Microbiological and Clinical Evaluation of the Isolator Lysis-Centrifugation Blood Culture Tube

NANCY K. HENRY,* CAROL A. McLIMANS, ALAN J. WRIGHT, RODNEY L. THOMPSON, WALTER R. WILSON, AND JOHN A. WASHINGTON II

Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55905

In a controlled evaluation of 6,010 blood cultures, the yield of clinically significant microorganisms was greater from a lysis-centrifugation system (Isolator, Du Pont Co.) than from a nonvented vacuum bottle containing tryptic soy broth with sodium polyanetholesulfonate and CO_2 and a vented bottle containing biphasic brain heart infusion medium with sodium polyanetholesulfonate. The Isolator significantly increased the frequency of isolation of *Staphylococcus aureus* and *Candida* spp. and significantly decreased the time required for the detection of *S. aureus, Pseudomonas aeruginosa*, and *Candida* spp.; however, anaerobic bacteria were recovered significantly more frequently from nonvented bottles with tryptic soy broth, and pneumococci were recovered significantly more frequently from both bottle systems. Contamination of cultures was significantly greater with the Isolator system than with either bottle system. Regardless of the number of blood cultures obtained per septic episode, the Isolator detected microbiologically proven bacteremia or fungemia in a significantly greater number of patients and significantly decreased the time required for detection.

For many years the detection of septicemia has been based upon the isolation of microorganisms from cultures of blood in broth media. Recovery of microorganisms has been contingent upon the detection of growth macroscopically or radiometrically, by blind subculture, or by acridine orange-stained smears of broth samples (6). Lysis-centrifugation and lysis-filtration were introduced to increase the sensitivity of methods for detecting microorganisms in the blood. These techniques permitted the rapid removal of microorganisms from microbicidal serum factors and antibiotics in serum, the selection of optimal culture media and atmosphere of incubation, and the recovery of an isolate in a shorter time. Early methods of lysis-centrifugation and lysis-filtration were cumbersome (2, 5), but improvements have produced a practical blood culture system which has increased the yield of microorganisms and decreased the time required for their detection (3, 7).

A lysis-centrifugation blood culture tube, the Isolator (Du Pont Co.), is now being marketed. Previous studies of this system have compared the yield from 7.5 ml of Isolator-processed blood with that from a conventional broth culture system inoculated with either 10 ml of blood (5 ml into each of two 45-ml broth bottles) (3) or 20 ml of blood (10 ml into each of two 100-ml broth bottles) (M. F. Fojtasek, T. M. Abbott, J. M.

Matsen, and M. T. Kelly, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C129, p. 293).

Because of the direct relationship between the volume of blood cultured and the yield (6), we compared the yield from 10 ml of Isolator-processed blood cultures with yields from 10 ml in each of two conventionally processed blood cultures. The microbiological and clinical results were analyzed to determine whether the Isolator increased the yield of microorganisms and decreased their detection time and whether this information was useful in the treatment of septicemic patients.

MATERIALS AND METHODS

Blood for cultures was collected by members of a venipuncture team from adult patients with suspected septicemia. At least two separate blood samples were ordinarily obtained per septic episode from each patient. After preparation of the skin with povidone-iodine, 30 ml of blood was obtained with a single syringe and needle. A 10-ml sample of blood was inoculated into each of the following: (i) a 10-ml Isolator (Du Pont Co.); (ii) a nonvented bottle of 100 ml of tryptic soy broth with 0.025% sodium polyanetholesulfonate under vacuum in an atmosphere containing CO_2 (Difco Laboratories) (NV-TSB bottle); and (iii) a transiently vented bottle containing a biphasic brain heart infusion medium with 0.025% sodium polyanetholesulfonate, prepared in our laboratory (V-BiBHI bottle).

Bottles containing broth were incubated at 35°C for 14 days. Each bottle was examined twice daily for visible evidence of growth on day 1, once daily on days 2 through 7, and finally on day 14. Samples from the NV-TSB bottles were subcultured onto chocolate agar plates between 6 and 17 h and after 48 h of incubation. Subculture plates were incubated at 35°C in 10% CO_2 for 48 h. The V-BiBHI bottles were not subcultured but instead were tilted daily to inoculate the agar slants.

