Comparison of Three Blood Culture Media with Tryptic Soy Broth

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Four consecutive studies comparing commercially prepared bottles that contained brain heart infusion, brucella (vented and unvented), and Trypticase soy broths with Tryptic soy broth failed to demonstrate significant differences in rates of isolation of organisms from blood. Statistically significant differences between bottles were limited to the average times required to detect growth of viridans streptococci in one study and of all organisms in another.

Despite the increasing number of reports concerning blood culture media and systems, much has yet to be learned about their relative efficacy. Reports are often difficult to assess because of inadequate control of the multiple variables associated with cultures of blood. Volumes of blood cultured, anticoagulants, atmospheres of incubation, as well as the timing and frequency of examinations and subcultures, represent the most important variables to be considered in any blood culture study. Finally, the number of cultures performed in any evaluation should be sufficiently large to permit proper statistical analysis of the isolates.

The purpose of this study was to compare two commercially prepared blood culture bottles containing soybean-casein digest broth and then to compare one of these with bottles containing brain heart infusion broth and brucella broth.

MATERIALS AND METHODS

Blood was collected aseptically by a phlebotomy team and inoculated (10%, vol/vol) into each of three vacuum blood culture bottles, two of which were the same and contained soybean-casein digest broth (Tryptic soy broth [TSB-D]; Difco Laboratories, Detroit, Mich.), and the third of which contained the test medium. Unless approved by prior consultation with the laboratory staff, at least two and no more than three sets of blood cultures were collected within a 24-h interval.

Upon their receipt in the laboratory, one of the two bottles containing TSB-D was transiently vented with a sterile, cotton-plugged needle to release its vacuum, whereas the other remained unvented. The test bottle was transiently vented in all but one study. All bottles were routinely subcultured within the first 15 h (in most instances, within the first 5 h) onto quadrants of chocolate blood agar plates, which were incubated in 10% CO₂ for 48 h (1). Routine subcultures of previously negative bottles were repeated 48 h later. All bottles were incubated at 35°C and examined macroscopically

later in the day of their collection, then daily for 7 days and, finally, after 14 days of incubation. Broth from any bottle found to be positive macroscopically or by routine subculture was examined microscopically and subcultured onto appropriate media, including a blood agar plate that was incubated anaerobically.

Two of the test broths, brain heart infusion and brucella, were prepared by Difco. Soybean casein digest broth (Trypticase soy broth [TSB-B]) was obtained from BioQuest, Div. of Becton, Dickinson & Co., Cockeysville, Md.). Each bottle used in these studies contained CO₂ and 100 ml of medium with 0.025% sodium polyanetholsulfonate. Brucella broth was tested initially in transiently vented bottles and then in unvented bottles. Statistical analysis was carried out by procedures described elsewhere (1). Paired comparisons were made of the transiently vented bottles in three studies and of the unvented bottles in one study.

RESULTS

Of 3,000 sets of cultures received between 8 March and 24 May 1976, in which transiently vented TSB-D and brain heart infusion broth bottles were compared, there were 264 isolates (Table 1). No statistically significant differences between these two media were noted, either in numbers of isolates or in the average time required for their detection. From 3,000 sets of cultures received between 6 April and 8 July 1977, in which transiently vented TSB-D and brucella broth were compared, there were 274 isolates (Table 2). No statistically significant differences occurred between these two media. Between 8 July and 18 September 1977, 3,000 additional pairs of TSB-D and brucella broth were received but were not vented before incubation (Table 3). The only statistically significant difference ($P < 0.01$) between these two media was that viridans streptococci were recovered from unvented brucella broth an aver-

