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Controlled Evaluation of BACTEC PLUS 27 and Roche Septi-Chek Anaerobic Blood Culture Bottles

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Becton Dickinson Diagnostic Instrument Systems (Sparks, Md.) recently introduced BACTEC high-volume aerobic and anaerobic bottles that accept up to 10 ml of blood for use on their nonradiometric blood culture instruments. Both bottles contain 25 ml of tryptic soy broth, 0.05% sodium polyanetholesulfonate, and mixed resins. We compared the anaerobic bottle, designated BACTEC PLUS 27 (BP27), with the Roche Septi-Chek (RSC) Columbia broth anaerobic bottle in a collaborative evaluation at three university hospitals. A total of 5,152 adequately filled blood cultures were obtained from adult patients with suspected bacteremia or fungemia. *Staphylococcus aureus* was recovered significantly more often ($P < 0.03$) from BP27 bottles alone; there were no other significant differences in yield. When microorganisms were recovered from both anaerobic bottles, growth was detected earlier in BP27 than it was in RSC ($P < 0.001$), especially for *S. aureus* ($P < 0.001$) and *Staphylococcus epidermidis* ($P < 0.02$). We conclude that the yield from BP27 bottles is equivalent to or better (*S. aureus*) than that from RSC anaerobic bottles with Columbia broth and that speed of detection is superior with BP27 bottles.

Successful recovery of microorganisms from the blood of patients with bacteremia and fungemia is dependent upon several factors, the most important of which is volume (1, 5, 6, 10, 13, 14). It is now recognized that optimal recovery of microorganisms occurs when 20 to 30 ml is cultured from each venipuncture (6, 10). Until recently, however, the only commercial broth blood culture systems with bottles designed to accept inocula of 10 ml of blood were manual systems. Becton Dickinson Diagnostic Instrument Systems (Sparks, Md.) recently introduced high-volume (i.e., 10-ml) BACTEC PLUS 26 (BP26) aerobic and PLUS 27 (BP27) anaerobic bottles for use with their nonradiometric 660, 730, and 860 systems. These bottles are manufactured in the same size and configuration as BACTEC bottles that accept only 5 ml of blood. Therefore, to culture 10 ml of blood, the volume of medium in each bottle was reduced from 30 to 25 ml. The resultant ratio of blood to broth in each bottle was reduced from 1:7 to as low as 1:3.5. Since the ratio of blood to broth medium is also important in maximizing recovery of microorganisms (2, 11), proprietary resins were added to overcome the reduced ratio.

We compared these high-volume BACTEC bottles with the Roche Septi-Chek (RSC) blood culture system. The RSC system was chosen as a reference method because (i) the bottles accept blood inocula of up to 10 ml, (ii) the blood to broth ratio is in the range (1:8) known to maximize recovery of microorganisms (2, 11), (iii) both aerobic and anaerobic media are available, and (iv) the media do not contain resins or other lysing agents. Results of a comparison of BP26 and RSC aerobic bottles were reported recently (15).

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MATERIALS AND METHODS

Blood culture systems. We compared BP27 bottles processed on the BACTEC 660/HPS against RSC anaerobic blood culture bottles. BP27 bottles contain 25 ml of tryptic soy broth, 0.05% sodium polyanetholesulfonate, and mixed resins. RSC anaerobic bottles contain 70 ml of Columbia broth and 0.05% sodium polyanetholesulfonate. A BP26 bottle was used in all cultures to ensure optimal recovery of aerobic microorganisms for patient care.

Blood culture collection. Skin disinfection was by standard techniques (11). Thirty milliliters of blood was collected at the bedside and was inoculated sequentially into one BP26 bottle, one BP27 bottle, and one RSC anaerobic bottle.

Laboratory processing. All bottles received in the microbiology laboratory were compared against standards with known volumes, and the adequacy of fill was recorded. Blood volumes of <8 ml were recorded as inadequate, volumes of 8 to 12 ml were recorded as adequate, and volumes of >12 ml were recorded as overfilled. Only bottles inoculated with 8 to 12 ml of blood (i.e., adequately filled) were included in the study, although all bottles containing any blood were processed to maximize recovery of microorganisms for patient care. All bottles were processed according to the recommendations of the manufacturers. In particular, RSC bottles were subcultured at the end of the 7-day incubation period (terminal subcultures); BACTEC bottles were not subcultured. Microorganisms were identified by standard procedures (8).

Clinical assessment. Patient charts were reviewed to assess the clinical importance of microorganisms isolated from cultures. Isolates were categorized as true causes of bacteremia or fungemia, contaminants, or indeterminate if their etiologic significance could not be ascertained on the basis of published criteria (16).

