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Practical Aerobic Membrane Filtration Blood Culture Technique: Clinical Blood Culture Trial

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During the course of preliminary clinical trials of an improved membrane filter blood culture system, filter plugging produced by a gelling of the lysing solution was observed when the patients had high leukocyte counts. A solution of streptokinase-streptodornase (Varidase, Lederle Laboratories) dissolved or prevented the gel and permitted rapid filtration without plugging. With streptokinase-streptodornase incorporated in the filtration procedure, a comparison of several culture systems was carried out on 176 blood cultures. Brucella broth with and without sodium polyanethol sulfonate, a prereduced osmotically stabilized broth, pour plates, and an improved aerobic membrane filter system were compared. The membrane filter system yielded 29 of the total of 37 positive cultures, far surpassing all other systems. Eight of these cultures were detected first by the filter technique, and 13 were positive only in this system. Nineteen of the 37 positive cultures were from patients on antimicrobial agents. Fourteen of these were detected by the filter, twice the number detected by any of the other systems.

The need for more efficient blood culturing techniques has been recognized for many years. It is important that bacteremia be detected and the infecting organism identified rapidly to guide proper antimicrobial therapy. Standard methods for recovery of bacteria from blood are inadequate in that they usually require several days for growth and identification of the infecting organism.

The advantages of membrane filtration for blood culturing have been recognized for many years, but practical, efficient membrane filter procedures have not been available. The accompanying paper (5) describes a very effective and simple membrane filtration system.

This paper reports details of a clinical study initiated to compare this improved membrane filter-lysing procedure with conventional broth and pour plate procedures.

Filter plugging, not encountered in studies with blood artificially seeded with bacteria, became a problem in a preliminary clinical study. Data will be presented to show a relationship between elevated leukocyte counts in blood samples and such plugging. Further information bearing on this problem and its solution by use of streptokinase-streptodornase (SK-SD) will also be presented. The clinical study reported in this paper utilized SK-SD in the filter procedure.

MATERIALS AND METHODS

Studies related to filter plugging. Filter plugging related to gelling of the lysing solution was noted in patients with elevated leukocyte counts in a preliminary clinical blood culture study. Accordingly, the following studies were initiated.

Blood (10 ml) was drawn from patients with leukocyte counts of greater than 10,000/mm^3. The erythrocytes were sedimented in a solution of 3% dextran in sodium lactate (2, 6). The supernatant (plasma and leukocytes) was placed into 190 ml of 0.08% Na_2CO_3-0.005% Triton X-100 solution and was filtered by the usual membrane filter technique.

A solution of SK-SD (Varidase, Lederle Laboratories) was prepared by diluting the commercial product to 20 ml with sterile water. This gave 5,000 U of SK and 1,250 U of SD per ml. When plasma supernatants placed into the lysing solution formed a gel, 3 ml of SK-SD was added.

The two components of SK-SD were studied separately. Four Vacutainer tubes (BD, Inc.) of blood from the same patient were collected, and the erythrocytes from each were sedimented by the dextran procedure. Supernatants were added to four separate bottles of lysing solution. One served as a control, one received 3 ml of SK-SD solution, and the other received, respectively, the corresponding amount of either SK or SD. Each solution was then filtered in the usual way.

Artificial seeding of blood in Vacutainer sodium polyanethol sulfonate (SPS) tubes was carried out with *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and viridans streptococci. The blood was then added to the lysing solution, and 3 ml of SK-SD was added and mixed for 1 min. The solution was filtered and incubated aerobically at 35 to 37 C. Brucella agar pour plates with no enzyme were used to quantitate the inocula used in the tests. Colony counts were made at 24 h.

Clinical blood culture study. Patients selected for the study, on the basis of suspected bacteremia, were from the Wadsworth and Sepulveda Veterans Hospitals and the UCLA Medical Center. Patients were evaluated clinically and only clinically significant bacteremias were considered as positive.

A 35-ml amount of blood was drawn, using a previously described three-way stopcock procedure (4). This provides a fresh sample for each system. Figure 1 shows the distribution of the blood and the incubation scheme. A 5-ml amount of blood was injected aseptically into two bottles, each containing 100 ml of Brucella broth (Pfizer), one of which contained SPS; both bottles contained 10% CO_2 in their atmosphere. A 5-ml amount of blood was also introduced into a bottle containing 100 ml of prereduced osmotically stabilized (OS) broth (McGaw Laboratories pilot batch) (4). Two Vacutainer tubes containing 0.35% SPS each received 8.3 ml of blood. One of these tubes was used for the membrane filter technique described in detail in the accompanying paper (5). The other was used to make 10 pour plates; 1 ml of blood was added to 19 ml of cooled, melted Brucella agar for the pour plates. The broths were incubated in a conventional incubator. The pour plates were incubated as follows: four were placed into a GasPak jar, three were placed into jars with 10% CO_2 in air, and three were incubated aerobically. The blood agar and chocolate agar plates containing the filters were placed under 10% CO_2, and the eosin-methylene blue plate with the filter was incubated aerobically. All systems were incubated at 35 to 37 C. Whenever feasible, observations of cultures were made at frequent intervals to determine which system yielded positive results earliest.

