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Clinical Laboratory Comparison of the 10-ml Isolator Blood Culture System with BACTEC Radiometric Blood Culture Media

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The efficiency of the 10-ml Isolator (E. I. du Pont de Nemours & Co., Inc.) for recovery of pathogens from blood was compared with that of BACTEC 6B and 7C media (Johnston Laboratories) by using 4,195 cultures from 1,662 patients. During the first phase of the study, BACTEC bottles were inoculated with 3 ml of blood; in the second phase, bottles were inoculated with 5 ml. The objectives were to compare results with similar blood volumes used for the detection of anaerobes as well as similar overall volumes and to determine the relative sensitivity of BACTEC media inoculated with the minimum and maximum volumes suggested by the manufacturer. From 180 patients, 391 significant isolates were recovered, 354 (91%) with the Isolator and 304 (78%) with the bottles. Isolators recovered 31 (15%) and 19 (18%) more pathogens overall than did the two-bottle system inoculated with 3 and 5 ml of blood, respectively, including 30 (36%) and 10 (34%) more *Enterobacteriaceae*. Recovery of anaerobes was greater in the BACTEC anaerobic medium, but only when its inoculum was increased to 5 ml. No significant differences existed between the two systems in pathogen detection times or detection of polymicrobic bacteremia. The Isolator contamination rate (8.3%) was approximately 4 times that of the bottles. The number of CFU of pathogen per milliliter of blood, blood volume sampled, and number of Isolators collected were more important than antimicrobial agent pretreatment in contributing to patient bacteremia or fungemia undetected by the Isolator. The Isolator appeared to be a practical alternative for recovery of aerobic and facultatively anaerobic pathogens from the blood.

The lysis-centrifugation technique, as first developed (7), has been improved (8) and adapted for marketing as the Isolator (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The device is available in two sizes to accommodate either 7.5 or 10 ml of blood. Earlier reports indicated improved detection with the 7.5-ml Isolator compared both with BACTEC media (12, 16) and nonradiometric bottles (1, 13, 15). The 10-ml Isolator was found to detect *Staphylococcus aureus* and *Candida* spp. more frequently and faster, but *Streptococcus pneumoniae* and anaerobes less often, than bottles inoculated with 10 ml of blood (10).

Because of reports indicating significantly greater yields of pathogens with increasing volumes of blood (9, 18), we elected to study the larger (10-ml) Isolator, which was compared with BACTEC radiometric media (Johnston Laboratories, Inc., Cockeysville, Md.). During the first phase of the study, the aerobic and anaerobic bottles were inoculated with 3 ml of blood each because this volume not only provided an effective 1:10 dilution of blood in the broth (3), but also yielded an inoculum for the anaerobic bottles similar in volume to the Isolator sediment from approximately 2.5 ml of blood used for inoculation of each anaerobically incubated agar plate. If the Isolator were to be used routinely for recovery of all pathogens from blood, its ability to detect anaerobes should be at least comparable to that of BACTEC media when similar volumes of blood are processed by both methods. It was understood that there would be a blood volume bias in the detection of aerobic and facultatively anaerobic pathogens in favor of the Isolator. The Isolator was next compared with aerobic and anaerobic BACTEC bottles inoculated with 5 ml of blood each to evaluate overall efficiency of the two systems when the total blood volumes introduced into each were identical, realizing that there would be a blood volume bias in the detection of anaerobes in favor of the BACTEC media. The effect of increasing the bottle inoculum from 3 to 5 ml on sensitivity and speed of detection relative to the Isolator was of further interest since these volumes were the minimum and maximum suggested in BACTEC media package inserts. The results were analyzed to determine whether there were microbiological or clinical advantages that would justify the routine use of the Isolator and whether there was a relationship between those organisms missed by the Isolator and antimicrobial agent pretreatment of the patients.

