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Controlled Evaluation of BACTEC Plus 26 and Roche Septi-Chek Aerobic Blood Culture Bottles

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Blood culture bottles that accept up to 10 ml of blood have recently been introduced for the BACTEC nonradiometric blood culture system. The new formulation, designated BACTEC Plus, contains 25 ml of tryptic soy broth, 0.05% sodium polyanetholesulfonate, and mixed resins. In a collaborative study conducted at three university hospitals, we evaluated the BACTEC Plus 26 (BP26) aerobic bottle and the Roche Septi-Chek aerobic bottle with its agar slide paddle in 5,293 paired blood cultures. Significantly more microorganisms ($P < 0.001$), especially *Staphylococcus aureus* ($P < 0.001$), *Staphylococcus epidermidis* ($P < 0.01$), enterococci ($P < 0.005$), and members of the family *Enterobacteriaceae* ($P < 0.005$), were detected by the BP26 bottle. When both bottles detected growth, BP26 did so earlier ($P < 0.001$). In particular, *S. epidermidis* ($P < 0.001$), streptococci ($P < 0.005$), enterococci ($P < 0.05$), and members of the family *Enterobacteriaceae* ($P < 0.001$) were detected earlier by the BP26 bottle. We conclude that the BP26 bottle provides a yield and speed of detection of microorganisms superior to those of the Roche Septi-Chek aerobic blood culture bottle.

During the past decade, the importance of the volume of blood cultured as a critical variable in the detection of bacteremia and fungemia has been emphasized (3, 8, 16, 18, 20, 21). New blood culture systems have been developed to accept blood volumes of 20 ml or more (i.e., 10 ml per bottle) from adults (6, 14, 22). The instrumented BACTEC blood culture systems, however, have been limited by bottle size and have continued to use culture bottles that accept a maximum of 5 ml each (or 10 ml per two-bottle blood culture set). Recently, BACTEC nonradiometric media were introduced that allow inoculation of up to 10 ml of blood per bottle. The new media, named BACTEC Plus 26 aerobic and BACTEC Plus 27 anaerobic, contain 25 ml of tryptic soy broth, 0.05% sodium polyanetholesulfonate, and mixed resins. We evaluated the new aerobic formulation and compared it with the Roche Septi-Chek (RSC) aerobic bottle containing tryptic soy broth with its slide agar paddle. RSC was chosen because it accepted a blood inoculum of up to 10 ml and had performed well against the BACTEC radiometric system in an earlier comparative study (23). We report here the results of 5,293 paired comparisons of the BACTEC Plus 26 and RSC aerobic blood culture bottles at three collaborating university hospitals that used identical methods of obtaining and processing blood cultures.

MATERIALS AND METHODS

Collection of samples. During the study period, two 25-ml BACTEC Plus bottles (BACTEC Plus 26 aerobic and BACTEC Plus 27 anaerobic) containing tryptic soy broth with 0.05% sodium polyanetholesulfonate and mixed resins and one 70-ml RSC aerobic bottle containing tryptic soy broth with 0.05% sodium polyanetholesulfonate were used to culture blood from adult patients at the Robert Wood Johnson University Hospital, the Duke University Medical Center, and the Vanderbilt University Medical Center. Blood for culture was obtained at the bedside after preparation of the skin with 10% povidone-iodine (1% available iodine) followed by 70% isopropyl alcohol. Blood (30 ml) from each separate venipuncture was distributed as follows: 10 ml to the two aerobic study bottles (RSC and BACTEC Plus 26) and 10 ml to a BACTEC Plus 27 bottle. Thus, the volume of blood inoculated into each bottle was the same. However, the blood:broth ratio in the study bottles was not the same: BACTEC Plus 26, 1:2.5; RSC, 1:7.

Volume standards. To ensure that the culture bottles were inoculated with the specified volume of blood, we measured the level of fluid in each container after it was filled with blood. Although all blood-containing bottles were incubated and processed, those containing less than 8 ml or more than 12 ml of blood were excluded from subsequent comparative analyses.

