JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1984, p. 283–285
0095-1137/84/020283-03$02.00/0

Vol. 19, No. 2

# Effect of Storage of the Du Pont Lysis-Centrifugation System on Recovery of Bacteria and Fungi in a Prospective Clinical Trial

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Received 11 August 1983/Accepted 31 October 1983

A commercially available lysis-centrifugation system was compared with a conventional biphasic brain heart infusion medium in a prospective clinical study of 5,125 fungal blood cultures. Recovery rates were compared between two time periods to assess the effect of 25°C storage before processing by the lysis-centrifugation system. The lysis-centrifugation tubes processed within 9 h showed a significantly higher yield (3.4 versus 1.49%) for yeasts (*Candida glabrata*), filamentous fungi (*Histoplasma capsulatum*), and bacteria (8.84 versus 7.34%) (*Klebsiella pneumoniae* and *Serratia marcescens*) than did those processed after 9 h.

Sepsis is a serious and often life-threatening event and warrants immediate attention by clinical microbiology laboratories. Until recently, the microbiological evidence of sepsis was dependent on the inoculation of blood into appropriate liquid or biphasic media and choosing the appropriate incubation conditions and the detection system (5). One disadvantage to this is the necessity for separate cultures when fungal septicemia is suspected, since bacterial cultures are not optimal for the recovery of fungi and vice versa (1). A lysis-centrifugation (LC) method, the Isolator system (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.), is commercially available and has shown promise in the earlier detection of bacterial and fungal sepsis (4). Since this system has the ability to detect both fungal and bacterial sepsis concurrently, a comparative prospective study of 5,125 fungal blood cultures was performed at our institution (2). Conventional blood cultures inoculated at the patient's bedside contain the broth which acts as a "holding medium" while the culture is transported to the laboratory. Since the LC method depends on centrifugation to concentrate blood, the tube must be transported to the laboratory before processing. Before this report, only studies using simulated blood cultures studied the effect of storage of the LC system before processing (J. C. Richards and C. Bentsen, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, C124, p. 292). One objective of this present study was to determine the effect of storage of Isolator tubes at room temperature on the recovery of fungi from blood.

After the skin of the patient was cleansed with povidone-iodine, 20 ml of blood was collected from patients suspected of having fungal sepsis. One 10-ml portion of blood was inoculated into a biphasic brain heart infusion bottle (Diagnostics, Inc., St. Paul, Minn.) and another into the LC tube at the patient's bedside. The order of inoculation of bottle and LC tube was interchanged weekly. The brain heart infusion bottle used was previously described (1).

After Isolator tubes were inoculated, the blood and chemical components (sodium polyanetholesulfonate, saponin, polypropylene glycol, disodium ethylenedinitrilotetraacetate, and a dense, inert fluorochemical) were mixed by inverting the tube several times. Cultures were processed in the laboratory at 8:00 a.m. and at 12:00 and 4:00 p.m., and any cultures arriving after 4:00 p.m. were held at room temperature until the next morning; holding times were recorded. Briefly, tubes were centrifuged at 3,000 × *g* in a Sorvall GLC-4 fixed-angle rotor centrifuge for 30 min, and the supernatant was discarded as recommended by the instructions of the manufacturer. Equal amounts of sediment were streaked onto the surface of chocolate blood, inhibitory mold, Sabouraud 2% glucose, and brain heart infusion agars. Culture dishes were taped to prevent inadvertant opening, incubated at 30°C for 30 days, and examined daily for the first 14 days and triweekly thereafter. All bacteria and fungi were identified by established methods (6).

To assess the effect of delay in the processing of cultures, precentrifugation times were calculated by subtracting the time tubes were centrifuged from the time blood was drawn. As seen in Fig. 1, the range of delay in processing the LC tubes ranged from less than 1 to 22 h, and the median time was 9 h. Recovery rates were defined as the ratio of the number of LC tubes positive for bacteria or fungi to the number of LC tubes processed during the same time period. Figures 2 and 3 show the recovery of bacteria and fungi by the hour of delay in processing. Two time periods were chosen and compared by the chi-square method (3). The median time of 9 h was chosen such that time period A (0 to 9 h) contained 2,546 cultures processed within 9 h, and time period B (9 to 22 h) contained the remaining 48.4%.

