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Controlled Clinical Comparison of the BacT/ALERT FN and the Standard Anaerobic SN Blood Culture Medium

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To determine the optimal anaerobic companion bottle to pair with the BacT/ALERT (bioMérieux, Durham, N.C.) nonvented aerobic FA (FA) medium for recovery of pathogenic microorganisms from adult patients with bacteremia and fungemia, we compared the BacT/ALERT FN (FN) anaerobic bottle with the standard BacT/ALERT SN (SN) anaerobic bottle. Each bottle, FA, FN, and SN, was filled with 8 to 12 ml of blood. Of 11,498 blood culture sets received in the clinical microbiology laboratories at two university medical centers, 7,945 sets had all three bottles filled adequately and 8,569 had both anaerobic bottles filled adequately. Of 686 clinically important (based on previously published criteria) isolates detected in one or both adequately filled anaerobic bottles, more staphylococci ($P < 0.001$), including *Staphylococcus aureus* ($P < 0.001$); members of the family *Enterobacteriaceae* ($P < 0.001$); and all microorganisms combined ($P < 0.001$) were detected in FN bottles. In contrast, more *Pseudomonas aeruginosa* isolates ($P < 0.01$) and yeasts ($P < 0.001$) were detected in SN bottles. More *Bacteroides fragilis* group bacteremias were detected only in the FN (six) than in the SN (one) anaerobic bottle (P = not significant). Overall, the mean time to detection was shorter with FN (16.8 h) than with SN (18.2 h). This difference in time to detection was greatest for the *B. fragilis* group: FN, 28 h, versus SN, 60.0 h. Many of the facultative microorganisms recovered in either FN or SN were also found in the companion FA. When microorganisms found in the companion FA bottle were omitted from the analysis, significantly more staphylococci ($P < 0.001$), including *S. aureus* ($P < 0.001$), and *Enterobacteriaceae* ($P < 0.005$) still were detected in FN bottles, whereas there were no significant differences for *P. aeruginosa* and yeasts, which were found as expected in FA bottles. We conclude that the companion anaerobic FN bottle detects more microorganisms than does the anaerobic SN bottle when used in conjunction with the nonvented aerobic FA bottle in the BacT/ALERT blood culture system.

An anaerobic blood culture medium is commonly used with a companion aerobic medium for detection of bacteremia. The BacT/ALERT FN medium (bioMérieux, Durham, N.C.) is a newly formulated anaerobic medium that contains activated charcoal and other ingredients designed to improve the detection of microorganisms from the blood of patients suspected of clinical sepsis. In contrast to the original BacT/ALERT anaerobic FAN medium (FAN) (10), the new FN formulation has had much of the brain heart infusion solids in FAN replaced with tryptic soy broth (Table 1). Additionally, the Ecosorb, which contained a combination of activated charcoal and fuller's earth, in FAN was replaced in FN with activated charcoal at a slightly increased concentration. Finally, the redox potential of FN medium was reduced to provide a better environment for strictly anaerobic microorganisms and to make it a more complementary medium to the BacT/ALERT aerobic FA (FA) formulation. There have been no controlled clinical comparisons of FN with the present BacT/ALERT standard anaerobic medium (SN), which differs from FN mainly in the presence of activated charcoal in FN (Table 1). Therefore, we compared FN and SN anaerobic bottles as a companion bottle to FA for detection of bacteremia and fungemia in adult patients at two university hospitals.

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MATERIALS AND METHODS

Blood culture and collection. Blood cultures were collected from adult patients hospitalized at Duke University Medical Center and Robert Wood Johnson University Hospital between September 2000 and May 2001. Institutional Review Board approval was obtained before the study, and all blood cultures were performed as part of routine patient care. Venipuncture sites were disinfected with alcohol followed by povidone iodine or 2% iodine tincture and allowed to dry. Up to 30 ml of blood was obtained with a sterile needle and syringe. Needles were not changed before or between inoculations of blood culture bottles. Ten milliliters of blood was placed into each of three blood culture bottles: an FA aerobic bottle, an FN anaerobic bottle, and an SN anaerobic bottle.

