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Specimen Volume Versus Yield in the BACTEC Blood Culture System

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During a 24-month period, 5,625 blood culture specimens were collected at the Seattle Veterans Administration Medical Center in 20-ml volumes and divided into separate 10-ml aliquots. The two aliquots were processed as duplicate sets (set 1, set 2) by the BACTEC system (Johnston Laboratories, Inc., Towson, Md.). Specimens (5 ml) from each set were inoculated into aerobic (6B) and anaerobic (7C/7D) vials. A total of 434 significantly positive blood cultures were found. In 342 of these positive cultures, yielding 379 isolates (112 members of the family *Enterobacteriaceae*, 104 staphylococci, 87 streptococci, 27 anaerobes, 20 yeasts, 14 pseudomonads, and 15 miscellaneous organisms), there was adequate specimen volume to fill all four vials. The utilization of set 1 would have resulted only in the failure to detect 65 of 379 (17.2%) significant isolates, 52 of 342 (15.2%) positive cultures, and 20 of 198 (10.1%) bacteremic episodes. There were no significant differences in the recovery of individual species in sets 1 and 2. Although the range of isolates recovered by the aerobic and anaerobic vials of each set differed, the percent yield of total isolates was similar, indicating total isolate yield was predominantly a function of specimen volume. The addition of set 2 most dramatically increased the recovery of *Escherichia coli* (30%), yeasts (33%), and anaerobes (42%).

There have been a number of reports in the literature concerning the relationship between the volume of blood drawn for culture and the resulting yield of significant isolates (2, 4, 8–11). In all, however, one or more extraneous variables that might bear on the outcome of the study, such as bottle type (2, 4, 10, 11), medium composition (4, 9), atmosphere of incubation (4, 11), and blood-to-broth ratio (8), remained uncontrolled. Moreover, no previous studies have been conducted which specifically addressed this issue in the BACTEC radiometric detection system (Johnston Laboratories, Inc., Towson, Md.). To assess the relationship between blood volume cultured and the sensitivity of detection of significant bacteremia by the BACTEC system, the volume of blood samples drawn for culture at the Seattle Veterans Administration Medical Center was doubled to 20 ml. This was divided equally into two identical sets of BACTEC vials for a period of 2 years.

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MATERIALS AND METHODS

During the period of the study, house officers and nurses were instructed to collect three 20-ml blood specimens at 60-min intervals from all patients with suspected sepsis. Aliquots (5 ml) were injected into each of two aerobic (6B) and two anaerobic (7C/D) vials at the bedside. For specimens of less than 20 but more than 12 ml, the phlebotomists were instructed to aliquot the specimen equally into each of the four vials. Specimens of less than 12 ml resulted in incomplete sets and were excluded from the study. The vials were sent to the laboratory within 30 min of collection.

In the laboratory, sets of vials were visually inspected. Those containing four inoculated vials were randomly assigned to set 1 or 2 (each set consisting of one aerobic and one anaerobic vial) to control for volume differences in the four vials. The BACTEC vials were tested on the BACTEC 460 Radiometric Analyzer. Aerobic vials (6B) were shaken during incubation and read twice daily for the first 48 h. All vials were subsequently tested daily for the remainder of the 7-day period. Negative vials were incubated for 7 additional days without testing and visually inspected before being discarded. The threshold for the aerobic and anaerobic vials was set at 40 and 13 growth index units, respectively.

The date, time, and growth index of each positive vial as well as the identification, biochemical characteristics, and antimicrobial susceptibility profile of all isolates were recorded. Demographic data were collected from the specimen request form. The diagnosis and antibiotic status of the patient at the time of specimen collection were provided by physicians of the infectious disease section of the medical service. The clinical significance of each isolate was determined by the method of Tenney et al. (11). All specimens drawn from a patient within 72 h of a positive blood culture were considered to have been collected during that bacteremic episode. Organism identification was done by conventional methods described in the Manual of Clinical Microbiology (6). Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion technique (7).

