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SIGNAL Blood Culture System for Detection of Bacteremia in Neonates

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In the SIGNAL (Oxoid Ltd., Basingstoke, United Kingdom) blood culture system, gas produced by bacterial metabolism displaces medium from the culture bottle into an upper reservoir via a hollow needle. Displacement of media may provide a visual indication of the presence of both aerobic and anaerobic organisms in a single medium. The single-bottle SIGNAL system was compared with paired BACTEC 16B and 7D (Johnston Laboratories, Inc., Towson, Md.) radiometric system bottles by using bacterial inocula and conditions which simulated those found in neonatal and pediatric populations. The single SIGNAL bottle was as good as the combined BACTEC media for *Escherichia coli* and *Staphylococcus aureus*, but was slower for *Candida* spp., *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, group B streptococci, alpha-streptococci, and pneumococci. The SIGNAL system failed to detect four of five isolates of *Neisseria meningitidis* and four of eight anaerobic organisms. The SIGNAL system is not suitable for neonatal blood cultures at its present state of development.

The SIGNAL (Oxoid Ltd., Basingstoke, United Kingdom) blood culture system is based on the principle that bacterial growth may produce gas which creates pressure in a sealed container. After inoculation of the SIGNAL culture bottle with blood, a secondary upper chamber is attached via a hollow needle. The apparatus is designed so that an increase in pressure in the culture bottle will force medium through the needle to the upper chamber, which equilibrates with the atmosphere via a semipermeable membrane. The membrane permits pressure equilibration but prevents contamination (11). After incubation, the presence of medium at or above an engraved mark on the upper chamber provides a visual indication of bacterial growth. The SIGNAL culture medium (80 ml) is claimed to support the growth of aerobic, anaerobic, and microaerophilic organisms and has been described elsewhere (11). The sensitivity of the SIGNAL system was compared with that of the BACTEC (Johnston Laboratories, Inc., Towson, Md.) radiometric culture system by using known inocula of bacteria with the addition of small volumes of human blood. The BACTEC blood culture system is based on the principle that bacteria produce $^{14}CO_2$ from the labeled substrates that can be used to detect growth. Separate aerobic and anaerobic culture media (30 ml in each bottle) are required. Conditions and organisms were largely chosen to reflect those which are likely to be found in the etiology of neonatal infections.

MATERIALS AND METHODS

Blood culture systems. The main features of the SIGNAL blood culture system (11) were described above. The manufacturers initially recommended that SIGNAL bottles be gently shaken by hand for a few seconds on each of the first 3 days of incubation. Results of preliminary studies indicated that the sensitivity of the system could be improved if cultures were shaken for the first 24 h at 140 oscillations per min and then kept static; the latter procedure was used for these studies. Cultures were interpreted as being positive when medium displaced from the main culture bottle reached an engraved mark on the reservoir. All BACTEC 16B bottles were gently shaken on an orbital shaker at 200 oscillations per min for the first 24 h of incubation, as recommended by the manufacturer. BACTEC cultures were interpreted as being positive when the growth index (numerical indicator of $^{14}CO_2$ production from the labeled medium) exceeded cutoff points determined by the manufacturer. Viable count determinations were performed by surface inoculation of serial dilutions of BACTEC and SIGNAL bottles onto appropriate agar plates to confirm that bottles which gave a positive indication of growth contained the expected numbers of viable bacteria. The individual viable counts were not relevant to the overall findings and are not presented.

Organisms. Pediatric blood cultures provided five of each of the following aerobic organisms: *Candida* spp. (four *C. albicans* isolates, one *Candida parapsilopsis* isolate), *Escherichia coli*, *Haemophilus influenzae* type b, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, alpha-hemolytic streptococci, and group B streptococci. Cultures of *Candida* spp., *E. coli*, *P. aeruginosa*, and *S. aureus* were stored at −80°C in Mueller-Hinton broth containing dimethyl sulfoxide (10%; vol/vol); the remaining organisms were stored at this temperature in a 20% (wt/vol) skim milk medium. Isolates of *Peptostreptococcus micros* (two isolates), *Fusobacterium nucleatum* (two isolates), *Clostridium butyricum* (one isolate), and *Veillonella parvula* (one isolate) from pediatric blood cultures were supplemented with stock laboratory cultures of *Bacteroides fragilis* (one isolate) and *Peptostreptococcus asaccharolyticus* (one isolate). These anaerobes were stored at −80°C in sheep blood containing dimethyl sulfoxide (5%; vol/vol) and glycerol (10%; vol/vol).