The Isolator was processed as follows. After inoculation, the blood was mixed thoroughly with the Isolator contents (sodium polyanetholesulfonate, saponin, polypropylene glycol, disodium ethylenedinitrilotetraacetate, and a dense, inert fluorochemical) and kept at 4°C for up to 4 h to facilitate processing in the centrifuge, which had a maximum capacity of 10 Isolators. To determine the number of CFU per milliliter of blood, Isolator tubes were held at 4°C to minimize the multiplication of microorganisms. After 30 min of centrifugation at 3,000 × g, the Isolators were processed as previously described (3). The concentrate was inoculated and streaked with a plastic disposable loop onto four agar plates: sheep blood agar, chocolate blood agar, brucella blood agar, and brain heart infusion agar. The sheep blood agar and chocolate blood agar plates were incubated at 35°C in an atmosphere containing 10% CO_2 for 4 days. The brucella blood agar was incubated at 35°C in anaerobic conditions for 2 days, and the deep-pour brain heart infusion agar plate was incubated at 30°C in humidified air for 30 days. The last-mentioned plate was placed in an air-permeable polyethylene bag to decrease contamination by saprophytic fungi. The sheep blood agar, chocolate blood agar, and brain heart infusion agar plates were examined twice on day 1 and once daily thereafter. The brucella blood agar plate was examined once on day 2. When growth was detected in a bottle or on any one of the plates, the corresponding bottle(s) or plates were reexamined. Positive plates or bottles were not reincubated.

The contamination rate of the Isolator was based on colonies located within the streaked area on the plates (on streak). Colonies outside the streaked area of the plate (off streak) were not identified except by morphology and were not included in the calculation of the Isolator contamination rate.

TABLE 1. Recovery of 483 pathogens, by system, from 6,010 cultures

System(s) positive[a]	No. of positive cultures[b]		
	Isolator	NV-TSB	V-BiBHI
Isolator	80 (17)		
NV-TSB		43 (9)	
V-BiBHI			54 (11)
Isolator + NV-TSB	30 (6)	30 (6)	
Isolator + V-BiBHI	65 (13)		65 (13)
NV-TSB + V-BiBHI		44 (9)	44 (9)
All three	167 (35)	167 (35)	167 (35)

[a] System or combination of systems positive for each pathogen.
[b] Numbers in parentheses show percentages.

TABLE 2. Contamination, by system, in 6,010 cultures

System(s) positive[a]	No. of cultures containing contaminants[b]		
	Iso-lator[c]	NV-TSB	V-BiBHI
Isolator	562		
NV-TSB		20	
V-BiBHI			61
Isolator + NV-TSB	4	4	
Isolator + V-BiBHI	7		7
NV-TSB + V-BiBHI		10	10
All three	5	5	5

[a] System or combination of systems positive for each pathogen.
[b] Percentages of contaminated cultures by system: Isolator, 9.6%; NV-TSB bottle, 0.6%; V-BiBHI bottle, 1.3%.
[c] Contamination for the Isolator is defined as on-streak contamination.

The number of CFU per milliliter was calculated on the first day of positive culture by using the following formula: CFU/ml = [(total CFU on all plates)/(total plates on which growth was expected)] × [(number of plates inoculated)/(milliliters of blood)].

Statistical analyses of the microbiological results were restricted to those culture sets in which all three systems were inoculated. The Isolator was compared with the NV-TSB and the V-BiBHI bottles on a volume-to-volume basis. The microbiological results were analyzed by the Sign test (1) to determine whether there were significant differences in the frequency of positivity and whether the bottle or the Isolator plates became positive earlier.

The clinical histories of all patients with positive blood cultures were reviewed. Only those patients with positive blood cultures in association with physical findings and laboratory evidence of septicemia were included in the analysis of the clinical data. All patients had a minimum of two separate blood culture sets with all three systems. Each episode of bacteremia or fungemia and each microorganism in cases of polymicrobial septicemia were evaluated independently. A blood culture system was judged to be useful clinically if it was the only positive system or if it was positive earlier than the other two systems at the onset of the septic episode. The clinical results were analyzed on a system-to-system basis by the Sign test (1).