TABLE 1. Numbers of isolates in positive cultures, by medium^a

Organism	TSB-D and BHI	TSB-D only	BHI only	Total positive
<i>Bacillus</i>	0	0	2	2
<i>Corynebacterium</i>	7	9	11	27
<i>Escherichia</i>	41	11	14	66
<i>Citrobacter</i>	2	0	0	2
<i>Klebsiella</i>	11	0	2	13
<i>Enterobacter</i>	1	2	1	4
<i>Serratia</i>	2	3	2	7
<i>Proteus</i>	8	1	3	12
<i>Haemophilus</i>	4	0	1	5
<i>Streptococcus</i>				
<i>S. pneumoniae</i>	4	2	1	7
Viridans	11	2	3	16
Group B	1	0	0	1
Group D	0	5	2	7
<i>Acinetobacter</i>	1	0	0	1
<i>Flavobacterium</i>	1	0	1	2
<i>Neisseria</i>	2	0	0	2
<i>Bacteroidaceae</i>	8	3	1	12
<i>Staphylococcus</i>				
<i>S. aureus</i>	16	4	3	23
<i>S. epidermidis</i>	4	7	16	27
<i>Pseudomonas</i>	15	5	3	23
<i>Candida</i>	5	0	2	7
<i>Cryptococcus</i>	0	0	1	1
Total	144	54	69	267

^a BHI, Brain heart infusion.TABLE 2. Numbers of isolates in positive cultures, by medium^a

Organism	TSB-D and brucella	TSB-D only	Brucella only	Total positive
<i>Bacillus</i>	2	3	2	7
<i>Corynebacterium</i>	2	10	4	16
<i>Escherichia</i>	44	12	12	68
<i>Klebsiella</i>	5	4	4	13
<i>Enterobacter</i>	7	3	1	11
<i>Serratia</i>	2	0	2	4
<i>Proteus</i>	3	1	0	4
<i>Streptococcus</i>				
<i>S. pneumoniae</i>	7	4	0	11
Viridans	11	3	4	18
Group A	6	0	0	6
Group B	3	1	1	5
Group C	1	0	0	1
Group D	4	1	3	8
<i>Acinetobacter</i>	1	0	0	1
<i>Brucella</i>	1	0	1	2
<i>Bacteroidaceae</i>	13	7	3	23
<i>Staphylococcus</i>				
<i>S. aureus</i>	14	1	5	20
<i>S. epidermidis</i>	4	10	13	27
<i>Peptococcus</i>	0	1	0	1
<i>Pseudomonas</i>	16	3	5	24
<i>Listeria</i>	2	0	0	2
<i>Candida</i>	0	2	0	2
Total	148	66	60	274

^a Both media were transiently vented.

age of 1.3 days sooner than from unvented TSB-D.

Between 19 September and 26 December 1977, TSB-D and TSB-B were compared in 2,632 pairs of transiently vented blood cultures. There were 207 isolates with no statistically significant differences in isolation rates by medium (Table 4). When the time required to detect isolates that were present in both media was examined, isolates were detected in TSB-D an average of 0.4 day sooner ($P < 0.005$) than from TSB-B.

DISCUSSION

Because of the direct relationship between volume of blood cultured and positivity of cultures, it has been our practice to collect 30 ml of blood from adults and to distribute it equally into three vacuum blood culture bottles on a 10% (vol/vol) basis (1). Although previous studies from this laboratory have failed to demonstrate the superiority of any of the several media compared with TSB-D, it has been shown that there are statistically significant differences between unvented and transiently vented bottles

containing TSB-D (1). We have, therefore, routinely used three bottles of TSB-D as a blood culture set and transiently vented two of them before incubation.

Additional media have been compared with TSB-D in this study by replacing one of the three customary bottles containing TSB-D with one containing another medium. The volumes of blood and media were the same in all instances, as was the concentration of sodium polyanetholsulfonate. All bottles were incubated, examined, and routinely subcultured in an identical fashion. By limiting analyses to paired, transiently vented or unvented bottles in each study, we were able to reduce the test variables to a minimum. Under these conditions, differences between TSB-D and the other media were, for all practical purposes, virtually nonexistent. The difference between TSB-D and TSB-B was limited to approximately a 0.5-day advantage of one in the average time required for detection of growth. There were no differences between time intervals for detecting positive cultures in TSB-D and those observed in previous studies (1).

