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Evaluation of the Fully Automated BACTEC MGIT 960 System for Testing Susceptibility of *Mycobacterium tuberculosis* to Pyrazinamide, Streptomycin, Isoniazid, Rifampin, and Ethambutol and Comparison with the Radiometric BACTEC 460TB Method

Claudio Scarparo,* Paolo Ricordi, Giuliana Ruggiero, and Paola Piccoli

Regional Mycobacteria Reference Centre, Laboratory of Clinical Microbiology and Virology, San Bortolo Hospital, Vicenza, Italy

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The performance of the fully automated BACTEC MGIT 960 (M960) system for the testing of *Mycobacterium tuberculosis* susceptibility to streptomycin (SM), isoniazid (INH), rifampin (RMP), ethambutol (EMB), and pyrazinamide (PZA) was evaluated with 100 clinical isolates and compared to that of the radiometric BACTEC 460TB (B460) system. The agar proportion method and the B460 system were used as reference methods to resolve the discordant results for SM, INH, RMP, and EMB (a combination known as SIRE) and PZA, respectively. The overall agreements were 96.3% for SIRE and 92% for PZA. For SIRE, a total of 26 discrepancies were found and were resolved in favor of the M960 system in 8 cases and in favor of the B460 system in 18 cases. The M960 system produced 8 very major errors (VME) and 10 major errors (ME), while the B460 system showed 4 VME and 4 ME. No statistically significant differences were found. Both systems exhibited excellent performance, but a higher number of VME was observed with the M960 system at the critical concentrations of EMB and SM. For PZA, a total of eight discrepancies were observed and were resolved in favor of the M960 system in one case and in favor of the B460 system in seven cases; no statistically significant differences were found. The M960 system showed four VME and three ME. The mean times to report overall PZA results and resistant results were 8.2 and 9.8 days, respectively, for the M960 system and 7.4 and 8.1 days, respectively, for the B460 system. Statistically significant differences were found. The mean times to report SIRE results were 8.3 days for the M960 system and 8.2 days for the B460 system. No statistically significant differences were found. Twelve strains tested for SIRE susceptibility and seven strains tested for PZA susceptibility had been reprocessed because of contamination. In conclusion, the M960 system can represent a valid alternative to the B460 for *M. tuberculosis* susceptibility testing; however, the frequent contamination of the tests needs to be improved.

Tuberculosis (TB) is a significant public health problem for both industrialized and developing nations. The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* in many geographic areas and the increased migratory flux from higher-prevalence to lower-prevalence countries underline the great importance of rapid identification and timely detection of drug resistance in the optimal management of patients with TB.

A multidrug-resistant *M. tuberculosis* strain is currently defined as one that is resistant to at least isoniazid (INH) and rifampin (RMP) or more antituberculosis drugs. The timely and systematic monitoring of the susceptibility of *M. tuberculosis* isolates to front-line drugs is essential for (i) rapid detection of drug-resistant strains, (ii) effective treatment of patients, and (iii) prompt and adequate public health measures to prevent or reduce the spreading of drug-resistant TB.

The BACTEC 460TB (B460) system (Becton Dickinson Biosciences, Sparks, Md.) has been widely validated for approximately 20 years and is regarded as the best method in clinical laboratories for reliable and rapid testing of susceptibility of *M. tuberculosis* isolates to front-line drugs such as streptomycin (SM), INH, RMP, ethambutol (EMB), and pyrazinamide (PZA), in accordance with the Centers for Diseases Control and Prevention recommendations (12). PZA is one of the most important agents in the effective management of patients with TB, representing an integral component of the short-course chemotherapy regimen. However, PZA susceptibility testing is technically difficult to perform because the bactericidal activity of this drug is optimal only in an acid environment that inhibits the growth of most of *M. tuberculosis* isolates (10). At present, the radiometric B460 method with a modified broth at pH 6.0 has been validated and considered to be the reference method for PZA susceptibility testing by the National Committee for Clinical Laboratory Standards (NCCLS) (6). Recently, the BACTEC MGIT 960 (M960), a newly developed nonradiometric, fully automated, continuous-monitoring system (Becton Dickinson), has been introduced as an alternative to the radiometric B460 system. A few studies have reported the performance of the automated M960 system for testing of susceptibility to four drugs: SM, INH, RMP, and EMB (a combination known as SIRE) (1, 2, 3, 13). After the recent commercial availability of the new M960 PZA medium at pH 5.9, only one study

