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Evaluation of BACTEC Mycobacteria Growth Indicator Tube (MGIT 960) Automated System for Drug Susceptibility Testing of *Mycobacterium tuberculosis*

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The reliability of the BACTEC MGIT 960 system, an automated version of the Mycobacteria Growth Indicator Tube (MGIT), for antimicrobial susceptibility testing of *Mycobacterium tuberculosis* was evaluated on 78 clinical isolates. Rifampin (RMP), isoniazid (INH), streptomycin (SM), and ethambutol (EMB) were tested at the following concentrations: 1.0 μg/ml for RMP, 0.1 and 0.4 μg/ml for INH, 1.0 and 4.0 μg/ml for SM, and 5.0 and 7.5 μg/ml for EMB. Results were compared with those obtained by the BACTEC 460 TB radiometric system. Initially the reproducibility study showed 99.5% agreement on repeat testing with all the four drugs. With susceptibility testing of clinical isolates, excellent agreement between the two systems was found for all the drugs. A total of nine major errors were observed for only three isolates, resistant according to BACTEC MGIT 960 and susceptible according to BACTEC 460 TB, to SM (4.0 μg/ml), INH (0.1 μg/ml), and EMB (5.0 μg/ml) (one isolate) and to SM (1.0 μg/ml), INH (0.4 μg/ml), and EMB (5.0 μg/ml) (two isolates). When these isolates were tested by using the conventional proportion method on Löwenstein-Jensen medium, agreement with BACTEC MGIT 960 was found for five results and with BACTEC 460 TB for the remainder. The time to report results was 7.9 days by MGIT 960 and 7.3 days by BACTEC 460 TB, which was not found statistically significant ($P > 0.05$). In conclusion, the performance of BACTEC MGIT 960 was found similar to that of BACTEC 460 TB and this new system can be considered a good alternative to the radiometric method for routine susceptibility testing of *M. tuberculosis*.

Drug-resistant *Mycobacterium tuberculosis* strains represent a serious public health problem. Resistance to the four primary drugs, streptomycin (SM), isoniazid (INH), rifampin (RMP), and ethambutol (EMB) (a combination known as SIRE), makes tuberculosis difficult to treat (19). Multidrug-resistant strains have emerged within the last decade, and the rapid detection of these isolates is critical for the effective treatment of patients (28). As recommended by the National MDR TB Task Force, to combat multidrug-resistant tuberculosis (7), antimicrobial susceptibility testing (AST) must be performed on all initial and follow-up *M. tuberculosis* isolates from each patient. Among the methods used for drug susceptibility testing, the agar proportion method (MOP) is universally accepted as the "gold standard" (18, 33). However, it requires a long time to report (generally 21 days after the test is set up). Since 1980 the BACTEC 460 TB radiometric system (Becton Dickinson Diagnostic Instruments, Sparks, Md.), which is based on the modified version of the proportion method (26), has been introduced to perform AST. The BACTEC 460 TB method provides results within 5 to 6 days, with a significant time savings. Several studies (25) have demonstrated that AST results obtained by BACTEC 460 TB were comparable with those of MOP, thus suggesting that the former method could be adopted for routine laboratory purposes. In 1995, the Mycobacteria Growth Indicator Tube (MGIT, 4 ml) (Becton Dickinson) was introduced for the growth and detection of mycobacteria from clinical specimens (3, 8, 12). This method overcomes the drawbacks of the BACTEC 460 TB system, such as the use of radioactive substance and needles. The new MGIT medium consists of modified Middlebrook 7H9 broth in a test tube with silicon rubber impregnated with a fluorescence-quenching oxygen sensor. The reliability of this manual MGIT for AST of *M. tuberculosis* was recently evaluated and compared with the BACTEC 460 TB system, suggesting that the MGIT system could be considered a good alternative to the BACTEC 460 TB radiometric system (3, 5, 6, 20–22, 31).

With use of the MGIT technology, a fully automated system (BACTEC MGIT 960 [Becton Dickinson]), which is able to continuously monitor the fluorescence due to growing mycobacteria (1, 11, 14, 29, 32) has been introduced recently. Also for this system, a modified version of the conventional MOP has been developed, in order to test the susceptibility of *M. tuberculosis* to the four frontline drugs, SIRE.