Since all four vials were inoculated from a single 20-ml sample and then pairs of vials (6B, 7C/D) were arbitrarily assigned to set 1 or set 2 in the laboratory, the two sets were considered equivalent and randomized. The enhanced yield afforded by the addition of set 2 was calculated as: (Yield of set 1 + set 2)/(Yield of set 1) × 100. Statistical analysis was performed by the nonparametric McNemar test (3).

RESULTS

A total of 5,625 blood specimens was processed during the course of the study. Of the 434 significant positive cultures, 342 had all vials of both sets filled. The 379 isolates recovered from these complete sets represented 198 bacteremic

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TABLE 1. Yield of clinically significant blood isolates from two identical sets of BACTEC vials

Microorganism	No. of isolates from[a]:		
	Both sets (%)	Only set 1 (%)	Only set 2 (%)
Aerobic gram-positive bacteria[b]			
Staphylococci	76 (73)	15 (14)	13 (13)
Streptococci	61 (70)	14 (16)	12 (14)
Other	0 (0)	1 (33)	2 (67)
Aerobic gram-negative bacteria[b]			
E. coli	39 (60)	11 (17)	15 (23)
Other enteric bacteria	35 (74)	5 (11)	7 (15)
Pseudomonas spp.	10 (72)	2 (14)	2 (14)
Other	9 (75)	2 (17)	1 (8)
Anaerobic bacteria	14 (52)	5 (18)	8 (30)
Yeast[c]	6 (30)	9 (45)	5 (25)
Total %	66	17	17

[a] P values for all isolates were not significant at $P > 0.05$. Sets 1 and 2 each had one 6B vial and one 7C/D vial.
[b] Facultative or aerobic microorganisms.
[c] *Candida albicans* (16 isolates), *Candida krusei* (2 isolates), *Candida stellatoidea* (1 isolate), and *Torulopsis glabrata* (1 isolate).

episodes in 177 patients. During 89 of the bacteremic episodes, three or more sets suitable for inclusion in the study were collected. In 81 and 28 of the episodes, two and one complete set(s), respectively, were received.

The total number of isolates and the number of isolates recovered in each organism category were similar for both sets (Table 1). There was no significant difference in the time to detection of 250 organisms recovered from both sets (Table 2). Although the range of isolates recovered from the aerobic (6B) and anaerobic (7C/D) vial in each set differed (Fig. 1), the percent yield of total isolates for each set was similar.

The addition of the second set of BACTEC vials to the routine blood culture procedure resulted in the detection of an additional 20 bacteremic episodes, 52 positive cultures, and 65 significant bacterial isolates. Two or more culture sets were collected from 17 bacteremic episodes that would have been missed by the deletion of set 2, and three or more were collected from 7 of the episodes. An additional 25 isolates were recovered at least 12 h earlier in set 2 than in set 1. If the yield of set 1 is taken as 100%, set 2 enhanced the detection of bacteremic episodes by 10.1%, positive cultures by 15.2%, and microbial isolates by 17.2%. The increased isolate yield was particularly notable for *Escherichia coli* (30.0%), anaerobes (42.1%), and yeasts (33.3%) (Fig. 2). When the earlier detection of bacterial isolates afforded by the addition of set 2 was considered, 90 (23.7%) of the total 379 isolates were recovered first or exclusively by set 2.

DISCUSSION

There have been a number of reports in the literature concerning the relationship between specimen volume and yield of conventional blood culture systems. All have shown the sensitivity of the culture procedure to be volume dependent, particularly in the recovery of gram-negative bacilli. Two groups of investigators, Sandven and Hoiby (9) and Tenney et al. (11), evaluated the comparative yields of 2- and 5-ml samples of blood. They reported that isolate recovery increased 6.2 and 5.8%, respectively, for each additional milliliter of blood cultured. Hall and co-workers (2) cultured 5- and 10-ml aliquots in similar vented bottles and demonstrated a 2.6% increase in yield per additional milliliter of specimen volume. A more recent analysis from the Mayo Clinic, with three 10-ml aliquots, showed a similar incremental yield (4). In none of these studies, however, have all variables affecting the sensitivity of the culture procedure, such as blood-to-broth ratio, medium, atmosphere of incubation, and processing methods, been rigidly controlled. In the best controlled study (11), the type of broth container used for the two blood aliquots differed, resulting in the vent of the smaller tube of medium being closer to the surface of the broth than that of the larger bottle. The tube of medium with the better aeration of the exposed surface area recovered significantly more fungal isolates per volume of blood cultured, emphasizing that, even in this study, factors other than specimen volume affected the study outcome.