The overall recovery of bacteria, yeasts, and filamentous fungi was 10.5%. Although our blood culture system was optimal for the detection of yeasts and filamentous fungi,

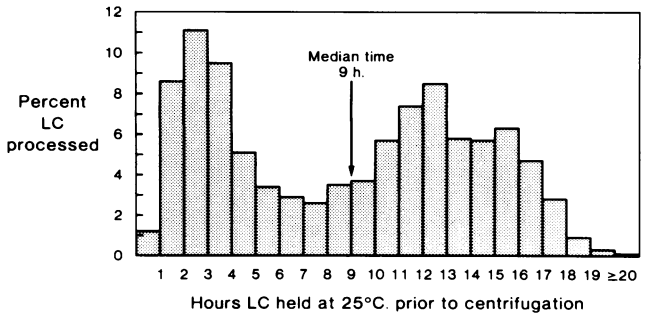


FIG. 1. Distribution of LC tubes processed.

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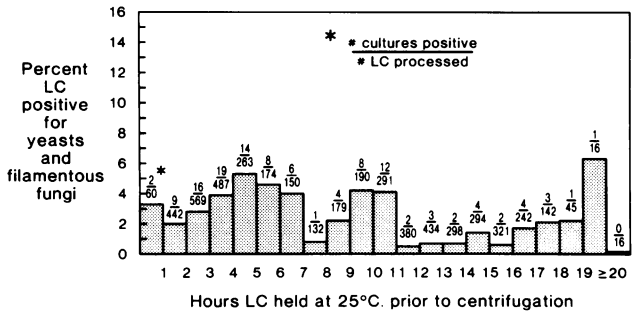


FIG. 2. Distribution of LC tubes positive for yeasts and filamentous fungi.

bacteria constituted 416 isolates, 76.7% of the total (Table 1). By comparing bacteria recovered in LC tubes processed within 9 h (8.84%) with those processed after 9 h (7.34%), there was a significant decrease in positive cultures ($P < 0.05$) (Table 3). The gram-positive bacteria collectively exhibited a decrease in the LC tubes held longer than 9 h, with *Mycobacterium avium-intracellulare* demonstrating the most significant difference ($P < 0.001$). Conversely, the gram-negative bacteria collectively did not show a statistically significant decrease until analyzed by species. The frequency of recovery of *Serratia marcescens* was significantly lower in time period B, and *Klebsiella pneumoniae* exhibited a probable decline. It is interesting to note that not all species showed a decline in frequency of recovery as the tubes were held longer. *Enterobacter aerogenes*, *Escherichia coli*, *Staphylococcus aureus*, and the viridans group streptococci exhibited a minor increase in frequency in time period B, although none were statistically different.

The fungi, representing 121 isolates (Table 2), also demonstrated a statistically significant decrease in frequency in time period B ($P < 0.001$) (Table 3). The two species most adversely affected were *Histoplasma capsulatum* ($P < 0.002$) and *Candida glabrata* ($P < 0.01$) (Table 3). Interestingly, *C. glabrata* constituted 41.7% of the yeast isolates.

The contamination rate of the LC tubes was 8.5% (2), and none of these isolates was included in this analysis. Most were *Staphylococcus epidermidis* (one colony) and were observed during the first 4 days of incubation. The fungal contaminants usually appeared after 8 days. The contamination rate appeared not to be related to the storage time of the LC tubes at room temperature before processing.

In conclusion, the LC tubes processed within 9 h showed a significantly higher yield for yeasts (*C. glabrata*), filamentous fungi (*H. capsulatum*), and bacteria (*K. pneumoniae*, *S. marcescens*, and *M. avium-intracellulare*) than did those LC tubes processed after a 9-h delay. Despite processing the LC tubes after 9 h of blood collection, the overall recovery rate for yeasts and filamentous fungi increased by 37% during this study (2). To ensure and perhaps enhance the recovery and the sensitivity of the LC system, we recommend processing all blood within 9 h of collection. However, in the average clinical microbiology laboratory where few cases of bacteremia or fungemia are seen, processing the cultures during the evening may not be feasible.

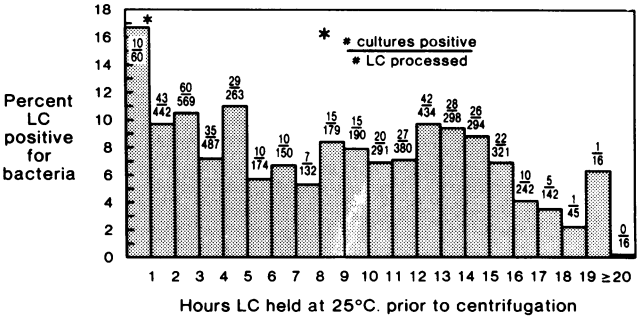


FIG. 3. Distribution of LC tubes positive for bacteria.

