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Controlled Clinical Comparison of BacT/ALERT Standard Aerobic Medium with BACTEC Standard Aerobic Medium for Culturing Blood

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Standard aerobic media are widely used for culturing blood with the BacT/ALERT (BioMérieux, Inc., Durham, N.C.) (BM) and BACTEC 9240 (BD Diagnostic Systems, Sparks, Md.) (BD) automated continuously monitoring instrument systems. Although similarly composed of soybean-casein digest broths, the formulations of the standard aerobic media available for these instruments differ from each other in supplements and in sodium polyanetholesulfonate concentration. Therefore, we compared the standard aerobic media available for these systems at two university hospitals. Blood samples from adult patients with suspected bloodstream infection were inoculated at the bedside into nonvented BM and BD standard aerobic blood culture bottles and incubated in their respective instruments. The laboratories received 6,743 pairs of bottles that were each filled with 8 to 12 ml of blood. A total of 523 isolates representing true infections were recovered from 257 patients; of these isolates, 348 were recovered from both the BD and the BM bottles, 108 were recovered from the BM bottles only, and 67 were recovered from the BD bottles only ($P < 0.005$). More staphylococci ($P < 0.05$), especially coagulase-negative staphylococci ($P < 0.05$), and yeasts ($P < 0.01$) were recovered from BM bottles than from BD bottles. Of 291 unimicrobial episodes of bloodstream infection, 220 were detected with both bottles, 41 were detected with the BM bottles only, and 30 were detected with the BD bottles only (difference not significant). Among 335 cultures that were positive in both bottles within the first 72 h of incubation, the median times to detection were 14 h for BM bottles and 13 h for BD bottles. Rates for false-positive results were 0.5% for BM bottles and 0.1% for BD bottles. One BM bottle and seven BD bottles yielded false-negative results. We conclude that the BM medium provides improved recovery of microorganisms, especially staphylococci and yeasts, compared with that provided by the BD medium.

Standard aerobic media are widely used for culturing blood with the BacT/ALERT (BioMérieux, Inc., Durham, N.C.) (BM) and BACTEC 9240 (BD Diagnostic Systems, Sparks, Md.) (BD) automated continuously monitoring instrument systems. Although similarly composed of soybean-casein digest broths, the formulations of the standard aerobic media available for these instruments differ in supplements and sodium polyanetholesulfonate concentration. There have been several studies evaluating the standard medium formulations for these systems (1, 4, 5, 8, 9). However, there has not been a published controlled clinical comparison of the two standard aerobic media used in these systems. Therefore, we compared the BM and BD standard aerobic medium formulations for detection of bacteremia and fungemia in patients at two university hospitals who were suspected of having clinical sepsis.

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MATERIALS AND METHODS

Blood culture and collection. Blood samples were collected from adult patients hospitalized at Duke University Medical Center and Robert Wood Johnson University Hospital between February and November 2000. Institutional Review Board (IRB) approval was obtained before the study began, and all blood cultures were performed as part of routine patient care. Venipuncture sites were disinfected with alcohol followed by povidone iodine or 2% iodine tincture and allowed to dry. Up to 30 ml of blood was obtained from each patient with a sterile needle and syringe. Needles were not changed before or between inoculations of blood culture bottles. Ten milliliters of blood was placed in random order into each of three blood culture bottles: a BM standard aerobic bottle (BacT/AlertSA), a BD standard aerobic bottle (BACTEC Standard 10 Aerobic/F) and a BM anaerobic FAN bottle (which was not part of the evaluation but was included for improved recovery of microorganisms).

Adequacy of blood volume. Upon receipt of the bottles in the laboratory, the volume of fluid in each bottle was measured against a volume standard to determine how many milliliters of blood had been inoculated into each of the bottles. All bottles were processed regardless of the volume of blood received. A bottle pair (one BM and one BD bottle) was included in the data analysis if both bottles contained 8 to 12 ml of blood.

