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Evaluation of Mycobacteria Growth Indicator Tube (MGIT) for drug susceptibility testing of *Mycobacterium tuberculosis* isolates from clinical specimens

Clin Microbiol Infect 1999; 5: 227–230

*Pablo Zapata, Miguel Arbeloa and Javier Aznar**

Departmento de Microbiología. Facultad de Medicina, Apdo 914, 41080 Sevilla, Spain

*Tel: +34 95 4557448 Fax: +34 95 4377413 E-mail: jaznar@cica.es

Accepted 20 August 1998

INTRODUCTION

The resurgence of tuberculosis and outbreaks of multidrug-resistant *Mycobacterium tuberculosis* has increased the emphasis on rapid turn-round time for mycobacterial cultures and susceptibility testing for effective treatment and control of the disease [1–3].

The two methods most commonly used in clinical laboratories for susceptibility testing of *M. tuberculosis* are the proportion method performed on Löwenstein-Jensen (LJ) egg medium and the Bactec TB 460 system (Becton Dickinson Microbiology System, Cockeysville, MD, USA) [4]. Because growth of colonies is necessary for interpretation, the agar proportion method requires 3 weeks of incubation. The Bactec TB system uses a broth medium containing radiolabeled palmitic acid substrate. Growth is detected by measuring $^{14}CO_2$ released during substrate utilization. With this method, results can be reported in as few as 4 days, but it is still labor-intensive and expensive, and generates radioactive waste [5].

A new method, the Mycobacterial Growth Indicator Tube (MGIT) (Becton Dickinson Microbiology System, Sparks, MD, USA), uses a fluorescence quenching-based oxygen sensor to detect the growth of mycobacteria. Recently, the MGIT system has been evaluated as a non-radiometric alternative to the Bactec TB system for the rapid growth and detection of mycobacteria [6]. The reliability of the MGIT tubes for susceptibility testing of *M. tuberculosis* isolates to isoniazid and rifampin in comparison to those obtained by the Bactec system has been evaluated [7]. We report the results obtained by using the MGIT system to test the susceptibilities of *M. tuberculosis* clinical isolates to streptomycin, isoniazid, rifampin and ethambutol in comparison to those obtained with the Bactec system.

MATERIALS AND METHODS

Strains

Fifty-two clinical isolates, including 22 strains resistant to one drug and 13 multiresistant, were tested in the MGIT system; the results were compared with those obtained with the radiometric method. Twenty-four strains were obtained from frozen clinical cultures (isolated during 1993–95) and 28 fresh clinical cultures from clinical specimens sent to our laboratory during the first 6 months of 1997. *M. tuberculosis* H37rv, susceptible to all antimicrobials, and four strains of *M. tuberculosis,* ATCC 35838, 35822, 35820 and 35837, resistant to each one of the antimicrobials tested, were included as control strains. All isolates and control

strains were subcultured on LJ slants and incubated for 2–3 weeks prior to testing.

Susceptibility testing

Inoculum preparation

Colonies from LJ slants were transferred to a tube containing Middlebrook 7H9 broth and five to eight sterile glass beads. Tubes were vigorously agitated on a vortex mixer and clumps were allowed to settle for 20 min. The supernatant was transferred to a sterile tube and clumps were again allowed to settle for 15 min. This supernatant was removed and adjusted with Middlebrook 7H9 broth to equal the density of a 0.5 McFarland standard for use as the standard inoculum in the Bactec TB 460 and MGIT systems and adjusted to equal the density of 1.0 McFarland for use as the standard inoculum for the proportion method [7].

MGIT procedure

Susceptibility testing was performed according to the protocol provided by the manufacturer. Tubes were prepared by adding 0.5 mL of MGIT OADC enrichment (Becton Dickinson) and 0.5 mL of a 1:5 dilution (with sterile saline) of the inoculum prepared as described previously. For each sample, four drug-containing MGITs and one control tube without drug were inoculated. Antimycobacterial drugs were adjusted in the MGITs to final concentrations of 0.8 mg/L for streptomycin, 0.1 mg/L for isoniazid, 1 mg/L for rifampin and 3.5 mg/L for ethambutol, as recommended by the manufacturer. The tubes were incubated at 37°C and were examined daily for fluorescence with a 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 growth control tube fluorescing [7].

