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Clinical Comparison of Nonvented Aerobic BacT/Alert Blood Culture Bottle and Standard Aerobic Bottle for Detection of Microorganisms in Blood

JAMES W. SNYDER,[1,2]* KAREN S. BENZING,[2] GINA K. MUNIER,[2] GRACE D. BOSTIC,[3] PAMELA S. BOZIGAR,[3] RAMI HANNA,[3] AND MARCUS J. ZERVOS[3]

Department of Pathology, Division of Laboratory Medicine, University of Louisville School of Medicine,[1] and University of Louisville Hospital,[2] Louisville, Kentucky 40202, and Department of Microbiology, William Beaumont Hospital, Royal Oak, Michigan 48073[3]

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The current BacT/Alert standard aerobic (VA) blood culture bottle was redesigned and designated a nonvented aerobic (NVA) culture bottle; this bottle does not require venting. A total of 3,873 sets of blood samples for culture were obtained from adult patients with suspected bacteremia or fungemia. The NVA bottle showed performance equivalent to that of the VA bottle for recovery and speed of detection of microorganisms from blood without the need for venting the bottle.

For febrile patients, with or without localizing signs or symptoms, blood culture is the most useful and most frequently performed test to detect systemic infection. In addition to the diagnostic value of blood culture, recovery of an infectious agent from the blood provides an invaluable aid for guiding antimicrobial therapy. Advances in blood culture technology, primarily due to the introduction of instrument-based continuous-monitoring blood culture systems, have decreased the time needed to detect bacteremia and, to some extent, fungemia, compared to non-instrument-based conventional methods. Accompanying the advancements in detection technology has been the addition of several choices of media for the recovery of aerobic and anaerobic bacteria and fungi, particularly yeasts. However, transient venting of aerobic blood culture bottles continues to be practiced, posing the risk of needle stick injury for personnel and increasing the associated costs of testing personnel for blood-borne pathogens and/or complicating factors. Additionally, the need for venting adds an extra step in the process that is time-consuming and allows for the possibility of introducing contaminants into the bottle.

The BacT/Alert Microbial detection system (Organon Teknika Corporation, Durham, N.C.) is a fully automated colorimetric blood culture system with membrane sensors for detecting microbial growth, an instrument to incubate, agitate, and scan the bottles for positivity, and a computerized database management system to record and report results.

The current aerobic blood culture bottle was redesigned and designated a nonvent aerobic (NVA) culture bottle; the NVA bottle does not require venting, provides a larger headspace for the proper atmospheric conditions (i.e., increased oxygen content), and contains a liquid emulsion sensor.

The purpose of this investigation was to determine if the performance of the NVA culture bottle is, at a minimum, equivalent to that of the manufacturer's current standard aerobic (VA) bottle for the recovery of and speed of detection of microorganisms in suspected cases of septicemia or fungemia.

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This study was conducted in two tertiary-care medical centers, the University of Louisville Hospital (ULH), Louisville, Ky., and the William Beaumont Hospital (WBH), Royal Oak, Mich. Blood samples were collected by phlebotomists and nursing personnel at ULH and by the phlebotomy team at WBH. An aliquot of ca. 20 ml was obtained from patients with clinically suspected bacteremia or fungemia following preparation of the venipuncture site with 70% isopropyl alcohol and 10% povidone (2, 4). Equal aliquots of blood were aseptically inoculated into both NVA and VA bottles in a random fashion. Blood volumes were measured in the laboratory by comparing each bottle to known volume standards. Only blood culture sets with differences in fill volumes between bottles of ≤20% of the larger volume were included in the analysis. Bottle pairs which did not meet this criterion were excluded from the study but were processed to maximize the recovery of microorganisms from each culture. All VA bottles were transiently vented to air for at least 30 s prior to placement into the BacT/Alert incubator module; NVA bottles were loaded directly. Cultures were incubated for a total of 5 days, with terminal subcultures performed at random on 20% of the instrument-negative NVA bottles. When a bottle signaled positive, broth from the vial was gram stained and subcultured into the appropriate medium. All isolates were identified by standard microbiologic procedures (3). False-positive bottles (i.e., those which had a positive instrument signal but were Gram-stain negative and subculture negative) were reincubated until growth occurred, whereupon the bottles were reflagged as suspected positives, or until the original 5-day incubation period had expired. All bottles were processed independently of the other bottles in a given set, that is, a negative bottle was not examined when the other bottle in the set was flagged as a suspected positive. If these bottles remained negative for 5 days, they were terminally subcultured. Instrument-negative bottles that grew an organism on terminal subculture were categorized as false negative. The clinical significance of recovered microorganisms was determined using published criteria (5).

