

Controlled Comparative Evaluation of BacT/Alert FAN and ESP 80A Aerobic Media as Means for Detecting Bacteremia and Fungemia

GARY V. DOERN,* ANN BARTON, AND SUDAH RAO

University of Massachusetts Medical Center, Worcester, Massachusetts 01655

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During a one-year period, a total of 6,305 blood cultures were processed in a tertiary-care teaching hospital; 6 to 12 ml of blood was inoculated into both a BacT/Alert Fan aerobic bottle and an ESP 80A aerobic bottle. The FAN aerobic bottle contains an antimicrobial-absorbing material; the 80A aerobic bottle does not. Bottles were processed on their respective continuous-monitoring blood culture instruments for up to five days of incubation. Four hundred thirty-three cultures (6.9%) representing 301 septic episodes in 235 different patients yielded 490 bacteria or yeasts thought to be clinically significant. Two hundred seventy-five of the 433 presumed clinically significant positive cultures (63.5%) representing 195 septic episodes and yielding 301 isolates were positive in both FAN and 80A bottles. One hundred nine significant positive cultures (25.2%) (i.e., cultures positive with an organism judged to be of probable clinical significance) from 70 septic episodes yielded 126 isolates only in FAN bottles. Conversely, the 80A bottle was exclusively positive in 49 instances (11.3%), representing 36 septic episodes and yielding 63 isolates. The higher rates of significant positive blood cultures, numbers of septic episodes documented, and numbers of isolates recovered in FAN bottles versus 80A bottles were all statistically significant ($P < 0.05$). Enhanced rates of detection of presumed clinically significant isolates in FAN bottles were largely accounted for by *Staphylococcus aureus*, members of the *Enterobacteriaceae*, and non-*Pseudomonas aeruginosa* miscellaneous gram-negative bacilli from patients receiving antimicrobial therapy at the time blood cultures were obtained. Enhanced recovery of one organism group, the β -hemolytic streptococci, occurred in 80A. With one exception, detection times were essentially equivalent in the two systems. The single exception pertained to streptococci and enterococci, which were recovered significantly faster in 80A bottles. Three hundred thirty-eight of the 6,305 blood cultures evaluated in this study (5.4%) were judged likely to be contaminated. The percentages of probable contaminated cultures were as follows: 26.6% FAN and 80A; 42.3% FAN only; 31.1% 80A only ($P < 0.05$). Finally, the instrument false-positive rates for the two systems were 0.7% with FAN and 3.0% with 80A ($P < 0.05$). We conclude that while contamination rates were slightly higher with FAN than with 80A, use of FAN aerobic bottles in conjunction with the BacT/Alert system will yield significantly higher numbers of clinically significant blood culture isolates than 80A bottles and the ESP system. Furthermore, this enhanced detection is most conspicuous in patients receiving antimicrobial therapy at the time blood cultures are performed, probably due to the presence of an antimicrobial-absorbing material in FAN aerobic bottles.

Three instrument-based continuous monitoring blood culture systems have been introduced for use in the United States: the BacT/Alert system (Organon Teknika, Durham, N.C.), the BacTec 9240 System (Becton Dickinson Microbiology Systems, Cockeysville, Md.), and the ESP System (Accumed Diagnostics, Cleveland, Ohio). Detection of bacteremia and fungemia by use of a continuous-monitoring blood culture device is commonly exploited in clinical microbiology laboratories. Each of the three systems noted above has been evaluated extensively, often in controlled clinical trials, and the results have been published in the literature (2, 5, 7–9, 11–17, 19, 20–25). In general, it can be said that continuous-monitoring blood culture systems afford more rapid detection of bacteremia and possibly fungemia than is possible with non-instrument-based manual methods (2, 8, 9, 11, 12, 15, 17, 19). In addition, differences in detection sensitivity have been noted when these systems have been compared with each other (5, 7, 13, 16, 21, 22, 25).

