Comparative Evaluation of Three Different Commercial Blood Culture Media for Recovery of Anaerobic Organisms

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A comparison of three different commercial media was made to assess their recovery of anaerobic organisms from the blood stream. The three media used were the 50-ml brain heart infusion broth with added CO₂ (Pfizer), the 50-ml Thiol broth with added CO₂ (Difco), and the 50-ml prereduced, supplemented peptone broth in a Vacutainer tube with added CO₂ (Becton-Dickinson). During a period of 17 consecutive months, 12,216 specimens of blood were processed with each broth. Aerobic or anaerobic bacteria were recovered from 913 specimens (7%). Seventy-four specimens (8%) of the total positive cultures contained anaerobic organisms. When potential contaminants were removed from the totals, 7% of the positive cultures contained anaerobic organisms and 7% of the patients with positive cultures had bacteremia with anaerobic bacteria. Of the three commercial blood culture media studied, the prereduced, supplemented peptone broth recovered more anaerobic organisms than did either the brain heart infusion or Thiol broths.

The optimum conditions for the recovery of anaerobic organisms from the blood stream have not been clearly established. Theoretically, a prereduced medium should provide the ideal means for recovering anaerobic organisms from blood cultures because it contains few reducing agents and minimal formation of organic peroxides (2, 6, 7, 10). However, the studies of Hall et al. (4), Rosner (9), and Washington and Martin (15) showed that prereduced media did not increase measurably the recovery rates of anaerobic organisms from blood cultures when compared with conventional media.

Washington and Martin (15) reported that prereduced brain heart infusion broth (BHI; Scott Laboratories) failed to yield a significant increase in recovery rate and did not reduce the length of time required to obtain a positive blood culture when compared with nonprereduced media. Other studies (4, 9) with a Vacutainer containing 18 ml of prereduced, supplemented peptone (PRS-peptone) broth (Becton-Dickinson & Co.) showed this medium to be less efficient than the other media tested. However, in these studies the observed differences may have been due to the fact that the prereduced media tubes contained 18 ml of broth, allowing only 2 ml of whole blood to be inoculated, whereas, the other media tested were in bottles that provided space for a 10-ml blood sample.

Few studies have been reported using the newer 50-ml-size Vacutainer tube (8).

The present study employed the 50-ml PRS-peptone Vacutainer tube to assess the efficiency of the larger-size bottle and larger blood sample size (5 ml) to recover anaerobic organisms from the blood stream. The three commercial blood culture media chosen for this investigation were: 50-ml BHI broth with added CO₂, 50-ml Thiol broth with added CO₂, and 50-ml PRS-peptone in a Vacutainer tube with added CO₂. The media were assessed for differences in rate of recovery of anaerobic organisms and for length of time required for each of the media to become positive for anaerobic organisms.

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MATERIALS AND METHODS

Blood specimens. All blood specimens for culture during the period of March 1974 through July 1975 were collected aseptically by venipuncture from the antecubital vein with a sterile 20-ml plastic syringe and a 20-gauge disposable needle (Styles disposable hypodermic syringe and needle, Pharmaseal Laboratories, Glendale, Calif.). Skin antisepsis of the venipuncture site was accomplished by either a 70% isopropyl alcohol pledget (Alco-Wipe, Milwaukee, Wis.) or by Betadine Swab Aids (Purdue Frederick Co., Norwalk, Conn.). All specimens were collected

by a special phlebotomy team.

Culture media. The blood specimen was distributed on a 10% (vol/vol) basis directly into each of the following bottles: 50-ml BHI broth under vacuum and with CO₂ (Pfizer Diagnostics Division, New York, N.Y.), 50-ml Thiol broth under vacuum and with CO₂ (Difco Laboratories, Detroit, Mich.), and 50-ml Vacutainer PRS-peptone broth under vacuum and with CO₂ (Becton-Dickinson Co., Rutherford, N.J.). All three media contained between 0.025 and 0.05% sodium polyanetholsulfonate.