Preparation of simulated infected blood cultures. Stock frozen organisms were subcultured three times on appropriate conventional media to establish consistent growth patterns and then were checked for purity and growth characteristics before use.

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TABLE 1. Comparison of the sensitivity of the SIGNAL[a] and BACTEC blood culture systems for the detection of 50 aerobic and facultatively anaerobic organisms

Organism and inoculum (mean no. of CFU [range for five organisms])	Blood culture system	No. of cumulative positive cultures at the following times (h)[b]:									No. of positive terminal subcultures by 168 h
		15	21	39	45	63	87	111	135	168	
Candida spp. 11.7 (6.8–19)	BACTEC 16B	0	0	4	5	5	5	5	5	5	5
	BACTEC 7D	0	0	0	0	0	0	0	0	0	0
	SIGNAL	0	0	0	0	2	4	4	5	5	5
Escherichia coli 5.9 (2.2–9.5)	BACTEC 16B	5	5	5	5	5	5	5	5	5	5
	BACTEC 7D	5	5	5	5	5	5	5	5	5	5
	SIGNAL	5	5	5	5	5	5	5	5	5	5
Haemophilus influenzae 34 (14–92)	BACTEC 16B	2	5	5	5	5	5	5	5	5	5
	BACTEC 7D	0	1	5	5	5	5	5	5	5	5
	SIGNAL	0	1	4	4	4	4	4	4	4	5
Neisseria meningitidis 14.6 (8.2–18)	BACTEC 16B	1	5	5	5	5	5	5	5	5	5
	BACTEC 7D	0	0	0	0	0	0	0	0	0	0
	SIGNAL	0	0	0	0	1	1	1	1	1	5
Pseudomonas aeruginosa 6.9 (3.3–11)	BACTEC 16B	0	5	5	5	5	5	5	5	5	5
	BACTEC 7D	0	0	3	5	5	5	5	5	5	5
	SIGNAL	0	1	5	5	5	5	5	5	5	5
Staphylococcus aureus 2.5 (1.6–2.9)	BACTEC 16B	2	5	5	5	5	5	5	5	5	5
	BACTEC 7D	0	1	5	5	5	5	5	5	5	5
	SIGNAL	2	5	5	5	5	5	5	5	5	5
Staphylococcus epidermidis 7.7 (2.7–13)	BACTEC 16B	0	5	5	5	5	5	5	5	5	5
	BACTEC 7D	0	0	5	5	5	5	5	5	5	5
	SIGNAL	0	1	5	5	5	5	5	5	5	5
Group B streptococci 14.8 (7.5–22)	BACTEC 16B	5	5	5	5	5	5	5	5	5	5
	BACTEC 7D	3	4	5	5	5	5	5	5	5	5
	SIGNAL	3	5	5	5	5	5	5	5	5	5
Alpha-streptococci 7.1 (5.2–17)	BACTEC 16B	2	4	5	5	5	5	5	5	5	5
	BACTEC 7D	0	4	5	5	5	5	5	5	5	5
	SIGNAL	1	4	5	5	5	5	5	5	5	5
Streptococcus pneumoniae 13.6 (5.0–19)	BACTEC 16B	5	5	5	5	5	5	5	5	5	5
	BACTEC 7D	2	5	5	5	5	5	5	5	5	5
	SIGNAL	0	3	3	3	4	4	4	4	4	4
All 50 aerobic organisms	BACTEC 16B	22	44	49	50	50	50	50	50	50	50
	BACTEC 7D	10	20	38	40	40	40	40	40	40	40
	SIGNAL	11	25	38	38	41	43	43	44	44	49

[a] The SIGNAL system was shaken for the first 24 h.
[b] Cultures giving positive results with the BACTEC or SIGNAL system which were subsequently confirmed to be positive by culture.

