Drug-resistant strains of *Mycobacterium tuberculosis*, though not a novel phenomenon, are emerging worldwide. According to the latest figures of the World Health Organization and the International Union against Tuberculosis and Lung Diseases, drug resistance, in particular acquired resistance, has poured additional fuel on the fire of global tuberculosis (TB) (18). Several outbreaks of multidrug-resistant TB (7) were characterized by delayed diagnoses, inadequate treatment regimens, high rates of mortality, and significant rates of transmission and have taught us two lessons: first, the days are definitely gone where full susceptibility of TB bacilli to front-line drugs can be taken for granted. Second, rapid detection of drug resistance is paramount, not only for effective treatment of TB patients but also for initiating adequate public health measures.

In the quest for new nonradiometric, culture-based strategies which allow both rapid detection of acid-fast bacilli and testing of susceptibility to antimicrobial agents, new liquid medium-based systems, such as the MB/BacT (Organon-Teknika, Durham, N.C.), ESP Culture System II (AccuMed International, Westlake, Ohio), MB Redox (Biotest, Dreieich, Germany), and the Mycobacteria Growth Indicator Tube 960 (MGIT 960, Becton Dickinson Microbiology Systems, Sparks, Md.), have become available. They all aim not only at recovering mycobacteria from clinical specimens but also at generating antimicrobial susceptibility testing (AST) data with a shorter turnaround time than that observed with the current “gold standard,” the agar proportion method (11). The performance of a new system should be comparable with that of the BACTEC 460 TB system, with elimination of the two core problems associated with the old BACTEC 460 TB technology, i.e., the risk of needle punctures and disposal of radioactive waste. Preliminary studies utilizing those new systems report good overall agreement of AST results with those generated with established methods (1, 3–5, 8, 10, 12–13, 15).

Recent automation of the MGIT 960 technology was another step forward, as it allows continuous monitoring of positive fluorescence, which is based on bacterial growth. It is noninvasive and eliminates potential reading difficulties during visual judging of the tubes, apart from saving labor. The threshold algorithms help in determining the susceptibility automatically.

In this multicenter study we have evaluated the reproducibility and reliability of the BACTEC MGIT 960 instrument for testing of *M. tuberculosis* susceptibility to isoniazid (INH), rifampin (RIF), ethambutol (EMB), and streptomycin (STR) and have compared the results to those obtained by the radiometric procedure. Discordant results were resolved by testing the strains with the agar proportion method using Löwenstein-Jensen (LJ) medium (6). This was done by an additional site which thus acted as an independent arbiter. Last, in order to address safety, we performed drug susceptibility testing in plastic MGITs, in addition to the glass tubes.
MATERIALS AND METHODS

Evaluation sites. Susceptibility testing results were generated by two mycobacteriology laboratories, the Mycobacteriology Laboratory, University of Nantes, Nantes, France (center 1), and the Swiss National Center for Mycobacteriology, Department of Medical Microbiology, University of Zurich, Zurich, Switzerland (center 2). A third laboratory, the National Reference Center for Mycobacteriology, Research Center, Borstel, Germany (center 3), acted as an arbiter site for the resolution of discrepant results.

Strains. A total of 110 M. tuberculosis strains were evaluated in this study. A total of 64 strains were fresh clinical isolates grown in MGIT (41 and 23 from centers 1 and 2, respectively). Another 46 strains (44 from center 1 and 2 from center 2) were selected from the culture collections in Nantes and Zurich, respectively. These strains were grown on LJ medium prior to inoculation to the MGIT medium. Accuprobe culture confirmation kits (GenProbe, San Diego, Calif.) and biochemical methods were used for identification.

Preparation of inoculum. For strains grown in the MGIT medium and incubated at 37°C in the BACTEC MGIT 960 instrument with ambient air, each culture was used for susceptibility testing within 1 to 5 days after the instrument flagged a positive signal. On days 1 and 2 following positivity, cell suspensions were used undiluted; on days 3 through 5, suspensions were diluted 1:5 with sterile saline. Tubes which had been positive for more than 5 days had first to be subcultured again into a new MGIT medium. As for strains initially grown on LJ medium and incubated at 37°C in ambient air, colonies no older than 14 days were suspended in 4 ml of Middlebrook 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). Growth control (GC) and drug-containing MGITs (see below) were inoculated with 0.5 ml.

