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Detection of Bacteremia by Difco ESP Blood Culture System

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In a multicenter study, the Difco ESP blood culture system (Difco Laboratories, Detroit, Mich.) was compared with the BACTEC NR660 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.). The ESP system monitors each blood culture bottle every 12 to 24 min to detect changes in oxygen consumption and gas production by microbes. Equal volumes of blood were inoculated into aerobic ESP-80A and BACTEC 6A, 16A, or PEDS Plus broths and anaerobic ESP-80N and BACTEC 7A or 17A broths and were incubated for up to 7 days. ESP bottles contain supplemented tryptic soy broth without antimicrobial agent-adsorbing resins. From 7,532 aerobic compliant sets, the ESP system detected 356 clinically significant positive cultures and the BACTEC NR660 system detected 329. From 6,007 anaerobic cultures, the ESP system detected 234 clinically significant positive cultures and the BACTEC NR660 system detected 198. In aerobic broths, 292 organisms were isolated from both systems and 78 organisms were isolated from the ESP system alone, whereas 54 organisms were isolated from the BACTEC NR660 system alone ($P < 0.05$). Among individual organisms, pneumococci were isolated significantly more often in ESP aerobic broths. In anaerobic broths, 180 organisms were isolated from both systems and 68 organisms were isolated from the ESP system alone, whereas 35 organisms were isolated from the BACTEC NR660 system alone ($P < 0.05$). Aerobic gram-positive organisms as a group and *Candida* spp. were isolated significantly more often in ESP anaerobic broths. Both systems detected 207 clinically significant bacteremic episodes and the ESP system alone detected 63, whereas the BACTEC NR660 system alone detected 32 ($P < 0.05$). Significantly more episodes of bacteremia caused by *Staphylococcus epidermidis* and anaerobes were detected by the ESP system. The differences in the numbers of organisms detected >6 h earlier in ESP broths compared with BACTEC NR660 broths were significant, as were earlier times to detection. Although the total number of organisms detected was not significantly different, the ESP system alone detected more organisms in a shorter time than did the BACTEC NR660 system alone. The continuous monitoring capability of the ESP system makes it an attractive alternative to the BACTEC NR660 system.

During the past 20 years, several systems for the rapid detection of nonviral blood-borne pathogens have been devised (2, 11). For three of these, BACTEC NR660 and BACTEC 9240 (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) and BacT/Alert (Organon Teknika Corporation, Durham, N.C.), culture bottles are monitored for positivity by instruments that detect the CO_2 released from the culture broth during organism growth and metabolism. A primary difference between these systems is the detection of CO_2 by infrared spectroscopy by the BACTEC NR660 system, fluorescent CO_2 sensors by the BACTEC 9240 system, and a colorimetric method by the BacT/Alert system. In addition, the BACTEC 9240 and BacT/Alert systems allow continuous monitoring of culture bottles (every 10 min), whereas the BACTEC NR660 system permits only one or two readings each day.

A new blood culture system, the ESP system, has been developed by Difco Laboratories (Detroit, Mich.). This system differs from the BACTEC and the BacT/Alert instruments because it detects the consumption and/or production of gases by microbes growing in the culture broth rather than the production of CO_2 only. Bottles are monitored by the instrument every 12 to 24 min. In a three-center study, we evaluated the performance of a prototype Difco ESP system in comparison with that of the BACTEC NR660 system to determine the recovery of significant organisms by each system and the times to detection of significant positive cultures.

(This work was presented in part at the 92nd General Meeting of the American Society for Microbiology, 26 to 30 May 1992, New Orleans, La. [4].)

MATERIALS AND METHODS

Specimens. A total of 6,007 blood specimens were drawn from adult patients in the following three hospital centers: the University of Chicago Medical Center (Chicago), Sparrow Hospital (Sparrow), and Rush-Presbyterian St. Luke's Medical Center (Rush). At Rush and Sparrow, aerobic and anaerobic culture bottles were inoculated directly at the patient's bedside with equal volumes of blood from a single venipuncture. The bottles were then sent to the laboratory for incubation in the ESP and BACTEC NR660 instruments. At Chicago, blood from adult patients was collected in Vacutainer tubes (Becton Dickinson Vacutainer Division, Cockeysville, Md.) containing 0.35% sodium polyanetholsulfonate. After transport to the laboratory, equal volumes of specimens were inoculated into culture bottles, which were then incubated in the two systems. In addition, 1,525 blood specimens were drawn from pediatric patients at Chicago. These were inoculated in equal amounts directly at the bedside into aerobic blood culture bottles.

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Throughout the study, the volume of blood inoculated into each bottle of a set varied between 0.5 and 5 ml. These volumes are within the ranges recommended by the manufacturers.

