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Controlled Evaluation of 5 versus 10 Milliliters of Blood Cultured in Aerobic BacT/Alert Blood Culture Bottles

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Bottles developed for use in the BacT/Alert automated blood culture system (Organon Teknika Corp., Durham, N.C.) can accept up to 10 ml of blood without falling below a 1:5 ratio of blood to broth. We compared the yield and speed of detection of microorganisms in 13,128 adequately filled, paired, aerobic bottles inoculated with 5 versus 10 ml of blood at three university hospitals. A total of 798 microorganisms causing sepsis grew in one or both bottles. The overall recovery of microorganisms from 10-ml samples exceeded that from 5-ml samples ($P < 0.001$); the increased yield attributed to the additional 5 ml in the samples was 7.2%. The increased yield from 10-ml inocula was most marked for *Escherichia coli* ($P < 0.01$) and other members of the family *Enterobacteriaceae* ($P < 0.001$). Ten-milliliter samples did not yield more gram-positive bacteria, nonfermentative gram-negative rods, or yeasts. When both bottles were positive, the bottles inoculated with 10 ml of blood showed growth sooner ($P < 0.001$). Earlier detection with 10-ml inocula was especially notable for coagulase-negative staphylococci ($P < 0.001$), streptococci ($P < 0.001$), *E. coli* ($P < 0.025$), and other members of the family *Enterobacteriaceae* ($P < 0.025$). We conclude that an increase in the volume of blood inoculated into BacT/Alert aerobic blood culture bottles from 5 to 10 ml will increase the overall yield and the speed of detection of clinically important blood pathogens.

The BacT/Alert automated blood culture system (Organon Teknika Corp., Durham, N.C.) recently has been shown to provide equivalent yields and earlier times to detection of positive blood cultures in comparison with those for the BACTEC 660/730 nonradiometric blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) (15). Although the BacT/Alert blood culture bottles are designed to accept up to 10 ml of blood, the aforementioned study used a blood inoculum of only 5 ml per bottle. To date, no data from an evaluation of the BacT/Alert system after inoculation of 10 ml of blood into its blood culture bottles have been published. It might be expected that a larger inoculum of blood per bottle, e.g., 10 ml, would result in an enhanced yield of microorganisms (3, 8, 11). To test this hypothesis, we compared the yield and speed of detecting microorganisms when 5 versus 10 ml of blood was inoculated into BacT/Alert aerobic blood culture bottles at three university hospitals that use identical methods of obtaining and processing blood cultures.

MATERIALS AND METHODS

Collection of samples. During the study period, two aerobic BacT/Alert blood culture bottles and one anaerobic BacT/Alert blood culture bottle were used to culture blood from adult patients at the Robert Wood Johnson University Hospital, Duke University Medical Center, and the Salt Lake City Veterans Affairs Medical Center. Blood for culture was obtained at the bedside after preparation of the skin with 10% povidone-iodine and then 70% isopropyl alcohol. Blood (20 ml) from each separate venipuncture was distributed as follows: 10 ml to one aerobic bottle, 5 ml to the other aerobic bottle, and 5 ml to the anaerobic bottle.

Volume standards. To ensure that the culture bottles were inoculated with the specified volume of blood, we measured the level of fluid in each container after it was filled with blood. Although all blood-containing bottles were incubated and processed for patient care, only bottle pairs which met volume standards for an adequate fill were included in the subsequent analyses of data. An adequately filled 10-ml bottle was required to contain 8 to 12 ml of blood, and an adequately filled 5-ml bottle was required to contain 4 to 6 ml of blood.

Processing of samples. Identical methods were used to process blood cultures in the clinical microbiology laboratories at all hospitals. Bottles with macroscopic evidence of growth upon arrival in the laboratory were not placed in the BacT/Alert instrument but were Gram stained and subcultured. All other bottles were placed in the BacT/Alert instrument and were processed according to the manufacturer's instructions. When growth was detected, all microorganisms were identified by standard microbiologic procedures (2). Each bottle was treated independently, and only bottles flagged as positive by the instrument were processed further. Companion bottles of those flagged as positive were not removed and were further processed unless they, too, were flagged as positive by the instrument.