MGIT GC. One hundred microliters of a positive MGIT 960 broth was pipetted into 10 ml of sterile saline to prepare a 1:100 dilution of the growth suspension for the GC tube. Half a milliliter of the diluted suspension was inoculated into an MGIT without drug.

Drug solutions. For drug susceptibility testing using the BACTEC MGIT 960 system, 4 ml of sterile distilled water was added to a lyophilized vial of the drug in question (stock solution). Part of the stock solution (0.1 ml) was added to an MGIT. The final critical concentrations were 0.1 µg/ml for INH, 1.0 µg/ml for RIF, 5.0 µg/ml for EMB, and 1.0 µg/ml for STR. For testing at the higher drug concentrations (0.4 µg/ml for INH, 7.5 µg/ml for EMB, and 4.0 µg/ml for STR), 2 ml of sterile distilled water was added to the lyophilized vial of the respective higher-concentration drug vial, and 0.1 ml was added to an MGIT. Drug susceptibility testing in the BACTEC MGIT 960 system, final drug concentrations were 0.1 µg/ml for INH, 2.0 µg/ml for RIF, 2.5 µg/ml for EMB, and 2.0 µg/ml for STR. Only those strains which showed a resistance to one or more drugs at the critical drug concentrations were tested at the higher concentrations in BACTEC MGIT 960. For EMB (0.4 µg/ml for INH, 7.5 µg/ml for EMB, and 6.0 µg/ml for STR).

Drug susceptibility testing. (i) BACTEC MGIT 900 system. BACTEC MGIT 960 drug susceptibility testing supplement (0.8 ml) (oleic acid-albumin-dextrose-catalase), 100 µl of the drug stock solution, and 0.5 ml of the suspension containing M. tuberculosis were added to an MGIT. The GC did not contain any drugs. Drug susceptibility testing sets were entered into the BACTEC MGIT 960 instrument and continuously monitored until a susceptible or resistant result was obtained. The drug susceptibility testing sets were reported by the instrument (determined by the software algorithms, once the GC became positive). Drug susceptibility testing was done in glass MGITs; center 2 used, in addition, plastic MGITs for 10 AST sets.

(ii) BACTEC 460 TB system. Half a milliliter of a positive MGIT 960 sample was inoculated into a 12B vial and was inoculated till the growth index was ≥ 500. Drug susceptibility testing was done following the standard procedure (S. H. Siddiqi, product and procedure manual, revision D, for BACTEC 460 TB system, Becton Dickinson Microbiology Systems, Sparks, Md.). Organisms initially grown on solid medium were inoculated in 12B vials and were tested as soon as the growth index was ≥ 500.

Reproducibility testing. Prior to testing clinical strains, a blinded panel of 10 strains of M. tuberculosis were sent to each center for reproducibility testing with the BACTEC MGIT 960 system by Becton Dickinson. Expected results had been generated by Becton Dickinson with the reference method (BACTEC 460 TB system, center 1 tested the 10 strains in duplicate at three cycles [thus, six replicates per strain]). Center 2 did reproducibility testing in triplicate at three cycles (thus, nine replicates per strain). Center 3 tested the 10 strains in duplicate (thus, two replicates per strain).

Quality control. Reference strains of M. tuberculosis (ATCC 27294 and ATCC 35822) were used as a batch quality control on a weekly basis.

| Drug (concn in µg/ml) | No. of tests | S by both tests | R by MGIT 960; S by 460 TB | S by MGIT 960; R by 460 TB | R by both tests | Agreement (%) |
|-----------------------|--------------|----------------|--------------------------|--------------------------|----------------|---------------|
| INH (0.1)             | 110          | 81             | 25                       | 96.4                     |
| INH (0.4)             | 29           | 5              | 21                       | 89.7                     |
| RIF (1.0)             | 110          | 92             | 17                       | 99.1                     |
| EMB (5.0)             | 110          | 93             | 13                       | 96.4                     |
| EMB (7.5)             | 17           | 7              | 7                        | 82.4                     |
| STR (1.0)             | 110          | 76             | 25                       | 91.8                     |
| STR (4.0)             | 34           | 12             | 12                       | 70.6                     |
| Total of tests        | 520          | 366            | 120                      | 93.5                     |

* S, susceptible; R, resistant.
in liquid media and tested at critical concentrations and from 89.8 to 98% at the higher concentrations.