Recently, a new medium, FAN, has been introduced in both

aerobic and anaerobic formulations for use with the BacT/Alert system (4, 20, 24). FAN medium contains adsorbent material referred to as Ecosorb (i.e., Fuller's earth plus charcoal), which binds antimicrobial agents, thus facilitating detection of bacteremia and fungemia in patients receiving antimicrobial therapy at the time blood cultures are performed. FAN media and other resin-based antimicrobial-binding blood culture systems have previously been shown in several investigations to accomplish such enhanced detection (1, 3, 12, 15, 20). A second notable feature of FAN bottles is their ability to accommodate 10-ml volumes of blood despite containing only 40 ml of broth medium.

The intent of the current investigation was to compare rates of recovery and detection times in FAN aerobic medium processed with the BacT/Alert system with those obtained in a second, high-volume aerobic medium, 80A, processed with the ESP continuous-monitoring blood culture system. The 80A bottles contain 80 ml of broth medium and rely on dilution as a means of minimizing antibiotic suppression of blood culture growth.

(Preliminary results of this investigation were presented at the 97th Annual Meeting of the American Society for Microbiology held in New Orleans [4].)

* Corresponding author. Present address: Department of Pathology, C606 GH, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 356-8616. Fax: (319) 356-4916.

MATERIALS AND METHODS

Specimen collection. This study was conducted between November 1995 and August 1996. An aliquot of ca. 20 ml of blood per culture was routinely collected by house officers, nurses, or phlebotomists following preparation of the venipuncture site with 70% isopropyl alcohol and 10% povidone iodine. By aseptic technique, equal aliquots of the blood specimen were inoculated immediately into both a BacT/Alert aerobic bottle containing 30 ml of FAN medium and an ESP 80A aerobic bottle containing 80 ml of tryptic soy-based medium. Both bottles are designed to accommodate at least 10 ml of blood. In selected cases, an additional 10-ml aliquot of blood was obtained and a third blood culture bottle, containing ESP 80N anaerobic medium, was inoculated. This investigation, however, was restricted to a comparison of FAN versus 80A. Blood culture bottles were transported to the laboratory within 1 h of specimen collection. Review of computer records of patient medication(s) was used to determine if patients were receiving antimicrobial therapy at the time blood cultures were drawn.

Processing in the laboratory. Upon receipt in the laboratory, between the hours of 7:00 a.m. and 12:30 a.m., blood culture bottles were processed immediately; between the hours of 12:30 a.m. and 7:00 a.m., bottles received in the laboratory were incubated at 35°C in ambient air, and these were batch processed between 7:00 a.m. and 7:30 a.m. To ensure that adequate volumes of blood had been cultured, the weights of all bottles were determined and compared with those of uninoculated bottles. Only culture sets with evidence of an inoculum of 6 to 12 ml of blood in both FAN and 80A were considered evaluable and were included in the analysis presented here.

FAN bottles were processed with the BacT/Alert blood culture instrument; 80A bottles (and 80N anaerobic bottles) were processed on the ESP machine. Both systems were used explicitly as instructed by the manufacturer. Cultures were incubated for a total of five complete days prior to being discarded as negative. Length of time to detection was defined as the length of time elapsed between placement of bottles on the instruments and the first signal indication of positivity.

When a bottle signaled positive, it was removed from the instrument and a Gram stain and subcultures were performed. If the Gram stain result was negative, the bottle was placed back on the instrument for further incubation. If the subcultures of smear-negative bottles yielded growth, they were then removed from the instrument. If the subcultures remained negative after 72 h of incubation, the bottles were retained on the instrument through the end of their 5-day incubation cycle; if they were still negative at that time, they were judged to have been an instrument false-positive. When only one bottle of a FAN-80A pair signaled positive, the companion bottle was retained on the instrument until it too signaled positive, at which point it was processed as described above. If the companion bottle remained negative throughout its 5-day incubation cycle, a blind subculture was performed on day 5 with subculture plates incubated for 72 h prior to being discarded as negative. In no case did such a blind subculture turn positive.

Organisms were identified by standard methods. Statistical analysis was performed with the McNemar test with correction for continuity and without adjustment for multiple tests (10). All positive cultures were reviewed and an assignment of clinical significance versus probable contamination was made according to the criteria of Weinstein et al. (18). Under circumstances in which the clinical significance of an isolate was uncertain on the basis of the criteria, chart review and discussions with the patient's primary clinicians were undertaken. Organisms deemed not to be the etiologic agent of sepsis in an individual patient were classified as probable contaminants.