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MATERIALS AND METHODS

Between April and October 1982, 16 ml of blood was aseptically collected by phlebotomists with a syringe from each patient with suspected bacteremia or fungemia. After the needle was replaced, 10 ml of blood was transferred to a 10-ml Isolator, and then 3 ml each was inoculated first to an aerobic (6B) and then to an anaerobic (7C) BACTEC bottle. From October 1982 until February 1983, 20 ml of blood was collected, 10 ml for the Isolator and 5 ml each for the two blood culture bottles. During alternating months, the aerobic bottle was inoculated first and the Isolator was inoculated last. Blood specimens for culture were usually collected at least twice, 30 min apart, from each patient. On receipt by the laboratory, the bottles were incubated immediately at 35°C. Within 60 min of collection (24 h a day), the Isolators were centrifuged at 3,000 × *g* for 30 min and processed according to the manufacturer's instructions, and the sediment (about 1.5 ml) was equally inoculated with a 3-ml

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syringe onto four agar plates: one reduced laked sheep blood agar (GIBCO Laboratories, Lawrence, Mass.) and one chocolate agar and two RS agar (Bionics Inc., King of Prussia, Pa.). RS agar is a modification of the noninfusion blood agar medium developed by Casman (5) and was selected because of its ability to support the growth of fastidious organisms like *Haemophilus influenzae* while still allowing the demonstration of hemolytic activity associated with staphylococci and streptococci.

Inoculated bottles were incubated at 35°C for 8 days, with continuous agitation of the aerobic bottle for the first 2 days. The aerobic bottles were both visually and radiometrically (with the BACTEC 460) examined twice daily on days 1 and 2, inspected only visually on days 3 through 7, and scanned once again visually and radiometrically on day 8. Anaerobic bottles were visually examined daily and checked radiometrically once on day 8. We have found from previous observations that, with careful daily visual inspection of the bottles, additional radiometric analysis of the BACTEC media has not been cost effective. A growth index of ≥30 or ≥20 was considered as radiometric evidence of a positive aerobic or anaerobic bottle, respectively. Each aerobic bottle was routinely subcultured to an aerobically incubated chocolate agar plate after 1 day of incubation, and each anaerobic bottle was subcultured to an anaerobically incubated chocolate agar plate after 2 days. All subculture plates were incubated at 35°C for 2 days. Bottles with visual or radiometric evidence of growth were Gram stained and subcultured and, when organisms were observed microscopically, differentially sedimented (2) for preliminary identification and antimicrobial agent susceptibility testing. After 3 additional days of incubation (or on day 8), all positive bottles were subcultured again for evidence of additional pathogens.

The Isolator-inoculated RS and chocolate plates were incubated aerobically (5 to 10% CO_2) at 35°C, the RS agar cultures for 4 days and the chocolate agar cultures for 8 days. The laked sheep blood plates were incubated anaerobically at 35°C for 6 days. All plates were inspected once daily, usually between 7:00 and 9:00 a.m. All positive cultures were reincubated for the specified time intervals to detect additional pathogens. When colony volume permitted, the initial colonies were used to undertake identification and antimicrobial agent susceptibility studies. Small numbers of poorly developed colonies were subcultured before further testing.

The CFU of pathogens per milliliter of blood recovered from Isolator-inoculated plates was determined as previously described (10), except that the maximum number of colonies recovered from each plate was used, whether that number was achieved on the first day of the positive culture or thereafter. Up to 250 colonies were counted per plate. Results in excess of this number were recorded as greater than 250 colonies per plate and greater than 100 CFU/ml of blood (when similar high recovery was obtained on all four inoculated plates). Each isolate was classified as either a contaminant or a pathogen. The clinical significance of the isolates was established by thoroughly reviewing the patients' charts as well as determining the number of Isolator plates (or bottles) positive with the same strain and the recovery of the same organism from other body sites. The Isolator contamination rate was calculated from colonies found only on the streaked area of the plates. The statistical significance of the results was established by using the Sign test (6).