Clinical assessment. Positive cultures were reviewed by a

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TABLE 1. Comparative yields of clinically important bacteria and fungi in BacT/Alert aerobic blood culture bottles inoculated with 5 and 10 ml of blood

Microorganism	No. of isolates recovered by:			P
	Both bottles	10-ml bottle only	5-ml bottle only	
Staphylococcus aureus	142	20	24	NS[a]
Coagulase-negative staphylococci	76	25	17	NS
Streptococci[b]	55	8	2	NS
Enterococcus spp.	40	11	12	NS
Other gram-positive bacteria[c]	2	4	0	NS
Escherichia coli	64	17	4	<0.01
Other members of the family *Enterobacteriaceae*	64	32	10	<0.001
Pseudomonas aeruginosa	28	7	7	NS
Other gram-negative bacteria[d]	9	4	4	NS
Gram-positive anaerobic bacteria[e]	8	1	1	NS
Gram-negative anaerobic bacteria[f]	2	2	1	NS
Yeasts and fungi[g]	59	17	19	NS
All microorganisms	549	148	101	<0.005

[a] NS, not significant ($P > 0.05$).
[b] Includes 26 *Streptococcus pneumoniae*, 13 group B streptococci, 13 viridans group streptococci, 4 nonhemolytic streptococci, 3 group A streptococci, 3 group F streptococci, 2 group G streptococci, and 1 group C streptococcus.
[c] Includes two *Listeria monocytogenes*, two *Corynebacterium* spp., and two unidentified gram-positive cocci.
[d] Includes nine *Acinetobacter baumanii*, three *Pseudomonas cepacia*, one *Pseudomonas maltophilia*, one *Acinetobacter lwoffi*, one *Flavobacterium meningosepticum*, one *Haemophilus influenzae*, and one *Neisseria meningitidis*.
[e] Includes seven *Clostridium* spp., two *Bifidobacterium* spp., and one *Peptostreptococcus* sp.
[f] Includes three *Bacteroides fragilis*, one *Bacteroides vulgatus*, and one *Fusobacterium* sp.
[g] Includes 38 *Candida albicans*, 37 *Torulopsis (Candida) glabrata*, 11 *Candida krusei*, 5 *Candida tropicalis*, 1 *Candida parapsilosis*, 1 *Cryptococcus neoformans*, and 1 *Scedosporium inflatum*.

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi in BacT/Alert aerobic blood culture bottles inoculated with 5 and 10 ml of blood

Microorganism	No. of isolates from:			P
	Both bottles at same time	10-ml bottle earlier	5-ml bottle earlier	
Staphylococcus aureus	41	54	47	NS[a]
Coagulase-negative staphylococci	9	48	19	<0.001
Streptococci[b]	17	30	8	<0.001
Enterococcus spp.	7	20	13	NS
Other gram-positive bacteria[c]	0	1	1	NS
Escherichia coli	19	31	14	<0.025
Other members of the family *Enterobacteriaceae*	15	33	16	<0.025
Pseudomonas aeruginosa	6	15	7	NS
Other gram-negative bacteria[d]	4	3	2	NS
Gram-positive anaerobic bacteria[e]	1	4	3	NS
Gram-negative anaerobic bacteria[f]	0	1	1	NS
Yeasts and fungi[g]	6	23	30	NS
All microorganisms	125	263	161	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Includes 17 *Streptococcus pneumoniae*, 13 group B streptococci, 12 viridans group streptococci, 4 nonhemolytic streptococci, 3 group A streptococci, 3 group F streptococci, 2 group G streptococci, and 1 group C streptococcus.
[c] Includes one *Listeria monocytogenes* and one *Corynebacterium jeikeium*.
[d] Includes five *Acinetobacter baumanii*, one *Pseudomonas cepacia*, one *Pseudomonas maltophilia*, one *Haemophilus influenzae*, and one *Neisseria meningitidis*.
[e] Includes two *Bifidobacterium* spp., two *Clostridium perfringens*, two *Clostridium septicum*, one *Clostridium tertium*, and one *Peptostreptococcus* sp.
[f] Includes one *Bacteroides fragilis* and one *Fusobacterium* sp.
[g] Includes 25 *Torulopsis glabrata*, 21 *Candida albicans*, 7 *Candida krusei*, 3 *Candida tropicalis*, 1 *Cryptococcus neoformans*, and 1 *Scedosporium inflatum*.

