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# Multicenter Evaluation of the Mycobacteria Growth Indicator Tube for Testing Susceptibility of *Mycobacterium tuberculosis* to First-Line Drugs

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**In a multicenter study involving three reference centers for mycobacteria, the reliability of the Mycobacteria Growth Indicator Tube (MGIT) for rapid antimicrobial susceptibility testing (AST) of *Mycobacterium tuberculosis* was evaluated and compared to the radiometric method (BACTEC 460TB). Test cultures for which the results of the MGIT and BACTEC 460TB tests were discordant were checked by the conventional proportion method on solid medium. Four hundred forty-one isolates have been tested for susceptibility to isoniazid (INH), rifampin (RMP), ethambutol (EMB), and streptomycin (SM). Discordant results were obtained for three isolates (0.7%) with INH (susceptible by MGIT, resistant by BACTEC 460TB), for four isolates (0.9%) with RMP (susceptible by MGIT, resistant by BACTEC 460TB), for six isolates (1.9%) with EMB (four susceptible by MGIT, resistant by BACTEC 460TB; two resistant by MGIT, susceptible by BACTEC 460TB), and for four isolates (0.9%) with SM (two susceptible by MGIT, resistant by BACTEC 460TB; two resistant by MGIT, susceptible by BACTEC 460TB). When cultures with discordant results were tested by the conventional proportion method, about half of the cultures yielded results similar to the BACTEC 460TB results, while the other half yielded results similar to the MGIT results. Turnaround times were 3 to 14 days (median, 8.8 days) for MGIT and 3 to 15 days (median, 7.8 days) for BACTEC 460TB. There was no statistically significant difference between the susceptibility testing results of the two methods ( $P > 0.05$ ). These data demonstrate that the MGIT system is an accurate, nonradiometric alternative to the BACTEC 460TB method for rapid susceptibility testing of *M. tuberculosis*.**

Due to the recent increase of the incidence in tuberculosis (TB) in certain parts of the world, the need for rapid diagnosis has become paramount. According to the latest figures of the World Health Organization (17), one-third of the world's population is infected with *Mycobacterium tuberculosis*, and there are 8 to 10 million new TB cases every year. In addition, multidrug-resistant (MDR) strains have been emerging. Because of the emerging resistance to isoniazid (INH) and rifampin (RMP), the two most important antituberculosis drugs, laboratories are challenged to provide rapid identification and antimicrobial susceptibility testing (AST) for effective treatment of the disease.

The radiometric BACTEC 460TB method has been proven to be both sensitive and rapid for detecting mycobacteria. Similarly, it provides the most rapid method for AST. The major drawbacks of the BACTEC 460TB system are well known, however, and are related to the use of a radioactive medium.

The Mycobacteria Growth Indicator Tube (MGIT) is, at present, a manual system and has been reported as a sensitive and rapid method for the growth and detection of mycobacteria from clinical specimens (2, 4, 5, 10, 12, 13, 15). The MGIT contains a modified Middlebrook 7H9 broth in conjunction with a fluorescence quenching-based oxygen sensor (silicon rubber impregnated with ruthenium pentahydrate). Prelim-

inary studies have reported that the MGIT can also be used for AST, but those evaluations were carried out with a limited number of strains and with two drugs, at best (3, 9, 11, 14, 16).

In this European multicenter study, we have evaluated the reliability of the MGIT for testing the susceptibility of *M. tuberculosis* to the four frontline drugs: INH, RMP, ethambutol (EMB), and streptomycin (SM). We have compared the results to those obtained by the BACTEC 460TB method. Discordant results were analyzed by the proportion method using Löwenstein-Jensen (LJ) or Middlebrook 7H10 agar.

## MATERIALS AND METHODS

**Strains.** A total of 441 *M. tuberculosis* strains were evaluated in this study. The evaluation included 391 strains obtained by primary isolation (342 isolates grown in the MGIT medium and 49 grown in Myco/F BACTEC 9000MB medium [Becton Dickinson Microbiology Systems, Cockeysville, Md.]) and 50 strains with known susceptibility patterns from the culture collection in Borstel, Germany. Each clinical center verified the identification of the isolates from primary isolation sources prior to their inclusion in the study. Colony morphology, routine biochemical methods (catalase, nitrate, and niacin reactions [7]), and/or the Accuprobe culture confirmation kits (Gen-Probe, San Diego, Calif.) were used for identification.

