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Clinical Comparison of an Agar Slide Blood Culture Bottle with Tryptic Soy Broth and a Conventional Blood Culture Bottle with Supplemented Peptone Broth

MELVIN P. WEINSTEIN,[1,2]* L. BARTH RELLER,[3,4] STANLEY MIRRETT,[3,4] WEN-LAN L. WANG,[5,6] AND DAVID V. ALCID[2]

Microbiology Laboratory, Middlesex General-University Hospital,[1] and University of Medicine and Dentistry of New Jersey-Rutgers Medical School,[2] New Brunswick, New Jersey 08903; Clinical Microbiology Laboratory, University of Colorado Hospital,[3] and Departments of Medicine[4] and Pathology,[5] University of Colorado School of Medicine, Denver, Colorado 80262; and Denver Veterans Administration Medical Center, Denver, Colorado 80220[6]

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The Roche Septi-Chek biphasic blood culture system with tryptic soy broth was compared with a conventional blood culture bottle with supplemented peptone broth in 6,956 paired blood cultures from adult patients. Both systems were inoculated with equal volumes of blood (5 ml) and incubated aerobically (vented) for 2 weeks. More clinically important bacteria and fungi, including *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli* and other *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Candida albicans* and *C. tropicalis* were recovered from the biphasic system ($P < 0.001$). In contrast, more clinically important anaerobic bacteria ($P < 0.001$) and *Gardnerella vaginalis* ($P < 0.05$) were recovered in conventional supplemented peptone broth. Staphylococci ($P < 0.01$), *Enterobacteriaceae* other than *E. coli* ($P < 0.05$), and fungi ($P < 0.001$) were detected 1 or more days earlier in the biphasic system, whereas streptococci ($P < 0.001$) were detected earlier in the conventional bottle. The overall superiority of the agar slide blood culture system compared with conventional blood culture bottles was confirmed by this evaluation. For optimal detection of anaerobic bacteremia, however, the agar slide bottle should be paired with an anaerobic bottle.

Several recent reports evaluating the Roche Septi-Chek agar slide blood culture system (Roche Diagnostic Systems, Nutley, N.J.) have shown it to be an attractive alternative to conventional blood culture methodologies (1, 2, 5, 8a). In only one study was the agar slide system with tryptic soy broth (RSC-TSB) compared with a conventional system with supplemented peptone broth (C-SPB) (1). In that evaluation of 1,209 blood cultures, the RSC-TSB detected more clinically important microorganisms than did C-SPB; however, the agar slide system benefited from a 20% volume advantage (8 ml for RSC-TSB versus 6.6 ml for C-SPB). Because two variables, medium and blood volume, were present, it was not possible to determine which variable was responsible for the increased yield in the RSC-TSB system. To better assess whether or not volume alone was responsible for the increased yield in RSC-TSB, we compared the two systems in 6,956 cultures of equal volumes of blood done at three collaborating hospitals.

MATERIALS AND METHODS

Collection of samples. During the study period a 45-ml bottle of supplemented peptone broth (C-SPB) with 0.03% sodium polyanetholsulfonate (Becton-Dickinson Vacutainer Systems, Rutherford, N.J.) and a 70-ml bottle of tryptic soy broth (RSC-TSB) with 0.05% sodium polyanetholsulfonate (Roche Diagnostic Systems) were used for all blood cultures from adult patients at Middlesex General-University Hospital, the University of Colorado Hospital, and the Denver Veterans Administration Medical Center. Blood cultures were obtained at the bedsides of patients after preparation of the skin with 10% povidone iodine (1% available iodine) followed by 70% isopropyl alcohol. Blood from each separate venipuncture was distributed by needle and syringe as follows: 5 ml inoculated into the bottle with 45 ml of SPB and 5 ml of blood inoculated into the bottle with 70 ml of TSB. Thus, the volume of blood was the same for both culture bottles (7), although the ratio of blood to broth was not the same (1:10 and 1:14, respectively). Both blood culture bottles had been evacuated and backflushed with 10% carbon dioxide and nitrogen at stoppering in the manufacturing process.

Volume standards. To ensure that the culture bottles actually received the specified amounts of blood, we measured the level of fluid in each container after it was filled with blood. Although all blood-containing bottles were incubated, those with fluid levels below or above the standards were coded as inadequate and were excluded from subsequent analyses. Fluid level standards were set to ensure that at least 4 ml but no more than 6 ml of blood was added to each bottle.