physician who specialized in infectious diseases. The microorganisms isolated were judged on the basis of published criteria (13) to be the etiologic agents of bacteremia and fungemia, contaminants, or indeterminate as the cause of sepsis.

Analysis of data. Only results from aerobic bottle pairs adequately filled with blood and in which microorganisms judged to represent true bacteremia or fungemia grew were analyzed. Comparisons were evaluated statistically by the modified chi-square test described by McNemar (4). When appropriate, the Yates correction for small numbers of observations was used.

RESULTS

A total of 13,128 adequately filled paired bottles were received during the study period. Of these, 1,184 (9.0%) were positive, including 712 (5.4%) that grew 798 microorganisms that caused illness, 405 (3.1%) that grew one or more contaminants, 41 (0.3%) that grew one or more microorganisms that were indeterminate as a cause of sepsis, 19 (0.1%) that grew a pathogen mixed with a contaminant or indeterminate isolate, and 7 (0.05%) that grew a contaminant mixed with an indeterminate isolate. Of the 798 clinically important microorganisms, 549 (68.8%) grew in both aerobic bottles, 148 (18.5%) grew only in the aerobic bottle inoculated with 10 ml of blood, and 101 (12.7%) grew only in the aerobic bottle inoculated with 5 ml of blood.

Significantly more microorganisms ($P < 0.005$) grew from the 10-ml bottle than from the 5-ml bottle (Table 1). The increased yield that could be attributed to the larger volume of blood was 7.2%. Of individual microorganisms or groups thereof, *Escherichia coli* ($P < 0.01$) and other members of the family *Enterobacteriaceae* ($P < 0.001$) were detected significantly more often in the 10-ml than in the 5-ml bottle (Table 1). Ten-milliliter samples did not yield significantly more gram-positive bacteria, nonfermentative gram-negative rods, or yeasts than 5-ml samples.

Of the 549 clinically important microorganisms that grew in both the 5- and 10-ml bottles, 125 (22.8%) were detected at the same time, 263 (47.9%) were detected earlier in the 10-ml bottle, and 161 (29.3%) were detected earlier in the 5-ml bottle ($P < 0.001$) (Table 2). The coagulase-negative staphylococci ($P < 0.001$), streptococci ($P < 0.001$), *E. coli* ($P < 0.025$), and other members of the family *Enterobacteriaceae* ($P < 0.025$) all were detected earlier from the 10-ml bottle than from the 5-ml bottle (Table 2).

The cumulative proportions of positive blood cultures by day were as follows: 24 h, 82% in both bottles; 48 h, 92% in 10-ml bottles and 95% in 5-ml bottles; and 72 h, 96% in 10-ml bottles and 98% in 5-ml bottles. When the mean time to positivity for bottles in which microorganisms were detected within the first 72 h of incubation was examined, paradoxically, there was a faster time to detection for the 5-ml bottle than the 10-ml bottle for some microorganisms, notably *Staphylococcus aureus* and the enterococci (Table 3), and the speed advantage for the 10-ml bottle for detecting members of the family *Enterobacteriaceae* was no longer evident. The explanation for the paradox lies in the magnitude of the differences in detection times. Although more positive cultures were detected first in the 10-ml bottle, the magnitude of this advantage tended to be small. By contrast, when the 5-ml bottle detected growth first,

TABLE 3. Comparison of mean time to detection of clinically important bacteria and fungi isolated within 72 h[a] of incubation in BacT/Alert aerobic blood culture bottles inoculated with 5 and 10 ml of blood