Processing of samples. Identical methods were used for processing blood cultures in the clinical microbiology laboratories at all hospitals. All bottles were incubated in an air incubator at 35°C for 7 days. Upon receipt in the laboratory, the agar slide paddle was attached to the RSC blood culture bottle, and an immediate subculture was done by inverting the bottle and allowing the blood-broth mixture to cover the agar-coated paddle. The bottle then was placed in a shaker-incubator (model 3527; Lab Line) at 160 rpm for 24 to 48 h, after which it was incubated without agitation. The BACTEC Plus 26 bottle was placed on a BACTEC orbital shaker at 280 rpm in the incubator for 24 to 48 h and then incubated without agitation.

Each RSC bottle was examined for macroscopic evidence of growth in broth and for growth on the agar slide paddle twice daily for the first 2 days of incubation and once daily

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thereafter through day 7. If a bottle was positive macroscopically in the broth but no growth was evident on the agar paddle, the slide chamber was removed and a sterile Pasteur pipette was used to obtain broth for Gram staining and subcultures. If growth on the agar paddle was noted at the same time that the broth became positive macroscopically, the initial mode of detection was considered to be the growth on the agar paddle, i.e., subculture.

The BACTEC Plus 26 bottle was examined macroscopically and by infrared spectrophotometry for CO_2 production twice daily for the first 2 days of incubation and once daily thereafter through day 7. BACTEC Plus 26 bottles with growth value readings above the established threshold or with sufficient growth value increases between consecutive readings were examined by Gram staining and were subcultured. Terminal subcultures were not done on negative BACTEC bottles (15).

All microorganisms were identified by standard microbiologic procedures (10).

Clinical assessment. Patients with positive blood cultures were evaluated by an infectious disease specialist who defined pathogens (clinically important microorganisms causing sepsis) and contaminants by established criteria (24).

Analysis of data. Paired comparisons of the two blood culture systems were done only on adequately filled bottles that grew microorganisms that cause true bacteremia or fungemia. Significance testing was done by the modified chi-square test described by McNemar (13). When appropriate, the Yates correction for small numbers of observations was used.

RESULTS

A total of 5,293 adequately filled paired bottles were received during the study period. Of these, 656 (12.4%) were positive, including 414 (7.8%) that grew 485 microorganisms that cause illness, 153 (2.9%) that grew 1 or more contaminants, 73 (1.4%) that grew 1 or more microorganisms that were indeterminate as a cause of sepsis, and 16 (0.3%) that grew a pathogen mixed with a contaminant or indeterminate isolate. Of the 485 clinically important microorganisms, 257 (53.0%) grew in both blood culture bottles, 163 (33.6%) grew only in the BACTEC Plus 26 bottle, and 65 (13.4%) grew only in the RSC bottle.

Significantly more microorganisms ($P < 0.001$), especially *Staphylococcus aureus* ($P < 0.001$), *Staphylococcus epidermidis* ($P < 0.01$), enterococci ($P < 0.005$), and members of the family *Enterobacteriaceae* ($P < 0.005$), were detected by the BACTEC Plus 26 bottle (Table 1). Although not significant statistically, *Candida* spp. tended to be detected more often in the BACTEC Plus 26 bottle, whereas *Cryptococcus* spp. were detected more often in the RSC bottle.

Of the 257 microorganisms that grew in both bottles, 129 (50.2%) were detected at the same time, 115 (44.7%) were detected earlier by the BACTEC Plus 26 bottle, and 13 (5.1%) were detected earlier by the RSC bottle (Table 2). In particular, *S. epidermidis* ($P < 0.001$), streptococci ($P < 0.005$), enterococci ($P < 0.05$), and members of the family *Enterobacteriaceae* ($P < 0.001$) were detected earlier by the BACTEC Plus 26 bottle, especially during the initial 48 h of incubation (Fig. 1). Of all positive BACTEC Plus 26 cultures, 62% were detected after 24 h of incubation and 91% were detected after 48 h of incubation. By contrast, of all positive RSC cultures, 42% were detected after 24 h of incubation and 74% were detected after 48 h of incubation.

Since blood for culture is obtained from many patients after antimicrobial agents have already been administered, and since the BACTEC Plus 26 bottle contained resins (whereas the RSC bottle did not), we evaluated, when data were available for review, the use of antimicrobial agents in 56 instances in which only the BACTEC Plus 26 bottle was positive for growth. Table 3 shows that in 36 of 56 instances, patients were not receiving antimicrobial agents that were active in vitro against the microorganisms isolated.