In this study, we evaluated the performance of the BACTEC MGIT 960 in testing antimicrobial susceptibility to SIRE in comparison to that of the BACTEC 460 TB system by analyzing 78 *M. tuberculosis* clinical isolates. Discrepant results were resolved by the conventional MOP using Löwenstein-Jensen (LJ) medium.

MATERIALS AND METHODS

Study design. The reliability of BACTEC MGIT 960 in testing of *M. tuberculosis* susceptibility to the first-line drugs SIRE was evaluated and compared to that of BACTEC 460 TB. The study consisted of three phases: reproducibility,

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TABLE 1. Results of testing of clinical isolates[a]

Drug (concn[b])	No. of isolates	No. found susceptible by both	No. found resistant by 960 and susceptible by 460	No. found susceptible by 960 and resistant by 460	No. found resistant by Both	Accuracy	Sensitivity (%)	Specificity (%)
SM (1.0)	78	74	2		2	0.974	100	97.3
SM (4.0)	78	77	1			0.987	NV	98.7
INH (0.1)	78	60	1		17	0.987	100	98.3
INH (0.4)	78	73	2		3	0.974	100	97.3
RMP (1.0)	78	78				1.000	NV	100
EMB (5.0)	78	64	3		11	0.962	100	95.5
EMB (7.5)	78	76			2	1.000	100	100

[a] 960, BACTEC MGIT 960; 460, BACTEC 460 TB; NV, not valuable.
[b] Values are in micrograms per milliliter.

clinical isolate testing, and resolution of discordant results. With the exception of RMP, two concentrations (low and high) were used for all of the drugs tested.

Strains and inoculum. A total of 78 *M. tuberculosis* isolates were included in the study. Sixty-six strains were obtained from primary isolation cultures in the MGIT medium, while 12 came from the stock culture collection. Strains were identified by conventional biochemical methods (15) and by PCR-reverse cross blot hybridization (23). For isolates initially grown in MGIT medium, inoculum was prepared from the positive culture on the 1st day of positivity as detected by the BACTEC MGIT 960 instrument. After being mixed well, 0.5 ml of the positive broth cultures was used for BACTEC MGIT 960 AST (see below). For isolates initially grown on LJ medium, a suspension of the microorganism was prepared in 7H9 medium at a density of 0.5 McFarland and was then diluted (1:5) with sterile saline. One-half milliliter of this dilution was used for BACTEC MGIT 960 AST.

Drug solutions. For AST using BACTEC MGIT 960, 4 ml of sterile distilled water was added to a lyophilized vial containing the low concentration of each drug (Becton Dickinson). Part of this solution (0.1 ml) was aseptically pipetted into an MGIT tube to obtain the following final drug concentrations in the medium (low or critical concentrations): 1.0 μg/ml for SM, 0.1 μg/ml for INH, 1.0 μg/ml for RMP, and 5.0 μg/ml for EMB. In addition, for SM, INH, and EMB, stock solutions at higher concentrations were prepared by dissolving each high-concentration lyophilized drug (Becton Dickinson) in 2 ml of sterile distilled water. Part of this antibiotic solution (0.1 ml) was transferred into the MGIT tube, yielding the final drug concentrations (high) of 4.0 μg/ml for SM, 0.4 μg/ml for INH, and 7.5 μg/ml for EMB. AST using the BACTEC 460 TB system was performed according to the manufacturer's instructions (see below). Final drug concentrations were 2.0 and 6.0 μg/ml for SM, 0.1 and 0.4 μg/ml for INH, 2.0 μg/ml for RMP, and 2.5 and 7.5 μg/ml for EMB.

BACTEC MGIT 960 AST. To each 7-ml MGIT tube, 0.8 ml of MGIT 960 Growth Supplement and 0.1 ml of the drug stock solution were aseptically added, and finally 0.5 ml of the test inoculum was added. For each isolate, a growth control (GC) tube with Growth Supplement and without drug was included. For this GC the inoculum was prepared by pipetting 0.1 ml of the test inoculum with 10 ml of sterile saline to make a 1:100 dilution; 0.5 ml of GC inoculum was added to a drug-free MGIT tube. All of the inoculated tubes (seven drug-containing tubes and one drug-free tube for each isolate) were placed into the BACTEC MGIT 960 instrument on the same day of inoculation. The relative growth ratio between the drug-containing tube and drug-free GC tube was determined by the system's software algorithm. If the relative growth in the drug-containing tube was equal to or exceeded that of the GC tube, the isolate was considered drug resistant; if the relative growth was less than in the GC tube, the isolate was considered drug susceptible. The instrument did the final interpretation and reported the susceptibility results automatically.

