Evaluation of Automated BACTEC MGIT 960 System for Testing Susceptibility of *Mycobacterium tuberculosis* to Four Major Antituberculous Drugs: Comparison with the Radiometric BACTEC 460TB Method and the Agar Plate Method of Proportion

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We evaluated the performance of BACTEC MGIT 960 for automated testing of the susceptibility of 133 strains of *Mycobacterium tuberculosis* to streptomycin, isoniazid, rifampin, and ethambutol. The BACTEC MGIT 960 results were compared with those obtained with the radiometric BACTEC 460TB system, and when there was disagreement, the method of proportion on agar plates was used as a reference method. Strains resistant to the critical concentration of streptomycin, isoniazid, or ethambutol were also tested with a second, higher concentration. The overall agreement between the two systems was 96.7%, and the 18 discrepancies were resolved in favor of BACTEC 460TB in 11 cases and in favor of BACTEC MGIT 960 in 7, a difference which was not statistically significant. Apart from the assay's low specificity for ethambutol, which was low for the radiometric assay as well, good sensitivity and specificity values characterized BACTEC MGIT 960. The average time required for completion of the test was 2.5 days shorter with BACTEC 460TB. In conclusion, BACTEC MGIT 960 appears to be a suitable replacement for the radiometric method of antimicrobial susceptibility testing of *M. tuberculosis*. The problem of frequent contamination of BACTEC MGIT 960 tests needs to be quickly resolved; in fact, 14 strains had to be reprocessed because of contamination.

A threatening increase in *Mycobacterium tuberculosis* drug resistance has been registered in the last few years in many great metropolises in industrialized parts of the world (3). While the prompt restoration of suitable measures macroscopically scaled down this problem in western countries, it exploded in nations of the former Soviet Union (9), where the efforts to fight this problem, which were successful in the western part of the world, are difficult to adopt due to the financial constraints. This stalemate leads to the belief that this problem will not be rapidly solved. On the other hand, the mounting migratory flux from eastern countries toward western European nations poses a threat of spreading drug-resistant tuberculosis in those countries that have little of this problem at present.

Rapid detection of *M. tuberculosis* strains resistant to antituberculosis drugs is probably the most important factor in taking suitable measures to minimize the spread of contagion. In this view, the time necessary for completion of susceptibility testing is critical. The adoption of liquid media is, at present, the most important measure by which to achieve the laboratory result reporting deadlines recommended by the Centers for Disease Control and Prevention (11).

In this study, we evaluated the reliability of a novel susceptibility testing technique that uses the automated nonradiometric BACTEC MGIT 960 system (Becton Dickinson, Sparks,

Md.) by comparing it with the radiometric BACTEC 460TB system (Becton Dickinson), the performance of which is well established.

MATERIALS AND METHODS

The majority of the 133 strains on which susceptibility testing was performed were obtained from clinical isolates in the routine laboratory testing of clinical specimens. All of the test cultures had been identified as belonging to the species *M. tuberculosis* by combining DNA-probe hybridization (AccuProbe *M. tuberculosis*; Gen-Probe, San Diego, Calif.), niacin accumulation, and nitrate reduction tests (4). Besides 120 fresh clinical isolates, eight resistant strains were retrieved from the laboratory culture collection as well. Four reference strains, each resistant to one of the major antimycobacterial drugs (ATCC 35820, streptomycin resistant; ATCC 35822, isoniazid resistant; ATCC 35838, rifampin resistant; ATCC 35837, ethambutol resistant), and the fully susceptible H37Rv reference strain (ATCC 27294) were also included in this study. The antimicrobial susceptibility of 120 of the strains in our study was unknown at the time when the comparison was done. At the end, after the discordant results were resolved, we had 19 strains resistant to isoniazid (14 of them at a high level), 12 resistant to streptomycin (7 at a high level), 7 resistant to ethambutol (4 at a high level), and 8 resistant to rifampin. Twelve strains were resistant to two or more drugs, and eight of them were multidrug resistant (i.e., resistant at least to isoniazid and rifampin).