Bottle processing. Procedures at both hospitals were done according to the same IRB-approved study protocol. Upon receipt in the laboratory, one bottle from each culture set was placed in its respective BM or BD instrument; each was incubated for 5 days or until the instrument signaled a positive result. A bottle flagged by the instrument as positive was removed, and an aliquot of the blood-broth mixture was removed from the bottle with a sterile needle and syringe. A portion was used for a Gram stain, and the remainder was subcultured onto solid plate medium according to the results of the Gram stain. Subsequent microbial isolation and identification and antimicrobial susceptibility testing were performed according to standard techniques (3a). Bottles with results that were Gram stain negative were returned to the instrument for the remainder of the 5-day incubation period or until reflagged by the

TABLE 1. Comparative yields of clinically important microorganisms in BM and BD blood culture bottles

Microorganism(s)	No. of isolates detected in:			*P* value
	Both bottles	BM bottles only	BD bottles only	
Gram-positive cocci				
Staphylococcus aureus	109	23	12	NS[a]
Coagulase-negative staphylococci	32	16	6	<0.05
Streptococcus spp.[b]	16	3	4	NS
Enterococcus spp.[c]	66	15	13	NS
Gram-positive bacilli[d]	2	0	1	NS
Gram-negative bacilli				
Enterobacteriaceae[e]	66	17	14	NS
Pseudomonas aeruginosa	11	4	2	NS
Other gram-negative bacteria[f]	19	7	5	NS
Anaerobic bacteria[g]	0	0	2	NS
Fungi[h]	27	23	8	<0.01
All microorganisms	348	108	67	<0.005

[a] NS, not significant ($P > 0.05$).
[b] Includes eight viridans group *Streptococcus*, seven *Streptococcus bovis*, six *Streptococcus pneumoniae*, and two group G beta-hemolytic *Streptococcus* isolates.
[c] Includes 55 *Enterococcus faecalis*, 36 *Enterococcus faecium*, and 2 *Enterococcus avium* isolates and 1 *Enterococcus* sp. isolate.
[d] Includes one isolate each of *Aerococcus viridans*, *Bacillus* sp., and *Corynebacterium* sp.
[e] Includes 36 *Klebsiella pneumoniae*, 27 *Escherichia coli*, 7 *Enterobacter cloacae*, 6 *Serratia marcescens*, 6 *Proteus mirabilis*, 4 *Klebsiella oxytoca*, 2 *Morganella morganii*, 2 *Proteus vulgaris*, and 2 *Salmonella enterica* serovar *Typhi* isolates and 1 isolate each of *Cedecea* sp., *Citrobacter freundii*, *Enterobacter aerogenes*, *Klebsiella ozaenae*, and *Proteus rettgeri.*
[f] Includes 11 *Acinetobacter baumannii*, 7 *Burkholderia cepacia*, 8 *Stenotrophomonas maltophilia*, 2 *Acinetobacter lwoffii*, and 2 *Alcaligenes faecalis* isolates and 1 *Aeromonas hydrophila* isolate.
[g] Includes one isolate each of *Clostridium perfringens* and *Bacteroides thetaiotaomicron.*
[h] Includes 28 *Candida albicans*, 7 *Candida tropicalis*, 18 *Candida glabrata*, 2 *Cryptococcus neoformans*, and 2 *Hansenula anomala* isolates and 1 *Scedosporium prolificans* isolate.

TABLE 2. Comparative yields of clinically important microorganisms in BM and BD blood culture bottles containing samples from patients receiving antimicrobial therapy

Microorganism(s)	No. of isolates detected in:			*P* value
	Both bottles	BM bottles only	BD bottles only	
Gram-positive cocci				
Staphylococcus aureus	22	10	7	NS[a]
Coagulase-negative staphylococci	11	4	2	NS
Streptococcus viridans group	5	0	0	NS
Enterococcus spp.[b]	22	6	3	NS
Gram-negative bacilli				
Enterobacteriaceae[c]	19	4	2	NS
Other gram-negative bacilli[d]	2	3	2	NS
Anaerobic bacteria[e]	0	0	1	NS
Yeasts[f]	9	12	2	<0.025
All microorganisms	90	39	19	<0.01

[a] NS, not significant ($P > 0.05$).
[b] Includes 16 *Enterococcus faecalis*, 13 *Enterococcus faecium*, and 2 *Enterococcus avium* isolates.
[c] Includes 15 *Klebsiella pneumoniae*, 3 *Escherichia coli*, 2 *Proteus vulgaris*, and 2 *Serratia marcescens* isolates and 1 isolate each of *Enterobacter aerogenes*, *Proteus mirabilis*, and *Proteus rettgeri*.
[d] Includes three *Burkholderia cepacia* and two *Acinetobacter baumannii* isolates and one isolate each of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*.
[e] Includes one *Bacteroides thetaiotaomicron* isolate.
[f] Includes 14 *Candida albicans*, 5 *Candida tropicalis*, 2 *Candida glabrata*, and 2 *Cryptococcus neoformans* isolates.