Turnaround times for AST ranged from 4.6 to 11.7 days (median, 6.5 days) for BACTEC MGIT 960 and from 4.0 to 10.0 days (median, 7.0 days) for BACTEC 460 TB (Table 5). There was no significant difference between center 1 and center 2 for BACTEC MGIT 960. Turnaround times for resistant strains ranged from 5.0 to 10.9 days (median, 6.3 days) for BACTEC MGIT 960 and from 5.0 to 10.0 days (median, 7.0 days) for BACTEC 460 TB.

**TABLE 3. Resolution of discrepant results by proportion method on solid LJ medium**

| Drug (concn in μg/ml) | Initial results for: | Resolved results<sup>a</sup> for: |
|-----------------------|----------------------|----------------------------------|
|                       | R by MGIT 960; S by 460 TB | S by MGIT 960 and PM (true resistant) |
| INH (0.1)             | 4                    | 1                                |
| INH (0.4)             | 3                    | 1                                |
| RIF (1.0)             | 1                    | 1                                |
| EMB (5.0)             | 3                    | 1                                |
| STR (1.0)             | 9                    | 1                                |
| STR (4.0)             | 10                   | 1                                |
| Total of tests        | 33                   | 1                                |

<sup>a</sup> Susceptible; R, resistant; PM, proportion method.

<sup>b</sup> Arbiter results based on the proportion method.

**DISCUSSION**

The purpose of this multicenter study was to evaluate the reliability of the newly introduced BACTEC MGIT 960 system for testing the susceptibility of *M. tuberculosis* to the three front-line drugs (INH, RIF, and EMB) and STR. We have compared the results to those obtained by the radiometric BACTEC 460 TB system. Most previous evaluations of newer AST systems have not included reproducibility testing (3, 4). In this study, excellent agreement was obtained for all four drugs at both concentrations and, thus, assured quality of the results.

Initial susceptibility testing yielded an overall agreement of 93.5%. There was a very good correlation for each of the drugs at the critical concentrations. After the 34 discrepant cases were retested by an independent arbiter site utilizing the proportion method, there were 22 major errors (ME) but no very ME (VME) by the BACTEC MGIT 960.

In the past few years, most of the studies comparing new systems with the agar proportion method or the BACTEC 460 TB system found discordant results (2–5, 15). When comparing the manual MGIT with the agar proportion method, Walters and Hanna (17) reported three VME of the manual MGIT among 117 strains of *M. tuberculosis* (two strains against INH and one against RIF). Similarly, in a large European multicenter study involving 441 strains of *M. tuberculosis*, Rüscher-Gerdes et al. (15) found 11 strains which yielded VME by the manual MGIT (one against INH, three against RIF, five against EMB, and two against STR) when it was compared to the BACTEC 460 TB system. Comparing the fully automated MB/BacT system with the agar proportion method, Diaz-Infantes et al. (5) reported five VME of 83 *M. tuberculosis* strains tested with the MB/BacT System (three strains with EMB and two with STR). By using the same system, Brunello and Fontana (4) found two VME out of 120 *M. tuberculosis* strains tested against INH, when it was compared with BACTEC 460 TB and the agar proportion method. Bergmann and Woods (3)
found three VME out of 20 \textit{M. tuberculosis} strains tested with the ESP Culture System II (two strains against INH and one against STR) when it was compared with the proportion method. The absence of VME in our study indicates that the fully automated MGIT 960 system is reliable in detecting true-resistant strains. Nevertheless, additional studies are required to confirm our preliminary results.