RESULTS

A total of 13,640 blood cultures were performed during the one-year period of this study. In 9,131 cases, both a FAN bottle and an 80A bottle were inoculated. In 6,305 instances, both FAN and 80A bottles were judged to have been inoculated with adequate volumes of blood (i.e., 6 to 12 ml) and thus were included in the analysis that follows. These 6,305 blood cultures were used to evaluate 3,002 presumed septic episodes in 2,612 patients.

A total of 433 of these 6,305 cultures (6.9%) were positive with an organism(s) judged to be of probable clinical significance (significant positive cultures). These 433 presumed significant positive blood cultures yielded a total of 490 bacteria or yeasts and had been obtained during the evaluation of 301 septic episodes in 235 patients. Two hundred seventy-five of the 433 positive blood cultures (63.5%), representing 195 septic episodes, yielding a total of 301 isolates, were positive in both FAN and 80A. One hundred nine of the significant positive blood cultures (25.2%) representing 70 septic episodes

TABLE 1. Comparison of numbers of presumed clinically significant bacteria and yeasts recovered in FAN and 80A blood culture bottles

Taxon	No. of isolates recovered by:			P value
	FAN and 80A	FAN only	80A only	
<i>Staphylococcus aureus</i>	44	36	4	<0.005
Non- <i>S. aureus</i> staphylococci	69	23	16	0.34
<i>Streptococcus pneumoniae</i>	6	2	2	0.63
Viridans group streptococci	10	2	1	1.00
β -Hemolytic streptococci ^a	4	0	5	0.07
Enterococci	28	14	9	0.40
<i>Corynebacterium</i> spp.	4	0	0	
<i>Escherichia coli</i>	21	8	3	0.23
<i>Klebsiella</i> spp. ^b	29	9	1	0.03
<i>Enterobacter</i> spp. ^c	17	8	2	0.11
<i>Serratia marcescens</i>	10	2	1	1.00
Other <i>Enterobacteriaceae</i> ^d	5	0	0	
<i>Pseudomonas aeruginosa</i>	15	3	7	0.34
<i>Acinetobacter</i> spp. ^e	10	1	0	1.00
Miscellaneous GNB ^f	6	5	0	0.07
Anaerobes ^g	3	1	2	1.00
Yeasts ^h	20	12	10	0.83
Totals	301	126	63	<0.005

^a Five group B, four group G β -hemolytic streptococci.

^b Thirty-five *K. pneumoniae* and four *K. oxytoca* isolates.

^c Seventeen *E. cloacae*, eight *E. sakazakii*, and two *E. aerogenes* isolates.

^d Three *Proteus mirabilis*, one *Morganella morganii*, and one *Citrobacter freundii* isolate.

^e Nine *A. baumannii* and two *A. lwofii* isolates.

^f Six *Pseudomonas*, two *Haemophilus influenzae*, one *Capnocytophaga*, and one *Stenotrophomonas maltophilia* isolate and one unidentified gram-negative bacillus (GNB).

^g Two *Bacteroides fragilis*, two *Clostridium perfringens*, one *Clostridium* (not *C. perfringens*) and one *Peptostreptococcus* isolate.

^h Twenty-two *Candida albicans*, seven *C. glabrata*, six *C. parapsilosis*, four *C. tropicalis*, and three *C. krusei* isolates.

were positive only in the FAN bottle. One hundred twenty-six organisms were recovered from these cultures. In contrast, 49 significant positive blood cultures (11.3%), representing 36 septic episodes, yielded 63 isolates and were positive exclusively in the 80A bottle of a pair. The higher rates of significant positive cultures and numbers of septic episodes documented in FAN bottles versus 80A bottles were statistically significant ($P < 0.05$).

