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Controlled Evaluation of Blood Culture Medium Containing Gelatin and V-Factor-Analog for Detection of Septicemia in Children

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Both *Neisseria meningitidis* and *Haemophilus influenzae* are important isolates recovered in blood cultures from septicemic children. Sodium polyanetholsulfonate is present in most blood culture media and can inhibit the growth of certain bacteria, including *N. meningitidis*. The addition of gelatin to blood culture media neutralizes this inhibition. The growth of *H. influenzae* is enhanced by specific growth factors such as hemin and NAD. The addition of gelatin and V-factor-analog (a proprietary supplement for enhancing the growth of *H. influenzae*) might have a positive effect on the yield and on the speed of detection of septicemia in children. To evaluate this possibility, we did 4,565 paired comparisons of blood cultured in BACTEC 6B (aerobic) medium with and without the addition of both 1.2% gelatin and V-factor-analog. More aerobic and facultative bacteria grew in the 6B than in the 6B-gelatin-V-factor-analog medium ($P < 0.01$). Only seven isolates of *Neisseria* spp. were recovered during this study period, with the 6B medium performing as well as the supplemented medium. When microorganisms grew in both bottles, they did so at the same time except for *H. influenzae* and *Candida albicans*. *H. influenzae* was recovered earlier from the 6B-gelatin-V-factor-analog bottle ($P < 0.01$), with a mean time to detection of 8.5 h compared with 15.9 h for the 6B bottle. *C. albicans* was recovered earlier from the 6B bottle ($P < 0.02$), with a mean time to detection of 34.9 h compared with 71.6 h for the 6B-gelatin-V-factor-analog bottle. We conclude that the 6B medium in its present formulation is superior to 6B supplemented with gelatin and V-factor-analog.

Both *Neisseria meningitidis* and *Haemophilus influenzae* are important isolates recovered in blood cultures from septicemic children (13). The formulation of blood culture media used in a pediatric population should reflect the need to isolate these pathogens among others. However, the anticoagulant sodium polyanetholsulfonate (SPS) is added routinely to virtually all commercial blood culture media (11). The effects of this anticoagulant include inhibition of phagocytosis, complement, and lysozyme activity, as well as inactivation of aminoglycoside antimicrobial agents (1, 11, 16). An undesirable effect of SPS is the inhibition of the growth of some bacteria, including *N. meningitidis*, *Neisseria gonorrhoeae*, *Peptostreptococcus anaerobius*, *Streptobacillus moniliformis*, and *Gardnerella vaginalis* (2, 4, 6, 10, 14). This inhibitory effect on bacterial growth can be countered by supplementing blood culture medium with 1.2% gelatin (3, 9, 10, 12, 19). The addition of gelatin to blood culture medium used in a pediatric population might be useful to ensure the recovery of *N. meningitidis*. The growth of *H. influenzae* is enhanced by specific growth factors such as hemin and NAD (5). Supplementation of blood culture media used in a pediatric population with specific growth factors such as hemin and NAD might be useful to ensure the recovery of *H. influenzae*.

However, the effect of the addition of gelatin and V-factor-analog (a proprietary supplement for enhancing the growth of *H. influenzae*) on the overall detection of bacteremia and fungemia in children has never been studied in a large field trial. We compared the yield and speed of detection of microorganisms, from 4,565 paired blood cultures from pediatric patients at four hospitals that use identical radiometric methods for culturing blood, from February to August 1986.

MATERIALS AND METHODS

Collection of samples. During the 6-month study period, two 30-ml BACTEC bottles (aerobic 6B and aerobic 6B with 1.2% gelatin and V-factor-analog) containing tryptic soy broth with 0.025% SPS were used for all blood cultures from pediatric patients (neonates to 18 years) at the Vanderbilt University Hospital, the Robert Wood Johnson University Hospital, the University of Colorado Hospital, and Denver General Hospital. Pediatric blood cultures were obtained at the bedside after preparation of the skin and povidone-iodine and isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe equally to a BACTEC 6B bottle and a BACTEC 6B bottle with gelatin-V-factor-analog.

Processing of samples. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at all four hospitals. Both bottles were incubated in an air incubator at 35°C for 7 days. The 6B and 6B medium with gelatin-V-factor-analog bottles were examined macroscopically and radiometrically twice daily on days 1 and 2 of incubation and once daily thereafter through day 7. Both bottles were shaken for the first 24 to 48 h of incubation. All bottles with a positive growth index were examined by Gram stain and subcultured. In addition, bottles with an

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increase of 10 or more growth index units between readings were examined by Gram stain and subcultured. All patients with positive blood cultures were evaluated by an infectious-disease specialist who defined pathogens (clinically important microorganisms causing sepsis) and contaminants by established criteria (18). These included the clinical status of the patient, the assessment by the attending physicians, and the use of and response to antibiotics.