TABLE 1. Comparative yield of clinically important bacteria and fungi in BACTEC Plus 26 and RSC aerobic blood culture bottles

Microorganism	No. of isolates recovered by:			P
	Both systems	BACTEC Plus 26 only	RSC only	
Staphylococcus aureus	45	41	3	<0.001
Staphylococcus epidermidis	31	30	13	<0.01
Streptococci[a]	15	6	5	NS[b]
Enterococci	12	15	1	<0.005
Other gram-positive bacteria[c]	5	3	4	NS
Enterobacteriaceae[d]	87	37	15	<0.005
Pseudomonas aeruginosa	15	9	5	NS
Other gram-negative bacteria[e]	13	6	4	NS
Anaerobic bacteria[f]	0	4	1	NS
Candida spp.	33	12	6	NS
Cryptococcus spp.	1	0	5	NS
Other fungi[g]	0	0	3	NS
All microorganisms	257	163	65	<0.001

[a] Includes 4 *Streptococcus pyogenes*, 2 *Streptococcus agalactiae*, 5 *Streptococcus pneumoniae*, 12 viridans group streptococci, and 3 untyped beta-hemolytic streptococci.

[b] NS, Not significant ($P > 0.05$).

[c] Includes nine *Corynebacterium* spp., two *Listeria monocytogenes*, and one *Bacillus* spp.

[d] Includes 63 *Escherichia coli*, 21 *Klebsiella pneumoniae*, 2 *Klebsiella oxytoca*, 24 *Enterobacter* spp., 7 *Serratia* spp., 7 *Citrobacter* spp., and 15 *Proteus mirabilis*.

[e] Includes nine *Acinetobacter calcoaceticus*, one *Alcaligenes* sp., one *Eikenella corrodens*, one *Haemophilus influenzae*, one *Neisseria meningitidis*, five *Xanthomonas maltophilia*, one *Pseudomonas* sp., and four unidentified gram-negative rods.

[f] Includes two *Clostridium tertium*, one *Clostridium septicum*, one *Bacteroides fragilis*, and one *Bacteroides thetaiotomicron*.

[g] Includes one *Aspergillus* spp. and two *Torulopsis glabrata*.

DISCUSSION

In this multicenter evaluation of the BACTEC Plus 26 and RSC aerobic blood culture bottles inoculated with equal volumes of blood, the BACTEC Plus 26 bottle detected significantly more microorganisms than did the RSC bottle. Although the volume of blood inoculated into each bottle was the same, the BACTEC Plus 26 and RSC bottles differed in other ways, perhaps accounting for the higher yield of microorganisms in the BACTEC bottle.

The most prominent variable that may have enhanced the yield in the BACTEC Plus 26 bottle was the presence of resins. Although the value of antibiotic-binding resins in broth blood culture media has been controversial (2, 18, 21, 25), previous studies of both the radiometric and nonradiometric BACTEC systems have suggested that yields in resin-containing BACTEC media are enhanced (1, 4, 5, 7, 12, 19).

Most clinicians and microbiologists have assumed that the potential advantage of resin-containing media, if any, has been due to the binding of antibiotics present in the blood of

TABLE 2. Comparison of speed of detection of clinically important bacteria and fungi in BACTEC Plus 26 and RSC aerobic blood culture bottles

Microorganism	No. of isolates recovered by:			P
	BACTEC Plus 26 and RSC at same time	BACTEC Plus 26 earlier	RSC earlier	
Staphylococcus aureus	34	9	2	NS[a]
Staphylococcus epidermidis	14	17	0	<0.001
Streptococci[b]	5	10	0	<0.005
Enterococci	6	6	0	<0.05
Other gram-positive bacteria[c]	2	3	0	NS
Enterobacteriaceae[d]	39	43	5	<0.001
Pseudomonas aeruginosa	5	8	2	NS
Other gram-negative bacteria[e]	7	6	0	<0.05
Candida spp.	16	13	4	NS
Cryptococcus spp.	1	0	0	NS
All microorganisms	129	115	13	<0.001

[a] NS, Not significant ($P \geq 0.05$).

[b] Includes four *Streptococcus pyogenes*, one *Streptococcus agalactiae*, four *Streptococcus pneumoniae*, four viridans group streptococci, and two other beta-hemolytic streptococci.

[c] Includes three *Corynebacterium* spp., one *Bacillus* sp., and one *Listeria monocytogenes*.

