Multicenter evaluation of mycobacteria growth indicator tube (MGIT) compared with the BACTEC radiometric method, BBL biphasic growth medium and Löwenstein–Jensen medium

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Objective: To evaluate the new BBL mycobacteria growth indicator tube (MGIT) in comparison with other media.

Methods: MGIT was evaluated in 10 Italian centers on 433 clinical samples, mainly of respiratory origin and mainly smear positive, in comparison with Löwenstein–Jensen and with one or more other methods represented, according to participating centers, by the BACTEC radiometric method or by the biphasic BBL Septi-Chek AFB system. While MGIT and Löwenstein–Jensen were used for all the samples, 285 of them were also inoculated in BACTEC vials and 274 in biphasic bottles. Of these samples, 132 were investigated with all the four methods.

Results: Although less rapid and sensitive than the radiometric method, the results of MGIT were equal when compared with the other two media with respect to overall isolation yield; furthermore, it allowed the detection of growth in significantly shorter times.

Conclusions: The results of this study indicate the value of MGIT for the detection of mycobacteria and, thanks to its extreme simplicity of use, its suitability for small and large laboratories. Its combined use with a solid medium can substantially improve the diagnosis of mycobacterial infection.

Key words: *M. tuberculosis*, culture media, non-tuberculous mycobacteria

The need for rapid diagnosis of tuberculosis has always been considered important, and it is now even more pressing because of the recent increase in cases [1]. It represents the most efficient measure in control of the outbreaks of multidrug-resistant tuberculosis that have become a major problem in urban communities [2]. Mycobacteria other than tuberculosis (MOTT) are also of increasing concern [3], as these infections, particularly in immunocompromised patients, reduce life-expectancy and greatly worsen the quality of life.

Microscopic examination is rapid, but its sensitivity is low, so that it often fails to detect small numbers of mycobacteria. Important progress has been made with the introduction of genetic amplification techniques

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[4–8], which in many instances allow a diagnosis in hours; their reliability, however, has not yet been assessed definitively [9], and culture still provides the mainstay for diagnostic detection, and is a necessary prerequisite for further investigations, such as susceptibility testing. The radiometric BACTEC system [10] is an important advance, in that it shortens, even by weeks, the time of culture for mycobacteria, but the presence of radioactivity is not well accepted in the majority of laboratories.

A recent attempt to exploit the rapid growth supported by liquid media is represented by the BBL mycobacteria growth indicator tube (MGIT) (Becton Dickinson, USA), where a fluorescence-quenching oxygen sensor gives early evidence of bacterial growth. A field assessment of this new medium has been carried out in 10 Italian centers skilled in mycobacteriology, testing it in parallel with conventional solid media and with the radiometric (BACTEC, Becton Dickinson, USA) and the biphasic (BBL Septi-Chek AFB, Becton Dickinson, USA) systems.

METHODS

MGIT consists of tubes of Middlebrook 7H9 broth containing a silicon-embedded O_2-sensitive fluorescence sensor (ruthenium metal complex). Before use the tubes are supplemented with oleic acid–albumin–dextrose and with the antimicrobial combination PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin). A positive signal is represented by the bright orange fluorescence emitted by the silicon-coated base when the tube is exposed to 365-nm UV light.

The test was performed in different centers on samples digested and decontaminated according to the standard NALC-NaOH procedure [11]. All types of specimens, except blood, were considered acceptable; multiple samples from the same patient were also accepted. In order to increase the expected prevalence of positive cultures, samples were selected which either were smear positive or came from patients known to have excreted mycobacteria in the previous 2 months. Auramine-stained smears were observed by fluorescence microscopy and positivity was scored as +, ++ or +++ according to the presence of 1, 2–10 or more than 10 acid-fast bacilli (AFB) per microscope field.