Data analysis. Only positive blood cultures drawn from adult patients with true bacteremia or fungemia and with adequate blood volumes in both anaerobic bottles were analyzed for the bottle versus bottle (BP27 versus RSC anaerobic) comparison. A subset of cultures in which all three bottles (BP26, BP27, and RSC anaerobic) were received and inoculated with an adequate volume of blood from septic patients was analyzed to determine the relative contribution of each bottle to the recovery of microorganisms. This subset was further analyzed to determine the relative contribution of each bottle to the recovery of strictly aerobic microorganisms, including those found only by terminal subculture of the RSC bottle. Statistical comparison between bottles was made with the McNemar chi-square test, including Yates' correction for small numbers when appropriate (9). Data from the three study sites were collated and analyzed at the Clinical Microbiology Laboratory at Duke University Medical Center.

RESULTS

A total of 5,152 blood cultures with both anaerobic bottles adequately filled with blood were received during the study period. Of these, 432 (8.4%) were positive and yielded a total of 506 microorganisms; 282 (65.3%) cultures grew 326 microorganisms that caused illness, 85 cultures (19.7%) grew one or more contaminants, and 65 cultures (15.0%) grew one or more microorganisms indeterminate as a cause of illness. Of the 326 microorganisms that caused illness, 148 (45.4%) were recovered from both bottles, 96 (29.4%) were recovered from BP27 bottles only, and 82 (25.2%) were recovered from RSC anaerobic bottles only (Table 1). *Staphylococcus aureus* was recovered significantly more often from BP27 bottles alone ($P < 0.03$). With this exception, yields from the two bottles were not significantly different.

Anaerobic bacteria accounted for 23 of 326 (7.1%) isolates from patients with bacteremia. Eight of 23 (34.8%) anaerobic bacteria were recovered from both bottles, 11 of 23 (47.8%) were recovered from BP27 bottles only, and 4 of 23 (17.4%) were recovered from RSC bottles only; the difference in recovery of strict anaerobes from the two anaerobic bottles was not statistically significant.

For the 148 microorganisms isolated from both anaerobic bottles, growth of 75 (50.7%) was detected simultaneously in the two bottles, growth of 55 (37.2%) was detected earlier in BP27 bottles, and growth of 18 (12.2%) was detected earlier in RSC bottles (Table 2). Growth of *S. aureus* ($P < 0.001$), *Staphylococcus epidermidis* ($P < 0.02$), and all organisms combined ($P < 0.001$) was detected earlier with BP27 bottles.

A total of 404 isolates were recovered from one or more of the three study bottles in that subset of cultures in which all three bottles (BP26, BP27, and RSC anaerobic) were received and inoculated with an adequate volume of blood from septic patients. When each two-bottle combination was compared, it was found that 373 of 404 (92.3%) isolates were recovered from a combination of BP26 aerobic and BP27 anaerobic bottles, 363 of 404 (89.9%) isolates were recovered from a combination of BP26 aerobic and RSC anaerobic bottles, and 303 of 404 (75.0%) isolates were recovered from a combination of BP27 and RSC anaerobic bottles. By using the same data set, it was possible to assess the comparative recovery by bottle of 10 ml of blood; of the 404 isolates, 318 (78.7%) were recovered from BP26 bottles, 230 (56.9%) were recovered from BP27 bottles, and 213 (52.7%) were recovered from RSC anaerobic bottles.

TABLE 1. Comparative yield of clinically important bacteria and fungi in BP27 and RSC anaerobic blood culture bottles

Microorganism	No. of isolates recovered from:			P
	Both bottles	BP27 only	RSC only	
S. aureus	39	20	8	<0.03
S. epidermidis	18	23	12	NS[a]
Enterococci	9	6	2	NS
Streptococci[b]	15	6	4	NS
Other gram-positive bacteria[c]	2	3	5	NS
Members of the family *Enterobacteriaceae*[d]	51	16	21	NS
P. aeruginosa	2	3	10	NS
Other gram-negative bacteria[e]	2	1	3	NS
Anaerobic bacteria[f]	8	11	4	NS
C. albicans	2	4	10	NS
Other fungi[g]	0	3	3	NS
All microorganisms	148	96	82	NS

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

[b] Includes one *Streptococcus pyogenes* isolate, three group B and four group D streptococci, five *Streptococcus pneumoniae* isolates, three *Streptococcus bovis* isolates, six viridans group streptococci, and one unidentified nonhemolytic and two unidentified (not group A) beta-hemolytic streptococci.

[c] Includes three *Bacillus* spp., three *Corynebacterium* spp., three *Corynebacterium* group J-K isolates, and one unidentified gram-positive bacillus.

