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Clinical Comparison of BACTEC 9240 Plus Aerobic/F Resin Bottles and the Isolator Aerobic Culture System for Detection of Bloodstream Infections

FRANK R. COCKERILL III,[1,2]* GAY S. REED,[1] JOHN G. HUGHES,[1] CATHERINE A. TORGERSON,[1] EMILY A. VETTER,[1] WILLIAM S. HARMSEN,[3] JANE C. DALE,[4] GLENN D. ROBERTS,[1] DUANE M. ILSTRUP,[3] AND NANCY K. HENRY[5]

Division of Clinical Microbiology, Department of Laboratory Medicine and Pathology,[1] Division of Infectious Diseases, Department of Internal Medicine,[2] Department of Laboratory Medicine and Pathology, Division of Clinical Biochemistry and Immunology,[4] Section of Biostatistics, Department of Health Sciences Research,[3] and Department of Pediatric and Adolescent Medicine,[5] Mayo Clinic, Rochester, Minnesota 55905

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The Plus Aerobic/F resin bottle of the BACTEC 9240 automated blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) was compared with aerobic culture of the Isolator system (Wampole Laboratories, Cranbury, N.J.) for the detection of bloodstream microorganisms from 6,145 blood cultures collected from adult patients with suspected septicemia. The BACTEC resin bottles were incubated for 7 days, and the sediment from the Isolator tube was inoculated to sheep blood and chocolate agars which were incubated for 72 h and to inhibitory mold, brain heart infusion, and Sabouraud agars which were incubated for 21 days. A total of 622 microorganisms were recovered from 583 blood cultures. The BACTEC resin bottle recovered statistically significantly more pathogens overall than the Isolator system ($P = 0.0006$). When individual pathogens isolated from either system for a 7-day study period were assessed, it was determined that the BACTEC resin bottle detected statistically significantly more isolates of *Staphylococcus aureus* ($P = 0.0113$) and coagulase-negative *Staphylococcus* spp. ($P = 0.0029$) than the Isolator system. The BACTEC resin bottle also detected statistically significantly more bloodstream infections (septic episodes) caused by coagulase-negative *Staphylococcus* spp. ($P = 0.0146$). The Isolator system recovered statistically significantly more contaminants overall ($P < 0.0001$), and among this group of microorganisms, recovered statistically significantly more *Bacillus* spp. ($P < 0.0001$), coagulase-negative *Staphylococcus* spp. ($P < 0.0001$), and viridans group *Streptococcus* spp. ($P = 0.0156$). The Isolator system detected statistically significantly more isolates of *Histoplasma capsulatum* ($P = 0.004$), but all of these isolates were detected at ≥7 days of incubation of fungal plates, i.e., after the system to system comparison study period (7 days). In blood culture sets which produced growth of the same pathogen in both systems, there was a statistically significant difference in median time to detection for all pathogens combined favoring the BACTEC resin bottle over the Isolator tube ($P < 0.05$). When assessing individual microorganisms, the median times for detection of *S. aureus*, *Enterococcus* spp., and *Pseudomonas* spp. were all statistically significantly less for the BACTEC system ($P < 0.05$). The BACTEC instrument had 79 (1.3%) false positive signals. The BACTEC system required less processing time than the Isolator system and eliminates the hands-on time for detection of positive cultures required with the Isolator system.

A manual aerobic blood culture system used routinely at the Mayo Clinic, Rochester, Minn., the Isolator system (Wampole Laboratories, Cranbury, N.J.), was compared with an aerobic resin bottle (Plus Aerobic/F) of the BACTEC 9240 automated blood culture system (Becton Dickinson Diagnostic Instrument Systems, Sparks, Md.) for the recovery of microorganisms from blood in adult patients. The BACTEC 9240 instrument monitors increases in CO_2 concentration produced by growing microorganisms by means of a fluorescent sensor located in the bottom of each bottle (6). Detection frequencies and time to detection of bloodstream microorganisms and detection frequencies of bloodstream infections (septic episodes) for these two aerobic blood culture systems were compared.