RESULTS

Studies related to filter plugging. When injected into the lysing solution, the plasma and leukocyte supernatants from patients with high leukocyte counts all formed gels which plugged the filter (Fig. 2 shows leukocyte count versus filtration time).

SK-SD (3 ml) (15,000 U of SK, 3,750 U of SD) added to the gelled solution resulted in breaking up of the gelled mass and filtration without plugging. Gelling could be prevented by adding SK-SD prior to gel formation.

In Table 1, a comparison of filter times with and without the enzymes is shown. In all cases the filtration times were under 4 min when SK-SD was used, whereas they were usually quite prolonged without the enzymes.

The two components of SK-SD, tested separately (Table 2), were clearly much less effective than the combination. With SK, there was little effect on the gelled mass and only a slight improvement in filtration. SD was distinctly better; the gelled mass appeared to dissolve and filtration was improved, but not nearly as much

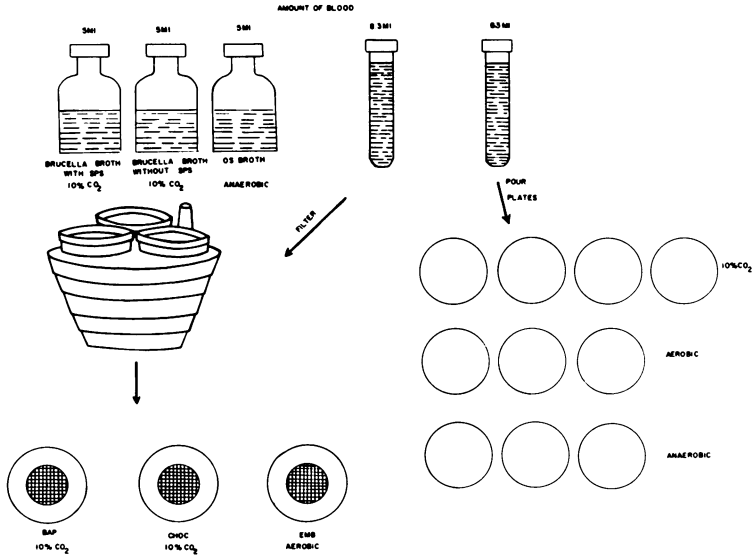

FIG. 1. *Distribution of blood in various culture systems.*

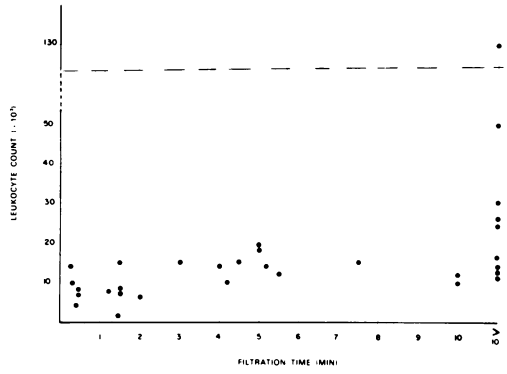

FIG. 2. *Leukocyte count versus filtration time.*

TABLE 1. *Filtration times with and without SK-SD in system*

Leukocyte count	Filtration time (min)	
	Without SK-SD	With SK-SD
6,900	2	1.33
10,900	10	2.50
14,300	4	1.67
14,300	0.75	1.75
16,000	18	1.33
17,550	15	1.33
18,800	5	2.50
24,000	>10	2.50
24,000	12	3.50
50,000	15	2.42
50,000	>10	2.50
136,000	20	0.67

as when the two enzymes were used in combination.

Simulated bacteremias using various test organisms failed to show any significant inhibitory effect of the SK-SD solution (Table 3).

Clinical blood culture study. The specific organisms recovered by each system, counts (on the filters and in the pour plates), the speed of detection, and the antimicrobial agents patients were receiving at the time of the culture are listed in Table 4.