The tests with the radiometric BACTEC 460TB system were performed in accordance with the manufacturer's recommendations (10). A radiometric vial with a growth index ranging from 500 to 800 was used for direct inoculation of drug-containing BACTEC 12B (12B) bottles and, after 1:100 dilution, for the drug-free control. Since the BACTEC MGIT 960 system is routinely used for primary isolation in our laboratory, 100 µl from a positive MGIT tube was subcultured in a 12B vial for radiometric susceptibility testing. The drug solutions were prepared by reconstitution of the provided lyophilized drugs (SIRE; Becton Dickinson) with distilled water. The BACTEC 460TB instrumentation was used for daily reading of the 12B vials until the control had reached a growth index of ≥30.

The tests with the automated BACTEC MGIT 960 instrumentation were performed with MGIT cultures that tested positive at least 1, but no more than

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TABLE 1. Comparison of results obtained with the BACTEC 460TB and MGIT systems

Drug (concn)[a]	No. of isolates			
	Susceptible with both systems	Susceptible with BACTEC 460TB and resistant with BACTEC MGIT 960	Resistant with BACTEC 460TB and susceptible with BACTEC MGIT 960	Resistant with both systems
Streptomycin (L)	120	2	0	11
Streptomycin (H)	5	1	0	6
Isoniazid (L)	111	3	0	19
Isoniazid (H)	5	3	0	14
Rifampin	124	1	0	7
Ethambutol (L)	124	3	2	4
Ethambutol (H)	3	1	2	0

[a] L, lower concentration; H, higher concentration.

2, days before. The MGIT tubes were supplemented with 0.8 ml of the provided enrichment (BACTEC MGIT 960 SIRE Supplement; Becton Dickinson). After the lyophilized drugs (BACTEC MGIT 960 SIRE; Becton Dickinson) were rehydrated in accordance with the recommended procedure, 100 μl of antibiotic solution was added to a labeled MGIT tube for each drug. Only the MGIT supplement was added to the growth control tube. All of the drug-containing tubes were then inoculated with 0.5 ml of the positive broth culture, while for the drug-free control, the culture was diluted 1:100 in distilled water before addition to the control tube. The tubes were placed in the proper MGIT rack in a fixed sequence (control, streptomycin, isoniazid, rifampin, and ethambutol); the rack was incubated in the cabinet drawer and left there until the conclusion of the test was signaled by the instrument.

Tests with both methods were performed with the standard critical concentrations (5) of streptomycin (2 μg/ml for 12B and 1 μg/ml for MGIT), isoniazid (0.1 μg/ml for 12B and MGIT), rifampin (2 μg/ml for 12B and 1 μg/ml for MGIT), and ethambutol (2.5 μg/ml for 12B and 5 μg/ml for MGIT). When a strain turned out to be resistant, the tests with both methods were repeated with the drug in question, regardless of whether the results were in agreement or not. When the tests were repeated they were also done with the higher drug concentrations (BACTEC SIRE; Becton Dickinson): streptomycin, 6 μg/ml for 12B and 4 μg/ml for MGIT; isoniazid, 0.4 μg/ml for 12B and MGIT; ethambutol, 7.5 μg/ml for 12B and MGIT. A single concentration of rifampin was tested.

Resolution of discrepancies remaining after test repetition was achieved by testing the susceptibility of the strains with discordant results by the method of proportions. For this purpose, Middlebrook 7H11 quadrant plates and impregnated antibiotic disks (16) (Sensi-Disc; Becton Dickinson) were employed with the following final drug concentrations: streptomycin, 2 and 10 μg/ml of agar; isoniazid, 0.2 and 1 μg/ml of agar; rifampin, 5 μg/ml of agar; ethambutol, 5 and 10 μg/ml of agar.

The purity of mycobacterial cultures was checked by placing a few microliters of the inoculum broth on blood agar and Middlebrook 7H11 plates, which were incubated and read daily (the 7H11 plates were also read with the aid of a low-magnification microscope) to detect the presence of possible contaminants. Likewise, broth cultures showing drug resistance were retrospectively checked for purity as well.