To date, no controlled prospective study has been published which specifically deals with the relation of specimen volume to yield in the BACTEC system. The increased yield associated with larger specimen volume in conventional culture systems has been largely restricted to the recovery of gram-negative bacilli, a category of organism for which the BACTEC has proven to be highly satisfactory (1). Nevertheless, concern has been expressed that the limited capacity of the BACTEC vials (recommended sample size, 3 to 5 ml per vial) might render the system less sensitive than methods accommodating larger specimen volumes (1). Indirect data bearing on the relation of yield to specimen volume in the BACTEC system have been reported recently by two groups of investigators (5, 12). Kellogg and co-workers (5) found that the yield of isolates from BACTEC vials inoculated with 3 and 5 ml of blood relative to that achieved with the 10-ml Isolator (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) did not differ substantially. In contrast, Wicher and Koscinski (12) found that the addition of either a BACTEC 8A (hypertonic) vial or a thioglycolate medium to the routine 6A (aerobic) and 7B (anaerobic) vials significantly enhanced the recovery of blood isolates. As the increased yields afforded by the 8A and thioglycolate media were similar, the author concluded that they were largely related to the increased volume of cultured blood. We undertook this study to test this latter hypothesis. By having the clinical staff inoculate 5 ml of blood into each of two identical aerobic and anaerobic vials at the bedside, arbitrarily assigning one aerobic and one anaerobic vial to each of two sets in

TABLE 2. Detection time differences in 250 isolates recovered in both sets of BACTEC vials

Microorganism	No. of isolates (%) with both sets detected at same time (%)	No. of isolates (%) detected >12 h earlier by[a]:	
		Set 1	Set 2
Aerobic gram-positive bacteria[b]			
Staphylococci	64 (84)	3 (4)	9 (12)
Streptococci	52 (85)	4 (7)	5 (8)
Aerobic gram-negative bacteria[b]			
E. coli	32 (82)	5 (13)	2 (5)
Other enteric bacteria	30 (86)	2 (6)	3 (8)
Pseudomonas spp.	9 (90)	1 (10)	
Other	4 (44)	1 (11)	4 (44)
Anaerobic bacteria	12 (86)		2 (14)
Yeast	6 (100)		

[a] Values were not significant at $P > 0.05$. Sets 1 and 2 each had one 6B vial and one 7C/D vial.
[b] Facultative or aerobic microorganisms.