Adequacy of blood volume. Upon receipt in the laboratory, the volume of fluid in each bottle was measured against a volume standard to determine how many milliliters of blood had been inoculated into each of the bottles. All bottles were processed regardless of the volume of blood received. Only bottle sets containing 8 to 12 ml of blood were included in the data analysis.

Bottle processing. Bottles from each culture set were placed in the BacT/ALERT instrument and incubated for 5 days or until they signaled positive. Bottles flagged by the instrument as positive were removed, and an aliquot of the blood broth mixture was removed from the bottle with a sterile needle and syringe. A portion was used for a Gram stain, and the remainder was subcultured onto solid plate medium according to the results of the Gram stain. Subsequent microbial isolation, identification, and antimicrobial susceptibility testing were

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TABLE 1. Comparison of medium formulations for BacT/ALERT FAN, FN, and SN anaerobic media

Feature	Anaerobic FAN	FN	SN
Volume (mL)	40	40	40
Tryptic soy broth (%, wt/vol)		2.0	
Pancreatic digest of casein (%, wt/vol)			1.70
Brain heart infusion solids (%, wt/vol)	1.5	0.1	
Papaic digest of soybean meal (%, wt/vol)			0.3
Sodium polyanetholesulfonate (%, wt/vol)	0.05	0.044	0.035
Pyridoxine HCl (%, wt/vol)	0.001	0.001	
Menadione (%, wt/vol)	0.00005	0.00005	0.00005
Hemin (%, wt/vol)	0.0005	0.0005	0.0005
Activated charcoal (%, wt/vol)		8.5	
Ecosorb (%, wt/vol)	7.0		
L-Cysteine and other complex amino acid and carbohydrate substrates in purified water	As needed	As needed	As needed
Atmosphere	Carbon dioxide and nitrogen under vacuum	Nitrogen under vacuum	Carbon dioxide and nitrogen under vacuum
Other proprietary adjustments	As needed	As needed	As needed

performed according to standard techniques (5). Gram stain-negative bottles were returned to the instrument for the remainder of the 5-day incubation period or until reflagged by the instrument. These Gram stain-negative bottles that were flagged by the instrument were considered false-positive bottles if no microorganisms were isolated on subculture. Negative companion bottles from positive sets were subcultured at the end of the 5-day protocol. Bottles that were instrument negative but grew a microorganism on subculture were considered false-negative bottles.

Clinical assessment. Each positive culture was reviewed by one of the physician investigators and coded as a true positive, a contaminant, or an isolate of unknown clinical importance. These assessments were made in accord with published criteria (7). True positives were defined as microorganisms that are considered pathogens when isolated from patients with signs and symptoms of disease or potential pathogens that were isolated from multiple cultures within a 48-h period. Contaminants were defined as single positive cultures for a microorganism usually considered a contaminant in the absence of a plausible source (e.g., coagulase-negative staphylococci from a febrile patient without a central venous catheter), single positive cultures for a microorganism usually considered a contaminant when there was a plausible source (e.g., central venous catheter) but the patient was clinically well (surveillance cultures), or single positive cultures for a microorganism usually considered a contaminant when several others drawn within the same time frame were negative. Isolates of unknown significance were defined as single cultures for a potential pathogen (e.g., coagulase-negative staphylococci, viridans streptococci, or enterococci) or a usual contaminant (e.g., *Bacillus* spp., diphtheroids, *Lactobacillus* spp., or *Micrococcus* spp.) in a symptomatic patient who had a plausible source but for whom only one culture was submitted to the laboratory.

An episode of bacteremia or fungemia was defined as a period beginning with the first positive blood culture and ending when 7 days (2 days for coagulase-negative staphylococci) had passed without another positive blood culture with the same microorganism, regardless of whether negative cultures were done in the intervening days (7). When a different clinically significant isolate was detected within 3 days of the first isolate, the episode was considered polymicrobial. Patients were considered to be on effective therapy if the antimicrobial agent given at the time that the blood culture was drawn was either known or presumed (based on usual in vitro susceptibility patterns if testing was not routinely done) to inhibit the microorganism isolated.

