CONCISE COMMUNICATION

Evaluation of the BacT/ALERT 3D system for recovery and drug susceptibility testing of *Mycobacterium tuberculosis*

K. A. K. Ängeby[1,2,], J. Werngren[1], J. C. Toro[1], G. Hedström[3], B. Petrini[3] and S. E. Hoffner[1]*

[1]Department of Bacteriology, Swedish Institute for Infectious Disease Control, 171 82 Solna, [2]Division of International Health, Department of Public Health Sciences, Karolinska Institute, Stockholm and [3]Department of Clinical Microbiology, Karolinska Hospital and Institute, Stockholm, Sweden

*Tel: + 46 8 457 24 79 Fax: + 46 8 30 17 97 E-mail: kristian.angeby@smi.ki.se

We evaluated the BacT/ALERT 3D system for recovery and drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (MTB). Of 2659 clinical specimens, MTB was detected in 92 using BacT/ALERT, compared to 94 using Löwenstein–Jensen culture. Detection time was 25% shorter with BacT/ALERT. Sensitivities were 92%, 96%, 78% and 100% for resistance to rifampicin, isoniazid, streptomycin and ethambutol, respectively, while specificity was 100% for all antibiotics, when BacT/ALERT was compared with the BACTEC 460 method on 50 MTB isolates. The BacT/ALERT system is fully automated and creates no radioactive waste. It seems to be a valid alternative for primary isolation, but further evaluation is needed regarding DST.

Keywords Tuberculosis, diagnosis, drug susceptibility testing, new methods

Accepted 14 April 2003

Clin Microbiol Infect 2003; 9: 1148–1152

Mycobacterium tuberculosis (MTB) is a slow-growing organism, which makes recovery and drug susceptibility testing (DST) on solid media laborious and time-consuming [1]. The introduction of the liquid medium semi-automatic radiometric BACTEC 460 system (Becton Dickinson, Sparks, MD, USA) (B460) [2] for culture and DST has significantly shortened turnaround time. However, this system has limitations, such as the necessity of daily manual loading, the possibility of cross-contamination due to the invasive reading, manual data management, and the creation of radioactive waste. Novel non-radiometric systems based on liquid culture have been developed with the aim of avoiding these drawbacks [3–5]. One of them is the BacT/ALERT 3D system [6] (bioMérieux, Durham, NC, USA), which is based on the detection of CO_2 released by actively proliferating mycobacteria into the liquid media. CO_2 lowers the pH in the media, which in turn produces a color change in a sensor in the vial, detected by a reflectometric unit in the instrument. The first version of this system, MB/BacT 240, was introduced in 1995. In 1998, the next generation, BacT/ALERT 3D, was launched. This system has been evaluated previously, but recently the manufacturer has introduced a new protocol for DST. The aim of our study was to evaluate the implementation of this technique compared to (1) culture on solid Löwenstein–Jensen (L-J) media for recovery of mycobacteria and (2) B460 for drug DST of MTB against four first-line antituberculosis drugs using the new protocol.

The study was performed at the Department of Clinical Microbiology, Karolinska Hospital, which receives all specimens for mycobacterial culture from central Sweden. During the period from January to July 2002, consecutive routine clinical specimens were cultured using the BacT/ALERT 3D system concomitantly with conventional solid L-J substrates. The specimens consisted of bronchoalveolar lavage fluids, fluids from various joints, pleural, peritoneal and cerebrospinal fluid, secretions, blood, bone marrow and biopsies, but not sputum, gastric aspirates, urine or feces.

Specimens from non-sterile sites were decontaminated using the NaOH–sodium lauryl sulfate method, which is the routine technique used in Sweden, supplemented with *N*-acetyl-L-cysteine 0.5% [7,8]. The material was centrifuged at 3500 *g*