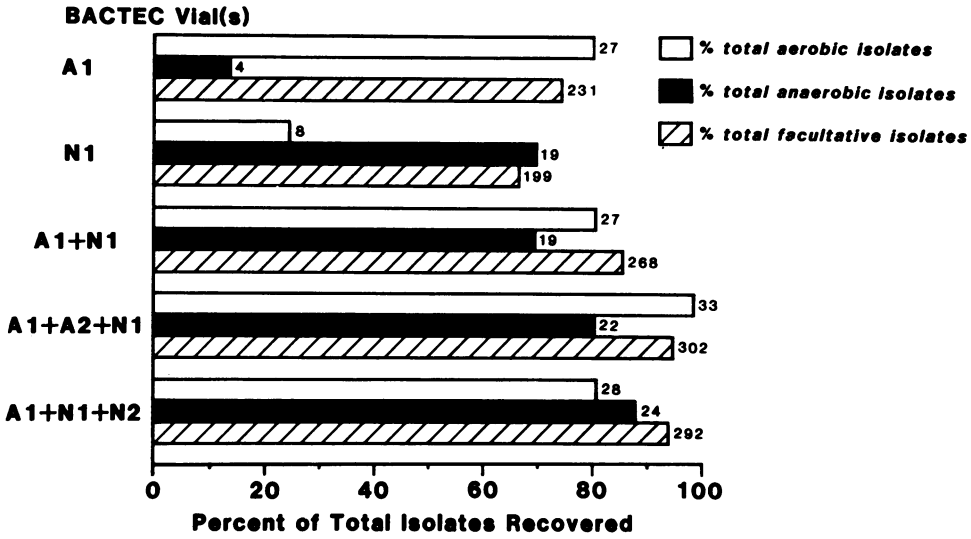

FIG. 1. The horizontal bars indicate the percentage of total aerobic, anaerobic, or facultative organisms detected by a single BACTEC vial or combination of BACTEC vials. The numbers at the end of each column represent the actual number of isolates recovered. A-1 and A-2 designations refer to the aerobic (6B) vials in set 1 and 2, respectively. N-1 and N-2 represent the anaerobic (7C/D) vials in the two sets.

the laboratory, and then processing the two sets in an identical manner, we hoped to avoid the confounding variables present in the volume studies described above. Our results suggest this was accomplished. The overall recovery rates for the two sets were the same (82.8 and 83.1%), and no isolate or group of isolates was recovered significantly more frequently by either blood set. Furthermore, for the organisms recovered in both sets of blood, detection time in each set was similar.

The addition of the second set of vials increased detection of bacteremic episodes by 10.1%, significant positive cultures by 15.2%, and significant microbial isolates by 17.2%. A total of 90 of the 379 (23.7%) total isolates were detected first or exclusively by the second set of BACTEC vials. Culture of the additional 10 ml of blood particularly enhanced the recovery of *E. coli* (30.0%), anaerobes (42.1%), and yeasts (33.3%). Interestingly, only 14.3% more pseudomonads were recovered. In both the study reported by Hall et al. (2) and that of Tenney et al. (11), the increased recovery of gram-negative bacilli was due largely to the enhanced detection of bacteremia caused by pseudomonads. In the BACTEC system, the aerobic vials are shaken for the first 24 h of incubation. It is conceivable that this enhances the recovery of obligate aerobes such as the pseudomonads and minimizes the advantage of additional specimen volume. Alternatively, it may reflect the advantage of the larger initial volume of blood cultured in our study.

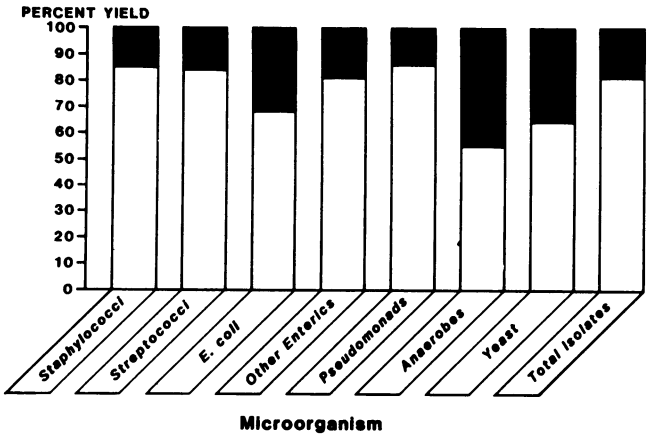

FIG. 2. Comparison of increased yield of various isolate groups afforded by a second set of BACTEC vials. The percent increase is indicated in black.

The incremental increase in isolate yield (1.76%/ml of blood) seen in our study is smaller than that reported by other investigators. This may be related, at least in part, to the fact that we used larger volumes of blood than did Sandven and Hoiby (8), Tenney et al. (11), and Hall et al. (2). However, our incremental yield was also smaller than that reported by the one study with comparable volume (4), suggesting that the BACTEC system may be slightly less volume sensitive than many conventional systems.