Table 5 summarizes the positive blood culture obtained in each of the systems compared in the study. In all, there were 37 positive cultures (one double bacteremia was counted as two positives), representing 27 episodes of illness in 25 patients. The membrane filter far surpassed any other system, with 29 positive results. Furthermore, eight cultures were detected earliest in this system and 13 were detected only by the filter technique.

Nineteen cultures in this study were from patients on antimicrobial agents. Table 6 shows the results of the cultures from these patients. The membrane system yielded 14 positive cultures, twice the number of the next best system. There were seven instances in which only the filter system was positive and five additional cultures in which growth was first detected in the filter system.

TABLE 3. *Effect of SK-SD[a] on organisms*

Organisms	No. of organisms recovered in pour plate	No. recovered on filter	Percent recovered
Viridans *Streptococcus*	45	41	91
		44	98
K. pneumoniae	64	72	100+
S. aureus	10	18	100+
P. aeruginosa	87	61	70
E. coli	75	85	100+
		76	100+

[a] SK-SD, 3 ml of a 1:20 dilution in water.

TABLE 2. *Filtration times with SD, SK, and the combination*

Leukocyte count	Filtration times			
	Control	SD	SK	SK-SD
24,000	Instant plugging	Incomplete after 10 min (15 ml left)	Incomplete after >16 min (100 ml left)	3.5 min
24,000	Incomplete filtration even after 10 min (20 ml left)	7 min	Incomplete after 10 min (15 ml left)	2.5 min
55,000	Instant plugging	Incomplete after 12 min (20 ml left)	Incomplete after 10 min (100 ml left)	4 min

TABLE 4. *Speed of detection and quantitation of organisms recovered from 37 positive blood cultures*

Organism recovered[a]	Antimicrobial agents	Avg no. of colonies/ml		Broths		
		Pour plate	Filter	Brucella with SPS	Brucella without SPS	OS
P. aeruginosa	Gentamicin Carbenicillin	—[b]	—	—	—	+ (21 days)
Candida albicans	Amphotericin B	—	0.1 (7 days)[c]	—	—	—
Viridans *Streptococcus*	Methicillin	—	0.1 (7 days)	—	—	—
Viridans *Streptococcus*	Ampicillin	—	1.2 (5 days)	—	—	—
Viridans *Streptococcus*	Clindamycin	—	—	—	+ (5 days)	—
S. aureus	Cephalothin Gentamicin	—	0.1 (11 days)	—	—	—
P. aeruginosa	Polymyxin Sulfadiazine	—	3.4 (15 h)	—	+ (96 h)	+ (64 h)
P. aeruginosa	Trimethoprim	1.0 (48 h)	1.8 (24 h)	+ (5 days)	—	+ (4 days)
E. coli	Penicillin G	—	0.1 (24 h)	—	—	—
P. aeruginosa	Penicillin G	—	—	+ (14 days)	—	—
S. aureus	Penicillin G Gentamicin	0.1 (7 days)	—	—	—	—
Candida sp.	Gentamicin Polymyxin Carbenicillin	0.2 (7 days)	0.4 (4 days)	—	—	—
S. aureus	Clindamycin	—	0.3 (48 h)	—	—	—
S. aureus		—	0.2 (24 h)	—	—	—
S. aureus		15.0 (20 h)	>300 (20 h)	+ (20 h)	+ (20 h)	+ (20 h)
Staphylococcus epidermidis	Penicillin G	0.2 (3 days)	—	—	—	+ (3 days)
C. albicans	Gentamicin	0.2 (24 h)	0.3 (24 h)	+ (24 h)	+ (24 h)	—
C. albicans	Carbenicillin	1.1 (48 h)	4.8 (19 h)	+ (24 h)	+ (24 h)	—
P. aeruginosa	Cephalothin	—	0.1 (24 h)	—	+ (48 h)	—
C. albicans	—	2.0 (37 h)	0.2 (37 h)	—	—	—
Serratia marcescens	—	2.0 (4 days)	3.4 (31 h)	+ (48 h)	+ (48 h)	+ (48 h)
S. marcescens	—	25.0 (92 h)	30.7 (92 h)	+ (24 h)	+ (92 h)	+ (92 h)
S. marcescens	—	59.0 (3 days)	94.4 (3 days)	+ (3 days)	+ (3 days)	+ (3 days)
E. coli	—	0.4 (14 days)	—	+ (5 days)	—	+ (24 h)
S. epidermidis	—	0.1 (3 days)	—	—	—	—
S. epidermidis	—	0.2 (3 days)	—	—	—	—
S. epidermidis	—	0.2 (2 days)	0.6 (7 days)	—	—	—
E. coli	—	—	0.1 (18 h)	—	—	—