instrument. The Gram-stain-negative bottles that were flagged by the instrument were considered false-positive bottles if no microorganisms were isolated on subculture. Bottles that were not flagged by the instrument were incubated for a total of 5 days. Negative companion bottles from positive sets were subcultured at the end of the 5-day protocol. Bottles that were negative according to the instrument but in which a microorganism grew on subculture were considered false-negative bottles.

Clinical assessment. Each positive culture was reviewed by one of the physician investigators and coded as a true positive, a contaminant, or an isolate of unknown clinical importance. These assessments were made in accordance with published criteria and were done without knowledge of the identity of the bottles (6). True positives were defined as microorganisms that are considered pathogens when isolated from patients with signs and symptoms of disease or potential pathogens that were isolated from multiple cultures within a 48-h period. Contaminants were defined as (i) single positive cultures of a microorganism that is usually considered a contaminant in the absence of a plausible source (e.g., coagulase-negative staphylococci from a febrile patient without a central venous catheter), (ii) single positive cultures of a microorganism that is usually considered a contaminant when there is a plausible source (e.g., a central venous catheter) but that is from a clinically well patient (as in surveillance cultures), or (iii) single positive cultures of a single microorganism that is usually considered a contaminant when several other cultures performed within the same time frame are negative. Isolates of unknown significance were defined as (i) single cultures of a potential pathogen (e.g., coagulase-negative staphylococci, viridans group streptococci, or enterococci) from a symptomatic patient for which there was a plausible source but in which case only one sample was submitted to the laboratory or (ii) single cultures of a usual contaminant (e.g., a *Bacillus* sp., a diphtheroid, a *Lactobacillus* sp., or a *Micrococcus* sp.) in a sample from a symptomatic patient for which there was a plausible source but in which case only one sample was submitted to the laboratory. An episode of bacteremia or fungemia was defined as a period beginning with the first positive blood culture and ending when 7 days (2 days for coagulase-negative staphylococci) had passed without another blood culture positive for the same microorganism, regardless of whether negative cultures resulted in the intervening days. When a different clinically significant isolate was detected within 3 days of the first isolate, the episode was considered polymicrobial. Patients were considered to be on therapy at the time the blood sample was drawn if they were receiving an antimicrobial agent with activity against the microorganism isolated. Susceptibility was documented for isolates actually tested by NCCLS methods or presumed for those isolates not ordinarily tested because the antimicrobial agent given is known to be active.

Data analysis. Comparison of recovery rates for the bottles was done with the chi-square test. Yates' correction was used when the number of comparisons was less than 20 (2). When both bottles in a set were positive within 72 h, the times to detection were compared by using the Wilcoxon signed rank sum test for continuous paired data that are not normally distributed.

RESULTS

The laboratory received a total of 8,630 blood culture sets that each contained both bottles. Of these sets, 6,743 (78%) met the criteria for adequacy of filling (3,378 from Robert Wood Johnson University Hospital and 3,365 from Duke University Medical Center). There were 523 isolates classified as clinically significant isolates from 257 patients. The BM bottles detected more coagulase-negative staphylococci ($P < 0.05$), yeasts ($P < 0.01$), and all microorganisms combined ($P < 0.005$) (Table 1). The results of a subset of blood cultures from patients who were being treated with antimicrobial agents at the time the blood cultures were done are summarized in Table 2. Yeasts ($P < 0.025$) and all microorganisms combined ($P < 0.01$) were detected more often in BM bottles than in BD bottles in this subgroup. For the subset of patients not receiving antimicrobial therapy, there were no

TABLE 3. Comparative times to detection in BM and BD blood culture bottles when both bottles were positive within 72 h