Bactec TB procedure

Control Bactec 12B vials contained 0.1 mL of a 1:100 dilution of the inoculum. Antimycobacterial drugs were adjusted in the Bactec 12B vials to final concentrations of 2.0 mg/L for streptomycin, 0.1 mg/L for isoniazid, 2 mg/L for rifampin and 2.5 mg/L for ethambutol. Vials were read on the Bactec 460 daily, starting on day 3 after inoculation, until the growth index of the control vial reached 30. An isolate was defined as resistant if the change in the growth index (ΔGI) of the drug-containing vial was greater than the ΔGI of the drug-free control [4].

The differences between mean time (from the day of inoculation) to test results for the MGIT and BACTEC systems were compared by the Student *t*-test.

When results from the MGIT and Bactec systems disagreed, strains were tested by the indirect proportion method on LJ slants (bioMerieux España, SA, Barcelona, Spain) at concentrations of 4 and 10 mg/L for streptomycin, 0.2 and 0.1 mg/L for isoniazid, 20 and 40 mg/L for rifampin and 2 and 3 mg/L for ethambutol [8].

Analysis of the data

The values for sensitivity (ability to detect true resistance), specificity (ability to detect true susceptibility) and efficiency (ratio between the number of correct results and the total number of results), and predictive values for resistance (PVR, the ratio of true resistances to total resistance) and for susceptibility (PVS, rate of true susceptibility to total susceptibility), were calculated according to Laszlo et al [9] in order to interpret the data.

RESULTS

Comparative results of drug susceptibility testing by the MGIT and Bactec 460 systems are summarized in Table 1. Streptomycin results agreed for 48 of the 52 isolates (92%). The method of proportion agreed with MGIT in two cases, and agreed with the Bactec system in the other two cases (Table 1). On repeat testing in the MGIT system with a similar streptomycin final concentration 2 mg/L to that used in the Bactec system, all four strains became susceptible to streptomycin, and isoniazid results agreed for 49 of the 52 isolates (94%). The method of proportion agreed with MGIT in two cases (both susceptible strains), and agreed with the Bactec system in one case. On repeat testing, one of the strains became resistant, but the other two strains

Table 1 Analysis of susceptible and resistant *M. tuberculosis* isolated by the MGIT and Bactec 460 systems: analysis of the discrepant results between the MGIT and the Bactec systems in comparison with those obtained by the LJ proportion method

Drug	MGIT/Bactec S	MGIT/Bactec R	MGIT R/Bactec S	MGIT S/Bactec R	LJ S	LJ R
Streptomycin	47	1	4	0	2	2
Isoniazid	23	26	1	2	2	1
Rifampin	33	18	0	1	1	0
Ethambutol	50	2	0	0	0	0

S, susceptible; R, resistant.

Table 2 Comparative evaluation among the results for the four drugs obtained with the MGIT system with those obtained with the Bactec system and the LJ proportion method

	Bactec system / Proportion method				
Drug	Sensitivity	Specificity	Efficiency	PVR	PVS
Streptomycin	100/100	92/100	92/100	20/100	100/100
Isoniazid	93/96	96/100	94/98	96/100	92/96
Rifampin	95/100	97/100	98/100	100/100	97/100
Ethambutol	100/100	100/100	100/100	100/100	100/100

PVR, predictive value of resistance; PVS, predictive value of susceptibility.

remained susceptible. However, among these three isoniazid-resistant strains, one strain had a *katG* gene mutation and the other two strains had *inhA* gene mutations [10]. Rifampin results agreed for 51 of the 52 isolates (98%), and disagreed for one isolate in which the result of the method of proportion agreed with that of the MGIT, although this isolate has a *rpoB* gene mutation [11]. Ethambutol results agreed (100%) for all isolates, 50 susceptible strains and two resistant strains. The sensitivity, specificity and efficiency values [9] when the MGIT results are compared with those obtained with the Bactec system and the proportion method for the four antimicrobials are summarized in Table 2.

The mean time (±standard error of the mean) from inoculation to results for MGIT was 5.4±2.5 (range, 2–10) days for streptomycin, 5.6±2.0 (range, 2–11) days for isoniazid, 5.5±2.1 (range, 2–11) days for rifampin and 5.4±2.0 (range, 2–10) days for ethambutol, compared to a mean of 6.7±1.7 days for all drugs with the Bactec system ($p<0.001$).

