Comparative Evaluation of BacT/ALERT 3D and BACTEC Systems for the Recovery of Pathogens Causing Bloodstream Infections

Wafaa Jamal    Gigette Tamaray    Aleyamma Pazhoor    V.O. Rotimi
Microbiology Unit, Department of Laboratory Medicine, Mubarak Al-Kabeer Hospital, Kuwait

Key Words
BacT/ALERT blood culture system · BACTEC 9240 blood culture system · Bacteraemia

Abstract
Objective: To compare BacT/ALERT (BTA) and BACTEC 9240 (BAC), two continuously monitoring automated blood culture systems, for the recovery of bloodstream pathogens and standard media available for these systems. Materials and Methods: Blood samples from 100 adults and 50 paediatric patients suspected of having bloodstream infections were inoculated at the bedside into non-vented BTA and BAC standard blood culture bottles and incubated in their respective instruments. The time to growth detection (TD) was recorded for each bottle that became positive. A quantitative assay was also carried out with 5 standard bloodstream pathogens to assess TD of each pathogen as well as the quantity of organisms recovered. Results: A total of 23 isolates representing true infections were recovered by both BTA and BAC bottles, indicating a blood culture positivity rate of 15.3%, 18 (78.3%) by BTA bottles and 13 (56.5%) by BAC. Proteus mirabilis, Pseudomonas aeruginosa and Clostridium perfringens were recovered only by the BTA system. The average TDs were 19.0 and 24.6 h for BTA and BAC, respectively. Analysis of the quantitative growth of known pathogens in both systems was more or less the same for Staphylococcus aureus, Escherichia coli and P. aeruginosa but slightly different for Haemophilus influenzae and Streptococcus pneumoniae. The anaerobic bottle of the BTA did not support the growth of H. influenzae below an inoculum of $10^9$ CFU/ml whereas the BAC did so at a lower inoculum of $10^6$ CFU/ml. TD for S. pneumoniae in the BTA was about half of that in the BAC. Conclusions: The BTA system appears to be more efficient in detecting common bloodstream pathogens as a higher inoculum is needed for the BAC system to detect the same organism.

Introduction
Blood cultures are considered to be one of the most significant specimen types that a microbiology laboratory processes, and every laboratory has a strict notification policy to ensure that positive blood cultures are promptly reported to the physician [1]. Septicaemia-attributable mortality is estimated to be 15%, and it represents the 8th most frequent cause of death in the USA [2]. These deaths, in most cases, are preventable. It is therefore important
to detect the causative micro-organism as early as possible so that an appropriate empirical therapy may be instituted promptly. The most efficient method of detecting these pathogens is blood culture.

Blood-culturing methods have progressed from the days of conventional bottles of nutrient broths cultured in ordinary incubators and subcultured contingent upon visual judgements to modern automated systems. Undoubtedly, continuously monitored automated instruments have greatly improved the efficiency of blood cultures. For example, about 89% of positive blood cultures are detected within the first 24 h by these systems [3]. However, due to clinical importance, each hour of earlier detection is crucial. BacT/ALERT 3D (BTA; Bio Mérieux Inc., France) and BACTEC 9240 (BAC; BD Diagnostic Systems, Sparks, Md., USA) are the most commonly used continuously monitored automated blood culture systems in many diagnostic microbiology laboratories. Both systems utilize soybean-casein digest broths. However, standard aerobic media available for them differ in supplements and polyanethol sulphonate concentrations. In most published reports, only scanty information is available concerning clinically controlled comparison between these two systems for the detection of bloodstream pathogens as well as the quantitative evaluation of the bacterial growth in each system [4]. The objective of this study was to compare the efficiency of the recovery of pathogens obtained directly from patients as well as in vitro quantitative growth assessment in the recently improved BTA in comparison with the BAC.

Materials and Methods

Blood Sample

Blood was collected from 100 consecutive adult and 50 paediatric patients with fever suspected of having bacteraemia or septicaemia. All blood culture collections were performed as part of routine patient care. Venepuncture sites were disinfected with alcohol followed by povidone-iodine and allowed to dry. A sample of 40 ml of blood was collected, 10 ml injected aseptically into aerobic and anaerobic BTA and BAC bottles for adults and, depending on the age, 0.5–5 ml into the paediatric bottles for children.

Handling of Blood Culture Bottles

Once the bottles had been received in the laboratory, they were placed into their respective BTA and BAC instruments and incubated until a positive signal was obtained from the instrument or for a maximum of 7 days. The flagged bottle was removed and processed as per the manufacturer’s instructions, i.e. an aliquot of blood-broth mixture was removed with a sterile needle and syringe. A portion was used for Gram staining and the remainder was sub-cultured onto a set of selective and non-selective media. Subsequent isolation, identification and susceptibility testing of the organism were performed according to the Standard Operating Procedures of the laboratory.