Processing of specimens. Identical methods were used for processing the blood cultures in the clinical microbiology laboratories at all three hospitals. Both bottles were incubated aerobically at 35°C for 14 days. When the paired samples were received, a sterile open venting unit that allowed continuous exchange of air was placed on the C-SPB bottle (8), and an agar slide paddle was attached to the RSC-TSB bottle. These steps and all subsequent manipulations were done at the laboratory bench. Cultures were examined macroscopically twice during the first 24 h and daily thereafter for 7 days; they were then reincubated until the final subcultures were done on day 14 of incubation.

In the C-SPB bottles subcultures were done through the

* Corresponding author.

TABLE 1. Comparison of yield of clinically important bacteria and fungi from 5-ml samples of blood cultured in C-SPB and in RSC-TSB

Microorganisms	No. of isolates from:			P
	Both C-SPB and RSC-TSB	C-SPB only	RSC-TSB only	
Aerobic and facultative bacteria	339	78	164	<0.001
Gram-positive	144	48	64	NS[a]
Staphylococcus aureus	70	9	24	<0.05
Staphylococcus epidermidis	5	2	17	<0.01
Streptococci[b]	64	30	22	NS
Other[c]	5	8	1	<0.05
Gram-negative	195	30	100	<0.001
Escherichia coli	59	5	35	<0.001
Other *Enterobacteriaceae*	92	16	39	<0.05
Pseudomonas aeruginosa	30	9	22	<0.05
Other[d]	14	0	4	NS
Anaerobic bacteria	13	33	6	<0.001
Gram-positive[e]	1	16	2	<0.01
Gram-negative[f]	12	17	4	<0.01
Bacteroides fragilis group	8	4	4	NS
All bacteria	352	111	170	<0.001
Fungi[g]	54	6	22	<0.01
Candida albicans	26	4	16	<0.05
Candida tropicalis	23	1	2	NS

[a] NS, Not significant ($P > 0.05$).
[b] Includes (number) group A streptococci (6), group B streptococci (11), enterococci (27), non-enterococcal group D streptococci (5), *Streptococcus pneumoniae* (32), viridans streptococci (25), and other streptococci (10).
[c] Gram-variable *Gardnerella vaginalis* (7), *Listeria monocytogenes* (7).
[d] *Eikenella* sp. (1), *Acinetobacter* spp. (7), *Pseudomonas* sp. (1), *Cardiobacterium hominis* (4), and *Haemophilus influenzae* (5).
[e] *Clostridium* (6), *Bifidobacterium* (2), *Eubacterium* (2), *Peptococcus* (5), and *Peptostreptococcus* (4) spp.
[f] *Bacteroides* (28), *Fusobacterium* (2), and *Veillonella* (3) spp.
[g] *Candida albicans* (46), *Candida tropicalis* (26), *Candida parapsilosis* (9), and *Torulopsis glabrata* (1).

needle of the open venting unit after 1 and 14 days of incubation. On day 1 aerobic subculture was done on chocolate agar incubated in 5% CO_2 at 35°C. On day 14, aerobic (chocolate agar) and anaerobic (5% sheep blood agar or brucella agar supplemented with vitamin K_1 and hemin) subcultures were done. In addition, acridine orange stain was performed after 12 to 24 h of incubation (4) on all macroscopically negative C-SPB bottles.

After attachment of the slide paddle, RSC-TSB bottles were immediately subcultured by inverting the bottle and allowing the blood-broth mixture to cover the agar-coated paddle. This process was repeated after 4 to 8 h of incubation, daily through day 7 at the time of macroscopic examination of the paddles and broth, and again on day 14. If a bottle was positive macroscopically in broth but no growth was evident on the agar paddles, the slide chamber was removed, and a sterile Pasteur pipette was used to obtain broth for Gram stain and anaerobic subcultures. If growth on the agar paddle was noted at the same time the broth became positive macroscopically, the initial mode of detection was considered to be the growth on agar, i.e., subculture.

Clinical assessment. All patients with positive blood cultures were evaluated by an infectious disease specialist who defined pathogens (clinically important bacteria causing sepsis) and contaminants by established criteria (9).

Analysis of data. Paired comparisons of the two blood culture methods were done only on adequately filled (≥4 and ≤6 ml of blood) bottles that grew microorganisms causing true bacteremia or fungemia. Significance testing was done by using the modified chi-square test described by McNemar (3).

RESULTS

A total of 6,956 adequately filled blood culture sets were received during the study period. Of these, 988 (14.2%) were positive including 635 (9.1%) that grew microorganisms causing illness, 269 (3.7%) that grew contaminants, 12 (0.2%) that grew one or more contaminants and pathogens, and 72 (1.0%) that grew organisms that were indeterminate as a cause of sepsis. A total of 715 isolates associated with sepsis were detected; 407 isolates grew in both systems and 220 of these were detected on the same day.

