

# Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* using the mycobacteria growth indicator tube (MGIT) system

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## Abstract

The emergence of multidrug-resistant strains of *Mycobacterium tuberculosis* has increased the need for rapid drug susceptibility tests, which are needed for adequate patient treatment. The objective of the present study was to evaluate the mycobacteria growth indicator tube (MGIT) system to detect multidrug-resistant *M. tuberculosis* strains. The MGIT system was compared with two standard methods (proportion and resistance ratio methods). One hundred clinical *M. tuberculosis* isolates [25 susceptible to isoniazid (INH) and rifampicin (RIF), 20 resistant to INH, 30 resistant to INH-RIF, and 25 resistant to INH-RIF and other drugs] obtained in the State of São Paulo were tested for INH and RIF susceptibility. Full agreement among the tests was found for all sensitive and all INH-resistant strains. For RIF-resistant strains results among the tests agreed for 53 (96.4%) of 55 isolates. Results were obtained within 6 days (range, 5 to 8 days), 28 days and 12 days when using MGIT, the proportion method and the resistance ratio methods, respectively. The MGIT system presented an overall agreement of 96% when compared with two standard methods. These data show that the MGIT system is rapid, sensitive and efficient for the early detection of multidrug-resistant *M. tuberculosis*.

## Key words

- Multidrug-resistant *Mycobacterium tuberculosis*
- Susceptibility tests
- Mycobacteria growth indicator tube
- Proportion method
- Resistance ratio method
- Multidrug resistance

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## Introduction

Tuberculosis has once again become a reason for concern in almost all parts of the world and especially in developing countries. Due to the shortage of laboratory resources, the diagnosis of tuberculosis in these countries is based on microscopic examination. This practice does not permit the isolation of the agent and the determination of the infectious strain's susceptibility profile. Therefore, the treatment usually begins in

the absence of information concerning drug susceptibility (1).

About 95% of the *Mycobacterium tuberculosis* isolates obtained from new cases of tuberculosis without previous treatment are susceptible to standard antituberculosis drugs. For these cases, treatment with first-line drugs usually leads to cure (2). However, in the presence of drug resistance, susceptibility tests must be done as soon as possible to allow the physician to control the dissemination of multidrug-resistant *M. tuberculosis*.

Therefore, the emergence of multidrug-resistant tuberculosis has increased the need for rapid drug susceptibility tests. The methods currently available use solid media (proportion, resistance ratio, and absolute concentration) and the radiometric BACTEC 460TB system (Becton-Dickinson Microbiology System, Sparks, MD, USA) (3-5). Methods that use solid media take 3 to 4 weeks to produce conclusive results, while the BACTEC 460TB system, which was the first method based on a liquid medium, provides results more rapidly. The system, however, is radiometric and due to its radioactive nature requires special equipment and radioactivity safety measures.

More recently, a new mycobacteria growth indicator tube (MGIT, Becton-Dickinson) was developed as an alternative, non-radiometric method for the detection of mycobacteria using a fluorescent oxygen-quenched sensor embedded in silicone at the bottom of tubes (6-10). The MGIT system is read manually, requiring only a UV lamp, and the tubes are easily inoculated. Preliminary studies of drug susceptibility testing of *M. tuberculosis* strains have shown promising results (11-13).

We have evaluated the use of the MGIT system specifically to detect strains of *M. tuberculosis* resistant to isoniazid (INH) and rifampicin (RIF) in 100 clinical isolates obtained in the State of São Paulo.

## Material and Methods

### Microorganisms

One hundred *M. tuberculosis* clinical isolates with previously known resistance profiles were used: 25 INH- and RIF-susceptible, 20 INH-resistant, and 55 multidrug-resistant strains (30 of them INH-RIF resistant and 25 resistant to INH-RIF and other drugs). The strain was considered to be multidrug resistant when it was resistant to INH and RIF.

### Inoculum preparation

Several colonies from a 3-week-old Lowenstein-Jensen (LJ) slant were subcultured in 4.0 ml of 7H9 broth, and incubated at 37°C for 10 days. After this period of time 2.0 ml of this culture was transferred to a tube and the turbidity adjusted with 7H9 broth to a No. 1 McFarland standard. This suspension was further diluted 1:5 with sterile distilled water.

### MGIT susceptibility testing

Susceptibility testing was performed according to the protocol provided by the manufacturer. The final concentrations of each antibiotic in the test tubes were 0.1 mg/ml INH and 1.0 mg/ml RIF. The reading of the test was started at day 3 after inoculation, using a BACTEC MicroMGIT Reader (Becton-Dickinson). The growth control tube was compared to the positive and negative controls. The day when the growth control tube gave a positive result was considered day 0 for the purpose of interpretation of the drug-containing tubes; if the growth control tubes remained negative, the reading was continued until day 12 after inoculation. On the day the growth control tube became positive, the drug-containing tubes were read and interpreted according to manufacturer recommendations. A strain was considered to be susceptible if the drug-containing tube did not fluoresce within two days of the date of the positivity of the growth control, and resistant if the drug-containing tube was positive within 2 days of the date of the positivity of the growth control.

