

## Differentiation of *Mycobacterium tuberculosis* from other mycobacteria with $\rho$ -nitrobenzoic acid using MGIT960

C. M. S. Giampaglia,\* M. C. Martins,\* E. Chimara,\* R. S. Oliveira,\* G. B. de Oliveira Vieira,†  
A. G. Marsico,† F. C. Q. Mello,† L. de Souza Fonseca,† A. Kritski,† M. A. da Silva Telles\*

\* Instituto Adolfo Lutz, São Paulo, † Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

### SUMMARY

**SETTING:** Mycobacteria growth in media with the addition of inhibitory substances has been used in species identification. Growth of the *Mycobacterium tuberculosis* complex (MTC) is inhibited by  $\rho$ -nitrobenzoic acid (PNB), whereas non-tuberculous mycobacteria (NTM) are resistant.

**OBJECTIVE:** To develop a rapid PNB test using the automated BACTEC MGIT960 system and to evaluate its usefulness in the screening of mycobacterial isolates.

**DESIGN:** PNB tests were performed in 93 MTC strains and 61 NTM strains from the Instituto Adolfo Lutz Culture Collection. PNB was added to Löwenstein-Jensen (LJ) medium and to BACTEC MGIT960 medium.

**RESULTS:** The MTC strains were all PNB-susceptible, confirming the original identification. Among 10 NTM

species, all were found to be resistant to PNB, except for one strain of *M. kansasii* and another of *M. marinum*. The median time to obtain presumptive identification of MTC by inhibition test in the BACTEC MGIT960 system was 6.3 days and for NTM it was 2.5 days. The presumptive identification of MTC in LJ was mostly obtained after day 20.

**CONCLUSION:** The key finding of this analysis was the possibility of combining the traditionally accepted method proposed by Tsukamura and Tsukamura in 1964 with the modern, safe and rapid BACTEC MGIT960 methodology.

**KEY WORDS:** *M. tuberculosis*; NTM; differentiation; PNB

IN SPITE OF THE PROGRESS made in promoting public health, the increase in tuberculosis (TB) has taken communities in many countries by surprise in different regions of the world. Even in the twenty-first century, this old disease continues to be a problem.

Rapid and precise diagnosis of each case is necessary for appropriate disease control. Isolation, identification and susceptibility testing are essential procedures that should be performed as quickly as possible so that adequate treatment can be prescribed. The use of liquid media has been suggested as the most efficient and fastest procedure for the isolation of mycobacteria and susceptibility testing. However, in addition to being isolated, these microorganism species should also be promptly identified. Although *Mycobacterium tuberculosis* infection is most common, infection due to mycobacteria other than *M. tuberculosis*, or non-tuberculous mycobacteria (NTM), is on the increase in many countries.<sup>1</sup> It is important to detect *M. tuberculosis* infection at an early stage, to establish adequate treatment for TB patients who follow treatment regimens different from those of patients infected with other mycobacteria.

The BACTEC 460TB system (Becton Dickinson Diagnostic Systems, Sparks, MD, USA) enables differentiation between the *M. tuberculosis* complex (MTC) and other mycobacteria by means of the NAP ( $\rho$ -nitro- $\alpha$ -acetylamino- $\beta$ -hydroxypropionophenone) test, which produces results in 4–6 days, but uses radioactive media.<sup>2</sup> Other methods, such as molecular probes and high performance liquid chromatography (HPLC), have been proposed for the differentiation of mycobacterial species. These methods, however, require procedures that are technically complex, laborious and costly, and countries with few resources may not be able to perform these procedures. Simple but rapid tests are therefore required.<sup>3</sup>

A recently developed non-radiometric, fully automated, continuous monitoring system has been introduced as an alternative to the radiometric BACTEC 460 for growth and detection of mycobacteria. This system uses a modified Middlebrook 7H9 broth and oxygen-quenching fluorescent technology to detect the amount of oxygen consumption induced by growing microorganisms. Culture vials are monitored hourly by the instrument and flagged as positive on the basis of specific growth algorithms. The system is reported

to be efficient in the rapid isolation and detection of drug resistance for these microorganisms.<sup>4</sup>