**Preparation of inocula.** For isolates initially grown in MGIT medium, each positive culture was used within 2 days of showing fluorescence. It was vortexed for 10 s, and 1 ml of medium was pipetted into 4 ml of sterile saline (1:5 dilution). For isolates initially grown in Myco/F, 0.5 ml of culture from each positive Myco/F bottle was transferred to an MGIT. AST was carried out within 1 to 3 days after the MGIT became positive by fluorescence. The MGIT test was performed as described under "AST" below. For isolates initially grown in an LJ slant, colonies no older than 14 days were used to prepare a suspension in 7H9 broth (adjusted to a McFarland standard of 0.5). One milliliter of this suspension was diluted with 4 ml of sterile saline (1:5 dilution).

**Drug solutions.** For AST using the MGIT, 4 ml of sterile distilled water was added to a lyophilized vial of the respective drug (stock solution). From the

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TABLE 1. Susceptibility patterns of clinical isolates of *M. tuberculosis* (n = 441)

Susceptibility pattern <sup>a</sup>	No. of strains tested <sup>b</sup>			
	BO	MI	ZH	Total
Fully susceptible	49	158	81	288
Inh <sup>r</sup>	8	8	8	24
Rmp <sup>r</sup>	3	3		6
Emb <sup>r</sup>		2	1	3
Sm <sup>r</sup>	5	6	2	15
Inh <sup>r</sup> Rmp <sup>r</sup>	2	1	4	7
Sm <sup>r</sup> Rmp <sup>r</sup>		3		3
Inh <sup>r</sup> Sm <sup>r</sup>	7	1	1	9
Inh <sup>r</sup> Rmp <sup>r</sup> Sm <sup>r</sup>	3	21		24
Inh <sup>r</sup> Emb <sup>r</sup> Sm <sup>r</sup>	7	1		8
Inh <sup>r</sup> Rmp <sup>r</sup> Emb <sup>r</sup>	1	1		2
Inh <sup>r</sup> Rmp <sup>r</sup> Emb <sup>r</sup> Sm <sup>r</sup>	13	38	1	52
Total	98	245	98	441

<sup>a</sup> Generated by BACTEC 460TB.

<sup>b</sup> BO, Borstel; MI, Milan; ZH, Zurich.

solubilized antibiotic, 0.1 ml was aseptically pipetted into an MGIT. The final drug concentrations were 0.1 µg/ml for INH, 1.0 µg/ml for RMP, 3.5 µg/ml for EMB, and 0.8 µg/ml for SM. AST using the BACTEC 460TB system was carried out according to the instructions of the manufacturer. Final drug concentrations were 0.1 µg/ml for INH, 2.0 µg/ml for RMP, 2.5 µg/ml for EMB, and 2.0 µg/ml for SM. Particular emphasis was put on EMB, as this drug was also tested at 3.75 µg/ml. The Borstel center tested 3 strains of *M. tuberculosis* with 2.5 µg of EMB/ml, 84 strains with 3.75 µg of EMB/ml, and 11 strains with EMB at both concentrations; the Milan center tested 28 strains with EMB at 2.5 µg/ml and 217 isolates with EMB at 2.5 as well as at 3.75 µg/ml.

**MGIT controls.** For the growth control (GC), an MGIT without any drug was inoculated with the test culture. For the positive control, the broth from a sterile, uninoculated MGIT was discarded and 5 ml of a 0.4% sodium sulfite solution was added to the empty tube. This positive control was stored at room temperature and used within 30 days. An uninoculated MGIT was used as the negative control.

