

Clinical Comparison of the Resin-Containing BACTEC 26 Plus and the Isolator 10 Blood Culturing Systems

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The new resin-containing BACTEC 26 Plus blood culturing system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.) was compared with the Isolator 10 system (Wampole Laboratories, Cranbury, N.J.). Blood samples were drawn by syringe, and equal 10-ml volumes were evaluated in each blood culture system by the recommended methods. Both systems were incubated aerobically with 5% CO₂. Of 11,506 acceptable study specimens, 1,788 aerobic isolates were recovered. Overall, recoveries were similar for the two systems, with 626 bacteria or fungi recovered in the BACTEC 26 Plus system only, 499 recovered in the Isolator system only, and 663 recovered in both systems. Of 345 gram-negative rods, 62 grew in the BACTEC system only and 109 grew in the Isolator system only ($P < 0.001$). Thirty-three of these Isolator-only gram-negative organisms were *Acinetobacter* spp. Of 209 yeasts, 38 grew in BACTEC only and 81 grew in Isolator only ($P < 0.001$). Of 200 streptococci and enterococci, 98 were recovered in BACTEC only and 26 grew in Isolator only ($P < 0.001$). Two hundred twenty-eight independent episodes of gram-negative rod bacteremia occurred. Isolator was the first system positive in 59 of 197 episodes, compared with 45 of 197 for BACTEC when *Acinetobacter* episodes were excluded. Times to detection were similar for the two systems. High colony counts correlated with repeat positive blood cultures. Isolator and BACTEC had similar overall recoveries, with individual merits and deficiencies for both systems. The additional quantitative information derived from Isolator had utility in our institution.

Blood culturing is probably the single most useful and important test performed in clinical microbiology laboratories (5, 9, 24, 25). Not only does the result identify a potential medical emergency, but important therapeutic choices can be made on the basis of preliminary information (18). Approximately 200,000 cases of sepsis occur per year in the United States, and mortality remains at approximately 40% (6, 25). Blood culture reports must be generated rapidly if they are to affect therapeutic outcome (18).

Several systems have been developed which are more rapid than conventional blood culture systems (4, 19, 24). The Isolator system (Wampole Laboratories, Cranbury, N.J.) uses lysis of blood cells, followed by centrifugation, to prepare an inoculum that is directly plated on agar media. Colonies are usually available rapidly, facilitating further identification and susceptibility testing. Another, more automated instrument that yields rapid detection is the BACTEC system (Becton Dickinson Diagnostic Instrument Systems, Towson, Md.). Carbon dioxide generated by microbial growth is detected spectrophotometrically or radiometrically in the BACTEC system. Usually, a 1:7 to 1:10 dilution ratio is required for optimal blood culturing. However, by using the new BACTEC 26 Plus resin-containing bottle, this ratio can be decreased to 1:3.5, allowing for 10 ml of blood to be sampled and potentially shortening the time before detection. In the current study, these two rapid blood culture systems were directly compared by using blood samples that were equally divided into the BACTEC 26 Plus and the Isolator tube.

MATERIALS AND METHODS

Clinical specimens. During a 10-month period, a total of 24 ml of blood per culture set was collected by syringe from patients at the M. D. Anderson Cancer Center. Blood was collected after treatment of the skin with 70% isopropyl alcohol, a 2% povidone-iodine scrub, and a 10% povidone-iodine scrub for 30 s, 2 min, and 3 to 5 min, respectively. All blood cultures were drawn only by our laboratory liaison team. Four milliliters of blood was inoculated into an anaerobic tryptic soy broth bottle (NR7 BACTEC), and the remainder was divided equally in random order into the du Pont Isolator 10 tube (Isolator) and the tryptic soy-based BACTEC 26 Plus (BACTEC Plus) resin-containing aerobic bottle.

Accuracy of blood volume distribution was ensured by using the graduations on the syringe barrel. After the blood was divided between the study systems, the volume was written on each label and each set was placed in a separate plastic bag for transport to the laboratory. Culture sets with a volume difference of more than 1 ml were not included in the study. Similarly, sets with less than 8 ml in the 26 Plus and Isolator systems or sets delayed more than 3 h in processing were not included in the study. Also, results from growth in the anaerobic bottle were not included.