and washed once with phosphate buffer, pH 6.8. Of the homogenized specimen, 0.2–0.3 mL was inoculated into one tube of L-J medium and one tube of L-J with glycerol replaced by 0.6% sodium pyruvate. Moreover, 0.5–0.6 mL was inoculated into one BacT/ALERT MP process bottle, which contained 10 mL of modified Middlebrook 7H9 broth supplemented with bovine serum albumin, catalase and casein. Before inoculation, MB/BacT antibiotic supplement (amphotericin B, azlocillin, nalidixic acid, polymyxin B, trimethoprim, vancomycin) in 0.5 mL of reconstitution fluid (amaranth, glycerol, Tween-80, purified water) (bioMérieux) was added to the process bottle, following the manufacturer's instructions. Both substrates were incubated for 7 weeks at 37 °C. L-J tubes were read for mycobacterial growth once weekly, and colonies were examined by microscopy. The BacT/ALERT instrument performed readings automatically every 10 min, and the results were easily available on a computer screen connected using the BacT/VIEW data management system (bioMérieux). The preliminary microscopic diagnosis of *M. tuberculosis* complex was confirmed by DNA probe testing (AccuProbe, GenProbe Inc., San Diego, CA, USA).

DST was carried out at the Swedish Institute for Infectious Disease Control. A panel of 50 clinical isolates (20 of which were fully susceptible and 30 of which had different resistance patterns), selected from the SMI strain collection, and the susceptible MTB reference strain H37Rv (ATCC 25618), were used. All strains were kept at −70 °C and grown on L-J medium prior to use.

For DST in the BacT/ALERT 3D system, an inoculum was prepared by dispensing and vortexing two 1-μL loops of bacteria from fresh L-J cultures in 7.5-mL screw-cap bottles containing 3 mL of phosphate-buffered saline (PBS) and a few 3-mm glass beads. Of the bacterial suspension (turbidity approximately McFarland no. 1), 0.5 mL was injected into a BacT/ALERT MP process bottle already enriched with 0.5 mL of BacT/ALERT reconstitution fluid, and the bottles were then incubated in the BacT/ALERT 3D instrument until it flagged positive for growth. Thereafter, DST took place according to the manufacturer's new protocol, with 0.5 mL of the positive BacT/ALERT process bottle as inoculum. Antibiotic stock solutions were prepared using the BacT/ALERT RISE susceptibility kit consisting of bottles with lyophilized antibiotics, which were dissolved in BacT/ALERT restoring fluid (glycerol, oleic acid, sodium salt) before use. After vortexing of the positive process bottle for 30 s, 0.5 mL was injected into (1) five process bottles with 0.5 mL of the antibiotic stock solutions added, (2) a process bottle supplemented with 0.5 mL of BacT/ALERT restoring fluid added to form a direct control, and (3) a BacT/ALERT dilution blank to form a 1 : 10 dilution, which was then inoculated into another restoring fluid-enriched process bottle to form a proportional control. The final antibiotic concentrations were rifampicin 0.9 mg/L, isoniazid 0.09 mg/L and 0.4 mg/L, streptomycin 0.9 mg/L, and ethambutol 3.5 mg/L. The bottles were incubated at 37 °C in the instrument until it flagged positive for growth in the proportional control bottle and the susceptibility results could be interpreted. As per the manufacturer's definitions, a strain was resistant to a given antibiotic if growth was detected in the antibiotic-containing bottle before or at the same time as the proportional control. Consequently, a strain was considered susceptible if no growth was detected in the antibiotic bottle before or at the same time as the proportional control. The test was regarded as invalid if one or both controls were positive for growth in less than 2 days or in more than 15 days, or if there was less than 1 day between the growth in the direct and proportional controls. Moreover, a test was judged invalid if there was growth in an antibiotic bottle more than 0.5 days before growth in the direct control. All invalid tests were repeated. B460 results had been achieved previously by standard procedures [2] using the following critical concentrations: rifampicin 2.0 mg/L, isoniazid 0.2 mg/L, streptomycin 4.0 mg/L and ethambutol 5.0 mg/L. The two methods were compared with regard to DST results and turnaround time. The lower isoniazid concentration used in the BacT/ALERT system was the one used in the comparison with BACTEC.

Of 2659 mycobacterial cultures from various routine specimens, equivalent numbers of MTB and non-tuberculous mycobacteria (NTM) were detected on BacT/ALERT and L-J substrates (Table 1). The mean detection time for MTB was 18 days with BacT/ALERT compared to 24 days with L-J. NTM were detected twice as fast by BacT/ALERT as by L-J culture. In both BacT/ALERT and L-J, fewer than 1% of cultures were contaminated by irrelevant microorganisms.

