Journal of Pediatric Infectious Diseases 3 (2008) 1–3
IOS Press

Editorial

Automated colorimetric blood culture systems in the diagnosis of neonatal sepsis

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Received /Accepted 21 December 2007

Blood cultures are one of the most significant single tests performed in the clinical microbiology laboratory and can account for up to 10% of the total microbiology supplies budget [1]. Despite advances in polymerase chain reaction-and antigen-based technologies, the blood culture remains the gold standard for diagnosis of bacteremia. In newborns, evaluation for bacteremia ("rule-out sepsis") frequently is prompted by one of two circumstances. In the first instance, there may be perinatal risk factors for sepsis. Often prematurity alone can be a sufficient risk. In the second instance, the neonate appears ill. The limited repertoire of neonatal signs possibly indicating infection may include derangements of the cardiovascular, neurologic, gastrointestinal or respiratory systems.

Beginning in the 1990s, an increasingly popular blood culture system in developed countries has become the BacT/Alert and BacT/Alert 3D (bioMérieux, Marcy l'Étoile, France; Organon Teknika, Inc., Durham, N.C., USA). These automated microbial detection systems are based on the colorimetric detection of CO_2 produced by growing microorganisms. The original BacT/Alert proprietary soybean-casein broth media was designed to support growth of aerobic, microaerophilic, and fastidious bacteria and common yeasts; a second broth was optimized for growing anaerobic bacteria. More recently, the manufacturer has developed new aerobic (FA) and anaerobic (FN) blood culture media (soybean-casein broth supplemented with brain-heart infusion) that have shown a higher percentage of positivity for many microorganisms, in particular for *Candida* species and obligate anaerobes [2]. Smaller pediatric PF (glass) and PPF (plastic) culture bottles are modified for use with the smaller inocula volumes required for very small patients. Many hospital laboratories, including our own, continue to use the larger standard bottles, albeit with smaller blood inocula, e.g., 1 mL, and inoculate only a FA bottle.

Given the increasing usage of the BacT/Alert systems, it is helpful to review the automated design and performance characteristics. A CO_2 sensor separated from the broth by a semi-permeable membrane is bonded to the bottom of each bottle. CO_2 produced by growing microorganisms diffuses across the membrane into the sensor and dissolves in water, generating hydrogen ions. As the pH decreases, sensor blue to dark green turns lighter green to yellow, which results in an increase of red light reflected. The BacT/Alert system incubates, shakes, and scans CO_2 production every 10 min using a computerized database management system to record and report results.

BacT/Alert offers several significant advantages over manual blood cultures for microbial detection from body fluids [3]. First, it and other fully automated systems are entirely self-contained, eliminating repeated manipulations of bottles and, consequently, significantly reducing workload and errors. The system is non-

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radiometric, eliminating need for disposal of low-level radioactive wastes. Since the detector is external to the bottles and does not require a sample of gas from the bottle headspace, bottle cross-contamination during repeated aspirations is eliminated. Finally, the repeated automated testing of culture bottles can reduce time needed to detect microbial growth [4].

However, despite the critical nature and high cost of blood cultures, hospitals have needed to rely on manufacturers' test site data [1]. This situation is beginning to be rectified by clinical laboratory performance studies targeting different populations. During the past dozen years, several observational and experimental trials have indicated that continuous blood culture monitoring permits sensitive and speedy confirmation of bacteremia and fungemia in adults [3,5] and children [6–9]. A key gap that remains is the paucity of data for neonates.

In adults, the BacT/Alert system shows improved positivity rate due to the large volume of blood sampled [1]. Fortunately, it appears that use of very small inoculation volume (down to 0.5 mL) does not compromise isolation rate [8,10], supporting the evidence-based assertion that microbial culture blood volume may be less important in neonates and small children than in adults [11].

Critical data for newborns is only now emerging. Janjindamai and Phetpisal [12] in Thailand evaluated time to blood culture positivity using the BacT/Alert system in 75 newborn infants with suspected sepsis. When definite and possible bacterial pathogens were considered, the time to positivity was 71% at 24 h, 95% at 36 h and 97% at 48 h. Sensitivity, specificity and negative predictive values were 70.3%, 100% and 93.3%, respectively. These findings are similar to those of a second retrospective evaluation of neonatal blood culture system performance by Kumar et al. [13] in more than 400 cultures obtained in a UK neonatal intensive care unit (NICU). When definite and probable bacterial pathogens were considered, the time to positivity was 89% at 36 h and 97% at 48 h. Importantly, negative predictive values were 99.7% and 99.8% at 36 h and 48 h, respectively.

In this issue of the *J Pediatr Infect Dis*, Hasan et al. [14] have made a significant contribution to the literature on automated detection of positive blood cultures in newborns. In a prospective trial, the manual method was compared to the BacT/Alert 3D system in 101 matched pairs of blood cultures obtained from patients in a New Delhi NICU. The results were the BacT/Alert 3D system proved to be considerably more sensitive and had shorter times to culture positivity. In this study, 2 mL of blood was inoculated into each BacT/Alert PF bottle. Unlike the two previous studies [12,13], Hasan at al. [14] found that mean and median times to positivity were closer to 24 h than 36 h.

In summary, shorter time to positivity decreases costs and medication errors. The substantial initial costs of conversion to an automated system such as BacT/Alert, which may appear prohibitive for clinical laboratories in developing countries [15], are offset over time by decreased labor costs, shorter hospital stays and lower morbidities. Of course, data on time to positivity is most meaningful when the laboratory is open 24 h a day for constant monitoring of semi- or fully automated blood culture systems. A recent study at Ankara University indicated the effects of delayed transport of inoculated blood cultures to the laboratory and incubation only became statistically significant after 24 h [16]. Nevertheless, a shorter interval from culture bottle inoculation to incubation is probably an optimal practice.

Finally, specific diagnostic laboratory challenges in neonatal sepsis include limited volumes and the presence of bloodstream antibiotics. As in adults [17,18] and children [6], the presence of antibiotics in any blood culture system might cause false negative blood cultures in the neonate. The BacT/Alert system contains activated charcoal and Fuller's earth for removal of antibiotics, although performance is dependent on the antibiotic. Kumar et al. [13] found no effect of administration of antibiotics before blood collection on the time to culture positivity. Therefore, current evidence suggests intrapartum maternal antibiotics may not significantly compromise growth in neonatal blood cultures.

Lessons for the clinician include a scrupulous attention to technique in obtaining a neonatal blood culture, sending inoculated blood culture bottles to the laboratory as soon as possible, and effective communication between NICU and laboratory. Use of automated colorimetric blood culture systems permits confident identification of the presence or absence of bloodstream bacteria within 36 h. Future research will need to define further the minimal volume requirements for blood culture inoculum in newborns.

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