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Comparison of the Roche Septi-Chek Blood Culture Bottle with a Brain Heart Infusion Biphasic Medium Bottle and with a Tryptic Soy Broth Bottle

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In a comparison of 1,368 positive blood cultures, a vented Roche Septi-Chek (V-RSC) blood culture bottle was superior to an unvented tryptic soy broth-containing bottle (Difco) for the recovery of all aerobic and facultatively anaerobic microorganisms. Anaerobic bacteria were recovered more frequently and earlier in the unvented tryptic soy broth-containing bottle. A separate comparison of 529 positive blood cultures was conducted to examine the performance of the V-RSC bottle with that of a vented brain heart infusion biphasic medium. The V-RSC bottle recovered significantly more isolates of *Enterobacteriaceae* and of anaerobic bacteria than did the vented brain heart infusion biphasic medium. The V-RSC bottle is a reliable blood culture system for all aerobic and facultatively anaerobic microorganisms. Because of its suboptimal recovery of anaerobic bacteria, it is recommended that the V-RSC bottle be used in combination with an unvented vacuum blood culture bottle.

Routine subculture as a means of providing initial detection of positive blood cultures is a traditional blood culture procedure (5). The introduction of a Roche Septi-Chek (RSC) blood culture bottle with its attachable agar-containing slide chamber offers the opportunity for repeated rapid subcultures. Hall et al. (2) reported that a brain heart infusion (BHI) biphasic medium bottle, which was prepared at Mayo Clinic and which did not contain CO_2, recovered more *Staphylococcus aureus* isolates than did a tryptic soy broth-containing bottle (TSB bottle; Difco) which was vented transiently. The TSB bottle recovered more anaerobic bacteria; this difference may have been due to differences in the production of the two bottles, particularly the relative degrees of vacuum and anaerobic conditions and the relative amounts of CO_2 and other gases present in each bottle. Pfaller et al. (4) evaluated the RSC bottle and compared it with a vented TSB blood culture bottle. The RSC bottle recovered significantly more gram-negative and gram-positive aerobic and facultatively anaerobic bacteria (4). We compared the RSC bottle with the vented biphasic BHI medium bottle described by Hall et al. (2) and with an unvented TSB bottle.

MATERIALS AND METHODS

Thirty milliliters of blood from patients with suspected bacteremia or fungemia was collected aseptically by venipuncture teams and inoculated equally (10 ml) into three blood culture bottles: an RSC bottle containing 70 ml of TSB with 0.05% sodium polyanetholesulfonate in an atmosphere of CO_2 (April through July 1982; January through May 1983); a biphasic bottle, prepared at Mayo Clinic, containing 60 ml of BHI broth with 0.025% sodium polyethanolesulfonate and a BHI agar slant (BiBHI bottle; April through July 1982); and a bottle containing 100 ml of TSB with 0.025% sodium polyethanolesulfonate and an initial atmosphere of CO_2 (April through July 1982; January through May 1983). For the period between January and May 1983, a 10-ml Isolator blood culture tube (Du Pont Co.) replaced the BiBHI bottle. The RSC and BiBHI bottles were vented transiently upon arrival in the laboratory (V-RSC and V-BiBHI bottles); the TSB bottle remained unvented (UV-TSB bottle). All bottles were incubated in an upright position at 35°C.

All macroscopically negative blood culture bottles were subcultured at 6 to 17 h in the following manner. The RSC slide chamber, containing chocolate, MacConkey, and malt agars, was attached to the bottle at this time according to Roche guidelines, and the bottle was tipped horizontally to inoculate all agar surfaces with the blood-broth mixture. The BiBHI bottle was tipped to allow the blood-broth mixture to flow over the agar slant, and the TSB bottle was subcultured by aseptically aspirating a sample of broth and inoculating a chocolate blood agar plate, which was incubated at 35°C in 5 to 10% CO_2 for 48 h.