All centers involved performed the test in parallel on MGIT and on two standard Löwenstein–Jensen slants; furthermore, laboratories which had the BACTEC system available extended the comparison at least to this radiometric method, and the remaining centers chose the biphasic system Septi-Chek as a further arm of comparison. The sample size was three drops for each of a pair of Löwenstein–Jensen slants and 0.5 mL for all other cases. The incubation was carried out at 37°C in normal atmosphere and the cultures were inspected visually (or using the BACTEC 460TB instrument, for radioactivity) twice a week, with the exception of MGIT, which was read daily. A positive result was recorded when mycobacterial colonies were detected on Löwenstein–Jensen or on the slides of the biphasic Septi-Chek system and, for MGIT and radiometric system, at the moment of microscopic confirmation of the presence of acid-fast bacilli in vials which had yielded a positive signal (visible fluorescence, or a growth index >20). Cultures which grew no mycobacteria after 60 days of incubation were considered negative. For each clinical sample, the first mycobacterial isolate detected in a culture was identified at various levels, according to the center; in every instance, however, the isolates belonging to the *Mycobacterium tuberculosis* complex were differentiated from MOTT.

The coded results of each procedure were filed in a relational database (Microsoft Access), with a record for each specimen; separate fields were created to label samples as truly positive for mycobacteria, *M. tuberculosis*, or MOTT, according to their identification on any of the seeded media. For the statistical analysis, Statistica (Statsoft Inc.) software was used. Isolation rates for mycobacteria were compared with the McNemar test on paired observations; diagnostic sensitivities for *M. tuberculosis* and MOTT, and the selective power of the media, were compared between independent groups (data not reported); isolation times achieved with the various methods were reported on a per group base, but compared on a pairwise base (that is, considering only isolates from the same specimen, on different media).

RESULTS

Respiratory specimens (372 sputa, 15 bronchial aspirates and six bronchial washings) were predominant; the other samples comprised 32 urine samples, three gastric aspirates, three lymph nodes and two pleural fluid samples.

Of the 433 specimens, only 50 were negative for AFB microscopy.

All samples were cultivated in parallel on Löwenstein–Jensen and MGIT; 285 were also seeded in BACTEC radiometric bottles and 274 in biphasic Septi-Chek bottles, 132 of the latter being tested in parallel with all four of the methods; six specimens were put through MGIT and Löwenstein–Jensen only.

Of the 433 samples thus processed, 74 did not grow any organism and two yielded a contaminating overgrowth on all media they had been seeded on. In 357 samples, a mycobacterium was isolated in at least one of the media: an organism belonging to the *M. tuberculosis* complex was isolated in 294 cases, while in 63 cases a MOTT was grown (Table 1). Of the 76 culture-negative specimens, 26 were also smear negative. The relationship between the microscopic density of AFB and the isolation rates of mycobacteria is described in Table 2; as expected, the isolation rates increase with AFB density, very significantly so for all mycobacteria and for *M. tuberculosis*, but not significantly for MOTT.

The overall diagnostic yield level clearly depends on the selection criteria of the test panel. It is of interest, however, to compare the yields on different media; the yield on MGIT (73.44% of positive cultures) was very similar to that on Septi-Chek (73.72%) (McNemar $\chi^2=0.41$; $p=0.52$) and on Löwenstein–Jensen (73.21%) (McNemar $\chi^2\approx0.00$; $p\approx1.00$); in contrast, the yield of the radiometric method, which grew mycobacteria in 78.25% of cases, was very significantly higher (McNemar $\chi^2=16.68$; $p<=0.0001$) (Table 3).

Table 1 *Mycobacterium* spp. isolated from 433 clinical samples

Organism	Number of isolates
M. tuberculosis complex	294
Unidentified MOTT	30
M. xenopi	8
M. avium complex	5
M. avium	4
M. celatum	4
M. malmoense	4
M. intracellulare	3
M. kansasii	3
M. chelonae	2

In the following analysis, we assume that the specificity of our mycobacterial isolations was absolute —i.e. we consider that there were no false positives. In order to assess the sensitivity of the various methods, all samples yielding a *Mycobacterium* isolate on any of the media seeded were considered as positive; the sensitivity of each medium was then computed as the percentage of positive samples being isolated on that medium. Similarly, separate sensitivities were calculated for the isolation of *M. tuberculosis* complex and of MOTT. Table 4 shows the sensitivities of the four media under comparison for the isolation of mycobacteria; the range of sensitivities is rather narrow, with a leading 95.7% for the radiometric method, followed by the Septi-Chek, the MGIT and the Löwenstein–Jensen. The same table allows a comparative estimate of the selective power of the various media, which may be inferred from the number and proportion of positive samples failing isolation because of contaminating overgrowth; the latter have been kept separate from the false negatives, as they point to a distinct determinant of the lack of sensitivity. Considering separately the performance of samples positive for *M. tuberculosis* complex (Table 5) or for MOTT (Table 6), the four media all show greater sensitivity for the isolation of the former; again, MGIT performs more or less like Löwenstein–Jensen, but not as well as the radiometric BACTEC.