* Corresponding author. Mailing address: Regional Mycobacteria Reference Centre, Microbiology and Virology Laboratory, San Bortolo Hospital, Viale Rodolfi 37, Vicenza I-36100, Italy. Phone: 39 0444 993507. Fax: 39 0444 993963. E-mail: claudio.scarparo@tin.it.

has reported the performance of the M960 for susceptibility testing, and this was limited to PZA (7). The testing of susceptibility of *M. tuberculosis* to SIRE and PZA by the M960 system has been cleared by the Food and Drug Administration (6).

This report summarizes the results of a study comparing the performances of the M960 and B460 systems in testing the susceptibility of 100 *M. tuberculosis* clinical isolates to all five front-line drugs (PZA and SIRE). Resolution of discrepancies was achieved by retesting the PZA susceptibility with the B460 method (reference method), and by testing the SIRE susceptibility by the proportion method with Middlebrook 7H11 agar.

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MATERIALS AND METHODS

Strains. A total of 100 *M. tuberculosis* isolates were evaluated in this study. Seventy strains were fresh clinical isolates grown in the B460 system, while 30 strains were selected from the laboratory culture collection. The strains were identified by combining colony morphology with results from Accuprobe *M. tuberculosis* complex culture confirmation kits (Gen-Probe, San Diego, Calif.) and biochemical tests (niacin accumulation and nitrate reduction tests) (5).

M960 system. All strains were inoculated in the M960 tubes and incubated in the M960 instrument. Each culture was used for susceptibility testing within 1 to 5 days after the instrument flagged a positive signal. For the preparation of the inoculation procedure, mycobacterial suspensions were used undiluted from day 1 to 2 following positivity, while the suspensions were diluted 1:5 with sterile saline from day 3 to 5. The culture in any tube which had been positive for more than 5 days was subcultured into a fresh, new M960 tube.

Susceptibility testing using the M960 system was performed with the following final drug concentrations: 1.0 and 6.0 μg/ml for SM, 0.1 and 0.4 μg/ml for IHN, 1.0 μg/ml for RMP, and 5.0 and 7.5 μg/ml for EMB. Susceptibility testing for PZA, using a modified broth at pH 5.9, was performed with a final drug concentration of 100 μg/ml.

The eight M960 tubes, seven for testing of susceptibility to SIRE and one for a growth control, were supplemented with 0.8 ml of the provided enrichment (BACTEC MGIT 960 SIRE supplement; Becton Dickinson). The two tubes of M960 PZA medium, one for testing of susceptibility to PZA and one for a growth control, were supplemented with 0.8 ml of the provided enrichment (BACTEC MGIT 960 PZA supplement; Becton Dickinson). After the lyophilized drugs (BACTEC MGIT SIRE and BACTEC MGIT PZA; Becton Dickinson) were rehydrated in accordance with the manufacturer's recommended procedure, 100 μl of antibiotic solution of SIRE was added to the labeled M960 tube for each concentration of drug and 100 μl of antibiotic solution of PZA was added to the labeled M960 PZA tube. All of the drug-containing tubes (including the M960 PZA tube) were then inoculated with 0.5 ml of the positive broth culture. The SIRE drug-free control was inoculated with 0.5 ml of a 1:100 dilution of the positive culture broth in sterile saline, while the PZA drug-free control was inoculated with 0.5 ml of a 1:10 dilution of the positive culture broth in sterile saline.

The eight M960 tubes for testing of susceptibility to SIRE were placed in an M960 set carrier in the following fixed order: growth control, SM at 1.0 μg/ml, SM at 6.0 μg/ml, INH at 0.1 μg/ml, INH at 0.4 μg/ml, RMP at 1.0 μg/ml, EMB at 5 μg/ml, and EMB at 7.5 μg/ml. The two M960 PZA tubes for testing of susceptibility to PZA were placed in an M960 PZA set carrier in the following fixed order: growth control and PZA at 100 μg/ml. Both of the set carriers were incubated in the M960 instrument and continuously monitored until the results indicating susceptibility or resistance were automatically interpreted and reported by using predefined algorithms (which compared growth in the drug-containing tube to that in the growth control tube).

