ELSEVIER

Diagnostic Microbiology and Infectious Disease
38 (2000) 83–86
Mycobacteriology

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

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Comparison of *Mycobacterium tuberculosis* susceptibility testing performed with BACTEC 460TB (Becton Dickinson) and MB/BacT (Organon Teknika) systems

Enrico Tortoli[a,*], Romano Mattei[b], Arnaldo Savarino[b], Laura Bartolini[a], Jörg Beer[c]

[a]*Laboratorio di Microbiologia e Virologia, Ospedale di Careggi, viale Morgagni 85, 50134 Firenze, Italy*
[b]*U.O. Analisi Chimico-cliniche e Microbiologiche, Ospedale Campo di Marte, Lucca, Italy*
[c]*Institut für Medizinische Mikrobiologie und Infektionsepidemiologie, Leipzig, Germany*

Abstract

The recently introduced automated culture systems MB/BacT (Organon Teknika, Belgium) was compared with radiometric BACTEC 460TB (Becton Dickinson, USA) to test antimicrobial susceptibility of *Mycobacterium tuberculosis* to first line drugs. On 113 strains 97.5% agreement was obtained, with the difference being not significant. Concordance was practically complete for the most important drugs, isoniazid and rifampin. The two methods however significantly differed for the time needed to complete the test; in fact MB/BacT required on the average five days more than BACTEC 460TB. Despite the delay in the completion of the test, the excellent reliability along with the elimination of radioactivity and full automation make MB/BacT an attractive alternative for susceptibility testing of *M. tuberculosis*.
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1. Introduction

Tuberculosis, with three million deaths each year, is still the infectious disease with the highest morbidity and mortality (Kochi, 1991); besides, tuberculosis control programs are hampered by the steady emergence of drug-resistant strains of *Mycobacterium tuberculosis* (Drobniewski, 1997).

A main role in the control of the spread of tuberculosis is played by diagnostic mycobacteriology; the need for rapid methods of diagnosis and determination of drug susceptibility are particularly important.

Following the increased isolation of multiresistant strains susceptibility testing is now strongly recommended, not only for *M. tuberculosis* strains obtained from patients who fail treatment, but also on primary isolates (Tenover et al., 1993). It is most important to detect resistant and multidrug-resistant strains, whose frequency has greatly increased in recent years and whose rapid detection is of extreme importance to avoid community outbreaks often characterized by high death rates (Drobniewski et al., 1995).

At present there are two major means to assay the drug susceptibility of *M. tuberculosis*, the proportion method performed on Lowenstein-Jensen (Canetti et al., 1963) or on Middlebrook media plates (Wayne & Krasnow, 1966) and the BACTEC 460TB system (Becton Dickinson, USA) (Siddiqi, 1992).

The susceptibility testing using the BACTEC 460TB system has become more and more common in recent years as the only method yielding reliable results within 5–12 days.

The BACTEC 460TB system, which is based on the detection of $^{14}CO_2$ produced by the utilization of a ^{14}C-labeled substrate, has recently been joined by several other rapid and automated culture methods exploiting different technologies. Among them, the MB/BacT system (Organon Teknika, Belgium) (Rohner et al., 1997; Brunello et al., 1999) is based on the measurement of produced CO_2 by using a colorimetric sensor and reflected light technology.

A standardized method, using MB/BacT, has been proposed by the producer to test the susceptibility of *M. tuberculosis* to ethambutol, isoniazid, pirazinamide rifampin and streptomycin. This method was developed on the basis of a fundamental study and the result of a multicenter evaluation (Beer et al., 1977; 1978). We evaluated this procedure in comparison with the standard BACTEC 460TB susceptibility method.

* Corresponding author. Tel.: +39-055-4279199; fax: +39-055-4279830.
E-mail address: tortoli@dada.it (E. Tortoli).

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PII: S0732-8893(00)00176-0

Table 1
Resistance patterns of WHO strains

Drugs	No. of strains
Ethambutol, isoniazid, rifampin, streptomycin	5
Ethambutol, isoniazid, rifampin	2
Isoniazid, rifampin, streptomycin	3
Ethambutol, isoniazid	2
Isoniazid	2
Streptomycin	2
none	4

2. Materials and methods

All the mycobacterial strains used were confirmed to belong to the species *M. tuberculosis* by hybridizing with AccuProbe *M. tuberculosis* complex (Goto et al., 1991), and being positive for niacin accumulation and nitrate reduction (Metchock et al., 1999). They included 87 strains obtained by primary isolation (all coming from different patients), a group of 20 World Health Organization (WHO) strains with known susceptibility (Table 1) sent around the word in 1994 as external quality control for susceptibility testing (Espinal, 1999) and the American Type Culture Collection strains of susceptible *M. tuberculosis* H37Rv (ATTC 27294) and of *M. tuberculosis* resistant to ethambutol (ATTC 35837), isoniazid (ATCC 35822), pyrazinamide (ATCC 35828), rifampin (ATCC 35838), and streptomycin (ATCC 35820).