BACTEC 460 TB AST. From each positive MGIT tube, 0.5 ml was inoculated into a BACTEC 12B vial. When the broth culture reached a growth index of ≥500, the BACTEC 460 TB AST was performed according to the manufacturer's recommendations (24).

Quality controls. To test each new lot of MGIT tubes, BACTEC 460 TB vials, MGIT, and Growth Supplement, we used three reference strains for growth performance: *M. tuberculosis* ATCC 27294, *Mycobacterium kansasii* ATCC 12478, and *Mycobacterium fortuitum* ATCC 6441. Quality control of each new batch of drug was performed with two reference strains, the H37Rv strain of *M. tuberculosis* (ATCC 27294), susceptible to all standard antituberculosis agents, and an *M. tuberculosis* INH-resistant strain (ATCC 35822).

Reproducibility testing. To assess the reproducibility of BACTEC MGIT 960 AST, a panel of 10 strains of *M. tuberculosis* with well-known susceptibility patterns was tested at three separate cycles from two different sources of inoculum (six replicates per strain). For each strain, the BACTEC MGIT 960 AST results at both low and high drug concentrations were compared to the expected results.

Resolution of discrepant results. Isolates for which BACTEC MGIT 960 AST results were discordant with those of BACTEC 460 TB were sent blind to an arbiter site (National Reference Center of Mycobacteria, Forschungszentrum Borstel) for confirmation. These strains were tested by MOP on LJ medium, according to a standard protocol (13).

Genetic analysis of drug resistance. The isolates that had discrepant results after BACTEC MGIT 960 AST and BACTEC 460 TB testing were also examined to detect mutations in the genes responsible for resistance to SM (*rpsL* and *rrs*), INH (*katG, inhA*, and *oxyR-ahpC*), RMP (*rpoB*), and EMB (*embB*), as previously described (9).

Statistical analysis. The statistical analyses were performed using the Epi Info computer package (version 6.03; Centers for Disease Control and Prevention, Atlanta, Ga.). *P* values of ≤0.05 were considered significant.

RESULTS

Reproducibility of BACTEC MGIT 960 testing. At first, reproducibility assays were performed by testing 10 well-characterized *M. tuberculosis* strains in triplicate from two separately prepared inocula. Agreement of 99.5% was obtained for all four drugs in a total of 420 tests, with only two incorrect results for low-concentration EMB.

Testing of clinical isolates. In the second phase of the study, 78 *M. tuberculosis* clinical isolates were tested by BACTEC MGIT 960 and BACTEC 460 TB for susceptibility to SM, INH, RMP, and EMB. Out of a total of 546 tests, we found 9 (1.6%) discordant results (Table 1). Of the 78 strains tested for susceptibility to low-concentration SM, agreement between the methods was found for 76 (97.4%) isolates (74 susceptible and 2 resistant). We found two strains determined to be resistant by BACTEC MGIT 960 and susceptible by BACTEC 460 TB. When all the strains were tested against SM (high concentration), they were found susceptible by BACTEC 460 TB, while one isolate remained resistant according to BACTEC MGIT 960 and susceptible according to BACTEC 460 TB. For low-concentration INH, results by BACTEC 460 TB and BACTEC MGIT 960 agreed for 77 (98.7%) isolates (60 susceptible and 17 resistant). One strain was resistant by BACTEC MGIT 960 and susceptible by BACTEC 460 TB. When the strains were tested against INH (high concentration), we found 73 strains susceptible and 3 resistant according to both methods, while two isolates were found resistant by BACTEC MGIT 960 only.

