INT J TUBERC LUNG DIS 3(4):344–348

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

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SUMMARY

SETTING: Mycobacteria Supranational Reference Laboratory.

OBJECTIVE: To evaluate the Mycobacteria Growth Indicator Tube (MGIT) method for drug susceptibility testing of *Mycobacterium tuberculosis*.

DESIGN: One hundred and one clinical strains of *M. tuberculosis* were evaluated for their susceptibilities to isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and streptomycin (SM) by MGIT and by the proportion method on Löwenstein-Jensen (L-J) medium. The concentrations of drugs in MGIT were: 0.1, 1.0, 3.5 and 0.8 μg/ml for INH, RIF, EMB and SM, respectively.

RESULTS: The results for individual drugs showed a good correlation: the specificity was 100% for INH, RIF and EMB and 92% for SM; the sensitivity was 100%, 94.6%, 96.1% and 89.7% for INH, RIF, EMB and SM, respectively, and the accuracy values 1.0, 0.98, 0.99 and 0.91.

CONCLUSION: The MGIT system appears to be a reliable and simple non-radiometric method for the drug susceptibility testing of *M. tuberculosis*.

KEY WORDS: MGIT; *M. tuberculosis*; drug susceptibility testing

AMONG COMMUNICABLE DISEASES, tuberculosis by itself, although preventable, kills many more people than any other infectious disease. The concern about the world-wide magnitude of the modern tuberculosis epidemic is so great that in April 1993 the World Health Organization (WHO) declared tuberculosis to be a global emergency, the first declaration of its kind in the history of the WHO.[1]

According to the WHO one third of the world's entire population is now infected with *Mycobacterium tuberculosis*. In the next decade it is estimated that 300 million more people will become infected, that 90 million people will develop the disease, and that 30 million people will die from it. Tuberculosis currently kills more adults each year than the acquired immunedeficiency syndrome (AIDS), malaria and tropical diseases combined, and almost 170 000 children.[1]

Of particular concern for the control of tuberculosis is the emergence of drug-resistant strains, which threatens to make the disease incurable again, as it was before the discovery of antibiotics in 1944. There is no cure for certain multidrug-resistant strains of *M. tuberculosis* (MDR-TB), and there is concern that they may spread rapidly around the world. Although reliable data are scarce, researchers estimate that more than 50 million people might now be infected with strains that are resistant to at least one of the anti-tuberculosis drugs. A global surveillance project has been completed by WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) to assess the real extent of multidrug resistance in tuberculosis.[2]

The emergence of MDR-TB underscores the need to rapidly determine the susceptibility of isolates to antimicrobial agents. The two methods currently in use for susceptibility testing of *M. tuberculosis* are the proportion method on agar or Löwenstein-Jensen (L-J) media, and the radiometric Bactec TB 460 system (Becton-Dickinson Microbiology Systems, Sparks, MD, USA).[3–5] The proportion method is considered as the gold standard, but requires a minimum of 3 to 4 weeks of incubation to obtain results; the Bactec method, although it can give results in a shorter period of time, has the disadvantage of requiring special instrumentation and the means for disposal of radioactive materials.

More recently Becton Dickinson has developed the mycobacteria growth indicator tube (MGIT) as an alternative, non-radiometric method for the detection of mycobacteria using a fluorescent oxygen-quenched sensor embedded in silicone at the bottom of the tubes. Previous reports on the evaluation of this method for primary isolation of mycobacteria from clinical and laboratory cultures have shown promising results.[6–10]

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Article submitted 9 March 1998. Final version accepted 5 November 1998.

Preliminary studies for drug susceptibility testing on a limited number of *M. tuberculosis* strains have shown a good correlation with the conventional proportion method.[11–14] In the present study we performed a thorough evaluation of this method on 101 clinical and laboratory strains of *M. tuberculosis*, and compared it to the proportion method on L-J against the first line anti-tuberculosis drugs isoniazid (INH), rifampicin (RIF), ethambutol (EMB) and streptomycin (SM).

MATERIALS AND METHODS

Mycobacterial strains

A total of 101 *M. tuberculosis* strains were evaluated in the present study. Fifty two strains were fresh strains isolated in our laboratory from clinical samples, and 49 laboratory strains belonged to a collection of strains evaluated by a multicentre study under a supranational surveillance on drug resistance, as reported elsewhere.[15] All the strains were maintained as fresh cultures on L-J slants at the time of the evaluation. In addition the following four ATCC strains were used for quality control: *M. tuberculosis* ATCC 35822, *M. tuberculosis* ATCC 35838, *M. tuberculosis* ATCC 35837 and *M. tuberculosis* ATCC 35820. These strains are each resistant to INH, RIF, EMB and SM, respectively.