[d] Includes 3 *Citrobacter freundii* isolates, 7 *Enterobacter aerogenes* isolates, 1 *Enterobacter agglomerans* isolate, 9 *Enterobacter cloacae* isolates, 38 *Escherichia coli* isolates, 2 unidentified gram-negative bacilli, 2 *Klebsiella oxytoca* isolates, 14 *Klebsiella pneumoniae* isolates, 1 *Morganella morganii* isolate, 6 *Proteus mirabilis* isolates, 1 *Proteus vulgaris* isolate, 2 *Serratia marcescens* isolates, and 2 *Salmonella* spp.

[e] Includes one *Actinetobacter* sp., one *Branhamella* sp., and four *Haemophilus influenzae* isolates.

[f] Includes five *Clostridium tertium* isolates, three *Clostridium* spp., one *Peptococcus* sp., one *Propionibacterium* sp., one *Bacteroides fragilis* isolate, one *Bacteroides vulgatus* isolate, seven *Bacteroides thetaiotaomicron* isolates, two *Fusobacterium* spp., and two *Fusobacterium nucleatum* isolates.

[g] Includes one *Cryptococcus neoformans* isolate, two *Candida parapsilosis* isolates, two *Candida tropicalis* isolates, and one *Torulopsis glabrata* isolate.

Nineteen *Pseudomonas aeruginosa* and 24 *Candida albicans* isolates were recovered from those cultures in which all three bottles were adequately filled with blood. Seventeen of 19 *P. aeruginosa* and 23 of 24 *C. albicans* isolates were recovered from BP26 bottles alone or in combination with one or both anaerobic bottles; 8 *P. aeruginosa* and 9 *C. albicans* isolates were recovered only from BP26 bottles, 9 *P. aeruginosa* and 14 *C. albicans* isolates were recovered from one or both anaerobic bottles in addition to BP26 bottles, and 2 *P. aeruginosa* and 1 *C. albicans* isolates were recovered only from RSC anaerobic bottles. No *P. aeruginosa* or *C. albicans* isolates were recovered only from BP27 bottles. Within this subset of cultures, 7 of 9 *P. aeruginosa* and 3 of 11 *C. albicans* isolates recovered from RSC anaerobic bottles were recovered by terminal subculture.

In the same subset of cultures, 25 total isolates were recovered from RSC anaerobic bottles by terminal subculture. Eighteen of 25 (72.0%) isolates were also recovered from one of the other two bottles in the same blood culture set. Seven of 25 (28.0%) isolates were recovered only from RSC bottles. Of these seven isolates, three were from patients who had other blood cultures from which the same microorganism was isolated without terminal subcultures. Thus, if terminal subcultures had not been performed on

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi in BP27 and RSC anaerobic blood culture bottles

Microorganism	Growth detected in:			P
	BP27 and RSC at same time	BP27 earlier	RSC earlier	
S. aureus	13	22	4	<0.001
S. epidermidis	7	10	1	<0.02
Streptococci[a]	6	7	2	NS[b]
Enterococci	5	3	1	NS
Other gram-positive bacteria[c]	1	1	0	NS
Members of the family *Enterobacteriaceae*[d]	35	9	7	NS
P. aeruginosa	2	0	0	NS
Other gram-negative bacteria[e]	1	1	0	NS
Anaerobic bacteria[f]	5	1	2	NS
C. albicans	0	1	1	NS
All microorganisms	75	55	18	<0.001

[a] Includes two group B and three group D streptococci, four *Streptococcus pneumoniae* isolates, one *Streptococcus bovis* isolate, three viridans group streptococci, and two unidentified (not group A) beta-hemolytic streptococci.
[b] NS, not significant ($P > 0.05$).
[c] Includes one *Bacillus* sp. and one *Corynebacterium* sp.
[d] Includes 2 *Citrobacter freundii* isolates, 6 *Enterobacter aerogenes* isolates, 1 *Enterobacter agglomerans* isolate, 4 *Enterobacter cloacae* isolates, 22 *Escherichia coli* isolates, 2 *Klebsiella oxytoca* isolates, 10 *Klebsiella pneumoniae* isolates, 3 *Proteus mirabilis* isolates, and 1 *Salmonella* sp.
[e] Includes two *Haemophilus influenzae* isolates.
[f] Includes three *Clostridium tertium* isolates, one *Clostridium* sp., one *Bacteroides fragilis* isolate, two *Bacteroides thetaiotaomicron* isolates, and one *Fusobacterium* sp.

RSC bottles, 4 of 404 (1.0%) total isolates would not have been recovered. These isolates included one each of *S. epidermidis*, a *Bacillus* sp., *P. aeruginosa*, and *C. albicans*.