TABLE 2. Resolution of discordant results by MOP on LJ medium

Strain and drug (concn) used with discordant results	Results obtained by following methods:			Resolved results
	BACTEC MGIT 960	BACTEC 460 TB	MOP	
R076				
SM (high)	R[a]	S	R	Yes
INH (low)	R	S	S	No
EMB (low)	R	S	S	No
R077				
SM (low)	R	S	R	Yes
INH (high)	R	S	R	Yes
EMB (low)	R	S	S	No
R078				
SM (low)	R	S	R	Yes
INH (high)	R	S	R	Yes
EMB (low)	R	S	S	No

[a] R, resistant; S, susceptible.

For results against EMB (low concentration), the two methods agreed for 75 (96.2%) isolates (64 susceptible and 11 resistant). For three isolates (resistant according to BACTEC MGIT. 960 and susceptible according to BACTEC 460 TB), results were discordant. With EMB (high concentration), 76 isolates were susceptible and only 2 were resistant according to both methods, with no discordant results. Obviously, the strains resistant to high concentrations of drugs were resistant to low concentrations too. Finally, full agreement (100%) between both methods was found for RMP results, with all of the isolates susceptible (Table 1).

Table 1 also shows the accuracy and reliability of BACTEC MGIT 960, compared with BACTEC 460 TB, for the four drugs tested. Sensitivity, i.e., the ability to detect true resistance, was 100% for all drugs (data for RMP and high-concentration SM were not valuable because resistant isolates were not found); specificity, i.e., the ability to detect true susceptibility, ranged from 95.5% (for EMB at 5.0 μg/ml) to 100% (for EMB [7.5 μg/ml] and RMP). When the nine discordant results were further analyzed by isolate, we found that these disagreements came from only three isolates. All the strains were found resistant by BACTEC MGIT 960 and susceptible by BACTEC 460 TB; in particular, discrepancies were observed with high-concentration SM, low-concentration INH, and low-concentration EMB for the isolate R076 and with low-concentration SM, high-concentration INH, and low-concentration EMB for the other two isolates, R077 and R078. These isolates with discordant results were tested by conventional MOP on LJ medium. As shown in Table 2, MOP agreed with BACTEC MGIT 960 for five of the nine discordant results and with BACTEC 460 TB for the remaining discrepancies. Particularly, by MOP testing the R076 isolate was found resistant to high-concentration SM, while the isolates R077 and R078 were resistant to low-concentration SM and high-concentration INH. Thus, five of the nine false-resistant results by BACTEC MGIT 960 were resolved as "true resistant." With the resolved results, the specificity of BACTEC MGIT 960 was raised to 100% for low- and high-concentration SM and high-concentration INH compared to BACTEC TB 460, while there were still four discrepant results (one low-concentration INH and three low-concentration EMB).

TABLE 3. Mutations detected in the 3 *M. tuberculosis* isolates with discordant results

Strain	Genes involved in resistance to:					
	INH			SM		EMB
	katG	*inhA*	*oxyR-ahpC*	*rrs*	*rpsL*	*embB*
R076	WT[a]	WT	WT	WT	K43R[b]	WT
R077	S315T[b]	WT	WT	WT	WT	WT
R078	S315T	WT	WT	WT	WT	WT

[a] WT, wild-type genotype.
[b] Amino acid mutation.

In addition, for these isolates, we analyzed the genes involved in the resistance to SM, INH, and EMB (Table 3). We found mutations in *rpsL* (K43R) for one isolate (R076) and in *katG* (S315T) for the other two isolates (R077 and R078).

Finally, the time to report results ranged from 4.6 to 13.2 days (median, 7.9 days) for BACTEC MGIT 960 and from 6 to 10 days (median, 7.3 days) for BACTEC 460 TB; the observed differences were statistically not significant ($P > 0.05$).

DISCUSSION

In the last decade, the increasing number of drug-resistant strains of *M. tuberculosis* has stimulated several efforts to develop rapid and accurate nonradiometric methods for AST (33). The MGIT can be considered the materialization of these efforts, and at present in Europe, the manual version of the system is used as a reliable method for susceptibility testing of *M. tuberculosis* (4). It is conceivable that the fully automated BACTEC MGIT 960, recently developed, could be a promising alternative to the BACTEC 460 TB radiometric system, which is still the widely used method providing susceptibility results in the shortest possible time. The instrument's software algorithms evaluate relative growth in the drug-containing tube and compare it to the drug-free GC tube, and the results are interpreted automatically. A strain is defined as resistant if the relative growth in the drug-containing tube equals or exceeds that in the GC tube and susceptible if the relative growth in the tube with drug is less than in the GC tube. MGIT has several advantages over the BACTEC 460 TB system, having no radioactivity (special safety and regulatory practices such as radioactive disposal are not required) and being noninvasive (using screw-cap tubes eliminates the need for needles) and labor saving (it is completely automatic and easy to use, with a few handling requirements).

