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Evaluation of Mycobacteria Growth Indicator Tube for Recovery and Drug Susceptibility Testing of *Mycobacterium tuberculosis* Isolates from Respiratory Specimens

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The new BBL mycobacteria growth indicator tube (MGIT) was evaluated for its ability to detect mycobacteria directly from patient specimens and to determine the drug susceptibility of *Mycobacterium tuberculosis* isolates. A total of 85 respiratory specimens were tested. Specimens were digested, concentrated, examined microscopically for acid-fast bacilli, and inoculated into MGITs and onto Lowenstein-Jensen slants by standard procedures. The tubes were incubated at 37°C and were examined daily for fluorescence to 365-nm UV light. All 25 specimens smear positive for acid-fast bacilli were tested for drug susceptibility in MGITs containing 1.0 μg of rifampin per ml, 0.1 μg of isoniazid per ml, 2.0 μg of streptomycin per ml, and 2.0 μg of ofloxacin per ml. These results were compared with those obtained by testing the same *M. tuberculosis* isolates by the indirect proportion method at drug concentrations of 4.0 μg of rifampin per ml, 0.2 μg of isoniazid per ml, 2.0 μg of ethambutol per ml, 4.0 μg of streptomycin per ml, and 2.0 μg of ofloxacin per ml. No significant difference in the sensitivity of detection of *M. tuberculosis* isolates was found between the two methods. However, the time to detection was significantly shorter in MGITs. Drug susceptibility test results for *M. tuberculosis* isolates by the two methods demonstrated an excellent correlation. The mean time to reporting of drug susceptibility results was 5 days for MGITs versus 16 days for Lowenstein-Jensen slants. The results of this preliminary study indicate that the MGIT system appears to have potential for routine use in mycobacteriology for both the detection and the drug susceptibility testing of *M. tuberculosis* isolates. However, it is important to emphasize that simple nonautomated equipment should be developed to improve the accuracy of fluorescence detection.

The incidence of mycobacterial infection, particularly tuberculosis, has increased in recent years in developed countries and still remains a serious public health problem in developing countries (1, 13). The reemergence of tuberculosis in conjunction with the increasing number of multi-drug-resistant strains of *Mycobacterium tuberculosis* has increased the need for rapid diagnostic methods.

The technology for the rapid diagnosis of tuberculosis has not advanced as rapidly as necessary to meet the changing needs of the clinical mycobacteriology laboratory. Application of molecular and/or immunologic tools to ease recognition and drug susceptibility evaluation of *M. tuberculosis* directly in clinical specimens has remained an unrealized promise (2, 12). Radiometric systems such as the BACTEC 460 TB system reduce the test time considerably, but they are still labor-intensive and expensive.

A new method has been developed by Becton Dickinson Microbiology Systems for the detection of mycobacteria by using silicon rubber impregnated with a ruthenium metal complex as a fluorescence quenching-based oxygen sensor (BBL mycobacteria growth indicator tube [MGIT]). The first reports of the primary isolation of mycobacteria from clinical and stock cultures and of the detection of *M. tuberculosis* resistance to isoniazid (INH) and rifampin (RIF) demonstrated that the MGIT technique has promise (5, 8, 14). A study of respiratory specimens was therefore initiated to compare the sensitivity and speed of mycobacterial detection by this new method with that by the standard Lowenstein-Jensen (LJ) culture method. In addition, we report preliminary results obtained by using MGITs to test the susceptibilities of *M. tuberculosis* isolates to INH, RIF, streptomycin (SM), ethambutol (EMB), and ofloxacin (Oflo).

MGITs. The tubes used in the study are BBL MGITs (catalog no. 4345111; BBL). The tubes contain 4.0 ml of Middlebrook 7H9 broth with an O_2-sensitive fluorescence sensor to indicate microbial growth. Added to the MGIT prior to use is 0.5 ml of oleic acid-albumin-dextrose (OADC) as a nutritional supplement and 0.1 ml of the PANTA mixture of antimicrobial agents.