**AST. (i) MGIT system.** A 0.5-ml portion of MGIT oleic acid-albumin-dextrose (OADC) and 100 µl of the drug stock solution were aseptically added to each MGIT. The GC tube did not contain antibiotics. One-half milliliter of the 1:5 diluted suspension of the test culture was inoculated into each MGIT. All tubes were incubated at 36 to 37°C. MGITs were read daily from day 3 to day 12 by placing them on a 365-nm UV transilluminator. In ambiguous cases (no clear fluorescence), MGITs were incubated for another 24 h and were read again next day. An isolate was considered susceptible if the drug-containing tube did not fluoresce within 2 days of positivity in the GC tube; it was considered resistant if the drug-containing tube showed growth on the day of GC positivity or within 2 days.

**(ii) BACTEC 460TB method.** Each MGIT showing fluorescence (within 2 days) was vortexed for 10 s, and 0.1 ml of medium from that tube was inoculated into a 12B vial. At a growth index (GI) of ≥500 the BACTEC 460TB susceptibility test was performed according to the standard procedure. Organisms initially grown on solid medium were inoculated into a 12B vial. At a GI of ≥500 the BACTEC 460TB susceptibility test was performed.

**(iii) Proportion method.** In cases of discordant results, AST was performed by the classical proportion method using Middlebrook 7H10 agar medium (Zurich) or an LJ slant (Borstel and Milan). The Borstel and Milan centers each followed their national guidelines (6, 8). The Zurich center followed the procedure described by Kent and Kubica (7).

**Internal quality controls.** Four reference strains of *M. tuberculosis* (ATCC 35820, ATCC 35822, ATCC 35837, and ATCC 35838) were used to test each new lot of MGITs and BACTEC 460TB vials, as well as each new lot of OADC and of drugs. All centers found that all materials met the quality standards.

**Reproducibility testing.** Prior to AST of clinical isolates, 20 strains of *M. tuberculosis* were sent by Becton Dickinson to each study center. These strains were blinded, i.e., AST results were known to the company but not to the three centers. The AST results obtained by the three centers for these strains were concordant for each method (MGIT, BACTEC 460TB, and proportion [solid media]) (data not shown).

**Statistical analysis.** The McNemar chi-square test was used for the comparison between the MGIT and BACTEC 460TB methods.

## RESULTS

In this multicenter study, AST was performed with the MGIT and BACTEC 460TB systems on a total of 441 clinical isolates of *M. tuberculosis*. The susceptibility patterns of the strains are listed in Table 1. Full agreement of results for all four drugs (including two EMB concentrations) was found in 1,965 tests (98.6%). Overall, 27 MGIT results (1.4%) were discordant with the BACTEC 460TB results. Twelve of these 27 MGIT results did, however, agree with those obtained on solid media.

Of 441 isolates tested for susceptibility to INH, results obtained by the two methods studied agreed for 438 isolates (99.3%; 315 susceptible and 123 resistant). Three strains tested susceptible with the MGIT but resistant with the BACTEC 460TB. RMP results agreed for 437 (99.1%) isolates (347 susceptible and 90 resistant). Four strains tested susceptible with the MGIT and resistant with the BACTEC 460TB. Of the 357 isolates tested against EMB at a concentration of 2.5 µg/ml, complete agreement between the MGIT and BACTEC 460TB results was found for 347 (97.2%) strains (302 were susceptible and 45 were resistant). Among the 10 isolates showing discrepant results, 3 tested resistant with the MGIT but susceptible with the BACTEC 460TB, and 7 tested susceptible with the MGIT and resistant with the BACTEC 460TB. When tested at a concentration of 3.75 µg of EMB/ml (312 isolates in total), 254 isolates were susceptible and 52 were resistant by both methods (98.1% agreement). Two isolates tested resistant with the MGIT but susceptible with the BACTEC 460TB, while four tested susceptible with the MGIT and resistant with the BACTEC 460TB. SM results of the two methods agreed for 437 (99.1%) isolates (328 susceptible and 109 resistant). Four discordant results were obtained: two strains tested resistant with the MGIT but susceptible with the BACTEC 460TB, and two isolates tested susceptible with the MGIT but resistant with the BACTEC 460TB (Table 2).

The false-resistant and false-susceptible rates for all four drugs with the MGIT compared with those with the BACTEC 460TB are shown in Table 3. Specificity, i.e., the ability to detect true susceptibility, was 100% for INH and RMP and 99% for EMB and SM. Sensitivity, i.e., the ability to detect true resistance, ranged from 86% (for EMB at 2.5 µg/ml) to 98% (for INH and SM).