RESULTS

Microbiological. Of 6,010 sets of blood cultures, 446 (7.4%) were positive, yielding 483 pathogens from 192 patients. Of these 446 cultures, 31 (7%) were polymicrobial. During the 5-month study, the most prevalent pathogens recovered (by culture) were *Staphylococcus aureus* (14%), *Candida* spp. and *Escherichia coli* (11% each), *Pseudomonas aeruginosa* (7%), and

TABLE 3. Number of isolates recovered

Organism	Isolator vs NV-TSB				Isolator vs V-BiBHI			
	Isolator only	NV-TSB only	Both	P^a	Isolator only	V-BiBHI only	Both	P
S. aureus	26	4	57	<0.001	32	5	51	<0.01
Enterobacteriaceae	37	32	65	NS[b]	30	36	72	NS
P. aeruginosa	12	10	15	NS	10	15	17	NS
S. pneumoniae	0	6	7	<0.05	0	6	7	<0.05
Enterococci	12	4	9	NS	13	3	8	<0.05
Anaerobic bacteria	2	20	10	<0.01	6	8	6	NS
Candida spp.	50	0	10	<0.001	20	13	40	NS

[a] P computed for Isolator only versus NV-TSB bottle only or V-BiBHI bottle only.
[b] NS, Not significant.

anaerobic bacteria (4%). During the study period, no system recovered any isolates of the genera *Listeria, Haemophilus, Campylobacter*, or *Brucella*; of other fastidious gram-negative bacilli such as those of the genera *Actinobacillus* or *Eikenella*; or of nutritionally variant viridans streptococci. The distribution of the recovery of the 483 pathogens by system is shown in Table 1. The number of pathogens (80) recovered only in Isolator-processed cultures was significantly higher than the numbers recovered in the NV-TSB bottle only (43; $P < 0.01$) and in the V-BiBHI bottle only (54; $P < 0.01$). Overall, the Isolator recovered 71% of the total number of pathogens, the NV-TSB bottle recovered 59% ($P < 0.01$), and the V-BiBHI bottle recovered 68% (not statistically significant).

The distribution of contaminants is shown in Table 2. The contamination rate of the Isolator was approximately nine times higher than that of either of the two bottles. *Staphylococcus epidermidis*, diphtheroids, *Bacillus* spp., *Neisseria* spp., and viridans streptococci were considered to be contaminants unless multiple bottles within a blood culture set or multiple sets were positive and they were judged to be pathogens after a review of the histories of the patients. *S.*

epidermidis accounted for 66, 43, and 36% of all contaminants in the Isolator, NV-TSB bottle, and V-BiBHI bottle, respectively.

Data on the frequency of recovery and the time to detection of pathogens are shown in Tables 3, 4, and 5. There were no statistically significant differences between the Isolator and either bottle system regarding the recovery of the *Enterobacteriaceae* as a group or as members of individual genera. *Streptococcus pneumoniae* was more frequently isolated from the NV-TSB and V-BiBHI bottles than from the Isolator ($P < 0.05$); enterococci were recovered from the Isolator more frequently than from the V-BiBHI bottle ($P < 0.05$). Anaerobic bacteria were more frequently isolated from the NV-TSB bottle than from the Isolator ($P < 0.01$). Specifically, a statistically significant difference was detected with *Bacteroides* spp. ($P < 0.05$) and *Clostridium* spp. ($P < 0.05$). No significant difference in the recovery of anaerobic bacteria was found between the Isolator and the V-BiBHI bottle. The Isolator detected *Candida* spp. more frequently than the NV-TSB bottle did ($P < 0.001$) and as frequently as the V-BiBHI bottle did.

The Isolator detected *S. aureus* and *P. aeru-*

TABLE 4. Time for detection of pathogens (Isolator versus NV-TSB bottle)

Organism	Days to positive[a]			P^c
	Isolator	NV-TSB	Difference[b]	
S. aureus	1.69 ± 0.14 (83)	2.57 ± 0.33 (61)	1.19 ± 0.29 (57)	<0.001
Enterobacteriaceae	1.64 ± 0.09 (102)	1.71 ± 0.21 (97)	0.19 ± 0.23 (65)	NS[d]
P. aeruginosa	2.00 ± 0.18 (27)	2.44 ± 0.87 (25)	0.60 ± 0.21 (15)	<0.05
S. pneumoniae	5.29 ± 0.30 (7)	1.54 ± 0.18 (13)	3.57 ± 3.35 (7)	NS
Enterococci	2.29 ± 0.24 (21)	3.38 ± 1.12 (13)	2.56 ± 1.60 (9)	NS
Anaerobic bacteria	2.92 ± 0.38 (12)	3.47 ± 0.47 (30)	0.40 ± 0.45 (10)	NS
Candida spp.	1.98 ± 0.10 (60)	6.10 ± 1.56 (10)	3.90 ± 1.63 (10)	NS

[a] Mean ± 1 standard error. Numbers in parentheses show numbers of isolates analyzed.
[b] Calculated when both systems were positive.
[c] P computed for difference in time to positivity of Isolator versus NV-TSB bottle.
[d] NS, Not significant.