For systems currently used to determine the susceptibility of *M. tuberculosis* to antimycobacterial agents, such as the radiometric method (BACTEC 460 TB) or MOP, two critical concentrations (low and high) of the primary drugs (INH, SM, and EMB) have been defined (13). In many laboratories only the low (critical) concentration is usually used in routine testing. However, it should be favorable to test both concentrations of the drugs, because the report of resistant at the low level, especially for INH, could erroneously lead the clinician to believe that the use of that drug would result in a therapeutic failure, while in fact that isolate could be susceptible at the

higher concentration of INH (13). Moreover, in many instances the testing of the low concentration does not correlate well if it is compared with molecular testing for resistance. For EMB, previous reports (2, 27) have demonstrated that mutations in the *embB* gene are associated only with resistance at the high concentration, although a subset of clinical isolates were found with no *embB* mutation, while laboratory screening showed resistance at the high EMB concentration.

The MGIT concentrations of SM, INH, RMP, and EMB have been established on the basis of MOP concentrations in 7H10 medium. Based on NCCLS recommendations (18, 33), using MGIT with two concentrations of all primary drugs, with the exception of RMP, in two steps, is foreseeable. First, test *M. tuberculosis* clinical isolates at the low or critical concentrations, and then test only those strains which are found resistant in the first round.

In this study, we evaluated the performance (reproducibility, accuracy, and reliability) of BACTEC MGIT 960 for determining the susceptibility of *M. tuberculosis* to the four first-line drugs (SIRE), by comparing its results with those of BACTEC 460 TB. Previously, Pfyffer et al. reported preliminary results of a similar evaluation (Abstr. 100th Gen. Meet. Am. Soc. Microbiol., abstr. C16, 2000). In their study, the susceptibility data obtained by using BACTEC MGIT 960 compared well with those for BACTEC 460 TB, suggesting that the new system was able to provide the clinician with rapid and reliable AST results.

There were only 3 strains, out of a total 78 tested, with nine discordant results: one isolate for SM (4.0 μg/ml), INH (0.1 μg/ml), and EMB (5.0 μg/ml) and two isolates for SM (1.0 μg/ml), INH (0.4 μg/ml), and EMB (5.0 μg/ml). All the discordant results showed resistance with BACTEC MGIT 960 and were found susceptible by the radiometric method. The arbiter results by MOP confirmed resistance and resolved the discrepancies in five cases, to high-concentration SM for the first strain and to low-concentration SM and high-concentration INH for the other two. Interestingly, the genetic analysis performed on these strains in order to detect mutations in the genes involved in the resistance against SM and INH reinforced the finding of resistant strains obtained by BACTEC MGIT 960 and MOP (in LJ medium). In fact, we found an S315T mutation in the *katG* gene of the R077 and R078 strains and a K43R mutation in the *rpsL* gene of the R076 strain. These mutations are known to be associated with resistance to INH and SM, respectively (10, 16, 17, 30). On the other hand, no mutations were observed in the *embB* gene of the three strains, in the *rrs* and *rpsL* genes of R077 and R078 strains, and in the *inhA, oxyR-ahpC*, and *katG* genes of strain R076; these findings were in agreement with the MOP results. These data indicate that it is possible to define the R077 and R078 strains as truly resistant to INH and the R076 strain as truly resistant to SM, while the three strains can be considered truly susceptible to low-concentration EMB and the R076 strain can be considered truly susceptible to INH. After resolution by the arbiter results, the specificity of BACTEC MGIT 960 increased. The turnaround times for reporting results for the two systems were also found very close, with no significant difference.

In conclusion, our data demonstrate that the BACTEC MGIT 960 gave good reproducibility; it performed as well as BACTEC 460 TB in AST and can be a valid alternative to the radiometric system under routine laboratory testing.

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