The ability of mycobacteria to grow in the presence of inhibitory substances in a suitable medium has been widely used in the identification of different species.<sup>2,5</sup> It has been reported that growth of the MTC is inhibited by *p*-nitrobenzoic acid (PNB) 500 µg/ml, whereas NTM are resistant to this concentration,<sup>6</sup> although, as suggested by Rastogi et al.<sup>7</sup> and Tsukamura & Tsukamura,<sup>5</sup> a small percentage of these bacteria may be susceptible to the substance. The study developed by Martins et al. showed that mycobacterial growth in PNB-containing medium may be used as a presumptive test for NTM.<sup>8</sup>

The objective of the present study was to develop a test adding PNB to the medium used in the automated non-radiometric Mycobacterium Growth Indicator Tube 960 system (MGIT 960, Becton Dickinson Microbiology Systems, Sparks, MD, USA) and verify its usefulness to differentiate MTC from other mycobacteria. Apart from saving labour and eliminating potential reading difficulties during visual judging of the tubes in traditional methods, the proposed methodology would generate data with a shorter turnaround time than that observed with current tests that use solid media to differentiate between species.

## MATERIAL AND METHODS

### Evaluation sites

This study was performed in the South-east region of Brazil at a University Reference Laboratory, Laboratório de Micobacteriologia, Programa Academico de Tuberculose, Universidade Federal Rio de Janeiro (Site 1), and a State Public Health Laboratory, Laboratório de Referencia Estadual de Micobacterias, Instituto Adolfo Lutz, Secretaria Estadual de Saude do Estado de Sao Paulo (Site 2). These reference laboratories work together with the Brazilian Tuberculosis Research Network (REDE-TB) to pursue the establishment of integrated clinical and laboratory evaluation of new diagnostic methods for TB.

### Microorganisms

A panel of 86 clinical isolates of *M. tuberculosis* and a panel of 53 clinical isolates of NTM were selected from the culture collection of the Laboratório de Referencia Estadual de Micobacterias, Instituto Adolfo Lutz. The selection took into account the isolation rates of each species from clinical samples.

The strains were maintained at -70°C in glass beads humidified with Sauton medium with 10% glycerol added. The frozen beads were inoculated in Löwenstein-Jensen (LJ) medium tubes and each of the participating laboratories received one of them. *M. tuberculosis* strains were identified by their culture characteristics and by classical biochemical testing.<sup>9,10</sup> Clinical NTM isolates were identified by culture char-

acteristics, classical biochemical testing and by polymerase chain reaction and restriction enzyme analysis of the *hsp 65* gene.

Five *M. tuberculosis* reference strains from the American Type Culture Collection (ATCC®) were included in the study: ATCC® 35822, isoniazid-resistant; ATCC® 35838, rifampicin-resistant; fully susceptible H37Rv, ATCC® 27294, H37Ra, ATCC® 25177, Erdman strain ATCC® 35801, one *M. africanum* (ATCC® 25420) and one *M. bovis* (ATCC® 19210).

The study also included reference strains of other mycobacteria species: *M. kansasii* (ATCC® 12478); *M. chelonae* (ATCC® 35752); *M. avium* subsp. *avium* (ATCC® 25291); *M. intracellulare* (ATCC® 23434); *M. xenopi* (ATCC® 19250); *M. gordonae* (ATCC® 14470); *M. terrae* (ATCC® 15755); *M. marinum* (ATCC® 927).

### Tests

Site 1 performed the niacin production test, nitrate reduction test and the heat stable catalase test (68°C), which are the classical phenotypic methods for the identification of MTC in accordance with Kent & Kubica,<sup>10</sup> as well as the PNB inhibition test in BACTEC MGIT960 medium.

Site 2 performed the PNB inhibition test using LJ medium as proposed by Tsukamura & Tsukamura,<sup>5</sup> and the PNB inhibition test using BACTEC MGIT960 medium. In addition to the strains tested by Site 1, Site 2 tested 53 NTM clinical isolates.

### *p*-nitrobenzoic acid inhibition tests

PNB was incorporated into LJ medium and BACTEC MGIT960 medium at a final concentration of 500 µg/ml.

Growth control tubes were LJ medium and BACTEC MGIT960 medium, without inhibitory substances.

