

Evaluation of a Novel Kit for Use with the BacT/ALERT 3D System for Drug Susceptibility Testing of *Mycobacterium tuberculosis*

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We evaluated a new protocol for the BacT/ALERT MB susceptibility test (bioMérieux Inc., Durham, NC) using 80 *Mycobacterium tuberculosis* WHO challenge panel strains. The drug susceptibility profiles of these strains are well characterized, and consensus drug resistance results have been established after tests were performed at around 20 international reference laboratories using recommended reference drug susceptibility techniques. Strains were tested according to the bioMérieux protocol using the following critical concentrations: rifampin (RIF), 0.9 mg/liter; isoniazid (INH), 0.4 and 0.09 mg/liter; and ethambutol (EMB), 1.8 mg/liter. The BacT/ALERT system detected 36/37 RIF-resistant strains. For INH (low concentration), 59/59 resistant strains were detected, and for EMB, 34/34 resistant strains were detected. Thus, the sensitivities were 97%, 100%, and 100% for RIF, INH, and EMB, respectively. The corresponding specificities were 100%, 95%, and 98%, respectively, for the same drugs. As soon as the BacT/ALERT MP seed bottle flagged positive, the median time to obtain a susceptibility results was 7.8 days. The results show good concordance with the consensus results of the international reference laboratories and demonstrate that BacT/ALERT 3D should be considered as an alternative method for rapid and automated drug susceptibility testing of *M. tuberculosis*.

Mycobacterium tuberculosis is slow growing, which makes recovery and drug susceptibility testing on solid media laborious and time-consuming (6).

For drug susceptibility testing (DST) of *M. tuberculosis* complex isolates, three reference techniques, using egg base, agar base, or liquid medium, are recommended by the World Health Organization (WHO): the resistance ratio method, the absolute concentration method, and the proportion method (13). For the four first-line antituberculosis drugs, rifampin (RIF), isoniazid (INH), streptomycin, and ethambutol (EMB), the liquid medium-based radiometric BACTEC 460 system (Becton Dickinson, Sparks, MD) is also a recommended reference DST system for *M. tuberculosis* (10). For pyrazinamide, the fifth important agent in an effective treatment regimen, the BACTEC 460 system provides a modified 7H12 medium at pH 6.0 that has been recommended for DST of *M. tuberculosis* by the Clinical and Laboratory Standards Institute (formerly NCCLS) (8).

The broth-based technique has considerably reduced the turnaround time of DST of *M. tuberculosis*. The BACTEC 460 system, however, is semiautomated and generates radioactive waste. In recent years, new liquid medium-based nonradiometric DST systems for *M. tuberculosis* have been developed and evaluated (3, 4, 9, 11).

The BacT/ALERT 3D system (bioMérieux Inc., Durham, NC), previously designated MB/BACT (Organon Teknica, Bostel, The Netherlands), is based on the detection of carbon dioxide (CO₂) released by actively proliferating mycobacteria. The elevated CO₂ concentration lowers the pH in the medium,

which in turn produces a color change in a sensor in the vial, which is detected by a reflectometric unit in the instrument. The BacT/ALERT automatically performs readings every 10 min, and all data are transferred to and saved in the BacT/VIEW data management system.

The BacT/ALERT 3D and MB/BacT systems have been evaluated with previous versions of the BacT/ALERT MB susceptibility reagents against conventional reference techniques (1, 2, 5, 7, 12, 14). The studies of Angeby et al., Bemer et al., and Brunello and Fontana, however, concluded that the testing of some drugs could be optimized using the BacT/ALERT system (1, 2, 5).

This study is the first evaluation of a novel BacT/ALERT MB kit for testing the susceptibility of *M. tuberculosis* to RIF, INH (low and high concentrations), and EMB, comparing the BacT/ALERT MB susceptibility test to the consensus susceptibility profiles for 80 WHO challenge panel strains. The drug susceptibility of these strains was based on results from approximately 20 reference laboratories using various DST techniques. The critical test concentrations of the antibiotics used in the BacT/ALERT MB susceptibility kit were 0.9 mg/liter for RIF, 0.09 and 0.4 mg/liter for INH, and 1.8 mg/liter for EMB. The reason for including two concentrations of INH was to detect resistance (the lower concentration) as well as to give an indication of the level of the resistance (the higher concentration). Strains resistant to the lower concentration but susceptible to the higher concentration of INH might still be inhibited by the drug. For strains resistant to both concentrations, the administration of INH is most likely not meaningful. In this study, we used 0.09 mg/liter INH to indicate resistance in the comparison with the previously obtained consensus results.

The major change in the new protocol was that the 10-fold-diluted growth control used in the previous test was excluded,

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and a strain is exclusively interpreted as drug resistant when a drug-containing test bottle gives a positive signal no later than 3.5 days after the undiluted control has flagged positive. In contrast to the case with previous BacT/ALERT MB susceptibility kits, streptomycin is no longer included.

MATERIALS AND METHODS

***M. tuberculosis* strains.** Eighty *M. tuberculosis* WHO panel strains were used in the study. The drug susceptibility profile of each strain was predetermined by the DST results from approximately 20 different laboratories using various WHO-recommended reference techniques. Of these, 37/80 were resistant to RIF, 59/80 were resistant to INH, and 34/80 were resistant to EMB.