B460 system. All strains were inoculated into 12B vials, incubated at 37°C, and tested daily in the B460 instrument. The broth of 12B vials with a growth index ranging from 500 to 800 was used for direct inoculation of SIRE drug-containing B460 12B vials and, after a 1:100 dilution, also for the SIRE drug-free control.

For the preparation of the inoculation procedure, the broth of 12B vials with a growth index ranging from 300 to 499 was used for the direct inoculation of PZA drug-containing B460 PZA vials and PZA drug-free controls, while if the growth index was greater than 499, the suspension broth was diluted in accordance with the manufacturer's recommended procedure.

After the lyophilized drugs (SIRE and PZA; Becton Dickinson) were rehydrated in accordance with the manufacturer's recommended procedure, 100 μl of antibiotic solution of SIRE was added to the labeled 12B vials for each concentration of the drug, and 100 μl of antibiotic solution of PZA was added to the labeled PZA vial.

The test was performed with the following final drug concentrations: 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. Testing of susceptibility to PZA, using a modified broth at pH 6, was performed with a final drug concentration of 100 μg/ml.

The susceptibility tests (SIRE and PZA) with the radiometric B460 system were performed in accordance with the standard procedures, and the readings were evaluated according to the established criteria for calculating susceptible, resistant, and borderline results (11).

Reproducibility testing. Reproducibility testing was performed prior to testing mycobacterial isolates from clinical specimens. A panel of five strains of *M. tuberculosis* with known susceptibility patterns was tested in triplicate at three cycles (nine replicates per strain). The M960 performance was compared to the expected results at both low and high drug concentrations.

Quality control (QC). Six reference strains of *M. tuberculosis* (H37Rv [ATCC 27294], fully susceptible; ATCC 35828, PZA resistant; ATCC 35820, SM resistant; ATCC 35822, INH resistant; ATCC 35838, RMP resistant; ATCC 35837, and EMB resistant) were used to test each new lot of M960 tubes and B460 vials, M960 PZA and B460 PZA media, medium components (e.g., growth supplement), and each new lot of drugs (SIRE and PZA).

Resolution of discrepant results. The proportion method was applied, according to a standard protocol with Middlebrook 7H11 agar (6), to strains for which discrepant results with SIRE were observed. Susceptibility testing was performed with the following final drug concentrations: 2.0 and 10 μg/ml of agar for SM, 0.2 and 1.0 μg/ml of agar for IHN, 1.0 μg/ml of agar for RMP, and 5.0 and 10 μg/ml of agar for EMB. The strains for which discrepant results with PZA were observed were retested by the B460 method (reference method).

Purity checks. All mycobacterial suspensions used for SIRE and PZA susceptibility tests were checked for purity on sheep blood agar and Middlebrook 7H11 agar plates, as were, retrospectively, all broth cultures showing drug resistance.

Statistical analysis. The differences in susceptibility results were evaluated by using the chi-square test (Epi Info version 6.04; Centers for Disease Control and Prevention, Atlanta, Ga.), while the differences in the number of days required to complete the test were determined by the paired *t* test. *P* values of ≤ 0.05 were considered to be statistically significant.

TABLE 1. Reproducibility testing for SIRE and PZA by the M960 system

Drug (μg/ml)	Strains[a]	No. of tests performed[b]	No. of results agreeing with reference method[c]	Agreement (%)
INH (0.1)	S	27	27	100
	R	18	18	100
INH (0.4)	S	27	27	100
	R	18	17	94.4
RMB (1.0)	S	27	27	100
	R	18	18	100
EMB (5.0)	S	27	26	94.4
	R	18	18	100
EMB (7.5)	S	27	27	100
	R	18	18	100
SM (1.0)	S	36	35	97.2
	R	9	9	100
SM (4.0)	S	36	36	100
	R	9	9	100
Total		315	312	99
PZA (100)	S	36	36	100
	R	9	8	88.9
Total		45	44	97.8

[a] S, susceptible; R, resistant.
[b] With five strains of *M. tuberculosis* in triplicate from three separate inocula (thus, nine replicates per strain)
[c] Agar proportion method for SIRE and B460 system for PZA.