The final concentration of PNB was selected on the basis of previous reports of studies performed using other broth systems such as BACTEC 460 and the BBL-MGIT manual system.<sup>11,12</sup> Preliminary experiments were conducted to establish the PNB concentration effective for BACTEC MGIT960 testing. Different concentrations of PNB (125 µg/ml, 250 µg/ml and 500 µg/ml) were prepared in MGIT960 media.

### Inoculum preparation for PNB tests on LJ and in BACTEC MGIT960 medium

The inoculum was prepared by scraping freshly grown colonies from the surface of the LJ medium. A few colonies were emulsified in one flask containing glass beads and 2 ml of sterile distilled water to obtain turbidity greater than McFarland 1 standard. The content of the flask was homogenised, allowed to stand for 15 min to allow larger clumps to settle, and then 1 ml of the supernatant suspension was transferred to another tube, where the turbidity was adjusted to McFarland 1

standard adding sterile distilled water. This bacterial suspension was used as the work suspension.

#### Inoculation

LJ medium was inoculated with 5 µl of the work suspension; BACTEC MGIT960 medium was inoculated with 500 µl of 1:5 dilution of the work suspension.

#### Incubation

LJ medium tubes were incubated into an incubator at 37°C. BACTEC MGIT960 tubes were loaded into the BACTEC MGIT960 instrument within 2 h of inoculation. Before loading the tubes, their barcodes were scanned and the instrument assigned them to their stations through the tube entry operation.

#### Reading

Reading of the LJ tubes was started on day 12 after incubation at 37°C. The strain was considered resistant when the tube containing the inhibitory substance presented a growth pattern similar to that observed in the drug-free control tube.

The growth in the control BACTEC MGIT960 medium tubes was automatically detected by the BACTEC MGIT960 instrument, which is able to continuously monitor the fluorescence due to growing mycobacteria. The positive stations were illuminated and the instrument reported the day the tubes became positive.

Readings of tubes containing the inhibitor were manually interpreted using the algorithm established by Giampaglia et al.<sup>12</sup> A strain was considered susceptible when the tube containing the inhibitor remained negative 2 days after a positive result was observed in the drug-free control tube. A strain was considered resistant when the tube containing the inhibitor became positive within 2 days after a positive result was observed in the drug-free control tube.

## RESULTS

The results of the preliminary tests performed in BACTEC MGIT960 showed that all 17 tested *M. tuberculosis* strains were susceptible to PNB at concentrations of 250 µg/ml and 500 µg/ml, and that four strains were resistant to PNB at 125 µg/ml but susceptible at 500 µg/ml. Based on these results, it was confirmed that the optimal testing concentration was 500 µg/ml.

The results for PNB inhibition tests of the *M. tuberculosis* strains in LJ and BACTEC MGIT960 system are summarised in Table 1 and those for NTM strains in Table 2.

The time to final results ranged from 4.6 to 16.3 days (median 6.3) for *M. tuberculosis* strains and from 2.5 to 10 days (median 2.5) for NTM strains.

## DISCUSSION

Laboratory services are very important in the diagnosis, management and epidemiological investigation of TB and other mycobacterial diseases. The roles of the laboratory differ depending on local requirements and financial constraints. In Brazil, as in most countries, bacteriological services are arranged in hierarchical fashion. State health authorities determine the level of TB services that each laboratory can afford or considers necessary. In most of the laboratories, mycobacteria culture is based on conventional techniques and use LJ medium.

Reference laboratories receive cultures, mainly in solid medium, from several laboratories to continue with mycobacteria identification to species level and perform antimicrobial drug susceptibility tests.

Presumptive differentiation between MTC and NTM can be made by growth characteristics (rough and cream colonies) and by microscopic observation of cording formation on Ziehl-Neelsen (ZN) stain<sup>13,14</sup>

**Table 1** Evaluation of the PNB inhibition test on LJ and in BACTEC MGIT960 medium for the identification of *M. tuberculosis* complex