There was no statistically significant difference between the two AST methods for all four drugs (for INH,  $P = 0.2482$ ; for RMP,  $P = 0.1336$ ; for EMB at 2.5 µg/ml,  $P = 0.3428$ ; for EMB at 3.75 µg/ml,  $P = 0.6831$ ; and for SM,  $P = 0.6171$ ).

TABLE 2. Susceptibilities of *M. tuberculosis* isolates as determined by the MGIT and the BACTEC 460TB systems

Drug <sup>a</sup>	Total no. of strains tested	No. of isolates with the following results <sup>b</sup> :			
		Both S	BACTEC S, MGIT R	BACTEC R, MGIT S	Both R
INH	441	315		3	123
RMP	441	347		4	90
EMB					
Expt 1	357	302	3	7	45
Expt 2	312	254	2	4	52
SM	441	328	2	2	109

<sup>a</sup> Drugs were used at the following concentrations (in micrograms per milliliter): INH, 0.1 (both systems); RMP, 1.0 (MGIT) and 2.0 (BACTEC 460TB); EMB, 3.5 (MGIT) and 2.5 (BACTEC 460TB) in experiment 1 and 3.5 (MGIT) and 3.75 (BACTEC 460TB) in experiment 2; and SM, 0.8 (MGIT) and 2.0 (BACTEC 460TB).

<sup>b</sup> S, susceptible; R, resistant.

TABLE 3. Accuracy and reliability of the MGIT compared with the BACTEC 460TB system for the four drugs tested

Drug <sup>a</sup>	Specificity (%)	Sensitivity (%)	NPV <sup>b</sup> (%)	PPV <sup>c</sup> (%)	Accuracy
INH	100	97.6	99.1	100.0	0.99
RMP	100	95.8	99.9	100.0	0.99
EMB					
Expt 1	99.0	86.5	97.7	93.8	0.97
Expt 2	99.2	92.9	98.5	96.3	0.98
SM	99.4	98.2	99.4	98.2	0.99

<sup>a</sup> Drugs were used at the concentrations given in Table 2, footnote a.

<sup>b</sup> NPV, negative predictive value.

<sup>c</sup> PPV, positive predictive value.

Results of the conventional proportion method for isolates with discordant results by the MGIT and BACTEC 460TB methods are shown in Table 4. In nearly 50% of the strains with discordant results, the results of the proportion method on solid medium agreed with the MGIT results.

Turnaround times for AST ranged from 3 to 14 days (median, 8.8 days) for MGIT and from 3 to 15 days (median, 7.8 days) for BACTEC 460TB.

## DISCUSSION

Multidrug resistance of *M. tuberculosis* has recently become a serious public health issue. Clinicians should not only receive prompt smear and culture results but should also know about the susceptibility pattern of the *M. tuberculosis* isolate.

The methods most widely used for antimycobacterial drug susceptibility testing are the proportion method on LJ or Middlebrook agar medium and the modified proportion method using the BACTEC 460TB system. For the proportion method, results are generally obtained within 3 weeks. The BACTEC 460TB system is the first broth-based method which provides a more rapid result. However, the system is radiometric and requires instrumentation, radiometric material disposal, and needle inoculation. MGITs are read manually, requiring only a UV light, and because they have caps, they can be inoculated with pipettes. Therefore, syringes are not required and the risk of needle puncture is considerably reduced. Results are easy to read. Strains are resistant if growth is detected in the GC tube and in the drug-containing tube. They are susceptible if the drug-containing tube shows no fluorescence within 2 days of growth in the control tube, while resistance is defined by fluorescence within this period of time.

TABLE 4. Analysis of discordant results tested by the proportion method on solid medium<sup>a</sup>

Drug <sup>b</sup>	No. of isolates with the following results <sup>c</sup> :	
	MGIT R, BACTEC S	MGIT S, BACTEC R
INH		3 (1 R, 2 S)
RMP		4 (3 R, 1 S)
EMB		
Expt 1	3 (2 S, 1 R)	7 (2 R, 5 S)
Expt 2	2 (2 S)	4 (3 R, 1 S)
SM	2 (2 R)	2 (2 R)

<sup>a</sup> LJ medium was used at the Borstel and Milan centers, and Middlebrook 7H10 agar was used at the Zurich center.

