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# Evaluation of Negative Results of BacT/Alert 3D Automated Blood Culture System

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**Although automated continuous-monitoring blood culture systems are both rapid and sensitive, false-positive and false-negative results still occur. The objective of this study, then, was to evaluate negative results occurring with BacT/Alert 3D blood culture systems. A total of 1032 samples were cultured with the BacT/Alert 3D automated blood culture system, using both aerobic (BPA) and anaerobic (BPN) media, and 128 of these samples yielded positive results. A total of 904 negative blood samples were then subcultured in 5% sheep blood agar, eosin methylene blue, chocolate agar, and sabouraud-dextrose agar. Organisms growing on these subcultures were subsequently identified using both Vitek32 (bioMerieux, Durham, NC) and conventional methods. Twenty four (2.6%) of the 904 subcultures grew on the subculture media. The majority (83.3%) of these were determined to be gram-positive microorganisms. Fourteen (58.3%) were coagulase-negative staphylococci, two (8.3%) were *Bacillus* spp., one (4.2%) was *Staphylococcus aureus*, and one (4.2%) was identified as *Enterococcus faecium*. *Streptococcus pneumoniae* and *Neisseria* spp. were isolated together in two (8.3%) vials. Gram-negative microorganisms comprised 12.5% of the subcultures, of which two (8.3%) were found to be *Pseudomonas aeruginosa*, and one (4.2%) was *Pseudomonas fluorescens*. The other isolate (4.2%) was identified as *Candida albicans*. We conclude that the subculture of negative results is valuable in the BacT/Alert 3D system, especially in situations in which only one set of blood cultures is taken.**

***Key words***: BacT/Alert 3D, false-negative results, subculture

Bacteremia is an extremely important clinical condition. If left untreated, bacteremia may result in sepsis and, ultimately, death. The identification of the causative agent is a prerequisite for accurate and effective antimicrobial therapy in cases of bacteremia (Nicholls *et al.*, 2000). Microorganisms in the blood can be detected by automated systems, variants of which are currently used worldwide. The introduction of these systems in a clinical context has resulted in a marked reduction in the time required to detect infections in the bloodstream (Gimenez *et al.*, 2002). Most of these automated systems are predicated on the detection, either by fluorescent or colorimetric methods, of carbon dioxide, the concentrations of which increase as the result of microorganism growth, changes in pH values, or changes in redox potential (Rohner *et al.*, 1995; Qian *et al.*, 2001). One such system, the BacT/Alert 3D automated blood culture system (bioMerieux, USA), monitors blood cultures with a colorimetric $CO_2$ sensor, which is internally attached to the bottom of the blood culture bottles. Positive cultures are recognized by a computer-driven algorithm, which monitors both initial and increased concentrations of $CO_2$ (Rohner *et al.*, 1995).

Blood culture media, subculture methods, and incubation time vary from one laboratory to another. Both true- and false-positive results in these systems have been previously evaluated and compared with each other, as well as with the results generated by other methods (Rohner *et al.*, 1995; Qian *et al.*, 2001; Mirrett *et al.*, 2003; Daxboeck *et al.*, 2004). The necessity of routine subculturing of negative results on automated blood cultures was evaluated more than twenty years ago, and the value of terminal subcultures was reported to be negligible, regardless of hospital setting (Campbell *et al.*, 1980; Araj *et al.*, 1981). However, extended incubation and terminal subculture have been reported to be worthwhile in selected conditions such as suspected endocarditis, or when symptoms of persistent or recurrent infection are detected in the absence of positive cultures (Campbell *et al.*, 1980). As false negative results can clearly affect the course of antibiotic therapy selected and the eventual disease outcome, our objective in organizing this study was to evaluate the occurrence of negative blood culture results on the BacT/Alert 3D automated blood culture system, and to accurately determine the rate of false negative results, by cul-

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turing these negative results using traditional classical methods.

## Materials and Methods

A total of 1032 blood cultures, collected between December 2003 and June 2004 at the Gaziantep University Hospital, were incubated with the BacT/Alert 3D automated blood culture system (bioMérieux, USA), using both aerobic (BPA) and anaerobic (BPN) media. All of the bottles were inoculated at bedside, with 5 to 10 ml of blood extracted by venipuncture of the antecubital veins of patients who exhibited signs and symptoms of systemic infection, including chills and fever (body temperature of >38°C). The bottles were then incubated for a period of seven days. After seven days, the negative bottles were removed from the instrument, and subcultured with 5% sheep blood agar, eosin methylene blue, chocolate agar, and sabouraud-dextrose agar. The subcultures were incubated for 24 - 48 h at 36°C in an atmosphere containing 5% $CO_2$. Microorganisms which had grown on the culture media were then identified with the Vitek 32 (bioMerieux, USA) system, along with conventional methods. Cultures which were negative according to the BacT/Alert 3D system but positive on terminal subculture were considered false negatives.

## Results

A total of 1032 samples were cultured with the BacT/Alert 3D automated blood culture system, 128 of which scored positive. Bacterial growth was detected in 24 (2.6%) of the 904 subcultures from the BacT/Alert 3D bottles. As is shown in Table 1, the majority of the isolates recovered from the false negative bottles were identified as gram-positive microorganisms (83.3%). Of these gram-positive microorganisms, 14 (58.3%) were found to be coagulase-negative staphylococci, two (8.3%) were *Bacillus* spp., one (4.2%) was *Staphylococcus aureus,* and one (4.2%) was ultimately identified as *Enterococcus faecium. Streptococcus pneumoniae* and *Neisseria* spp. were isolated together in two (8.3%) vials. Three subcultures (12.5%) contained gram-negative rods, two of which (8.3%) were *Pseudomonas aeruginosa*, and one (4.2%) was identified as *Pseudomonas fluorescens*. The remaining microorganism (4.2%) was identified as *Candida albicans.* One of the patients from whom *S. pneumoniae* was isolated died of meningitis two days after her blood culture was obtained. The second case of *S. pneumoniae* was diagnosed with community-acquired pneumonia, and that patient recovered after being treated with antimicrobials.

