JOURNAL OF CLINICAL MICROBIOLOGY, June 1996, p. 1565–1567 Vol. 34, No. 6
0095-1137/96/$04.00+0


# Testing of Susceptibility of *Mycobacterium tuberculosis* to Isoniazid and Rifampin by Mycobacterium Growth Indicator Tube Method

SHAUN B. WALTERS[1] AND BRUCE A. HANNA[1,2]*

*Department of Pathology, New York University School of Medicine,[1] and Bellevue Hospital Center,[2] New York, New York 10016*

Received 28 November 1995/Returned for modification 9 January 1996/Accepted 4 March 1996

**We tested isolates of *Mycobacterium tuberculosis* recovered from 117 patients for their susceptibilities to isoniazid (INH) and rifampin (RIF) by the Centers for Disease Control and Prevention's disk modification of the indirect method of proportions (MOP) test and a three-tube mycobacteria growth indicator tube (MGIT; BBL) antimycobacterial susceptibility test (AST). Sixty-seven of the *M. tuberculosis* isolates were recovered from Lowenstein-Jensen (BBL) subcultures, and 50 of the isolates were recovered from MGIT cultures of samples from various body sites. For the MGIT AST method, 0.5 ml of test organism suspension was inoculated into an MGIT with 0.1 μg of INH per ml, an MGIT with 1.0 μg of RIF per ml, and growth control MGIT. The tubes were incubated at 37°C and were examined daily. The MGIT AST results were interpreted as follows: susceptible if the tubes containing INH or RIF did not fluoresce within 2 days of the time that the positive growth control fluoresced and resistant if the tubes containing INH or RIF did fluoresce within 2 days of the time that the positive growth control fluoresced. The mean time fluorescence for the positive growth control was 5.5 days. The two methods were in agreement for 114 of the 117 isolates from patients, while for 3 isolates there were minor discordant results.**

The emergence of multidrug-resistant (MDR) *Mycobacterium tuberculosis* in New York City and other urban areas has altered the role of the clinical mycobacteriology laboratory. In general, MDR *M. tuberculosis* isolates are those strains resistant to isoniazid (INH), rifampin (RIF), and possibly, other drugs as well. The rapid detection of MDR *M. tuberculosis* is vital to the efforts to contain the incidence of tuberculosis. Patients with tuberculosis who may be infected with MDR *M. tuberculosis* should be identified as quickly as possible to ensure that appropriate therapy is begun. The current standards for performing susceptibility tests are the Centers for Disease Control and Prevention's method of proportions (MOP) test and the radiometric BACTEC 460TB system (Becton Dickinson Diagnostic Instrument Systems, Inc.). While both tests are reliable and reproducible, the conventional MOP test has the advantage of flexibility, i.e., the ability to test against many second-line drugs, but it is hindered by a laborious procedure and an incubation period of several weeks. In comparison, the BACTEC 460TB system is significantly faster than the conventional MOP test and is able to determine the susceptibilities of *M. tuberculosis* isolates to first-line antibiotics within 4 to 6 days. The BACTEC 460TB system, however, requires radioactive material, which must be disposed of, and lacks a protocol for testing second-line drugs (1).

The recently introduced mycobacterium growth indicator tube (MGIT; BBL) is a rapid detection method for the isolation of mycobacteria from clinical specimens. The MGIT uses a fluorescent indicator embedded in silicone at the bottom of a 16-by-100-mm round-bottom tube filled with an enriched 7H9 (modified Middlebrook) broth base with 0.25% glycerol and a 10% $CO_2$ environment (2, 3, 7). The fluorescent indicator is quenched in the presence of oxygen; however, an actively respiring mycobacterium will use the oxygen available within the tube, thereby exciting the fluorescence reaction. Fluorescence indicating microbial growth can be detected when the MGIT is viewed with a 365-nm UV light from a transilluminator. By adding *M. tuberculosis* to an MGIT containing antituberculosis agents, an antimycobacterial susceptibility test (AST) can be performed by comparing the fluorescence of an antibiotic-containing MGIT with that of a growth control MGIT without antibiotics.

The strains of *M. tuberculosis* chosen for the present study were selected from 117 different patient specimens processed in the Bellevue Hospital Center Mycobacteriology Laboratory from 1993 through 1995. First, 67 stock strains of *M. tuberculosis* stored at −40°C in saline and glycerol were subcultured onto a Lowenstein-Jensen agar slant (BBL). The Lowenstein-Jensen agar slant was used after 3 to 4 weeks of incubation at 37°C in an atmosphere of 7% $CO_2$ to prepare the MGIT AST inoculum by adding a significant portion of the growth from the slant to 4 ml of 7H9 broth in a 16.5-by-128-mm sterile glass tube containing 8- to 10 4-mm-diameter Pyrex beads (Corning, Inc.). After vortexing for 2 to 3 min, the suspension was allowed to sit for 20 min to permit the larger clumps of *M. tuberculosis* to settle. The supernatant was removed and transferred to another sterile glass tube. After 15 min the supernatant from the second tube was transferred to a final sterile glass tube, and its turbidity was adjusted to match the turbidity of a McFarland 0.5 standard by adding 7H9 broth. A 1:5 dilution of the suspension with turbidity equivalent to that of a McFarland 0.5 standard was made in saline and vortexed, and 0.5 ml of this was added to each tube of the MGIT AST (5).