TABLE 5. Time for detection of pathogens (Isolator versus V-BiBHI bottle)

Organism	Days to positive[a]			P^c
	Isolator	V-BiBHI	Difference[b]	
S. aureus	1.69 ± 0.14 (83)	2.14 ± 0.16 (56)	0.92 ± 0.17 (51)	<0.01
Enterobacteriaceae	1.64 ± 0.09 (102)	2.37 ± 0.26 (108)	0.79 ± 0.28 (72)	NS[d]
P. aeruginosa	2.00 ± 0.18 (27)	3.25 ± 0.55 (32)	1.47 ± 0.73 (17)	<0.05
S. pneumoniae	5.29 ± 0.30 (7)	1.54 ± 0.22 (13)	3.57 ± 3.44 (7)	NS
Enterococci	2.29 ± 0.24 (21)	1.73 ± 0.24 (11)	0.25 ± 0.25 (8)	NS
Anaerobic bacteria	2.92 ± 0.38 (12)	3.00 ± 0.45 (14)	0.50 ± 0.56 (6)	NS
Candida spp.	1.98 ± 0.10 (60)	4.21 ± 0.37 (53)	2.20 ± 0.38 (40)	<0.01

[a] Mean ± 1 standard error. Numbers in parentheses show numbers of isolates analyzed.
[b] Calculated when both systems were positive.
[c] P computed for difference in time to positivity of Isolator versus V-BiBHI bottle.
[d] NS, Not significant.

ginosa more rapidly than the NV-TSB bottle did. The differences were 1.2 and 0.6 days for S. aureus and P. aeruginosa, respectively (Table 4). S. aureus, P. aeruginosa, and Candida spp. were recovered more rapidly with the Isolator than with the V-BiBHI bottle, with differences of 0.9, 1.5, and 2.2 days, respectively (Table 5).

Quantitation of bacteria or fungi in blood cultures was possible with the Isolator. The distribution of CFU per milliliter of blood is shown in Table 6. The magnitude of bacteremia caused by S. aureus was greater than that caused by S. epidermidis; however, 25% of cultures yielding S. aureus contained <1 CFU/ml. In cultures contaminated by S. epidermidis, 96% contained <1 CFU/ml. The magnitude of bacteremia caused by S. aureus was greater than that caused by P. aeruginosa and E. coli.

Clinical. The Isolator was helpful clinically more often than either the NV-TSB (Table 7) or the V-BiBHI (Table 8) bottle. S. aureus septicemia was detected earlier and more often with the Isolator. The Isolator was helpful clinically in 60% of the S. aureus bacteremias compared with 3% for the NV-TSB bottle and in 59% compared with 8% for the V-BiBHI bottle. The Isolator detected enterococcal and Candida septicemia more often than the NV-TSB bottle did, and

Candida septicemia was identified earlier by the Isolator than by the V-BiBHI bottle. Not surprisingly, the Isolator was less helpful clinically than the NV-TSB bottle in cases of anaerobic bacteremia. Despite the statistically significant differences between the Isolator and the bottles for the recovery of S. pneumoniae, there were no significant differences among the systems in the clinical analysis of patients with S. pneumoniae bacteremia.

The impact of antibiotic therapy on the recovery of microorganisms by the three systems is shown in Table 9. Of the 225 septic episodes evaluated, one of the three systems was distinguished as clinically helpful in 127 (56%); the Isolator was helpful in 81 episodes, the NV-TSB bottle in 22 ($P < 0.01$), and the V-BiBHI bottle in 24 ($P < 0.01$). In these 225 septic episodes, 61 patients were receiving antibiotics when the blood cultures were drawn. The Isolator was helpful clinically more often than the V-BiBHI bottle ($P < 0.05$) but not more helpful than the NV-TSB bottle. In 33 of these 61 septic episodes, patients were receiving antibiotics that were active in vitro against the microorganism isolated. Among this group, the Isolator was helpful clinically more often than either the NV-TSB bottle (15 versus 4; $P < 0.05$) or the V-BiBHI bottle (15 versus 5; $P < 0.05$).