Specimens and culture methods. A total of 85 consecutive routine sputum specimens sent to the Instituto Adolfo Lutz (São Paulo State Reference Laboratory) for the diagnosis of mycobacterial infection were included in the analysis. All specimens were decontaminated, digested, and concentrated by the Petroff method (7). From the sediment, 0.2 ml was directly inoculated onto two LJ slants, and smears were prepared for examination by Ziehl-Neelsen acid-fast staining. The remaining sediment was vortexed, and 0.5 ml was inoculated into the MGIT. All media were incubated under nonshaking conditions for up to 8 weeks at 37°C and were examined daily for mycobacterial growth. The MGITs were examined by placing an entire rack of tubes on top of a 365-nm UV transilluminator. Fluorescence of the contents of the MGIT indicating microbial growth was manifested by a bright orange color on the bottom

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TABLE 1. Sensitivity and growth detection times of MGIT and LJ culture systems

Medium	No. (%) positive cultures (n = 26)	Detection time (days)	
		Mean	Range
MGIT	25 (96.1)	12.5	5–30
LJ	25 (96.1)	19.5	14–30

of the tube and at the meniscus. Positive and negative control tubes were used for comparison. The tubes were considered positive if the amount of fluorescence resembled most closely that of the sodium sulfite positive control. The presence of mycobacteria was confirmed by acid-fast microscopy.

Drug susceptibility tests and identification procedures. The 25 specimens which were positive for acid-fast bacilli after cultivation in MGITs were reincubated until turbidity was observed. The mycobacterial suspension was adjusted to a 1.0 McFarland standard by the addition of Middlebrook 7H9 by using a nephelometer. For each sample, six drug-containing MGITs and one control tube without drug were inoculated. Antimycobacterial drugs were adjusted in the MGITs to final concentrations of 1.0 μg/ml for RIF (Sigma), 0.1 μg/ml for INH (Sigma), 2.0 μg/ml for SM (Sigma), 2.0 μg/ml for EMB (Sigma), and 2.0 μg/ml for Oflo (Johnson and Johnson) by using concentrated stock solutions. Middlebrook OADC enrichment (0.5 ml) was added to each tube. Inoculations were performed in the following manner: 0.5 ml of a 1:5 dilution of a 1.0 McFarland standard was placed in drug-containing and control tubes, yielding a final concentration of 2×10^5 CFU/ml. The tubes were incubated at 37°C and were examined daily for fluorescence to 365-nm UV light.

The results were interpreted only after the growth control tube fluoresced. A test result was considered to indicate resistance if the drug-containing tube fluoresced within 2 days of the time that the growth control tube fluoresced. For the susceptible strains, the late emergence of drug-resistant mutants was controlled for by reading MGITs for 6 weeks.

All fresh clinical isolates were also tested for INH, RIF, SM, EMB, and Oflo susceptibility by the indirect proportion method on LJ slants (7) at concentrations of 0.2 μg/ml for INH, 4.0 μg/ml for RIF, 4.0 μg/ml for SM, 2.0 μg/ml for EMB, and 2.0 μg/ml for Oflo. All isolates were identified as *M. tuberculosis* by conventional methods (7, 11) and, in some cases, also by PCR with species-specific primers directed to the IS*6110* repetitive sequence element (4).

Statistical analysis. The rate of agreement of isolation between the two techniques was determined by the kappa statistical method (3). Data analysis of drug susceptibility tests involved a standard 2-by-2 contingency table. The results were interpreted in terms of sensitivity and specificity for each of the drugs used in the test system compared with those for each of the drugs used in the conventional system.

Mycobacteria were recovered from 26 of 85 (30.5%) sputum specimens submitted for mycobacterial culture during the study period. Culture sensitivity and growth detection times in LJ slants and MGITs are presented in Table 1. The kappa coefficient calculation showed an excellent agreement (0.94) between the MGIT and the LJ culture methods. The mean time for the detection of growth in the MGIT was 1 week earlier than that on LJ medium, whereas the presence of contaminants was more frequent in the MGIT system (seven versus four specimens).