* Corresponding author. Mailing address: Division of Clinical Microbiology, Mayo Clinic, Hilton 4, 200 First St. S.W., Rochester, MN 55905. Phone: 507-284-2901. Fax: 507-284-4272. E-mail: Cockerill.franklin@mayo.edu.

MATERIALS AND METHODS

All blood cultures were obtained from patients over 16 years of age at the Mayo Medical Center in Rochester, Minn. The Mayo Medical Center consists of two large teaching hospitals (combined beds, ~1,600) and a large subspecialty clinic. The same procedures for collection of blood, processing, and detection of microorganisms were used for all patients. Phlebotomists aseptically collected approximately 30 ml of peripheral blood using a needle and syringe. Equal volumes of this blood were inoculated into three blood culture receptacles at the patient's bedside by using an inoculation sequence which had been predetermined for each set of blood culture bottles by a randomization schedule. Therefore, blood was distributed equally into the two study receptacles, a 10-ml Isolator tube and a BACTEC 9240 Plus Aerobic/F resin bottle, and into a nonstudy receptacle, a nonvented Septi-Chek bottle (Becton Dickinson, Cockeysville, Md.). Due to limitations of the amount of blood collected per phlebotomy, the evaluation did not permit comparison of an anaerobic bottle (Plus Anaerobic/F or Lytic/10 Anaerobic/F) on the BACTEC 9240 system with the anaerobic blood culture bottle used during the study in our laboratory, the nonvented Septi-Chek bottle. A total collection volume of at least 15 ml (5.0 ml per receptacle) was required for inclusion in the study.

The Isolator tube was processed in the Clinical Microbiology Laboratory according to the manufacturer's instructions. Sediment from the Isolator tube was inoculated onto 5% sheep blood trypticase soy agar (SBA) and onto chocolate agar (CA), which were incubated at 35°C with 5 to 7% CO_2 for 72 h, and onto brain heart infusion agar, inhibitory mold agar, and Sabouraud dextrose agar, which were incubated at 30°C for 21 days. The nonvented Septi-Chek bottle

TABLE 1. Comparison of BACTEC Plus Aerobic/F resin bottle to Isolator system

Microorganism	No. of microorganisms detected with:				*P* value[b]
	Isolator system only	BACTEC resin bottle only	Both systems	Neither system[a]	
All microorganisms	198	130	294	73	0.0002
Pathogens					
All microorganisms	60	105	277	53	0.0006
S. aureus	10	26	70	0	0.0113
Staphylococcus spp. (coagulase-negative)	11	31	46	4	0.0029
Streptococcus pneumoniae	1	1	3	0	NS[c]
Streptococcus spp. (viridans group)	0	2	10	1	NS
Enterococcus spp.	3	8	18	6	NS (0.2266)
Enterobacteriaceae	13	13	39	7	NS
Escherichia coli	5	5	13	6	NS
Pseudomonas spp.	3	6	26	0	NS
Obligately anaerobic bacteria	0	0	0	16	NS
Other bacteria	1	3	11	0	NS
Candida spp.	12	10	41	3	NS (0.8318)
Other fungi[d]	2	0	0	0	NS
Contaminants					
All microorganisms	138	25	17	20	<0.0001
Bacillus spp.	18	0	2	0	<.0001
Corynebacterium spp.[e]	7	6	1	2	NS
Propionibacterium spp.	0	0	0	12	NS
Staphylococcus spp. (coagulase-negative)	99	17	7	5	<0.0001
Streptococcus spp. (viridans group)	7	0	2	3	.0156
Other bacteria[f]	7	2	5	0	NS

[a] Refers to the number of isolates detected only with the Septi-Chek anaerobic blood culture system.
[b] Refer to Methods section for calculation of *P* values.
[c] NS, not significant; *P* value of >0.05.
[d] Other fungi included *Cladosporium* spp., *Geotrichum* spp., and unidentified species. Not shown are 9 *H. capsulatum* isolates detected only with the Isolator system ($P = 0.004$). However, these isolates were detected after the 7-day two-system comparison study period.
[e] Does not include *Corynebacterium jeikeium.*
[f] Other bacteria included *Acinetobacter* spp. and nonfermenting gram-negative bacteria which were not further identified.

