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 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.

Adquacy 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 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.

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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 physicians, 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 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).

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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of isolates detected:</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both bottles</td>
<td>FN only</td>
</tr>
<tr>
<td>Gram-positive cocci</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>113</td>
<td>173</td>
</tr>
<tr>
<td>Coagulase-negative <em>staphylococci</em></td>
<td>65</td>
<td>53</td>
</tr>
<tr>
<td><em>Streptococcus</em> spp.</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp.</td>
<td>51</td>
<td>16</td>
</tr>
<tr>
<td>Gram-positive bacilli*</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative bacilli</td>
<td>65</td>
<td>37</td>
</tr>
<tr>
<td>Other gram-negative bacilli*</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Anaerobic bacteria*</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Yeasts*</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>All microorganisms</td>
<td>319</td>
<td>273</td>
</tr>
</tbody>
</table>

*NS, not significant (P > 0.05).
* Includes eight *viridans* group *Streptococcus*, seven *Streptococcus pneumoniae*, two *Streptococcus agalactiae* and four *Streptococcus pyogenes* isolates.
* Includes 53 isolates of *Enterococcus faecalis*, 22 of *Enterococcus faecium*, and 1 of *Enterococcus sp.*
* Includes one *Bacteroides caccae* and one *Bacillus cereus* isolate.
* Includes 34 Klebsiella pneumoniae, 29 Escherichia coli, 16 E. cloacae, 7 Salmonella spp., 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 aerogenes*, *Morganella morgani*, *Proteus retgeri*, and *Providencia stuartii*.
* Includes 15 isolates of *P. aeruginosa* and 1 isolate each of *A. baumannii*, *Oligella sp.*, *Haemophilus influenzae*, and *P. oxyrhinum*.
* 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*.
* 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*.
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 at the time of blood culture, the question becomes which anaerobic bottle is preferable when the blood culture system used offers more than one anaerobic medium.

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 Chrysobacterium 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

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of isolates detected by:</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Both bottles</td>
<td>FN only</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Yeasts</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>All microorganisms</td>
<td>106</td>
<td>48</td>
</tr>
</tbody>
</table>

* NS, not significant (P > 0.05).

* Includes six isolates of viridans streptococci, two of Streptococcus pneumoniae, and one of group B streptococci.

* Includes 12 of Enterococcus faecalis and 6 of Enterococcus faecium.

* Includes five of P. aeruginosa and one of P. oryzihabitans.

* Includes three of the Bacteroides thetaiotaomicron/violacein group, one of Bacteroides fragilis, and one of Bacteroides caccae.

* 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.
is to be expected, since these microorganisms are recovered optimally from an aerobic medium.

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.

**ACKNOWLEDGMENTS**

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