A breakdown of isolation rates of individual organisms judged to be clinically significant is provided in Table 1. Significantly enhanced recovery was noted with *Staphylococcus aureus* and *Klebsiella* spp. in FAN bottles. In all other cases, the differences between recovery rates in FAN bottles versus 80A bottles were not statistically significant. With certain organism groups, however, despite the fact that differences were not statistically significant, there was a clear trend towards enhanced rates of recovery in FAN bottles (e.g., *Enterobacter* spp. and miscellaneous gram-negative bacilli) and in 80A bottles (e.g., β -hemolytic streptococci). When the total numbers of organisms recovered in these two bottles were compared, i.e., 427 in FAN versus 364 in 80A, the difference was highly statistically significant ($P < 0.05$).

A conspicuous difference between FAN bottles and 80A bottles is the presence in FAN of an antimicrobial-adsorbing material. In view of this difference, it was of interest to know if the enhanced rate of recovery noted in FAN was accounted for by patients receiving antimicrobial therapy at the time blood cultures were obtained. This appears to have been the case. The numbers of presumed clinically significant isolates in FAN

TABLE 2. Lengths of time to detection of bacteria and yeast in FAN and 80A blood culture bottles yielding a single, presumed clinically significant organism

Organism group	No. of isolates ^a	Mean length of time to detection (h):		P value
		FAN	80A	
Staphylococci	95	18.7	18.8	NS ^b
Streptococci-enterococci	25	30.3	17.2	<0.05
Enterobacteriaceae	54	14.8	16.4	NS
Miscellaneous gram-negative bacilli	16	21.0	22.3	NS
Yeasts	18	35.1	38.3	NS

^a This analysis was restricted to isolates recovered in both FAN and 80A bottles.

^b NS, not significant.

and 80A, FAN only, and 80A only in patients not receiving antibiotic therapy were 158, 35, and 23, respectively ($P = 0.15$); in patients receiving therapy, these values were 143, 91, and 40, respectively ($P < 0.05$).

Another measure of detection sensitivity with blood culture systems is length of time to positivity. In Table 2, the average length of time to an instrument signal of positivity is listed for clinically significant isolates recovered in both FAN and 80A. The only significant differences between FAN and 80A with respect to detection times occurred with streptococci and enterococci, in which case 80A bottles became positive significantly faster than FAN bottles.

Three hundred thirty-eight of the 6,305 blood cultures included in this survey (i.e., 5.4%) were judged to be probably contaminated. Of these 338 blood cultures, in 90 cases (26.6%) both FAN and 80A bottles were contaminated, in 143 cases (42.3%) only the FAN bottle was contaminated, and in the remaining 105 cases (31.1%) only the 80A bottle was contaminated ($P < 0.05$). A listing of probable contaminants is presented in Table 3. The difference between overall recovery rates of probable contaminants in FAN bottles versus 80A bottles was largely accounted for by the large numbers of non-*S. aureus* staphylococci that were recovered only in FAN bottles. For the 90 probable contaminants recovered in both FAN and 80A bottles, the mean length of time to detection in FAN bottles was 28.7 h and in 80A bottles was 29.6 h. This difference was not statistically significant.

During the course of this study, 46 FAN bottles signaled positive with the BacT/Alert instrument yet failed to yield an organism on subculture. The Gram stain results for these 46 bottles were also negative. These FAN cultures were considered false-positives (i.e., false-positive rate with FAN bottles, 0.7%). Conversely, 191 false-positive 80A cultures (3.0%) were identified. The difference between the false-positive rates with the two blood culture systems was highly significant ($P < 0.05$).

DISCUSSION

It is clear from this and other studies that no single blood culture system optimizes recovery of all organism groups. In the current study, the BacT/Alert continuous-monitoring blood culture system with FAN aerobic medium was clearly superior to the ESP system with standard 80A aerobic medium as a means for detecting bacteremia in a tertiary-care referral hospital. Significantly greater numbers of positive blood cultures with *S. aureus* and *Klebsiella* sp. bacilli were obtained with this system. Conspicuous trends towards higher rates of recov-

TABLE 3. Comparison of recovery rates of probable contaminants in FAN and 80A blood culture bottles