RESULTS

During the 10-month study, 4,195 blood specimens from 1,662 patients were analyzed. Of 2,478 cultures processed during the comparison of the Isolator with the 3 ml-inoculated bottles, 225 (9%) were positive for 256 pathogens from 111 (12%) of the 951 patients. Of 1,717 cultures processed during the comparison with 5 ml-inoculated bottles, 129 (8%) were positive for 135 pathogens from 69 (10%) of the 711 patients. Obligate anaerobes were never recovered from aerobically incubated Isolator plates or aerobic bottles, but, as expected, facultatively anaerobic species were frequently recovered from anaerobically incubated Isolator plates and anaerobic bottles. The relative ability of each blood culture system to recover pathogens is shown in Table 1. The

TABLE 1. Pathogens recovered with Isolators and bottle sets

Organism	No. (%) of significant isolates							
	Isolator versus 3-ml BACTEC inoculum				Isolator versus 5-ml BACTEC inoculum			
	Isolator alone	BACTEC alone	Both systems	*P*[a]	Isolator alone	BACTEC alone	Both systems	*P*
Gram positive								
S. aureus	5 (14)	2 (5)	30 (81)	NS[b]	4 (25)	0	12 (75)	NS
Staphylococcus spp., coagulase negative	0	2 (14)	12 (86)	NS	5 (29)	0	12 (71)	<0.05
Streptococcus spp.								
Enterococci	0	0	4 (100)	NS	3 (75)	0	1 (25)	NS
S. pneumoniae	1 (17)	0	5 (83)	NS	1 (10)	0	9 (90)	NS
Other	1 (7)	4 (27)	10 (67)	NS	0	0	14 (100)	NS
Corynebacterium sp.	0	0	6 (100)	NS	0	0	2 (100)	NS
Gram negative								
Enterobacteriaceae	36 (30)	6 (5)	77 (65)	<0.001	16 (36)	6 (13)	23 (51)	<0.05
P. aeruginosa	2 (20)	3 (30)	5 (50)	NS	0	0	4 (100)	NS
Other	3 (23)	2 (15)	8 (62)	NS	0	2 (67)	1 (33)	NS
Anaerobes								
B. fragilis	4 (40)	1 (10)	5 (50)	NS	0	1 (50)	1 (50)	NS
Clostridium sp.	1 (11)	3 (33)	5 (56)	NS	0	1 (100)	0	NS
Other	0	0	4 (100)	NS	0	3 (50)	3 (50)	NS
Yeasts	2 (22)	1 (11)	6 (67)	NS	3 (27)	0	8 (73)	NS
Total	55 (21)	24 (9)	177 (69)	<0.001	32 (24)	13 (10)	90 (67)	<0.005

[a] *P* was calculated from the Isolator alone versus 3-ml BACTEC inoculum alone or 5-ml BACTEC inoculum alone.
[b] NS, Not significant.

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Isolator recovered significantly more pathogens than the bottles, regardless of the blood volume introduced into the latter. Compared to 3 ml-inoculated bottles, the Isolator detected 232 (91%) of the pathogens, whereas the bottles recovered 201 (79%) ($P < 0.001$); 15% more pathogens were detected with the Isolator. Compared with bottles with 5 ml of blood, the Isolator detected 122 (90%) of the pathogens and the bottles recovered 103 (76%) ($P < 0.005$); 18% more pathogens were detected with the Isolator. Overall, the Isolator detected 354 (91%) of the 391 total pathogens, whereas 304 (78%) were recovered with the bottles.

Isolators detected significantly more (36 and 34%) *Enterobacteriaceae* than did the 3 ml- and 5 ml-inoculated bottles, respectively. Neither the Isolator nor the bottles offered any clear advantage in the recovery of other gram-negative organisms, streptococci, *Corynebacterium* spp., or yeasts. When the Isolator was compared with 3 ml-inoculated bottles, the overall sensitivities of the two systems for anaerobes and staphylococci were similar. Compared with the 5 ml-inoculated bottles, however, the Isolator recovered less than half of the anaerobic isolates ($P < 0.05$), but more coagulase-negative staphylococci ($P < 0.05$).

