Antimicrobial Susceptibility Testing of *Mycobacterium tuberculosis* to First-Line Drugs: Comparisons of the MGIT 960 and BACTEC 460 Systems

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Abstract. The reliability of the Mycobacteria Growth Indicator Tube (MGIT) 960 system for rapid antimicrobial susceptibility testing (AST) of *Mycobacterium tuberculosis* was evaluated. Forty-seven isolates, including 10 fully susceptible and 37 resistant strains, were tested for susceptibility to the critical concentrations of streptomycin (STR), isoniazid (INH), rifampin (RMP), and ethambutol (EMB), as recommended by the manufacturer. Strains resistant to the critical concentrations were tested with higher concentrations. The results were compared to those obtained by a radiometric method (BACTEC 460TB) and by a conventional agar dilution method, which served as the reference method. Based on these data, we suggest that the following antibiotic concentrations give satisfactory results with the MGIT 960 system: STR, 4.0 μg/ml; INH, 0.1 μg/ml; RMP, 1.0 μg/ml; and EMB, 5.0 μg/ml. The time required to obtain susceptibility results averaged 6.9 days by the MGIT 960 system and 5.4 days by the BACTEC 460TB system; these intervals were not significantly different. This study shows that the MGIT 960 system is a reliable, rapid, automated method for testing the susceptibility of *M. tuberculosis* isolates to first-line drugs.
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Keywords: *Mycobacterium tuberculosis*, antimicrobial susceptibility testing, automated analysis

Introduction

Tuberculosis remains a major health threat, and the rapid emergence of drug-resistant mycobacteria has strengthened the demand for rapid diagnosis and effective treatment. Multidrug-resistant (MDR) strains have been emerging. Laboratories are challenged to provide rapid identification and efficient antimicrobial susceptibility testing (AST) for effective treatment of the disease [1,2].

The BACTEC 460TB procedure (Becton Dickinson Co., Towson, MD) is a well-established, semi-automated, broth-based method that provides rapid detection of mycobacteria within a closed system. Unfortunately, this system uses a radiometric method to detect the mycobacterial growth. The disposal of radioactive waste produced by the BACTEC 460TB technique poses a considerable logistical problem and increased costs.

The MGIT 960 system (Mycobacteria Growth Indicator Tube, Becton Dickinson Co.) detects the growth of mycobacteria from clinical specimens by utilizing a ready-to-use liquid medium [3-6] and an oxygen-quenching fluorescent sensor system, in conjunction with unique on-board test algorithms [3-6]. The MGIT 960 system has been reported to be an accurate, non-radiometric alternative to the BACTEC 460TB procedure for rapid susceptibility testing of *M. tuberculosis* to four first-line drugs [7-14].

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The MGIT 960 SIRE drug susceptibility kit is a 4-13 day qualitative test that is based on the growth of a *M. tuberculosis* isolate in a drug-containing tube, compared to a drug-free tube (ie, growth control, GC). The MGIT 960 instrument automatically interprets these results using predefined algorithms and reports the drug susceptibilities accordingly.

This study evaluated the reliability of the MGIT 960 system for testing the susceptibility of *M. tuberculosis* to the four first-line drugs: streptomycin (STR), isoniazid (INH), rifampin (RMP), and ethambutol (EMB). Results with the MGIT 960 system were compared to those by the BACTEC 460TB method, and by the standard agar dilution method, using Middlebrook 7H11 agar.

Materials and Methods

Specimen collection and processing. Fory-seven *M. tuberculosis* complex strains were evaluated following isolation by standard procedures [15]. Isolates were identified as *M. tuberculosis* complex by the NAP (*p*-nitro-α-acetyl-amino-β-hydroxy-propiophenone) test prior to their inclusion in this study. The antibiotic susceptibility patterns of the tested *M. tuberculosis* isolates are listed in Table 1.

Table 1. Antibiotic susceptibility patterns of the 47 isolates of *M. tuberculosis* that were tested in this study.

Drug resistance*	Number of strains
None (fully susceptible)	10
INH	11
INH, RMP	10
STR, INH	4
STR	3
EMB	2
INH, EMB	2
INH, RMP, EMB	2
RMP	1
STR, RMP	1
STR, INH, RMP	1

* Based on assays by the BACTEC 460TB system; STR, streptomycin; INH, isoniazid; RMP, rifampin; EMB, ethambutol.

Preparation of inocula. After each isolate was shown as positive by the MGIT 960 instrument for 1 to 2 days, the isolate was inoculated into fresh MGIT medium. After positivity for 3, 4, or 5 days, 1 ml of positive broth was added to 4 ml of sterile saline (1:5 dilution) and mixed well as the inoculum.

