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Superior Sensitivity and Decreased Time to Detection with the Bactec Peds Plus/F System Compared to the BacT/Alert Pediatric FAN Blood Culture System

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Here, we compare the sensitivities and times to detection (TTD) of BacT/Alert Pediatric FAN (PF) and Bactec Peds Plus blood culture bottles. Test bottles were inoculated with 2 ml of banked whole blood, 1-ml aliquots of antibiotic suspension, and organisms diluted to simulate a bacteremia level of 10 to 100 CFU/ml. The control bottles were inoculated with 3 ml of banked blood and organism suspensions only. The organism-drug combinations were *Staphylococcus epidermidis* and vancomycin, methicillin-resistant *Staphylococcus aureus* and vancomycin, *Streptococcus pneumoniae*, vancomycin, and ceftriaxone, *Streptococcus agalactiae*, ampicillin, and cefotaxime, *Escherichia coli*, cefotaxime, and cefepime, *Pseudomonas aeruginosa*, piperacillin-tazobactam, cefepime, and gentamicin, *Neisseria meningitidis* and ceftriaxone, and *Haemophilus influenzae* and ceftriaxone. The control and test bottle combinations were tested in duplicate. The bottles were incubated for 5 days; 32 control and 104 test bottles were incubated. Overall, the bacterial recovery rates for the PF and Peds Plus bottles were 37% and 62%, 94% and 100% in the controls, 19% and 50% in the test bottles, and 33% and 92% in the bottles with vancomycin, respectively. No bacteria were recovered from the bottles with *S. pneumoniae*, *S. agalactiae*, *E. coli*, *N. meningitidis*, or *H. influenzae* in combination with cefotaxime or ceftriaxone. The Peds Plus system detected *P. aeruginosa* in bottles with cefepime and piperacillin-tazobactam, but the PF system recovered bacteria only in bottles with trough levels of piperacillin-tazobactam. The mean TTD were shorter in the Peds Plus system controls (14.2 versus 18.0 h; $P = 0.001$) and the test bottles (14.3 versus 17.8 h; $P = 0.008$) than in the PF bottles. Overall, we demonstrated superior sensitivity, TTD, and antibiotic neutralization in the Bactec Peds Plus system compared to those in the Pediatric FAN system.

Clinical microbiology laboratories tasked with detecting bacteremia in young children frequently face the challenge of detecting bacterial cells from small blood draw volumes (1). In a 60-kg adult, the ideal 20- to 30-ml blood draw translates to approximately 1% of his or her total blood volume (2, 3). Comparatively, 1% of the total blood volume in a newborn weighing 3.4 kg (the mean birth weight in the United States) amounts to only 3 ml (4, 5).

The BacT/Alert Pediatric FAN (PF) (bioMérieux, Durham, NC) and Bactec Peds Plus/F (Peds Plus) (BD Diagnostics, Sparks, MD) are two widely used pediatric blood culture systems. Industry-derived data based on members of the Children's Hospital Association suggest that a large proportion of them use a combination of pediatric and "adult" blood culture bottles, and a minority use a pediatric or an adult system exclusively (K. Fordyce, personal communication). Both products were designed to support the growth of aerobic organisms and facultative anaerobes. In an effort to compensate for the smaller blood draw volumes from children, the PF system uses lower broth volumes than its adult counterpart. Pediatric media also contain lower concentrations of sodium polyanethol sulfonate (SPS), an anticoagulant that suppresses the growth of *Neisseria meningitidis*, an important causative agent of infantile bacteremia. Finally, both systems use antibiotic-neutralizing agents, an 8.5% charcoal suspension in the PF system and antibiotic-binding resins in the Peds Plus system.