Since the efficiencies of the three different culture media were being compared, each study required a total volume of 15 ml of whole blood. A 5-ml amount of the blood sample was injected in a random order into each type of blood culture medium at the bedside. The three bottles were taped together and processed in the Diagnostic Microbiology Laboratory as a single unit. All culture bottles were incubated without air venting at 35°C and were visually examined each day for 14 days for signs of bacterial growth.

Subcultures and identification of isolates. In the absence of visual growth, all bottles were routinely subcultured at 2 days and again at 7 days after initial inoculation. The rubber cap of each bottle was decontaminated with a 70% isopropyl alcohol pledget, and a 0.5-ml sample was withdrawn with a sterile 1-ml disposable syringe. Each sample was inoculated onto a freshly prepared Trypticase soy agar base (Baltimore Biological Laboratory, Cockeysville, Md.) enriched with 5% defibrinated sheep blood, 10.0 µg of vitamin K₁ (ICN Pharmaceuticals, Cleveland, Ohio) per ml, and 5.0 µg of hemin (Eastman Kodak Co., Rochester, N.Y.) per ml. The plates were placed into a vented GasPak jar (Baltimore Biological Laboratory, Cockeysville, Md.). The atmosphere of the jar was evacuated and replaced three times with a gaseous mixture of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen (Liquid Carbonic Corporation, San Carlos, Calif.). The jar was sealed and incubated at 35°C for 2 days.

With blood cultures suspected of being positive by visual observation or by blind subculture, 0.5 ml of the contents from the blood culture bottle was aspirated by needle and syringe for a Gram stain and subcultured to chopped-meat carbohydrate broth and BHI agar-supplemented roll streak tube (Scott Laboratories, Fiskeville, R.I.). The length of time before detection of bacterial growth was recorded for each blood culture medium. All colonial morphotypes were Gram stained and tested for oxygen tol-

erance. Once an isolate was determined to be an anaerobe, it was identified by the procedures described by Holdeman and Moore (6).

RESULTS

During the period from 1 March 1974 through 31 July 1975, 24,746 blood culture specimens were received. Of these, 12,216 met the criterion for this study, i.e., 5 ml of blood inoculated into all three blood culture systems. Either anaerobic or aerobic bacteria were recovered from 913 (7%) of the 12,216 specimens (Table 1). Seventy-four (8%) of the positive cultures contained anaerobic bacteria. The 74 positive anaerobic blood cultures were recovered from 50 (8%) of the 590 patients with positive blood cultures. If *Staphylococcus*, coagulase negative, *Bacillus* sp., *Corynebacterium* sp., and *Propionibacterium acnes* are assumed to represent contaminants (1% total cultures), then 749 (6%) of 12,216 specimens were positive; 56 (7%) of the 749 positive cultures contained anaerobic bacteria; and 33 (7%) of the 502 patients with positive cultures had bacteremia with anaerobic bacteria. A total of 6 (11%) of the 56 positive cultures with anaerobic organisms contained additional species of bacteria; 2 of the 6 contained more than one species of anaerobic bacteria.

Bacteroides fragilis was the most frequent anaerobe isolated and represented 46% of all anaerobes (Table 2). The next most frequent isolates were *P. acnes* (24%), *Clostridium perfringens* (7%), *C. septicum* (6%), *Peptococcus* sp. (5%), *Bacteroides* sp. (4%), *B. oralis* (3%), and *B. melaninogenicus*, *C. cadaveris*, *C. bif fermentans*, *C. tetanomorphum*, and *Peptostreptococcus* sp. (1% of all isolates).

