Comparative Evaluation of Different Types of Blood Culture Media for Isolation of Aerobes

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The possible advantage of hypertonic sucrose medium over isotonic medium for isolating aerobic organisms from blood was studied. Approximately 50 ml of medium and 5 ml of blood inoculum were present in each culture bottle. In the first phase of the study, supplemented peptone broth (SPB) was compared with brucella broth containing 10% sucrose (BB-10S). There were 194 significant clinical isolates in at least one of the two bottles in each set during a 7-month period; 160 (82%) of the isolates grew in SPB, whereas 191 (98%) grew in BB-10S ($P < 0.01$). Of the 158 isolates that grew in both media, 13 (8%) appeared earlier in BB-10S, whereas none did so in SPB. In the second phase of the study, SPB with 10% sucrose (SPB-10S) was compared with BB-10S. There were 187 isolates during a 9-month period; 173 (93%) grew in SPB-10S compared with 179 (96%) for BB-10S. In this comparison there was no apparent difference in the time interval required for recovery of organisms. The two hypertonic sucrose media (SPB-10S and BB-10S) were comparable for isolating organisms under aerobic conditions and superior to the nonhypertonic medium (SPB).

Numerous studies have shown that the addition of sucrose to blood culture media results in increased numbers of isolates and faster rates of recovery as compared with isotonic blood culture media (7, 12, 13, 14). Other studies, however, suggest that sucrose supplementation offers no advantage over the isotonic condition (3, 15). Comparison of these studies is difficult because of differences in volume of inoculated blood, venting procedures, time of inoculation of blood (bedside or laboratory), and the amount of shaking of cultures among the various studies. The present study was designed to compare recovery of aerobic organisms in hypertonic and isotonic media by using constant venting, blood inoculation at the patient's bedside, and no shaking for either medium. Because a significant difference was observed in isolation rates of the isotonic and hypertonic media, a second phase of study was conducted in which both media were made hypertonic and only the basal medium differed.

Hypertonic brucella broth (brucella broth containing 10% sucrose; BB-10S) was chosen for this study because it appeared to permit a high rate of isolation compared with other commercially available media (13). Isotonic-supplemented peptone broth (SPB) was selected for comparison because it was the most widely used medium available in Vacutainer bottles and be-

cause the Vacutainer system appeared to offer certain advantages in blood collection. Hypertonic SPB (SPB containing 10% sucrose; SPB-10S) in Vacutainer bottles was later substituted for isotonic SPB and directly compared with BB-10S.

MATERIALS AND METHODS

Culture media. The following three media were tested: (i) a 45-ml amount of SPB (Vacutainer culture tube, Becton, Dickinson & Co., Rutherford, N.J.) containing 0.03% sodium polyanetholsulfonate (SPS); (ii) 50 ml of BB-10S (Pfizer Inc., New York, N.Y.) containing 0.05% SPS; and (iii) 50 ml of SPB-10% sucrose (SPB-10S; Becton, Dickinson & Co.) with 0.07% sodium amylosulfate (SAS). All three bottles contained 10% CO₂.

In both phases of the study, two blood culture bottles were incubated aerobically by continuous venting. In phase I, SPB was compared with BB-10S. In phase II, SPB was replaced with SPB-10S, and the latter was compared with BB-10S.

In both phases of study, an unvented or Vasparg-plugged SPB (SPB-An) was incubated as a third bottle in each blood culture set to detect potential anaerobic organisms not found in the two aerobic bottles.

Collection of specimens. Blood was collected under aseptic conditions by either a venipuncture team or by house staff. Blood was drawn into a syringe, and then 5 ml was inoculated into each of three blood culture bottles at the bedside. When the bottles were received in the laboratory, they were included in the

study if, at least, both aerobic bottles contained the correct blood volumes, as determined by visual inspection. Venting was performed in the laboratory, and the venting needle remained in the bottle during incubation. The bottles were incubated at 35°C and were examined macroscopically every day for growth. Gram stains and subcultures were performed routinely at 24 h, and subculture was performed again at day 7. All bottles were kept for a total of 9 days. Subcultures were performed by inoculating both a sheep blood agar plate incubated under anaerobic conditions by the GasPak system and a chocolate blood agar plate incubated in 5% CO₂. Aerobic and anaerobic organisms were identified by conventional biochemical procedures; gas chromatography was not used.