Data analysis. Comparison of recovery rates from the bottles was done with the chi-square test of McNemar (3). Yates' correction was used when n was less than 20. Comparison of times to positivity between bottles was performed only where both bottles were positive within 72 h.

RESULTS

A total of 11,498 blood cultures were processed, of which 8,569 sets (75%) contained an adequate volume of blood in both anaerobic bottles. All three bottles were adequately filled in 7,945 sets (69%), and 1,238 (15.6%) were positive with one or more isolates. This included 747 (9.4%) cultures with clinically significant isolates and 415 (5.2%) with one or more contaminants. The remaining 76 cultures contained isolates of unknown significance. There were 686 isolates classified as clinically significant that were detected in one or both adequately filled anaerobic bottles (Table 2).

Only 17 (2.5%) of the 686 isolates were anaerobes. Isolates of *Staphylococcus aureus* ($P < 0.001$), members of the family *Enterobacteriaceae* ($P < 0.001$), and all microorganisms combined ($P < 0.001$) were detected more often in the FN bottle (Table 2). However, more isolates of nonenteric gram-negative

TABLE 2. Comparative yield of clinically important isolates in FN versus SN anaerobic blood culture bottles

Microorganism	No. of isolates detected by:			*P* value
	Both bottles	FN only	SN only	
Gram-positive cocci				
Staphylococcus aureus	113	173	12	<0.001
Coagulase-negative staphylococci	65	33	22	NS[a]
Streptococcus spp.[b]	14	2	5	NS
Enterococcus spp.[c]	51	16	9	NS
Gram-positive bacilli[d]	1	1	0	NS
Gram-negative bacilli				
Enterobacteriaceae[e]	65	37	11	<0.001
Other gram-negative bacilli[f]	5	1	13	<0.005
Anaerobic bacteria[g]	5	9	3	NS
Yeasts[h]	0	1	19	<0.001
All microorganisms	319	273	94	<0.001

[a] NS, not significant ($P > 0.05$).

[b] Includes eight *viridans* group *Streptococcus*, seven *Streptococcus pneumoniae*, two *Streptococcus agalactiae* and four *Streptococcus pyogenes* isolates.

[c] Includes 53 isolates of *Enterococcus faecalis*, 22 of *Enterococcus faecium*, and 1 of *Enterococcus* sp.

[d] Includes one *Bacillus cereus* and one *Bacillus* sp. isolate.

[e] Includes 34 *Klebsiella pneumoniae*, 29 *Escherichia coli*, 16 *E. cloacae*, 7 *Salmonella* sp., 6 *Klebsiella oxytoca*, 5 *Enterobacter aerogenes*, 5 *Serratia marcescens*, 3 *Proteus mirabilis*, 2 *Citrobacter freundii*, and 2 *Serratia liquefaciens* isolates and 1 isolate each of *Enterobacter americana*, *Morganella morganii*, *Proteus rettgeri*, and *Providencia stuartii*.

[f] Includes 15 isolates of *P. aeruginosa* and 1 isolate each of *A. baumanii*, *Oligella* sp., *Haemophilus influenzae*, and *P. oryzihabitans*.

[g] Includes four isolates of *Bacteroides fragilis*; four of *Bacteroides thetaiotaomicron*; three of *Clostridium clostridioforme*; two of the *Bacteroides fragilis* group; and one each of *Bacteroides caccae*, *Bacteroides distasonis*, *Clostridium difficile*, and *Clostridium ramosum*.

[h] Includes five of *Candida albicans*, five of *C. parapsilosis*, three of *Candida tropicalis*, three of *Candida glabrata*, two of *Candida* sp. and one each of *Candida lusitaniae* and *Malassezia furfur*.