**Table 1.** Positive terminal subcultures from 904 previously negative BacT/Alert3D blood cultures

| Microorganism | Number (%) |
|---|---|
| Coagulase negative *Staphylococci* | 14 (58.3%) |
| *Bacillus* spp. | 2 (8.3%) |
| *Staphylococcus aureus* | 1 (4.2%) |
| *Pseudomonas aeruginosa* | 2 (8.3%) |
| *Pseudomonas fluorescens* | 1 (4.2%) |
| *Enterococcus faecium* | 1 (4.2%) |
| *Candida albicans* | 1 (4.2%) |
| *Streptococcus pneumoniae*+ *Neisseria* spp. | 2 (8.3%) |
| Total | 24 (100.0%) |

## Discussion

This study demonstrated that 2.6% of results generated by the BacT/Alert 3D system are false-negatives. Culture and antimicrobial susceptibility tests of pathogens are crucial for the effective antimicrobial treatment of bacteremia. In addition, the early detection of causative agents allows for a reduction in the use of broad spectrum antibiotics, as well as concomitant savings with regard to both time and cost (Wellinghausen *et al.,* 2004). False-negative results constitute significant errors, which can radically affect clinical outcomes in bacteremic patients. The false negative rates of automated blood culture systems have been evaluated in several studies, and are normally reported to be less than 3%. In a study which compared the VITAL and BacT/Alert blood culture systems, false-negatives were not observed in association with the BacT/Alert system (Fontanals *et al.,* 1998). Studies which evaluated the accuracy of the BacT/Alert system, the previous version of the BacT/Alert 3D system, indicated a false-negative rate of between 0.2% and 0.4% (Hardy *et al.,* 1992, Alfa *et al.,* 1995). Other continuous monitoring blood culture systems, including BACTEC 9240, have evidenced false-negative rates of less than 0.1% (Schwabe *et al.* 1995). Currently, the routine subculturing of 5- to 7-day negative cultures has been considered unnecessary with continuous-monitoring blood culture systems, as the false-positive rates are remarkably low, and the majority of these have been attributed to the influence of contaminant organisms (Hardy *et al.,* 1992). However, Shigei *et al.* (Shigei *et al.,* 1995) reported an additional 6.0% of positive results were detected by terminal subculture, although this applied only to the BACTEC 9240 system. The false-negative cultures consisted primarily of *Pseudomonas* spp., *Staphylococcus* spp., and yeasts. Gimenez *et al.* (Gimenez *et al.,* 2002) reported a 0.6% false-negative rate with the VITAL system, which was attributed to serious difficulties in the ability of this system to detect *N. meningitidis*, *Brucella* spp., yeasts, and methicillin- and aminoglycoside-resistant *Staphylococcus aureus* (MARSA). Thus, it appears that a blind subculture protocol is, in fact, a necessity. To the best of our knowledge, this study is the first to evaluate false-negative results in the BacT/Alert3D system. The 2.6%

false-negative rate determined in this study is consistent with the results of the study conducted by Shigei *et al.* (1995), and appears slightly higher than has been reported in previous studies which have evaluated continuous-monitoring blood culture systems.

If common skin flora are classed as contaminants, the recovery rate of clinically relevant strains via terminal subculturing becomes 0.88% in this study. Positive results from terminal subcultures are frequently the result of contaminants, such as coagulase-negative staphylococci and *Propinobacterium* spp., *Bacillus cereus*, and *Corynebacterium* spp. (Araj *et al.*, 1981; Hardy *et al.*, 1992, Alfa *et al.*, 1995), rather than the result of pathogens actually present in the pre-culture bloodstream. However, false-negative cultures, when subjected to terminal subculturing, often yield significant pathogens, including beta-hemolytic streptococci, *Streptococcus anginosus* (Schwabe *et al.*, 1995), *Pseudomonas* spp., *Staphylococcus* spp., and yeasts (Shigei *et al.*, 1995; Gimenez *et al.*, 2002; Horvath *at al.*, 2004; Seegmuller *et al.*, 2004). In this study, one-third of our false-positive results yielded potential pathogens, including *S. aureus, E. faecium., S. pneumoniae, P. aeruginosa, P. fluorescens,* and *C. albicans.* As the two patients from whom *S. pneumoniae* was isolated were ultimately diagnosed with meningitis and pneumonia, respectively, the *S. pneumoniae* isolates were considered to be true pathogens. In order to evaluate the clinical relevance of the false-negative results yielded in this study, it was crucial to count the results of the other sets for the patients who exhibited false-negative results. Unfortunately, as only one set of blood cultures had been acquired from each of the study patients, we were unable to determine the significance of skin flora isolated only from terminal subcultures, and were also unable to determine whether other sets from the same patient would have generated a positive signal, or would have revealed the absence of potential pathogens.

Our study determined a 2.6% rate of false-negative results with the BacT/Alert 3D system, and one-third of these false-negative samples were ultimately found to harbor potential pathogens. Therefore, we conclude that the subculturing of negative results would prove valuable when using the BacT/Alert 3D system, especially in situations in which only one set of blood cultures has been obtained. Further studies with a larger sample will be necessary in order to accurately evaluate the efficacy of the subculturing of negative results in the BacT/Alert 3D system.

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