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Evaluation of a Lysis-Centrifugation System for Recovery of Yeasts and Filamentous Fungi from Blood

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A lysis-centrifugation system (Isolator; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) was compared with a biphasic brain heart infusion (BHI) medium in a prospective study of 5,125 fungal blood cultures. The Isolator recovered 90.3% of the positive cultures, compared with 63.4% recovered by the biphasic BHI medium. Overall, the detection of fungemia was increased 36.6% by the Isolator. Mean recovery times for yeasts were 2.12 and 4.90 days for the Isolator and BHI bottles, respectively. Cultures of *Histoplasma capsulatum* required 8.0 and 24.14 days for recovery by the Isolator and BHI bottles, respectively. The Isolator provided a more rapid and sensitive means of detecting organisms associated with fungemia.

Fungemia, a serious and often life-threatening infection for immunocompromised and surgical patients, warrants immediate attention. Laboratory efforts should be directed toward the rapid recovery and identification of the etiological agent(s) involved.

Previous reports have emphasized the importance of venting culture bottles to enhance the recovery rate of fungi from blood (1a, 3, 6). A vented biphasic brain heart infusion (BHI) medium is currently recommended to shorten the time for recovery of fungi (1, 4). A major disadvantage of the latter system is that a separate blood culture must be performed when fungemia is suspected, and conditions for incubation are suboptimal for the recovery of bacteria. Hence, a single system is not optimal for the detection of fungemia and bacteremia.

The recent introduction of a lysis-centrifugation blood culture tube, the Isolator, (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) offers the potential for detecting fungemia and bacteremia. Reports on the efficacy of the system for the recovery of bacteria have been encouraging (2, T. F. Kiehn, C. Capitolo, A. de la Cruz, and D. Armstrong, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C128, p. 292). The present study was undertaken to evaluate the Isolator for its ability to rapidly detect fungemia.

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MATERIALS AND METHODS

After cleaning the skin with povidone-iodine, we collected 20 ml of blood from patients in whom fungal sepsis was suspected. One 10-ml portion of blood was inoculated into a biphasic BHI bottle (Diagnostics, Inc., St. Paul, Minn.), another into a 10-ml Isolator tube.

Each biphasic BHI bottle contained a slant of 50 ml of BHI agar (BBL Microbiology Systems, Cockeysville, Md.) overlaid with 60 ml of BHI broth (Difco Laboratories, Detroit, Mich.). After inoculation at the patient's bedside, cultures were permanently vented with sterile cotton-plugged needles and incubated in an upright position for 30 days at 30°C. Cultures were examined daily for visual evidence of growth. Each examination was followed by a gentle washing of the agar slant surface with the blood-broth mixture, providing a daily subculture of broth onto the slant.

After Isolator tubes were inoculated, blood was mixed with the chemical components by inverting each tube several times. Isolator tubes were processed in our laboratory at 8:00 a.m. and at 12:00 and 4:00 p.m. Tubes that arrived after 4:00 p.m. were kept at room temperature and processed the next morning. Isolator tubes were centrifuged for 30 min at 3,000 × *g* in a Sorvall GLC-4 fixed-angle rotor centrifuge. After centrifugation, Isolator tubes were processed according to the recommendations of the manufacturer.

Equal amounts of sediment from the Isolator tubes were inoculated and streaked for isolation onto the surface of culture dishes containing chocolate agar, inhibitory mold agar, BHI agar, and Sabouraud 2% dextrose agar. Lids of the culture dishes were taped to prevent inadvertent opening during incubation. Cultures were incubated for 30 days at 30°C and examined daily for the first 14 days, followed by triweekly