The average time needed by mycobacteria to give positive cultures was 14.54 days on MGIT, 11.83 days on the radiometric BACTEC, 18.57 on the Septi-Chek and 24.87 on Löwenstein–Jensen. The advantage of BACTEC over all other media, and of MGIT over either Septi-Chek or Löwenstein–Jensen, is clearly shown in Table 7, which gives the results of the pairwise comparisons.

Table 2 Culture results of samples with increasing density of acid-fast bacteria (AFB)

AFB density[a]	Row totals	Isolation of *M. tuberculosis*		Isolation of MOTT		Total isolations	
		n	%	*n*	%	*n*	%
–	50	19	38.00	5	10.00	24	48.00
+	198	131	66.16	25	12.63	156	78.79
++	121	91	75.21	24	19.83	115	95.04
+++	64	53	82.81	9	14.06	62	96.88
Chi-square		30.28		4.15		65.3	
p		<0.0001		0.25		<0.0001	

[a]Using fluorochrome stain, smear-positive samples were scored as +, ++ or +++ when less than 1 AFB, 2–10 AFB and more than 10 AFB, per microscope field, were seen respectively.

Table 3 Comparison of culture results for mycobacteria on different media

Media	Results	MGIT results			Row totals	
		Positive	Negative	Contamination		
BACTEC	Positive	197	9	17	223	(78.25%)
	Negative	1	55	0	56	(19.65%)
	Contamination	2	0	4	6	(2.11%)
	Totals	200 (70.18%)	64 (22.46%)	21 (7.37%)	285	(100.00%)
Septi-Chek	Positive	189	4	9	202	(73.72%)
	Negative	7	53	3	63	(22.99%)
	Contamination	2	4	3	9	(3.28%)
	Totals	198 (72.26%)	61 (22.26%)	15 (5.47%)	274	(100.00%)
Löwenstein–Jensen	Positive	296	7	14	317	(73.21%)
	Negative	9	77	5	91	(21.02%)
	Contamination	13	3	9	25	(5.77%)
	Totals	318 (73.44%)	87 (20.09%)	28 (6.47%)	433	(100.00%)

Table 4 Sensitivity and selective power of different media for the isolation of mycobacteria

	MGIT		BACTEC		Septi-Chek		Löwenstein–Jensen	
	Count	%	Count	%	Count	%	Count	%
True positives	318	89.08	223	95.70	202	93.09	317	88.80
False negatives	16	4.48	5	2.15	12	5.53	17	4.76
Positives, overgrown	23	6.44	5	2.15	3	1.38	23	6.44
Total positives	357	100	233	100	217	100	357	100

Table 5 Sensitivities and selective power of different media for the isolation of *M. tuberculosis* complex

	MGIT		BACTEC		Septi-Chek		Löwenstein–Jensen	
	Count	%	Count	%	Count	%	Count	%
True positives	269	91.50	180	96.78	171	97.16	264	89.80
False negatives	11	3.74	3	1.61	4	2.27	10	3.40
Positives, overgrown	14	4.76	3	1.61	1	0.57	20	6.80
Total positives	294	100	186	100	176	100	294	100

Table 6 Sensitivities and selective power of different media for the isolation of MOTT

	MGIT		BACTEC		Septi-Chek		Löwenstein–Jensen	
	Count	%	Count	%	Count	%	Count	%
True positives	49	77.78	43	91.50	31	75.61	53	84.13
False negatives	5	7.94	2	4.25	8	19.51	7	11.11
Positives, overgrown	9	14.28	2	4.25	2	4.88	3	4.76
Total positives	63	100	47	100	41	100	63	100