Table 1 Recovery of mycobacteria from 2659 clinical routine specimens in BacT/ALERT cultures and on Löwenstein–Jensen (LJ) media

	M. tuberculosis complex ($n = 102$)		Non-tuberculous mycobacteria ($n = 48$)	
	BacT/ALERT	**L-J**	**BacT/ALERT**	**L-J**
Positive cultures (*n*)	92	94	37	34
Detection time (days), median (range)	18 (6–31)	24 (11–30)	11 (4–35)	20 (14–52)

Testing of 50/51 MTB strains for their susceptibilities to rifampicin, isoniazid, streptomycin and ethambutol gave an overall agreement of 96.5% between the B460 and the BacT/ALERT methods (193/200 individual susceptibility tests) (Table 2). One strain had to be excluded, since it could not be interpreted by the BacT/ALERT method because there was no growth in the proportional control, even though the test was repeated twice. The sensitivity (in detecting drug resistance) of the BacT/ALERT compared to that of the BACTEC 460 technique was 91% (69/76 tests), and the specificity was found to be 100% (124/124 tests). Full correlation was found in the results for ethambutol. Good agreement was also found for isoniazid, but some discrepancy was seen when sensitivity was tested for rifampicin resistance, and even more so for streptomycin resistance (Table 2). The outcome was entirely concordant for 44/50 strains, while results were conflicting for six strains in seven tests.

One ethambutol test had to be repeated because of contamination in the bottle, and isoniazid and rifampicin tests had to be repeated in two strains (following the manufacturer's instructions), because the tests were invalid due to growth in the antibiotic bottles more than 0.5 days earlier than in the direct control. The repeated tests were valid and concordant with B460. One strain that was resistant to the lower, but susceptible to the higher, isoniazid concentration using BacT/ALERT was classified as resistant by B460. BacT/ALERT results were ready in a median time of 11 days (range: 7.1–26 days), including preparation of inoculum in the MB/BacT process bottle and DST. The median time for inoculum preparation was 5.5 days (range: 2.5–19 days), and that for DST was 6.1 days (range: 4.2–10 days). The BACTEC 460 results had been obtained previously, and the results were normally evaluated after 7–8 days of incubation.

MTB was detected 25% earlier and with equivalent sensitivity with BacT/ALERT compared to L-J. NTM were detected even faster with BacT/ALERT. It should be noted, however, that L-J cultures were read by eye once a week, whereas

Table 2 Susceptibility results of the BacT/ALERT 3D system compared to the BACTEC 460 system

	BacT/ALERT 3D			
Drug and BACTEC 460 results	**Resistant**	**Susceptible**	**Sensitivity (%)**	**Specificity (%)**
Rifampicin				
Resistant	23	2	92	
Susceptible	0	25		100
Isoniazid				
Resistant	26	1	96	
Susceptible	0	23		100
Streptomycin				
Resistant	14	4	78	
Susceptible	0	32		100
Ethambutol				
Resistant	6	0	100	
Susceptible	0	44		100
Total				
Resistant	69	7	91	
Susceptible	0	124		100

growth of mycobacteria was signaled by the BacT/ALERT system as soon as the culture had reached the detection limit.

Manual work with cultures can be minimized by the BacT/ALERT system; only positive cultures need to be handled after signaling, and negative cultures, which are the majority, are reported automatically via the laboratory central computer system. L-J cultures, on the other hand, must be read and reported manually and entered into the laboratory computer, thus increasing the workload in the laboratory. There was a low rate of contamination in both the BacT/ALERT broth cultures and in L-J solid media, probably because most specimens were from sterile sites.

As far as we know, this is the first study evaluating the performance of the manufacturer's new protocol for DST of MTB in the BacT/ALERT system. In our study, the sensitivity in detecting drug resistance was satisfactory for ethambutol and isoniazid, but far too low to be acceptable for rifampicin and streptomycin. The specificity, however, was 100% for all tested antibiotics. One explanation for this seemingly systematic discordance may be that the new protocol utilizes a 1 : 10 diluted proportional control, instead of a 1 : 100 diluted one, which is used in B460, the proportion method on solid media, and also in bioMérieux's previous protocol. A 1 : 10 diluted control shortens turnaround time, since the results are read when this control turns positive, but there might consequently be a risk of false susceptibility results. Moreover, the new protocol utilizes one-tenth of the isoniazid concentration (0.09 versus 1.0 mg/L) of the old protocol. In our study, the specificities were at least as high as those reported by other groups who have evaluated the BacT/ALERT 3D with the previous protocol. Nevertheless, their sensitivity in detecting resistance to rifampicin and isoniazid was higher in all cases [9–11].