Culture processing. The two blood culture systems were processed as recommended by the manufacturers. BACTEC Plus bottles were processed by our routine 7-day protocol. Bottles were placed on an orbital-motion shaker with a 7-mm stroke at 280 rpm for 24 h. Bottles were incubated at 35°C and read by infrared spectroscopy on a BACTEC 730 instrument. Readings were made twice on days 1 and 2 and once on days 3, 4, and 7. At these times, the macroscopic

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appearance of the bottles was also noted. A bottle was subcultured and gram stained if it appeared macroscopically positive or if it exceeded a threshold of 30 arbitrary growth index units by BACTEC on day 1. On days 2, 3, 4, and 7, a growth threshold of 35 was used. Routine terminal subculture of BACTEC bottles was not performed. Positive Gram stain results or macroscopic appearance results were reported as the time of detection for this system. Susceptibility testing and identification were usually complete within 10 h of colony isolation on subculture. If growth was detected macroscopically on Isolator plates, the time of macroscopic detection was recorded as the time of detection for the Isolator system.

Isolator tubes were processed by the manufacturer's protocol. Study specimens were completely processed within 3 h of collection in a laminar-flow biosafety cabinet. Tubes were centrifuged at $3,000 \times g$ for 30 min, and the concentrate was equally distributed between two blood agar and two chocolate agar plates (Scott Laboratories, Fiskeville, R.I.). The plates were incubated at 35°C with 5% CO₂ and observed once per day for 4 days. Initial readings were made after overnight incubation. All plates with six or more representative colonies were Gram stained and processed for identification and susceptibility testing. If fewer than six colonies were detected, then presumptive isolates of coagulase-negative staphylococci, streptococci, micrococci, coryneform bacteria, and bacilli were not reported. The following screening procedures were used on all morphologically compatible colonies from Isolator: Gram stain, Staphaurex, L-pyrrolidonyl-*p*-naphthylamide, optochin susceptibility, oxidase, and motility. For fewer than six colonies, all gram-negative rods, screen-positive organisms, molds, yeasts, branching rods, and suspected acid-fast bacilli were sent for identification and susceptibility testing. Thus, for BACTEC any isolate was reported but for Isolator selected skin flora were not reported.

For both systems, most of the identifications and susceptibility tests were performed with a Vitek AMS Instrument (Vitek Instruments, St. Louis, Mo.).

Clinical assessment. An episode of bacteremia or fungemia was defined as the first positive blood culture or the result of a new positive blood culture occurring more than 7 days after the preceding positive result. Positive cultures occurring within 7 days and belonging to the same species were considered to originate from one episode.

Statistical analysis. Comparison of the two blood culture systems was made with acceptable paired study specimens only. Probability values were determined by using the McNemar modification of the chi-square test as recommended by Ilstrup (13). Correlations between mutually dependent variables were made with Pearson's coefficient of correlation (8).

RESULTS

During the study period, 11,506 acceptable study specimens were evaluated. Organisms grew in 1,759 (15.3%) culture sets from 720 patients. Of the 1,788 aerobic isolates (obligate aerobes or facultative anaerobes) recovered, 249 were from polymicrobial sets. A comparison of yields of aerobic isolates in paired culture sets is shown in Table 1. More organisms were recovered in the BACTEC Plus system (1,289) than in the Isolator system (1,162) ($P < 0.01$). Recovery rates of clinically significant isolates and contaminants are compared in Table 2. Both peripheral blood and central venous catheter sources showed significantly more

TABLE 1. Comparison of microbial recoveries in BACTEC 26 Plus and Isolator 10 tubes