Analysis of data. Paired comparisons of 6B and 6B with gelatin-V-factor-analog were done only on bottles that grew microorganisms causing the bacteremia or fungemia. Significance testing was done with the modified chi-square test described by McNemar (7).

RESULTS

A total of 4,565 blood culture sets were received during the study period. Of these, 261 isolates (5.7%) associated with sepsis were detected; 185 (68.5%) grew in both the 6B and gelatin-V-factor-analog-supplemented 6B bottles; 45 (16.7%) isolates grew in the 6B medium, whereas 31 (11.5%) grew only in the gelatin-V-factor-analog-supplemented 6B medium. Overall, more aerobic and facultative bacteria grew in the 6B medium than in the 6B-gelatin-V-factor-analog medium ($P < 0.01$; Table 1). Gelatin decreased the detection of *Staphylococcus epidermidis* ($P < 0.05$). A total of seven isolates of *Neisseria* spp. were recovered during the 6-month study period. Two isolates of *N. meningitidis* and one of *N. gonorrhoeae* were recovered from both media. Of interest was the detection of one isolate each of *N. meningitidis* and *N. gonorrhoeae* in only 6B medium. The yield of *H. influenzae* was the same in both media.

TABLE 1. Comparison of yield of clinically important bacteria and fungi from BACTEC 6B medium with and without 1.2% gelatin and V-factor-analog

Microorganism	No. of isolates recovered by:			P
	Both media	6B only	6B-gelatin-V-factor only	
Aerobic or facultative bacteria	171	42	28	<0.01
Gram-positive bacteria	109	22	20	NS[a]
Staphylococcus aureus	39	3	2	NS
Staphylococcus epidermidis	33	13	3	<0.05
Streptococcus pneumoniae	22	3	8	NS
Group B streptococcus	5	0	3	NS
Other *Streptococcus* spp.	10	2	3	NS
Other[b]	6	1	1	NS
Gram-negative bacteria	56	20	8	<0.05
Escherichia coli	9	7	3	NS
Other *Enterobacteriaceae*	16	7	3	NS
Haemophilus influenzae	23	1	1	NS
Pseudomonas aeruginosa	3	2	1	NS
Other[c]	5	3	0	NS
Anaerobic bacteria[d]	0	0	1	NS
Fungi[e]	14	3	2	NS

[a] NS, Not significant ($P > 0.05$).
[b] Includes *Bacillus* spp. (4 isolates) and unidentified gram-negative bacillus (4 isolates).
[c] Includes *Neisseria meningitidis* (3 isolates), *Neisseria gonorrhoeae* (2 isolates), *Acinetobacter* spp. (1 isolate), *Moraxella* spp. (1 isolate), and gram-negative diplococcus (1 isolate).
[d] Includes *Bacteroides fragilis* (1 isolate).
[e] Includes *Candida albicans* (14 isolates), *Candida parapsilosis* (1 isolate), *Candida tropicalis* (1 isolate), and *Torulopsis glabrata* (3 isolates).

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi from BACTEC 6B medium with and without 1.2% gelatin and V-factor-analog

Microorganism	No. of isolates recovered by:			P
	Both media at the same time	6B earlier	6B-gelatin-V-factor earlier	
Aerobic or facultative bacteria	131	8	18	NS[a]
Gram-positive bacteria	102	7	6	NS
Staphylococcus aureus	33	4	2	NS
Staphylococcus epidermidis	28	2	3	NS
Streptococcus pneumoniae	22	0	0	NS
Group B streptococcus	5	0	0	NS
Other *Streptococcus* spp.	10	0	0	NS
Other[b]	4	1	1	NS
Gram-negative bacteria	37	1	12	<0.01
Escherichia coli	8	0	1	NS
Other *Enterobacteriaceae*	15	1	0	NS
Haemophilus influenzae	14	0	9	<0.01
Pseudomonas aeruginosa	3	0	0	NS
Other[c]	2	0	2	NS
Anaerobic bacteria	0	0	0	NS
Fungi[d]	4	10	0	<0.02

[a] NS, Not significant ($P > 0.05$).
[b] Includes *Bacillus* spp. (2 isolates) and unidentified gram-positive rod (4 isolates).
[c] Includes *Neisseria meningitidis* (1 isolate), *Neisseria gonorrhoeae* (1 isolate), *Acinetobacter* spp. (1 isolate), and gram-negative diplococcus (1 isolate).
[d] Includes *Candida albicans* (11 isolates) and *Torulopsis glabrata* (3 isolates).

