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Improved Method for Recovery of Peritonitis-Causing Microorganisms from Peritoneal Dialysate

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The efficacy of recovery of peritonitis-causing microorganisms from peritoneal dialysate fluid by using the Septi-Chek blood culture system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.) was compared with those of other conventional techniques, such as the 20-ml culture and the filtration methods. The recovery of microorganisms by using the Septi-Chek system was found to be as effective as the filtration of 250 ml of dialysate that used a modified Millipore filtration technique (Millipore Corp., Bedford, Mass.). Both methods were found to be superior to the 20-ml culture method. We suggest using the Septi-Chek method as the standard protocol for the culture of dialysate as a relatively inexpensive and labor-saving recovery technique.

Continuous ambulatory peritoneal dialysis (CAPD) is becoming a feasible alternative to hemodialysis in the treatment of end-stage renal disease since it allows the patient mobility and an improved quality of life. Unfortunately, despite many technical improvements which have certainly lowered the high incidence of peritonitis associated with this technique, peritonitis remains a serious problem for these patients.

The diagnosis and treatment of peritonitis greatly depends on the quick recovery and identification of the peritonitis-causing organism from the dialysate fluid. Culturing of dialysis fluid from CAPD patients remains a time-consuming and costly procedure. Methods for the culturing of dialysis fluid continue to be refined. In the meantime, both the number of patients on CAPD and the number of hospitals involved in culturing dialysis fluid increase. Our laboratory is attempting to find methods to increase the recovery of microorganisms while decreasing the workload associated with these specimens (S. Fessia, B. Cocanour, and S. Ryan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1986, C204, p. 362). In the present study, the Septi-Chek blood culture system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.) was adapted for use with peritoneal dialysate. The recovery rate by using the enrichment system was compared with those by standard methods.

MATERIALS AND METHODS

Patient samples. Specimens were received by the Microbiology Department intact in the dialysis bags from patients receiving CAPD at St. Joseph's Dialysis Unit. The entire dialysis bag was received in the laboratory rather than an aliquot. In this way, mixing of the fluid in the bag could be performed in the laboratory and a more representative sample of fluid could then be aspirated for culture and cell count. Specimens included in this study had cell counts of >100 cells per mm^3, with a majority of polymorphonuclear leukocytes (10; S. J. Rubin, Clin. Microbiol. Newsl. **6**:3–5, 1984). The cell counts were performed by using a hemacytometer, and a differential was made by concentrating the specimen with Cytospin 2 (Shandon Southern Instruments, Sewickley, Pa.) and staining with Wright stain. Specimens (100 samples) were tested from patients seen in the hospital from January to September 1985.

Culture and isolation. Specimens were obtained aseptically by cleansing the port of the dialysate bag and removing the fluid with a 20-ml syringe. Specimens were cultured by routine culture techniques, by a modified filtration technique (1) with routine culture methods, and by the Roche Septi-Chek system. Briefly, our routine culture system used the sediment of 20 ml of centrifuged dialysate for Gram stain and culture onto a 5% sheep blood agar plate, a chocolate agar plate, a brain heart infusion broth, and a cooked meat-glucose broth. By use of the modified Millipore filtration system (Millipore Corp., Bedford, Mass.), 250 ml of dialysate was passed through a prefilter of gauze and a membrane filter (Millipore; 0.45-μm pore size). Each filter was placed on an agar plate of 5% sheep blood, eosin methylene blue, and Sabouraud dextrose agar. The Septi-Chek system is a two-bottle blood culture system that has an additional feature of an attached agar paddle. For dialysis fluid culture, a syringe was used to draw off a sample from the dialysis bag and this was transferred aseptically into the bottles. After the specimen was injected into the bottle, the cap was removed from the aerobic bottle and a plastic housing which contained an agar paddle was screwed onto the bottle. The fluid within the bottle could be made to pass over the agar by inverting the bottle. The agar slide was three sided and contained chocolate, MacConkey, and malt agars. The aerobic bottle contained Trypticase soy broth, and the anaerobic bottle contained Columbia broth. Each bottle was injected with 20 ml of dialysate fluid; another 20 ml was centrifuged, and the sediment was used for the Gram stain. The bottles were held for 7 days and checked daily for growth. They were incubated at 37°C in CO_2 and inverted daily.