Table 7 Pairwise comparisons of mean times to growth detection of mycobacteria in different media

	Mean days	*n*	Mean difference	SE of mean difference	*p*
MGIT versus BACTEC	15.13 11.04	197	4.09	0.292	<0.001
MGIT versus Septi-Chek	13.76 17.88	189	−4.12	0.360	<0.001
MGIT versus Löwenstein–Jensen	14.14 24.51	296	−10.37	0.462	<0.001

When *M. tuberculosis* complex and MOTT were considered separately, while on MGIT and BACTEC *M. tuberculosis* complex presented mean growth times shorter than those of MOTT, the opposite was true for Löwenstein–Jensen and the biphasic Septi-Chek system (data not shown). The variation of growth-times emerging when smear-negative and weakly, intermediately or heavily smear-positive samples were considered did not present substantial differences on the various media, with a mean delay of microscopically negative specimens, in comparison to the heavily positive ones, ranging between 6 and 8 days (data not shown).

DISCUSSION

Sensitivity, selective power and early detection of growth are major parameters in the evaluation of culture media. The features of MGIT emerging from this study reveal a sensitivity very similar to that of the most widely applied solid medium, Löwenstein–Jensen, and to that of the thoroughly validated biphasic method, Septi-Chek [12–16]. The lower rate of false-negative results presented by MGIT in comparison with Septi-Chek and Löwenstein–Jensen is in fact counterbalanced by a higher number of contaminations, a clue to its lower selective power. It is, however, the early detection of growth that makes the difference in favor of MGIT; this allows a saving of a substantial number of days (4–10) in comparison with both the above mentioned methods. These differences exceed any delays that could result from the less frequent reading of the comparative cultures. In comparison with the radiometric method, MGIT appeared clearly less competitive; in fact, BACTEC was shown to be significantly more sensitive, more selective and faster.

With respect to convenience, MGIT is characterized by several advantages; it does not require post-inoculation handling, like the transfer of Löwenstein–Jensen from the slanted to the upright position after the first week of incubation and the tightening of the loose screw caps 1 or 2 weeks later, or like the periodic flooding needed by the biphasic Septi-Chek system. On the other hand, the presence of radioactivity and the need for expensive instrumentation (BACTEC 460TB) are evident disadvantages of the radiometric system. The ease of reading is probably the most attractive advantage for the user: simply placing the transparent rack holding the MGIT tubes over a UV transilluminator allows immediate recognition of the fluorescent bases of the positive cultures; thus high workloads may be accommodated, which is not the case for the other systems.

Several evaluations of MGIT in comparison with alternative media have been recently reported; one of them [17], being restricted to blood samples, is not directly comparable with our study. Two papers [18,19] which tested in parallel MGIT with BACTEC and solid media gave results compatible with ours. In one of them [18] the similarity of data is particularly striking when results on smear-positive samples (as were the majority of ours) are compared. In the other [19], characterized by a high prevalence of *M. avium* complex, results closer to ours are represented by the *M. tuberculosis* isolations. In a further study too [20], where a limited number of respiratory specimens were tested in parallel with MGIT and Löwenstein–Jensen, the results presented are in agreement with ours. In the only paper [21] in which the MGIT is compared with Septi-Chek, a better sensitivity and a lower rate of contamination of the latter are reported, compatible with our data.

The use in parallel of solid and liquid media is today recommended for the achievement of greater sensitivity in the detection of mycobacteria [22,23]; no medium used alone is in fact sensitive enough to grow mycobacteria from all clinical samples in which such organisms are present. This recommendation seems endorsed by the results of our study, in which the combinations of MGIT, Septi-Chek and BACTEC with Löwenstein–Jensen increased the recovery of mycobacteria by 3.04%, 3.18%, and 6.84% respectively.

Because of the relevance of mycobacterial infections, sensitivity and rapidity of their detection are nowadays of paramount importance; in this respect, MGIT qualifies as an easy-to-use liquid medium which, as an adjunct to the universally employed solid slants, can significantly improve the reliability of mycobacterial investigations in laboratories not using a radiometric method.

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