Broths. Specimens from adults were inoculated into an ESP-80A aerobic broth culture bottle and an ESP-80N anaerobic broth culture bottle. The companion bottles were BACTEC 6A (aerobic) and 7A (anaerobic) broths, except that at Chicago and Sparrow, BACTEC 16A (aerobic, resin) and BACTEC 17A (anaerobic, resin) broths were inoculated when the patient was known to be receiving antimicrobial agents. For specimens from pediatric patients, only two bottles were inoculated: ESP-80A and BACTEC PEDS Plus (aerobic, resin). The volume of broth in each culture bottle was 80 ml for ESP bottles and 30 ml for all BACTEC bottles except BACTEC PEDS Plus, which contained 20 ml. Thus, the dilution of blood in broth varied from 1:16 (5 ml of blood) to 1:160 (0.5 ml of blood) in ESP broths and from 1:6 to 1:40 in BACTEC broths.

ESP-80A broth is a modified tryptic soy formulation composed of soy-casein peptone A, yeast extract, sodium chloride, glucose, divalent salts, supplement O, and 0.006% sodium polyanetholsulfonate. This medium is designed to enhance the growth and the production and consumption of gases by most microorganisms. ESP-80N is a highly enriched peptone digest broth containing proteose peptone N, yeast extract, sodium chloride, polysorbate 80, glucose, supplement AN, trisodium citrate, hemin, cysteine, vitamin K_1, and resazurin. It is designed for enhanced growth and the detection of fastidious anaerobes, but it also supports the growth of facultative anaerobes and some strict aerobes, such as yeasts. Neither ESP-80 broth contains resins.

ESP system. The prototype system consisted of eight microprocessor-controlled modules, each of which permitted monitoring of 16 bottles (a total of 128 bottles could be monitored). Four modules were agitated continuously at 160 rpm to optimize the growth of aerobic organisms, whereas the other four modules were stationary and designed for the incubation of anaerobic culture bottles. The principle of the ESP system is the measurement of pressure changes in the headspace of the sealed bottles. After inoculation with patient blood, each culture bottle is fitted with a disposable connector whose recessed needle penetrates the septum of the bottle closure. The connector eliminates residual vacuum or pressure and connects the bottle to the sensing probe located at the top of each position into which a bottle is placed. A unique accession number used to identify each bottle is entered into a computer terminal which assigns a location for the bottle if the technologist has not already done so. Once the bottle is placed in the ESP instrument, the sensor continuously monitors changes in the headspace pressure and records datum points of millivolts versus time. Each aerobic bottle is monitored once every 12 min, and each anaerobic bottle is monitored once every 24 min. When changes indicating microbial growth are detected, red lights are illuminated at three locations: on the front of the module in which the bottle is located, at the bottle location, and at the outside top of the instrument. The information is also transferred to the computer that continuously monitors the units, generates patient reports, and can be used for data management. The status of each bottle can be determined at any time on the computer terminal by viewing a graph of activity reflecting gas consumption and evolution.

Protocol. (i) ESP system. When the bottles arrived in the laboratory, a connector was inserted into each bottle, the specimen information was entered into the ESP computer system, and the bottle was placed in the assigned location in the ESP instrument. If entry into the system was delayed for more than 4 h, the bottles were kept incubated at 35°C. When a positive culture was detected by the instrument, aliquots of broth from the bottle were Gram stained and subcultured onto appropriate media. If no organisms were seen on the smear, a new connector was placed on the bottle and the incubation was continued. Companion bottles in a set were not processed as positive unless they were also detected as positive by the instrument. Isolates were identified to the species level by using the standard methods of each laboratory. Negative cultures were incubated for up to 7 days, at which time all aerobic bottles received a terminal subculture; 10% of negative anaerobic cultures received a terminal subculture.

(ii) BACTEC system. Bottles were tested according to the manufacturer's recommendations and the individual laboratory protocol. Aerobic bottles were tested twice on days 1 and 2 and once on days 3 to 6 (Chicago, Rush) or day 7 (Sparrow). Anaerobic bottles were tested once on days 2 to 5 or 7 according to the individual laboratory protocol. Bottles were considered presumptively positive when they showed visual changes, when the growth value reached a predetermined threshold, or when the change in growth value exceeded a predetermined range. All negative aerobic bottles received a terminal subculture, as did 10% of negative anaerobic bottles. Positive cultures detected with the instrument were treated in the same manner as ESP bottles.

Criteria for clinical significance. The criteria for clinical significance included one or more of the following: (i) both bottles of a set were positive, (ii) two or more positive specimens were obtained from the same patient, (iii) organism identity (rarely a contaminant), (iv) physician consultation, and (v) chart review.

Statistical analysis. Isolation rates were evaluated by the McNemar modification of the chi-square test. A continuity correction was used for small sample sizes. Times to positivity were evaluated by the paired t test (1).