TABLE 6. Quantitation of microorganisms

Organism[a]	No. of positive cultures with[b]:					
	Pathogens at CFU/ml:			Contaminants at CFU/ml:		
	<1	1–10	>10	<1	1–10	>10
S. aureus (76)	19 (25)	13 (17)	40 (53)	4 (5)	0	0
S. epidermidis (357)	1 (0.3)	2 (0.6)	3 (0.9)	342 (96)	8 (2)	1 (.3)
E. coli (47)	28 (60)	7 (15)	11 (23)	1 (2)	0	0
P. aeruginosa (22)	12 (55)	2 (9)	9 (36)	0	0	0
Candida spp. (61)	22 (36)	16 (28)	23 (36)	0	0	0

[a] Numbers in parentheses show numbers of cultures.
[b] Numbers in parentheses show percentages.

TABLE 7. Clinical utility of the Isolator compared with the NV-TSB bottle

| Organism[a] | No. of positive identifications by: | | | | | P^d | | |
| | Both systems[b] | Isolator | | NV-TSB | | Only | Early | Only or early |
		Only	Early[c]	Only	Early			
S. aureus (38)	14	11	12	1	0	<0.01	<0.01	<0.01
E. coli (37)	13	12	2	8	2	NS[e]	NS	NS
Other Entero-bacteriaceae (41)	18	8	3	9	3	NS	NS	NS
P. aeruginosa (16)	5	4	5	2	0	NS	NS	NS
S. pneumoniae (6)	3	0	0	2	1	NS	NS	NS
Enterococci (13)	5	6	2	0	0	<0.05	NS	<0.01
Anaerobic bacteria (21)	5	2	1	13	0	<0.01	NS	<0.01
Candida spp. (21)	1	14	5	1	0	<0.01	NS	<0.01

[a] Numbers in parentheses show numbers of septicemia episodes analyzed.
[b] Both systems positive simultaneously.
[c] Both systems positive with one positive earlier.
[d] P values for all isolates: Only, <0.05; early, <0.01; only or early, <0.001.
[e] NS, Not significant.

DISCUSSION

In our study, the Isolator blood culture system resulted in increased recovery or more rapid detection of microorganisms which comprised at least one-third of those isolated from blood cultures. The results of the analysis of patient histories to determine the clinical utility of the Isolator were similar to the microbiological results. The NV-TSB bottle was more helpful clinically than the Isolator in cases of anaerobic bacteremia. When one of the three blood culture systems was judged to be helpful clinically, the Isolator was the system most often helpful. The use of antibiotic therapy did not reduce the rapid recovery of pathogens by the Isolator, and for patients who were receiving effective antimicro-bial therapy, the Isolator was helpful clinically more often than either of the bottles.

There is no single ideal blood culture system currently available. The Isolator has several important advantages, such as increased recovery and decreased detection time for certain microorganisms. In our study, the 17% increase in the yield of pathogenic isolates was attributed to the Isolator. Specifically, this increase was significant for S. aureus, enterococci, and Candida spp. The Isolator decreased the times required for recovery of S. aureus, P. aeruginosa, and Candida spp. The Isolator was helpful in detecting polymicrobial blood cultures; of 31 such cultures, 10 would have been unrecognized had the Isolator not been used. This accounts for a 50% increase in the detection of polymicrobial

TABLE 8. Clinical utility of the Isolator compared with the V-BiBHI bottle

| Organism[a] | No. of positive identifications by: | | | | | P^d | | |
| | Both systems[b] | Isolator | | V-BiBHI | | Only | Early | Only or early |
		Only	Early[c]	Only	Early			
S. aureus (39)	13	11	12	3	0	NS[e]	<0.01	<0.01
E. coli (39)	11	11	4	8	5	NS	NS	NS
Other Entero-bacteriaceae (40)	17	5	7	9	2	NS	NS	NS
P. aeruginosa (20)	3	4	6	5	2	NS	NS	NS
S. pneumoniae (6)	1	0	1	2	2	NS	NS	NS
Enterococci (15)	6	7	0	2	0	NS	NS	NS
Anaerobic bacteria (10)	2	3	3	2	0	NS	NS	NS
Candida spp. (24)	4	4	12	4	0	NS	<0.01	<0.05

[a] Numbers in parentheses show numbers of septicemia episodes analyzed.
[b] Both systems positive simultaneously.
[c] Both systems positive with one positive earlier.
[d] P values for all isolates: Only, NS; early, <0.01; only or early, <0.001.
[e] NS, Not significant.