Taxon	No. of isolates recovered by:			P value
	FAN and 80A	FAN only	80A only	
Non- <i>S. aureus</i> staphylococci	75	132	91	0.01
<i>Micrococcus</i> spp.	0	5	1	0.22
<i>Staphylococcus aureus</i>	1	3	0	0.25
Viridans group streptococci	3	8	6	0.79
Enterococci	2	2	9	0.07
<i>Corynebacterium</i> spp.	2	6	12	0.24
<i>Bacillus</i> spp.	0	4	3	1.00
Enterobacteriaceae ^a	0	6	2	0.29
Other gram-negative bacilli ^b	4	3	4	1.00
<i>Moraxella-Neisseria</i>	1	4	0	0.13
Anaerobes ^c	1	3	3	0.68
Fungi ^d	1	1	3	0.62
Totals	90	177	134	0.02

^a Five *Escherichia coli*, one *Enterobacter cloacae*, one *Klebsiella pneumoniae*, and one *K. oxytoca* isolate.

^b Two *Acinetobacter*, one *Capnocytophaga*, one *Comamonas*, one *Eikenella corrodens*, one *Flavimonas*, one *Pseudomonas putida*, and one *Stenotrophomonas maltophilia* isolate and three miscellaneous gram-negative bacilli.

^c Four *Propionibacterium acnes*, one *Bacteroides fragilis* group, one *Clostridium perfringens*, and one *Peptostreptococcus* isolate.

^d Two *Candida albicans*, one *Alternaria*, one *Rhizopus*, and one *Penicillium* isolate.

ery in FAN (albeit lacking statistical significance) were noted with *Enterobacter* spp. and miscellaneous gram-negative bacilli. In contrast, enhanced detection of β -hemolytic streptococci was noted with the ESP system using 80A bottles.

The incremental increases in blood culture recovery rates noted with BacT/Alert were largely accounted for by patients receiving antimicrobial therapy at the time blood cultures were obtained. This is not surprising insofar as the FAN aerobic medium employed with the BacT/Alert system contains an antibiotic-neutralizing material that is not present in the ESP 80A blood culture bottles. These observations are similar to those of Welby-Sellenriek et al., who demonstrated similarly enhanced rates of detection of bacteremia in FAN aerobic bottles in comparison to ESP 80A bottles in a pediatric patient population (21).

Some controversy has existed as to the overall clinical value of using a blood culture system that attempts to diminish antibiotic effect as a means of facilitating detection of bacteremia (6). There is no doubt, however, that higher rates of bacteremia detection can be achieved by either pretreatment of blood specimens with a resin device or use of blood culture systems which employ bottles that contain either antimicrobial-binding resins or an absorbent material such as the Ecosorb that is present in aerobic FAN bottles (1, 3, 12, 15, 20). Two previous investigations that are particularly relevant to the current study compared the rates of detection of bacteremia and fungemia in FAN aerobic and anaerobic medium to those in standard O/T aerobic and anaerobic medium (20, 24). In both studies significantly enhanced detection rates were obtained by use of FAN media.

Length of time to detection is another important measure of the relative sensitivity of continuous-monitoring blood culture systems. Interestingly, in the current study, despite generally higher detection rates in FAN versus 80A, roughly comparable detection times were noted with the two systems. The only exception was significantly shorter times to detection of streptococci and enterococci observed in 80A bottles.

Overall rates of contamination in the current study were noted to be higher in FAN than in 80A. Non-*S. aureus* staphylococci, recovered exclusively in FAN, accounted for most of the difference in contamination rates seen between the two systems. It is possible that the use of what appears to be a more sensitive system for detecting clinically significant bacteremia, such as FAN, may also result in recovery of more contaminants.

Finally, one important consideration for laboratories which utilize continuous-monitoring blood culture systems is the frequency with which instruments generate false-positive signals. False-positive signals lead to needless expenditures of time, effort, and money in working up negative blood culture bottles. In the current study, the false-positive rates with the two systems that we evaluated were 0.7% (BacT/Alert-FAN) and 3.0% (ESP-80A). This difference in rates was statistically significant.

We conclude from the results of this study that the use of FAN aerobic bottles in conjunction with the BacT/Alert blood culture system generally provides higher recovery rates in a tertiary-care referral hospital laboratory than does the use of 80A bottles processed with the ESP system. In addition, fewer false-positive results are obtained. Use of the BacT/Alert system with FAN aerobic bottles is, however, associated with higher rates of contamination.

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