To date, various studies involving adult blood culture bottles have suggested superior sensitivities and/or swifter times to detection (TTD) of bacteria in Bactec Aerobic Plus/F bottles (BD Diagnostics, Sparks, MD) compared to those in BacT/Alert FA or FAN bottles (bioMérieux, Durham, NC) (6–11). However, to our knowledge, no data comparing the performance of the two pediatric systems are available in the literature. In this study, we compared the sensitivities and TTD in BacT/Alert Pediatric FAN (PF) and Bactec Peds Plus bottles and their respective abilities to neutralize therapeutic concentrations of antibiotics. A seeded blood culture methodology was used to simulate bacteremia in an infant.

MATERIALS AND METHODS

Preparation of antibiotic suspensions. Antibiotics were selected in consultation with the Children's Hospital of Philadelphia (CHOP) Antimicrobial Stewardship Program. The goal was to choose antibiotics that are recommended for empirical antimicrobial therapy through the hospital-wide clinical pathway program at the CHOP. Each antibiotic was prepared, stored, and used in the time frame recommended by the manufacturer. Each drug was reconstituted and then diluted using an intravenous formulation of 0.9% sodium chloride. The final drug concentrations were selected to reflect estimated therapeutic levels (7, 12); the peak and trough concentrations are summarized in Table 1.

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TABLE 1 Test organism MICs and drug concentrations

Organism	Test drug(s)	Organism MIC (μg/ml)[a]	Drug concn (μg/ml)	
			Peak	Trough
Staphylococcus epidermidis (ATCC 12228)	Vancomycin	1.5	50	10
Methicillin-resistant *Staphylococcus aureus* (ATCC 43300)	Vancomycin	1.5	50	10
Streptococcus pneumoniae (ATCC 49619)	Vancomycin	0.38	50	10
	Ceftriaxone	0.094	250	94
Streptococcus agalactiae (ATCC 12386)	Ampicillin	0.094	47	3
	Cefotaxime	0.094	215	94
Escherichia coli (ATCC 25922)	Cefotaxime	0.094	215	94
	Cefepime	0.064	164	10
Pseudomonas aeruginosa (ATCC 27853)	Piperacillin-tazobactam	4/4	240/24	5/0.7
	Cefepime	2	164	10
	Gentamicin	2	8	1
Neisseria meningitidis (ATCC 13077)	Ceftriaxone	ND[b]	250	94
Haemophilus influenzae (ATCC 49247)	Ceftriaxone	0.19	250	94

[a] MICs were determined using Etest (bioMérieux, Durham, NC).
[b] ND, MICs were not determined for the *N. meningitidis* strain used in this study due to concerns related to safety when handling this pathogen.

Preparation of organism suspensions. We used ATCC strains that are known to be susceptible to the test drugs, as recommended by the Clinical and Laboratory Standards Institute (13). The test isolates are summarized in Table 1. The MICs were verified using the Etest (bioMérieux, Durham, NC).

A digital pipettor was used throughout this study, and colony counts were performed to verify organism concentrations. Starting with a 1.0 McFarland standard suspension of an organism, we performed three successive 100-fold dilutions by placing 10 μl of the suspension into 990 μl of the diluent. These were followed by a 30-fold dilution by placement of 100 μl of the suspension into a blood culture bottle with 2 ml of the banked blood and 1 ml of the antibiotic suspension to achieve a final simulated bacteremia level of 10 to 100 CFU/ml (14, 15).

Blood culture bottle spiking and incubation. Each test bottle was inoculated with 2 ml of the refrigerated, banked whole blood obtained from the CHOP Division of Transfusion Medicine, followed by the organism suspension and then the antibiotic suspension in a 1-ml aliquot. The control bottles were inoculated with 3 ml of the banked blood and the organism suspension only. Each control and organism/drug/blood culture system combination was tested in duplicate. The 6-ml volume (3 ml per bottle, in duplicate) was chosen because it represents approximately 1% of the total blood volume of a 6-month-old boy at the 50th percentile for weight (7.8 kg) in the United States, assuming a total blood volume of 75 ml/kg (5, 15, 16).