TABLE 2. Initial results of testing of clinical isolates of *M. tuberculosis* for SIRE and PZA

Drug (μg/ml)	No. of tests	No. of tests with the following results[a]				Agreement (%)
		S by M960, S by B460	R by M960, S by B460	S by M960, R by B460	R by M960, R by B460	
INH (0.1)	100	51	3		46	97
INH (0.4)	100	56	5		39	95
RMB (1.0)	100	70		1	29	99
EMB (5.0)	100	78		3	19	97
EMB (7.5)	100	86	2		12	98
SM (1.0)	100	61	2	5	32	93
SM (4.0)	100	72	2	3	23	95
Total	700	474	14	12	200	96.3
PZA (100)	100	65	3	5	27	92

[a] S, susceptible; R, resistant.

RESULTS

Reproducibility testing. The results of reproducibility testing of the M960 system with five strains of *M. tuberculosis* are presented in Table 1. Agreement of 97.8% was observed for PZA in a total of 45 tests, with only one incorrect test result with a PZA-resistant strain. Agreement of 99% was observed for SIRE reproducibility in a total of 315 tests, with one incorrect test result at the high concentration of INH and one incorrect test result at the low concentrations of EMB and SM.

Testing of clinical isolates. A total of 100 clinical strains of *M. tuberculosis* were evaluated for susceptibility testing to five drugs: SM, INH, RMP, EMB, and PZA. All strains were tested at critical (low) and high concentrations for SM, INH, and EMB, and at critical concentrations for RMP and PZA.

Of the 100 strains tested for susceptibility to SIRE and to PZA, full agreement of results between the two methods was found for 74 isolates (74%). Twenty-seven strains were multidrug-resistant *M. tuberculosis*, as defined by resistance to at least INH and RMP. Twelve strains tested for susceptibility to SIRE and seven strains tested for susceptibility to PZA had been reprocessed because of contamination of the M960 broths.

Initial results of susceptibility testing of clinical isolates for SIRE are illustrated in Table 2. Of 100 strains tested for susceptibility to SIRE, results for INH obtained by the two methods agreed for 97 strains (97%) (51 susceptible and 46 resistant) at the critical concentration and for 95 strains (95%) (56 susceptible and 39 resistant) at the higher concentration. Results for RMP agreed for 99 strains (99%) (70 susceptible and 29 resistant). Results for EMB agreed for 97 strains (97%) (78 susceptible and 19 resistant) at the critical concentration and for 98 strains (98%) (86 susceptible and 12 resistant) at the higher concentration. Results for SM agreed for 93 strains (93%) (61 susceptible and 32 resistant) at the critical concentration and for 95 strains (95%) (72 susceptible and 23 resistant) at the higher concentration.

Out of a total of 700 tests for SIRE drugs, we observed 26 single-drug disagreements (3.7%). Fourteen discordant results were resistant according to the M960 system but susceptible according to the B460 system: three results for INH at the critical concentration, five results for INH at the high concentration, two results for EMB at the high concentration, two results for SM at the critical concentration, and two results for SM at the high concentration. Twelve results were susceptible according to the M960 system but resistant according to the B460 system: one result for RMP, three results for EMB at the critical concentration, five results for SM at the critical concentration, and three results for SM at the high concentration (Table 2).

Initial results of susceptibility testing of clinical isolates for PZA are shown in Table 2. Of 100 strains tested for susceptibility to PZA, results obtained by the two methods agreed for 92 strains (92%) strains (65 susceptible and 27 resistant). We observed eight disagreements (8%): (i) three results were resistant according to the M960 system but susceptible according to the B460 system, and (ii) five results were susceptible according to the M960 system but resistant according to the B460 system (Table 2).

For SIRE, the resolution of 26 discrepant results by the 7H11 base agar plate proportion method is illustrated in Table 3. The proportion method confirmed the results of the M960 system in 8 cases (4 false-susceptible and 4 false-resistant results for the B460 system), while it confirmed the results of the B460 system in 18 cases (8 false-susceptible and 10 false-resistant results for the M960 system).

For PZA, the results after resolution of eight discrepant results by repetition of the test with the B460 method (reference method) are presented in Table 3. The repetition of the test by the B460 method confirmed the previous results of the B460 system in seven cases (four false-susceptible and three false-resistant results for the M960 system), while it confirmed the result of M960 system in one case (one false-resistant result for the B460 system). This last case was retested twice with B460 system, and both results were susceptible.