TABLE 1. Bacteria recovered from the LC system

| Organism | No. | Maximum time (h) at 25°C before processing |
|---|---|---|
| *Acinetobacter calcoaceticus* | 2 | 11 |
| *Bacillus* species | 2 | 2 |
| *Citrobacter diversus* | 2 | 16 |
| *Citrobacter freundii* | 1 | 1 |
| *Corynebacterium* species | 6 | 11 |
| *Enterobacter aerogenes* | 11 | 15 |
| *Enterobacter agglomerans* | 1 | 11 |
| *Enterobacter cloacae* | 3 | 14 |
| *Escherichia coli* | 55 | 19 |
| *Haemophilus influenzae* | 5 | 16 |
| *Haemophilus parainfluenzae* | 1 | 6 |
| *Klebsiella pneumoniae* | 19 | 17 |
| *Klebsiella oxytoca* | 2 | 17 |
| *Lactobacillus* species | 2 | 3 |
| *Morganella morganii* | 3 | 16 |
| *Mycobacterium avium-intracellulare* | 34 | 15 |
| *Nocardia asteroides* | 1 | 12 |
| *Proteus mirabilis* | 5 | 14 |
| *Proteus vulgaris* | 1 | 4 |
| *Pseudomonas aeruginosa* | 40 | 15 |
| *Pseudomonas alcaligenes* | 2 | 15 |
| *Pseudomonas fluorescens* | 10 | 15 |
| *Pseudomonas maltophilia* | 1 | 9 |
| *Pseudomonas paucimobilis* | 2 | 12 |
| *Pseudomonas* species | 3 | 10 |
| *Salmonella enteritidis* | 1 | 12 |
| *Serratia marcescens* | 10 | 17 |
| *Staphylococcus aureus* | 91 | 17 |
| *Staphylococcus epidermidis* | 55 | 18 |
| *Streptococcus* group A | 2 | 3 |
| *Streptococcus* group D enterococcus | 23 | 15 |
| *Streptococcus* group G | 1 | 12 |
| *Streptococcus pneumoniae* | 3 | 16 |
| Viridans group streptococci | 16 | 16 |
| Total | 416 | |

TABLE 2. Fungi recovered from the LC system

| Organism | No. | Maximum time (h) at 25°C before processing |
|---|---|---|
| *Beauvaria* species | 6 | 10 |
| *Candida albicans* | 18 | 18 |
| *Candida glabrata* | 43 | 19 |
| *Candida guilliermondii* | 2 | 9 |
| *Candida parapsilosis* | 4 | 5 |
| *Candida tropicalis* | 24 | 16 |
| *Cryptococcus neoformans* | 4 | 12 |
| *Histoplasma capsulatum* | 18 | 16 |
| *Saccharomyces cerevisiae* | 1 | 8 |
| *Trichosporon beigelii* | 1 | 4 |

TABLE 3. Frequency of bacteria and fungi recovered during time period A and time period B

| Organism | Time period A (%) | Time period B (%) | *P* value |
|---|---|---|---|
| Bacteria | 8.84 | 7.34 | <0.05 |
| Gram-positive organisms | 5.22 | 4.00 | <0.04 |
| *M. avium-intracellulare* | 1.10 | 0.20 | <0.001 |
| *S. aureus* | 1.70 | 1.90 | NS[a] |
| *S. viridans* | 0.27 | 0.36 | NS |
| Gram-negative organisms | 3.62 | 3.40 | NS |
| *E. aerogenes* | 0.19 | 0.24 | NS |
| *E. coli* | 0.94 | 1.21 | NS |
| *K. pneumoniae* | 0.53 | 0.20 | <0.06[b] |
| *S. marcescens* | 0.34 | 0.04 | <0.05 |
| Yeasts and filamentous fungi | 3.40 | 1.49 | <0.001 |
| *C. glabrata* | 1.17 | 0.48 | <0.01 |
| *H. capsulatum* | 0.60 | 0.08 | <0.002 |

[a] NS, Not significant.
[b] Borderline.

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