### Proportion and resistance ratio methods

All the strains were also tested by two gold standard methods, i.e., the proportion and resistance ratio methods in LJ medium, based on standard procedures (14,15). The drug concentrations used were 0.2 µg/ml

INH and 40.0 µg/ml RIF for the proportion method, and 0.5 to 0.2 µg/ml INH and 2.5 to 10 µg/ml RIF for the resistance ratio method. We considered the strains to be resistant or sensitive to INH and RIF without considering resistance to other drugs that were not tested in the present study.

**Results and Discussion**

The MGIT method detected INH- and RIF-resistant strains, despite their different resistance patterns. Total agreement was found for the sensitive strains between the two standard methods and MGIT (Table 1). Among the 20 strains resistant only to INH, the MGIT method showed complete agreement, whereas there was disagreement between the two standard methods: one strain was found to be resistant to INH and RIF by the resistance ratio method and two strains were resistant to INH and RIF by the proportion method. Among the 55 strains resistant to INH and RIF there was disagreement between the standard methods and MGIT for two strains, which were sensitive to RIF by MGIT. Another strain resistant to both INH and RIF by the method originally used to identify it, was sensitive to INH by the resistance ratio method.

Table 2 shows a comparison of the results of the MGIT versus standard methods for INH-sensitive and INH-resistant strains and Table 3 shows a similar comparison of the results of the MGIT versus standard methods for RIF-sensitive and RIF-resistant strains. Whereas there was complete agreement between the tests with regard to INH sensitivity, discrepant results were obtained for two strains with regard to RIF sensitivity. Two strains identified as RIF resistant by both standard methods were sensitive to RIF by the MGIT method (Table 3).

Therefore, there was a 100% correlation between MGIT and standard methods concerning resistance to INH, while for RIF there was a 98% correlation. In a similar

Table 1. Susceptibility of *Mycobacterium tuberculosis* strains to isoniazid (INH) and rifampicin (RIF) tested by the mycobacteria growth indicator tube (MGIT) and the proportion and resistance ratio methods.

Resistance pattern	Number of strains			
	Previous test	Resistance ratio	Proportion	MGIT
INH + RIF sensitive	25	25	25	25
INH resistant	20	19	18	20
INH + RIF resistant	55	54	55	53

Table 2 - Comparison of results of the mycobacteria growth indicator tube (MGIT) versus standard methods for isoniazid (INH).

MGIT	Standard methods	
	INH resistant	INH sensitive
INH resistant	75	0
INH sensitive	0	25

Sensitivity: 75/75 = 100%; specificity: 25/25 = 100%; positive predictive value: 75/75 = 100%; negative predictive value: 25/25 = 100%; accuracy: 100/100 = 100%.

Table 3. Comparison of results of the mycobacteria growth indicator tube (MGIT) versus standard methods for rifampicin (RIF).

MGIT	Standard methods	
	RIF resistant	RIF sensitive
RIF resistant	54	0
RIF sensitive	2	44

Sensitivity: 54/56 = 96.4%; specificity: 44/44 = 100%; positive predictive value: 54/54 = 100%; negative predictive value: 44/46 = 95.6%; accuracy: 98/100 = 98%.

study by Palomino et al. (16), 100% agreement was found regarding INH, 98% for RIF, 99% for ethambutol and 91% for streptomycin. Similar results have been reported in other studies (11,17).

MGIT susceptibility results are usually obtained within 8 days (range: 5 to 13 days) (11,16,17). This speed is the main advantage

of the test when compared with standard methods that use solid medium, which are routinely used in developing countries.

The recommended treatment for tuberculosis includes a combination of RIF and INH; therefore, *M. tuberculosis* strains resistant to both of these drugs are designated multidrug resistant. The emergence of multidrug-resistant tuberculosis represents a major threat to the control of tuberculosis, and it has been shown that the major cause of multidrug resistance is the use of poor tuberculosis control strategies (18). As part of a tuberculosis control program, it is very important to monitor drug sensitivity patterns in the community and individual patients with chronic tuberculosis after treatment failure. Understanding drug resistance patterns in a community is also of great epidemiological

significance since it provides indicators of the existence and prevalence of primary and acquired drug resistance, essential to evaluate the quality of the tuberculosis control program (19).

The results of the present study indicate that a possible way to overcome the problem of the scarce resources of public health laboratories in developing countries like Brazil would be to adopt the MGIT system as a screening test for multidrug-resistant *M. tuberculosis* strains, thus allowing for rapid identification of patients who need special treatment and isolation conditions.

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