Despite the close similarity in this study between TSB-D and TSB-B, produced by two

TABLE 3. Numbers of isolates in positive cultures, by medium^a

Organism	TSB-D and brucella	TSB-D only	Brucella only	Total positive
<i>Bacillus</i>	0	1	0	1
<i>Corynebacterium</i>	4	9	8	21
<i>Escherichia</i>	32	21	12	65
<i>Citrobacter</i>	3	0	1	4
<i>Klebsiella</i>	18	5	3	26
<i>Enterobacter</i>	6	1	2	9
<i>Serratia</i>	4	1	2	7
<i>Proteus</i>	3	4	3	10
<i>Haemophilus</i>	4	0	0	4
<i>Streptococcus</i>				
<i>S. pneumoniae</i>	0	2	0	2
Viridans	45	5	4	54
Group B	4	2	0	6
Group C	5	0	0	5
Group D	10	8	4	22
<i>Acinetobacter</i>	1	0	0	1
<i>Bacteroidaceae</i>	11	2	1	14
<i>Staphylococcus</i>				
<i>S. aureus</i>	46	2	2	50
<i>S. epidermidis</i>	13	9	9	31
<i>Peptostreptococcus</i>	2	0	0	2
<i>Pseudomonas</i>	7	3	2	12
<i>Pasteurella</i>	1	0	0	1
<i>Vibrio</i>	0	1	0	1
<i>Clostridium</i>	5	1	0	6
<i>Lactobacillus</i>	2	0	0	2
<i>Listeria</i>	2	0	0	2
<i>Candida</i>	0	0	1	1
<i>Torulopsis</i>	0	1	1	2
Total	228	78	55	361

^a Neither medium was vented.

manufacturers, one might anticipate that differences may occur in the performance of blood culture media of the same generic description produced by different manufacturers because of differences related to (i) manufacturers' sources of components of the medium, (ii) unspecified and perhaps proprietary supplements, additives, anticoagulants, and chelating agents in the medium, and (iii) the atmospheric content of the bottle. These variables should, of course, not be confused with often substantive interlaboratory differences in blood culture technique. One must, therefore, be extremely cautious about generalizing from the results of a study of a particular generic product.

It is apparent from the data presented that the percentage of positive cultures varied appre-

TABLE 4. Number of isolates in positive culture, by medium

Organism	Both TSB-B and TSB-D	TSB-D only	TSB-B only	Total positive
<i>Bacillus</i>	0	1	3	4
<i>Corynebacterium</i>	0	5	1	6
<i>Escherichia</i>	24	4	10	38
<i>Citrobacter</i>	1	1	2	4
<i>Klebsiella</i>	8	4	0	12
<i>Enterobacter</i>	6	2	2	10
<i>Serratia</i>	0	2	0	2
<i>Proteus</i>	13	3	2	18
<i>Salmonella</i>	0	0	1	1
<i>Streptococcus</i>				
Viridans	11	1	2	14
Group A	0	1	0	1
Group B	2	1	0	3
Group D	8	0	0	8
<i>Acinetobacter</i>	4	1	0	5
<i>Moraxella</i>	0	1	0	1
<i>Peptococcus</i>	0	1	0	1
<i>Bacteroidaceae</i>	7	5	3	15
<i>Staphylococcus</i>				
<i>S. aureus</i>	17	2	3	22
<i>S. epidermidis</i>	5	6	2	13
<i>Pseudomonas</i>	6	5	3	14
<i>Aeromonas</i>	0	0	1	0
<i>Clostridium</i>	2	1	0	3
<i>Candida</i>	7	5	0	12
Total	121	52	35	207

ciably over the period of time encompassed by the four studies. Although it might be tempting to conclude that one particular set of media, e.g., those in Table 3, were superior to other sets studied, it should be emphasized that TSB-D was inoculated in each of the four studies and served as the basis for comparison and statistical analysis for each of the other media. Many factors can account for differences in positive rates of blood cultures. Populations of patients within hospitals vary over a period of time, as do hospital endemics or epidemics, the numbers of patients undergoing surgery and other procedures, antibiotic usage, and the ordering habits of physicians. These variables make consecutive (rather than parallel) comparisons of blood culture media or systems statistically invalid.

LITERATURE CITED

1. Washington, J. A. II (ed.). 1978. The detection of septicemia. CRC Press, Inc., West Palm Beach, Fla.