Species	Strains <i>n</i>	Site 1*						Site 2†					
		Niacin		Nitrate		Catalase 68°C		MGIT PNB		LJ PNB		MGIT PNB	
		P	N	P	N	P	N	S	R	S	R	S	R
<i>M. tuberculosis</i> clinical isolates	86	86	0	86	0	0	86	86	0	86	0	86	0
<i>M. tuberculosis</i> ATCC® 35838	1	1	0	1	0	0	1	1	0	1	0	1	0
<i>M. tuberculosis</i> ATCC® 35822	1	1	0	1	0	0	1	1	0	1	0	1	0
<i>M. tuberculosis</i> HRa ATCC® 25177	1	1	0	1	0	0	1	1	0	1	0	1	0
<i>M. tuberculosis</i> HRv ATCC® 27294	1	1	0	1	0	0	1	1	0	1	0	1	0
<i>M. tuberculosis</i> Erdman ATCC® 35801	1	1	0	1	0	0	1	1	0	1	0	1	0
<i>M. africanum</i> ATCC® 25420	1	0	1	0	1	0	1	1	0	1	0	1	0
<i>M. bovis</i> ATCC® 19210	1	0	1	0	1	0	1	1	0	1	0	1	0

\* University Reference Laboratory: Laboratorio de Micobacteriologia, Programa Academico de Tuberculose, Universidade Federal Rio de Janeiro.

† State Public Health Laboratory: Laboratorio de Referencia Estadual de Micobacterias, Instituto Adolfo Lutz, Secretaria Estadual de Saude do Estado de Sao Paulo.

PNB = p-nitrobenzoic acid; LJ = Löwenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tube; P = positive; N = negative; S = susceptible; R = resistant; ATCC® = American Type Culture Collection.

**Table 2** Evaluation of the PNB inhibition test on LJ and in BACTEC MGIT960 medium for the identification of non-tuberculous mycobacteria

Species	Strains (n = 61)	Site 1*		Site 2†			
		MGIT PNB		MGIT PNB		LJ PNB	
		S	R	S	R	S	R
<i>M. marinum</i> ATCC® 927	1	1	0	0	1	1	0
<i>M. chelonae</i> ATCC® 35752	1	0	1	0	1	0	1
<i>M. terrae</i> ATCC® 15755	1	0	1	0	1	0	1
<i>M. avium</i> ATCC® 25291	1	0	1	0	1	0	1
<i>M. intracellulare</i> ATCC® 23434	1	0	1	0	1	0	1
<i>M. kansasii</i> ATCC® 12478	1	0	1	0	1	0	1
<i>M. xenopi</i> ATCC® 19250	1	0	1	0	1	1	0
<i>M. gordonae</i> ATCC® 14470	1	0	1	0	1	0	1
<i>M. avium</i>	24	ND	ND	0	24	0	24
<i>M. kansasii</i>	14	ND	ND	2	12	0	14
<i>M. gordonae</i>	2	ND	ND	0	2	0	2
<i>M. fortuitum</i>	5	ND	ND	0	5	0	5
<i>M. chelonae</i>	4	ND	ND	0	4	0	4
<i>M. abscessus</i>	4	ND	ND	0	4	0	4

\* University Reference Laboratory: Laboratório de Micobacteriologia, Programa Acadêmico de Tuberculose, Universidade Federal Rio de Janeiro.

† State Public Health Laboratory: Laboratório de Referência Estadual de Micobacterias, Instituto Adolfo Lutz, Secretaria Estadual de Saúde do Estado de São Paulo.

PNB = p-nitrobenzoic acid; LJ = Löwenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tube; S = susceptible; R = resistant; ATCC® = American Type Culture Collection; ND = not done.

of a positive culture. This is not, however, a definitive differentiation, as some NTM also produce cording. Conclusive identification of MTC can be obtained using conventional tests, such as the niacin test, nitrate reduction or catalase production, but these procedures are time consuming.

The inhibition test results of 93 MTC strains using the BACTEC-MGIT960 system with 500 µg/ml PNB were in agreement with the results of conventional tests performed on LJ and with the results previously reported in the literature.<sup>5,11,12,15</sup>

All MTC strains, including seven standard strains from ATCC® and 86 clinical isolates, were susceptible to PNB on LJ and in BACTEC-MGIT960 medium.

Of the eight standard NTM strains tested by PNB, six were resistant on LJ medium. *M. xenopi* and *M. marinum* were found to be susceptible. When the results were compared with those obtained with BACTEC MGIT960, the *M. xenopi* strain was considered resistant at both participating sites. The standard *M. marinum* strain was found to be susceptible at Site 1 and resistant at Site 2.