The inoculated blood culture bottles were inserted in a synchronized fashion into the appropriate blood culture instrument (Bactec FX or BacT/Alert 3D) within 30 min of bottle inoculations. The bottles were incubated for a maximum of 5 days following routine procedures. After the signal of a positive blood culture, the bottle was removed and subcultured to 5% sheep's blood or chocolate agar to verify the growth of the appropriate organism.

Statistical analysis. The McNemar test for proportions was used to compare the recoveries of organisms in the two systems. The paired *t* test was used to compare the mean times to positivity in the PF/Peds Plus pairs in which there were bacterial recoveries from both systems.

RESULTS

In total, 32 control bottles without antibiotics and 104 test bottles containing antibiotics were incubated in the two blood culture systems. The recovery rates of organisms are summarized in Table 2. From all bottles, the bacterial recovery rates were 37% in the PF bottles and 62% in the Peds Plus bottles ($P = 0.004$).

Among the control bottles, the Peds Plus system recovered bacterial isolates in 16/16 bottles (100%). The PF system detected organisms in all but one bottle inoculated with *N. meningitidis*.

In the test bottles, the recovery rates were 19% in the PF system and 50% in the Peds Plus system. The Peds Plus system neutralized vancomycin well, recovering bacteria in 11/12 bottles spiked with *Staphylococcus epidermidis*, methicillin-resistant *Staphylococcus aureus* (MRSA), or *Streptococcus pneumoniae*. The PF system recovered organisms in 4/12 bottles (bottles with *Staphylococcus* spp. and trough vancomycin levels only).

The neutralization of ceftriaxone and cefotaxime was poor in both systems, even at trough levels. No bacterial isolates were recovered from bottles spiked with *S. pneumoniae*, *Streptococcus agalactiae*, *Escherichia coli*, *N. meningitidis*, or *Haemophilus influenzae* in combination with these antibiotics. A Peds Plus bottle inoculated with *H. influenzae* and ceftriaxone signaled positive at 25.2 h. Organisms were not seen on the Gram stain that was performed from the positive blood culture bottle. Also, a subculture of the blood culture broth did not yield growth of bacteria. This bottle was analyzed as a false-positive culture.

Pseudomonas aeruginosa was recovered from all Peds Plus bottles inoculated with cefepime and piperacillin-tazobactam. The PF system recovered organisms only in bottles with trough levels of piperacillin-tazobactam. Both systems failed to recover *E. coli* inoculated with cefepime. Both systems recovered organisms from all bottles with gentamicin present.

The mean TTD was shorter in the Peds Plus controls than in the PF controls (14.2 versus 18.0 h; $P = 0.001$) and in the test bottles (14.2 versus 17.8 h; $P = 0.008$).

TABLE 2 Recovery of inoculated organisms by antibiotic exposure

Organism and drug	No. of bottles recovering organisms/total no. tested in:			
	BacT/Alert PF		Bactec Peds Plus	
	Peak concn	Trough concn	Peak concn	Trough concn
S. epidermidis				
Vancomycin	0/2	2/2	1/2	2/2
MRSA				
Vancomycin	0/2	2/2	2/2	2/2
S. pneumoniae				
Vancomycin	0/2	0/2	2/2	2/2
Ceftriaxone	0/2	0/2	0/2	0/2
S. agalactiae				
Ampicillin	0/2	0/2	0/2	2/2
Cefotaxime	0/2	0/2	0/2	0/2
E. coli				
Cefotaxime	0/2	0/2	0/2	0/2
Cefepime	0/2	0/2	0/2	0/2
P. aeruginosa				
Piperacillin-tazobactam	0/2	2/2	2/2	2/2
Cefepime	0/2	0/2	2/2	2/2
Gentamicin	2/2	2/2	2/2	2/2
N. meningitidis				
Ceftriaxone	0/2	0/2	0/2	0/2
H. influenzae				
Ceftriaxone	0/2	0/2	1/2[a]	0/2[a]

[a] One Peds Plus bottle inoculated with *H. influenzae* and ceftriaxone at peak levels was positive. The quality control plate did not grow any organisms.

