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Evaluation of Isolator System and Large-Volume Centrifugation Method for Culturing Body Fluids

HARRY R. ELSTON,[1]* MARGHERITA WANG,[1] AND ABRAHAM PHILIP[2]

Clinical Microbiology Laboratory, Veterans Administration Hospital, Loma Linda, California 92357,[1] and Diagnostic Division, E. I. du Pont de Nemours & Co., Wilmington, Delaware 19810[2]

Received 5 June 1989/Accepted 22 September 1989

The Isolator system was compared with the large-volume centrifugation method for processing and recovering organisms from body fluids other than blood, cerebrospinal fluid, and urine. A total of 155 body fluid samples were processed for the recovery of clinically significant organisms. Of the 55 positive cultures, Isolator detected 94% and the large-volume centrifugation method detected 64%. The time necessary to indicate positivity was not significantly different in the two methods; however, in five cases, the Isolator system yielded clinically significant organisms 24 h sooner than the conventional method. The Isolator system was found to be a more sensitive alternative than the conventional large-volume centrifugation method.

A common problem in the culturing of body fluids is the low yield of positive cultures. Because of the small number of organisms in most of these fluids, a concentration method to recover the organisms is desirable (6, 10, 11). Of these methods, the centrifugation of large volumes of body fluids is probably the most widely used (3, 5, 6, 8, 10).

Most studies dealing with this problem have concentrated on improving the techniques for peritoneal and peritoneal-dialysate fluids (2, 4, 5, 8, 11), but little attention has been paid to other body fluids. Evaluations have included clear and cloudy fluids, a variety of protocols and media (1, 5, 7, 9, 10), and blood culture broth systems (5–7, 11). This report compares the Isolator lysis-centrifugation system (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) with the large-volume centrifugation method.

From August 1987 to July 1988, 155 body fluid samples (other than blood, cerebrospinal fluid, and urine) from 118 patients were submitted to the Microbiology Laboratory of the Loma Linda Veterans Administration Hospital. These fluids were from abdominal (n = 23), peritoneal (including dialysates) (n = 45), pleural (n = 70), synovial (n = 16), and biliary (n = 1) sources.

Fluid (7 to 10 ml) was added to each Isolator tube. These tubes were then centrifuged at 3,000 × g (model TJ-6, modified; Beckman Instruments, Inc., Fullerton, Calif.) for 30 min. The sediment was mixed; Gram stained for microscopy; and inoculated onto MacConkey agar, 5% sheep blood agar, and chocolate agar plates for aerobes and facultative anaerobes, Sabhi medium for fungi, and blood agar enriched with vitamin K and hemin for anaerobes. Aerobic, anaerobic, and fungal cultures were incubated for 4, 6, and 8 days, respectively, and were examined daily for growth. The large-volume method used was that established by Vas and Law for peritoneal fluids and was applied to all fluids used in this study (10). The volume processed ranged from 20 to 100 ml, with the majority of specimens being two 50-ml samples, except for synovial fluids, for which 7 to 20 ml was used. The samples were centrifuged at 2,350 × g (Beckman TJ-6) for 15 min. The sediment was Gram stained, inoculated, incubated, and examined for growth in the same manner as in the Isolator system.

A total of 155 body fluid samples were examined. Of these, 55 (35%) tested positive for microorganisms. Identical results were obtained by each method in 32 of 55 positive fluids, while 23 specimens yielded different results. Isolator was found to be more sensitive than the large-volume centrifugation method in detecting organisms in fluids. Of the 86 isolates, 49 were detected by both methods, 33 were detected by Isolator only, and 4 were detected by the large-volume centrifugation method only. This sensitivity was apparent even when fluids from patients receiving antimicrobial therapy were cultured; i.e., two of seven tested positive by Isolator only.

A total of 62 isolates were deemed significant after physicians and the charts on history and clinical diagnosis were consulted. Of these isolates, 42 were recovered by both methods, 18 were recovered by Isolator only, and 2 were recovered by large-volume centrifugation method only (Table 1).

Many of the isolates (39%) were aerobic gram-positive cocci, mostly represented by the coagulase-negative-staphylococcus group. Gram stain examination of sediments was not very rewarding, as only 6% of the specimens were positive by microscopy. The time necessary to indicate positivity was not significantly different in the two methods; however, in five cases, the Isolator system yielded clinically significant organisms 24 h sooner than the conventional method.

A number of methods have been used for the recovery of organisms from body fluids. The application of lysis-centrifugation methods to the processing of fluids other than blood is relatively new. In our study, the Isolator method was found to be more sensitive than the conventional method in detecting organisms in fluids when the concentration of organisms was low. However, the recovery rate of isolates was comparable for both methods when fairly large numbers of organisms were present, i.e., when colony counts were 50 to 100 CFU/ml. The greater ability of the Isolator system to recover organisms at low concentrations is most likely due to the lysis of leukocytes and the inactivation of antimicrobial agents during the lysis-centrifugation process (9; M. B. Coyle, P. A. Granato, J. A. Morello, and R. J. Zabransky, Clin. Microbiol. Newsl. **8**:141–142, 1986).

The Isolator system also produced more cultures with multiple isolates, although even in quantitative cultures, potential pathogens and contaminants cannot always be

* Corresponding author.