DISCUSSION

Data from this evaluation indicate that, with the exception of *S. aureus*, there was no significant difference between BP27 and RSC anaerobic bottles for recovering microorganisms from the blood of adult patients with bacteremia or fungemia. Microbial growth was detected significantly earlier with BP27 bottles ($P < 0.001$). In particular, growth of *S. aureus* ($P < 0.001$) and *S. epidermidis* ($P < 0.02$) was detected earlier with BP27 bottles.

The reason for the increased yield and earlier detection of microbial growth with BP26 (15) and BP27 bottles in comparison with those with equivalent RSC bottles is not known. The volume of blood cultured in each bottle was carefully controlled to between 8 and 12 ml. We did not assess the impact of antibiotic therapy on yield in this study, but in the companion evaluation (15), binding of antimicrobial agents by resins in BP26 bottles did not completely explain the difference in recovery between the two aerobic bottles. Although the ratio of blood to broth is greater in RSC bottles and therefore should have enhanced recovery of microorganisms from those bottles, such a difference in overall recovery was not observed. The most likely explanation for the increased recovery of microorganisms such as *S. aureus* from BP27 bottles is the presence of resins. The mechanism by which these resins increase recovery of microorganisms is not known, although a recent report (7) suggests it is by lysis of leukocytes and the subsequent release of phagocytized microorganisms into the blood-broth mixture. Although improved recovery of microorganisms might occur if an agar slant were part of the anaerobic Septi-Chek system, such slants are not available. Placing slants designed for use with aerobic Septi-Chek bottles on the anaerobic bottles would render the bottles nonanaerobic and is not recommended by the manufacturer.

Although the difference was not statistically significant, strictly aerobic microorganisms such as *P. aeruginosa* and *C. albicans* were recovered more often from RSC anaerobic bottles than from BP27 bottles. Improved recovery of such microorganisms was most likely secondary to the less anaerobic atmosphere present in the RSC bottle headspace relative to that of the BP27 bottle. After each BP27 sampling, the BP27 bottle is backflushed with anaerobic gas, maintaining the atmosphere and, hence, the broth medium in a more anaerobic state relative to that found in the RSC anaerobic bottle. In contrast, because the RSC bottle atmosphere is not sampled or replenished, the broth medium may become less anaerobic over time, allowing for enhanced recovery of aerobic microorganisms. It should be emphasized that when all three bottles were adequately filled with blood, 17 of 19 *P. aeruginosa* and 23 of 24 *C. albicans* isolates were recovered from BP26 bottles alone or in combination with one or both anaerobic bottles. Moreover, for isolates which were recovered from RSC anaerobic bottles, 7 of 9 *P. aeruginosa* and 3 of 11 *C. albicans* isolates were recovered only on terminal subculture. Consequently, both BP27 and RSC anaerobic bottles should be paired with an aerobic bottle for timely recovery of strict aerobes.

A total of 404 isolates were recovered from the subset of cultures in which all three bottles were received and were adequately filled with blood. Within this group a total of 25 isolates were recovered from RSC anaerobic bottles by terminal subculture only. Since 18 of these isolates were recovered from one of the two BP bottles in the same culture and 3 of the remaining 7 isolates were recovered from other blood cultures, only 4 of 404 isolates would have been missed if terminal subcultures had not been performed on RSC anaerobic bottles. Therefore, routine terminal subcultures cannot be recommended for RSC anaerobic bottles on the basis of the data from this study.

The relative paucity of strict anaerobes (7.1% of all isolates) isolated from bacteremic patients in this study is similar to that reported by others (3, 4, 12, 16). As a single bottle, the BP26 outperformed either anaerobic (BP27 or RSC) bottle with regard to total yield in this study. It might be inferred, given the primacy of volume of blood cultured in yield (1, 5, 6, 10, 13, 14), that more isolates would be detected overall if 20 ml was cultured aerobically rather than being divided equally between aerobic and anaerobic bottles. Whether or not selective cultures for anaerobes on the basis of clinical criteria (e.g., intraabdominal or gynecologic sepsis) would be prudent remains to be determined in a prospective trial. Our data show that blood cultured anaerobically has a lesser yield than an equal volume cultured aerobically. Since it is not possible to change the atmosphere and medium once the bottle has been inoculated with blood, a critical analysis of this tissue is needed. Specifically, there should be a comparison of selective blood cultures for anaerobes versus arbitrary splitting of samples into aerobic and anaerobic bottles for all patients and clinical situations. Until such a study has been done and data for a full analysis are available, the current use of an aerobic-anaerobic bottle

pair will continue. In this regard, this evaluation has shown the BP27 bottle to be an excellent companion bottle to the aerobic BP26 bottle when it is used with BACTEC nonradiometric blood culture instruments.

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