There are several practical points to address in this system. The DST procedure might be simplified by avoiding the preinoculation step. Some antibiotic concentrations might be changed for practical and logical reasons; for example, the isoniazid concentration of 0.09 mg/L could be changed to 0.1 mg/L. The computer program BacT/VIEW data management system could be developed to better suit the slow metabolism of mycobacteria, with better discrimination of the growth curves. The criteria might be changed so that antibiotic bottles are counted as valid even when positive 0.5 days before the direct control. Finally, it is unfortunate that the equipment and reagents are too expensive to be used in most settings with high tuberculosis incidence.

Taken together, our results suggest that the BacT/ALERT 3D system is a valid alternative for the recovery of mycobacteria. However, further development to improve the sensitivity of detection of drug resistance seems to be necessary. The new method provides computerized results, the automatic, non-invasive readings are likely to increase biosafety, and no radioactive waste is produced.

ACKNOWLEDGMENTS

We thank bioMérieux for temporarily providing the BacT/ALERT 3D instruments free of charge. Some of these results were presented at the 12th European Congress of Clinical Microbiology and Infectious Diseases in Milan, Italy, 24–27 April 2002.

REFERENCES

1. Canetti G, Froman S, Grosset J *et al.* Mycobacteria. Laboratory methods for testing drug sensitivity and resistance. *Bull WHO* 1963; 29: 565–78.
2. Siddiqi SH, Libonati JP, Middlebrook G. Evaluation of a rapid radiometric method for drug susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 1981; 13: 908–12.
3. Pfyffer GE, Welscher HM, Kissling P *et al.* Comparison of the Mycobacteria Growth Indicator Tube (MGIT) with radiometric and solid culture for recovery of acid-fast bacilli. *J Clin Microbiol* 1997; 35: 364–8.
4. Tortoli E, Cichero P, Chirillo MG *et al.* Multicenter comparison of ESP Culture System II with BACTEC 460TB and with Lowenstein–Jensen medium for recovery of mycobacteria from different clinical specimens, including blood. *J Clin Microbiol* 1998; 36: 1378–81.
5. Rohner P, Ninet B, Metral C, Emler S, Auckenthaler R. Evaluation of the MB/BacT system and comparison to the BACTEC 460 system and solid media for isolation of mycobacteria from clinical specimens. *J Clin Microbiol* 1997; 35: 3127–31.
6. Piersimoni C, Scarparo C, Callegaro A *et al.* Comparison of MB/Bact alert 3D system with radiometric BACTEC system and Lowenstein–Jensen medium for recovery and identification of mycobacteria from clinical specimens: a multicenter study. *J Clin Microbiol* 2001; 39: 651–7.

7. Isenberg HD, ed. *Clinical microbiology procedures handbook*, Vol. 1. Washington, DC: American Society for Microbiology, 1992: 3.4.1–3.4.2.
8. Kubicka GP, Dye E, Cohn ML, Middlebrook G. Sputum digestion and decontamination with N-acetyl-L-cysteine-sodiumhydroxide for culture of mycobacteria. *Am Rev Respir Dis* 1963; 87: 775–9.
9. Brunello F, Fontana R. Reliability of the MB/BacT system for testing susceptibility of *Mycobacterium tuberculosis* complex isolates to antituberculous drugs. *J Clin Microbiol* 2000; 38: 872–3.
10. Diaz-Infantes MS, Ruiz-Serrano MJ, Martinez-Sanchez L, Ortega A, Bouza E. Evaluation of the MB/Bact mycobacterium detection system for susceptibility testing of *Mycobacterium tuberculosis*. *J Clin Microbiol* 2000; 38: 1988–9.
11. Tortoli E, Mattei R, Savarino A, Bartolini L, Beer J. Comparison of *Mycobacterium tuberculosis* susceptibility testing performed with BACTEC 460TB (Becton Dickinson) and MB/BacT (Organon Teknika) systems. *Diagn Microbiol Infect Dis* 2000; 38: 83–6.