To determine any differences between the VA and NVA

* Corresponding author. Mailing address: Department of Pathology, Division of Laboratory Medicine, University of Louisville School of Medicine, Louisville, KY 40202. Phone: (502) 852-1777. Fax: (502) 852-1771. E-mail: jwsnyd01@gwise.louisville.edu.

TABLE 1. Comparative yields and total recovery of clinically significant microorganisms in BacT/Alert VA and NVA blood culture bottles[a]

Microorganism	No. of isolates detected by:		
	Both types of bottle	VA bottles only	NVA bottles only
Gram-positive cocci			
Staphylococcus aureus	48	6	7
Methicillin-resistant *S. aureus*	4	0	1
CoNS[b]	26	22	28
Enterococcus faecalis	4	1	1
Enterococcus faecium	2	0	0
Enterococcus spp.	6	0	0
Streptococcus pneumoniae	18	1	1
Beta-hemolytic streptococci[c]	9	0	0
Viridans group streptococci[d]	5	2	2
Gram-positive bacilli			
Bacillus cereus	1	0	0
Brevibacterium spp.	1	0	1
Corynebacterium jeikeium	0	1	0
Gram-negative bacilli			
Acinetobacter baumannii	0	1	2
Enterobacter aerogenes	1	1	0
Enterobacter cloacae	2	0	0
Escherichia coli	14	1	1
Klebsiella oxytoca	0	1	1
Klebsiella pneumoniae	8	0	1
Proteus mirabilis	1	1	0
Providencia rettgeri	1	0	1
Pseudomonas aeruginosa	4	1	2
Serratia marcescens	2	2	1
Anaerobic bacterium, *Bacteroides fragilis*	0	2	0
Fungi			
Candida albicans	6	0	2
Candida parapsilosis	0	1	0
Candida glabrata	3	1	1
Cryptococcus neoformans	0	0	1
All microorganisms	166	45	54

[a] Organisms were recovered from 236 monomicrobic and 17 polymicrobic cultures; organism totals exceed the numbers of cultures because some cultures contained more than one organism.
[b] Includes 8 *Staphylococcus epidermidis*, 4 *Staphylococcus haemolyticus*, 1 *Staphylococcus hominis*, 1 *Staphylococcus lugdunensis*, and 1 *Staphylococcus simulans* isolate and 61 isolates that were not identified to the species level.
[c] Includes six group A, one group B, and two group G beta-hemolytic streptococci.
[d] Includes four *Streptococcus sanguis*, and one *Streptococcus mitis* isolate and four viridans group streptococci that were not identified to the species level.

TABLE 2. Clinical isolates recovered from episodes of monomicrobial (172) and polymicrobial (12) bacteremia or fungemia detected by BacT/Alert VA and NVA culture bottles[a]

Microorganism	No. of isolates detected by:		
	Both types of bottle	VA bottles only	NVA bottles only
Gram-positive cocci			
Staphylococcus aureus	35	5	6
Methicillin-resistant *S. aureus*	3	0	1
CoNS[b]	23	16	20
Enterococcus faecalis	4	1	0
Enterococcus faecium	2	0	0
Enterococcus spp.	4	0	0
Streptococcus pneumoniae	13	0	1
Beta-hemolytic streptococci[c]	5	0	0
Viridans group streptococci[d]	3	2	2
Gram-positive bacilli			
Bacillus cereus	1	0	0
Brevibacterium spp.	1	0	0
Corynebacterium jeikeium	0	1	0
Gram-negative bacilli			
Acinetobacter baumannii	0	1	2
Enterobacter aerogenes	1	1	0
Enterobacter cloacae	1	0	0
Escherichia coli	10	1	0
Klebsiella oxytoca	1	0	0
Klebsiella pneumoniae	6	0	1
Proteus mirabilis	1	1	0
Providencia rettgeri	1	0	0
Pseudomonas aeruginosa	3	1	2
Serratia marcescens	1	2	1
Anaerobic bacterium, *Bacteroides fragilis*	0	1	0
Fungi			
Candida albicans	3	0	2
Candida parapsilosis	0	1	0
Candida glabrata	2	0	0
Cryptococcus neoformans	0	0	1
All microorganisms	124	34	39