Microorganism	No. of isolates recovered				P value
	BACTEC only	Isolator only	Both systems		
<i>Micrococcaceae</i>					
Coagulase-negative staphylococci	353	176	220	<0.001	
<i>Staphylococcus aureus</i>	26	25	83	NS ^a	
<i>Micrococcus</i> sp.	2	5	4	NS	
<i>Streptococcaceae</i>					
<i>Streptococcus</i> sp. group A	3	0	1	NS	
<i>Streptococcus</i> sp. group B	2	0	5	NS	
Viridans group streptococci	57	7	34	<0.001	
Other streptococci ^b	11	1	6	<0.05	
Enterococci	20	18	22	NS	
<i>Streptococcus pneumoniae</i>	5	0	8	NS	
Gram-negative rods					
<i>Citrobacter</i> sp.	3	7	2	NS	
<i>Enterobacter</i> sp.	14	17	22	NS	
<i>Escherichia</i> sp.	14	12	41	NS	
<i>Klebsiella</i> sp.	6	5	27	NS	
<i>Morganella</i> sp.	0	2	0	NS	
<i>Proteus</i> sp.	1	1	2	NS	
<i>Salmonella</i> sp.	3	0	3		
<i>Serratia</i> sp.	0	1	4	NS	
<i>Pseudomonas aeruginosa</i>	6	5	19	NS	
Other <i>Pseudomonas</i> spp.	5	19	36	<0.01	
<i>Achromobacter</i> sp.	1	0	3	NS	
<i>Acinetobacter calcoaceticus</i>	1	10	8	<0.05	
<i>Acinetobacter lwoffii</i>	1	23	4	<0.001	
<i>Aeromonas</i> sp.	1	0	0	NS	
<i>Agrobacter</i> sp.	0	1	0	NS	
CDC ^c groups VE2 and DF2	1	3	0	NS	
<i>Flavobacterium</i> sp.	1	1	0	NS	
<i>Haemophilus</i> sp.	3	0	2	NS	
<i>Moraxella</i> sp.	1	2	1	NS	
Gram-positive rods					
<i>Bacillus</i> sp.	19	11	6	NS	
Corynebacteria	26	16	0	NS	
<i>Listeria</i> sp.	0	1	2	NS	
Other positive rods	0	5	6	NS	
<i>Mycobacterium chelonae</i>	0	5	0	NS	
Yeasts					
<i>Candida albicans</i>	13	14	45	NS	
<i>Candida</i> sp.	22	40	40	<0.05	
<i>Torulopsis glabrata</i>	2	7	3	NS	
Other yeasts ^d	1	20	2	<0.001	
Molds					
	2	39	2	<0.001	

^a NS, not significant.

^b Other streptococci: group D non-Enterococci and gamma streptococci not grouped and in groups C, G, and F.

^c CDC, Centers for Disease Control.

^d Other yeasts: *Cryptococcus*, *Malassezia*, *Trichosporon*, *Rhodotorula*.

contaminants for BACTEC Plus culture than for Isolator ($P < 0.001$ and $P = 0.01$). This difference occurred when we rejected low colony count skin flora when using Isolator, a practice established in prior studies (4, 15, 16). Central venous catheter-derived cultures showed more contamination for Isolator ($P < 0.01$).

Significantly more *Micrococcaceae* and *Streptococcus*

TABLE 2. Recovery rates of significant clinical isolates by Isolator versus BACTEC 26 Plus

Culture system (blood source)	No. of organisms	
	Significant	Contaminant ^a
Isolator (peripheral blood)	484	105
BACTEC (peripheral blood)	454	194
Isolator (CVC) ^b	389	134
BACTEC (CVC)	408	197

^a Defined as any single isolate of *S. epidermidis*, *Corynebacterium* sp., *Bacillus* sp., or *Micrococcus* sp.

^b CVC, central venous catheter.

spp. were recovered by BACTEC Plus than by Isolator. Similarly, for the Isolator more gram-negative rods, yeasts, and molds were recovered. By using a screening procedure for *Staphylococcus aureus*, comparable recoveries were achieved with the two systems: 23 in Bactec Plus only and 25 in Isolator only (no significant difference). Screening prevented low colony count *S. aureus* from being rejected with coagulase-negative staphylococci. Isolator and BACTEC showed similar recoveries for enterococci but not for *Streptococcus pneumoniae*. The intensities of yeast fungemia and gram-negative bacteremia were both typically low (Fig. 1).

To demonstrate the clinical significance of yeasts recov-

TABLE 3. Clinical significance of Isolator-only yeasts^a

Microorganism	No. of isolates recovered	No. of patients	No. of patients without clinical evidence ^b	No. of patients treated
<i>C. parapsilosis</i>	18	11	2	10
<i>C. albicans</i>	15	11	1	11
<i>C. tropicalis</i>	10	5	0	4
<i>C. krusei</i>	6	1	0	1
<i>C. lusitaniae</i>	8	1	0	1
<i>C. neoformans</i>	1	1	0	1
<i>T. glabrata</i>	3	3	1	0

^a The first 54 serial isolates were evaluated.