RESULTS

Organism recovery. Table 1 displays a summary of the data for all blood cultures and for positive cultures with monomicrobic and polymicrobic isolates. Three hundred forty-three of the clinically significant monomicrobic and 13 clinically significant polymicrobic isolates were detected in aerobic cultures by the ESP system and 312 and 17, respectively, were detected by the BACTEC NR660 system. In anaerobic broth culture bottles, the ESP system detected 223 clinically significant monomicrobic and 11 polymicrobic isolates; the BACTEC NR660 system detected 186 and 12, respectively. None of these differences was statistically significant. The false-positive and false-negative rates were significantly higher for the ESP system than for the BACTEC NR660 system (46 and 12 versus 37 and 18, respectively), although the numbers for both systems were small. The contamination rates for ESP and BACTEC NR660 aerobic broth culture bottles were 2.0 and 1.5%, respectively, and for anaerobic broths they were 1.2 and 0.5%, respectively.

Table 2 lists the organisms or organism groups recovered in the aerobic broth cultures by each system. Although the total numbers of organisms detected by each system were not significantly different, the number of organisms detected by the ESP system alone was significantly greater than the number detected by the BACTEC NR660 system alone ($P < 0.05$). Among individual organisms, pneumococci were detected significantly more often by the ESP system alone than by the BACTEC NR660 system alone.

Table 3 lists the organisms or organism groups recovered in

TABLE 1. Summary of culture results

Culture (no.)	No. of cultures			
	ESP system		BACTEC NR660 system	
	Total	Clinically significant	Total	Clinically significant
Aerobic (7,532)				
Positive	455	356	390	329
Monomicrobic	434	343	367	312
Polymicrobic	21	13	23	17
Negative	7,035		7,128	
False-positive[a]	30		7[b]	
False-negative[c]	12		7[b]	
Anaerobic (6,007)[d]				
Positive	279	234	213	198
Monomicrobic	268	223	199	186
Polymicrobic	11	11	14	12
Negative	5,687		5,778	
False-positive[a]	16		5[b]	
False-negative[c]	25		11[b]	

[a] A false-positive result in either system was defined as a specimen flagged as positive by the instrument but that had no visible signs of growth and negative subcultures.

[b] $P < 0.05$.

[c] A false-negative result in either system was defined as a specimen that had a positive subculture but that was not flagged as positive by the instrument.

[d] A total of 1,525 specimens from pediatric patients were cultured aerobically only.

the anaerobic broth cultures by each system. Again, the number of organisms detected by the ESP system only was greater than the number detected by the BACTEC NR660 system only ($P < 0.05$). Although no organism was detected significantly more often by either system, when considered as groups, aerobic gram-positive organisms and *Candida* spp. were detected significantly more often by the ESP system alone.

Table 4 lists the organisms isolated from patients with clinically significant monomicrobic bacteremic episodes. If multiple specimens from the same patient yielded the same organism within 2 weeks, they were considered to compose a single episode. Among individual organisms or groups of organisms, aerobic gram-positive bacteria, coagulase-negative staphylococci, and anaerobes were detected significantly more often by the ESP system only than by the BACTEC NR660 system only. In addition, when considering all patient episodes, more were detected by the ESP system only ($P < 0.05$).

Times to detection. Times to detection for both the ESP and BACTEC NR660 systems were calculated from the time that the bottles were entered into the blood culture instrument until a positive signal was obtained. With the ESP system, the actual detection times were available on a graph generated through the computer. Table 5 compares the detection times for organisms isolated in both the ESP and BACTEC aerobic broths. Most organisms or organism groups were detected >6 h earlier by the ESP system than by the BACTEC NR660 system. The number of organisms detected earlier by the ESP system than by the BACTEC NR660 system (147 versus 33) was highly significant ($P < 0.001$). In addition, the overall times for detection of the total number of organisms as well as for isolates of *Streptococcus pneumoniae*, *Streptococcus agalactiae*, *Enterococcus* spp., *Enterobacter* spp., and anaerobes were significantly faster by the ESP system than by the BACTEC NR660 system.

Table 6 compares detection times for organisms isolated in ESP and BACTEC anaerobic broths. More coagulase-negative staphylococci, enterococci, *Escherichia coli*, *Klebsiella pneumoniae*, and other enteric organisms were detected >6 h earlier by the ESP system than by the BACTEC NR660 system. Times to detection were faster for the total number of organisms as well as for coagulase-negative staphylococci, most enteric organisms, and the anaerobes as a group.

The average time to detection of isolates in ESP aerobic medium was 21.1 h, and in BACTEC aerobic media the average time was 26.4 h. For ESP anaerobic medium, the average time to detection was 24.9 h; the time was 30.2 h for BACTEC anaerobic media. By the *t* test, the more rapid detection by ESP media was highly significant ($P < 0.001$)

False-negative cultures. As shown in Table 1, in ESP aerobic broth culture bottles, 12 positive cultures were detected by subculture only (falsely negative); that is, they failed to signal the presence of organisms. In the anaerobic ESP broth culture bottles, at least 25 cultures containing microorganisms failed to signal a positive result; the exact number is unknown, because only 10% of anaerobic bottles were subjected to a terminal subculture. For the BACTEC broths, 7 aerobic and at least 11 anaerobic cultures were falsely negative.