The BACTEC 460TB system was used following the recommendation of the manufacturer (Siddiqi, 1989), the modified critical concentrations of provided drugs (SIRE, Becton Dickinson) were adopted: 0.1 μg/ml for isoniazid, 2 μg/ml for rifampin, 2.5 μg/ml for ethambutol, and 2 μg/ml for streptomycin (N.C.C.L.S., 1995). In short, actively growing BACTEC 12B cultures which were obtained by inoculating vials with 100 μL of a 0.5 McFarland dense mycobacterial suspension in Diluting fluid (Becton Dickinson) and by incubating it until a growth index (GI) ranging from 500 to 800 was reached, were used to inoculate 12B vials containing single drug concentrations, while an antibiotic-free vial was seeded with an 1/100 dilution of the same culture. Cultures were read daily with BACTEC 460TB instrument until the achievement by the control bottle of a GI $>$ 30; at that point the daily increment of each drug-containing vial was compared with the one of the control and considered resistant if greater than it.

For MB/BacT lyophilized drugs (excluding pyrazinamide) are commercially available in Europe (SIRE, Organon Teknika) their solution with sterile water yields the following final concentrations in the medium: 2 μg/ml for ethambutol, and 1μg/ml for isoniazid, rifampin, and streptomycin. To each of four BacT bottles were added with 0.5 mL of provided Reconstitution solution (without antibiotic supplement) and with 0.5 mL of a single drug. In the control bottle 0.5 mL of distilled water were added, in place of the drug, along with the same volume of Reconstitution solution.

Each antibiotic-containing bottle was inoculated with 0.5 mL of a culture obtained by seeding a MB/BacT bottle with 100 μL of a 0.5 McFarland dense mycobacterial suspension and by incubating it in the MB/BacT cabinet until signaled positive or within next 36 h; the control bottle was inoculated with 0.5 mL of an 1/100 dilution of the same culture. The septum of bottles was disinfected with isopropanilic alcohol before each addition, which was accomplished using tuberculin syringes with non-removable needle, and after the final one. The bottles were then incubated in MB/BacT cabinet until the diluted control signaled positive; at that moment the strain was considered susceptible or resistant to single drugs according to the absence or the presence of signaled growth in the respective bottles.

A different protocol was adopted by both methods for determining the susceptibility to pyrazinamide. In the BACTEC 460TB system two vials of the provided acid medium (PZA test medium, Becton Dickinson) were used, to one of which provided pyrazinamide solution was added to the final concentration of 100 μg/ml, and both were seeded with 100 μL of the above mentioned inoculum culture at the moment it exhibited a GI between 300 and 500. When the GI of the antibiotic-free control exceeded 200 the test was considered concluded and the strain was defined susceptible if the GI in the pyrazinamide-containing medium was less then 9% that in the drug-free medium (Siddiqi, 1989).

In the MB/BacT system two bottles, added with 0.5 mL of Reconstitution solution were used. To one of them 0.5 mL of a pyrazinamide solution (purchased from Sigma) were added to a final concentration of 50 μg/ml, while in the second bottle this was replaced by 0.5 mL of distilled water. The pH of both bottles was lowered to 5.5 by adding 2 mL of filter-sterilized 1M KH_2PO_4 solution; and they were subsequently seeded with 0.5 mL of the above undiluted inoculum culture (Beer et al., 1997). They were than placed in the MB/BacT, along with the bottles prepared to test the susceptibility to the other drugs, until the growth of the non-acid control of the latter was positive. At that moment, according to presence or absence of growth in the pyrazinamide containing bottle, the strain was considered susceptible or resistant to the drug. The acid control, which had to be positive at least 3.5 days before, was only included to verify the adaptability of the strain to grow at acid pH.

In both methods the purity of the culture used for inoculum was checked by seeding a drop on a plate of Middlebrook 7H11 and observing, with a low magnification microscope, the morphology of colonies after a few days incubation in a 5% CO_2-containing atmosphere.

For WHO-certified strains the known susceptibilities were taken to be their susceptibility status. Specificity and sensitivity of MB/BacT system were calculated considering BACTEC 460TB results as the gold standard.

The McNemar χ^2 test was used for the comparison be-

Table 2
Results of susceptibility testing of 113 *M. tuberculosis* isolates determined by MB/BacT and BACTEC 460TB systems

Drug	Strains tested	Results achieved with methods under comparison[a]			
		both S	BactecS BacT R	BactecR BacT S	both R
Ethambutol	113	94	3	2	14
Isoniazid	113	86	—	1	26
Pyrazinamide	106 (+7)[b]	97	1	—	8
Rifampin	113	95	1	—	17
Streptomycin	113	95	3	1	14

[a] S, susceptible; R, resistant
[b] Seven strain were tested with Bactec 460TB only and were all susceptible

tween the two methods and the paired *t* test for the comparison of times needed for test completion.