The very major errors (VME), or false-susceptible results, and major errors (ME), or false-resistant results, of both methods are reported in Table 4. These parameters were computed by using the results of the agar proportion method for SIRE and the results of test repetition with the B460 method for PZA, which were considered the "gold standards."

The time to report results for SIRE ranged from 5.1 to 12.4 days (median, 8.3 days) for the M960 system and from 4 to 13 days (median, 8.2 days) for the B460 system (Table 5). The time to report results for PZA ranged from 4.2 to 19.3 days (median, 8.2 days) for the M960 system and from 4 to 20 days (median, 7.4 days) for the B460 system. The turnaround time

TABLE 3. Resolution of discrepant SIRE and PZA results by the proportion method on solid 7H11 medium and by repetition of the test with the B460 method (reference method), respectively

Drug (μg/ml)	No. of tests with the following results[a]:			
	R by M960, S by B460, R by 7H11	R by M960, S by B460, S by 7H11	S by M960, R by B460, R by 7H11	S by M960, R by B460, S by 7H11
INH (0.1)	1	2		
INH (0.4)	2	3		
RMB (0.1)			1	
EMB (5.0)			3	
EMB (7.5)	1	1		
SM (1.0)		2	4	1
SM (4.0)		2		3
Total	4	10	8	4
PZA (100)		3	4	1

[a] S, susceptible; R, resistant.

TABLE 4. VME and ME of both methods for SIRE and PZA susceptibility testing

Drug (μg/ml)	M960		B460	
	VME	ME	VME	ME
INH (0.1)		2	1	
INH (0.4)		3	2	
RMB (1.0)	1			
EMB (5.0)	3			
EMB (7.5)		1	1	
SM (1.0)	4	2		1
SM (4.0)		2		3
Total	8	10	4	4
PZA (100)	4	3		1

for PZA-susceptible strains ranged from 4.4 to 16.9 days (median, 7.5 days) for the M960 system and from 4 to 12 days (median, 7.0 days) for the B460 system. The turnaround time for PZA-resistant strains ranged from 4.2 to 19.3 days (median, 9.8 days) for M960 system and from 4 to 20 days (median, 8.1 days) for the B460 system (Table 5).

DISCUSSION

The aim of this study was to evaluate the performance of the fully automated M960 system for susceptibility testing of *M. tuberculosis* strains against SIRE and PZA and to compare it to that of the semiautomated B460 system. The use of radioactive materials, with the need for disposal of radioactive waste, represented the major disadvantage of the B460 system, and therefore the need for improvements in this field has motivated the development of other liquid medium systems. The radiometric B460 system remains, however, the benchmark against which new systems need to be measured.

The objective of a laboratory's QC program is to evaluate the precision and accuracy of test procedures, monitor reagent performance, and evaluate the proficiency of personnel performing tests. This is particularly important because the clinical mycobacteriology laboratory is responsible for providing accurate and reliable information that is necessary for management of the patient's therapy. A critical element of QC is the selection and use of reference strains that are genetically stable and for which susceptible or resistant results are well documented. We have used the fully susceptible *M. tuberculosis* H37Rv (ATCC 27294) strain for quality control of susceptibility testing, as recommended by NCCLS. Furthermore, when testing both the critical concentration and a higher concentration of a drug, a strain of *M. tuberculosis* that consistently demonstrates resistance to the low concentration but is susceptible to the higher concentration should be an ideal reference strain for quality control. However, at present, reference strains that perform optimally for this purpose are not available. Alternatively, in-house isolates with the same characteristics may be used for QC programs, but for safety considerations, the use of multiple-drug-resistant strains is not recommended (6). In the absence of these possibilities, for QC testing we have used strains of *M. tuberculosis* (American Type Culture Collection strains) that are resistant to INH, RMP, ETB, and PZA; however, these strains are resistant to high concentrations of the respective drugs and are not ideal for QC testing. Reference strains should be tested with each new lot of drugs, media, and medium components (e.g., growth supplement). In addition, QC tests should be performed at least once a week in laboratories that perform tests daily or weekly or when a patient isolate is tested if tests are performed less frequently. Participation in an external proficiency testing program that periodically includes *M. tuberculosis* strains with low-level resistance to INH and resistances to the other antituberculous drugs is strongly recommended.

