Reliability of Mycobacteria Growth Indicator Tube for Testing Susceptibility of Mycobacterium tuberculosis to Ethambutol and Streptomycin

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The reliability of mycobacterial growth indicator tubes (MGIT) for testing susceptibility of Mycobacterium tuberculosis to ethambutol and streptomycin was evaluated by comparing MGIT results to those obtained by the radiometric BACTEC TB system and the method of proportion. The method of proportion was considered the reference method. To resolve discrepancies, all three testing methods were repeated. For the 74 isolates evaluated, initial ethambutol results agreed by all three methods for 64 (86.5%) of them; 58 were susceptible and 6 were resistant. MGIT and method-of-proportion results agreed for 67 isolates, and BACTEC results agreed with method-of-proportion results for 71 (P = 0.096). Initial streptomycin results obtained by all three methods agreed for 69 (93.2%) isolates: 55 were susceptible and 14 were resistant. MGIT and method-of-proportion results were concordant for 69 isolates, and BACTEC and method-of-proportion results agreed for 73 (P = 0.049). The mean times to MGIT results were 5.58 ± 0.10 days (range, 3 to 9 days) for ethambutol and 5.47 ± 0.11 days (range, 3 to 9 days) for streptomycin, compared to a mean of 7.41 ± 0.20 days (range, 4 to 12 days) for both drugs with the BACTEC system (P < 0.001).

Tuberculosis remains an important public health problem in the United States today. To help control tuberculosis by ensuring appropriate therapy early in the course of disease, experts at the Centers for Disease Control and Prevention (CDC) recommend that Mycobacterium tuberculosis susceptibility test results be available within 28 days of receipt of the specimen in the laboratory (8). Of the two methods currently used in the United States for susceptibility testing of M. tuberculosis, i.e., the method of proportion and the BACTEC TB 460 system (Becton Dickinson, Sparks, Md.), only the BACTEC TB system has the potential to provide this suggested turnaround time.

Recently, the mycobacteria growth indicator tube (MGIT; Becton Dickinson) was introduced as a nonradiometric alternative to the BACTEC TB system for rapid growth and detection of mycobacteria (2, 4, 5, 7). The MGIT consists of a 16-by-100-mm round-bottom tube containing an enriched 7H9 broth with 0.25% glycerol. Embedded in silicone at the bottom of the tube is a fluorescent indicator that is quenched in the presence of oxygen. A mycobacterium or other organism actively growing consumes the oxygen in the broth, and the indicator fluoresces when exposed to 365-nm light. Results from a few studies suggest that the MGIT system also may be reliable for testing susceptibility of isolates of M. tuberculosis to isoniazid and rifampin (1, 6, 9). The purpose of this study was to evaluate the reliability of the MGIT system for determining susceptibility of M. tuberculosis to ethambutol and streptomycin.

Seventy-four isolates of M. tuberculosis, identified by DNA probe (Gen-Probe, Inc., San Diego, Calif.), were evaluated. Sixty-five isolates were recovered from clinical specimens, including one each from 61 consecutive patients diagnosed with tuberculosis at the University of Texas Medical Branch and 4 multidrug-resistant isolates (based on testing all primary and several secondary antituberculous agents) from separate patients kindly provided by Max Salfinger, State of New York Department of Health, Albany. The other 9 isolates had been included in proficiency testing panels provided by the CDC. Susceptibility test results for the 4 New York and 9 CDC isolates had been confirmed by both the BACTEC and method-of-proportion techniques. Expected results for these 13 isolates were unknown to the individual performing the testing for the study until the evaluation was completed. Control strains of M. tuberculosis (ATCC 27294 [susceptible to ethambutol and streptomycin], ATCC 35820 [streptomycin resistant and ethambutol susceptible], and ATCC 35837 [streptomycin susceptible and ethambutol resistant]) were included with each testing run. All isolates in the study were subcultured to Löwenstein-Jensen (LJ) slants and tested within 2 weeks after colonies were visible.

To prepare the inoculum, colonies from an LJ slant were transferred to a tube containing Middlebrook 7H9 broth and sterile glass beads. Tubes were vigorously agitated on a vortex mixer and then left undisturbed for 20 min to allow clumps to settle. The supernatant was transferred to a second sterile glass tube, and clumps again were allowed to settle for 15 min. The supernatant from this tube was transferred to a third sterile glass tube and adjusted with Middlebrook 7H9 broth to equal the density of a 0.5 McFarland standard. This suspension served as the standard inoculum for all susceptibility testing by the MGIT, the BACTEC TB system, and the method of proportion. Lyophilized ethambutol and streptomycin (Becton Dickinson) were rehydrated with sterile water, aliquotted, and stored at −20°C until the day of testing by both the MGIT and BACTEC TB systems.