DISCUSSION

Overall, bacteria were recovered in 37% of the PF bottles and 62% of the Peds Plus bottles, which suggests superior sensitivity of the Bactec Peds Plus system over that of the BacT/Alert PF system. These results corroborated findings in the published literature for comparisons of the Bactec Plus Aerobic/F with the BacT/Alert FA and FAN systems (6–11).

Under our study conditions, both systems reliably recovered organisms in the absence of antibiotics (31 of 32 bottles). However, the TTD in the Peds Plus controls were, on average, 4 h faster than those for the PF controls. The discrepancy in the mean TTD was particularly pronounced in the bottles inoculated with *H. influenzae* and *N. meningitidis* (20.0 h in Peds Plus versus 29.5 h in PF). The hemin and SPS concentrations are similar in the two products (0.0005% and 0.02% in Peds Plus and 0.000625% and 0.025% in PF, respectively).

The superior vancomycin neutralization by the Bactec Plus Aerobic/F resins compared with that for the charcoal suspension in the BacT/Alert FA system has been documented in the published literature (7, 8). Our findings corroborated these data. The neutralization of vancomycin was satisfactory at the trough levels for *Staphylococcus* spp. in both systems, while the Peds Plus system recovered organisms even at the peak levels in blood cultures inoculated with *Staphylococcus* spp. and *S. pneumoniae*.

Despite the widespread availability of conjugate pneumococcal vaccines, *S. pneumoniae* continues to be a relevant agent of bacteremia in infants (17). Third-generation cephalosporins are frequently used empirically in infantile sepsis to provide antimicrobial activity against penicillin-resistant strains. With no organism recoveries in bottles inoculated with *S. agalactiae*, *S. pneumoniae*, *E. coli*, *N. meningitidis*, or *H. influenzae* and ceftriaxone or cefotaxime, the importance of collecting blood prior to the initiation of empirical antibiotics must be emphasized, regardless of the blood culture system in use.

In blood cultures inoculated with *P. aeruginosa*, the Peds Plus system neutralized piperacillin-tazobactam, cefepime, and gentamicin at peak and trough levels, while the PF system did not recover this organism with cefepime or peak levels of piperacillin-tazobactam. This suggests that particular care should be taken with the timing of blood draws in septic infants at risk of *P. aeruginosa* bacteremia in institutions using the PF system.

This study has a number of limitations. It must be underscored that observed differences in organism recovery rates and TTD in the two systems were relative, not absolute, due to the fact that bacteremia was simulated in this study. First, banked whole blood was used as a proxy for fresh blood. The pharmacologic effect of a given antibiotic is dependent, in part, on the proportion of the drug that remains unbound to the proteins present in the blood. The protein composition of banked blood may differ from that of fresh human blood, affecting the efficacy of the antibiotic. In addition, the citrate preservative present in banked blood may inhibit bacterial growth. Second, the 4 ml per simulated "blood draw" in the test blood cultures (2 ml in duplicate for each organism-drug combination) was relatively low but necessary to accommodate the 1-ml aliquots required for the antibiotic suspensions. Blood dilutes SPS, enabling the growth of SPS-susceptible organisms. Blood also supports the recovery of hemin-requiring bacteria, including *H. influenzae*. The recovery of organisms in blood cultures inoculated with *N. meningitidis* or *H. influenzae* with ceftriaxone may have been challenged by the blood volumes used in the test blood cultures. Third, 3 ml of whole blood was inoculated into the control bottles, while 2 ml was inoculated into the test bottles. The use of 2 ml of whole blood and 1 ml of saline in the control bottles was considered, but we wished to remain true to our initial goal of simulating bacteremia in a typical 6-month-old infant. The main analytic consequence of using different blood volumes was that it precluded us from comparing the detection and times to positivity in the control versus test bottles. This analysis was sacrificed in favor of preparing the control bottles with the optimal 3 ml of blood. Next, we did not use clinical strains of bacteria in this study. We opted for ATCC strains in an effort to support the reproducibility of our findings and to ensure the standardization of antibiotic susceptibility. Finally, the peak and trough antibiotic levels were estimates and were derived from adult pharmacology data due to a lack of analogous pediatric data.