Overall, clinically important bacteria and fungi were recovered more often ($P < 0.001$) in the agar slide system (Table 1). This improved yield could be attributed largely to better detection of staphylococci and gram-negative aerobic and facultatively anaerobic bacteria. Specifically, *Staphylococcus aureus* ($P < 0.05$), *S. epidermidis* ($P < 0.01$), *Escherichia coli* ($P < 0.001$), other *Enterobacteriaceae* ($P < 0.05$), and *Pseudomonas aeruginosa* ($P < 0.05$) were favored by the agar slide system. In addition, fungi ($P < 0.01$) were also detected more frequently with the agar slide system. In contrast, recovery of anaerobic bacteria ($P < 0.001$) and *Gardnerella vaginalis* ($P < 0.05$) was greater in SPB.

The speed with which the two systems detected positive cultures was variable (Table 2). Although there was no difference for bacteria overall, staphylococci ($P < 0.01$) and members of the family *Enterobacteriaceae* other than *E. coli* ($P < 0.05$) were detected 1 or more days earlier in the agar slide system. In contrast, streptococci ($P < 0.001$) were detected earlier in SPB. The most dramatic differences, however, were noted for fungi, which were found significantly sooner ($P < 0.001$) with the agar slide system.

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi from 5-ml samples of blood cultured in C-SPB and in RSC-TSB

Microorganisms	No. of isolates from:			P
	C-SPB + RSC-TSB positive same time	C-SPB positive ≥1 day earlier	RSC-TSB positive ≥1 day earlier	
Aerobic and facultative bacteria	205	63	71	NS[a]
Gram-positive	90	27	27	NS
Staphylococci[b]	44	6	25	<0.01
Streptococci[c]	43	19	2	<0.001
Other[d]	3	2	0	NS
Gram-negative	115	36	44	NS
Escherichia coli	32	16	11	NS
Other *Enterobacteriaceae*	55	11	26	<0.05
Pseudomonas aeruginosa	19	6	5	NS
Other[e]	9	3	2	NS
Anaerobic bacteria[f]	8	4	1	NS
All bacteria	213	67	72	NS
Fungi[g]	7	1	46	<0.001

[a] NS, Not significant ($P > 0.05$).
[b] *Staphylococcus aureus* (70), *S. epidermidis* (5).
[c] Group A streptococci (5), group B streptococci (5), enterococci (13), non-enterococcal group D streptococci (3), *Streptococcus pneumoniae* (22), viridans streptococci (14), and other streptococci (2).
[d] *Gardnerella vaginalis* (2), *Listeria monocytogenes* (3).
[e] *Acinetobacter* spp. (4), *Pseudomonas* sp. (1), *Cardiobacterium hominis* (4), and *Haemophilus influenzae* (5).
[f] *Bacteroides* spp. (12), anaerobic gram-positive rod (1).
[g] *Candida albicans* (26), *Candida tropicalis* (23), and *Candida parapsilosis* (5).

Contaminant isolates were detected with greater frequency in the agar slide system than in the conventionally processed SPB (Table 3). In particular, *S. epidermidis* ($P < 0.001$), *Bacillus* spp. ($P < 0.05$), and *Corynebacterium* spp. ($P < 0.001$) were detected more frequently in the agar slide system. Overall, 115 contaminant isolates were detected in the SPB processed conventionally versus 241 contaminant isolates in the agar slide system.

DISCUSSION

This study has confirmed earlier reports that the agar slide blood culture system improves the detection of microorganisms in blood in comparison with conventional broth systems (1, 2, 5, 8a). In addition, the data have shown that the agar slide system with TSB detects more microorganisms than does a conventional system with SPB. Since equal volumes of blood were used in this evaluation, it would seem that the advantage of the agar slide system demonstrated by Bryan (1) was not due solely to the volume of blood cultured but rather to medium and perhaps other factors (e.g., frequency of subculture, bottle configuration, and headspace).

TABLE 3. Comparison of yield of contaminant bacteria and fungi from 5-ml samples of blood cultured in C-SPB and in RSC-TSB

Microorganisms	No. of isolates from:			P
	Both C-SPB + RSC-TSB	C-SPB only	RSC-TSB only	
Staphylococcus epidermidis	45	32	114	<0.001
Bacillus spp.	2	0	7	<0.05
Corynebacterium spp.	8	7	32	<0.001
Propionibacterium spp.	2	7	14	NS[a]
Other[b]	5	7	11	NS

[a] NS, Not significant ($P > 0.05$).
[b] *S. aureus* (3), viridans streptococci (7), *G. vaginalis* (2), *Micrococcus* spp. (2), *Neisseria* spp. (2), and enterococcus, *Klebsiella* sp., *Haemophilus* sp., *Clostridium* sp., *Peptococcus* sp., *Fusobacterium* sp., and *Candida tropicalis* (1 each).