Of the 53 clinical isolates represented by six NTM species, all were found to be resistant except for one *M. kansasii* strain.

In agreement with Rastogi et al.,<sup>7</sup> discrimination

of *M. marinum* between *M. kansasii* was not fully achieved. However, these conflicting data do not impair the usefulness of the PNB inhibitory test combined with the BACTEC MGIT960 system, as *M. kansasii* requires a shorter time to give observable growth than *M. tuberculosis*, and *M. marinum* usually needs lower growth temperatures.

The median time to obtain presumptive identification of MTC by the inhibition test in BACTEC MGIT960 system was 6.3 days, while the results in solid medium were mostly obtained after a minimum incubation of 20 days.

The key finding of this analysis was the possibility of combining the traditionally recommended method proposed by Tsukamura & Tsukamura in 1964<sup>5</sup> with the modern, safe and rapid BACTEC MGIT960 methodology. In summary, it represents the possibility of providing rapid and reliable results in settings where a high prevalence of TB demands rapid procedures for identification and susceptibility testing against anti-tuberculosis drugs.

As previously suggested,<sup>12</sup> the following procedures should be observed: 1) establish presumptive identification of the MTC by growth characteristics (rough and cream colonies) added by microscopic examination of ZN smear from the positive culture (cording), and 2) perform combined susceptibility and PNB testing on strains presumptively identified as MTC. Set up one control tube, one additional tube of PNB and the tubes for the antimycobacterial susceptibility testing of BACTEC MGIT960 system.

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#### References

- Falkinham J O III. Epidemiology of infection by nontuberculous mycobacteria. Clin Microbiol Rev 1996; 9: 177-215.
- Laszlo A, Siddiqi S H. Evaluation of a rapid radiometric differentiation test for the *Mycobacterium tuberculosis* complex by selective inhibition with p-nitro-α-acetylamino-β-hydroxypropionophenone. J Clin Microbiol 1984; 19: 694-698.
- Levy-Frebault V V. Méthodes rapides de détection et de diagnostic des mycobactéries : actualité et perspectives. Méd Mal Infect 1992; 22: 391-406.
- Telles M A S, Bori A, Amorim A B R, Cruz A F, Pini M I T, Sato D N. Rapid detection of multidrug-resistant *Mycobacterium tuberculosis* using the growth indicator tube (MGIT) system. Braz J Med Biol Res 2002; 35: 1127-1131.
- Tsukamura M, Tsukamura S. Differentiation of *Mycobacterium tuberculosis* and *Mycobacterium bovis* by p-nitrobenzoic acid susceptibility. Tubercle 1964; 45: 64-65.
- Tsukamura M. Screening for atypical mycobacteria. Tubercle 1970; 51: 280-284.
- Rastogi N, Goh K S, David H I. Selective inhibition of the *Mycobacterium tuberculosis* complex by p-nitro-α-acetylamino-β-hydroxypropionophenone (NAP) and p-nitrobenzoic acid (PNB) used in 7H11 agar medium. Res Microbiol 1989; 140: 419-423.
- Martins M C, Ueki S Y M, Palhares M C A, et al. An alternative biphasic culture system for recovery of mycobacteria and

- for differentiation of species other than *M. tuberculosis* complex from blood specimens. *Rev Microbiol* 1997; 28: 183–189.
- 9 Collins C H, Grange J M, Yates M D. Tuberculosis bacteriology organization and practice. 2nd ed. Oxford, UK: Butterworth-Heinemann, 1997.
  - 10 Kent P T, Kubica G P. Public health mycobacteriology. A guide for a level III laboratory. Atlanta, GA, USA: Centers for Disease Control, 1985.
  - 11 Collins T, Levett P N. Radiometric studies on the use of selective inhibitors in the identification of *Mycobacterium* spp. *J Med Microbiol* 1989; 30: 175–181.
  - 12 Giampaglia C M S, Martins M C, Inumaru V T G, Butuem I V, Telles M A S. Evaluation of a rapid differentiation test for the *Mycobacterium tuberculosis* complex by selective inhibition with  $\rho$ -nitrobenzoic acid and thiophene-2-carboxylic acid hydrazide. *Int J Tuberc Lung Dis* 2005; 9: 206–209.
  - 13 Monteiro P H T, Martins M C, Ueki S Y M, Giampaglia C M S, Telles M A S. Cord formation and colony morphology for the presumptive identification of *Mycobacterium tuberculosis* complex. *Brazilian J Microbiol* 2003; 34: 171–174.
  - 14 Tu H Z, Chang S H, Huaug T S, Huaug W K, Liu Y C, Lee S S. Microscopic morphology in smears prepared from MGIT broth medium for rapid presumptive identification of *Mycobacterium tuberculosis* complex, *Mycobacterium avium* complex and *Mycobacterium kansasii*. *Ann Clin Lab Sci* 2003; 33: 179–183.
  - 15 Medeiros R F M, Melo J B, Caldas P C S, Santos L M, Barreto A M W. Utilização de agentes inibidores (PNB e TCH) para identificação de *M. tuberculosis* no sistema MB/Bact. Um estudo preliminar. *Laes & Haes* 2001; 131: 202–204.