Overnight cultures of aerobic organisms grown on blood or chocolate agar were suspended in brain heart infusion broth (Prepared Media Laboratories, Tualatin, Ore.). This medium was supplemented with X and V factors for experiments with *H. influenzae*. The broth suspensions were incubated at 35°C for between 1 and 4 h to yield a suspension with a density corresponding to a 0.5 McFarland standard (1). Phosphate-buffered saline (pH 7.4) was used to dilute the standardized suspensions. Preliminary experiments with each organism indicated the dilution necessary for a 0.5-ml fraction of a suspension to contain an appropriate inoculum for each blood culture bottle. After inoculation of the SIGNAL and BACTEC 16B (aerobic, with ion-exchange resin) and 7D (anaerobic) culture bottles with equal numbers of organisms, fresh heparinized (14.7 U/ml; VACUTAINER tubes; Beckton Dickinson Vacutainer Systems, Rutherford, N.J.) blood (0.5 ml) from adults was added to each bottle.

For experiments with anaerobic organisms, a loopful of each agar plate culture was inoculated into prereduced, anaerobically sterilized brain heart infusion broth (5 ml; Carr-Scarborough Microbiologicals, Inc., Decatur, Ga.) and incubated overnight at 35°C. The anaerobic infusion broth was diluted in thioglycolate broth without indicator (Difco Laboratories, Detroit, Mich.) to yield a suspension with a density equivalent to a 0.5 McFarland standard. Further dilutions were performed in thioglycolate broth to prepare suspensions that were appropriate for inoculation into the blood culture bottles in a manner analogous to that described above for the aerobic organisms. Viable counts of all bacterial suspensions which were used to inoculate the blood

TABLE 2. Comparison of the sensitivity of the SIGNAL and BACTEC blood culture systems for the detection of a selection of nine anaerobic organisms

Organism (no. of CFU inoculated into each bottle)	Time (h) at which the culture became positive with[a]:	
	BACTEC 7D	SIGNAL[b]
Peptostreptococcus asaccharolyticus (32)	63	87
Veillonella purvala (12)	39	NG
Bacteroides fragilis (22)	39	63
Fusobacterium nucleatum isolate A (43)	111	TS
Fusobacterium nucleatum isolate B (32)	39	63
Clostridium butyricum (11)	15	39
Peptostreptococcus micros isolate A (8)	87	NG
Peptostreptococcus micros isolate B (13)	63	NG

[a] Cultures giving positive results with the BACTEC or SIGNAL system which were subsequently confirmed to be positive by culture. NG, No growth on terminal subculture; TS, growth on terminal subculture only.
[b] The SIGNAL system was shaken for the first 24 h.

culture bottles were determined by the surface inoculation of serial 10-fold dilutions onto appropriate agar media.

Treatment of simulated blood cultures. Bottles were incubated at 35°C and examined at the intervals given in Table 1. A Gram stain and quantitative culture were performed on each positive culture to confirm that growth had occurred. No false-positive reactions or contamination with extraneous organisms occurred with either the SIGNAL or BACTEC systems in this study. Terminal cultures and Gram stain studies were performed at 168 h on all media which did not appear to have supported growth.

RESULTS

Equal numbers of CFU of organisms were inoculated in parallel into BACTEC 16B and 7D and SIGNAL blood culture bottles. The data summarized in Table 1 show that for aerobic and facultative anaerobic organisms the SIGNAL system was generally slower than the BACTEC 16B system and was especially poor at detecting *N. meningitidis*. As expected, the BACTEC 7D anaerobic medium was less sensitive than the BACTEC 16B medium for these organisms.

The behaviors of a small selection of anaerobic organisms in the three culture media are compared in Table 2. No anaerobic organism survived in the BACTEC 16B medium. The SIGNAL medium was slower and detected fewer organisms than the BACTEC 7D medium under these test conditions.

Aerobic organisms such as *P. aeruginosa* were inoculated into the anaerobic BACTEC 7D medium because it is a routine clinical procedure to use paired aerobic and anaerobic media for blood cultures. A similar rationale was applied for the use of aerobic BACTEC 16B medium for anaerobes. The SIGNAL system has the potential to avoid the use of irrelevant media for strict aerobes and strict anaerobes.

DISCUSSION

Blood culture is an important diagnostic tool in neonates in whom infections may not be associated with specific signs. Much of the erythrocyte transfusion requirements of ill, premature babies are a direct effect of the blood loss from essential laboratory monitoring (3). Thus, blood culture in premature babies is frequently limited to a single specimen of 0.1 to 3 ml (5, 8); less stringent limitations may apply to larger infants. Although large concentrations of organisms may be found in the blood of children with bacteremia (2, 6, 12), a proportion of patients may have only small populations. In one study, 23% of neonates with *E. coli* bacteremia had <4 organisms per ml of blood and 54% had <49 organisms per ml of blood (6). Similar values have been obtained for other pathogens in a general pediatric population aged less than 2 years (2, 12). A sensitive, rapid method for the diagnosis of bacteremia is therefore pertinent in the diagnosis of infection in this patient population.