With the adoption of a new test method, the laboratories should validate test results by performing the current test method and the new method in parallel for a series of patient isolates; furthermore, after this first step in validation, it should be important to check test results for several months by confirming results with selected isolates by another method, if available, or in a referral laboratory. The choice of selected isolates should include both susceptible and resistant *M. tuberculosis*, permitting checking for the presence of VME and ME. False-susceptible results are considered a serious problem, because they can result in treatment failure in the patient.

Mycobacterial purity checks of suspensions used for SIRE and PZA susceptibility tests are important to ensure that the test is performed with a pure culture of a single mycobacterial species. When the test is performed in liquid medium, if the strain is resistant to any of the drugs tested, a mycobacterial purity check from at least one of the vials showing an unexpected drug resistance is recommended (6). This approach permits checking for any possible contamination of the broths during the inoculation procedure, avoiding possible false-resistant results (ME). We have observed that bacterial contamination could occur in only one tube during the inoculation procedure, particularly when working with MGIT tubes, which use screw cups instead of rubber septa as with the B460. When more than one tube in the set showed resistant result, the different turbidities of the broths (contaminated broth and broth with growth of *M. tuberculosis*) could help to identify a possible contaminated tube, but for safety's sake, in this study we preferred to check all broths showing drug resistance.

In our evaluation, for SIRE drugs, the overall agreement between the results obtained with the M960 and B460 systems was 96.3% upon initial testing. Of the 26 discordant results, 8 results were in agreement with the M960 system and 18 results were in agreement with the B460 system when these strains were tested by the agar proportion method on 7H11 medium.

TABLE 5. Turnaround time to susceptibility results for SIRE and PZA tests

Drug	Strains[a] (n)	Turnaround time (days)				P (paired t test)
		M960		B460		
		Mean	Range	Mean	Range	
SIRE	Total (100)	8.3	5.1–12.4	8.2	4–13	0.319[b]
	S (44)	7.8	5.4–12.3	7.6	5–12	0.280[b]
	R (56)	8.7	5.1–12.4	8.6	4–13	0.433[b]
PZA	Total (100)	8.2	4.2–19.3	7.4	4–20	0.016
	S (69)	7.5	4.4–16.9	7.0	4–12	0.122[b]
	R (31)	9.8	4.2–19.3	8.1	4–20	0.026

[a] S, susceptible; R, resistant.
[b] Not statistically significant.

The M960 system showed 8 VME, or false-susceptible results, and 10 ME, or false-resistant results, while the B460 system showed 4 VME and 4 ME. No statistically significant difference between the two methods was found for any of the four drugs ($P > 0.05$).

Of the eight VME observed with the M960 system, one was with RMP, four were with SM at the critical concentration, and three were with EMB at the critical concentration. These last seven false-susceptible results were susceptible at the higher concentration with both systems, and the strains were considered partially or moderately resistant to these drugs (low-level-resistant strains).

Of the four VME observed with the B460 system, only one was reported with INH at the critical concentration (at the higher concentration the strains were susceptible), while the others were observed with INH in two cases and with EMB in one case at the higher concentrations. Four studies comparing the automated M960 system with the B460 system for susceptibility testing to *M. tuberculosis* has been published so far. Our results do not confirm the findings of Bemer et al. (3) and Ardito et al. (2), who did not report VME with the M960 system. Our results instead are similar to those of Tortoli et al. (13) and of Adjers-Koskela and Katila (1), as well as to those in other studies published in the past few years which compared the manual MGIT or other available automated systems (MB/BacT system [Organon Teknika, Turnhout, Belgium] and ESP culture system II [Accumed International, Westlake, Ohio]) with the B460 system (4, 8, 9).

In our study we observed 10 ME with the M960 system, i.e., 4 false-resistant results at the critical concentration (2 with INH and 2 with SM) and six at the high concentration (three with INH, one with EMB, and two with SM). These later false-resistant results were resistant at the critical concentration with both the systems (low-level-resistant strains). The four ME observed with the B460 system were all with SM, one at the critical concentration and three at the higher concentration; these three false-resistant results were resistant at the critical concentration with both systems (low-level-resistant strains).

Evaluating the results of the combination of low and high drug concentrations, no cases were found in which the same drug was fully susceptible with one system and fully resistant with the other.