Examination of these cultures revealed that the isolates from nine of the ESP aerobic cultures (containing three *Corynebacterium* spp., two coagulase-negative staphylococci, and one each of *Micrococcus* sp., *Aspergillus* sp., an unidentified gram-positive bacillus, and both a coagulase-negative staphylococcus and a viridans group streptococcus) were considered contaminants; no other cultures in the sets were positive. For three cultures containing *Xanthomonas maltophilia*, either the aerobic bottle of the set or an aerobic bottle in a companion set signaled a positive result, and therefore, the bacteremic episode would have been detected.

Among the 25 false-negative anaerobic broth cultures in the ESP system, 6 were considered contaminants (2 *Propionibacterium* spp. and 1 each of *Candida albicans*, coagulase-negative staphylococcus, *Bacillus* sp., and *Cladosporium* sp.), and for 17 broth cultures (5 each of *X. maltophilia* and *Pseudomonas aeruginosa*, 3 *Candida parapsilosis*, 2 *C. albicans*, and 1 each of coagulase-negative staphylococcus and *Alcaligenes xylosoxidans* subsp. *xylosoxidans*), the bacteremic episode would have been detected by a positive companion bottle or set. The two remaining isolates were a *P. aeruginosa* detected in the BACTEC NR660 system only (no signal and no positive subculture in the ESP aerobic bottle) and a *Sphingobacterium multivorum* isolated only in the subculture of the ESP anaerobic bottle.

For the seven false-negative BACTEC aerobic bottles, one coagulase-negative staphylococcus and one *Aspergillus flavus* isolate were considered contaminants, 1 *Lactobacillus* sp. was isolated from the anaerobic bottle of another blood specimen, and one clinically significant *C. albicans* isolate was detected by subculture from a BACTEC bottle only. In addition, one isolate each of *Candida glabrata*, *Pseudomonas paucimobilis*, and *Listeria monocytogenes* that were considered clinically significant were isolated from ESP cultures only. Among the 11 false-negative BACTEC anaerobic cultures, one *Propionibacterium* sp. and one *Staphylococcus aureus* isolate were considered contaminants; for two each of *Streptococcus sanguis*, *P. aeruginosa*, coagulase-negative staphylococci, and *X. maltophilia*, and one *S. aureus*, the companion aerobic bottle signaled a positive result.

ESP versus resin-containing BACTEC broth culture bottles. The performances of the resin-containing BACTEC PEDS Plus, 16A, and 17A broths were examined and compared with

TABLE 2. Clinically significant organisms isolated from ESP-80A and BACTEC NR660 aerobic broth cultures

Microorganism	Total no. of isolates	No. of isolates positive in:		
		ESP-80A and BACTEC NR aerobic broth cultures[a]	ESP-80A broth cultures only	BACTEC NR660 aerobic broth cultures only[a]
Aerobic gram-positive organisms	256	178	45	33
Coagulase-negative staphylococci[b]	93	67	16	10
Staphylococcus aureus	63	47	5	11
Streptococcus pneumoniae	27	16	10[c]	1
Streptococcus agalactiae	14	12	0	2
Other streptococci[d]	21	17	1	3
Enterococcus spp.	23	15	4	4
Corynebacterium spp.[e]	13	4	7	2
Listeria monocytogenes	2	0	2	0
Aerobic gram-negative bacilli	133	89	26	18
Escherichia coli	43	30	6	7
Klebsiella pneumoniae	22	13	6	3
Enterobacter spp.[f]	14	10	3	1
Other enteric organisms[g]	16	12	3	1
Pseudomonas aeruginosa	22	17	3	2
Other nonfermenters[h]	14	7	4	3
Haemophilus influenzae	2	0	1	1
Anaerobes	10	6	4	0
Clostridium spp.[i]	7	6	1	0
Other[j]	3	0	3	0
Fungi	25	19	3	3
Candida spp.[k]	24	19	2	3
Fusarium sp.	1	0	1	0
Total, all organisms	424	292	78[c]	54