was incubated for 7 days at 35°C. The SBA and CA Isolator sediment plates and the Septi-Chek bottle were manually examined twice daily during the first 48 h after collection and daily thereafter. Brain heart infusion agar, inhibitory mold agar, and Sabouraud dextrose agar plates were examined once daily. The BACTEC resin and the Septi-Chek bottles were discarded after 7 days and therefore were not further evaluable. The Isolator fungal plates were evaluable for 14 days beyond the time the BACTEC bottles were evaluable (21 days in all). However, isolates detected on Isolator fungal plates after more than 7 days were not considered in the system to system comparison.

Upon receipt in the Clinical Microbiology Laboratory, BACTEC Plus Aerobic/F resin bottles were loaded into the instrument into the computer-assigned position and incubated for 7 days with continuous agitation. The BACTEC 9240 instrument was observed at 4-h intervals for positive signals. Whenever a positive signal occurred, the bottle was removed from the instrument to be Gram stained and subcultured. If the Gram stain was negative, the bottle was returned to the instrument. Bottles that produced positive signals but were negative on Gram stain and subculture on CA incubated at 35°C with 5 to 7% CO_2 and on SBA incubated under anaerobic conditions were recorded as instrument false positives.

Microorganisms isolated from positive cultures were identified by standard biochemical techniques. Time to detection was defined as the time elapsed from collection until detection of a positive Gram stain. This was dependent on the routine examination schedules for both the manual and automated systems.

Microorganisms isolated from blood were classified as pathogens if the identity of the organism rarely characterized it as being a contaminant. If microorganisms were either viridans group *Streptococcus* spp. or coagulase-negative *Staphylococcus* spp., chart reviews were conducted by one of the authors, an infectious diseases specialist (F.R.C.), to determine if these isolates were clinically significant. Bloodstream infections (septic episodes) were defined by criteria modified from those previously published by Kirkley and colleagues (5). To summarize, a bloodstream infection was defined as the initial isolation of a pathogen, the subsequent isolation of a different pathogen, or the isolation of the same pathogen after at least a 5-day interval since the first positive culture with that organism. If more than one pathogen was isolated from a blood culture set, each individual pathogen was counted as a separate bloodstream infection.

For each organism species detected (and overall), comparisons of the detection rates of the two systems were assessed by using the sign test. Paired comparisons of the time to detection between the two systems were made by using the Wilcoxon signed rank test. All calculated *P* values were two sided, and *P* values of ≤0.05 were considered to be statistically significant.

RESULTS

Results are provided in Tables 1 to 3. A total of 6,145 blood cultures met the criteria for inclusion in the study. Microbial growth was produced by 583 cultures (9.5%), yielding a total of 622 microorganisms. Based on our criteria for pathogens and contaminants, 442 of 622 microorganisms were considered pathogens; 180 microorganisms were considered contaminants.

The BACTEC Plus Aerobic/F resin bottle detected statistically significantly more pathogens overall than the Isolator system (Table 1). When assessing individual pathogens, the BACTEC resin bottle detected statistically significantly more *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. isolates than the Isolator system (Table 1). The BACTEC resin bottle detected statistically significantly more bloodstream infections (septic episodes) caused by coagulase-negative *Staphylococcus* spp. (Table 2). The Isolator system recovered statistically significantly more contaminants overall and among this group of microorganisms recovered statistically significantly more *Bacillus* spp., coagulase-negative *Staphylococcus* spp., and viridans group *Streptococcus* spp.

In blood culture sets which produced growth of the same pathogens in both systems, there was a statistically significant difference in median time to detection for all pathogens com-

TABLE 2. Summary of bloodstream infections detected with BACTEC Plus Aerobic/F resin bottle and/or the Isolator system[a]