TABLE 9. Effect of antibiotics on clinical utility of blood culture systems

Antibiotics[a]	Septicemias with clinical help from one system only[b]			P^c for Isolator vs:	
	Isolator	NV-TSB	V-BiBHI	NV-TSB	V-BiBHI
None (164)	58	11	16	<0.01	<0.01
Active[d] (33)	15	4	5	<0.05	<0.05
Inactive[d] (28)	8	7	3	NS[e]	NS

[a] Numbers in parentheses show numbers of septicemias analyzed.

[b] Positive result yielded exclusively or earliest by one system.

[c] P values for all septicemias, <0.01 versus each bottle system; for all antibiotic-treated septicemias, NS versus NV-TSB bottle system, <0.05 versus V-BiBHI bottle system.

[d] Antibiotics active or inactive, as indicated, in vitro against blood culture isolate.

[e] NS, Not significant.

blood cultures. In our study, the Isolator was not the optimal system for recovering *S. pneumoniae* or anaerobic bacteria. The reasons for the decrease in the recovery of these bacteria from Isolator-processed blood are unclear. Holding the Isolators at 4°C before processing may affect the recovery of anaerobic bacteria, although no reduction was reported in the recovery of *Bacteroides* spp. or *Peptostreptococcus anaerobius* from Isolators kept at 4°C for 8 h before processing (J. C. Richards and C. Bentsen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C124, p. 292). We held the blood culture tubes at 4°C to reduce multiplication of the microorganisms so that the number of CFU per milliliter could be determined. We do not believe that incubation of the brucella blood agar anaerobic plate for only 2 days accounts for the lower recovery of anaerobes.

The incidence of contamination (on streak) was greater with the Isolator than with either of the bottles. A disproportionate number of the contaminating organisms were *S. epidermidis*, most often present at <1 CFU/ml. Although a study by Dorn et al. (4) suggested that bacterial counts of <1 CFU/ml for common contaminants are not indicative of septicemia, 25% of our clinically significant cultures with *S. aureus* had counts of <1 CFU/ml. Cultures with low counts must, therefore, be examined and subcultured to verify their species identification; this is time consuming. The most likely sources of *S. epidermidis* are the skin of the patients and laboratory processing of Isolators. The majority of the

Isolator plates in our study were inoculated and streaked on a benchtop. Proper disinfection procedures and processing Isolator tubes in a laminar flow hood should decrease the contamination rate (R. B. Thomson, Jr., S. J. Vanzo, K. L. Guenther, and N. K. Henry, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, C77, p. 324).

The time and cost of using the Isolator was compared with those for the NV-TSB and V-BiBHI bottles. Processing an Isolator culture which later is reported as negative requires 7 min, compared with 5 and 2.5 min for the NV-TSB and V-BiBHI bottles, respectively. For positive cultures, the Isolator requires 11 min, the NV-TSB bottle requires 17 min, and the V-BiBHI requires 15 min. The cost of supplies, excluding overhead, was calculated to be $7.60 for the Isolator, $1.80 for the NV-TSB bottle (plus $1.25 for media if the bottle is positive), and $1.60 for the V-BiBHI bottle (plus $1.25 for media if the bottle is positive). The cost effectiveness of the Isolator is enhanced when one considers that an Isolator culture may be used to isolate fungi and bacteria, thereby eliminating the cost of a separate fungal blood culture bottle.

We found the Isolator lysis-centrifugation blood culture tube to be a sensitive blood culture system which results in increased recovery and earlier detection of microorganisms which are common causes of septicemia. The Isolator may be an important adjunct to a conventional broth blood culture system. Because of the decreased recovery of *S. pneumoniae* and anaerobic bacteria, the Isolator should be used together with a bottle containing broth suitable for the recovery of these microorganisms.

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