A total of 28 (8%) of the positive cultures contained more than one pathogen, and neither system showed a significant advantage in their recovery. Of 53 isolates detected from 22 polymicrobic cultures during the comparison with bottles containing 3 ml of blood, the Isolator recovered 47 (89%) of the pathogens and the bottles recovered 45 (85%). Of 12 isolates found in 6 polymicrobic cultures during the comparison with 5 ml-inoculated bottles, Isolator plates grew out 11 (92%) of the organisms and the bottles yielded 8 (67%).

Although the ability of the 2 blood culture systems to detect pathogens in at least one blood specimen from most patients with bacteremia or fungemia was often similar, the Isolator proved to be more effective in detecting bacteremia due to *Enterobacteriaceae*, especially when compared with 3 ml-inoculated bottles (Table 2). The Isolator detected 62 (95%) of such episodes compared with only 44 (68%) for the 3 ml-inoculated bottles. The Isolator missed only 3 (5%) while finding 41% more of these episodes. Although the Isolator detected 32% more of the *Enterobacteriaceae* episodes than the two-bottle system inoculated with an identical blood volume (5 ml each), it also missed 17%.

The mean detection time for total pathogens was 29 h for both systems and did not vary significantly for the bottles when the inoculum volume was increased from 3 to 5 ml. Although the mean detection time for the 23 anaerobes recovered by the Isolator was 40 h, the range was 8 to 91 h. Nine (39%) of these anaerobes were detected within the first 24 h of incubation, and 5 (22%) were recovered by the device on day 4. Only one aerobic or facultatively anaerobic pathogen was detected by the Isolator at 5 days, and none was recovered after longer periods of incubation. One pathogen was recovered by the bottles only after 7 days of incubation, and three were recovered at 8 days. The mean time to availability of isolated colonies was 29 h for the Isolator and 52 h for the bottles.

TABLE 3. Quantitative range of recovery with Isolator

CFU/ml of blood	Detected pathogens	
	No. of positive cultures	% of total isolates
0.1	63	18
0.2	30	8
0.3	18	5
0.4	16	5
0.5	9	3
0.6 to 1.0	40	11
1.1 to 10	74	21
10.1 to 100	59	17
>100	45	13

Of the pathogens detected by the Isolator, 50% resulted in a total count of 10 colonies or less (≤1.0 CFU/ml of blood), and 18% yielded only one colony (Table 3). The quantitative recovery, however, varied substantially by species or group (Table 4). Only about 20% of the isolates of *Staphylococcus aureus* resulted in 1.0 CFU/ml or less as compared to 56% of the *Streptococcus pneumoniae*, 57% of the anaerobes, 60% of the *Enterobacteriaceae*, and 82% of the *Pseudomonas aeruginosa* encountered. Other streptococci, yeasts, and coagulase-negative staphylococci also were more frequently recovered in counts lower than those observed with *S. aureus*. As could be expected, the species or groups that were usually recovered with the largest counts were those rarely missed by the Isolator.

Of the 37 significant isolates from 30 patients which Isolators failed to detect (Table 5), 24 (65%) of the pathogens from 24 (80%) of the patients involved a single false-negative Isolator result per patient. From 12 of the patients, the negative Isolator culture was the only one collected. From the other 12 patients, additional Isolator cultures detected the pathogen. However, 13 (35%) of the missed pathogens from six (20%) of the patients involved two or three false-negative Isolator cultures per patient. There were eight false-negative Isolator cultures (from four patients), which represented the only Isolator samples drawn. From the other two patients, additional Isolator tests detected the pathogens. Of the patients with false-negative Isolator results, 20 (67%) had

TABLE 2. Detection of bacteremia or fungemia with Isolators and bottle sets

Pathogen	No. (%) of patients							
	Isolator versus 3-ml BACTEC inoculum				Isolator versus 5-ml BACTEC inoculum			
	Isolator alone	BACTEC alone	Both systems	P^a	Isolator alone	BACTEC alone	Both systems	P
S. aureus	2 (14)	1 (7)	11 (79)	NS[b]	1 (14)	0	6 (86)	NS
Streptococci	1 (8)	2 (17)	9 (75)	NS	2 (15)	0	11 (85)	NS
Enterobacteriaceae	21 (32)	3 (5)	41 (63)	<0.001	11 (37)	5 (17)	14 (47)	NS
P. aeruginosa	1 (14)	1 (14)	5 (71)	NS	0	0	3 (100)	NS
Anaerobes	3 (20)	2 (13)	10 (67)	NS	0	2 (40)	3 (60)	NS
Yeasts	0	0	4 (100)	NS	1 (20)	0	4 (80)	NS

[a] P was calculated from the Isolator alone versus 3-ml BACTEC inoculum alone or 5-ml BACTEC inoculum alone.
[b] NS, Not significant.