[a] BACTEC NR6A, NR16A, or PEDS Plus broth cultures.
[b] *Staphylococcus epidermidis*, $n = 47$; other coagulase-negative staphylococci or not identified to the species level, $n = 46$.
[c] $P \leq 0.05$; significant difference by chi-square test.
[d] *Streptococcus pyogenes*, $n = 5$; group F streptococcus, $n = 1$; *Streptococcus intermedius*, $n = 4$; *Streptococcus sanguis*, $n = 3$; *Streptococcus mitis*, $n = 3$; other viridans group streptococci, $n = 5$.
[e] *Corynebacterium jeikeium*, $n = 6$; other corynebacteria, $n = 7$.
[f] *Enterobacter cloacae*, $n = 12$; *Enterobacter aerogenes*, $n = 1$; *Enterobacter agglomerans*, $n = 1$.
[g] *Serratia marcescens*, $n = 6$; *Salmonella* spp., $n = 3$; *Citrobacter amalonaticus*, $n = 2$; *Citrobacter freundii*, $n = 3$; *Proteus mirabilis*, $n = 1$; *Yersinia enterocolitica*, $n = 1$.
[h] *Acinetobacter baumannii*, $n = 4$; *Acinetobacter lwoffii*, $n = 2$; *Xanthomonas maltophilia*, $n = 5$; *Alcaligenes xylosoxidans*, $n = 1$; *Chryseomonas luteola*, $n = 1$; *Pseudomonas paucimobilis*, $n = 1$.
[i] *Clostridium tertium*, $n = 6$; *Clostridium bifermentans*, $n = 1$.
[j] *Actinomyces viscosus*, $n = 1$; *Lactobacillus* sp., $n = 1$; *Streptococcus constellatus*, $n = 1$.
[k] *Candida albicans*, $n = 11$; *Candida parapsilosis*, $n = 9$; *Candida krusei*, $n = 2$; *Candida glabrata*, $n = 2$.

those of nonresin-containing ESP broth culture bottles. Of the 1,525 PEDS Plus and ESP-80A broth culture bottles inoculated with specimens from pediatric patients, 71 were positive. Of these, 49 positive cultures were detected by both systems, 16 were detected by the ESP system alone, and 6 were detected by the BACTEC NR660 system alone ($P < 0.05$). Although no single organism was detected significantly more often by one system than the other, among the important pediatric pathogens, the recovery rates in both broths, the ESP-80A broth, and the PEDS Plus broth for pneumococci were 10, 6, and 1, respectively; for *S. agalactiae* they were 4, 0, and 2, respectively, and for *Haemophilus influenzae* they were 0, 1, and 1, respectively.

A total of 106 organisms were isolated from the 2,179 BACTEC 16A and ESP-80A broth culture bottles inoculated: 72 in both broths, 13 in ESP-80A broth only, and 21 in BACTEC 16A broth only (P was not significant). Among these organisms, only *S. aureus* was isolated significantly more often in one system alone: 10 isolates in the BACTEC NR660 system only versus 1 isolate in the ESP system only ($P < 0.05$).

Thirty-two organisms were isolated from the 983 BACTEC 17A and ESP-80N broth culture bottles inoculated: 14 in both broths, 9 in ESP broths only, and 9 in BACTEC 17A broths only (P was not significant). Too few organisms of any one species were isolated to determine trends. Of the six *S. aureus* isolates, three grew in both broth culture bottles and three grew in the BACTEC 17A culture bottles only.

DISCUSSION

The use of current medical and surgical advances to treat previously fatal diseases and conditions has resulted in patient populations that are at increased risk for bacteremia and excess mortality (7). Rapid detection of nonviral blood-borne pathogens with subsequent early therapeutic intervention may aid in the survival of these immunocompromised hosts. Because of such clinical urgencies, in some large medical centers, the numbers of blood specimens received by the clinical microbiology laboratory have increased dramatically, often taxing personnel resources. Blood culture detection systems that frequently and automatically monitor culture broths for positivity could provide a marked advantage in such situations.

TABLE 3. Clinically significant organisms isolated from ESP-80N and BACTEC NR7A or 17A broth cultures

Microorganism	Total no. of isolates	No. of isolates positive in:		
		ESP-80N and BACTEC NR7A or 17A broth cultures	ESP-80N broth cultures only	BACTEC NR7A or 17A broth cultures only
Aerobic gram-positive organisms	179	116	40[a]	23
Coagulase-negative staphylococci[b]	64	42	15	7
Staphylococcus aureus	60	42	12	6
Streptococcus pneumoniae	11	6	1	4
Streptococcus agalactiae	8	8	0	0
Other streptococci[c]	13	4	5	4
Enterococcus spp.	18	14	2	2
Listeria monocytogenes	2	0	2	0
Corynebacterium spp.[d]	3	0	3	0
Aerobic gram-negative bacilli	78	53	14	11
Escherichia coli	31	24	1	6
Klebsiella pneumoniae	19	12	5	2
Enterobacter spp.[e]	8	7	1	0
Other enteric organisms[f]	12	10	2	0
Pseudomonas aeruginosa	7	0	5	2
Xanthomonas maltophilia	1	0	0	1
Anaerobes	16	9	6	1
Bacteroides spp.[g]	5	3	2	0
Clostridium tertium	6	5	1	0
Other[h]	5	1	3	1
Candida spp.[i]	10	2	8[a]	0
Total, all organisms	283	180	68[a]	35