The failure to detect an agent of bacteremia in septic patients can have negative consequences. Culture-negative sepsis is often managed with a continuation of broad-spectrum empirical antibiotics. In neonates, for example, the prolonged use of empirical antibiotics in culture-negative early-onset neonatal sepsis has been associated with an increased risk of mortality and later development of necrotizing enterocolitis (18). The time to detection of bacteremia can also impact clinical outcomes. Pediatric *S. aureus* bacteremia is associated with a high risk of mortality and

embolic complications (19, 20). The timely removal of central venous catheters (19) and early initiation of optimized antibiotics (e.g., nafcillin, oxacillin, or cefazolin in methicillin-susceptible *S. aureus* bacteremia) have been associated with a reduction in the number of treatment failures (21, 22). Similarly, delayed initiation of targeted enterococcal therapy has been associated with increased lengths of hospital stay and risk of mortality, regardless of the vancomycin susceptibility status (23, 24). This study provides evidence that the superior sensitivity and swifter time to detection attributed to the Bactec Aerobic Plus/F system also applies to the Bactec Peds Plus product. Investigation of the clinical impact of blood culture system choice on pediatric clinical outcomes is an important area for future research.

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REFERENCES

1. **Schifman RB, Bachner P, Howanitz PJ.** 1996. Blood culture quality improvement: a College of American Pathologists Q-Probes study involving 909 institutions and 289 572 blood culture sets. Arch. Pathol. Lab. Med. **120:**999–1002.
2. **Clinical and Laboratory Standards Institute.** 2009. Principles and procedures for blood cultures. Approved guideline, vol 27, no. 18. CLSI document M47-A. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Cockerill FR, III, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, Schleck CD, Ilstrup DM, Washington JA, II, Wilson WR.** 2004. Optimal testing parameters for blood cultures. Clin. Infect. Dis. **38:**1724–1730.
4. **Donahue SM, Kleinman KP, Gillman MW, Oken E.** 2010. Trends in birth weight and gestational length among singleton term births in the United States: 1990-2005. Obstet. Gynecol. **115:**357–364.
5. **Linderkamp O, Versmold HT, Riegel KP, Betke K.** 1977. Estimation and prediction of blood volume in infants and children. Eur. J. Pediatr. **125:**227–234.
6. **Zadroga R, Williams DN, Gottschall R, Hanson K, Nordberg V, Deike M, Kuskowski M, Carlson L, Nicolau DP, Sutherland D, Hansen GT.** 2013. Comparison of 2 blood culture media shows significant differences in bacterial recovery for patients on antimicrobial therapy. Clin. Infect. Dis. **56:**790–797.
7. **Flayhart D, Borek AP, Wakefield T, Dick J, Carroll KC.** 2007. Comparison of Bactec Plus blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J. Clin. Microbiol. **45:**816–821.
8. **Viganò EF, Vasconi E, Agrappi C, Clerici P, Melloni P.** 2004. Use of simulated blood cultures for antibiotic effect on time to detection of the two blood culture systems BacT/Alert and Bactec 9240. New Microbiol. **27:**235–248.
9. **Roh KH, Kim JY, Kim HN, Lee HJ, Sohn JW, Kim MJ, Cho Y, Kim YK, Lee CK.** 2012. Evaluation of Bactec Plus aerobic and anaerobic blood culture bottles and BacT/Alert FAN aerobic and anaerobic blood culture bottles for the detection of bacteremia in ICU patients. Diagn. Microbiol. Infect. Dis. **73:**239–242.