Clinical Assessment and Definitions

Each positive culture was reviewed by two clinical microbiologists to determine if it was true positive or contaminant. The assessment was done according to published criteria [5] and without knowledge of the identity of the bottles. ‘True-positive’ was taken as growth of a micro-organism that was considered a pathogen when isolated from a patient with signs and symptoms of bloodstream infection. ‘Contaminant’ was defined as a single positive culture of an organism that is usually considered a contaminant without a plausible source, a single positive culture of an organism that is considered as a contaminant with a plausible source in a clinically well patient or a mixed culture of 2 or more organisms from a clinically well patient. ‘False-positive’ was defined as the Gram-stain-negative and subculture-negative bottles flagged by the instrument. Terminal subculture was not done, as a pilot study in our laboratory had earlier failed to demonstrate any positive culture among the negative blood culture bottles at the end of 7 days’ incubation for a period of 1 year.

Quantitative Evaluation

0.1-ml amounts of 10-fold (serially) diluted overnight broth cultures of 5 standard bloodstream pathogens, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Haemophilus influenzae* and *Streptococcus pneumoniae*, were seeded into 10 ml broth of both BTA and BAC bottles labelled $10^{-1}$ to $10^{-8}$ and incubated in corresponding machines. The broth for BTA was soybean-casein digest with charcoal for paediatric bottles and pancreatic digest for adults while that of BAC was enriched soybean-casein digest. The specific bacterial strains were chosen because they represent over 80% of agents isolated from cases of bacteraemia in our hospital. Viable count of the pathogen in each inoculum was made according to a standard method [6] to ascertain the bacterial count of each pathogen in each inoculum seeded into individual bottles. Upon a positive signal by the instruments, time to detection (TD) was recorded and then plotted against concentration (viable counts, $\log_{10}$) of the inoculum. Two blood culture contaminants, *Staphylococcus epidermidis* (coagulate-negative staphylococci) and *Corynebacterium* sp. (‘diphtheroid’), were also tested in parallel.

Results

Clinically Significant Isolates

Of the 150 blood cultures, 27 were positive, 23 representing clinically significant isolates and 4 insignificant (contaminants) isolates. The recovery rates of clinically significant isolates by BTA and BAC were 18/150 (12%) and 13/150 (8.7%), respectively (table 1). Of the 23 significant isolates, 18 (78.3%) were recovered by BTA compared with 13 (56.5%) by BAC. Of the 50 paediatric patients investigated, 2 (4%) were positive according to BTA and 4 (8%) according to the BAC. Of the 16 and 9
isolates recovered in adults by BTA and BAC, respectively, 11 (68.8%) and 7 (77.8%) were Gram-positive bacteria. All of the 4 judged as contaminants were recovered by the BAC system; 2 of the 4 were recovered by BTA. The average TD in BTA was 19.0 h versus 24.6 h in BAC (table 1). The mean TD for *Enterococcus faecalis* and *Streptococcus mitis* was faster overall in BTA than in BAC. Interestingly, the BAC system failed to detect *Proteus mirabilis*, *P. aeruginosa* and *Clostridium perfringens* within 72 h; none of these patients had been on any antibiotic before blood was obtained for culture.

**In vitro Quantitative Evaluation of Growth Media**

Both systems supported the growth of wild strains of *S. aureus*, *P. aeruginosa* and *E. coli* down to the lowest inoculum of $10^4$ CFU/ml. The average TDs were 4 and 6 h faster in the BTA system than in the BAC system for these organisms, respectively. However, the TDs for *H. influenzae* and *S. pneumoniae* were different by both systems. The growth of *H. influenzae* was better supported by the BAC than by the BTA system. In the aerobic bottle, the BTA system supported growth at a concentration of $\geq 7.5 \log_{10}$ CFU/ml whereas in the BAC medium, the concentration was 2 logs lower ($5.1 \log_{10}$ CFU/ml). In the anaerobic bottle, the TD in the BTA medium was 96 h at the highest concentration (10.2 $\log_{10}$ CFU/ml) studied, whereas in the BAC medium, it was 8.75–15.0 h at 2 $\log_{10}$ and 8.2 $\log_{10}$ CFU/ml, respectively. The difference in the detection limit was 2 $\log_{10}$ lower in BAC than BTA. For *S. pneumoniae*, under aerobic incubation, the detection limits were the same for both BTA and BAC media, ranging from 5.3 to 8.5 $\log_{10}$ CFU/ml, but the TD was more than twice as long in the BAC (13.09–20.1 h) than the BTA system (6.16–10.7 h). In the anaerobic medium, BTA did not support growth of *S. pneumoniae* while BAC supported growth up to a concentration of $7.2 \log_{10}$ CFU/ml, detected at 22.08 h. Two known contaminants (*S. epidermidis* and *Corynebacterium sp.*) tested along with the other pathogens were detected by both the BTA and the BAC systems but were detected faster by the BTA (17 h) than the BAC (36 h).