[a] $P \leq 0.05$; significant difference by chi-square test.
[b] *Staphylococcus epidermidis*, $n = 33$; other coagulase-negative staphylococci or not identified to the species level, $n = 31$.
[c] *Streptococcus intermedius*, $n = 4$; *Streptococcus pyogenes*, $n = 3$; *Streptococcus sanguis*, $n = 3$; *Streptococcus mitis*, $n = 2$; other viridans group streptococci, $n = 1$.
[d] *Corynebacterium* spp., $n = 2$; *Corynebacterium jeikeium*, $n = 1$.
[e] *Enterobacter cloacae*, $n = 7$; *Enterobacter aerogenes*, $n = 1$.
[f] *Serratia marcescens*, $n = 6$; *Citrobacter amalonaticus*, $n = 2$; *Citrobacter freundii*, $n = 3$; *Proteus mirabilis*, $n = 1$.
[g] *Bacteroides fragilis*, $n = 2$; *Bacteroides bivius*, $n = 2$; *Bacteroides* spp., but not *Bacteroides fragilis*, $n = 1$.
[h] *Fusobacterium nucleatum*, $n = 1$; *Lactobacillus* sp., $n = 1$; *Peptostreptococcus* sp., $n = 1$; *Propionibacterium acnes*, $n = 1$; *Propionibacterium* sp., $n = 1$.
[i] *Candida albicans*, $n = 7$; *Candida glabrata*, $n = 2$; *Candida krusei*, $n = 1$.

Until recently, the BACTEC systems (in particular, the BACTEC 460 and NR660/730 systems) were the only commercially available instruments that could automatically detect positive blood cultures. These systems require manual loading of culture bottles into the instrument for monitoring and permit only a limited number of daily tests for microbial detection (maximum monitoring, twice per day). The BacT/Alert and BACTEC 9240 are newer systems that monitor blood culture bottles every 10 min. In a recent comparative study of the BACTEC NR660/730 and BacT/Alert systems (11), the microbial yields were comparable between the two systems, but the BacT/Alert instrument detected microbial growth earlier. In a study of a prototype BACTEC 9240 system compared with the BACTEC NR660 system (5), not only was microbial growth detected earlier with the BACTEC 9240 system but more clinically significant positive blood cultures were also detected. These systems use a tryptic soy broth base culture medium and detect positive cultures by monitoring the evolution of CO_2 by the microorganisms growing in the culture broth.

The ESP system is another new instrument that continuously monitors blood culture bottles for positivity (every 12 [aerobic] to 24 [anaerobic] min). Unlike the BACTEC and BacT/Alert systems, however, microbial growth is monitored by both consumption and evolution of gases (consumption of O_2 and evolution of N_2, H_2, and CO_2) rather than by evolution of CO_2 alone. The tryptic soy base culture media have been specially formulated to enhance microbial growth so that resulting changes in gas pressure are rapidly detected by the instrument's sensitive transducers.

In the present comparative study of the ESP and BACTEC NR660 systems, more organisms were recovered with the ESP system alone and the speed of detection was significantly better with the ESP system than with the BACTEC NR660 system. Among individual organisms isolated in aerobic broth cultures, only pneumococci were recovered significantly more often by the ESP system than the BACTEC NR660 system (Table 2), although significantly more episodes of bacteremia caused by coagulase-negative staphylococci and anaerobes were detected by the ESP system (Table 4). The former have emerged as a major cause of bacteremia in immunocompromised patients (6, 8), and therefore, their early detection is important. Although not significant for every organism group, more organisms were detected earlier (>6 h, Table 5) in the ESP-80A broth than in BACTEC aerobic broth cultures. Six hours was chosen for comparative purposes because it is the approximate time between the two BACTEC readings taken on days 1 and 2 of culture incubation. Thus, workup of cultures detected as positive by the ESP system could begin before they were detected as positive by the BACTEC NR660 system.

In ESP anaerobic broth, no single organism was recovered significantly more often than in BACTEC anaerobic broth (Table 3), but aerobic gram-positive organisms and *Candida* spp. each taken together as a group were recovered more often

TABLE 4. Clinically significant patient episodes with monomicrobic isolates from the ESP and BACTEC NR660 systems

Microorganism	Total no. of episodes	No. of isolates positive in:		
		ESP and BACTEC NR660 systems	ESP system only	BACTEC NR660 system only
Aerobic gram-positive organisms	179	127	34[a]	18
Coagulase-negative staphylococci[b]	67	53	12[a]	2
Staphylococcus aureus	44	32	6	6
Streptococcus pneumoniae	25	15	8	2
Streptococcus agalactiae	11	9	0	2
Other streptococci[c]	11	8	2	1
Enterococcus spp.	14	7	3	4
Corynebacterium spp.[d]	5	3	1	1
Listeria monocytogenes	2	0	2	0
Aerobic gram-negative bacilli	95	65	18	12
Escherichia coli	32	22	4	6
Klebsiella pneumoniae	15	11	2	2
Enterobacter spp.[e]	11	7	3	1
Other enteric organisms[f]	11	7	3	1
Pseudomonas aeruginosa	15	13	1	1
Other nonfermenters[g]	9	5	4	0
Haemophilus influenzae	2	0	1	1
Anaerobes	14	5	8[a]	1
Bacteroides spp.[h]	4	2	2	0
Clostridium spp.[i]	3	2	1	0
Other[j]	7	1	5	1
Fungi	14	10	3	1
Candida spp.[k]	13	10	2	1
Fusarium sp.	1	0	1	0
Total all organisms	302	207	63[a]	32