False resistance, in turn, is considered an ME, as it indicates a drug to be not effective for treatment, even though in reality, the drug could be successfully used. In our study there were only four discordant results at the low concentration and three at the high concentration of INH. One strain was confirmed resistant by the arbiter at both concentrations. This strain was multidrug resistant and was missed by the BACTEC 460 TB system. The two discordant results at the higher concentration of INH were resistant at the critical concentration with both systems and should be considered low-level resistant strains.

Out of the 19 discrepancies observed with STR, seven were found true resistant by the arbiter and eight were false resistant at the critical concentration with the MGIT 960 system. Overall sensitivity for STR was 100\%, and its specificity was the lowest of all drugs. There were seven VME of the BACTEC 460 TB when its results were compared to the arbiter results. Among the seven truly resistant strains, six showed a low level of resistance detected by both systems at the critical STR concentration. The moderately resistant strains were the one which gave the most discordant results. Such strains were not always detected by the BACTEC 460 TB system as described by Siddiqi et al. (16).

Among the primary drugs, EMB is considered a difficult drug to be tested that often yields less reproducible results. For the BACTEC 460 TB, Roberts et al. (14) observed a sensitivity value that did not exceed 66\%, when it was compared with the proportion method. In 1994, a quality assurance program for drug susceptibility testing \textit{of M. tuberculosis} was initiated by the World Health Organization in 16 laboratories across the world. The specificity values of EMB (mean, 98\%) were significantly higher than its sensitivity values (mean, 66\% [9]). As a consequence, the sensitivity of EMB leads to underreporting of drug resistance. With the MB/BacT System, Brunello and Fontana (4) utilizing the MB/BacT system (8.5 days). Automation of the MGIT method has thus reduced the median time by two more days (time for manual MGIT, 8.8 days [15]). The shorter median time observed for the BACTEC 460 TB at center 2 (5.0 days) might be due to the daily testing schedule, whereas at center 1, drug susceptibility was not read daily (nonweekend protocol [Siddiqi, manual, Becton Dickinson]). There was no statistically significant difference in reporting time ($P > 0.05$) between susceptible and resistant strains.

In summary, our study demonstrates that the BACTEC MGIT 960 system is a reliable method for testing the susceptibility of \textit{M. tuberculosis}. The overall excellent sensitivity suggests that the BACTEC MGIT 960 system is more efficient than the BACTEC 460 TB system in detecting true-resistant strains. Being as rapid as the results of BACTEC 460 TB, our results indicate that this system will easily replace the radiometric system.