Although yield overall was better in the agar slide system than in conventional SPB, this finding was not true for all microorganism groups. Thus, anaerobes and *G. vaginalis* were detected significantly more often in conventionally processed SPB. The improved yield of anaerobic bacteria in C-SPB may have been due in part to the low redox potential of this medium (8), whereas enhanced detection of *Gardnerella* spp. may have been due to the presence in C-SPB of 1.2% gelatin which counteracts the inhibitory effects of SPS on this bacterium (6). Although these microorganisms are relatively less common causes of bacteremia than are staphylococci and *Enterobacteriaceae*, they are important pathogens in some patients (6, 9). Therefore, clinical microbiology laboratories should use blood culture systems capable of detecting these bacteria as well as more common aerobic and facultative microorganisms. Indeed, evaluations of the agar slide system with TSB (2, 8a) and SPB processed conventionally (8) suggest that a two-bottle system utilizing these media would be a reasonable approach in many laboratories.

In designing this evaluation, we attempted to use each blood culture system to maximum efficiency within the limits of practical hospital laboratory microbiology. The time and effort involved in subculturing conventional broth blood culture bottles is well known and limits the frequency with which this procedure can be done. However, the agar slide system allows subculture to be done simply by inverting the bottle, thus enabling frequent subculture with a minimum of effort. The speed advantage of the agar slide system in detecting staphylococci, *Enterobacteriaceae* other than *E. coli*, and fungi may relate in part to this fact, namely, that more frequent subculturing in the agar slide system resulted in earlier detection of positivity. Frequent subculture cannot

represent the only factor, however, since streptococci were isolated earlier in the conventional bottle with SPB.

As noted in other comparisons with conventional broth blood culture bottles (5, 8a), the agar slide system has a higher contamination rate (3.5 versus 1.7% in this study). In all probability this problem relates to the additional manipulations associated with attachment of the paddle to the bottle and occasional need to remove the agar paddle from its plastic cylinder for complete examination. It is not known whether performing all maneuvers in a laminar air flow hood would reduce contamination sufficiently to warrant the extra effort and additional cost, but this possibility deserves study.

LITERATURE CITED

1. **Bryan, L. E.** 1981. Comparison of a slide blood culture system with a supplemented peptone broth culture method. J. Clin. Microbiol. **14**:389–392.
2. **Henry, N. K., C. M. Grewell, C. A. McLimans, and J. A. Washington II.** 1984. Comparison of the Roche Septi-Chek blood culture bottle with a brain heart infusion biphasic medium bottle and with a tryptic soy broth bottle. J. Clin. Microbiol. **19**:315–317.
3. **McNemar, Q.** 1962. Psychological statistics, 3rd ed., p. 209–239. John Wiley & Sons, Inc., New York.
4. **Mirrett, S., B. A. Lauer, G. A. Miller, and L. B. Reller.** 1982. Comparison of acridine orange, methylene blue, and Gram stains for blood cultures. J. Clin. Microbiol. **15**:562–566.
5. **Pfaller, M. A., T. K. Sibley, L. J. Westfall, J. E. Hoppe-Bauer, J. A. Keating, and P. R. Murray.** 1982. Clinical laboratory comparison of a slide blood culture system with a conventional broth system. J. Clin. Microbiol. **16**:525–530.
6. **Reimer, L., and L. B. Reller.** 1984. *Gardnerella vaginalis* bacteremia: a review of thirty cases. Obstet. Gynecol. **64**:170–172.
7. **Tenney, J. H., L. B. Reller, S. Mirrett, W.-L. L. Wang, and M. P. Weinstein.** 1982. Controlled evaluation of the volume of blood cultured in detection of bacteremia and fungemia. J. Clin. Microbiol. **15**:558–561.
8. **Tenney, J. H., L. B. Reller, S. Mirrett, M. P. Weinstein, and W.-L. L. Wang.** 1982. Controlled evaluation of the effect of atmosphere of incubation on detection of bacteremia and fungemia in supplemented peptone broth. J. Clin. Microbiol. **16**:437–442.

8a. **Weinstein, M. P., L. B. Reller, S. Mirrett, W.-L. L. Wang, and D. V. Alcid.** 1985. Controlled evaluation of Trypticase soy broth in agar slide and conventional blood culture systems. J. Clin. Microbiol. **21**:626–629.

9. **Weinstein, M. P., L. B. Reller, J. R. Murphy, and K. A. Lichtenstein.** 1983. The clinical significance of positive blood cultures: a comprehensive analysis of 500 episodes of bacteremia and fungemia in adults. I. Laboratory and epidemiologic observations. Rev. Infect. Dis. **5**:35–53.