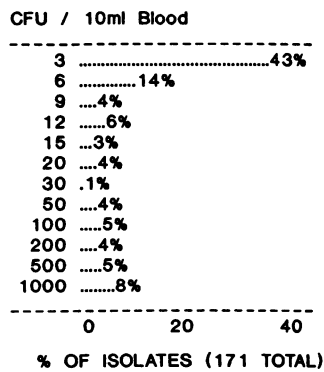
^b Required multiple blood cultures or blood plus two other sources and treatment.

ered only in the Isolator system, we reviewed the charts of the first 54 consecutive Isolator-only yeast isolates (Table 3). It was not feasible to review all of the yeasts. Of 54, 50 had additional microbiologic evidence of disease, usually including multiple blood cultures with additional positive cultures from tracheal, stool, urine, sputum, lung biopsy, ascites, pleural fluid, bronchoalveolar lavage, cerebrospinal fluid, catheter, or autopsy sources. To be considered significant, stools and urines required an additional source. In addition, 28 (85%) of 33 patients received treatment with either amphotericin B or fluconazole.

Times needed for initial detection of isolates recovered by both systems are compared in Table 4. Of 717 organisms recovered, Isolator was fastest by 1 or more days in 89 cases (12%) and BACTEC Plus was faster in 239 cases (33%; $P < 0.01$). However, the overall mean times to recovery were very similar at 1.90 days for Isolator and 1.96 days for BACTEC Plus. Yeasts were recovered, on average, 0.5 day faster by Isolator i.e., in 2.6 days by Isolator and in 3.1 days by BACTEC Plus.

Two hundred twenty-eight independent episodes of gram-negative rod bacteremia were identified. Isolator was the first system positive for 90 episodes, and BACTEC Plus recovered the isolate first in 49 episodes ($P < 0.001$). However, only 4 of 23 acinetobacters showed evidence of infection by chart review. When *Acinetobacter* spp. were removed from consideration, 59 of 197 independent episodes of gram-negative rod bacteremia were identified first by Isolator and 45 of 197 were identified first by BACTEC Plus ($P = 0.2$). Of 98 independent yeast episodes, 53 were recovered first by Isolator and 29 were recovered first by BACTEC Plus ($P < 0.05$).

YEAST HISTOGRAM



GNR HISTOGRAM

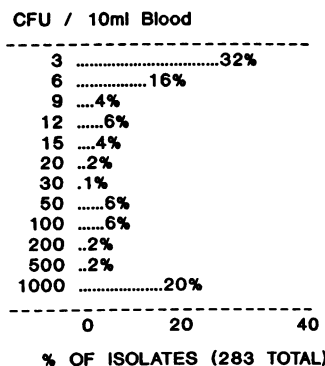


FIG. 1. Distribution of quantitative blood culture results.

TABLE 4. Speed of detection of isolates recovered in both culture systems

Microorganism(s) ^a	No. of isolates detected ^b :			P value
	First in BACTEC	First in Isolator	At the same time by both systems	
<i>Staphylococcus</i> sp.	138	32	162	<0.01
<i>Streptococcus</i> sp.	46	2	29	<0.01
Members of the family <i>Enterobacteriaceae</i>	10	3	95	NS ^c
<i>Pseudomonas</i> sp.	20	12	21	<0.05
Fungi	16	43	34	<0.01

^a Includes isolates different only by biotype recovered in both systems.

^b Number recovered one or more days faster.

^c NS, not significant.

TABLE 5. Incidence of multiple isolates versus solitary isolates of coagulase-negative staphylococci

Colony count ^a	No. of episodes ^b	
	Multiple positive isolates	Solitary isolates
<6	31	28
6-12	35	38
13-30	21	11
31-50	11	3
51-100	12	2
101-200	12	2
201-500	9	4
501-1,000	2	2
>1,000	33	3

^a Numbers of CFU found in 10 ml of blood.

^b Episodes were defined as ± 7 days from the index culture within inpatient days.

The incidence of multiple positive blood cultures correlated with the intensity of bacteremia. The number of repeat isolations of coagulase-negative staphylococci was compared with the number of solitary findings of coagulase-negative staphylococci for different quantitative categories (Table 5). High-intensity bacteremias had a significantly greater likelihood of showing multiple positive isolates (Pearson's correlation, $P < 0.05$).

