APPLIED MICROBIOLOGY, Apr. 1974, p. 699-702
Vol. 27, No. 4

Printed in U.S.A.

Comparison of Two Liquid Blood Culture Media Containing Sodium Polyanetholesulfonate: Tryptic Soy and Columbia

MARSHA HALL, EDWARD WARREN, AND JOHN A. WASHINGTON II
Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

Received for publication 26 December 1973

In a comparison of tryptic soy broth and Columbia broth, two blood culture media containing sodium polyanetholesulfonate, there were 589 positive cultures (excluding presumed contaminants). The two media were equivalent in performance except for lower detection rates for *Staphylococcus aureus* ($P < 0.01$) and *Pseudomonas aeruginosa* ($P = 0.05$) and a higher detection rate for *Bacillus* ($P < 0.01$) in Columbia broth. No significant differences were noted in time intervals to detection of positivity. Routine subcultures on the 1st and 5th days of incubation provided the initial detection of 18.1% of the positive cultures.

Because of the frequency of bacteremia and the morbidity and mortality associated with it, its detection remains of paramount importance to the clinical laboratory. Many different types of liquid blood culture media containing sodium polyanetholesulfonate (SPS) are now commercially available; however, reports of their relative performances are few. We previously compared tryptic soy broth (TSB) and thiol broth, both containing SPS (4). We now report a study comparing TSB and Columbia broth.

MATERIALS AND METHODS

Blood was collected with a sterile needle and syringe, from patients suspected of having bacteremia, by members of a venipuncture team; the skin site was prepared with alcohol followed by povidone-iodine (Swabsticks, Purdue Frederick Co.). Blood samples were inoculated (10%, vol/vol) into one bottle each of TSB (Difco) and Columbia broth (Difco), both under vacuum with CO_2 atmosphere. Each bottle contained 100 ml of medium and 0.05% SPS, and neither bottle was vented during incubation. All units were incubated at 35 C and were inspected daily for 14 days.

All units without visible evidence of growth were subcultured routinely within 24 h of blood collection, by sampling with a sterile needle and syringe through the stopper, inoculation of chocolate blood agar plates, and incubation in an atmosphere of 10% CO_2 for 48 h. This measure is recommended for the prompt detection of *Pseudomonas aeruginosa* (5), *Haemophilus influenzae*, *H. parainfluenzae*, *Moraxella*, and *Neisseria gonorrhoeae* (1). An additional routine subculture of 7,460 units that were negative on gross inspection was carried out in the same manner on the 5th day after collection. Finally, 1,258 units were routinely Gram stained at the time of the initial subculture. No further stains or subcultures were carried out unless there was gross evidence of growth, in which case stains and appropriate subcultures were performed.

Methods of statistical analysis have been reported previously (8) and are based on those described by Cochran (2).

RESULTS

During the period of study (1 June 1973 to 1 October 1973), 6,904 blood samples were cultured. There were 797 sets of positive cultures (11.5%); however, cultures containing *Bacillus*, *Corynebacterium* (including *Propionibacterium*), and *Staphylococcus epidermidis* (in a single culture only) comprised 208 sets of these cultures. Excluding these presumed contaminants, therefore, there were 589 positive cultures (8.5%). The numbers of isolates in positive cultures, by medium, are listed in Table 1. The two media performed equally well with only three exceptions: *Bacillus* was isolated more frequently in Columbia broth than in TSB, and *Staphylococcus aureus* and *P. aeruginosa* were isolated more frequently in TSB than in Columbia broth.

The time intervals to detection of positivity in each medium are listed in Table 2. There were no statistically significant differences between the two media in this respect.

Between 19 June 1973 and 4 September 1973, 4,258 samples were cultured; of these, 531 (12.4%) became positive. Of these positive cultures, 61 (11.5%) were first detected in the initial 24-h subculture, and 35 (6.6%) were first detected in the 5-day subculture. The remainder (81.9%) were first detected by visual inspection. Most cultures with *P. aeruginosa* were