TABLE 1. Number of microorganisms recovered from body fluids

Organism (*n*)	No. positive[a] by:		
	Both methods	Isolator only	Large-vol centrifugation only
Coagulase-negative staphylococci (17)	5	11	1
Staphylococcus aureus (4)	4	0	0
Viridans group streptococci (9)	5	3	1
Group D streptococci (4)	4	0	0
Escherichia coli (6)	3 (3)	3 (2)	0
Klebsiella pneumoniae (5)	4 (4)	1 (1)	0
Klebsiella oxytoca (1)	1 (1)	0	0
Enterobacter cloacae (3)	3 (3)	0	0
Morganella morganii (1)	1 (1)	0	0
Pseudomonas aeruginosa (4)	2 (2)	2 (2)	0
Pseudomonas spp. (3)	0	1 (1)	2 (1)
Acinetobacter calcoaceticus subsp. *anitratus* (5)	2 (2)	3 (2)	0
Bacillus spp. (1)	1	0	0
Diphtheroids (2)	0	2	0
Kingella spp. (1)	1	0	0
Neisseria spp. (1)	1	0	0
Peptostreptococcus prevotii (2)	2	0	0
Eubacterium limosum (2)	2	0	0
Fusobacterium spp. (3)	3	0	0
Bacteroides fragilis (2)	0	2 (2)	0
Bacteroides spp. (4)	2 (2)	2 (2)	0
Propionibacterium spp. (2)	0	2	0
Candida albicans (4)	3	1	0

[a] Numbers in parentheses indicate the number of clinically significant results.

differentiated, and there can be a certain amount of overlapping (6). We found two diphtheroids, two *Propionibacterium* species, and six instances when coagulase-negative staphylococci were considered frank contaminants. Of these coagulase-negative staphylococcus isolates, one was recovered by both methods, one was isolated by the large-volume method only, and four were isolated by Isolator only.

Some recently described methods to improve culture recovery have used enrichment broth to enhance the growth of organisms (5, 8, 11; T. J. Tinghitella and L. Buhlmann, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, C-11, p. 333). One of the difficulties with a broth system is that growth cannot be quantitated and organisms still have to be isolated. This is not a problem with the Isolator system because the initial inoculation after processing is on plates.

In addition to its greater sensitivity, the Isolator system has the advantage of requiring only 10 ml of fluid, which is helpful when large volumes of fluid are not available. The Isolator tubes can be inoculated at the bedside of the patient, thus decreasing the interval between obtaining and inoculating the specimens. Also, since we recovered 15 anaerobic isolates, 6 of which were detected by the Isolator system only, this study indicates that anaerobic cultures should be included in any microbiology protocol for fluids.

Compared with the large-volume centrifugation method, the Isolator system is somewhat more cumbersome to use and more prone to contamination during specimen processing, and the materials are slightly more expensive. However, the Isolator system yielded higher numbers of positive cultures for both aerobic and anaerobic organisms. We believe that this could result in earlier laboratory diagnosis and, ultimately, shorter hospitalizations for patients. Thus, the Isolator system is a feasible alternative to the conventional large-volume centrifugation method for culturing body fluids.

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LITERATURE CITED

1. **Dawson, M. S., A. M. Harford, B. K. Garner, D. A. Sica, D. M. Landwehr, and H. P. Dalton.** 1985. Total volume culture technique for the isolation of microorganisms from continuous ambulatory peritoneal dialysis patients with peritonitis. J. Clin. Microbiol. **22**:391–394.
2. **Fenton, P.** 1982. Laboratory diagnosis of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. J. Clin. Pathol. **35**:1181–1184.
3. **Finegold, S. M., and E. J. Baron.** 1986. Bailey and Scott's diagnostic microbiology, 7th. ed., p. 316–317. The C. V. Mosby Co., St. Louis.
4. **Gould, I. M., I. Reeves, and N. Chauhan.** 1988. Novel plate culture method to improve the microbiological diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. **26**:1687–1690.
5. **Males, B. M., J. J. Walshe, L. Garringer, D. Koscinski, and D. Amsterdam.** 1986. Addi-Chek filtration, BACTEC, and 10-ml culture methods for recovery of microorganisms from dialysis effluent during episodes of peritonitis. J. Clin. Microbiol. **23**: 350–353.
6. **Rubin, J., W. A. Rogers, H. M. Taylor, E. D. Everett, B. F. Prowant, L. V. Fruto, and K. D. Nolph.** 1980. Peritonitis during continuous ambulatory peritoneal dialysis. Ann. Intern. Med. **92**:7–13.
7. **Runyon, B. A., E. T. Umland, and T. Merlin.** 1987. Inoculation of blood culture bottles with ascitic fluid, improved detection of spontaneous bacterial peritonitis. Arch. Intern. Med. **147**:73–75.
8. **Ryan, S., and S. Fessia.** 1987. Improved method for recovery of peritonitis-causing microorganisms from peritoneal dialysate. J. Clin. Microbiol. **25**:383–384.
9. **Spencer, R. C., and W. K. Ahmad.** 1986. Laboratory diagnosis of peritonitis in continuous ambulatory peritoneal dialysis by lysis and centrifugation. J. Clin. Pathol. **39**:925–926.
10. **Vas, S. I., and L. Law.** 1985. Microbiological diagnosis of peritonitis in patients on continuous ambulatory peritoneal dialysis. J. Clin. Microbiol. **21**:522–523.
11. **Woods, G. L., and J. A. Washington II.** 1987. Comparison of methods for processing dialysate in suspected continuous ambulatory peritoneal dialysis-associated peritonitis. Diagn. Microbiol. Infect. Dis. **7**:155–157.