<sup>b</sup> Drugs were used at the concentrations given in Table 2, footnote a.

<sup>c</sup> S, susceptible; R, resistant. Results obtained by the proportion method are given in parentheses after the number of strains with discordant results.

The purpose of this evaluation was to establish the MGIT system as a method for testing the susceptibility of *M. tuberculosis* to INH, RMP, EMB, and SM and to compare it with the BACTEC 460TB system.

For the 441 strains tested, there was almost 100% agreement between the MGIT and BACTEC results for INH. To resolve the discrepancies of the three MGIT-susceptible but BACTEC 460TB-resistant strains, susceptibility testing was performed by the proportion method. Two of these strains were also susceptible when tested by the proportion method on LJ medium. In preliminary studies, Reisner et al. (11) found an agreement of 100% while Bergmann and Woods (3) detected two false-susceptible and two false-resistant isolates (taking the BACTEC 460TB method as the standard) in testing against INH. For all strains testing susceptible to RMP with the BACTEC 460TB, 100% correlation was found with the MGIT results. For isolates testing resistant to RMP with the BACTEC 460TB, a concordance of 96% was observed. Of the four false-susceptible strains, three were resistant but one was susceptible by the proportion method. With the MGIT, two false-susceptible and two false-resistant isolates were detected in testing against SM. All four strains were resistant by the proportion method. The overall agreement for susceptibility (to INH, RMP, and SM) between the MGIT and BACTEC methods was 99%.

EMB is a powerful antituberculosis drug and is increasingly used in cases of drug-resistant TB. In 1994, an external quality control for susceptibility testing (20 strains were sent to all participating laboratories) was initiated by the World Health Organization (WHO) in laboratories across the world (18). As a result, specificity has generally been higher than sensitivity. Testing for susceptibility to INH and RMP, the two antibiotics that define MDR TB, has shown the highest degree of overall accuracy within the network. For EMB, however, sensitivity was low (90% in 1996 [18]). One possible explanation for this phenomenon may be the heterogeneous nature of EMB resistance itself. Alcaide et al. (1) have shown that in *M. tuberculosis*, mutations in the *embB* gene were consistently associated with high resistance to EMB (MICs of >20 µg/ml), while strains with low resistance (MICs of ≤10 µg/ml) had no mutation in the conserved ethambutol resistance-determining region. As a consequence, strains without a mutation in *embB* may appear to be false-susceptible if they are tested at a high EMB concentration only (7.5 µg/ml). In the present study, we found 10 discordant results when isolates were tested against 2.5 µg of EMB/ml (total *n* = 357) in the BACTEC 460TB system and 6 discordant results when 3.75 µg/ml was used (total *n* = 312). Of the seven false-susceptible isolates (2.5 µg/ml), five were also susceptible by the proportion method. When isolates were tested against EMB at 3.75 µg/ml, only one of the four false-susceptible strains yielded the same result on solid medium. For EMB, two conclusions can be drawn: (i) the correctness of results depends on whether strains show high- or low-level resistance; (ii) to date, there is no EMB concentration for both methods (the BACTEC 460TB and the proportion method) which can safely be used as a "gold standard." However, comparison of the MGIT with the BACTEC 460TB method shows that we obtained good levels of accuracy, 97% with EMB at 2.5 µg/ml and 98% with EMB at 3.75 µg/ml.

For all four drugs and for all strains, results yielded full agreement in 1,965 tests (maximum number of test combinations, 1,992); only 1.4% discordant results could be found when MGIT results were compared with BACTEC 460TB results.

The mean time required to obtain susceptibility results was 8.8 days for MGIT, which is as rapid as that for the BACTEC

460TB system (7.8). These results confirm those reported previously (3, 11).

In conclusion, the results of this large multicenter study indicate that the MGIT system is as efficient as the BACTEC 460TB system for AST of *M. tuberculosis*, making this new technology a candidate to replace the radiometric method.

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