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TABLE 4. Quantitative recovery of pathogens by Isolator

Pathogen	Detection sensitivity[a]	No. of pathogens recovered at following no. of CFU/ml of blood:			
		0.1	0.2 to 1.0	1.1 to 10	>10
S. aureus	51 of 53 (96)	5 (10)	5 (10)	8 (16)	33 (65)
Staphylococci, coagulase negative	29 of 31 (94)	2 (7)	9 (31)	5 (17)	13 (45)
Yeasts	19 of 20 (95)	0	8 (42)	9 (47)	2 (11)
S. pneumoniae	16 of 16 (100)	3 (19)	6 (38)	2 (13)	5 (31)
Streptococci, other	33 of 37 (89)	5 (15)	7 (21)	10 (30)	11 (33)
Anaerobes	23 of 32 (72)	1 (4)	12 (52)	9 (39)	1 (4)
Enterobacteriaceae	152 of 164 (93)	37 (24)	54 (36)	34 (22)	27 (18)
P. aeruginosa	11 of 14 (79)	6 (55)	3 (27)	0	2 (18)

[a] Numbers within parentheses indicate percentages of total isolates of a species or group recovered with the Isolator during the comparison with both 3 ml- and 5 ml-inoculated BACTEC bottles.
[b] Numbers within parentheses indicate the percentages of isolates of a species or group recovered from the Isolator.

not been treated with appropriate antimicrobial agents for at least 1 week before specimen collection. Of the 14 patients with false-negative Isolator cultures, but with one or more positive Isolator cultures, 10 (71%) showed low recovery (≤1.0 CFU/ml) from each positive Isolator culture. In all, 15 of 23 (65%) of the positive Isolator cultures from these patients yielded pathogens in counts of ≤1.0 CFU/ml, 4 (17%) resulted in counts of >1 to ≤10 CFU/ml, and 4 (17%) showed counts of >10 CFU/ml.

Of the 87 pathogens missed with bottles inoculated with either 3 or 5 ml of blood, approximately half were each detected as only one colony (0.1 CFU/ml) with the Isolator (Table 6). Of 30 *Escherichia coli* isolates which the bottles failed to detect, the Isolator recovered just 1 colony from 17 (57%) and 2 colonies from another 6 (20%).

The contamination rate for the Isolator (8.3%) was approximately 4 times that of the bottles (2.2%) and did not fluctuate substantially over the months of the study. The contaminants were most frequently seen as one or two colonies of coagulase-negative staphylococci, diphtheroids, or viridans streptococci.

DISCUSSION

In this study, the 10-ml Isolator resulted in the detection of 16% more pathogens (mostly *Enterobacteriaceae*) than did the BACTEC media. A significantly improved overall recovery by bottles inoculated with the larger (5-ml) blood volume might have been anticipated from previous studies (9, 11, 18), but was never observed at any time during the second phase of the study. Perhaps the inoculum volume increase from 3 ml (1:10 dilution) to 5 ml (1:6 dilution) was insufficient to provide enough additional microorganisms to offset the lesser dilution of blood and antimicrobial agents. Blood cultures diluted 1:10 have been found to provide greater and faster recovery than those diluted 1:5 (3).