Microorganism(s)	No. of isolates detected	Time to detection (h)				*P* value
		BM bottles		BD bottles		
		Median	Range	Median	Range	
Staphylococcus aureus	108	13	4–74	13	2–57	NS[a]
Coagulase-negative staphylococci	32	21	4–55	19	3–68	NS
Enterococcus spp.[b]	66	13	4–70	12	4–57	0.003
Other gram-positive bacteria[c]	16	13	6–48	11	4–39	0.0001
Escherichia coli	13	12	7–36	10	5–68	NS
Klebsiella pneumoniae	26	11	5–65	10	1–46	0.003
Other *Enterobacteriaceae*[d]	23	15	6–58	13	4–44	NS
Pseudomonas aeruginosa	11	18	11–26	15	9–30	NS
Other gram-negative bacteria[e]	16	17	7–38	15	4–49	0.008
Candida albicans	13	25	16–47	27	15–54	0.014
Other yeasts[f]	10	34	13–65	36	12–66	NS
Mold	1	62		57		
All microorganisms	335	14	4–70	13	1–68	<0.0001

[a] NS, not significant ($P > 0.05$).
[b] Includes 40 *Enterococcus faecalis*, 23 *Enterococcus faecium*, and 2 *Enterococcus avium* isolates and 1 *Enterococcus* sp. isolate.
[c] Includes five *Streptococcus bovis*, five viridans group streptococcus, and three *Streptococcus pneumoniae* isolates and one isolate each of group G streptococcus, *Corynebacterium* sp., and *Aerococcus viridans*.
[d] Includes five *Serratia marcescens*, four *Enterobacter cloacae*, four *Proteus mirabilis*, three *Klebsiella oxytoca*, and two *Proteus vulgaris* isolates and one isolate each of *Citrobacter freundii*, *Klebsiella ozaenae*, *Morganella morganii*, *Providencia rettgeri*, and *Salmonella enterica* serovar Typhi.
[e] Includes six *Acinetobacter baumannii*, six *Stenotrophomonas maltophilia*, two *Acinetobacter lwoffii*, and two *Burkholderia cepacia* isolates.
[f] Includes five *Candida glabrata*, three *Candida tropicalis*, and two *Hansenula anomala* isolates.

statistically significant differences between the results for any microorganism group.

The median times to detection by the instrument for 335 clinically significant isolates when both bottles were positive within 72 h are shown in Table 3. The overall median times to detection were similar the for BM and BD bottles (14 and 13 h, respectively). Although statistical significance was shown for the results of some of the subgroups of microorganisms, the difference in median times to detection was 3 h or less in all cases.

There were no differences in numbers of unimicrobial episodes of bacteremia and fungemia detected when all microorganisms were combined (Table 4). However, there were significantly more episodes of yeast fungemia detected in BM bottles ($P < 0.025$) than in BD bottles.

Of the 377 contaminant isolates detected during this study, 115 were detected in both bottles, 149 were detected in BM bottles only, and 113 were detected in BD bottles only ($P < 0.05$). There were eight (one BM and seven BD) false-negative bottles. The BM instrument failed to detect one isolate of *Enterococcus faecium*. The BD instrument failed to detect two isolates of *Staphylococcus aureus*, one isolate of *E. faecium*, one isolate of group G streptococcus, two isolates of *Candida albicans*, and one isolate of *Candida glabrata*.

Among the 6,743 paired blood culture sets, false-positive bottles were seen more with BM (31 [0.5%]) than with BD (6 [0.1%]) bottles.

DISCUSSION

Clinical microbiology laboratory directors and supervisors frequently must decide on the optimal blood culture system for use in their institutions. The decision process includes evaluations of the price, the service of the manufacturer, the institutional contracts, the available medium for the instrument, the medium performance, the patient populations, and other parameters. In this controlled clinical evaluation, we addressed the performance of two proprietary formulations of common media used in the two most widely used blood culture instruments, namely, the BM and the BD blood culture systems.

This study, in which we examined more than 6,000 pairs of

TABLE 4. Comparative yields of clinically important episodes of infection detected in BM and BD blood culture bottles

Microorganism(s)	No. of episodes detected in:			*P* value
	Both bottles	BM bottles only	BD bottles only	
Staphylococcus aureus	75	10	4	NS[a]
Coagulase-negative staphylococci	28	4	2	NS
Streptococcus spp.[b]	7	1	1	NS
Enterococcus spp.[c]	31	4	10	NS
Enterobacteriaceae[d]	39	5	4	NS
Other gram-negative bacteria[e]	19	4	4	NS
Yeasts[f]	19	13	3	<0.025
All microorganisms	220[g]	41	30[h]	NS