TABLE 1. Recovery of fungi in Isolator and biphasic BHI medium

Species (no. of isolates)[a]	Matched and unmatched pairs	No. (%) of organisms recovered (matched pairs) by:						
		Isolator only		BHI only		Isolator + BHI		P value
C. albicans (30)	18	6	(33.3)	4	(22.2)	8	(44.4)	NS[c]
C. glabrata (56)	27	7	(25.9)	3	(11.1)	17	(63.0)	NS
C. guilliermondii (2)	2					2	(100)	NS
C. parapsilosis (5)	4	1	(25)			3	(75)	NS
C. tropicalis (27)	21	9	(42.9)	1	(4.8)	11	(52.3)	<0.05
C. neoformans (5)	4	2	(50)	1	(25)	1	(25)	NS
Saccharomyces spp. (1)								
T. beigelii (2)	1					1	(100)	NS
Beauvaria spp. (6)	1	1	(100)					NS
H. capsulatum (18)	15	8	(53.3)			7	(46.7)	<0.01
N. asteroides[b] (1)								

[a] Matched and unmatched pairs.
[b] Acid-fast bacterium.
[c] NS, Not significant.

observation for the rest of the incubation period. All fungi recovered during the study were identified by previously published methods (5).

Since Isolator tubes were not processed during the evening hours, the effect of holding tubes at 25°C before processing was determined. The positivity rate for cultures processed during four time periods, 0 to 2, 3 to 8, 9 to 12, and ≥13 h was compared with that of the total number of tubes received and held during the same time periods.

The contamination rate for the Isolator was defined as on-streak if colonies were within the streaked line of inoculation and as off-streak if colonies were away from the streaked line of inoculation. Only the on-streak contaminants recovered during the first 14 days of incubation were considered, and these were identified to the species level.

Statistical analysis for all comparisons between Isolator and biphasic BHI bottles was made only when both were inoculated (i.e., matched pairs). Data obtained from all Isolators were used to study the parameters of that system and to compare the different media used.

RESULTS

Of the 5,125 fungal blood cultures performed from March to October 1982, 153 (2.98%) (Table 1) were positive for pathogenic yeasts, filamentous fungi, or *Nocardia asteroides*. Only 93 matched pairs of positive cultures were available for a comparison of the Isolator system to the biphasic BHI bottle. Overall, the Isolator recovered 84 (90.3%) of the positive cultures, whereas the biphasic BHI bottle recovered 59 (63.4%) ($P < 0.01$). The Isolator increased the positivity rate during the study by 36.6% (34 of 93) and detected 42.9 and 53.3% of the *Candida tropicalis* ($P < 0.05$) and *Histoplasma capsulatum* ($P < 0.01$) isolates, respectively (Table 1).

The Isolator detected yeasts more rapidly than the BHI biphasic bottle; mean recovery times for each were 2.12 and 4.90 days, respectively. Specific mean and median recovery times for individual species are shown in Table 2. *H.*

TABLE 2. Detection times of fungi recovered by Isolator and biphasic BHI medium

Species	Time to detection (days)									
	Isolator					BHI				
	Total recovered			Matched pairs		Total recovered			Matched pairs	
	Mean	Median	Range	Mean	Median	Mean	Median	Range	Mean	Median
C. albicans	2.37	2.0	1–6	1.71	2.0	4.0	3.0	2–8	3.14	3.0
C. glabrata	2.95	2.0	1–10	2.82	2.0	7.47	4.0	2–30	7.71	5.0
C. guilliermondii	1.0	1.0		1.0	1.0	2.0	2.0	1–3	2.0	2.0
C. parapsilosis	2.0	2.0		2.0	2.0	4.75	4.5	4–6	5.0	5.0
C. tropicalis	1.96	2.0	1–6	1.55	1.0	2.57	2.5	2–3	2.45	2.0
C. neoformans	2.25	2.0	2–3	2.0	2.0	4.5	4.5	4–5	4.0	4.0
Saccharomyces spp.	3.0	3.0								
T. beigelii	2.0	2.0		2.0	2.0	2.5	2.5	2–3	3.0	3.0
Beauvaria spp.	8.5	7.5	7–12							
H. capsulatum	9.39	8.0	7–14	8.0	8.0	15.67	23.0	15–40	24.14	23.0
N. asteroides[a]	14.0	14.0								