Despite the fact that results from over 4,000 blood cultures were analyzed, relatively small numbers of some groups of pathogens, such as yeasts and anaerobes, were recovered. Although various blood culture systems have been explored within the last 10 years by this laboratory in an attempt to isolate more yeasts from the blood, the yearly percentage of patients from whom that group of pathogens has been recovered has never exceeded 5% of the total number of patients with bacteremia or fungemia in our community hospital's patient population. A medium such as SABHI, designed primarily for recovery of pathogenic fungi, was therefore not included in the Isolator study protocol.

When the inoculum volumes for the recovery of anaerobes by the two systems were similar, the overall recovery of anaerobes was equivalent. However, increasing the inoculum of the anaerobic bottle to 5 ml resulted in a superior recovery of anaerobes with the bottles. The effect of blood volume on the detection of pathogens in the blood has been well established (9, 11, 18) and may explain the poorer performance of the Isolator in recovering anaerobes when 2 (16) and 4 times (10) the amounts of blood were used in anaerobic bottles as were inoculated to anaerobically incubated plates from the Isolator. An increased risk of missing anaerobes with the Isolator could be predicted from the relatively small volume of blood processed for anaerobes, the use of only one anaerobic plate per specimen, and the low number of colonies obtained from most anaerobic isolates. The risk of missing an anaerobe should be 4 times that of missing an organism recoverable from all 10 ml of blood on all four inoculated plates, unless anaerobes are usually present in the blood in significantly larger numbers. Our findings agree with those previously reported (15) that the

TABLE 5. Relationship of false-negative Isolators to episodes of bacteremia or fungemia

No. of false-negative Isolator cultures per patient	Pathogens missed by Isolators[a]	
	Versus 3-ml BACTEC inoculum	Versus 5-ml BACTEC inoculum
1	18 (18)	6 (6)
2	6 (3)	4 (2)
3	0	3 (1)

[a] Numbers within parentheses indicate numbers of patient episodes.

TABLE 6. Relationship of false-negative bottle sets to Isolator recovery

Isolator results (CFU/ml)	No. (%) of isolates missed by BACTEC bottle sets	
	3-ml inoculum	5-ml inoculum
0.1	24 (44)	17 (53)
0.2	8 (15)	8 (25)
0.3	3 (5)	1 (3)
0.4	5 (9)	2 (6)
0.5	1 (2)	1 (3)
0.6 to 1.0	8 (15)	1 (3)
1.1 to 10	6 (11)	2 (6)
>10	0	0

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majority of anaerobes are recovered in counts of ≤1 CFU/ml. The potential for missing anaerobes with the Isolator, their relative infrequency (32 [8%] of the total pathogens were anaerobes), the large number of anaerobe jars required (an average of 12 were used each day), and the labor involved in working with and maintaining the jars appeared to negate the value of the Isolator for the detection of this group of pathogens.

Our results demonstrating a superior detection sensitivity of the Isolator are similar to the reports from previous studies with the 7.5-ml Isolator (compared with BACTEC media [12, 16] and non-radiometric media [1, 13, 15]) and the 10-ml device (10). The improved capability of the Isolator in the detection of isolates of and bacteremic patients with *Enterobacteriaceae* has already been noted (15). The previous findings that the Isolator results in diminished recovery of *S. pneumoniae* (10, 13) or streptococci in general (15) and increased detection of *S. aureus* (10) were not observed during this study. However, since three (19%) of our isolates of *S. pneumoniae* were recovered as a single colony and nine (56%) were found as ≤1 CFU/ml (Table 4), it appears reasonable to expect that the organism, apparently often present in the blood in small numbers, could be missed by the Isolator. *P. aeruginosa*, the species recovered by the Isolator in the lowest counts, was the organism for which the device had the lowest detection sensitivity.

As has been previously reported (15), the lower the number of CFU per milliliter of a positive Isolator culture, the greater the chance of missing a significant isolate with the bottles. From our study, 18% of the pathogens detected by the Isolator were recovered as 0.1 CFU/ml, but approximately half of the isolates missed by the bottles were detected in that amount by the Isolator. Since 60% of the *Enterobacteriaceae* were recovered in amounts of 1 CFU/ml or less, it could be expected that pathogens within that family would be more frequently missed with the bottles than would isolates of other groups or species more frequently recovered in larger amounts. Since about 70% of the organisms recovered by the Isolator yielded 10 CFU/ml or less and 100% of the isolates missed by the bottles were detected within that range by the Isolator, the failure of the bottles to detect significant isolates should be (and was) a frequent occurrence.