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**REFERENCES**

1. Ardito, F., M. Sanguineti, L. Sechi, B. Posteraro, L. Masucci, G. Padda, and S. Zanetti. 2000. Comparison of the Mycobacteria Growth Indicator Tube with radiometric and solid culture for isolation of mycobacteria from clinical specimens and susceptibility testing of \textit{Mycobacterium tuberculosis}. New Microbiol. 23:151–158.
2. Bergmann, J. S., and G. L. Woods. 1997. Mycobacterial Growth Indicator Tube for susceptibility testing of \textit{Mycobacterium tuberculosis} to isoniazid and rifampin. Diagn. Microbiol. Infect. Dis. 28:153–156.
3. Bergmann, J. S., and G. L. Woods. 1998. Evaluation of the ESP Culture System II for testing susceptibilities of \textit{Mycobacterium tuberculosis} isolates to four primary antitubercular drugs. J. Clin. Microbiol. 36:2940–2943.
4. Brunello, F., and R. Fontana. 2000. Reliability of the MB/BacT system for testing susceptibility of \textit{Mycobacterium tuberculosis} complex strains to antitubercular drugs. J. Clin. Microbiol. 38:872–873.
5. Diaz-infantes, M. S., M. J. Ruiz-Serrano, L. Martínez-Sánchez, A. Ortega, and E. Bouza. 2000. Evaluation of the MB/BacT Mycobacterium detection system for susceptibility testing of \textit{Mycobacterium tuberculosis}. J. Clin. Microbiol. 38:1988–1989.
6. DIN Deutsches Institut für Normung eV. 1996. Empfindlichkeitsprüfung von Tuberkulosebakterien gegen Chemotherapeutika. Norm 58943-8. Beuth Verlag, Berlin, Germany.
7. Fischl, M. A., R. B. Uttamchandani, G. L. Daikos, R. B. Poblete, J. N. Moreno, R. R. Reyes, et al. 1992. An outbreak of tuberculosis caused by multiple-drug-resistant tubercle bacilli among patients with HIV infection. Ann. Intern. Med. 117:177–183.
8. Goloubeva, V., M. Lecocq, P. Lassowsky, F. Matthys, P. Portaels, and L. Bastian. 2001. Evaluation of \textit{Mycobacteria Growth Indicator Tube} for direct and indirect drug susceptibility testing of \textit{Mycobacterium tuberculosis} from respiratory specimens in a Siberian prison hospital. J. Clin. Microbiol. 39:1501–1505.
9. Laszló, A., M. Rahman, M. Raviglione, and F. Bustreo. 1997. Quality assurance programme for drug susceptibility testing of \textit{Mycobacterium tuberculosis} in the WHO/IUATLD Supranational Laboratory Network: first round of proficiency testing. Int. J. Tuberc. Lung Dis. 4:231–238.
10. Macondo, E. A., F. Ba, A. Gaye-Diallo, N. C. Touré-Kane, O. A. Kaire, A. Gueye-Ndiaye, C. S. Boye, and S. Mhoup. 2000. Rapid susceptibility testing of \textit{Mycobacterium tuberculosis} by the \textit{Mycobacteria Growth Indicator Tube} (MGIT AST SIRE). Clin. Microbiol. Infect. 6:363–367.
11. National Committee on Clinical and Laboratory Standards. 2000. Susceptibility testing of \textit{Mycobacteria}, \textit{Nocardia}, and other aerobic Actinomycetes. Tentative standard, 2nd ed. M24-T2. NCCLS, Wayne, PA.
12. Palaci, M., S. Y. Ueki, D. N. Sato, M. A. Da Silva Telles, M. Curcio, and E. A. Silva. 1996. Evaluation of \textit{Mycobacteria Growth Indicator Tube} for recovery and drug susceptibility testing of \textit{Mycobacterium tuberculosis} isolates from respiratory specimens. J. Clin. Microbiol. 34:762–764.
13. Palomino, J. C., H. Traore, K. Fissette, and F. Poertaels. 1999. Evaluation of \textit{Mycobacteria Growth Indicator Tube} (MGIT) for drug susceptibility testing
of *Mycobacterium tuberculosis*. Int. J. Tuberc. Lung. Dis. 3:344–348.

14. Roberts, G. D., N. L. Goodman, L. Heifets, H. W. Larsh, T. H. Lindner, J. K. McClatchy, M. R. McGinnis, S. H. Siddiqi, and P. Wright. 1983. Evaluation of the BACTEC radiometric method for recovery of mycobacteria and drug susceptibility testing of *Mycobacterium tuberculosis* from acid-fast smear-positive specimens. J. Clin. Microbiol. 18:689–696.

15. Rüsch-Gerdes, S., C. Domehl, G. Nardi, M. R. Gismondo, H. M. Welscher, and G. E. Pfifer. 1999. Multicenter evaluation of the Mycobacteria Growth Indicator Tube for testing susceptibility of *Mycobacterium tuberculosis* to first-line drugs. J. Clin. Microbiol. 37:45–48.

16. Siddiqi, S. H., J. P. Libonati, and G. Middlebrook. 1981. Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*. J. Clin. Microbiol. 13:908–912.

17. Walters, S. B., and B. A. Hanna. 1996. Testing of susceptibility of *Mycobacterium tuberculosis* to isoniazid and rifampin by mycobacterium growth indicator tube method. J. Clin. Microbiol. 34:1565–1567.

18. World Health Organization. 1997. Anti-tuberculosis drug resistance in the world. The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance, 1994–1997. WHO/TB/97.229. World Health Organization, Geneva, Switzerland.