[a] Acid-fast bacterium.

capsulatum was detected significantly earlier by the Isolator; mean recovery times for the Isolator and biphasic BHI bottles were 8.0 and 24.14 days, respectively. Overall, 123 clinically important yeasts and filamentous fungi were processed in the Isolator tubes. Inhibitory mold agar, BHI, Sabouraud 2% dextrose agar, and chocolate agar each recovered 11.4, 4.1, 7.3, and 7.3% of the organisms, respectively. Specifically, inhibitory mold agar recovered the only isolate of *Trichosporon beigelii* as well as 25% of the isolates each of *Cryptococcus neoformans* and *Candida parapsilosis*. BHI recovered 21.1% of all the isolates of *Candida albicans* whereas Sabouraud 2% dextrose agar recovered the only isolate of *N. asteroides*. Interestingly, chocolate agar recovered 16.7% of the isolates of *H. capsulatum*.

The effect of holding Isolator tubes containing blood at 25°C before processing was determined by comparing the positivity rate and total number of tubes processed within four time quartiles: 0 to 2, 3 to 8, 9 to 12, and ≥13 h. The distribution of the 5,125 tubes processed within the four quartiles was equivalent. Overall, 20.9, 27, 27.1, and 26.8% were submitted for culture and held for the previously mentioned times, respectively. The overall positivity rates for tubes held during the time quartiles of 0 to 2, 3 to 8, 9 to 12, and ≥13 h were 2.6, 3.9, 2.2, and 1.3%, respectively. In general, the positivity rate decreased after 8 h of incubation at 25°C, ($P < 0.001$). Specifically, fewer isolates of *Candida glabrata* ($P < 0.01$) and *H. capsulatum* ($P < 0.002$) were recovered in tubes held for ≥9 h at 25°C.

On-streak contamination of media used with the Isolator was 8.4%, compared with a 0.4% contamination rate for the biphasic BHI bottles. The highest rate of bacterial contamination was observed during the first 4 days of incubation, and most fungal contamination appeared after 8 days. *Staphylococcus epidermidis* was responsible for 44% of the contamination; other bacteria, including *Bacillus* spp., accounted for 16%. Fungi, primarily *Cladosporium, Aspergillus, Geotrichum*, and *Ustilago* spp., accounted for 40% of the contaminants. No single organism other than *S. epidermidis* was consistently seen; others were randomly distributed throughout the duration of the study.

DISCUSSION

Results of our study revealed that the Isolator system increased the detection rate of fungemia by 36.6% in our institution. Significantly, more isolates of *C. tropicalis* and *H. capsulatum* were detected with the Isolator. Moreover, the Isolator markedly decreased the recovery time of yeasts; most were recovered within the first 3 days of incubation. Isolates of *H. capsulatum* were detected within 8 days.

Analysis of the different media used with the Isolator for the recovery of fungi showed that all four were necessary for optimal recovery. However, inhibitory mold agar detected more fungi than the other media evaluated. The use of fewer media and a larger inoculum for each is a possibility that should be evaluated.

Data on the effect of holding Isolator tubes before processing suggest that, for optimal recovery, cultures should be processed within 9 h of collection. Adhering to that time frame might further enhance the recovery rate of fungi with the Isolator system.

Contamination of media used with the Isolator had little effect on the recovery of pathogenic fungi. Most fungal contaminants were single colonies of airborne molds, and none are among the clinically significant fungi shown in Table 2. Isolates of *Beauvaria* spp., which might have been considered a contaminant, were present in several cultures.

Our study showed that the Isolator system is superior to the biphasic BHI bottle in its ability to recover fungi from blood. In addition, it provided a more rapid means of detecting organisms associated with fungemia. We recommend the Isolator as an optimal system for fungal blood cultures.

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