All bottles were examined macroscopically twice on day 1, daily for the next 6 days, and once on day 14 before they were discarded. Agar surfaces of the biphasic bottles were examined at the time of each macroscopic examination; the agar surfaces were reinoculated with the blood-broth mixture at this time. Chocolate agar subculture plates from UV-TSB bottles were examined after 6, 12, 24, and 48 h; subcultures of negative UV-TSB bottles were repeated at 48 h.

The V-RSC bottle was compared with the UV-TSB or V-BiBHI bottle on a volume-to-volume basis. The microbiological data were analyzed to determine whether significant differences were present in the frequency of positivity and to ascertain which bottle became positive earlier when both were positive. Statistical analysis was performed using McNemar's chi-square test (1).

RESULTS

Blood culture sets (5,121) collected from April through July 1982 were entered into a comparison of the two biphasic blood culture bottles, i.e., V-RSC and V-BiBHI bottles (Table 1). Because of the relatively small number of anaerobic bacterial isolates between April and July 1982, the V-RSC and UV-TSB bottles from these culture sets were added to an additional 9,916 blood culture sets collected between January and May 1983 for a comparison of the V-RSC bottle with the UV-TSB bottle (Table 1).

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TABLE 1. Overall recovery of microorganisms

Comparison	Blood cultures				Isolates			
	Total	No. (%) positive			Total	No. (%) recovered[a]		
		UV-TSB	V-RSC	V-BiBHI		UV-TSB	V-RSC	V-BiBHI
V-RSC vs V-BiBHI	5,121[b]		414 (8.1)	387 (7.6)	571		440 (77)	410 (72)
V-RSC vs UV-TSB	15,037[c]	853 (5.7)	1,206 (8.2)		1477	904 (61)	1,280 (87)	

[a] For V-RSC versus UV-TSB, $P < 0.001$; for V-RSC versus V-BiBHI, P is not significant.
[b] A total of 529 (10.3%) cultures were positive in this comparison.
[c] A total of 1368 (9.1%) cultures were positive in this comparison.

Of the 5,121 blood culture sets in the V-RSC/V-BiBHI comparison, 529 (10.3%) were positive: 8.1% in the V-RSC bottle and 7.6% in the V-BiBHI bottle (Table 1). There was no significant difference between the V-RSC and the V-BiBHI bottles in the recovery of the 571 isolates; the V-RSC bottle recovered 440 (77%), and the V-BiBHI bottle recovered 410 (72%). There were significant differences in the recovery of several microorganism groups. Among the aerobic and facultatively anaerobic bacteria, the V-RSC bottle recovered significantly more gram-negative bacteria, primarily members of the family *Enterobacteriaceae* (Table 2). There was no difference in the recovery of *Pseudomonas aeruginosa* between the two bottles. The biphasic bottles were comparable in their recovery of all gram-positive bacteria in general, although the V-RSC bottle recovered significantly more isolates of *Streptococcus* spp.

The V-RSC bottle recovered significantly more anaerobic bacteria ($P < 0.01$) than did the V-BiBHI bottle. With yeasts, primarily *Candida* spp., and fungi, there was a trend in favor of the V-BiBHI bottle, but this difference was not statistically significant.

The contamination rate for each bottle was determined by defining a contaminated culture as a single blood culture positive with *Staphylococcus epidermidis*, *Bacillus* spp., *Corynebacterium* spp., or *Propionibacterium* spp. The rates for the biphasic bottles were similar: 1 and 1.2% for the V-RSC and V-BiBHI bottles, respectively.

Of the 15,037 blood culture sets in the V-RSC/UV-TSB comparison, 1,368 (9.1%) were positive: 5.7% in the UV-TSB bottle and 8.2% in the V-RSC bottle (Table 1). There were 1,477 isolates recovered. The UV-TSB bottle recovered 904 (61%), and the V-RSC recovered 1,280 (87%; $P < 0.001$). There were significant differences in the recovery of almost all microorganism groups (Table 3). Among the *Streptococcus* spp., the V-RSC bottle was superior to the UV-TSB bottle for the recovery of *Streptococcus pneumoniae* ($P < 0.01$; $n = 63$) and of viridans streptococci ($P < 0.001$; $n = 96$). The comparison of the V-RSC bottle with the UV-TSB bottle for the recovery of anaerobic bacteria was of interest. The UV-TSB bottle recovered a significantly greater number of anaerobic bacteria than did the V-RSC bottle ($P < 0.05$; $n = 83$; Table 3), in particular, *Bacteroides* spp. ($P < 0.05$; $n = 57$) and *Clostridium* spp. ($P < 0.05$; $n = 12$). Not surprisingly, the UV-TSB bottle did not perform as well as the V-RSC bottle for the detection of yeasts and fungi (Table 3). There were no statistically significant differences be-

