The Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., has developed recommendations for laboratory practice (5) that include the provision of acid-fast bacillus smear results within 24 h, isolation and identification of *M. tuberculosis* within 10 to 14 days, and provision of susceptibility test results within a total of 15 to 30 days of specimen collection. The most widely used methods for antimycobacterial drug susceptibility testing are the agar proportion method and the modified proportion method with the BACTEC 460 MTB system. The former procedure involves the inoculation of mycobacteria onto a solid medium (Lowenstein-Jensen, Middlebrook 7H11, or Middlebrook 7H11 medium) with incubation at 35 to 37°C in a 10% CO2 atmosphere. Under such conditions, colonies of *M. tuberculosis* cannot be detected until 21 days after inoculation, which is too long for adherence to the CDC guidelines for efficiency. The BACTEC 460 MTB system was the first broth-based system which provided a more rapid result. The BACTEC bottles can be read radiometrically in as short a time as 5 days, depending on the inoculum size (3). The BACTEC 460 MTB instrument has been in use for many years but has the drawbacks of the use of radioactivity (it uses radioactive 14C for detection of the CO2 produced by microbial growth) and the fact that it is a semiautomated system, which therefore requires constant supervision. However, the MB/BacT mycobacterium detection system has the advantages over the BACTEC 460 MTB system of being fully automated (it offers continuous automated monitoring of the growth signal), which is translated into time savings for the technical staff. The procedure used with the MB/BacT system is rapid, and due to its noninvasive colorimetric detection, the need for radioisotopes and the risk of cross-contamination during readings are eliminated.

Our study consists of the evaluation of the MB/BacT system (Organon Teknika) for testing the susceptibility of *M. tuberculosis* to the frontline drugs (except pyrazinamide). A selection of 83 *M. tuberculosis* strains isolated from clinical specimens was evaluated with the MB/BacT culture system. For comparison, susceptibility analysis was also performed by a reference method (the agar proportion method) in the Reference Mycobacteriology Laboratory, Hospital Carlos III. Control strain *M. tuberculosis* H37Rv (ATCC 27294) was tested by both methods as a quality control. The method used was a modification of the agar proportion method (2, 4) with Middlebrook 7H11 agar medium supplemented with casein hydrolysate. The drug concentrations used were derived from the criteria for resistance: strains of *M. tuberculosis* whose growth on drug-containing media represents more than 1% of the colonies that develop on drug-free media (2, 4). The final concentrations of antimicrobial agents used in the agar proportion method were as follows: isoniazid (INH), 0.2 μg/ml; rifampin (RMP), 1 μg/ml; streptomycin (SM), 4 μg/ml; and ethambutol (EMB), 8 μg/ml. For the susceptibility testing of mycobacteria with the MB/BacT culture system, we followed the methodology recommended by the manufacturer, Organon Teknika. The different MB/BacT process bottles were supplemented with the following final concentrations of drugs: INH, 1 μg/ml; RMP, 1 μg/ml; SM, 1 μg/ml; and EMB, 2 μg/ml. The bottles were then inoculated with 0.5 ml of a mycobacterial test suspension (MTS) adjusted to a McFarland no. 2 standard. Two bottles without antibiotics were used as controls, one with 0.5 ml of MTS (control 1) and the other with 0.5 ml of MTS diluted 1:100 (control 2). At the time that the MB/BacT system recognized growth in the control 2 bottle, the tests were terminated and the growth status of the bottles containing antimicrobial agents was determined. The isolate was reported as “resistant” when the drug-containing bottle was positive prior to or on the same day as the corresponding diluted control bottle, and it was reported as “susceptible” when the drug-containing bottle remained negative or became positive later than the control 2 bottle. Confirmation of *M. tuberculosis* growth in positive MB/BacT bottles was made with a Ziehl-Neelsen-stained smear.

Of the 83 *M. tuberculosis* strains tested with the MB/BacT system, we detected 36 *M. tuberculosis* strains resistant to one or more drugs and 47 *M. tuberculosis* strains susceptible to all four drugs (Table 1). The results for all INH-, RMP-, and
showed 90.4% agreement (8 of 83 both methods), and for EMB-resistant strains both methods showed 96.4% agreement. We detected 26 INH-resistant and 20 RMP-resistant strains, the agreement detected by both methods are summarized in Table 2. For agar proportion method. The different patterns of susceptibility test results with the MB/BacT system agreed 100% with those obtained by the agar proportion method. The time necessary for detection of resistant strains obtained with the MB/BacT system was resistant to EMB by the agar proportion method. Only one strain found to be fully susceptible with the MB/BacT system was resistant to EMB by the agar proportion method. The degree of overall accuracy within the network was shown for INH and RMP. For EMB, however, sensitivity was low (90% in 1996 [8]). One possible explanation for this may be the heterogeneous nature of EMB resistance itself (1).

In conclusion, the MB/BacT system is a novel, completely automated system which is useful for susceptibility testing of M. tuberculosis isolates in routine mycobacteriology laboratories. With this system we can obtain susceptibility results in an amount of time as short as that recommended by CDC (5).

REFERENCES