The number, percentage, and type of anaerobic organism detected in each of the three test media are shown in Table 3. Most striking was the 100% recovery of *B. fragilis* in the PRS-peptone broth, 88% in Thiol Broth, and 47% in BHI broth. The portion of the 72 anaerobic isolates detected by each medium was 67 (90%) for PRS-peptone broth, 59 (80%) for Thiol broth, and 30 (40%) for BHI broth. Deletion of *P. acnes*

TABLE 1. Incidence of anaerobic bacteria in blood specimens meeting the study criteria

Total no. of blood cultures tested	Total no. of positive cultures	No. of cultures yielding anaerobic bacteria	Positive cultures with anaerobic bacteria (%)	Total no. of patients with positive cultures	No. of patients yielding anaerobic bacteria	Patients with anaerobes in cultures (%)
12,216	913 ^a	74	8	590	50	8
12,216	749 ^b	56	7	502	33	7

^a All isolates, regardless of whether they were aerobic or anaerobic.

^b Potential contaminants removed (*Staphylococcus*, coagulase negative, *Bacillus* sp., *Corynebacterium* sp., and *P. acnes*).

TABLE 2. Incidence of anaerobes isolated from positive blood cultures

Organism	No. of patients	No. of isolates	Total anaerobic isolates (%)
<i>B. fragilis</i>	16	34	46
<i>B. melaninogenicus</i>	1	1	1
<i>B. oralis</i>	2	2	3
<i>Bacteroides</i> sp.	2	3	4
<i>C. cadaveris</i>	1	1	1
<i>C. bifermentans</i>	1	1	1
<i>C. perfringens</i>	3	5	7
<i>C. septicum</i>	2	4	6
<i>C. tetanomorphum</i>	1	1	1
<i>Peptococcus</i> sp.	3	3	5
<i>Peptostreptococcus</i>	1	1	1
<i>P. acnes</i>	17	18	24

increased the differences among BHI and the other two media.

The mean time until recognition of cultures containing anaerobes (Table 4) was 3.8 days for PRS-peptone broth, 4.2 days for Thioli broth, and 4.3 days for BHI broth. The range of time until detection of positive cultures was 1 to 8 days for 27 isolates in BHI broth, 1 to 9 days for 54 isolates in Thioli broth, and 1 to 9 days for 63 isolates in PRS-peptone broth. These data also reveal that anaerobes frequently could be recovered from the different media within 2 to 3 days.

DISCUSSION

Prompt recovery and identification of anaerobic organisms from blood are imperative due to the high incidence of anaerobic bacteremia (12,

TABLE 3. Organisms detected by each medium

Organism	No. of isolates	BHI		Thioli		Peptone	
		No.	%	No.	%	No.	%
<i>B. fragilis</i>	34	16	47	30	88	34	100
<i>B. melaninogenicus</i>	1	0	0	1	100	1	100
<i>B. oralis</i>	2	0	0	2	100	1	50
<i>Bacteroides</i> sp.	3	1	33	2	66	2	66
<i>C. cadaveris</i>	1	0	0	0	0	1	100
<i>C. bifermentans</i>	1	1	100	0	0	1	100
<i>C. perfringens</i>	5	2	40	5	100	5	100
<i>C. septicum</i>	4	2	50	4	100	4	100
<i>C. tetanomorphum</i>	1	0	0	1	100	1	100
<i>Peptococcus</i> sp.	3	0	0	2	66	2	66
<i>Peptostreptococcus</i>	1	0	0	0	0	1	100
<i>P. acnes</i>	18	8	46	12	66	14	78
Total	74	30	40	59	80	67	90
Total without <i>P. acnes</i>	56	22	39	47	84	53	95

TABLE 4. Mean time required to detect a positive culture

Organism	BHI		Thioli		Peptone	
	No.	Mean days	No.	Mean days	No.	Mean days
<i>B. fragilis</i>	15	3.1	29	2.7	34	2.6
<i>B. melaninogenicus</i>	0		1	3.0	1	3.0
<i>B. oralis</i>	0		2	4.0	1	5.0
<i>Bacteroides</i> sp.	1	5.0	2	5.0	2	3.5
<i>C. cadaveris</i>	0		0		1	2.0
<i>C. bifermentans</i>	1	4.0	0		1	4.0
<i>C. perfringens</i>	2	2.5	5	2.6	5	2.4
<i>C. tetanomorphum</i>	0		1	3.0	1	3.0
<i>Peptococcus</i> sp.	0		2	5.5	2	4.5
<i>Peptostreptococcus</i>	0		0		1	4.0
<i>P. acnes</i>	8	6.9	12	8.5	14	8.0
Mean recovery for all organisms	27	4.3	54	4.2	63	3.8