The similarity of both systems in the detection of pathogens from cases of polymicrobic bacteremia was surprising, considering the Isolator's overall detection superiority. This similarity has been noted previously (15), but others have reported that the Isolator (10, 13) or bottles (16) recovered significantly more of these pathogens. The incubation of positive bottles for 3 additional days after the first detection was not a significant factor in bottle performance, since all multiple pathogens were detected at the time the bottles were first found to be positive.

It appears useful to inspect the Isolator-inoculated anaerobic plates within the first 24 h, since more than one-third of the anaerobes were recovered in that time period, some after only 8 h. Because more than 20% of the Isolator-recovered anaerobes were detected on day 4 of incubation, retention of anaerobically incubated plates for 5 or 6 days seems appropriate. Since no pathogens were recovered by the Isolator after 5 days of incubation, routine incubation and analysis of any Isolator-inoculated plates after 6 days appear not to be cost effective, except when pathogenic fungi (4), agents such as *Brucella* sp., or conditions such as bacterial endocarditis are suspected.

The clinical impact of a 1-day-earlier time to availability of isolated colonies, noted in this and other studies (1, 15), depends on the type of technology available in the laboratory. In most cases, accurate identification or susceptibility studies (or both) can be initiated soon after bottles are first found to be positive either by direct inoculation of test media (14) or by inoculation after centrifugation of the blood culture broth (2, 17). On the other hand, the obvious asset of having isolated colonies available for testing when the Isolator cultures were first positive was offset, at least some of the time in this study, when only one or two small colonies were recovered and subculture for 6 to 24 h was required before undertaking most identification and antimicrobial agent susceptibility procedures.

The failure of Isolators to detect 37 pathogens did not appear to be due primarily to antimicrobial agent pretreatment. Bacteremia or fungemia undetected by the device appeared due primarily to low numbers of organisms in the blood, blood volume sampled, and the use of only one or two Isolators per episode.

The relatively high Isolator contamination rate was similar to that of most previous reports (1, 4, 10, 15, 16), but higher than one (13). Although the Isolator plates were inoculated and frequently read in a laminar-flow hood, frequent hand washing was stressed, and other precautions were taken, the contamination rate of about 8% per month could not be lowered.

The 10-ml Isolator was found to be as sensitive for the detection of aerobic and most facultatively anaerobic pathogens as the BACTEC media and more sensitive for the recovery of *Enterobacteriaceae*. It was, however, determined to be neither especially sensitive nor practical for recovery of anaerobes. The improved performance of 5 ml-inoculated bottles, at least in the detection of anaerobes (although only a small number were recovered), and bacteremic episodes due to *Enterobacteriaceae* indicated that a 3-ml inoculum volume for the BACTEC media was inadequate. As has been previously observed (10, 13, 15), no one blood culture system appears to fulfill all of the needs of a laboratory for the detection of agents of bacteremia and fungemia. The combination of the 10-ml Isolator (for detection of aerobic and facultatively anaerobic pathogens only) with an anaerobic, 5 ml-inoculated BACTEC bottle would seem to provide an acceptable blood volume (15 ml) as well as a comprehensive means of detection of each type of bacterial and fungal pathogen.

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LITERATURE CITED

1. **Abbott, T. M., M. Fojtasek, J. R. Dizikes, D. C. Hale, R. Boshard, D. Bruckner, W. J. Martin, M. T. Kelly, and J. M. Matsen.** 1983. Enhanced septicemia detection with the lysis-centrifugation technique in comparison with a two-bottle conventional blood culture method: a collaborative study, p. 31–37. *In* A. Balows and A. C. Sonnenwirth (ed.), Bacteremia. Laboratory and clinical aspects. Charles C Thomas, Publisher, Springfield, Ill.
2. **Appelbaum, P. C., S. F. Schick, and J. A. Kellogg.** 1980. Evaluation of the four-hour Micro-ID technique for direct identification of oxidase-negative, gram-negative rods from blood cultures. J. Clin. Microbiol. **12**:533–537.
3. **Auckenthaler, R., D. M. Ilstrup, and J. A. Washington II.** 1982. Comparison of recovery of organisms from blood cultures diluted 10% (volume/volume) and 20% (volume/volume). J. Clin. Microbiol. **15**:860–864.