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

Organism	TSB and Columbia	TSB only	Columbia only	Total positive	P[a]
Bacillus	0	7	24	31	<0.01
Clostridium	6	0	2	8	NS[b]
Corynebacterium	23	55	40	118	NS
Lactobacillus	2	0	1	3	NS
Escherichia	79	27	21	127	NS
Salmonella	0	0	1	1	NS
Citrobacter	2	0	0	2	NS
Klebsiella	37	5	5	47	NS
Enterobacter	2	2	1	5	NS
Serratia	10	1	0	11	NS
Proteus	11	8	7	26	NS
Haemophilus	10	1	0	11	NS
Listeria	2	0	2	4	NS
Streptococcus					
S. pneumoniae	3	4	2	9	NS
Viridans	36	5	4	45	NS
Group A	0	0	1	1	NS
Group B	0	2	2	4	NS
Group D	19	5	1	25	NS
Eubacterium	0	1	0	1	NS
Acinetobacter	0	2	1	3	NS
Alcaligenes	0	1	3	4	NS
Flavobacterium	1	0	0	1	NS
Bacteroidaceae	18	11	15	44	NS
Micrococcus	0	0	1	1	NS
Staphylococcus					
S. aureus	60	25	5	90	<0.01
S. epidermidis	25	33	19	77	NS
Peptostreptococcus	3	1	0	4	NS
Peptococcus	0	0	1	1	NS
Veillonella	1	0	0	1	NS
Pseudomonas	35	15	6	56	0.05
Aeromonas	3	1	1	5	NS
Candida	6	1	1	8	NS
Torulopsis	0	3	0	3	NS

[a] For difference between media.
[b] NS, Not significant.

detected in the initial subculture. Potentially significant organisms detected in the 5-day subcultures were *P. aeruginosa* (five times), *Candida albicans* (seven times), *S. aureus* (three times), and *P. cepacia* (once). The remainder detected in 5-day subcultures were presumed contaminants.

There were 629 cultures, representing 1,258 bottles, that were negative on gross inspection within the first 24 h after collection. At the time of the initial routine subculture, a smear of each was prepared, Gram stained, and examined microscopically. The only positive findings were a gram-negative bacillus that ultimately proved to be *Bacteroides fragilis* in two bottles and a gram-negative bacillus that proved to be *P. aeruginosa* in one bottle. During this same period of study, there were seven cultures in which *P. aeruginosa* was first detected in the 24-h subculture, but smears had been negative.

DISCUSSION

Columbia broth was initially described by Morello and Ellner (6), who compared it in parallel with TSB in 1,019 blood cultures; both media were bottled in 50-ml volumes and contained SPS. Although not specified in the report, it is assumed that CO_2 was added to each bottle, in view of the authors' reference to their method as that described in an earlier article by Ellner (3). On the basis of 95 isolates, these authors concluded that, although isolation rates in the two media did not differ significantly, the time interval to detection of growth did and was shorter in Columbia broth.

TABLE 2. *Time intervals to detection of positivity*

Organism	TSB		Columbia	
	No.	Mean ± SD[a] (days)	No.	Mean ± SD (days)
Bacillus	7	3.9 ± 4.6	24	4.5 ± 2.7
Clostridium	6	1.0 ± 0	8	1.0 ± 0
Corynebacterium	78	8.2 ± 3.1	63	9.3 ± 4.0
Lactobacillus	2	6.5 ± 0.7	3	5.0 ± 2.6
Escherichia	106	1.6 ± 1.6	100	1.6 ± 1.5
Salmonella	0		1	1.0
Citrobacter	2	1.0 ± 0	2	1.0
Klebsiella	42	2.3 ± 2.7	42	1.9 ± 2.1
Enterobacter	4	1.0 ± 0	3	1.0 ± 0
Serratia	11	1.2 ± 0.4	10	1.5 ± 1.0
Proteus	19	1.9 ± 1.2	18	2.1 ± 1.6
Haemophilus	11	2.5 ± 1.5	10	3.7 ± 4.4
Listeria	2	2.0 ± 0	4	2.0 ± 0
Streptococcus				
S. pneumoniae	7	1.3 ± 0.5	5	1.6 ± 0.5
Viridans	41	2.1 ± 1.8	40	1.9 ± 1.3
Group A	0		1	2.0
Group B	2	1.0 ± 0	2	1.5 ± 0.7
Group D	24	2.3 ± 1.6	20	2.4 ± 1.6
Eubacterium	1	7.0	0	
Acinetobacter	2	2.0 ± 0	1	2.0
Alcaligenes	1	7.0	3	9.6 ± 5.7
Flavobacterium	1	5.0	1	7.0
Bacteroidaceae	29	4.5 ± 3.7	33	3.8 ± 3.5
Micrococcus	0		1	5.0
Staphylococcus				
S. aureus	85	2.9 ± 2.9	65	1.9 ± 1.2
S. epidermidis	58	3.8 ± 2.1	44	3.9 ± 2.2
Peptostreptococcus	4	1.5 ± 0.6	3	1.3 ± 0.6
Peptococcus	0		1	7.0
Veillonella	1	3.0	1	3.0
Pseudomonas	50	3.5 ± 2.6	41	3.1 ± 1.7
Aeromonas	4	1.3 ± 0.5	4	1.5 ± 0.6
Candida	7	8.3 ± 2.6	7	7.3 ± 0.9
Torulopsis	3	4.3 ± 1.5	0	

[a] SD, Standard deviation.