[a] $P \leq 0.05$; significant difference by chi-square test.
[b] *Staphylococcus epidermidis*, $n = 37$; other coagulase-negative staphylococci or not identified to the species level, $n = 30$.
[c] *Streptococcus pyogenes*, $n = 3$; group F streptococcus, $n = 1$; *Streptococcus intermedius*, $n = 4$; *Streptococcus sanguis*, $n = 1$; *Streptococcus mitis*, $n = 2$.
[d] *Corynebacterium jeikeium*, $n = 3$; other corynebacteria, $n = 2$.
[e] *Enterobacter cloacae*, $n = 9$; *Enterobacter aerogenes*, $n = 1$; *Enterobacter agglomerans*, $n = 1$.
[f] *Serratia marcescens*, $n = 4$; *Salmonella* spp., $n = 3$; *Citrobacter amalonaticus*, $n = 1$; *Citrobacter freundii*, $n = 1$; *Proteus mirabilis*, $n = 1$; *Yersinia enterocolitica*, $n = 1$.
[g] *Acinetobacter baumannii*, $n = 2$; *Acinetobacter lwoffii*, $n = 2$; *Xanthomonas maltophilia*, $n = 2$; *Alcaligenes xylosoxidans*, $n = 1$; *Chryseomonas luteola*, $n = 1$; *Pseudomonas paucimobilis*, $n = 1$.
[h] *Bacteroides fragilis*, $n = 2$; *Bacteroides bivius*, $n = 1$; *Bacteroides* spp. but not *Bacteroides fragilis*, $n = 1$.
[i] *Clostridium tertium*, $n = 2$; *Clostridium bifermentans*, $n = 1$.
[j] *Actinomyces viscosus*, $n = 1$; *Fusobacterium nucleatum*, $n = 1$; *Lactobacillus* sp., $n = 1$; *Peptostreptococcus* sp., $n = 1$; *Propionibacterium acnes*, $n = 1$; *Propionibacterium* sp., $n = 1$; *Streptococcus constellatus*, $n = 1$.
[k] *Candida albicans*, $n = 7$; *Candida parapsilosis*, $n = 3$; *Candida glabrata*, $n = 2$; *Candida krusei*, $n = 1$.

($P < 0.05$). In addition, when comparing all organisms, recovery was faster in ESP anaerobic broth (Table 6).

It is not unexpected that, as with the BacT/Alert (11) and BACTEC 9240 (5) systems, frequent monitoring by the ESP system (60 to 120 times per day) leads to the more rapid detection of microorganisms in blood cultures than monitoring only once or twice per day, as with the BACTEC NR660 system. In addition, the ability of the ESP system to detect oxygen consumption and the evolution of gases other than CO_2 by organisms growing in the enriched broths may be responsible for the decreased time for organism detection, especially of organisms that do not produce large amounts of CO_2.

The improved detection of microorganisms and in particular pneumococci and yeasts by the ESP system in comparison with that by the BACTEC NR660 system may be related to different medium formulations, different detection systems, or both. None of the ESP media contain antimicrobial agent-inactivating resins, whereas resin-containing BACTEC 16A and 17A media were used at Chicago and Sparrow when patients were receiving antimicrobial agents at the time that blood was drawn for culture. Except for *S. aureus*, the absence of resins in ESP media did not appear to significantly affect organism detection by the ESP system, and among pediatric patients, more organisms were isolated from ESP-80A broth cultures than from the resin-containing PEDS Plus broth cultures. Washington and Ilstrup (10) have noted that resins appear to improve the detection of *S. aureus*. When considering all clinically significant episodes of *S. aureus* bacteremia (Table 4), however, an equal number was detected by each test system alone. Therefore, factors other than resins are important for the recovery of this microorganism, even in patients receiving antimicrobial therapy.

Of note is that for each *S. aureus* bacteremic episode detected by the ESP system alone, the anaerobic medium was always positive, whereas the aerobic medium was positive in conjunction with anaerobic medium in three of six instances (data not shown). In some patients with *S. aureus* bacteremia who are receiving antimicrobial agents, therefore, the ESP anaerobic medium formulation may substitute for the positive effect of antimicrobial agent-adsorbing resins.