MGIT

The mycobacteria growth indicator tubes were those provided by Becton Dickinson; these tubes contain 4.0 ml of an enriched 7H9 broth with an O_2 sensitive fluorescence sensor indicating microbial growth; prior to their use, the tubes were supplemented with 0.5 ml of oleic acid-albumin-dextrose-catalase (OADC).

Inoculum preparation

Several colonies from a 4-week-old L-J slant were transferred into a tube containing 4 ml of 7H9 broth and a few glass beads, and vortexed for 2 to 3 minutes to break larger clumps; the suspension was left to stand for 20 minutes and the supernatant transferred to another tube and left for another 15 minutes; the supernatant was again transferred into a third tube and the turbidity adjusted with 7H9 broth to a 0.5 McFarland standard using a nephelometer. This suspension was further diluted 1:5 with sterile saline.

MGIT susceptibility testing

Susceptibility testing was performed according to the protocol provided by the manufacturers. Briefly, MGIT tubes were supplemented with 0.5 ml of OADC enrichment and 0.1 ml of test antibiotics added with a calibrated pipette; the final concentration of each antibiotic in the test tubes was INH 0.1 μg/ml, RIF 1.0 μg/ml, EMB 3.5 μg/ml and SM 0.8 μg/ml. A growth control tube was prepared without any antibiotic. The tubes were then inoculated with 0.5 ml of the 1:5 dilution prepared previously. A positive control tube was prepared by adding 5 ml of a 0.4% sodium sulfite solution to an empty MGIT tube; an uninoculated enriched MGIT tube served as negative control. All tubes were incubated at 37 °C in normal atmosphere.

Beginning at day 3 after inoculation, the MGIT tubes were removed from the incubator and placed on a 365 nm UV transilluminator. The growth control tube was compared to the positive and negative controls; positivity was indicated by bright orange fluorescence on the bottom of the tube and an orange reflection at the meniscus; negative tubes on the other hand showed very little or no fluorescence. If fluorescence appeared on the growth control tube it was considered as positive for growth and that day was considered as day 0 for the purpose of interpretation of the drug-containing tubes; if the growth control tubes were 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 the recommendations of the manufacturers: a strain was considered susceptible if the drug-containing tube did not fluoresce within two days of positivity of the growth control, and resistant if the drug-containing tube was positive on or within 2 days of positivity of the growth control.

Proportion method

All the evaluated strains were also tested by the proportion method on L-J medium, according to standard procedures,[4] at concentrations of INH 0.2 μg/ml, RIF 40 μg/ml, EMB 2 μg/ml and SM 4 μg/ml.

Data analysis

Data analysis involved a standard 2-by-2 contingency table. Results were interpreted for each drug under evaluation in terms of sensitivity and specificity, and compared with those obtained by the standard proportion method. Predictive and accuracy values were also determined for each drug under evaluation.

RESULTS

The comparative results obtained for INH, RIF, EMB and SM are shown in Table 1. From the 101 strains of *M. tuberculosis* evaluated, 38, 64, 75 and 57 were susceptible to INH, RIF, EMB and SM, respectively, by both methods; resistant strains by both methods to INH, RIF, EMB and SM were 63, 35, 25 and 35, respectively.

For INH, the results of both methods agreed for all

Table 1 Susceptibility and resistance of *M. tuberculosis* strains by the MGIT and the proportion method

Test drug	Total count	No. of strains with the following results			
		Both-S (A)	PRO-S MGIT-R (B)	PRO-R MGIT-S (C)	Both-R (D)
INH	101	38	0	0	63
RIF	101	64	0	2	35
EMB	101	75	0	1	25
SM	101	57	5	4	35

MGIT = Mycobacteria Growth Indicator Tube; INH = isoniazid; RIF = rifampicin; EMB = ethambutol; SM = streptomycin; S = susceptible; R = resistant; PRO = proportion method.

the strains tested: 38 were susceptible and 63 were resistant. For RIF, two strains were found resistant by the proportion method, but susceptible by the MGIT method. For EMB only one strain was found resistant by the proportion method but susceptible by the MGIT method. For SM, disagreement was found for nine strains evaluated: four were resistant by the proportion method but susceptible by the MGIT method and five were susceptible by the proportion method but resistant by the MGIT method.

The specificity was found to be 100% for INH, RIF and EMB and 92% for SM; the sensitivity on the other hand was 100%, 94.6%, 96.1% and 89.7% for INH, RIF, EMB and SM, respectively. The predictive value for susceptibility (PPV) was 100%, 97%, 98.7% and 93.4% for INH, RIF, EMB and SM, respectively, while the predictive value for resistance (NPV) was 100% for INH, RIF and EMB, and 87.5% for SM. The accuracy values were 1.0, 0.98, 0.99 and 0.91 for INH, RIF, EMB and SM, respectively.