Microorganism	No. of microorganisms detected with:				*P* value[c]
	Isolator system, BACTEC resin bottle, and both systems (total)	Isolator system only	BACTEC resin bottle only	Both systems	
All microorganisms	241	34	52	155	0.0662
S. aureus	49	6	9	34	NS[d]
Staphylococcus spp. (coagulase-negative)[b]	56	6	19	31	0.0146
Streptococcus pneumoniae	3	1	0	2	NS
Streptococcus spp. (viridans group)[b]	8	0	2	6	NS (0.50)
Enterococcus spp.	17	3	3	11	NS
Enterobacteriaceae	36	6	8	22	NS
E. coli	13	3	2	8	NS
Pseudomonas spp.	24	2	5	17	NS
Other bacteria	7	0	2	5	NS
Candida spp.	27	6	2	19	NS (0.2891)
Other fungi[e]	1	1	0	0	NS (1.0)

[a] Refer to Methods section for definition of bloodstream infection.
[b] Refer to Methods section for categorization of these isolates as pathogens.
[c] Refer to Methods section for calculation of *P* values.
[d] NS, not significant; *P* value of >0.05.
[e] Two bloodstream infections caused by *H. capsulatum* were detected only with the Isolator system but at >7 days, which was after the two-system comparison study period (7 days).

bined, favoring the BACTEC resin bottle over the Isolator system (Table 3).

The Isolator system detected statistically significantly more isolates of *Histoplasma capsulatum* (nine versus zero), but all were identified after more than 7 days of incubation, i.e., after the two-system comparison study period. Eight *H. capsulatum* isolates were from one patient, and the ninth isolate was from a different patient. In addition, two isolates of *Candida glabrata* from one patient and one isolate of *Geotrichum* sp. from a different patient were recovered only with the Isolator system but after 7 days of incubation.

The BACTEC 9240 instrument had 79 (1.3%) false positive signals. That is, the Gram stain of broth from bottles that produced a positive signal on the BACTEC 9240 instrument was negative and subculture of broth to solid medium produced no growth.

DISCUSSION

In the current study, we compared two aerobic blood culture systems simultaneously. The results of our study are generally in agreement with those from another study, by Pohlman and colleagues (7), which also compared the performance of the Isolator aerobic culture system to the BACTEC Plus Aerobic/F resin bottle. Like our study, Pohlman and colleagues showed that statistically significantly more isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. were recovered from the BACTEC resin bottle compared to the Isolator system. However, the Pohlman study also showed that statistically significantly more isolates of *Pseudomonas aeruginosa* and members of the family *Enterobacteriaceae* were recovered with the BACTEC resin bottle. In our study, the Isolator system and BACTEC resin bottle recovered equivalent numbers of *Enter-*

TABLE 3. Comparison of detection times of pathogens for matched BACTEC Plus Aerobic/F resin bottle and Isolator components

Microorganism (no.)	Detection time (h)			*P* value[b]
	Isolator system [median (mean)]	BACTEC resin bottle [median (mean)]	Difference in detection time (h) between Isolator and BACTEC systems [median (mean)], IQR[a]	
E. coli (13)	16.0 (20.7)	17.0 (17.3)	0.0 (3.4), 0 to 4	NS[c] (>0.10)
Enterobacteriaceae (39)	20.0 (23.5)	19.0 (21.4)	0.0 (2.1), 0 to 10	NS (>0.10)
Pseudomonas spp. (26)	37.5 (38.2)	25.5 (31.4)	6.0 (6.8), 0 to 16	<0.02
S. aureus (70)	23.5 (31.9)	23.5 (26.5)	0.0 (5.5), 0 to 9	0.02
Coagulase-negative *Staphylococcus* spp. (46)	25.0 (31.8)	24.0 (27.6)	0.0 (4.2), −3 to 10	NS (0.4177)
Enterococcus spp. (18)	20.0 (24.4)	17.0 (18.0)	0.0 (6.4), 0 to 8	<0.01
Streptococcus spp. (viridans group) (10)	19.5 (20.8)	21.0 (21.0)	0.0 (0.2), −6.0 to 0.0	>0.10
Streptococcus pneumoniae (3)	13.0 (15.7)	13.0 (15.7)	0.0 (0.0), 0.0 − 0.0	—[d]
Other bacteria (11)	18.0 (22.0)	12.0 (12.8)	8.0 (9.2), 3 to 10	<0.01
Candida spp. (43)	42.0 (45.3)	41.0 (47.9)	−3.0 (2.8), −11 to 7	NS
All pathogens (277)	24.0 (31.3)	23.0 (27.7)	0.0 (3.5), −1 to 10	<0.0004