MGIT susceptibility testing was performed according to the manufacturer’s recommendations. All tubes were examined by only one observer. For each isolate tested, three tubes were prepared: two contained the antituberculous drugs, and one was a drug-free growth control. To all tubes, 0.5 ml of MGIT OADC (oleic acid, bovine serum albumin, dextrose, and catalase) growth supplement was added, and 0.1 ml of the antibi-
otic solution was added to each drug-containing tube, to give final concentrations of 3.5 μg of ethambutol per ml and 0.8 μg of streptomycin per ml. All three tubes then were inoculated with 0.5 ml of a 1:5 dilution (in sterile saline) of the standard inoculum, tightly capped, and incubated at 37°C for up to 9 days. To test the sterility of the standard inoculum, an aliquot was inoculated onto a sheep blood agar plate, which was incubated at 37°C and examined after 48 h. A positive MGIT control tube, which emits a bright orange fluorescence on the bottom of the tube and an orange reflection at the meniscus, was prepared by removing the broth and replacing it with a 0.4% (wt/vol) sodium sulfite solution. An uninoculated MGIT tube, which shows minimal or no fluorescence, served as the negative control.

Starting on day 3 after inoculation, MGIT tubes were examined daily for fluorescence by placing them on a 365-nm UV transilluminator. If organisms failed to grow, oxygen remained in the broth, quenching the fluorescent indicator, but if growth occurred, organisms consumed the oxygen and the pattern of fluorescence resembled that in the positive control tube. An isolate was considered susceptible to the drug tested if the drug-containing tube did not fluoresce within 2 days after the growth control tube fluoresced. Conversely, the isolate was considered resistant to the drug if the drug-containing tube showed fluorescence within the same period. If the growth control tube showed no fluorescence by day 12 after inoculation, results for the test isolate could not be evaluated, and the MGIT assay was repeated.

BACTEC TB susceptibility testing and the method of proportion were performed according to standard procedures (3). Concentrations of ethambutol tested were 2.5 μg/ml for the BACTEC method and 5.0 μg/ml for the method of proportion. For streptomycin, a 2.0-μg/ml concentration was tested by each method. If the results of MGIT or BACTEC testing did not agree with the results obtained by the method of proportion, which was considered the reference method, all three assays were repeated. Assuming that the results obtained by the method of proportion were correct, the performance of the MGIT was compared to that of the BACTEC by the t test based on the normal approximation of the binomial. The differences between mean time (from day of inoculation) to test results for the MGIT and BACTEC systems were compared by the Student t test.

Susceptibility testing results are summarized in Table 1. In all cases, ATCC control strains performed as expected, and the MGIT growth control fluoresced by day 12, obviating the need for repeat MGIT testing. With ethambutol, initial results for 64 (86.5%) of the 74 isolates obtained by all three methods agreed (58 isolates were susceptible; 6 were resistant). MGIT and method-of-proportion results were concordant for 67 (90.5%) isolates, and BACTEC and method-of-proportion results agreed for 69 (93.2%) (one-tailed test, P = 0.096 [not significant]). After repeat testing, ethambutol results obtained by all three methods agreed for 73 (98.6%) isolates. One isolate remained resistant by MGIT but susceptible by BACTEC testing and the method of proportion.

Initial streptomycin results obtained by all three methods agreed for 69 (93.2%) isolates (55 were susceptible; 14 were resistant). Of the remaining isolates, four were resistant to streptomycin by MGIT but were susceptible by the BACTEC system and the method of proportion, and one was resistant by MGIT and BACTEC testing but susceptible by the method of proportion. Thus, MGIT and method-of-proportion results were concordant for 69 (93.2%) isolates, whereas BACTEC and method-of-proportion results agreed for 73 (98.6%) isolates (one-tailed test, P = 0.049). After repeat testing, streptomycin results obtained by all three methods agreed for 71 (95.9%) isolates (56 were susceptible; 15 were resistant). For the remaining three isolates, the streptomycin result was resistant by MGIT but susceptible by both the BACTEC system and the method of proportion. Streptomycin results obtained by the BACTEC system and the method of proportion agreed for all 74 isolates.

The mean time (± standard error of the mean) from inoculation to results for ethambutol was 5.58 ± 0.10 days (range, 3 to 9 days) for MGIT, compared to a mean of 7.41 ± 0.20 days (range, 4 to 12 days) for the BACTEC system (P < 0.001). For streptomycin, the mean time was 5.47 ± 0.11 days (range, 3 to 9 days) for MGIT, compared to a mean of 7.41 ± 0.20 days (range, 4 to 12 days) for the BACTEC system (P < 0.001).

In summary, our results suggest that for indirect testing of susceptibility of M. tuberculosis isolates to ethambutol and streptomycin, the fluorescence-based MGIT system did not perform as well as it did in previous evaluations with isoniazid and rifampin. Additional studies of the MGIT system for susceptibility testing of M. tuberculosis isolates against all four drugs are needed to confirm or refute these data.

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### REFERENCES