The disagreement of the susceptibility results achieved with the two methods was evaluated with the McNemar χ^2 test, while the paired t test was used to compare the times needed for test completion.

RESULTS

Out of 133 strains tested, 106 gave identical results with both methods for the four drugs tested. Forty-two single-drug disagreements were observed among the remaining 27 strains. A higher prevalence of resistance was observed with the BACTEC MGIT 960 tests than with the BACTEC 460TB tests. Sterility checks of broth cultures showing resistance and repeat testing of the discordant results indicated that the majority of differences were due to contamination of the MGIT broth. The proportion of tests found to be contaminated among those inoculated with culture collection isolates and those inoculated directly from primary isolation tubes was not

substantially different from the contaminated proportion of the whole culture panel tested; it was therefore not possible to compare the weight of possibly contaminated primary cultures with that of contaminations that may have occurred during test set-up. When results of repeat testing were considered, the agreement between the two methods rose to 120 while only 18 discrepancies remained among the overall 571 single-drug tests. Among the strains that were tested with the higher drug concentrations, 33 out of 40 agreed (Table 1). After resolution of the discrepant results, the drug most frequently involved in disagreements was ethambutol (total, eight tests), followed by isoniazid (six tests), streptomycin (three tests), and rifampin (one test) (Table 2). When the combination of low and high drug concentration results was analyzed, four cases were found in which the same drug (streptomycin once, rifampin once, and ethambutol twice) was fully effective with one method and fully resistant with the other. Along with such major discrepant results, 12 minor discrepancies were observed in which partial resistance to a drug by one method corresponded to either full susceptibility or full resistance by the other. Compared with the agar proportion method, the errors concerning isoniazid occurred mostly with the BACTEC MGIT 960 system while those concerning the other drugs were almost equally distributed between the two methods. In the majority of cases (8 out of 11), the BACTEC MGIT 960 errors were false resistances while BACTEC 460TB errors appeared to be prevalently false susceptibilities (5 out of 7). The specificity, i.e., the ability to detect susceptibility, and sensitivity, i.e., the ability to detect resistance, computed by using the agar proportion method as

TABLE 2. Resolution of discrepant results in comparison with the proportion method

Drug (concn)[a]	No. of false susceptibility results		No. of false resistance results	
	BACTEC 460TB	BACTEC MGIT 960	BACTEC 460TB	BACTEC MGIT 960
Streptomycin (L)	1	0	0	1
Streptomycin (H)	1	0	0	0
Isoniazid (L)	0	0	0	3
Isoniazid (H)	1	1	0	1
Rifampin	0	0	0	1
Ethambutol (L)	2	1	1	1
Ethambutol (H)	0	1	1	1

[a] L, lower concentration; H, higher concentration.

TABLE 3. Sensitivity and specificity of the BACTEC MGIT 960 and 460TB systems

Drug	% Sensitivity (% specificity)	
	BACTEC MGIT 960	BACTEC 460TB
Streptomycin	99.2 (100)	100 (88.9)
Isoniazid	96.6 (97.1)	100 (97.1)
Rifampin	99.2 (100)	100 (100)
Ethambutol	98.5 (81.8)	98.5 (81.8)

the "gold standard" are reported for single drugs and for both methods in Table 3.

The resolution of 18 discrepancies on the basis of the proportion method confirmed the 12B results in 11 cases and the MGIT results in 7 cases.

The average time to a final result was 6.9 (range, 5 to 16) days for 12B and 9.4 (range, 5 to 14) days for MGIT.

On the basis of the McNemar χ^2 test, the difference in performance between the two methods turned out not to be statistically significant ($\chi^2 = 0.89$) while the 2.5-day difference between the times to test completion was significant ($P < 0.01$).

DISCUSSION

The importance of rapid availability of *M. tuberculosis* drug resistance results is universally acknowledged. Detection of resistance at the genetic level by the presence of mutations in certain loci, although promising, is still far from finding its place among the techniques of diagnostic mycobacteriology, the major hindrance being the presence of multiple resistance mechanisms for the majority of antimycobacterial drugs (14).