[a] IQR, interquantile range, i.e., the range of values between the 25th and 75th percentiles.
[b] Refer to Methods section for *P* value calculations.
[c] NS, not significant; *P* value of >0.05.
[d] Significance test not done ($n = 3$).

obacteriaceae. Though more *Pseudomonas* spp. isolates were recovered from the BACTEC resin bottle in our study, this difference did not achieve statistical significance. In both studies, more *Candida* spp. isolates were recovered with the Isolator system, but these differences were not statistically significant. In our study, statistically significantly more isolates of *H. capsulatum* were recovered with the Isolator system, but these isolates were identified after the 7-day two-system comparison study period. Incubation of BACTEC resin bottles for more than 7 days may have produced positive results for these organisms, but it has been our experience that broth-based systems are less sensitive for detecting *H. capsulatum* regardless of the length of incubation period.

When assessing bloodstream infection (septic episodes), the Pohlman study demonstrated no statistically significant differences for any microorganism group. Pohlman and colleagues did observe that more bloodstream infections with coagulase-negative *Staphylococcus* spp. organisms were diagnosed with the BACTEC resin bottle than with the Isolator system (29 versus 17), but this difference was not statistically significant. In contrast, our study demonstrated statistical significance for the same comparison. Both studies showed that candidemias were more frequently diagnosed with the Isolator system, but statistically significant differences were not observed. We subsequently reviewed all of the medical records of patients who had candidemias diagnosed by using only one system. In all cases, these candidemias were clinically significant.

A concern about the Isolator blood culture system has been its relatively high rate of recovery of contaminating microorganisms, which is likely the result of the additional processing steps required for the Isolator system compared to broth systems (1, 4). Contaminated blood cultures significantly increase resource utilization and therefore add unnecessarily to the cost of medical care (1). For the current study, we considered any isolate of *S. aureus* to be a probable pathogen. This was based on two prior studies by us which determined that in the majority of cases the isolation of *S. aureus* only from the Isolator tube was clinically significant (2, 3). Based on our definition for contaminants, which excluded *S. aureus* isolates, our results showed that the Isolator system recovered statistically significantly more contaminants than the BACTEC resin bottle.

The continuous monitoring feature of the BACTEC 9240 system resulted in statistically significantly better median times of detection for all pathogens combined and for certain subgroups of organisms including *S. aureus*, *Enterococcus* spp., and *Pseudomonas* spp. Because the Isolator system must be visually inspected for growth, it is not practical to monitor the system with the same frequency as the BACTEC system. Had we monitored the Isolator system more frequently, these median time differences may have been smaller. The amount of technologist time required for pre-analytical processing of blood cultures, placement of culture plates or bottles into incubators, and screening and evaluation of positive cultures varied considerably between the two systems. For example, we have previously determined that processing of the Isolator system (centrifugation and inoculating sediment onto agar plates) requires 3.25 min (unpublished data). The Isolator system also requires manual screening of culture plates for growth. These two procedures are not required for the automated BACTEC system. Considering all required procedures, the BACTEC resin bottles required less processing time compared to the Isolator system.

In conclusion, for the two aerobic blood culture systems prospectively evaluated (Isolator and BACTEC Plus Aerobic/F resin bottle in the BACTEC 9240 system), the BACTEC resin bottle detected statistically significantly more pathogens overall and statistically significantly fewer contaminants overall. The BACTEC resin bottle detected statistically significantly more isolates of *S. aureus* and coagulase-negative *Staphylococcus* spp. and more bloodstream infections caused by coagulase-negative *Staphylococcus* spp. The Isolator system detected statistically significantly more *H. capsulatum* isolates, but this occurred after the 7-day two-system comparison period. The BACTEC system had statistically significantly shorter median detection times for all pathogens combined as well as for *S. aureus*, *Enterococcus* spp., and *Pseudomonas* spp. The BACTEC system had relatively few false positive signals and by virtue of automation required less hand-on time than the Isolator system.

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