We also evaluated the MGIT AST in a real time frame by performing the susceptibility test with 50 newly positive *M. tuberculosis* MGIT cultures of specimens from a wide variety of body sites, including respiratory sites, lymph node biopsy specimens, and aspirates. All MGIT cultures were screened daily for fluorescence, and on the day of detection, an MGIT with a positive result was examined by using a Kinyoun-stained smear to identify the presence of acid-fast bacilli (AFB). By the time that an MGIT fluoresces, the population density is more than

* Corresponding author. Mailing address: Department of Pathology, NYU Medical Center, Bellevue 4W1, New York, NY 10016. Phone: (212) 263-6444. Fax: (212) 263-8284. Electronic mail address: hannab@is2.nyu.edu.

sufficient for the detection by using smears for AFB and DNA probe analysis and is comparable to that in a BACTEC 12B vial with a growth index of approximately 500 (6). All AFB-positive MGITs were tested by the AccuProbe DNA hybridization probe (Gen-Probe, Inc.) for *M. tuberculosis* and *M. avium* complex within 2 days, and those cultures identified as *M. tuberculosis* were selected for susceptibility testing. In a fashion similar to that for the BACTEC 12B protocol, the MGIT AST was performed within 1 to 3 days of the first identification of *M. tuberculosis* by AccuProbe directly from an MGIT culture (1). After 1 to 2 days of reincubation at 37°C to increase the biological load of *M. tuberculosis* isolates, the positive MGIT was vortexed for 1 min to thoroughly mix the inoculum. A 1:5 dilution of the contents of the vortexed MGIT was made in saline, and 0.5 ml was inoculated into each tube for the MGIT AST.

The MGIT AST contains MGIT (4.0 ml), oleic acid-albumin-dextrose (0.5 ml), inoculum (0.5 ml), and an antibiotic (0.1 ml) dispensed into one of the following three tubes: (i) a growth control MGIT, (ii) an MGIT containing INH at a final concentration of 0.1 μg/ml, and (iii) an MGIT containing RIF at 1.0 μg/ml. To ensure that the proper antibiotic concentrations were achieved in the appropriate MGITs, lyophilized antibiotics from the BACTEC 460TB system, containing 0.02 mg of INH per vial and 0.4 mg of RIF per vial, were used. Stock solutions of INH and RIF at concentrations of 5 and 50 μg/ml, respectively, were obtained by adding 4 and 8 ml of sterile distilled water to the respective vial. The final concentrations of INH and RIF in the MGITs were created by adding 0.1 ml of the stock antibiotic to the appropriate MGIT with a sterile pipette. Unused portions of the antibiotics were stored at −40°C in 0.5- or 1.0-ml aliquots.

The absence of bacterial contamination was verified by inoculating a TSAII blood plate (BBL) with 0.5 ml of the MGIT AST inoculum, and the plate was incubated at 37°C in an atmosphere of 7% $CO_2$. The plates were read daily for 5 days for visible colonial growth, and the result was interpreted as negative if no growth occurred. We did not observe any bacterial contamination of the MGIT AST during the present study. All primary isolates used as inocula were further identified by DNA hybridization with both AccuProbe *M. tuberculosis* and AccuProbe *M. avium* complex systems so that mixed infections could be identified. In addition, mycobacterial contamination or mixed cultures were ruled out by observation of the colonial morphology of *M. tuberculosis* on solid medium that was inoculated for the MOP test.

All tubes in the MGIT ASTs were incubated at 37°C and were read daily for the detection of fluorescence. The growth control tube was identified as positive by observing a visible difference in fluorescence levels compared with that in an uninoculated MGIT. Two days after the growth control was observed to fluoresce the test was terminated and the organisms in all antibiotic-containing MGITs which had not been interpreted as resistant by that time were marked as susceptible. The disk MOP test was performed by standard methods with INH at final concentrations of 0.2 and 1.0 μg/ml and RIF at a final concentration of 1.0 μg/ml (4, 8). The plates were incubated at 37°C with 7% $CO_2$, and the results were interpreted after 3 weeks.