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4. **Bille, J., L. Stockman, G. D. Roberts, C. D. Horstmeier, and D. M. Ilstrup.** 1983. Evaluation of a lysis-centrifugation system for recovery of yeasts and filamentous fungi from blood. J. Clin. Microbiol. **18**:469–471.
5. **Casman, E. P.** 1947. A noninfusion blood agar base for *Neisseriae*, pneumococci, and streptococci. Am. J. Clin. Pathol. **17**:281–289.
6. **Dixon, W. J., and F. J. Massey, Jr.** 1969. Introduction to statistical analysis, 3rd ed. McGraw-Hill Book Co., New York.
7. **Dorn, G. L., J. R. Haynes, and G. G. Burson.** 1976. Blood culture technique based on centrifugation: developmental phase. J. Clin. Microbiol. **3**:251–257.
8. **Dorn, G. L., G. A. Land, and G. E. Wilson.** 1979. Improved blood culture technique based on centrifugation: clinical evaluation. J. Clin. Microbiol. **9**:391–396.
9. **Hall, M. M., D. M. Ilstrup, and J. A. Washington II.** 1976. Effect of volume of blood cultured on detection of bacteremia. J. Clin. Microbiol. **3**:643–645.
10. **Henry, N. K., C. A. McLimans, A. J. Wright, R. L. Thompson, W. R. Wilson, and J. A. Washington II.** 1983. Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. J. Clin. Microbiol. **17**:864–869.
11. **Ilstrup, D. M., and J. A. Washington II.** 1983. The importance of volume of blood cultured in the detection of bacteremia and fungemia. Diagn. Microbiol. Infect. Dis. **1**:107–110.
12. **Isenberg, H. D.** 1983. Clinical laboratory comparison of the lysis-centrifugation blood culture technique with radiometric and broth approaches, p. 38–54. *In* A. Balows and A. C. Sonnenwirth (ed.), Bacteremia. Laboratory and clinical aspects. Charles C Thomas, Publisher, Springfield, Ill.
13. **Kelly, M. T., G. E. Buck, and M. F. Fojtasek.** 1983. Evaluation of a lysis-centrifugation and biphasic bottle blood culture system during routine use. J. Clin. Microbiol. **18**:554–557.
14. **Kiehn, T. E., C. Capitolo, and D. Armstrong.** 1982. Comparison of direct and standard microtiter broth dilution susceptibility testing of blood culture isolates. J. Clin. Microbiol. **16**:96–98.
15. **Kiehn, T. E., B. Wong, F. F. Edwards, and D. Armstrong.** 1983. Comparative recovery of bacteria and yeasts from lysis-centrifugation and a conventional blood culture system. J. Clin. Microbiol. **18**:300–304.
16. **McLaughlin, J. C., P. Hamilton, J. V. Scholes, and R. C. Bartlett.** 1983. Clinical laboratory comparison of lysis-centrifugation and BACTEC radiometric blood culture techniques. J. Clin. Microbiol. **18**:1027–1031.
17. **Moore, D. F., S. S. Hamada, E. Marso, and W. J. Martin.** 1981. Rapid identification and antimicrobial susceptibility testing of gram-negative bacilli from blood cultures by the AutoMicrobic system. J. Clin. Microbiol. **13**:934–939.
18. **Salvanti, J. F., T. A. Davies, E. L. Randall, S. Whitaker, and J. R. Waters.** 1979. Effect of blood dilution on recovery of organisms from clinical blood cultures in medium containing sodium polyanethol sulfonate. J. Clin. Microbiol. **9**:248–252.

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