Comparative results of drug susceptibility testing are provided in Table 2. The results obtained for the 25 clinical specimens of *M. tuberculosis* by using INH, RIF, SM, EMB, and Oflo were highly satisfactory: all sensitivities, specificities, and predictive values (susceptibility and resistance) were 100%. The length of time required for completion of drug susceptibility testing by the MGIT method was 3 to 14 days (mean, 5 days), whereas it was 15 to 20 days (mean, 16 days) for the proportion method, representing a net gain of 11 days by using MGITs.

TABLE 2. Analysis of susceptible and resistant *M. tuberculosis* isolates by the MGIT and proportion methods

Drug	No. of isolates with following results[a]:			
	Both S (A)	MP S, MGIT R (B)	MP R, MGIT S (C)	Both R (D)
INH	19	0	0	6
RIF	20	0	0	5
EMB	22	0	0	3
SM	23	0	0	2
Oflo	25	0	0	0

[a] S, susceptible; R, resistant; MP, method of proportion. Values were determined as follows: sensitivity = D/D + C, specificity = A/A + B, predictive value (susceptibility) = A/A + C, and predictive value (resistance) = D/D + C.

Our study demonstrated no statistical difference in the sensitivity of detection of *M. tuberculosis* between the MGIT method and the conventional method. However, it confirmed the findings of Stitt et al. (14) and Hanna et al. (6) of the more rapid detection of mycobacterial growth with the MGIT system than with cultures on LJ slants. The MGITs showed no advantage of speed of detection for two smear-negative specimens. Studies should be carried out to determine whether the MGIT system is more sensitive or more rapid in the detection of smear-negative specimens.

The high rate of contamination seen in the present study (4.5% for the cultures on LJ slants and 7.8% for the MGIT system) may have been due to delays in sample processing (in some cases, the sputa arrived at the study laboratory 1 to 3 days after collection).

It is widely known that drug susceptibility testing of *M. tuberculosis* is more rapid in liquid media such as the medium provided with the BACTEC system (9, 10); however, the BACTEC system is costly and requires radioactive material. Kodsi et al. (8) recently reported a comparison of MGIT, the BACTEC system, and the proportion method for RIF and INH susceptibility using 55 *M. tuberculosis* isolates. The comparison of the MGIT system with the BACTEC system suggested that the MGIT system has high degrees of sensitivity and specificity. On the other hand, the sensitivity (64.7%) of RIF-containing MGIT compared with that of the proportion method was lower than the sensitivity found in our study, perhaps because of the lower concentration of RIF (1.0 μg/ml) used in their trial (8).

Our study addressed the application of the MGIT system to the performance of indirect drug susceptibility testing. Our use of three drugs (SM, EMB, and Oflo) not yet studied by MGIT susceptibility testing was successful. The results showed excellent agreement between the MGIT and the proportion methods. Overall, drug resistance could be observed much earlier by the MGIT method (mean, 5 days) than by the proportion method (mean, 16 days). Walters et al. (15) and Kodsi et al. (8) noted a similar rapid detection of INH- and RIF-resistant *M. tuberculosis* strains using this novel susceptibility system.

In conclusion, the present work indicates that the MGIT system has great potential: it is a time-saving method, the results correlate well with those of conventional methods, and

the system is compact, safe, and simple to use and does not require exogenous gas or radioactive elements. However, it is important to emphasize that simple nonautomated and low-cost equipment should be developed to improve the accuracy of fluorescence detection. These advantages would make the use of the MGIT system for the detection of mycobacteria in clinical material and the testing of drug susceptibilities of *M. tuberculosis* isolates suitable for small laboratories, particularly in developing countries where tuberculosis has a higher incidence than in developed countries.

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