**Table 1. Comparative yield of clinically significant bacteria in BTA and BAC blood culture bottles**

<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>Isolates detected by</th>
<th>TD, h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>both bottles</td>
<td>BTA bottles only</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Streptococcus mitis</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa + Enterococcus faecalis</em></td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Escherichia coli + Enterococcus faecalis</em></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><em>Escherichia coli + Staphylococcus haemolyticus</em></td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

= = No growth.

**Discussion**

Blood cultures are among the most frequently ordered and clinically important tests performed in a clinical microbiology laboratory. Recognizing this importance, manufacturers have produced a wide variety of commercial blood culture products that may be evaluated in clinical trials. Therefore, clinical microbiology laboratory directors and supervisors have to decide on the optimal blood culture system for use in their institutions. This important decision depends on several factors, which include evaluation of performance of the system, its cost, safety, space, specimen capacity, patient population, degree of user maintenance required and the ability of the manufacturer or local supplier to support the system.

In this study, the performance of two commonly used blood culture instruments, the BTA and BAC systems, was compared. The study suggests that the BTA performed better than the BAC system for the detection of clinically important pathogens directly from the patients, except for *H. influenzae*, where the BAC system was the
more sensitive of the two. An explanation for this unexpected inhibitory effect on *H. influenzae* in BTA may have been due in part to a high level of CO₂ (>20%) in the bottles, which far exceeded the normal level of 5% required for its growth. In addition, some strains of *H. influenzae* are sensitive to the anticoagulant present in the media. This finding, with respect to BTA performance on other important pathogens, is similar to an earlier report by Mirrett et al. [4], who also found this system to be superior in the detection of coagulase-negative staphylococci and yeasts, which are important pathogens nowadays. At the same time, they found that BTA improved the recovery of contaminants like coagulase-negative staphylococci. In our study, yeasts were not recovered from the clinical samples by neither the BTA nor the BAC systems, but this may be due to the relatively small number of the patients studied compared to the larger study of Mirrett et al. [4].

Most importantly, the time to detection is crucial for the management of septicaemic patients. The earlier the pathogen is detected, the better, with respect to initiating appropriate empiric therapeutic regimes. A gain of 2 h or more for TD is therefore significant in this regard. The average TD by BTA of 19 h versus about 25 h by the BAC system (about 6 h difference) supports BTA as the superior system in this evaluation. Additionally, an advantage of the new BTA is its sleekness and much smaller size than the BAC, thereby saving considerable bench space in the laboratory.

Most blood culture investigations have originated from studies on clinical specimens. The bacterial load in adult bacteraemia can be very low, often less than 1 CFU/ml [7]. In children, the bacterial load is believed to be higher (10–100 CFU/ml), which is the justification for drawing half of the volume of adults' blood injected into the bottles. However, a report by Kellogg et al. [8] has shown that less than 10 CFU/ml, and even as low as 1 CFU/ml, resulted in positive blood cultures in 60 and 23%, respectively, of 137 paediatric septicaemic cases. The limit of detection in this study for the clinical specimens was similar in both systems.

The BAC system failed to detect bacteraemia due to *C. perfringens* which was detected by BTA. The explanation for this discrepancy is not clear at this time, but it is probably due to an inherent problem of the machine, its media, or it may possibly be related to the inoculum. It is also conceivable that the machine failed to detect the organism because of low numbers in the blood at the time of collection. Perhaps performing a terminal blood culture, which we did not do, might have been helpful. Although a decrease in anaerobic bacteraemia was observed in the early 1990s [9], recent publications have shown that anaerobes were considered an important cause of hospital-acquired bacteraemia. Garrouste-Orgeas et al. [10] found that 13.5% of all hospital-acquired bacteraemias were due to anaerobes. In addition, Warren et al. [11] once reported that 6% of 38 bacteraemic cases in the intensive-care unit were attributable to anaerobes, as did the report of Vigano et al. [12], which found that 4% of 916 bacteraemic episodes were due to anaerobes in 8 hospitals in the Lombardia region. Therefore, a system that cannot reliably detect the presence of anaerobes during anaerobic bacteraemia is not adequate for the general blood culturing system in a routine diagnostic hospital laboratory.

The quantitative assessment of the growth media in both bottles shows that they do support the growth of several blood-borne pathogens. However, the pathogens appear to grow faster in the BTA than in the BAC system.

**Conclusion**

The BTA system appears to be more efficient in detecting common bloodstream pathogens than the BAC system, except for the BTA anaerobic bottle, which was not as efficient in the isolation of *H. influenzae* as the BAC system.
References