Table 2 displays the average time and range for results with the four drugs; these data are also expressed in terms of susceptible or resistant strains alone. Table 3 shows the distribution of strains by the time needed to obtain a result for each drug separately or with all four drugs.

DISCUSSION

Our evaluation of the MGIT system for the drug susceptibility testing of INH, RIF, EMB and SM against

Table 2 MGIT drug susceptibility testing (DST) time to results in days

Drug	All strains		Susceptible strains		Resistant strains	
	Average	Range	Average	Range	Average	Range
INH	7.4	3–11	8.0	5–11	7.0	3–11
RIF	8.0	3–12	8.5	5–12	7.0	3–11
EMB	8.4	4–13	8.5	5–12	8.0	4–13
SM	8.3	3–13	8.5	5–12	8.0	3–13

INH = isoniazid; RIF = rifampicin; EMB = ethambutol; SM = streptomycin.

Table 3 Number of strains (%) positive in MGIT (n = 101) at time interval

	INH	RIF	EMB	SM	All 4 drugs
<5 days	14 (13.9)	4 (3.9)	1 (1)	5 (4.9)	0
5–10 days	83 (82.2)	90 (89.2)	91 (90.1)	87 (86.2)	92 (91.1)
>10 days	4 (3.9)	7 (6.9)	9 (8.9)	9 (8.9)	9 (8.9)

INH = isoniazid; RIF = rifampicin; EMB = ethambutol; SM = streptomycin.

101 clinical strains of *M. tuberculosis* has given very satisfactory results. In a recent study done by Suzuki et al.[13] on 56 strains of *M. tuberculosis* an overall agreement of 96.4% was observed when comparing the absolute concentration method on Ogawa medium with the MGIT system at the same concentrations of INH, RIF, EMB and SM in the MGIT system as used in the present study; drug resistance was detected on an average of 5.9 days in the MGIT system compared to 21 days on the Ogawa medium. A similar study done by Palaci et al.[11] on 25 *M. tuberculosis* strains, showed a sensitivity and specificity of 100% for INH, RIF, EMB, SM and ofloxacin; two previous studies have evaluated the activity of INH and RIF, with an agreement ranging from 96.5% to 99.1%.[12,13]

This is a larger study performed on the main four antituberculosis drugs. The general accuracy for the four drugs evaluated was 0.97; however, this value was due mainly to the accuracy obtained when considering SM (0.91). With the other three drugs the accuracy value was 0.99, which compares quite well with previous published studies. We found 12 discordant results, nine of which were with SM (Table 4). Of the two RIF discordant results, one strain (94-1173), which was resistant by the proportion method and sensitive by MGIT, was also sensitive with the Line-Probe assay (Inno-Lipa Rif-TB test),[16,17] and by the supranational evaluation of drug resistance.[15] The other RIF discordant strain (96-1302), resistant by the proportion method and sensitive by MGIT, was resistant by the Inno-Lipa Line-Probe assay. This strain had a resistance of 4% by the proportion method (Table 4), which could explain in some cases the cause of discrepancies when the percentage of resistant bacteria is not high. Thus only one result was a true discordant for RIF. The discordant strain for EMB (96-609), which was resistant by the proportion method but sensitive by MGIT, was also reported as sensitive by the supranational evaluation of drug resistance.[15] Concerning the nine discordant results for SM, five strains (96-1339, 94-1175, 95-850, 96-1040 and 96-1039) were sensitive by the proportion method but resistant by MGIT; of these, one (94-1175) was resistant and another (95-850) sensitive by the supranational evaluation of drug resistance.[15] Of the other four strains (96-603, 9161, 95-842 and 95-834) which were resistant by the proportion method but sensitive by MGIT, two

Table 4 MGIT versus proportion method discrepant strains

		MGIT method				Proportion method (% of resistance)			
Strain	Origin	INH	RIF	EMB	SM	INH	RIF	EMB	SM
9161	ITM	R	R	R	S	R (>10)	R (>10)	R (>10)	R (3)
94-1173	SNL 94-25	R	S	S	S	R (>10)	R (9)	S	S
94-1175	SNL 94-25	R	R	S	R	R (>10)	R (>10)	S	S
95-834	SNL 92-16	R	R	R	S	R (>10)	R (>10)	R (>10)	R (>10)
95-842	SNL 92-338	R	R	S	S	R (9)	R (>10)	S	R (2)
95-850	SNL DR-593	R	R	R	R	R (10)	R (>10)	R (>10)	S
96-1039	ITM	S	S	S	R	S	S	S	S
96-1040	ITM	S	S	S	R	S	S	S	S
96-1302	ITM	R	S	S	S	R (>10)	R (4)	S	S
96-1339	ITM	R	S	S	R	R (10)	S	S	S
96-603	SNL 16727	R	S	S	S	R (>10)	S	S	R (5)
96-609	SNL 6012	R	R	S	R	R (>10)	R (>10)	R (>10)	R (10)