TABLE 3. Comparative yield of clinically important isolates in FN versus SN anaerobic blood culture bottles from patients on antimicrobial therapy

Microorganism	No. of isolates detected by:			*P* value
	Both bottles	FN only	SN only	
Gram-positive bacteria				
Staphylococcus aureus	72	140	6	<0.001
Coagulase-negative staphylococci	45	24	16	NS[a]
Streptococcus spp.[b]	8	2	2	NS
Enterococcus spp.[c]	38	13	7	NS
Bacillus sp.	0	1	0	NS
Gram-negative bacilli				
Enterobacteriaceae[d]	35	25	7	<0.005
Other gram-negative bacilli[e]	4	1	8	<0.05
Anaerobic bacteria[f]	2	7	2	NS
Yeasts[g]	0	0	6	<0.05
All microorganisms	204	213	54	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Includes five isolates of *Streptococcus pneumoniae*, four of *Streptococcus pyogenes*, two of viridans group streptococci, and one of *Streptococcus agalactiae*.
[c] Includes 47 of *Enterococcus faecalis*, 10 of *Enterococcus faecium*, and 1 of *Enterococcus* sp.
[d] Includes 23 of *Klebsiella pneumoniae*; 16 of *Escherichia coli*; 8 of *E. cloacae*; 7 of *Salmonella* spp.; 3 of *Enterobacter aerogenes*; 3 of *Klebsiella oxytoca*; 3 of *Serratia marcescens*; and 1 each of *Ewingella americana*, *Morganella morganii*, *Providencia stuartii*, and *Serratia liquefaciens*.
[e] Includes 10 of *P. aeruginosa*, 1 of *A. baumannii*, 1 of *Oligella* sp., and 1 of *Haemophilus influenzae*.
[f] Includes three of *Bacteroides fragilis*; three of *Clostridium clostridioforme*; two of the *Bacteroides fragilis* group; and one each of *Clostridium difficile*, *Clostridium ramosum*, and *Bacteroides distasonis*.
[g] Includes two of *Candida glabrata*, two of *C. parapsilosis*, and one each of *Candida albicans* and *Candida tropicalis*.

bacteria ($P < 0.005$) and yeasts ($P < 0.001$) were detected in the SN bottle. These differences were similar for patients who were on antimicrobial therapy at the time of blood culture collection (Table 3). However, for patients who were not on antimicrobial therapy, only greater numbers of both *S. aureus* isolates in the FN bottle and yeasts in the SN bottle remained statistically different (Table 4).

When cultures positive in the FA bottle were omitted from the analysis (data not shown), more *S. aureus* ($P < 0.001$) and *Enterobacteriaceae* ($P < 0.005$) isolates were still detected more frequently in FN bottles, whereas there were no significant differences for *Pseudomonas aeruginosa* and yeasts, which were found as expected in FA bottles.

When analyzed by septic episode, *S. aureus*, *Enterobacteriaceae*, and all microorganisms combined were found more frequently in FN blood culture sets than in SN (Table 5).

When positive blood cultures were detected in both bottles within 72 h, which included 305 of 316 (96.5%) comparisons, the mean time to detection was 16.8 h in the FN bottle and 18.2 h in the SN bottle (Table 6).

Of the 8,569 paired anaerobic blood culture bottles, false-positive bottles were seen more often with SN (41, 0.5%) than with FN (23, 0.3%).

There were 31 (14 from FN and 17 from SN) clinically significant isolates detected when instrument-negative companion bottles from positive sets (false negative) were subcultured. Subcultures from FN detected *Chryseobacterium meningosepticum* (1 isolate) and *P. aeruginosa* (13 isolates). The isolates detected in subcultures from SN bottles were *S. aureus* (one isolate), coagulase-negative staphylococci (two isolates), *Enterobacter cloacae* (one isolate), *Acinetobacter baumannii* (two isolates), *Burkholderia cepacia* (two isolates), *C. meningosepticum* (one isolate), *P. aeruginosa* (three isolates), *Pseudomonas oryzihabitans* (two isolates), *Stenotrophomonas maltophilia* (one isolate), and *Candida parapsilosis* (two isolates). All false-negative isolates from FN or SN bottles were detected in the companion FA bottle.

Microorganisms determined to be contaminants (primarily coagulase-negative staphylococci) were isolated more frequently from FN than from SN (both bottles = 71, FN bottle only = 136, SN bottle only = 71; $P < 0.001$).