Drug solutions. For AST using the MGIT 960, 4 ml of sterile distilled water was added to a lyophilized vial of the respective drug. Then 0.1 ml of the antibiotic stock solution was aseptically pipetted into each MGIT. Final drug concentrations were 1.0 µg/ml for STR, 0.1 µg/ml for INH, 1.0 µg/ml for RMP, and 5.0 µg/ml for EMB. In case of discordant results, AST was performed using higher drug concentrations of 4.0 µg/ml for STR, 0.4 µg/ml for INH, and 7.5 µg/ml for EMB. For the BACTEC 460TB system, the final drug concentrations were 2.0 µg/ml for STR, 0.1 µg/ml for INH, 2.0 µg/ml for RMP, and 2.5 µg/ml for EMB. Table 2 lists the concentrations of the drugs that were used for antimicrobial sensitivity tests with the MIGT 960, BACTEC 460TB, and agar dilution assays.

AST by the MGIT 960 method. The MGIT 960 test was done according to the manufacturer's instructions. To each MGIT tube was aseptically added 0.8 ml of MGIT oleic acid-albumin-dextrose (OADC) and 100 µl of the drug stock solution. The growth control (GC) tube did not contain any

Table 2. Concentrations (µg/ml) of drugs in the MGIT 960, BACTEC 460TB, and agar dilution assays.

Drug*	Agar dilution		BACTEC 460TB		MGIT 960	
	critical	high	critical	high	critical	high
STR	2	10	2	6	1.0	4
INH	0.2	1.0	0.1		0.1	0.4
RMP	1.0		2		1.0	
EMB	5	10	2.5	7.5	5	7.5

* STR, streptomycin; INH, isoniazid; RMP, rifampin; EMB, ethambutol.

antibiotic. One-half ml of inoculum was added to each MGIT and mixed well. Vortexing the tubes after inoculation of the specimens was critical. All tubes were incubated at 37°C in the BACTEC MGIT 960 instrument and continually monitored for increased fluorescence. Fluorescence of the drug-containing tubes was analyzed by the instrument in comparison to the GC tube to determine the antibiotic susceptibility results.

AST by the BACTEC 460TB method. Each MGIT tube that gave positive results by the MGIT 960 instrument was vortexed and 0.1 ml of the medium was inoculated into a 12B vial. The BACTEC 460TB susceptibility test was performed according to the manufacturer's instructions.

AST by the agar dilution method. In cases of discordant results, AST was performed by the classical agar dilution method, following a reference microbiological technique [16] using Middlebrook 7H11 agar medium, which provides optimal growth for multiple-drug resistant strains [17].

Results

Antibiotic susceptibility tests for four first-line drugs were performed on 47 strains of *M. tuberculosis* using both the MGIT 960 and BACTEC 460TB systems. The results obtained by the two methods agreed in 38 (80.9%) and 44 (93.6%) strains for STR 1.0 µg/ml and 4.0 µg/ml; in 45 (95.7%) and 36 (76.6%) strains for INH 0.1 µg/ml and 0.4 µg/ml; in 45 (95.7%) strains for RMP 1.0 µg/ml; in 36 (76.6%) and 41 (87.2%) strains for EMB 5.0 µg/ml and 7.5 µg/ml (Table 3). Only 25 strains (53.2%) showed complete agreement of susceptibility results for all 4 drugs, when the manufacturer's suggested critical concentrations were used in the MGIT 960 system.

Compared to the agar dilution method, MGIT 960 results showed agreement in 41 (87.2%) and 46 (97.9%) strains for STR 1.0 µg/ml and 4.0 µg/ml; in 45 (95.7%) and 35 (74.5%) strains for INH 0.1 µg/ml and 0.4 µg/ml; in 46 (95.7%) strains for RMP 1.0 µg/ml; and in 40 (85.1%) and 41 (87.2%) strains for EMB 5.0 µg/ml and 7.5 µg/ml. Compared to the agar dilution method, BACTEC 460TB results showed agreement in 45 (95.7%) strains for STR and INH; in 44 (93.6%) strains for RMP; and in 37 (78.7%) strains for EMB. (Table 4).

Table 3. Susceptibility of *M. tuberculosis* isolates as determined by the MGIT 960 system, in comparison to the BACTEC 460TB system.

Drug*	No. of isolates with specified results†			
	Both S	Both R	BACTEC S MGIT R	BACTEC R MGIT S
STR				
1.0 µg/ml	30	8	8	0
4.0 µg/ml	35	9	3	0
INH				
0.1 µg/ml	16	29	1	1
0.4 µg/ml	18	18	11	0
RMP				
1.0 µg/ml	31	14	1	1
EMB				
5.0 µg/ml	34	2	7	4
7.5 µg/ml	40	1	5	1

* The drugs were tested in the MGIT 960 system at the concentrations listed in this column. The drugs were tested in the BACTEC 460TB system at the following concentrations: STR (streptomycin), 2.0 µg/ml; INH (isoniazid), 0.1 µg/ml; RMP (rifampin), 2.0 µg/ml; and EMB (ethambutol), 2.5 µg/ml.
† S = susceptible; R = resistant.