RESULTS

Phase I studies. The first phase of our study covered a 7-month period during which there were 1,923 matched sets of blood cultures acceptable for study. In this group, 10.2% of these blood cultures was positive for significant isolates. *Staphylococcus epidermidis*, *Bacillus* species, and diphtheroids were considered nonsignificant isolates or contaminants unless isolated repeatedly from the same patient. Contaminants occurred at similar rates in both aerobic bottles and were omitted from further analysis. Overall, there were 196 significant isolates (Table 1), of which 191 grew in BB-10S and only 160 grew

in SPB. In this phase of the study, there was an SPB-An bottle present in all sets. Only two anaerobic organisms recovered in SPB-An were not recovered from either SPB or BB-10S. Four anaerobic gram-positive rods and five *Bacteroides* also grew well in the aerobically incubated hypertonic medium. Eight of the total of nine pneumococcal isolates grew well in the hypertonic medium.

Of the 194 isolates recovered only in the aerobically incubated media, 160 (82%) were recovered in SPB, and 191 (98%) were found in BB-10S ($P < 0.01$, by the Chi-square method with the Yates correction). *Staphylococcus aureus*, group D enterococcus, anaerobic gram-positive rods, *Escherichia coli*, and *Candida* were found more often in BB-10S ($P < 0.01$ for each organism).

In addition to a discrepancy between the number of isolates recovered in the two media incubated aerobically, there was a discrepancy in the time interval required for recovery of organisms in these media. There were 158 isolates found in both SPB and BB-10S. Thirteen of these isolates were detected at least one day earlier in BB-10S by noting increased turbidity, gas formation and appearance of colonies on the cell layer. None was detected earlier in SPB. The isolates that appeared faster in BB-10S

TABLE 1. Comparison of blood culture media, phase I

Organism	Total positive	Positive in:		
		SPB	BB-10S	SPB-An
Gram positive				
<i>Staphylococcus aureus</i>	41	33	41	30
<i>S. epidermidis</i>	8	8	8	8
Streptococci				
Group D, enterococcus	7	3	7	3
Group D, nonenterococcus	8	8	8	8
Other groups	23	21	23	21
<i>S. pneumonia</i>	9	9	8	9
Clostridium	1	0	0	1
Anaerobic gram-positive rods	5	0	4	3
Gram negative				
<i>Escherichia</i>	45	38	44	38
<i>Klebsiella</i>	7	7	7	7
<i>Enterobacter</i>	1	1	1	1
<i>Serratia</i>	7	5	7	5
<i>Proteus</i>	9	9	9	5
<i>Salmonella</i>	1	1	1	1
<i>Pseudomonas</i>	4	4	4	3
<i>Haemophilus</i>	4	4	4	1
<i>Acinetobacter</i>	2	1	2	0
<i>Moraxella</i>	1	0	1	0
<i>Bacteroides</i>	6	4	5	4
Fungi				
<i>Candida</i>	7	4	7	3

were eight strains of *E. coli*, two strains of *Candida*, one strain of *S. pneumoniae*, one group D enterococcus, and one strain of *S. aureus*. In addition, when an isolate grew at the same time in both media, the number of colony-forming units on subculture was often greater in BB-10S.

Phase II studies. In an effort to determine if the difference between SPB and BB-10S noted in phase I was due to different basal media (SPB versus BB) or to the absence of 10% sucrose in SPB, a second phase of study was initiated in which SPB was replaced by SPB with 10% sucrose (SPB-10S). BB-10S was again used to maintain one medium as a constant factor in both phases of the study and also because it was the better of the two aerobic media used in phase I. SPB incubated aerobically was not included in phase II because results of the first study showed it to be inferior to BB-10S for detection of aerobic growth and because of limitations on the amount of blood that could be drawn per patient. The presence of BB-10S in both phases of study served as a common bench-mark for the detection of aerobic growth.