TABLE 2. Recovery of microorganisms from V-RSC versus V-BiBHI bottles

Microorganism	No. of isolates recovered			P
	V-BiBHI only	V-RSC only	Both	
Bacteria				
Aerobic and facultative	104	126	251	NS[a]
Gram negative	30	51	106	<0.05
Enterobacteriaceae	26	44	83	<0.05
P. aeruginosa	3	6	17	NS
Miscellaneous[b]	1	1	6	NS
Gram positive	74	75	145	NS
S. aureus	13	11	54	NS
S. epidermidis	29	26	28	NS
Streptococci	6	17	55	<0.05
Miscellaneous[c]	26	21	8	NS
Anaerobic[d]	4	18	3	<0.01
Yeasts and fungi	21	12	23	NS

[a] NS, Not significant.
[b] Includes *Haemophilus influenzae*, *Neisseria gonorrhoeae*, and *Acinetobacter* spp.
[c] Includes species of *Bacillus*, *Corynebacterium*, and *Lactobacillus*.
[d] Excludes *Propionibacterium* spp.

TABLE 3. Recovery of microorganisms from V-RSC versus UV-TSB bottles

Microorganism	No. of isolates recovered			P
	UV-TSB only	V-RSC only	Both	
Bacteria				
Aerobic and facultative	145	435	660	<0.001
Gram negative	64	147	254	<0.001
Enterobacteriaceae	51	83	208	<0.01
P. aeruginosa	5	32	30	<0.001
Miscellaneous[a]	8	32	16	<0.001
Gram positive	81	288	406	<0.001
S. aureus	20	37	135	<0.05
S. epidermidis	35	107	88	<0.001
Streptococci	14	50	153	<0.001
Miscellaneous[b]	12	94	30	<0.001
Anaerobic[c]	34	17	28	<0.05
Yeasts and fungi	1	86	10	<0.001

[a] Includes *Haemophilus influenzae* and species of *Pseudomonas*, *Campylobacter*, *Neisseria*, *Flavobacterium*, *Vibrio*, and *Cardiobacterium*.
[b] Includes species of *Bacillus*, *Corynebacterium*, *Lactobacillus*, and *Listeria*.
[c] Excludes *Propionibacterium* spp.

TABLE 4. Time for detection

Organism	Days to positive[a]				P
	V-RSC	V-BiBHI	UV-TSB	Difference[b]	
Candida spp.	4.9 (35)	3.9 (44)		1.2 (23)	<0.05
Bacteroides spp.	4.5 (32)		2.8 (46)	1.8 (22)	<0.01

[a] Numbers in parentheses show numbers of isolates analyzed.
[b] Calculated when both bottles were positive.

tween these two blood culture bottles for the recovery of isolates of *Listeria* ($n = 10$), *Neisseria* ($n = 5$), or *Haemophilus* ($n = 11$), but the numbers were too small for statistical analysis.

The contamination rate of each bottle was determined as described above. The rate for the UV-TSB bottle was 0.6%, and that for the V-RSC bottle was 1.5%.

Two groups of microorganisms were detected earlier with one system (Table 4). The difference in mean days to positive was determined when both bottles were positive for the same microorganism and may differ from the mean days to positive for that microorganism overall. *Candida* spp. were detected earlier in the V-BiBHI bottle than in the V-RSC bottle, and *Bacteroides* spp. were detected earlier in the UV-TSB bottle than in the V-RSC bottle. The time to detection of anaerobic bacteria is shown in Table 5. The mean time to detection of anaerobic bacteria was faster in the UV-TSB bottle, except with *Clostridium* spp.