DISCUSSION

Both the BACTEC system and the Isolator system yield high recovery and rapid detection of microorganisms compared with more conventional methods (11, 21, 22, 26). The BACTEC system is dependent on a semiautomated instrument, whereas the Isolator system is more labor intensive but requires little instrumentation. The data presented here demonstrate that the new BACTEC 26 Plus culture bottle has good overall recovery compared with the 10-ml Isolator tube. Isolator had significantly better recovery of fungi and gram-negative rods. BACTEC Plus achieved better recovery of *Streptococcus* and *Staphylococcus* spp. Although BACTEC recovered a higher total number of organisms, under conditions in which presumed skin flora contaminants were rejected Isolator recovered as many clinically significant isolates as BACTEC and fewer contaminants.

As had been reported previously (22), Isolator showed poor recovery of *Streptococcus* spp. The enterococci are an exception, in part because of our screening procedure. By our criteria, *S. pneumoniae* should not have been screened out. The lysis centrifugation system may have some direct toxicity for these organisms (22). However, screening procedures were used in this study as a basis for rejection of presumed skin flora found in low counts with the Isolator system. Although screening errors are not evident for *S. aureus*, gram-negative rods, yeasts, or molds, it is conceivable that some alpha-hemolytic streptococci with fewer than six colonies were rejected as coagulase-negative staphylococci. It should be noted that catalase could not be used as a screen because of lysed erythrocytes on Isolator plates.

Fungi are more frequently recovered in Isolator (2, 3, 10, 24). Yeast isolates were our third most common isolate and were frequently associated with neutropenia; 57 of 111 isolates came from patients with $\leq 1,000$ leukocytes at the time of culture. Eighty-eight percent of yeasts in the study had additional evidence of clinical significance. Mold recov-

ery must be interpreted with caution because Isolator is a plate-dependent system.

Rapid results were provided by both systems. Isolator provides rapid results relative to the earlier BACTEC system (4, 9, 15, 16, 18, 20, 22). The new BACTEC Plus bottle uses a large inoculum at a high blood-to-medium ratio. If toxic serum factors can be removed by the resin, the time to organism detection could potentially be shortened. Blood culture sets positive in both systems are more likely to occur in high-intensity bacteremias. A large initial inoculum would give more rapid detection for BACTEC but not for Isolator. Thus, for matched pairs, BACTEC Plus was faster for all subsets except yeasts; however, the overall mean times to detection were essentially equivalent for the two systems. Both the BACTEC and Isolator systems could potentially show faster recovery if the bottles or plates were examined more frequently.

The clinical relevance of an increased isolation rate associated with antimicrobial agent-removing blood culture systems has been questioned (7, 12, 14). If antimicrobial agent removal resulted in repeated isolation from the same patients, a low ratio of patient bacteremic episodes to number of isolates would be expected. However, we found that 68% of isolates were from independent episodes. These findings can be compared to the 1,093 episodes among 2,534 gram-negative rod isolates reported by Washington and Ilstrup for Septi-Check and Isolator (24); in most categories, Washington and Ilstrup found more episodes reported by Isolator (24). Broad empiric antibiotic coverage is commonly used in our hospital, yet repetitive isolation is not prominent. The issue of antimicrobial agent removal requires additional study before general utility is demonstrated. In the BACTEC Plus system, the main advantage of the resin may be that it allows inoculation of a 10-ml blood sample.

Our quantitative results show that many coagulase-negative staphylococcal isolates with fewer than six colonies are solitary findings. With the use of screening procedures, recovery of *S. aureus* in Isolator was equivalent to that of BACTEC Plus; however, many low colony count coagulase-negative staphylococci were not reported from Isolator. The cost to a patient of treating a false-positive blood culture is approximately \$4,300 (1). However, the definition of a contaminant based on fewer than six colonies is somewhat arbitrary and requires additional study (17, 20, 23).

In summary, culturing of 10 ml of blood in the BACTEC 26 Plus bottle generally gave recovery comparable to that of cultures performed from Isolator tubes. In our hospital, neither of these systems could be used alone, Isolator because of poor recovery of streptococci and BACTEC Plus because of poor recovery of yeasts. The two systems were equivalent in speed of detection. The quantitative information from Isolator was useful to predict which specimens would be associated with recurrent positive findings. Quantitative information may increase the prognostic utility of coagulase-negative staphylococcus isolation at our institution.

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