These results differ from our experience reported here, and there are several factors that may contribute to these differences. Morello and Ellner used a 10-ml sample of blood transported to the laboratory in Vacutainer tubes containing SPS, inoculating 5 ml into 50 ml of each of the two media, whereas in our study a member of a venipuncture team inoculated 10 ml of blood directly into 100 ml of each of the two media and these bottles were transported to the laboratory. It is not known how the performance of TSB manufactured by BBL might differ from that manufactured by Difco, so the significance of this difference between the two studies is not clear.

The optimal timing and frequency of routine subcultures and the value of routine Gram-stained smears of blood cultures are not known. Blazevic et al. (1) studied the role of routine staining on the 1st, 4th, and 7th days of incubation and of routine subcultures on the 1st and 4th days of incubation. They first detected positivity by visual inspection in 65%, by microscopy examination of the Gram-stained smear in 23%, and by subculture in 12% of their cultures. Their rate of positivity first detected by subculture is similar to that in our study (18.1%), but their rate of first detection by microscope examination of the stained smear is substantially in excess of ours and cannot be explained satisfactorily. In our estimation, the yield from a routine Gram-stained smear is so small that its use, except to confirm suspicion of positivity, is not warranted, particularly considering the effort involved.

It appears that at least one routine ("blind")

subculture within 24 h after collection of the blood culture is mandatory and that a second routine subculture 3 or 4 days later is advisable. Furthermore, it appears, on the basis of our data and those of Blazevic et al. (1), that routine subcultures should be inoculated onto chocolate blood agar incubated in CO_2 and that routine anaerobic subcultures are not warranted. Most anaerobic bacteria are easily detectable by visual inspection after a short period of incubation in unvented blood culture bottles.

Previous studies in this laboratory have compared TSB in an unvented bottle containing 100 ml of medium with SPS under vacuum and CO_2 with (i) supplemented prereduced brain heart infusion broth with SPS (9), (ii) the aerobic and anaerobic culture vials used in a radiometric system (7), (iii) thiol broth (4), and (iv) aerobic and anaerobic Vacutainer tubes with supplemented peptone broth (4). Under our conditions, TSB with SPS has been the best general-purpose medium for detection of bacteremia. We prefer not to vent the bottles, so that we maintain a sufficiently low redox potential in the medium to favor growth and prompt detection of anaerobic bacteria; we depend on routine subcultures on the 1st and 5th days of incubation for detection of *P. aeruginosa* and other organisms whose growth may not be evident macroscopically.

Selection of microbiological media is complex because there are few standards against which to compare new products. Moreover, there are many individual differences in practices among laboratories, including number of specimens collected, methods of collection and transport, types and volumes of media, use of additives, atmosphere of incubation, frequency of examinations and subcultures, and duration of incubation. All of these variables need to be identified and controlled in comparative evaluations.

ACKNOWLEDGMENT

The authors are indebted to Duane M. Ilstrup, Section of Medical Research Statistics, for statistical analysis of these data.

LITERATURE CITED

1. Blazevic, D. J., J. E. Stemper, and J. M. Matsen. 1974. Comparison of macroscopic examination, routine Gram strains, and routine subcultures in the initial detection of positive blood cultures. Appl. Microbiol. **27**:537-539.
2. Cochran, W. G. 1950. The comparison of percentages in matched samples. Biometrika **37**:256-266.
3. Ellner, P. D. 1968. System for inoculation of blood in the laboratory. Appl. Microbiol. **16**:1892-1894.
4. Hall, M., E. Warren, and J. A. Washington II. 1974. Detection of bacteremia with liquid media containing sodium polyanetholsulfonate. Appl. Microbiol. **27**:187-191.
5. Knepper, J. G., and B. F. Anthony. 1973. Diminished growth of *Pseudomonas aeruginosa* in unvented blood-culture bottles. Lancet **2**:285-287.
6. Morello, J. A., and P. D. Ellner. 1969. New medium for blood cultures. Appl. Microbiol. **17**:68-70.
7. Renner, E. D., L. A. Gatheridge, and J. A. Washington II. 1973. Evaluation of radiometric system for detecting bacteremia. Appl. Microbiol. **26**:368-372.
8. Washington, J. A., II. 1971. Comparison of two commercially available media for detection of bacteremia. Appl. Microbiol. **22**:604-607.
9. Washington, J. A., II, and W. J. Martin. 1973. Comparison of three blood culture media for recovery of anaerobic bacteria. Appl. Microbiol. **25**:70-71.