DISCUSSION

In our study, the turn-round time for the MGIT system was 4–9 days (median 6), which is similar to preliminary results previously reported [7,12], compared with 5–12 days for Bactec (median 8). The lack of one unique internationally accepted laboratory methodology for susceptibility testing of *M. tuberculosis* makes it very difficult to compare the results obtained with different testing methods. We found a better correlation between MGIT and the proportion method on LJ than with the Bactec 460 method when testing streptomycin, rifampin and ethambutol, but a poorer correlation when testing isoniazid. Overall, these results agreed with those obtained by Laszlo et al [9], except for those of isoniazid. However, three resistant strains (two isoniazid-resistant and one rifampin-resistant) confirmed by molecular methods [10,11] were detected only in the Bactec system, and had been missed by the former systems. Two isoniazid-resistant isolates were detected by the Bactec system after 15 days of incubation because the increase in GI was very slow. These strains might have been detected by the MGIT system with longer incubation. The correlation between MGIT and Bactec test results for testing susceptibility to rifampin was not 100% as previously reported [7,13]. Results for 51 of 52 isolates agreed; the MGIT system detected 18 of 19 rifampin-resistant isolates included in this study.

In contrast, for testing susceptibility to streptomycin, Palaci et al [14] found 100% correlation between MGIT and the indirect proportion method on LJ using a final concentration of 2 mg/L. Our results agreed with those obtained by Bergmann et al [12], and when repeat testing was performed by us with that concentration, we also found 100% agreement between the MGIT and Bactec systems. Again, Laszlo et al [9] found discordant results when testing streptomycin, as we did; however, no discrepant results were found by us when testing ethambutol, probably due to the small number of resistant strains tested in our study. It is noticeable that the results obtained by molecular methods do not always correlate with those obtained by the different microbiological methods, suggesting that these molecular methods should be included as a part of the evaluation of the different drug susceptibility testing of *M. tuberculosis.*

Too few data have been collected to determine the critical concentration of drugs required for susceptibility testing using the MGIT system; however, the need to use a critical concentration different from that used in the standard method would not be surprising, given the unique detection system and the test media. The reading of the signal and its interpretation as 'positive' or 'negative' remains subjective and may vary from person to person, so a scale for the interpretation of the fluorescence signal should be included in the MGIT system.

In summary, the MGIT system appears to be an acceptable alternative to the radiometric Bactec method for rapid and reliable susceptibility testing of

M. tuberculosis. Additional studies with all four drugs should be performed to confirm our results as well as to determine the critical concentration of drugs required, especially those of streptomycin and rifampin. If an automated system incorporating MGIT technology is made available, it may further enhance the performance.

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Hepatitis C virus RNA (HCV RNA) and viral types in dialysis patients in Dakar, Senegal

Clin Microbiol Infect 1999; 5: 230–232

Elisabetta Falasca[1]*, *George Raphenon*[2], *Corrado Pipan*[1], *Boucar Diouf*[3], *Paul Seignot*[4] *and Guiseppe A. Botta*[1]

[1]Institute of Microbiology, Medical School, University of Udine, 33100 Udine, Italy; [2]Institut Pasteur, Dakar, [3]Nephrology Department, Hôpital Le Dantec, Université, Cheik Anta Diop, and [4]Intensive Care, Hôpital Principal, Dakar, Senegal

*Tel: +39 0432 559226 Fax: +39 0432 545526 E-mail: giuseppe.botta@drmm.uniud.it

Accepted 12 August 1998

Patients undergoing renal dialysis are at increased risk of hepatitis C virus (HCV) infection, depending on the duration of treatment, extended exposure to transfusion and possible nosocomial transmission. The reported [1] incidence of HCV infection in dialysis units in southern Europe, Japan and the USA ranges from 10% to 30%, while in northern Europe the incidence is lower, ranging from 1% to 9%.

Information about HCV infections in the general African population is scant, with reported prevalence rates varying greatly in different countries, from more than 10% in the pygmy population in Cameroon to 0–1.5% in South Africa. Socio-economic factors as well as cultural tradition (such as cosmetic tattooing) are likely to be relevant to the observed variation. To our knowledge, no data are available for sub-Saharan Africa