10. **Ziegler R, Johnscher I, Martus P, Lenhardt D, Just HM.** 1998. Controlled clinical laboratory comparison of two supplemented aerobic and anaerobic media used in automated blood culture systems to detect bloodstream infections. J. Clin. Microbiol. **36:**657–661.
11. **Jorgensen JH, Mirrett S, McDonald LC, Murray PR, Weinstein MP, Fune J, Trippy CW, Masterson M, Reller LB.** 1997. Controlled clinical laboratory comparison of Bactec plus aerobic/F resin medium with BacT/Alert aerobic FAN medium for detection of bacteremia and fungemia. J. Clin. Microbiol. **35:**53–58.
12. **Amsden GW.** 2010. Tables of antimicrobial agent pharmacology, p 705–761. *In* Mandell GL, Bennett JE, Dolin R (ed), Principles and practice of infectious diseases, 7th ed, vol 1. Churchill Livingstone Elsevier, Philadelphia, PA.
13. **Clinical and Laboratory Standards Institute.** 2013. Performance standards for antimicrobial susceptibility testing; 21st informational supplement, vol 33, no. 1. CLSI document M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
14. **Miles AA, Misra SS, Irwin JO.** 1938. The estimation of the bactericidal power of the blood. J. Hyg. (Lond.) **38:**732–749.
15. **Baron EJ, Weinstein MP, Dunne WM, Yagupsky P, Welch DF, Wilson DM.** 2005. Cumitech 1C, blood cultures IV. Coordinating ed, Baron EJ. ASM Press, Washington, DC.
16. **Centers for Disease Control and Prevention.** Clinical growth charts. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/growthcharts/clinical_charts.htm.
17. **Laupland KB, Gregson DB, Vanderkooi OG, Ross T, Kellner JD.** 2009. The changing burden of pediatric bloodstream infections in Calgary, Canada, 2000-2006. Pediatr. Infect. Dis. J. **28:**114–117.
18. **Cotten CM, Taylor S, Stoll B.** 2009. Prolonged duration of initial empirical antibiotic treatment is associated with increased rates of necrotizing enterocolitis and death for extremely low birth weight infants. Pediatrics **123:**58–66.
19. **Carrillo-Marquez MA, Hulten CG, Mason EO, Kaplan SL.** 2010. Clinical and molecular epidemiology of *Staphylococcus aureus* catheter-related bacteremia in children. Pediatr. Infect. Dis. J. **29:**410–414.
20. **McNeil JC, Hulten KG, Kaplan SL, Mahoney DH, Mason EO.** 2013. *Staphylococcus aureus* infections in pediatric oncology patients: high rates of antimicrobial resistance, antiseptic tolerance and complications. Pediatr. Infect. Dis. J. **32:**124–128.
21. **Schweizer ML, Furuno JP, Harris AD, Johnson JK, Shardell MD, McGregor JC, Thom KA, Cosgrove SE, Sakoulas G, Perencevich EN.** 2011. Comparative effectiveness of nafcillin or cefazolin versus vancomycin in methicillin-susceptible *Staphylococcus aureus* bacteremia. BMC Infect. Dis. **11:**279. doi:10.1186/1471-2334-11-279.
22. **Stryjewski ME, Szczech LA, Benjamin DK, Jr, Inrig JK, Kanafani ZA, Engemann JJ, Chu VH, Joyce MJ, Reller LB, Corey GR, Fowler VG, Jr.** 2007. Use of vancomycin or first-generation cephalosporins for the treatment of hemodialysis dependent patients with methicillin-susceptible *Staphylococcus aureus* bacteremia. Clin. Infect. Dis. **44:**190–196.
23. **Cheah AL, Spelman T, Liew D, Peel T, Howden BP, Spelman D, Grayson ML, Nation RL, Kong DC.** 2013. Enterococcal bacteremia: factors influencing mortality, length of stay and costs of hospitalization. Clin. Microbiol. Infect. **19:**E181–E189. doi:10.1111/1469-0691.12132.
24. **Vergis EN, Hayden MK, Chow JW, Snydman DR, Zervos MJ, Linden PK, Wagener MM, Schmitt B, Muder RR.** 2001. Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia. A prospective multicenter study. Ann. Intern. Med. **135:**484–492.