Each clinical blood culture system has characteristic drawbacks and advantages and may have different sensitivities for individual organisms. The BACTEC system is widely used in clinical laboratories and was chosen as a convenient reference system. All organisms which were tested grew in at least one BACTEC medium, and the sensitivity of this system was comparable to that of the recently introduced lysis-centrifugation method (4). As expected, the use of paired bottles, which is recommended in clinical practice, proved redundant for strict aerobes and some strict anaerobes. These results are, in general, consistent with the limited data provided in a previous assessment (11) of the SIGNAL system in which an accepted clinical blood culture system was not used as a reference. In a direct comparison of the BACTEC system with a prototype of the SIGNAL system with clinical blood cultures, the systems detected 94 and 81%, respectively, of known positive cultures (7). There is broad agreement between the findings with these simulated cultures in the BACTEC system and those of a clinical investigation of the incubation period that is necessary to detect bacteremia in neonates (10).

Although pediatric blood cultures frequently contain large numbers of organisms, an appreciable proportion contain bacterial populations within the range chosen for the studies reported in Tables 1 and 2. Although heparin has been reported to inhibit the growth of some bacteria (9), the amounts that were present after dilution in culture media were likely to be insignificant. Furthermore, growth occurred more commonly and more quickly in BACTEC media, in which the final concentration of heparin was higher than that in the SIGNAL bottles (0.47 versus 0.18 units/ml). The potential advantages of a nonradiometric, single-bottle system in pediatrics should encourage further work to overcome the relatively slow response of the SIGNAL system with some common organisms and its especially poor performance with *N. meningitidis* and anaerobes.

LITERATURE CITED

1. **Barry, A. L., and C. Thornsberry.** 1985. Susceptibility tests: diffusion test procedures, p. 978–987. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
2. **Bell, L. M., G. Albert, J. M. Campos, and S. A. Plotkin.** 1985. Routine quantitative blood cultures in children with *Haemophilus influenzae* or *Streptococcus pneumoniae* bacteremia. Pediatrics **76**:901–904.
3. **Blanchette, V. S., and A. Zipursky.** 1984. Symposium on perinatal hematology. Clin. Perinatol. **11**:489–513.
4. **Carey, R. B.** 1984. Clinical comparison of the Isolator 1.5 microbial tube and the BACTEC radiometric system for detection of bacteremia in children. J. Clin. Microbiol. **19**:634–638.
5. **Davies, P. A., and L. Gothefors.** 1984. Bacterial infections in the fetus and newborn infant, p. 77–97. *In* M. Markowitz (ed.), Major problems in clinical pediatrics, vol. 26. The W. B. Saunders Co., Philadelphia.

Saunders Co., Philadelphia.
6. **Dietzman, D. E., G. W. Fischer, and F. D. Schoenknecht.** 1974. Neonatal *Escherichia coli* septicaemia—bacterial counts in blood. J. Pediatr. **85**:128–130.
7. **King, A., G. Bone, and I. Phillips.** 1986. Comparison of radiometric and gas capture system for blood cultures. J. Clin. Pathol. **39**:661–665.
8. **Neal, P. R., M. B. Kleimar, J. K. Reynolds, S. D. Allen, J. A. Lemons, and P.-L. Yu.** 1986. Volume of blood submitted for culture from neonates. J. Clin. Microbiol. **24**:353–356.
9. **Reller, L. B., P. R. Murrary, and J. D. MacLowry.** 1982. Cumitech 1A, Blood cultures II. Coordinating ed., J. A. Washington II. American Society for Microbiology, Washington, D.C.
10. **Rowley, A. M., and E. R. Wald.** 1986. Incubation period necessary to detect bacteremia in neonates. Pediatr. Infect. Dis. **5**:590–591.
11. **Sawhney, D., S. Hinder, D. Swaine, and E. Y. Bridson.** 1986. Novel method for detecting micro-organisms in blood cultures. J. Clin. Pathol. **29**:1259–1263.
12. **Sullivan, T. D., J. L. Leonard, Jr., and E. Neter.** 1982. Relationship between the magnitude of bacteremia in children and the clinical disease. Pediatrics **69**:699–702.