M. tuberculosis strains found to be EMB resistant by both methods were always resistant to INH and less frequently resistant to other first-line drugs, thereby confirming the considerations reported in the M24-A document recently published by NCCLS (6).

Both systems showed excellent performance, but more VME were observed with the M960 system at the critical concentrations of EMB and SM. Similar problems with EMB and SM susceptibility testing were previously reported (1). It must be pointed out that most of the discrepant results observed with both methods in this study are related to strains with a low level of resistance that are difficult to correctly classify because they are represented by different rates of susceptible and resistant mycobacterial subpopulations. Better concordance of results obtained from the different methods could be achieved by adjusting the drug concentrations according to the concentrations used in the conventional agar proportion method, always supposing that this method could safely be used as a gold standard for the *M. tuberculosis* strains with borderline drug susceptibility results; further studies will be required to validate this approach.

The mean times required to obtain susceptibility results for the two methods were found to be very close, and no statistically significant difference was found between susceptible and resistant strains. These results confirm those reported by Bemer et al. (3) and Ardito et al. (2), but they contrast with those of Tortoli et al. (13), who obtained the susceptibility test results, on average, 2.5 days earlier with the B460 system (a statistically significant difference). They also disagree with those of Adjers-Koskela and Katila (1), who obtained the susceptibility test results, on average, 2.3 days earlier with the M960 system.

In our evaluation for PZA, the overall agreement between the results obtained with the M960 and the B460 systems was 92% upon initial testing. When these strains were retested with the B460 system (reference method), the M960 system showed four VME and three ME. The B460 system showed only one ME, and no statistically significant difference between the two methods was found ($P > 0.05$). These results do not confirm those of the only previous study, by Pfyffer et al. (7), who observed a tendency of the M960 method to generate ME rather than VME.

We believe that these initial discrepant results may be related to the recommended method for the MGIT inoculation procedure, which is too sensitive to the nonhomogeneous distribution of the *M. tuberculosis* strains in the liquid medium. Three differences between the M960 and B460 procedures must be highlighted: (i) for the M960 method, it is necessary to use a pipette, which can collect large clumps more easily than a fine needle of a tuberculin syringe; (ii) for M960 method, it is necessary to take the suspensions for the inoculation procedure at two different times and probably at two different depths from a positive tube, instead of only at one time (B460 system); and (iii) for the M960 method, it is necessary to dilute 1:5 the suspension for the PZA control tube, which is not required for the B460 procedure. These differences can probably explain most of the ME and VME initially observed.

In contrast with the results for the SIRE, we have found a statistically significant difference in the mean time required to obtain PZA susceptibility results with the two methods. A shorter median time was observed with the B460 system, particularly when testing resistant strains (1.7 days), while no statistically significant difference was found for susceptible strains. According to the manufacturer's procedure, an M960 tube should be used for the preparation of the susceptibility test inoculum on the day after it first becomes positive on the M960 instrument, while the reporting of the complete susceptibility testing results of the M960 system was further extended, on average, for about 1.5 days for PZA-resistant strains (8.3 days for overall SIRE results and 9.8 days for PZA-resistant results). Vials flagged positive by the B460 instrument require 2 days or more to reach the correct growth index for the preparation of the susceptibility test inoculum, and these additional days must be added to the mean time necessary to perform susceptibility testing with the B460 system. With these considerations in mind, the M960 system appears on the whole to be slightly more rapid than the B460 system.

A high rate of contamination of the M960 system was pre-

viously reported (13), and this was observed both with the M960 tubes and with the M960 PZA tubes. It is reasonable to suppose that the use of screw caps instead of rubber septa and the inoculation procedures may be responsible for this problem. This complication forced us to perform an expensive repetition of the tests, with a delay in the reporting of the results to the clinician.

The fully automated nonradiometric M960 system is less labor-intensive than the B460 system because tubes are placed in the M960 instrument only once, whereas B460 vials are incubated off line in an incubator and manually loaded and unloaded every day during the total incubation period.

In conclusion, this study has demonstrated that the M960 system performs as well as the B460 system for testing the susceptibility of *M. tuberculosis* to SIRE and PZA and that it appears to be an accurate and suitable replacement for the radiometric B460 method. In our opinion, better performance of the M960 system could be obtained by improving the inoculation procedures, particularly for PZA, and resolving the problem of frequent contamination of the M960 broths.

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