[a] Organism totals exceed the numbers of cultures because some cultures contained more than one organism.
[b] Includes 5 *Staphylococcus epidermidis*, 1 *Staphylococcus haemolyticus*, 1 *Staphylococcus hominis*, 1 *Staphylococcus lugdunensis*, and 1 *Staphylococcus simulans* isolate and 50 isolates that were not identified to the species level.
[c] Includes three group A, one group B, and one group G beta-hemolytic streptococcus.
[d] Includes two *Streptococcus sanguis*, and two *Streptococcus mitis* isolates and three viridans group streptococci that were not identified to the species level.

bottles with respect to recovery of organisms, McNemar's test for paired samples was applied to the positive-yield data generated for compliant sets (1). A probability value of less than 0.05 would indicate a statistically significant difference in the recovery of the two bottles. The signed-rank test was used to determine the differences between the VA and NVA bottles with respect to speed of detection (1). For each compliant culture that was positive in both bottles, the difference in detection times for the systems was computed. Differences were analyzed using a separate signed-rank test for each of the bacterial species. A probability value of less than 0.05 would indicate a significant difference in the speed of detection between both bottles.

A total of 3,873 paired VA and NVA bottles were received for culture, of which 2,984 met the fill volume criterion for inclusion in the analysis. There were 463 positive cultures detected in one or both bottles. The VA bottles resulted in 319 (10.7%) positives, 26 (0.9%) false positives, and 3 (0.1%) false negatives, while the NVA bottles resulted in 348 (11.7%) positives, 32 (1.1%) false positives, and 2 (0.1%) false negatives. Terminal subcultures of the two false negatives detected in the NVA bottles yielded *Streptococcus pneumoniae* and coagulase-negative staphylococci (CoNS); the corresponding VA bottles were positive for *S. pneumoniae* and negative for CoNS. The

three false negatives detected in the VA bottles yielded *Pseudomonas aeruginosa* (two isolates) and *Staphylococcus epidermidis* (one isolate); these organisms were also detected in the corresponding NVA bottles.

Of the 253 (236 monomicrobic and 17 polymicrobic) positive cultures with clinically significant organisms, 156 (62%) were detected by both bottles, 43 (17%) were detected by the VA bottles only, and 54 (21%) were detected by the NVA bottles only. The comparative yields of clinically significant bacteria and fungi from the two aerobic culture bottles are summarized in Table 1. There was no statistically significant difference between bottles in recovery of clinically significant organisms. The recovery of anaerobes from the VA bottles and not from the NVA bottles was expected, due to the higher oxygen content in the headspace of the NVA bottles, which likely inhibited the growth of anaerobic organisms.

The recovery of clinically significant microorganisms in each culture system with blood from patients with septic episodes is depicted in Table 2. A septic episode was defined as the initial isolation of a significant organism, the subsequent isolation of a different significant organism after 3 days, or the isolation of the same significant organism after an interval of at least 7 days since the last positive culture. Isolation of a different significant organism within 3 days of the last positive culture constituted a polymicrobic episode. Of the 184 (172 monomicrobic and 12 polymicrobic) septic episodes, representing 176 patients, 116 (63%) were detected in both bottles, 32 (17%) were detected in the VA bottles only, and 36 (20%) were detected in the NVA bottles only. There was no statistically significant difference between bottles in recovery of clinically significant organisms from septic episodes.

The comparative mean times to detection for the different organism groups are summarized in Table 3 and include only bottle pairs from which the same, single, clinically significant organism was recovered. There was no statistically significant difference in detection times between bottles.

The performance of the new NVA blood culture bottle was comparable to that of the current VA bottle for the recovery and speed of detection of microorganisms. The use of the NVA bottles in conjunction with the BacT/Alert blood culture instrument precludes the need for venting and thus reduces the risk of needle stick injury and the associated mental and medical costs. Additional cost savings could be realized by the reduction of technologist processing time and the elimination of needle-associated contamination.

TABLE 3. Comparison of mean times for detection of growth when the same clinically significant microorganism was isolated from both bottles[a]

Microorganism	No. of isolates	Detection time (h)	
		VA bottles	NVA bottles
Gram-positive cocci	110	15.2	14.8
Gram-positive bacilli	2	21.9	30.8
Gram-negative bacilli	22	21.2	23.7
Fungi	9	37.3	34.6
Total	143	17.6	17.6

[a] Differences in mean detection times were not significant at a P value of 0.05 for any of the organisms tested.

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