When a microorganism grew in both media, there was little difference in the speed of recovery for most isolates (Table 2). Of the 185 isolates which grew in both media, 149 (80.5%) were detected at the same time. Gram-negative bacteria were isolated more rapidly in the 6B-gelatin-V-factor-analog media (Table 2) solely because of the number of isolates of *H. influenzae*. Notable exceptions were *H. influenzae* and fungi. *H. influenzae* was recovered earlier from the 6B-gelatin-V-factor-analog bottle ($P < 0.01$), with a mean time to detection of 8.5 h compared with 15.9 h for the 6B bottle (Table 3). *Candida albicans* was recovered earlier from the 6B bottle ($P < 0.02$), with a mean time to detection of 34.9 h compared with 71.6 h for the 6B-gelatin-V-factor-analog bottle.

TABLE 3. Comparison of mean time to detection for frequently isolated bacteria and fungi from BACTEC 6B medium with and without 1.2% gelatin and V-factor-analog

Microorganism	No. of isolates	Mean time to detection (h)	
		6B	6B-gelatin-V-factor-analog
Staphylococcus aureus	34	13.3	13.7
Streptococcus pneumoniae	20	9.6	9.6
Haemophilus influenzae	23	15.9	8.5
Escherichia coli	9	11.4	9.9
Candida albicans	10	34.9	71.6

DISCUSSION

The addition of gelatin to blood culture media containing SPS has been shown to improve the yield of certain bacteria, including *N. meningitidis* (9), which is an important pathogen in the pediatric population. Moreover, the addition of specific growth factors such as hemin and NAD can enhance the growth of *H. influenzae*, which is another important pathogen in the pediatric population. However, the effects of gelatin and V-factor-analog (a proprietary supplement containing, in part, hemin and NAD) on the detection of other common microorganisms causing bacteremia and fungemia in children has not been studied in a large field trial. We evaluated in a pediatric population the widely used BACTEC 6B medium in its currently available formulation (tryptic soy broth containing SPS) and in a medium that differed only by the addition of 1.2% gelatin and V-factor analog.

The results clearly favored the current formulation of yield (Table 1). An important role of SPS is the inactivation of complement (11). We speculate that the greater availability of complement in gelatin-supplemented media results in inhibition of serum-sensitive members of the family *Enterobacteriaceae* as well as other organisms (15) and may explain the lower yield. Of the bacteria reported to be recovered with greater frequency in gelatin-supplemented media, only three *N. meningitidis* and two *N. gonorrhoeae* isolates were recovered during the 6-month study period; none were isolated only in the gelatin-supplemented media.

A similar study with adults (17) during the same time period revealed that more aerobic and facultative bacteria grew in the 6B medium than in 6B medium supplemented with only 1.2% gelatin ($P < 0.001$). Unfortunately, none of the bacteria reported to be recovered with greater frequency in gelatin-supplemented media were recovered during the 6-month study period from adult blood cultures.

The increased speed of recovery for *H. influenzae* (7 of 16 were found earlier in the supplemented 6B) is most likely due to the addition of V-factor-analog. The mean time of detection (8.5 h for 6B-gelatin-V-factor-analog) was such that most clinical microbiology laboratories would be aware of the positive blood culture on the second day of culture (8). The decreased speed of recovery for *C. albicans* is unexplained. The clinical implications of this difference in recovery time are important, as most clinical laboratories would be aware of the positive blood culture on day 3 for 6B versus day 6 for 6B-gelatin-V-factor-analog (8).

Our results differ from those of Pai and Sorber (9), who noted that the addition of 1% gelatin to Columbia broth enhanced the detection of *N. meningitidis* but did not influence the recovery of other microorganisms from the blood. There were multiple differences between that study and this one, including the medium (Columbia broth versus tryptic soy broth), the number of cultures studied (1,662 versus 4,565), and the number of positive cultures evaluated (45 versus 442). Our results support the concept that the effect of gelatin may be medium dependent. Most importantly, the effect of gelatin (or for that matter any additive) on any specific medium used for diagnostic studies in a clinical microbiology laboratory should be evaluated in a large clinical field trial.

In summary, the presence of 1.2% gelatin and V-factor-analog in 6B medium did not significantly enhance the isolation of pathogenic bacteria and fungi from the blood of children and appeared detrimental to some organism groups. The currently marketed 6B medium outperformed the experimental medium and, therefore, remains the recommended formulation for pediatric use.

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