Phenotypic susceptibility testing, therefore, remains the method of choice. In this area, the use of liquid media is the only possibility of speeding up this testing. The radiometric BACTEC 460TB method has brilliantly performed this task in the last 2 decades (7), but due to an increasing concern about radioactivity and its disposal, there is a growing tendency to eliminate radioactivity from diagnostic laboratories.

Although there is no impediment to *M. tuberculosis* susceptibility testing with nonautomated liquid media, it is evident that only automatic systems have the potential to replace the semiautomated BACTEC 460TB method.

Among automated mycobacterial culture systems, BACTEC MGIT 960 has shown good sensitivity and an excellent ability to shorten the time required for detection of growth from clinical specimens (12).

In many ways, BACTEC 460TB susceptibility testing is considered the gold standard and every new alternative system has to be measured against it. In comparative tests, for resolution of discrepant results, the reference proportion method performed on agar-based solid media is generally used.

In our evaluation, 96.7% agreement between the two systems was observed and in 11 out of 18 discrepant results, the radiometric BACTEC 460TB method turned out to be correct. This difference, however, was found not to be statistically significant. When disagreements occurred, the BACTEC MGIT 960 system had a tendency to overestimate resistance while the BACTEC 460TB system showed the opposite tendency.

Specificity and sensitivity values were high with both methods, with better sensitivity values for BACTEC 460TB and better specificity for BACTEC MGIT 960. Both of the systems yielded the lowest sensitivity values with ethambutol.

The time needed for test completion was, on average, 2.5 days shorter with BACTEC 460TB, which was a statistically significant difference.

The workload was overlapping, as far as preparation of antibiotic-containing media and inoculation are concerned, but was clearly different for subsequent steps, which are fully automatic with BACTEC MGIT 960, while the radiometric method is substantially labor-intensive.

No evaluation of susceptibility testing performed with BACTEC MGIT 960 has been published so far. Although several evaluations concerning antimicrobial susceptibility testing using MGIT medium have been reported previously (1, 6, 15), they were all done with a tentative method that is no longer supported by the producer. That method, which, being fully manual, did not use the BACTEC MGIT 960 instrumentation, did not allow for the universally accepted basic concept of the proportion method, which discriminates susceptibility from resistance according to a proportion of resistant mutants lower or higher then 1%. The data emerging from such studies are therefore not comparable to ours.

Comparisons like ours have been reported, however, for the other available automated systems. Two papers have compared the MB/BacT (Organon Teknika, Turnhout, Belgium) with the radiometric BACTEC 460TB method. Slightly higher isoniazid specificity of the first (2) and lower ethambutol sensitivity of the second (13) were reported. Only one study has compared ESP System II (Accumed, Westlake, Ohio) with BACTEC 460TB (8). The results of that study do not substantially differ from ours. The turnaround time was reported to be slightly shorter for ESP II, and there was a tendency of the new system to give false resistances with the lower concentration of isoniazid. Therefore, except for the lower specificity for ethambutol, our data are close to those of other automated systems.

In conclusion, based on our results, BACTEC MGIT 960 appears to be a good replacement for the BACTEC 460 radiometric system. However, the high BACTEC MGIT 960 contamination rate was a problem in our study. In fact, it requires repetition of the test and thus delays the results and, if ignored, leads to false resistance reports, which may affect the therapeutic regimen. It must, however, be stressed that false resistance is not as serious an error as false susceptibility; it does not lead to treatment with inactive drugs.

Two major factors that are, in our opinion, responsible for the higher rate of contamination of MGIT tubes are the richness of the medium and the use of screw caps instead of rubber septa. Modification of the medium seems not to be possible due to the present BACTEC MGIT 960 growth detection principle, while the use of a perforated screw cap with a rubber septum may be possible. With this option, the operator would be free to choose the route of inoculation. On the other hand, we realize that the use of needles is a serious safety issue and may encounter strong and not unjustified resistance.

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