TABLE 5. Comparison of detection times for organisms isolated from both ESP-80A and BACTEC NR aerobic broths

Microorganism	Total no. of isolates	No. of isolates detected in:		
		ESP-80A and BACTEC NR aerobic broth cultures[a] at same time	ESP-80A broth culture earlier (>6 h)	BACTEC NR aerobic broth culture[a] earlier (>6 h)
Aerobic gram-positive organisms	178	66	87[b,c]	25
Coagulase-negative staphylococci	67	23	39[b]	5
Staphylococcus aureus	47	16	20	11
Streptococcus pneumoniae	16	8	8[b,c]	0
Streptococcus agalactiae	12	4	7[b,c]	1
Other streptococci	17	7	3	7
Enterococcus spp.	15	7	8[b,c]	0
Corynebacterium spp.	4	1	2	1
Aerobic gram-negative bacilli	89	43	41[b,c]	5
Escherichia coli	30	15	13[b]	2
Klebsiella pneumoniae	13	4	9[b]	0
Enterobacter spp.	10	4	6[b,c]	0
Other enteric organisms	12	6	4	2
Pseudomonas aeruginosa	17	11	5	1
Other nonfermenters	7	3	4[b]	0
Anaerobes	6	0	6[b,c]	0
Fungi	19	3	13[b]	3
Total, all organisms	292	112	147[b,c]	33

[a] BACTEC NR6A, NR16A, or PEDS Plus broth.
[b] $P \leq 0.05$; significant difference for number of isolates detected >6 h earlier by chi-square test.
[c] $P \leq 0.001$; significant difference for times to positivity by t test analysis.

Another factor responsible for the increased level of detection of microorganisms, even from patients receiving antimicrobial agents, may be the large volume of blood in the ESP bottles (80 ml) and therefore the greater dilution of inhibitory substances in blood than in BACTEC media (20 or 30 ml). The range of dilutions in ESP broth culture bottles was 1:16 to 1:160, as opposed to 1:6 to 1:40 in BACTEC broth culture bottles. Although all broth culture bottles except ESP-80N contain sodium polyanetholsulfonate, the concentration in ESP-80A bottles (0.006%) is lower than that in BACTEC broth culture bottles (0.035%, except 0.025% in BACTEC PEDS Plus bottles). In media containing low concentrations of sodium polyanetholsulfonate, enteric gram-negative bacilli might be inhibited by the greater availability of complement

TABLE 6. Comparison of detection times for organisms isolated from both ESP-80N and BACTEC NR7A or 17A anaerobic broths

Microorganism	Total no. of isolates	No. of isolates detected in:		
		ESP-80N and NR7A or 17A broth cultures at same time	ESP-80N broth culture earlier (>6 h)	NR7A or 17A broth culture earlier (>6 h)
Aerobic gram-positive organisms	116	46	48[a,b]	22
Coagulase-negative staphylococci	42	13	24[a,b]	5
Staphylococcus aureus	42	16	11	15
Streptococcus pneumoniae	6	4	1	1
Streptococcus agalactiae	8	6	1	1
Other streptococci	4	1	3	0
Enterococcus spp.	14	6	8[a]	0
Aerobic gram-negative bacilli	53	30	23[a,b]	0
Escherichia coli	24	15	9[a,b]	0
Klebsiella pneumoniae	12	4	8[a,b]	0
Enterobacter spp.	7	7	0	0
Other enteric organisms	10	4	6[a,b]	0
Anaerobes	9	6	2[b]	1
Bacteroides spp.	3	1	1	1
Clostridium tertium	5	5	0	0
Lactobacillus spp.	1	0	1	0
Total, all organisms	178	82	73[a,b]	23

[a] $P \leq 0.05$; significant difference for number of isolates detected >6 h earlier by chi-square test.
[b] $P \leq 0.001$; significant difference for times to positivity by t test analysis.

(9), but no inhibitory effect on enteric organisms was seen in the present study.

The false-positive culture rate was significantly higher for the ESP system than for the BACTEC NR660 system, although it was relatively low with both systems. In most instances, examination of the curves generated by the ESP system indicated hardware problems peculiar to the prototype instrument. Electronic improvements in the production models and fine-tuning of the algorithms in the ESP data base are expected to decrease this false-positive rate.

Most of the false-negative ESP cultures were due to the presence of contaminants or aerobic organisms growing in the anaerobic bottles. Two episodes of bacteremia would have been missed if subcultures had not been performed, whereas four episodes of bacteremia would have been missed by false-negative BACTEC cultures. Thus, as with the BACTEC systems (3), it does not appear that blind terminal subcultures are warranted with the ESP system. At Sparrow, cultures were incubated for 7 days rather than 6 days as at Chicago and Rush. Only three additional isolates were found: two contaminants and one isolate that had already been recovered in another culture set. Thus, incubation past 6 days does not appear to be warranted.

In summary, the ESP system is an attractive alternative to the BACTEC NR660 system. When considering the total number of organisms recovered by the two systems, more clinically significant organisms were recovered by the ESP system alone and they were detected in a significantly shorter time by the ESP system.

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