Of the 117 isolates tested by both the MGIT AST and the MOP test, the results for 114 isolates were concordant by both methods and the results for 3 isolates were discordant. Eighty-three isolates were susceptible to both INH and RIF by both methods, and 17 isolates were resistant to INH and RIF by both methods. Eight isolates were noted to be resistant to INH and susceptible to RIF, while six isolates were susceptible to INH but resistant to RIF. Among the three isolates for which discordant results were observed, two were interpreted as being susceptible to INH by MGIT AST and 100% resistant to INH by the MOP test. A third strain was found to be susceptible to RIF by MGIT AST and 35% resistant to RIF by the MOP test. The results are summarized in Table 1.

TABLE 1. Comparison of susceptibilities of 117 *M. tuberculosis* isolates to INH and RIF by MGIT AST and MOP test

| No. of patients | Susceptibility by the indicated test[a] | | | |
|---|---|---|---|---|
| | MGIT AST | | MOP test | |
| | INH (0.1 μg/ml) | RIF (1.0 μg/ml) | INH (0.2 μg/ml) | RIF (1.0 μg/ml) |
| 83 | S | S | S | S |
| 17 | R | R | R | R |
| 8 | R | S | R | S |
| 6 | S | R | S | R |
| 1 | S | S | R | S |
| 1 | S | R | R | R |
| 1 | R | S | R | R |

[a] S, susceptible; R, resistant.

For the 117 *M. tuberculosis* isolates tested by MGIT AST, the mean time to detection of fluorescence in the growth control tube was 5.5 days, with a range of 3 to 13 days. Although our 2-day rule for determining the test outcome resulted in a mean time of 7.5 days to determine susceptible strains, the fluorescence of 94% of the isolates (32 of 34) that were resistant to INH or RIF was detected by the MGIT AST on the same day that the fluorescence of the growth control was detected. Among the 50 real-time tests performed, the growth control was interpreted as positive as early as 4 days and as late as 10 days after inoculation. The time to fluorescence of the growth control appeared to be related to the period of incubation allowed between performing the Kinyoun staining and Gen-Probe identification and inoculating the tube for the MGIT AST. This interval ranged from 1 to 2 days to replenish the organisms that were removed and to allow the fluorescence levels in the MGITs to return to the values originally observed. All of the MDR *M. tuberculosis* strains in the tubes containing either INH or RIF were detected within 1 day that growth in the growth control MGIT was detected, and 86% of the tubes (six of seven tubes) were positive on the same day that growth in the growth control tube was detected. When compared with the 3 weeks required to complete the MOP test, this is a considerable improvement. Our results suggest that the MGIT AST has the accuracy of the MOP test and the speed of the BACTEC 460TB system. Studies currently under way with streptomycin, ethambutol, and pyrazinamide indicate that the MGIT AST may be a suitable alternative to these methods, particularly in clinical microbiology laboratories which do not serve as mycobacteriology reference laboratories.

We thank Stanley Bonk, Al-Nasir Mussa, and the members of the Bellevue Hospital Center Mycobacteriology Laboratory for their thoughtful comments and assistance.

The study was supported in part by Becton Dickinson & Co.

## REFERENCES

1. **Becton Dickinson Diagnostic Instrument Systems.** 1995. BACTEC TB System. Product and procedure manual, MA-0029. Becton Dickinson Diagnostic Instrument Systems, Towson, Md.
2. **Hanna, B. A.** 1995. Diagnosis of tuberculosis by microbiologic techniques, p. 149–159. *In* W. N. Rom and S. M. Garay (ed.), Tuberculosis. Little, Brown & Co., Boston.

3. **Hanna, B. A., S. B. Walters, S. E. Kodsi, D. T. Stitt, P. M. Tierno, and L. J. Tick.** 1994. Detection of *Mycobacterium tuberculosis* directly from patient specimens with the Mycobacteria Growth Indicator Tube, abstr. C-112, p. 510. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
4. **Kent, P. T., and G. P. Kubica.** 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control, Atlanta, Ga.
5. **Kodsi, S. E., S. B. Walters, D. T. Stitt, and B. A. Hanna.** 1994. Rapid detection of MDRTB from culture using a novel susceptibility system, abstr. C-115, p. 510. *In* Abstracts of the 94th General Meeting of the American Society of Microbiology 1994. American Society for Microbiology, Washington, D.C.
6. **Siddiqi, S. H.** Personal communication.
7. **Stitt, D. T., and S. E. Kodsi.** 1994. A rapid method for the growth and detection of mycobacteria in clinical and stock cultures, abstr. C-114, p. 510. *In* Abstracts of the 94th General Meeting of the American Society of Microbiology 1994. American Society for Microbiology, Washington, D.C.
8. **Wayne, L. G., and I. Krasnow.** 1966. Preparation of tuberculosis susceptibility testing mediums by means of impregnated discs. Am. J. Clin. Pathol. **45:**769–771.