3. Results

The comparison was carried out on 113 strains of *M. tuberculosis* including the WHO and the ATCC strains. In seven cases (6%) susceptibility to pyrazinamide could not be determined with MB/BacT system because of the failure of such strains to grow in the acidified BacT medium.

With all the mycobacteria with known susceptibility (WHO and ATCC strains) results identical to the expected ones were obtained by both methods.

The proportions of resistant strains, with the BACTEC 460TB system, were as follows: ethambutol 14%, isoniazid 24%, pyrazinamide 7%, rifampin 15% and streptomycin 13%.

The overall agreement between the two methods was 96% and it increased to 97% ignoring the pyrazinamide for the strains in which this drug could not be tested with the MB/BacT method.

Only one discrepancy was obtained for isoniazid, pyrazinamide, and rifampin, four in the case of streptomycin and five for ethambutol (Table 2). In eight cases a susceptibility was reported as a resistance while in four cases the opposite was true. Neither the differences concerning the single drugs nor the one concerning the whole tests were statistically significant on the basis of the McNemar χ^2 test.

Specificity and sensitivity of MB/BacT, i.e., its ability of achieving respectively susceptibility and resistance results overlapping to the ones of BACTEC 460TB are reported in Table 3.

In no case the susceptibility test could not be interpreted because of contamination.

Turnaround times ranged from four to 14 days with BACTEC 460TB and from 4 to 26 for MB/BacT, with an average of 7.6 and 11.6 respectively, ($p < 0.01$, paired *t* test).

4. Discussion

Owing to the increasing frequency of multidrug resistant *M. tuberculosis* rapid knowledge on antimicrobial susceptibility is important. Methods using solid media, either agar- or egg-based, require long times to be read, and radiometric susceptibility testing has been the only rapid method available so far.

The suitability of MB/BacT system for the sensitive and early detection of mycobacteria in culture has been recently tested (Rohner et al., 1997; Brunello et al., 1999) and a good performance, in comparison with BACTEC 460TB, emerged.

In our study a very good agreement between the two systems was obtained, it was almost absolute for the major drugs isoniazid and rifampin and very few were the discrepancies for the remaining first line agents. For the drugs which presented more than one disagreement (streptomycin and ethambutol) discrepancies were present in both directions. This suggests a fortuitous nature of discordance and tends to exclude a systematic tendency of the systems compared here to overestimate susceptibility or resistance to any of the drugs tested. Ethambutol was the drug for which more frequently disagreement was noticed; a similar problem was detected when the adaptability of a manual culture method, MGIT (Becton Dickinson), to test antimicrobial susceptibility of *M. tuberculosis*, was assayed in comparison with radiometric BACTEC 460TB (Rüsch-Gerdes et al., 1999). The presence of two levels of resistance to ethambutol, to

Table 3
Specificity and sensitivity of MB/BacT method to single drugs when BACTEC result was considered the gold standard

	Ethambutol	Isoniazid	Pyrazinamide	Rifampin	Streptomycin
Specificity	96.9	100	98.9	98.9	96.9
Sensitivity	87.5	96.3	100	100	93.3

which were imputed the disagreement in that occasion, may well explain present discrepancies.

The assay of pyrazinamide, although accurate, is limited, in the MB/BacT, by the not yet optimal setting up of the medium which hampered the growth of 6.2% of isolates that, on the contrary, grew well in the slightly less acidic medium (pH 6) used by BACTEC 460TB.

Turnaround times required by two methods significantly differed as, in average, five days more were required to achieve the final results with MB/BacT.

Only one paper, so far, investigated the possible use of MB/BacT for *M. tuberculosis* susceptibility testing (Brunello & Fontana, 2000). The results of the two studies present a very good agreement despite the higher number of resistances in our strains (83 versus 30) and in both the ethambutol testing was the less satisfactory. Only turnaround times differed in the two evaluations with both methods, but particularly with MB/BacT, requiring, in our hands, longer incubation periods. Pyrazinamide had not been investigated previously.

We conclude that MB/BacT system is suitable to test the antimicrobial susceptibility of *M. tuberculosis* to first line drugs. The delay in attainment of final reading is compensated by the elimination of radioactivity and the attendant disposal and by the full automation; the radiometric BACTEC 460TB, being in fact semi-automated, requires the daily loading and reading of vials, which implies important and not fully resolved problems for the laboratories which are closed on Sunday or even in the week-end.

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