DISCUSSION

Although the routine use of anaerobic blood culture media has been questioned (4), many laboratories continue to use a combination of an aerobic and an anaerobic medium in their routine blood culture system. The addition of an anaerobic medium, however, results in the culturing of a larger volume of blood and provides a milieu that facilitates the growth of facultative microorganisms such as staphylococci as well as the growth of anaerobes. For laboratories using an anaerobic bottle, the question becomes which anaerobic bottle is preferable when the blood culture system used offers more than one anaerobic medium.

The original anaerobic FAN medium for the BacT/ALERT system was formulated to improve the recovery of microorganisms over that with the anaerobic standard medium when inoculated with blood from adult patients. The overall improved

TABLE 4. Comparative yield of clinically important isolates in FN versus SN anaerobic blood culture bottles from patients not on antimicrobial therapy

Microorganism	No. of isolates detected by:			*P* value
	Both bottles	FN only	SN only	
Gram-positive bacteria				
Staphylococcus aureus	35	26	6	<0.001
Coagulase-negative staphylococci	19	8	6	NS[a]
Streptococcus spp.[b]	6	0	3	NS
Enterococcus spp.[c]	13	3	2	NS
Bacillus cereus	1	0	0	NS
Gram-negative bacilli				
Enterobacteriaceae[d]	28	9	4	NS
Other gram-negative bacilli[e]	1	0	5	NS
Anaerobic bacteria[f]	3	1	1	NS
Yeasts[g]	0	1	12	<0.01
All microorganisms	106	48	39	NS

[a] NS, not significant ($P > 0.05$).
[b] Includes six isolates of viridans streptococci, two of *Streptococcus pneumoniae*, and one of group B streptococcus.
[c] Includes 12 of *Enterococcus faecium* and 6 of *Enterococcus faecalis*.
[d] Includes 12 of *Escherichia coli*; 10 of *Klebsiella pneumoniae*; 7 of *E. cloacae*; 3 of *Klebsiella oxytoca*; 3 of *Proteus mirabilis*; 2 of *Enterobacter aerogenes*; and 1 each of *Citrobacter freundii*, *Providencia rettgeri*, *Serratia liquefaciens*, and *S. marcescens*.
[e] Includes five of *P. aeruginosa* and one of *P. oryzihabitans*.
[f] Includes three of the *Bacteroides thetaiotaomicron/ovatus* group, one of *Bacteroides fragilis*, and one of *Bacteroides caccae*.
[g] Includes four of *Candida albicans*; three of *C. parapsilosis*; two of *Candida tropicalis*; and one each of *Candida glabrata*, *Candida lusitaniae*, *Candida* spp., and *Malassezia furfur*.

TABLE 5. Comparative yield of clinically important unimicrobial episodes of bacteremia and fungemia by FN versus SN anaerobic blood culture bottles paired with an FA bottle

Microorganism	No. of isolates detected by:			*P* value
	Both sets	FA-FN only	FA-SN only	
Gram-positive bacteria				
Staphylococcus aureus	109	21	3	<0.001
Coagulase-negative staphylococci	55	1	0	NS[a]
Streptococcus spp.[b]	11	1	1	NS
Enterococcus spp.[c]	32	1	0	NS
Bacillus sp.	0	1	0	NS
Gram-negative bacilli				
Enterobacteriaceae[d]	55	8	0	<0.025
Other gram-negative bacilli[e]	10	0	2	NS
Anaerobic bacteria[f]	5	5	0	NS
Yeasts[g]	16	0	1	NS
All microorganisms	293	38	7	<0.001

[a] NS, not significant ($P > 0.05$).
[b] Includes six isolates of viridans group streptococci, five of *Streptococcus pneumoniae*, and two of *Streptococcus pyogenes*.
[c] Includes 20 of *Enterococcus faecalis* and 13 of *Enterococcus faecium*.
[d] Includes 21 of *Escherichia coli*; 15 of *Klebsiella pneumoniae*; 11 of *E. cloacae*; 4 of *Serratia marcescens*; 3 of *Enterobacter aerogenes*; 3 of *Klebsiella oxytoca*; 2 of *Proteus mirabilis*; and 1 each of *Ewingella americana*, *Morganella morganii*, *Serratia liquefaciens*, and *Salmonella* sp.
[e] Includes nine of *P. aeruginosa* and one each of *A. baumannii*, *Oligella* sp., and *Haemophilus influenzae*.
[f] Includes three of *Bacteroides fragilis*, two of the *Bacteroides fragilis* group, and one each of *Clostridium clostridioforme*, *Clostridium ramosum*, *Bacteroides caccae*, *Bacteroides distasonsis*, and *Bacteroides thetaiotaomicron/ovatus*.
[g] Includes five of *Candida albicans*, five of *Candida glabrata*, three of *C. parapsilosis*, three of *Candida tropicalis*, and one of *Candida lusitaniae*.