The overall results by the BACTEC 460TB, MGIT 960, and agar dilution methods are shown in Table 4, including parameters for specificity, sensitivity, negative predictive value, and positive predictive value. For INH and RMP, the overall performance was >90% for all parameters when tested for susceptibility at the critical concentrations. For EMB, the overall performance for MGIT 960 was better than BACTEC 460TB when tested at the critical and high concentrations. Seven and 6 strains, respectively, gave discrepant results in the MGIT 960, when tested at 5.0 µg/ml and 7.5 µg/ml of EMB. Three of 7 strains in the former group were susceptible by MGIT 960, but resistant by the agar dilution method. At the higher concentration

Table 4. Antimicrobial susceptibility test results and diagnostic performance indices as determined by the MGIT 960 system and the BACTEC 460TB system, compared to the agar dilution method, based on 47 isolates of *M. tuberculosis.*

Drug, method, & drug concentration *	No. of isolates with specified results†				Diagnostic performance indices			
	Both S	Both R	Agar S other R	Agar R other S	Specificity (%)	Sensitivity (%)	NPV† (%)	PPV† (%)
STR								
MGIT 1.0 µg/ml	30	11	6	0	83.3	100	100	64.7
MGIT 4.0 µg/ml	35	11	1	0	97.2	100	100	91.7
BACTEC 2.0 µg/ml	36	9	0	2	100	81.8	94.7	100
INH								
MGIT 0.1 µg/ml	16	29	1	1	94.1	96.7	94.1	96.7
MGIT 0.4 µg/ml	17	18	0	12	100	60	58.6	100
BACTEC 0.1 µg/ml	16	29	1	1	94.1	96.7	94.1	96.7
RMP								
MGIT 1.0 µg/ml	31	15	0	1	100	93.8	96.9	100
BACTEC 2.0 µg/ml	30	14	1	2	96.8	87.5	93.8	93.3
EMB								
MGIT 5.0 µg/ml	35	5	4	3	89.7	62.5	92.1	55.6
MGIT 7.5 µg/ml	39	2	0	6	100	25	86.7	100
BACTEC 2.5 µg/ml	35	2	4	6	89.7	25	85.4	33.3

* In the agar dilution method, the drugs were tested at the following concentrations: STR, 2 µg/ml; INH, 0.2 µg/ml; RMP, 1.0 µg/ml; and EMB, 5 µg/ml.
† S = susceptible; R = resistant; NPV = negative predictive value; PPV = positive predictive value.

of STR in the MGIT 960 system, overall performance was better than at the lower concentration. Only 1 strain gave results that were discrepant with the agar dilution method.

The turn-around times for the automated antibiotic susceptibility tests ranged from 4 to 12 days (mean, 6.9 days; median, 6.4 days) for the MGIT 960 system and from 3 to 12 days (mean, 5.4 days; median, 5.0 days) for BACTEC 460TB system. These intervals did not differ significantly,.

Discussion

Efficient methods for anti-mycobacterial drug susceptibility testing have recently become more important because of an increase of mutidrug-resistant strains of *M. tuberculosis*. In addition to rapid detection time, other important parameters, such as convenience, high-capacity, automation, and cost should be considered in selecting an assay system for a clinical setting. In the present study, the MGIT 960 system was compared with the well-established BACTEC 460TB system and the agar dilution method, which is a standard technique.

The drug concentrations used for the BACTEC 460TB system were suggested by the manufacturer to yield susceptibility results comparable to those at critical concentrations in the agar dilution method (Table 2). The results obtained by the MGIT 960 and BACTEC 460TB systems gave better agreement when higher concentrations of STR and EMB were used in the MGIT 960 tests (Table 3).

In Table 4, the results of both automated methods were compared with the agar dilution method. The MGIT 960 gave comparable results with the BACTEC 460TB when the suggested critical concentration were used for INH and RMP. The overall performance of INH and RMP was >90% for all parameters. For EMB, it was best to use the critical concentration as suggested, since it yielded the lowest number of strains with susceptible results that were actually resistant by the agar dilution method. The higher concentration of STR should be used in the MGIT 960 system in order to obtain the most comparable results and best overall performance.

Based on these data, we recommend that the drug concentrations for the MGIT 960 system should be STR 4.0 μg/ml, INH 0.1 μg/ml, RMP 1.0 μg/ml, and EMB 5.0 μg/ml to give satisfactory performance. The turn-around time to obtain susceptibility results averaged 6.9 days for the MGIT 960, which is comparable to the BACTEC 460TB system (5.4 days).

We found that the overall performance of the MGIT 960 system for antimicrobial susceptibility tests of four first-line drugs is comparable to the BACTEC 460 system, as well as the agar dilution method, if the suggested drug concentrations are used.

In addition, the MGIT 960 system has the advantages of requiring the least amount of labor, and being easiest to use, with high-capacity, fully-automated, continuous monitoring; importantly, it avoids the radiometric waste disposal that is required for the BACTEC 460TB system.

In conclusion, based on limited results, we conclude that the MGIT 960 system is as reliable as the BACTEC 460TB system for antimicrobial susceptibility testing of *M. tuberculosis*.

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