The initial means of detection of microorganisms in each of the three blood culture bottles were recorded. Whereas the biphasic bottle agar slant(s) detected 62% (V-RSC) to 66% (V-BiBHI) of all microorganisms, the subculture of the TSB bottle detected only 30%.

DISCUSSION

A biphasic blood culture bottle offers a convenient and time-saving method for subculturing blood cultures (2, 3). The increased volume subcultured in a biphasic bottle, compared with that subcultured from a broth-containing bottle, and the easily repeatable subculture procedure may account for the >60% initial recovery of microorganisms on the agar surface(s). Pfaller et al. (4) observed similar findings. The V-RSC blood culture bottle was similar to our biphasic bottle for the recovery of gram-positive bacteria, but was superior for the recovery of gram-negative aerobic and facultatively anaerobic bacilli, especially members of the family *Enterobacteriaceae*. The V-RSC bottle, although vented transiently like the V-BiBHI bottle, recovered a greater number of anaerobic bacteria than did the latter bottle. This was attributed to differences in production of these bottles, including the amounts of CO_2 and vacuum initially present in the V-RSC bottle.

TABLE 5. Time for detection of anaerobic bacteria

Organism	V-RSC			UV-TSB		
	No. of isolates	Days to positive		No. of isolates	Days to positive	
		Mean	Median		Mean	Median
Bacteroides spp.	32	4.5	4.0	46	2.8	2.0
Clostridium spp.	2	2.5	2.5	10	3.0	2.0
Eubacterium spp.	3	3.7	4.0	3	2.0	2.0
Fusobacterium spp.	3	11.0	14.0	1	8.0	8.0
Peptococcus spp.	3	7.7	5.0	2	5.0	5.0

Anaerobic bacteria account for 6.9% of all microorganisms isolated from blood at our institution, and it would be desirable to have a single blood culture bottle that could recover all microorganisms. The V-RSC bottle appeared to have this potential. It contained an aerobic environment suitable for the growth of *P. aeruginosa* and yeasts, and an environment conducive to the recovery of anaerobic bacteria. Unfortunately, the V-RSC bottle was significantly less effective than was the UV-TSB bottle for recovery and time to detection of anaerobic bacteria.

The V-RSC bottle was superior to the UV-TSB bottle for the recovery of *Enterobacteriaceae*, *P. aeruginosa*, *S. aureus* and *S. epidermidis*, *Streptococcus* spp., and yeasts and fungi. These results are not surprising for a bottle that includes agar surfaces and an aerobic environment.

The V-RSC bottle is a blood culture bottle which enables rapid detection of aerobic and facultatively anaerobic bacteria. It has a rate of contamination similar to that of the V-BiBHI bottle that we have been using and slightly higher than that of the UV-TSB bottle. Although it recovers some anaerobic bacteria, the V-RSC bottle cannot be recommended as a substitute for an unvented vacuum blood culture bottle. The latter bottle should be used in combination with the V-RSC bottle.

LITERATURE CITED

1. **Dixon, W. J., and F. J. Massey, Jr.** 1969. Introduction to statistical analysis, 3rd ed. McGraw-Hill Book Co., New York.
2. **Hall, M. M., C. A. Mueske, D. M. Ilstrup, and J. A. Washington II.** 1979. Evaluation of a biphasic medium for blood cultures. J. Clin. Microbiol. **10**:673–676.
3. **Henry, N. K., C. A. McLimans, A. J. Wright, R. L. Thompson, W. R. Wilson, and J. A. Washington II.** 1983. Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. J. Clin. Microbiol. **17**:864–869.
4. **Pfaller, M. A., T. K. Sibley, L. M. Westfall, J. E. Hoppe-Bauer, M. 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.
5. **Reller, L. B., P. R. Murray, and J. D. MacLowry.** 1982. Cumitech 1A, Blood cultures II. Coordinating ed., J. A. Washington II. American Society for Microbiology, Washington, D.C.