INH = isoniazid; RIF = rifampicin; EMB = ethambutol; SM = streptomycin; ITM = Institute of Tropical Medicine; SNL = Supranational laboratory evaluation.[7]

(95-842, 95-834) were resistant and one (96-603) sensitive by the supranational evaluation of drug resistance.[15]

As has been published recently under the Quality Assurance Programme for drug susceptibility testing of *M. tuberculosis* by the WHO/IUATLD Supranational Laboratory Network, drug susceptibility testing was reliable for INH and RIF, which is important for the detection of MDR-TB.[15] However, some discrepancies were still detected with SM and EMB which could be corrected by adopting standard critical concentrations, critical proportion and reading time frames that define drug resistance.[15] In the present study nine out of 12 discrepant results were found with SM, comparing very favourably with the results of the supranational evaluation. Additional adjustments to the MGIT system, such as the concentration of SM employed as well as evaluation of a greater number of strains, could help to explain the cause of discrepancies and improve the results obtained in the present study.

The time to detection with the MGIT system found in our study was on average 8 days, with a range of 3 to 13 days, comparing favourably with other reported studies,[11–13] and is shorter than the time usually required when performing the proportion method.[3,4]

CONCLUSION

The MGIT system is currently a reliable method for the susceptibility testing of *M. tuberculosis* to the four major antituberculosis drugs; it has the advantage of being non-radiometric, simple to perform, and does not require expensive equipment.

Acknowledgements

This work was partially supported by the Damien Foundation, Brussels, Belgium, by Becton Dickinson who provided us with the MGIT tubes, and by the Fonds National de la Recherche Scientifique, Belgium (grant 15.192.95F).

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RÉSUMÉ

CADRE : Un laboratoire de référence supranational en matière de mycobactéries.
OBJECTIF : Evaluation de la méthode du Tube Indicateur de Croissance Mycobactérienne (MGIT) pour tester la sensibilité de *Mycobacterium tuberculosis* à l'égard des médicaments.
SCHÉMA : Cent et une souches cliniques de *M. tuberculosis* ont fait l'objet d'une évaluation de sensibilité à l'égard de l'isoniazide (INH), la rifampicine (RIF), l'ethambutol (EMB) et la streptomycine (SM) par le MGIT et par la méthode des proportions sur milieu de Löwenstein-Jensen (L-J). Dans le système MGIT, les concentrations des médicaments furent les suivantes : 0,1, 1,0, 3,5, et 0,8 μg/ml respectivement pour INH, RIF, EMB et SM.
RÉSULTATS : Les résultats pour les drogues individuelles ont montré une bonne corrélation : la spécificité est de 100% pour INH, RIF et EMB, et de 92% pour SM ; la sensibilité est de 100%, 94,6%, 96,1% et 89,7% respectivement, pour INH, RIF, EMB et SM, et les valeurs de précision de 1,0, 0,98, 0,99 et 0,91.
CONCLUSION : Le système MGIT apparaît être une méthode fiable et aisée pour tester la sensibilité médicamenteuse de *M. tuberculosis* d'une manière non radiométrique.

RESUMEN

MARCO DE REFERENCIA : Laboratorio de Referencia Supranacional de Micobacterias.
OBJETIVO : Evaluar el método del Tubo Indicador de Crecimiento de Micobacterias (MGIT) para estudiar la sensibilidad de *Mycobacterium tuberculosis*.
MÉTODO : Se evaluó la sensibilidad de 101 cepas de *M. tuberculosis* a la isoniacida (INH), rifampicina (RIF), etambutol (EMB) y estreptomicina (SM) por el método del MGIT y por el método de las proporciones en medio de Löwenstein-Jensen (L-J). La concentración de las drogas en el MGIT fue de 0,1, 1,0, 3,5 y 0,8 μg/ml para INH, RIF, EMB y SM, respectivamente.
RESULTADOS : Los resultados para cada droga mostraron una buena correlación : la especificidad fue del 100% para INH, RIF y EMB y 92% para SM ; la sensibilidad fue del 100%, 94,6%, 96,1% y 89,7% para INH, RIF, EMB y SM, respectivamente ; los valores de precisión fueron 1,0, 0,98, 0,99 y 0,91.
CONCLUSIÓN : El sistema MGIT parece ser un método no radiométrico confiable y simple para estudiar la sensibilidad de *M. tuberculosis*.