performance of the anaerobic FAN versus the standard medium was shown in a controlled multicenter clinical comparison by our group in an earlier publication (10). The study presented herein showed that the new FN formulation also outperformed the present SN medium. Both the anaerobic FAN and FN showed improved isolation of staphylococci, *Enterobacteriaceae*, and all microorganisms overall compared with standard media; however, the new FN medium also showed improved performance for recovery of anaerobic bacteria. In both the earlier and present studies the standard anaerobic media gave higher yields for nonfermenters and yeasts. However, in the present study when the yields from the combined FA-FN set and from FA-SN were considered, these differences were no longer present for either nonfermenters or yeasts. This is to be expected, since these microorganisms are recovered optimally from an aerobic medium.

TABLE 6. Comparative time to positivity in FN versus SN blood culture bottles detected within 72 h

Microorganism	No.	Mean time (h)	
		FN	SN
Gram-positive bacteria			
Staphylococcus aureus	105	16.5	19.9
Coagulase-negative staphylococci	64	22.0	23.6
Streptococcus and *Enterococcus* spp.	65	14.2	13.8
Gram-negative bacilli			
Enterobacteriaceae	63	14.3	13.2
All microorganisms[a]	305	16.8	18.2

[a] Includes four isolates of *P. aeruginosa*; two of *Bacteroides fragilis*, one of *Haemophilus influenzae*, and one of *Bacillus* sp.

In our earlier study (10), the mean time to detection of positive culture results was delayed in FAN by almost 2 h compared with the standard anaerobic medium, whereas the present FN medium detected microorganisms overall sooner by a mean of 1.4 h than did the standard anaerobic medium. This difference was most marked for isolates of *S. aureus*, which were detected a mean of 3.4 h sooner. In both studies the same criterion of comparing positives in the first 72 h (more than 95% of comparisons) was used in order to avoid the bias of outliers.

Analysis of septic episodes in controlled clinical comparisons of blood culture media reduces potential bias from multiple positive cultures on an individual patient that consistently favors one bottle or the other. Both the earlier anaerobic FAN (10) study and the present FN study showed that more episodes of bacteremia were detected when either the anaerobic FAN or FN bottle was used than when standard medium was used. In addition, in the present study, we evaluated the detection of septic episodes by comparing results from FA-FN and from FA-SN sets, which also showed the superiority of the FN to the SN. These results are consistent with the improved recovery of charcoal-containing media that was shown to have clinical importance by McDonald et al. (2).

There were fewer false positives with FN than were previously found with anaerobic FAN bottles (10), which suggests that medium modifications or instrument algorithms have been modified successfully by the BacT/ALERT system to minimize this problem. Moreover, in the present study, the FN bottle showed fewer false-positive results and had fewer false-negative results than did the SN bottle. False-negative FN bottles grew primarily *P. aeruginosa*, which is known to grow poorly, if at all, in anaerobic media and is recovered most often from aerobic bottles. In contrast, isolates from false-negative SN bottles represented a wide range of species.

Isolates determined to be contaminants were found significantly more frequently in the FN medium. This was not seen in our previous evaluation of the anaerobic FAN medium (10) but was noted in earlier studies of the aerobic FAN medium (6). The enhanced detection of positive blood cultures, especially with staphylococci, in both charcoal-containing (1, 6, 10) and resin-containing (8, 9) media includes not only clinically important isolates but also contaminants (2). Thus, clinical microbiologists must weigh both the benefits and limitations of various blood culture medium formulations for continuously monitored instruments when selecting a blood culture system for routine use.

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