[d] Includes 41 *Escherichia coli*, 14 *Klebsiella pneumoniae*, 16 *Enterobacter* spp., 5 *Serratia* spp., 7 *Proteus mirabilis*, and 4 *Citrobacter* spp.

[e] Includes five *Acinetobacter calcoaceticus*, three unidentified gram-negative rods, one *Haemophilus influenzae*, one *Neisseria meningitidis*, two *Xanthomonas maltophilia*, and one *Pseudomonas* sp.

patients from whom blood samples for culture are obtained during therapy. However, data from the subset of patients for whom detailed information about antimicrobial therapy was available (Table 3) found that in 36 of 56 instances in which only the BACTEC Plus 26 bottle was positive, patients were not receiving antimicrobial agents to which the microorganisms isolated were susceptible. It may be that subinhibitory concentrations of antimicrobial agents prevent enough growth for detection (11) and that resin-containing media enhance yields by binding to these agents. Alternatively, binding of antimicrobial agents by the resins in the

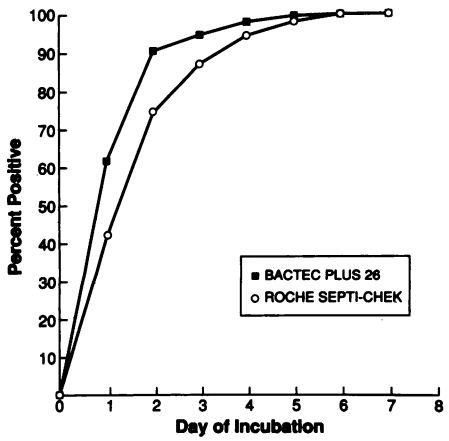

FIG. 1. Cumulative yield of microorganisms from BACTEC Plus 26 and RSC aerobic blood culture bottles during 7 days of incubation.

TABLE 3. Evaluation of antimicrobial therapy in patients with positive blood cultures detected only by the BACTEC Plus 26 blood culture bottle

Microorganism	No. of cultures	
	Effective therapy	No effective therapy
Staphylococci	9	15
Enterococci	1	4
Enterobacteriaceae	5	5
Pseudomonas aeruginosa	1	4
Other bacteria	2	4
Candida spp.	2	4

BACTEC Plus 26 medium may not be the sole explanation for the advantage in yield. For example, Jungkind et al. (9) showed that lysis of leukocytes was increased three- to fourfold in BACTEC radiometric resin-containing medium compared with that in non-resin-containing radiometric medium. Moreover, leukocyte lysis was increased threefold when the BACTEC orbital shaker speed was increased from 200 to 300 rpm. Enhanced lysis of leukocytes containing phagocytized microorganisms by mechanical action of the resin beads might account, therefore, for the greater yield in the resin-containing BACTEC Plus 26 medium.

Other differences in the two bottles studied should be mentioned. The BACTEC Plus 26 bottle had a blood-to-broth ratio less than the normally recommended minimum of 1:5. The potential negative effect of the narrow ratio (1:2.5) on the yield in this bottle probably was countered, at least in part, by the presence of the resins. Variables such as additives to the basal tryptic soy broth culture medium and bottle configuration and headspace also may have influenced the yield of microorganisms. The latter, in particular, seems less likely to be responsible for the observed differences based on our earlier evaluation of BACTEC and RSC systems that showed comparability (23).

Lastly, the earlier detection of microorganisms in the BACTEC Plus 26 bottle was not surprising, in light of previous studies showing a speed advantage for BACTEC versus other broth-based culture systems (17, 22, 23). Of interest, however, was the similar magnitude of the speed advantage in the current study, in which the RSC bottle was agitated for the first 24 to 48 h of incubation, versus our earlier study in which the RSC bottle was incubated without agitation (23). This finding suggests that the speed advantage of the BACTEC system is not solely a function of agitation; the type of motion and cycle speed also may be important.

In summary, this multicenter comparative evaluation of the BACTEC Plus 26 and RSC aerobic blood culture bottles demonstrated significantly better yield and earlier detection of microorganisms by the BACTEC Plus 26 bottle. The enhanced yield may be due to lysis of leukocytes that contain viable microorganisms and the speed and character of agitation as well as to inactivation of antimicrobial agents by the resins.

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