Inoculum preparation. The strains were taken from the *M. tuberculosis* strain collection at the Swedish Institute for Infectious Disease Control (SMI) and were grown in Löwenstein-Jensen medium at 37°C. Cultures less than 4 weeks old were used to prepare a homogeneous suspension at a 1.0 McFarland standard in Middlebrook 7H9 medium. This suspension was inoculated into the BacT/ALERT MP seed bottle. When the seed bottle flagged positive by the system, it was used as the standard inoculum for the subsequent DST, as stated in the protocol provided with the BacT/ALERT MB susceptibility kit. All positive BacT/ALERT MP seed bottles were checked for contamination by subculture on blood agar plates.

BacT/ALERT drug susceptibility testing. The BacT/ALERT MB susceptibility reagents and the glass BacT/ALERT MP (Mycobacteria Process) bottles were provided by bioMérieux. Drug susceptibility testing was performed according to the bioMérieux protocol. Briefly, 0.5 ml of the lyophilized antibiotic solutions and 0.5 ml restoring fluid were added to the glass BacT/ALERT MP test bottles and the undiluted direct control bottle, respectively. The final drug concentrations in the test bottles were 0.9 mg/liter for RIF, 0.4 and 0.09 mg/liter for INH, and 1.8 mg/liter for EMB.

Half a milliliter of the seed inoculum was added to all BacT/ALERT MP test bottles. Bottles were loaded into the BacT/ALERT 3D system simultaneously, and the maximum test time was automatically limited to 15 days.

Susceptibility testing interpretation. An organism was determined to be resistant to an antibiotic when the drug-containing bottle had a time to detection (TTD) that was less than or equal to the sum of the TTD of the positive direct control plus 3.5 days. If the drug-containing bottle had a TTD that was more than the sum of the TTD of the positive direct control bottle plus 3.5 days, or remained negative, the organism was interpreted as being susceptible to the drug. If a test bottle flagged positive less than 2 days after inoculation, it was checked for contamination.

RESULTS

Good concordance was seen between the BacT/ALERT results and the previously determined consensus results from internationally recommended DST techniques, with 316/320 tests in agreement. The four discordant test results were distributed among three strains.

One INH-susceptible strain was falsely determined to be resistant to both test concentrations (0.09 mg/liter and 0.4 mg/liter) by the BacT/ALERT 3D. Another strain, resistant to RIF, was detected 1 day beyond the 3.5-day cutoff time. According to the BacT/ALERT MB protocol, this strain is interpreted as being drug sensitive. For EMB, one susceptible strain was falsely found to be resistant by the system.

The specificity, i.e., the ability to detect true susceptibility, was 100% for RIF, 95% for INH (0.09 mg/liter), and 98% for EMB. The sensitivity, i.e., the ability to detect true resistance, was 97% for RIF, 100% for INH (0.09 mg/liter), and 100% for EMB. For INH, four strains were found to be resistant to 0.09 mg/liter and sensitive to 0.4 mg/liter.

The drug susceptibility results for the 80 panel strains of *M. tuberculosis* as determined by BacT/ALERT 3D are shown in Table 1.

Almost all drug-resistant strains gave a positive signal near

TABLE 1. Accuracy of the BacT/ALERT 3D system for the antituberculosis drugs tested

Drug (concn [mg/liter])	No. of strains identified correctly/total no. of strains		Sensitivity (%)	Specificity (%)
	Resistant	Susceptible		
RIF (0.9)	36/37	43/43	97	100
INH (0.09)	59/59	20/21	100	95
EMB (1.8)	34/34	45/46	100	98

the point of TTD of the direct control. Two INH-resistant strains, however, had a TTD of 3.2 days after the positive direct controls, which is close to the 3.5-day detection cutoff time.

The median time for a positive signal of the seed bottles was 6 days (range, 3 to 21 days). The median time for the undiluted direct control to give a positive signal was 4.3 days (range, 2.5 to 10.8 days). Turnaround times for DST ranged from 6.0 to 14.7 days (median, 7.8 days).

DISCUSSION

Due to the automated reading, color-coded reagents, and prepackaged DST reagents, we found this system easy to use, and it may reduce the laboratory workload. Also, the noninvasive readings are likely to improve biosafety, and in addition, no radioactive waste is produced. The concordance of equivalency of DST with previous consensus results was well within acceptable limits. However, the cost of equipment and reagents limits its use in low-income settings with high tuberculosis incidence rates.

The new BacT/ALERT MB protocol was shown to be valid for DST of *M. tuberculosis*. The 3.5-day limitation in the protocol generally worked well but may be too stringent for the detection of rifampin resistance in some *M. tuberculosis* strains. In our study, one strain was falsely found to be susceptible to rifampin while having a delay of 1 day for the positive signal, which remained delayed by 0.5 days in a repeated run. In contrast, true rifampin-susceptible strains typically did not flag positive within the 15-day test time. In this study, four INH-resistant strains were found to be susceptible to 0.4 mg/liter. For INH, tests with the lower concentration accurately reflected drug resistance, while the higher concentration gave additional information that identified the strains with high-level resistance. The three strains with discrepant results were retested in the BacT/ALERT system, and the results were confirmed. Two additional strains, both multidrug resistant, with initially poor growth had to be retested before evaluation was possible. We evaluated this system previously (1), and by comparison, the new, modified version offered an increased sensitivity, which was 97% for RIF (previously 92%) and 100% for INH (previously 96%), while the excellent 100% sensitivity was kept for EMB. Taken together, our results suggest that the BacT/ALERT 3D system should be considered a valid alternative for rapid drug susceptibility testing of *M. tuberculosis*.

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