Microorganism	No. of bottle pairs[b]	Mean time (h) to detection	
		10-ml inoculum	5-ml inoculum
Staphylococcus aureus	137	15.7	14.3
Coagulase-negative staphylococci	76	17.6	19.0
Streptococci	52	10.8	11.1
Enterococcus spp.	39	15.7	15.2
Other gram-positive bacteria	2	24.5	24.0
Members of the family *Enterobacteriaceae*	123	11.0	11.1
Pseudomonas aeruginosa	28	14.4	14.6
Other gram-negative bacteria	9	14.6	16.4
Gram-positive anaerobic bacteria	7	14.3	19.5
Yeasts and fungi	48	32.2	31.6
All microorganisms	521	15.8	15.7

[a] The 72-h cutoff was used in the analysis to reduce bias from late positives (i.e., outliers).

[b] The number of bottle pairs listed describes bottle pairs for which both bottles were adequately filled with blood and both bottles were positive by 72 h of incubation.

the magnitude of the time difference tended to be great. For example, in the case of *Serratia marcescens*, the 10-ml bottle detected growth earlier five times, on average 1.6 h earlier than the 5-ml bottle. The 5-ml bottle detected growth earlier only twice, but the time differences were 11 and 48 h, respectively. Thus, although more *S. marcescens* organisms were detected earlier in the 10-ml bottle, the calculated mean time to detection favored the 5-ml bottle.

DISCUSSION

Two of the key variables that influence successful blood cultures, blood volume and blood:broth ratio, as well as their complex interplay, have been addressed by and have affected the results of the present study in which one bottle had a volume (10 ml) advantage but a possible ratio disadvantage (1:5). Clearly, the volume of blood obtained from the patient influences the yield from cultures, and multiple studies now have shown that when larger volumes of blood are cultured, more bacteremias and fungemias are detected (1, 3, 5, 8, 11). The optimum blood:broth ratio appears to be 1:10 (12). Lower blood:broth ratios (e.g., $\leq$1:5) may be associated with reduced yields, particularly of staphylococci and other gram-positive bacteria, for blood from patients who are receiving antimicrobial agents (9, 10). Accordingly, although it was not surprising that there was an increased yield of positive cultures in the bottle that received the larger inoculum of blood in the present study, it is possible that the lower blood:broth ratio in the bottle with the larger blood inoculum may have moderated the yield advantage that could have been present because of volume alone. The increased positivity rate of 1.44% per ml of blood from 5 to 10 ml of blood in the current evaluation was less than might have been expected on the basis of our previous studies (11).

Overall, when cultures were positive in both study bottles, growth was detected earlier more frequently in the 10-ml bottle (Table 2). However, paradoxically, the mean time to positivity for some microorganisms was shorter in the 5-ml bottles (Table 3). Theoretically, the 10-ml bottle, with twice the blood inoculum of the 5-ml bottle, might have been expected to show growth earlier. The fact that this was not observed uniformly suggests that unknown factors, perhaps related to the reduced blood:broth ratio associated with the 10-ml blood inoculum, could have slowed the rate of growth of some microorganisms in these bottles. A logical, but not proved, explanation for the observed results could be the effect of antimicrobial agents in delaying but not necessarily ablating growth (7, 14) either by direct inhibition or perhaps by a postantibiotic effect. For example, a 5-ml inoculum with a greater dilution of antibiotic could be positive sooner than a 10-ml sample with twice the antimicrobial agent concentration per bottle and, hence, a longer delay until the surviving microorganism(s) could resume growth after degradation or inactivation of antibiotics (6, 9). Alternatively, in the 10-ml (i.e., less dilute) bottle, the lag phase of growth may have been prolonged, thereby allowing growth to be detected first in the 5-ml bottle, despite its lower inoculum of organisms. Although these findings are unexplained, their clinical significance in most cases is probably modest on the basis of the very small overall differences in the mean time to detection.

In conclusion, the results of the present study suggest that the yield of positive blood cultures in BacT/Alert aerobic blood culture bottles can be improved if the inoculum of blood is increased from 5 to 10 ml. The mean time to detection of positive cultures may be slightly prolonged for some microorganisms with 10-ml versus 5-ml inocula, but this difference is probably clinically insignificant.

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