Shanson et al. (10) have reported that the time to detection of viridans group streptococcal bacteremia is decreased by increasing the volume of cultured blood from 15 to 45 ml. In our study, 25 of the 250 isolates recovered from both sets of BACTEC vials were recovered at least 12 h earlier by the second set; no detection of a single isolate or group of isolates was particularly enhanced. Our study thus confirms the work of Shanson et al. and suggests that the effect reported is not species dependent.

Finally, we showed that the increased sensitivity afforded by larger specimen volumes was largely independent of the medium used. Although the spectrum of organisms recovered in the aerobic and anaerobic vials clearly differed (Fig. 1), the number of isolates recovered by each differed only slightly. In fact, the number of isolates recovered from any combination of two vials and any combination of three vials was essentially the same.

In conclusion we have shown, in a carefully controlled study, that the sensitivity and detection time of the BACTEC radiometric system are, like those of the conventional systems tested before it, volume dependent. An increase in the specimen size from 10 to 20 ml in the BACTEC system currently requires the addition of two extra vials to the blood culture procedure at a substantial increase in cost.

The overall cost-effectiveness of this system vis-à-vis conventional systems is, however, maintained by the significant decrease in the personnel cost of processing blood cultures afforded by this system. It is also possible that the larger volume of blood drawn may decrease the total number of samples required to detect most bacteremias (7a; J. J. Plorde, unpublished data).

LITERATURE CITED

1. **Anhalt, J. P.** 1978. New or experimental approaches to detection of bacteremia, p. 109–138. *In* J. A. Washington II. (ed.), The detection of septicemia. CRC Press, Inc., Boca Raton, Fla.
2. **Hall, M. M., D. M. Ilstrup, and J. A. Washington II.** 1976. Effect of volume of blood cultured on detection of bacteremia. J. Clin. Microbiol. **3**:643–645.
3. **Ilstrup, D. M.** 1978. Statistical methods employed in the study of blood culture media, p. 31–39. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, Inc., Boca Raton, Fla.
4. **Ilstrup, D. M., and J. A. Washington II.** 1983. The importance of volume of blood cultured in the detection of bacteremia and fungemia. Diagn. Microbiol. Infect. Dis. **1**:107–110.
5. **Kellogg, J. A., J. P. Manzella, and J. H. McConville.** 1984. Clinical laboratory comparison of the 10-ml Isolator blood culture system with BACTEC radiometric blood culture media. J. Clin. Microbiol. **20**:618–623.
6. **Lennette, E. H., A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.).** 1980. Manual of clinical microbiology, 3rd ed. American Society of Microbiology, Washington, D.C.
7. **National Committee for Clinical Laboratory Standards.** 1979. Performance standards for antimicrobic disc susceptibility tests, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.

7a. **Plorde, J. J.** 1985. Newer methods in microbial diagnosis, p. 147–167. *In* R. K. Root and M. A. Sande (ed.), Contemporary issues in infectious disease, vol. 4: septic shock. Churchill Livingstone, New York.

8. **Salventi, J. F., T. A. Davies, E. L. Randall, S. Whitaker, and J. R. Waters.** 1979. Effect of blood dilution on recovery of organisms from clinical blood cultures in medium containing sodium polyanethol sulfonate. J. Clin. Microbiol. **9**:248–252.
9. **Sandven, P., and A. E. Hoiby.** 1981. The importance of blood volume cultured on detection of bacteremia. Acta. Pathol. Microbiol. Scand. **89**:149–152.
10. **Shanson, D. C., F. Thomas, and D. Wilson.** 1984. Effect of volume of blood cultured on detection of *Streptococcus viridans* bacteremia. J. Clin. Pathol. **37**:568–570.
11. **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.
12. **Wicher, K., and D. Koscinski.** 1984. Laboratory experience with radiometric detection of bacteremia with three culture media. J. Clin. Microbiol. **20**:668–671.