Statistical methods. Statistical analysis was performed by the chi-square test (12).

RESULTS

After testing 100 patient specimens, we found that the recovery rate by using the routine 20-ml culture method for dialysis fluid was 51%. The modified filtration method increased recovery to 91%, whereas the Septi-Chek system

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TABLE 1. Microorganisms recovered in 100 specimens

Organism	No. of isolates recovered by method		
	20-ml culture	Septi-Chek	Modified Millipore filter
Staphylococcus aureus	17	22	22
Staphylococcus epidermidis	19	37	37
Viridans group streptococci	2	5	5
Streptococcus pneumoniae	0	2	2
Enterococci	1	3	3
Group D, nonenterococci	1	2	2
Peptostreptococcus spp.	0	1	0
Diphtheroids	3	4	4
Bacillus spp.	1	1	1
Pseudomonas aeruginosa	2	3	3
Klebsiella pneumoniae	1	2	2
Acinetobacter calcoaceticus	1	2	2
Escherichia coli	1	2	2
Bacteroides fragilis	0	1	0
Torulopsis glabrata	2	4	4
Candida albicans	0	2	2

was as or more efficient, with a recovery rate of 93%. The chi-square analysis comparing the results from the routine 20-ml culture method to the modified filtration or the Septi-Chek methods was significant to the 99.9% level ($P < 0.001$). The organisms recovered by using each method are listed in Table 1. The percentage of types of bacteria found in peritonitis episodes agrees with the reports of others (70% gram-positive cocci, 10% gram-negative bacilli, 5% gram-positive bacilli) (4). Staphylococci were the most frequently isolated. *Staphylococcus epidermidis* was encountered more often than *Staphylococcus aureus*. An unusual isolate of *Acinetobacter calcoaceticus* reported in only a few studies (4, 8, 9, 13) was also found in this study. Of the yeasts encountered, *Torulopsis glabrata* was found in 4% of peritonitis episodes.

DISCUSSION

The literature suggests that improved, standardized methods for laboratory isolation of peritonitis-causing microorganisms from peritoneal fluid are needed (2, 5, 7). Attempts using costly equipment, such as the BACTEC system developed by Johnston Laboratories, Inc., Towson, Md., for blood cultures have been shown in some laboratories to be somewhat successful in increasing the recovery of microorganisms from dialysate fluid (14). On the other hand, recent studies show that this procedure is not more sensitive than the 10-ml culture method or the Addi-Chek filtration method developed by Millipore Corp. (11). Use of the BACTEC system for peritoneal fluid requires the purchase of costly equipment for what is basically a screening procedure for detecting the growth of microorganisms; further subculturing for isolation and identification must still be done. Reports indicate that the increased efficiency is worth the cost of the BACTEC system (3), but we believe that there are less costly methods which are still feasible alternatives. Another less costly method tried in our laboratory is the total volume culture technique reported by Dawson et al. (4). Concentrated culture medium stored in small blood transfer bags is added to drained dialysate exchange bags; both bags are then incubated, and the culture medium bag is observed for turbidity. We found this method to be very time-consuming owing to the requirement of medium preparation. Also we found this technique to be cumbersome because of the need to make transfers by using whole bags. Furthermore, the requirement of excessive incubator space for whole bags was a problem for this clinical laboratory, as it would be for other hospital laboratories with a high volume of specimens.

We use and recommend the Septi-Chek system for the routine isolation of peritonitis-causing microorganisms from peritoneal fluid. We showed it to have greater sensitivity than those of standard methods of plating, since the enrichment broth allows for enhanced recovery. This system requires minimal manipulation of the specimen, leading to decreased contamination; also, daily blind subculturing can be done without entering the system. This method offers significant workload reduction and requires no capital equipment investment.

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