## R É S U M É

**CONTEXTE :** Le développement des mycobactéries dans des milieux additionnés de substances inhibitrices a été utilisé pour l'identification des espèces. La croissance du complexe *Mycobacterium tuberculosis* (MTC) est inhibée par l'acide  $\rho$ -nitrobenzoïque (PNB), alors que les mycobactéries non-tuberculeuses (NTM) y sont résistantes.

**OBJECTIF :** Développer un test PNB rapide en utilisant le système automatisé BACTEC MGIT960 et évaluer l'intérêt de ce dépistage pour les isolats mycobactériens.

**SCHÉMA :** On a pratiqué le test PNB sur 93 souches du MTC et sur 61 souches NTM provenant de la collection de cultures de l'Instituto Adolfo Lutz. On a ajouté le PNB au milieu de Löwenstein-Jensen (LJ) et au milieu BACTEC-MGIT960.

**RÉSULTATS :** Toutes les souches MTC ont été sensibles au PNB, ce qui a confirmé leur identification originale. Parmi les 10 souches NTM, toutes se sont avérées résistantes au PNB, sauf une souche de *M. kansasii* et une autre de *M. marinum*. La durée médiane pour l'obtention d'une identification présomptive des MTC par le test d'inhibition dans le système BACTEC MGIT960 a été de 6,3 jours et de 2,5 jours pour les NTM. L'identification présomptive des MTC sur LJ a été le plus souvent obtenue après le 20ème jour.

**CONCLUSION :** L'observation-clé de cette analyse est la possibilité de combiner la méthode admise traditionnellement et proposée par Tsukamura et Tsukamura en 1964 avec la méthodologie moderne sûre et rapide BACTEC MGIT960.

## R E S U M E N

**MARCO DE REFERENCIA :** La adición de sustancias inhibitorias a los medios de cultivo se ha utilizado en la identificación de algunas especies de micobacterias. El ácido  $\rho$ -nitrobenzoico (PNB) inhibe el crecimiento del complejo *M. tuberculosis* (MTC), pero las micobacterias atípicas (NTM) son resistentes al mismo.

**OBJETIVO :** Poner a punto una prueba rápida con PNB utilizando el sistema automatizado BACTEC MGIT960 y evaluar su utilidad en la identificación de los aislados de micobacterias.

**MÉTODO :** Se realizó la prueba PNB con 93 cepas del MTC y 61 cepas de NTM de la colección de cultivos del instituto Adolfo Lutz. El PNB se agregó a los medios Löwenstein-Jensen (LJ) y BBL-MGIT960.

**RESULTADOS :** Todas las cepas del MTC fueron sensibles al PNB y se confirmó así la identificación inicial. De las 10 cepas de NTM, todas fueron resistentes al PNB, con excepción de una cepa de *M. kansasii* y otra de *M. marinum*. La mediana del tiempo necesario a la identificación presuntiva del MTC por la prueba de inhibición en el sistema BACTEC MGIT960 fue 6,3 días y para las NTM fue 2,5 días. La identificación presuntiva del MTC en medio LJ se obtuvo generalmente después de 20 días.

**CONCLUSIÓN :** El hallazgo central del estudio fue la posibilidad de combinar el método tradicional validado propuesto por Tsukamura y Tsukamura en 1964 con un método moderno, seguro y rápido que aplica la técnica del BACTEC MGIT960.