13, 15, 16) and the necessity for appropriate antibiotic therapy (14, 16). The choice of a blood culture medium for the recovery of anaerobic bacteria from the blood stream is a confusing yet essential decision for clinical microbiologists. Although the ideal blood culture medium for anaerobic organisms has not been defined, direct clinical comparisons of various media provide clinical microbiologists with some basis for the selection of a medium for their laboratories.

The present study was designed to assess the ability of three different commercial blood culture media to detect anaerobic bacteria under clinical conditions. Our study shows that a greater number of anaerobic organisms were recovered from blood with PRS-peptone and Thiol broth than with BHI. More anaerobic isolates were recovered from the PRS-peptone than from Thiol broth; however, the differences were not statistically significant ($0.10 > P < 0.15$). All anaerobic organisms, even possible contaminants, were included to determine the total number of anaerobes each medium was capable of recovering. Even if potential contaminants are disregarded, these media still were superior to BHI. With the exception of *P. acnes*, most positive blood cultures were detected within 2 to 3 days, and the majority were detected by 5 days. Preliminary studies from our laboratory with pure cultures of anaerobes have not presented adequate information to account for some of the potential advantages of PRS-peptone, e.g., the fact that it contains additional supplements and is prereduced with a minimal amount of reducing agents.

We have not examined the possibility that other commercial sources of the media tested in the present study might perform differently. However, until published information demonstrates whether such differences exist, the results of the present study suggest that a medium other than BHI broth should be used for the optimum recovery of anaerobic bacteria.

The results of our study differ from previous reports that have shown no differences in the recovery of anaerobic organisms when prereduced blood culture media were compared with conventional media (4, 9, 15). In the studies of Hall et al. (4) and Rosner (9), the 18-ml PRS-peptone tube, which allows only 2 ml of whole blood to be aspirated into the tube, gave significantly lower recovery rates than conventional media. However, these studies were almost certainly influenced by the differences in blood volume in the test systems. This is an important consideration since the levels of bacteremia with anaerobic bacteria are of a low order of magnitude (3, 11). Another study by Wash-

ington and Martin (15) compared a 50-ml volume of prereduced medium (prereduced, supplemented BHI) with a 50-ml volume of conventional medium and found no increase in yield or reduction of the time to obtain a positive culture. However, this medium was of different composition than the PRS-peptone broth used in this study, and the results cannot be directly compared.

The proportion of positive blood cultures containing anaerobic bacteria (7%) in our study is less than that reported from the Mayo Clinic (11-27%) (12, 13, 15, 16). Although we cannot account for this difference with certainty, it is unlikely that it can be attributed entirely to differences in media, blood volume sampled, or other technical factors since our study included at least one medium in common with their studies and divided a total of 10 ml of blood between our two best media. For example, if Washington and Martin (15) had only relied upon Thiol broth (Difco) into which they had inoculated 10 ml of blood, their incidence would have still been 21%, an incidence considerably higher than our experience. A more likely explanation would seem to be variations in the populations studied, particularly with respect to the proportion of patients with surgery or trauma involving the intestinal or genitourinary tracts. This uncontrolled factor may significantly influence the incidence of infections with anaerobic bacteria among different institutions and even among different time periods within the same institution.

In the absence of a suitable, all-purpose, single medium, the results of the present study emphasize the necessity of a two-bottle system for the recovery of both aerobic and anaerobic organisms from blood (1, 5). If a single, all-purpose system such as BHI, even though not vented, is used for culture of the blood, many anaerobes will not be detected. Although it has been reported (14) that commercially available blood culture media, if unvented, may have a sufficiently low redox potential for the recovery of clinically significant anaerobes, our study shows that BHI was not acceptable for the recovery of anaerobic organisms from blood specimens.

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