[a] NS, not significant ($P > 0.05$).
[b] Includes three viridans group streptococcus, three *Streptococcus pneumoniae*, and two *Streptococcus bovis* isolates and one group G streptococcus isolate.
[c] Includes 30 *Enterococcus faecalis* and 14 *Enterococcus faecium* isolates and 1 *Enterococcus avium* isolate.
[d] Includes 19 *Klebsiella pneumoniae*, 16 *Escherichia coli*, 4 *Enterobacter cloacae*, 2 *Proteus mirabilis*, and 2 *Serratia marcescens* isolates and 1 isolate each of *Cedecea* sp., *Citrobacter freundii*, *Enterobacter aerogenes*, *Klebsiella oxytoca*, and *Salmonella enterica* serovar Typhi.
[e] Includes 11 *Pseudomonas aeruginosa*, 8 *Acinetobacter baumannii*, 4 *Stenotrophomonas maltophilia*, and 2 *Burkholderia cepacia* isolates and 1 isolate each of *Acinetobacter lwoffii* and *Alcaligenes* spp.
[f] Includes 20 *Candida albicans*, 10 *Candida glabrata*, and 3 *Candida tropicalis* isolates and 1 isolate each of *Cryptococcus neoformans* and *Hansenula anomala*.
[g] Includes one isolate each of *Aerococcus viridans* and *Corynebacterium* sp.
[h] Includes one isolate each of *Clostridium perfringens* and *Bacteroides thetaiotaomicron*.

TABLE 5. Comparison of formulations for BM and BD standard aerobic media[a]

Characteristic	Formulation for:	
	BM medium	BD medium
Vol (ml)	40	40
Pancreatic digest of casein (% [wt/vol])	1.7	
Pancreatic digest of soybean meal (% [wt/vol])	0.3	
Soybean-casein digest broth (% [wt/vol])		3.0
Sodium polyanethole sulfonate (% [wt/vol])	0.035	0.025
Pyridoxine or pyridoxal HCl (% [wt/vol])	0.001	0.001
Other complex amino acids and carbohydrate substrates in purified water	As needed	
Atmosphere	CO_2 in oxygen under vacuum	CO_2
Other proprietary adjustments		As needed
Yeast extract (% [wt/vol])		0.3
Animal tissue digest (% [wt/vol])		0.01
Sucrose (% [wt/vol])		0.1
Hemin (% [wt/vol])		0.0005
Menadione (% [wt/vol])		0.00005
Sodium bicarbonate (% [wt/vol])		0.04

[a] Medium formulations derived from respective manufacturers' package inserts.

blood culture bottles containing comparable volumes of blood, showed more similarities than differences between the systems. The superior recovery of clinically important coagulase-negative staphylococci and yeasts in the BM system is noteworthy. Coagulase-negative staphylococci now represent one of the five most common blood culture isolates associated with bacteremia. This change is related to the increased use of intravascular catheters and other implantable devices for patient care (7). Although the BM medium detected more clinically important coagulase-negative staphylococci than did the BD medium, it also grew more contaminants. The improved recovery of coagulase-negative staphylococci in the BM medium was not demonstrated by episode analysis, suggesting that additional cultures of blood from these patients during this episode were also positive in BD medium.

Yeasts are also being detected more frequently in patients with bloodstream infections, and rapid diagnosis by modern blood culture techniques is critical to optimal patient care (7). Yeasts generally prefer a less nutritive medium than that preferred by bacteria for optimal growth. The BM medium is less nutritious than the BD medium (Table 5), which may explain the superior yeast recovery in the BM medium. This advantage was evident even in the presence of antimicrobial agents at the time the blood sample was drawn. There is also added oxygen in the BM medium, which provides a more aerobic atmosphere for growth of yeasts. Because significantly more episodes of bloodstream infections with yeasts were detected by the BM system, more appropriate therapeutic interventions could be instituted.

The times to detection of microorganisms showed a wide range in both the BM and the BD media (Table 3). It is unlikely that differences between median times to detection of less than 3 h for any group of microorganisms would be clinically important. One should be cautious about overinterpreting statistical differences in times to detection that have little or no clinical meaning because of the wide range of times to detection and the appreciable overlap in recovery between the two systems.

In conclusion, the results of this study suggest that clinical laboratories that use the current standard BM or BD aerobic media will find enhanced detection of coagulase-negative staphylococci and yeasts with the BM system.

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