In this phase, during a 9-month period, there were 1,948 acceptable blood culture sets submitted to the laboratory; 189 (9.7%) of these cultures yielded significant isolates. In contrast to the first phase of study, no significant differences were noted between the two hypertonic media incubated aerobically (Table 2). A total of 187 isolates were found in either one or both aerobic bottles; 173 of the isolates grew in SPB-10S, whereas 179 grew in BB-10S. This difference is not statistically significant. In addition, no difference was noted in the time interval required for recovery of organisms from either hypertonic medium. The incidence of contaminants in either aerobic bottle was the same. The actual contamination rate (percentage of positive culture sets) was 26.4% for phase II, which was similar to the rate of 18.9% observed in phase I.

The anaerobic medium again yielded significantly fewer isolates than either aerobic medium. The anaerobic medium failed to support growth of many common facultative gram-negative rods such as *Escherichia*, *Enterobacter*, and *Proteus*, which normally would be expected

TABLE 2. Comparison of blood culture media, phase II

Organism	Total positive	SPB-10S	BB-10S	SPB-An
Gram positive				
<i>Staphylococcus aureus</i>	19	17	18	14
<i>S. epidermidis</i>	11	10	11	8
Streptococci				
Group A	1	1	0	0
Group B	1	0	0	1
Group D, enterococcus	7	6	7	6
Group D, nonenterococcus	4	4	3	3
Other groups	14	12	12	11
<i>Clostridium</i>	1	0	0	1
<i>Peptostreptococcus</i>	1	0	1	0
<i>Bacillus</i>	1	1	1	1
Gram negative				
<i>Escherichia</i>	36	35	35	30
<i>Klebsiella</i>	16	16	16	13
<i>Enterobacter</i>	15	15	15	7
<i>Serratia</i>	2	2	2	1
<i>Proteus</i>	12	11	12	6
<i>Salmonella</i>	8	7	8	7
<i>Citrobacter</i>	1	1	1	1
<i>Pseudomonas</i>	20	17	19	6
<i>Pasturella</i>	2	2	2	0
<i>Haemophilus</i>	1	1	1	1
<i>Neisseria</i>	2	2	2	0
CDC group II ^a	1	1	0	0
CDC group Ve	2	2	2	0
<i>Bacteroides</i>	4	3	4	3
Anaerobic gram-negative rod	1	1	1	1
Fungi				
<i>Candida</i>	6	6	6	2

^a CDC, Center for Disease Control, Atlanta Ga.

to grow under anaerobic conditions. In this second phase, only two isolates (group B streptococcus and *Clostridium* species) were made in the anaerobic medium, which were not detected simultaneously in the aerobic medium.

DISCUSSION

Most reported blood culture studies comparing hypertonic and isotonic media have indicated that the use of hypertonic medium permits higher isolation rates of microorganisms and often permits more rapid detection. In an earlier study by Rosner (12), BB with 30% sucrose and 0.05% SPS was shown to be more effective for isolating most gram-positive and fastidious gram-negative organisms (121 total isolates) than isotonic BB with 0.05% SPS (103 organisms; 85% recovery) or BB without either additive (74 organisms; 61% recovery). This difference was most marked when the level of bacteremia as determined by the pour plate technique was less than 20 organisms per ml of blood. Gram-negative enteric bacilli were not included in this analysis. In a later study from the same laboratory (13) where all types of microorganisms (176 total isolates) were included for study, BB-10S with SPS (171 organisms; 97% recovery) was shown to be superior to isotonic thioglycolate medium with SPS (130 organisms; 74% recovery). In addition, all isolates from the hypertonic BB were observed within 48 h, whereas 66% of the isolates found in the isotonic medium required more than 48 h to appear. Initial studies by Ellner and associates using Columbia broth with and without 10% sucrose showed that the hypertonic medium yielded 16% more isolates and a 12% faster recovery rate than the isotonic medium (3). Henrichsen and Bruun (7) also demonstrated that hypertonic broth was significantly superior to isotonic broth with most bacterial species, especially the gram-negative enteric bacilli and *S. aureus*. In addition, their impression was that organisms present in both media grew more rapidly in the hypertonic medium.

Less clinical work has been done with hypertonic medium incubated under anaerobic conditions (4). Sullivan et al. (14) found that the addition of 16% sucrose and SPS to an anaerobic broth yielded more isolates than when both additives were excluded (43 versus 27 isolates). The effect of SPS alone was not studied.

In a study by Coleman et al. of the radiometric detection of bacteremia under aerobic conditions (2), hypertonic medium was responsible for the isolation of more organisms than was the isotonic medium, but it was necessary to use both hypertonic and isotonic aerobic media for max-

imum detection. Organisms often grew first in the hypertonic bottle, and fewer false-positive growth index readings occurred in this bottle.

Fewer studies have been done that are critical of the previously claimed advantages of hypertonic sucrose blood culture medium. Washington et al. (15) were unable to show any statistically significant differences in the isolation rates of Trypticase soy broth with SPS versus Trypticase soy broth with SPS and 15% sucrose. Their cultures were incubated without venting. According to a separate study from the same laboratory (6), venting is important only for the isolation of *Pseudomonas* and *Candida*. In contrast, Blazevic et al. (1) found that venting was generally important and resulted in a higher isolation rate, but that the unvented bottle was also important for detecting some isolates missed by the vented bottle. Our own studies with SPB also indicated that vented bottles (i.e., SPB) yielded significantly more isolates than unvented, Vaspar-plugged bottles (i.e., SPB-An). Thus, the lack of venting in Washington's study (15) makes difficult direct comparison with other studies on hypertonic media for aerobic isolates.

In further studies, Ellner and associates concluded that hypertonic medium yielded no additional isolates compared with isotonic medium when both media were shaken during incubation (3). In none of the previously described studies was shaking regularly practiced, except in Coleman's study (2) where it was done for the first 24 h. In this latter study, hypertonic medium did yield more positives than the isotonic medium. In the absence of shaking, Ellner's study (3) showed that a hypertonic-isotonic combination resulted in the recovery of 99.4 organisms per 1,000 cultures versus a recovery rate of 83.6 per 1,000 from duplicate isotonic bottles.

The mechanism of growth enhancement for hypertonic medium is not clear. At least three mechanisms have been proposed. First, sucrose may stabilize the bacterial wall in cell wall-defective mutants circulating in the blood of patients either previously treated with antibiotics or present because of damage inflicted by opsonins (12). Second, sucrose may inactivate the penicillins and permit the partially inhibited organisms to grow (9). Last, sucrose may be anticomplementary (10) or antagonistic to non-specific inhibiting substances in blood as occurs with SPS (12).

It should be noted that SAS was used in the SPB-10S medium in phase II of our study rather than SPS, which was present in the SPB used in phase I. SPS has been shown to interfere with the growth of peptostreptococci and pep-

tococci, whereas SAS does not (8). However, SAS and SPS have not been proven to be inhibitory to other organisms (5). Therefore, it is unlikely that the use of these different additives accounted for the difference in performance between SPB and SPB-10S.

SPB was employed as the aerobic isotonic medium in phase I of our study. Although some studies have indicated that SPB is inferior to Trypticase soy broth (13), the volumes of SPB (18 ml) and blood inoculum for SPB (2 ml) in these other studies have been less than half used for the comparative medium. The discrepancies in isolation rate may simply be due to the difference in inoculum size. When SPB bottles containing 45 ml were compared with the same volume of Eugon broth and the blood inocula were identical in both bottles, no important differences were noted in the isolation rates of gram-positive cocci or *Enterobacteriaceae* (11).

In conclusion, our studies confirm most reports in the literature indicating that there is an improved rate of isolation of microorganisms in hypertonic sucrose medium compared with isotonic medium when both are incubated under aerobic conditions. Therefore, it would appear to be worthwhile to include a hypertonic sucrose bottle maintained under aerobic conditions in each blood culture set. The addition of a second aerobic bottle with isotonic medium may possibly result in a small increase in isolation rates, but this has not been firmly established.

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