TABLE 4—*Continued*

Organism recovered[a]	Antimicrobial agents	Avg no. of colonies/ml		Broths		
		Pour plate	Filter	Brucella with SPS	Brucella without SPS	OS
S. aureus	—	>300 (19 h)	>300 (9 h)	+ (19 h)	+ (19 h)	+ (19 h)
Torulopsis sp.	—	0.2 (5 days)	0.1 (5 days)	+ (5 days)	+ (16 days)	—
Haemophilus parainfluenzae	—	—	1.7[d] (4 days)	+ (10 days)	+ (10 days)	—
H. parainfluenzae	—	—	0.3[d] (3 days)	—	—	—
H. parainfluenzae	—	—	0.3[d] (2 days)	—	—	—
Enterococcus	—	150 (24 h)	111.7 (24 h)	+ (24 h)	+ (24 h)	+ (24 h)
Candida tropicalis	—	ND[e]	0.1 (5 days)	—	—	—
S. aureus	—	—	0.1 (48 h)	—	—	—
S. aureus	—	—	0.2 (8 days)	—	—	—

[a] Organisms joined by bracket indicates multiple positive cultures from same patient (never drawn on same day, but often on successive days) plus one double bacteremia.
[b] —, No growth.
[c] Figures in parentheses, time growth was first detected.
[d] Only on filter on chocolate blood agar plate.
[e] ND, Not done.

TABLE 5. *Results of 37 positive blood cultures[a] by various blood culture systems*

Blood culture system	Total no. positive	No. of cultures positive only in this system	No. of cultures detected first[b]
Membrane filter	29	13	8
Pour plates	18	3	1
Broths			
Brucella with SPS	13	1	1
Brucella without SPS	13	1	0
Prereduced OS	11	1	1

[a] Two different organisms were isolated from one of these blood cultures (counted as two positives).
[b] When more than one setup was positive.

DISCUSSION

It was observed that there was a direct correlation between filter plugging and the patients' leukocyte counts. The blood of patients with high leukocyte counts gelled in the lysing solution, thereby causing the filter to plug before all of the solution could be filtered. The leukocytes of leukemic and other patients with high leukocyte counts were separated from the erythrocytes and placed, together with the plasma, into the lysing solution. Gelling of the solutions and plugging of the filters again occurred. Commercial SK-SD (3 ml; diluted to 20 ml with water) added to the lysing solution-

TABLE 6. *Results of 19 positive blood cultures[a] drawn from patients on antimicrobial agents*

Blood culture system	Total no. positive when patient on antimicrobial agent(s)	Total no. positive only in this system	Total no. of positive cultures detected first in this system
Membrane filter	14	7	5
Pour plates	7	1	0
Broths			
Brucella with SPS	5	1	0
Brucella without SPS	6	1	0
Prereduced OS	5	1	0

[a] Two different organisms were isolated from one of these blood cultures (counted as two positives).

blood mixture resulted in breaking up of the gelled mass and permitted filtration without problem. In further experiments, it was found that one of the components of the SK-SD, SD (a deoxyribonuclease), breaks up the gelled compound or prevents gelling if added in advance. Hydrochloric acid, trypsin, pancreatin, pancreatic dornase, and heating to 85 C were tried with little or no effect on the gel. At alkaline pH values deoxyribonucleic acid denatures, becoming a gel. The pH of the lysing solution is between 10.0 and 10.3.

When 3 ml of SK-SD is added to the system there is little or no effect on bacteria. It is not until 9 ml is added that a marked decrease in recovery is observed.

A comparison of three types of blood culturing systems was made with 176 cultures. Among a total of 37 positive cultures, 29 were positive in the improved membrane filter system, 18 in the pour plates, 13 in Brucella broth with SPS, 13 in Brucella broth without SPS, and 11 in a prereduced OS broth. In several instances, bacteremia was not detected by the filter system, but was detected by one or more of the other systems. In virtually all of these cases, there was evidence that the bacteremia was of a very low level (very slow growth in one broth only or pour plates with documented low level bacteremia), and therefore the bacteremia may have been missed simply because of chance distribution of the small number of organisms present. The membrane filter gave earliest detection of bacteremia in eight cultures; 13 positive were detected only by this system. Part of the difference between the systems is undoubtedly related to the different amounts of blood used (8.3 ml for the filter and the pour plates and 5 ml for each of the broths). The choice of the amounts of blood was based on the following considerations. Conventional blood cultures utilize broths and either 5 or 10 ml of blood (for adults). Whereas the larger amount of blood is desirable, this would provide a dilution of only 1:10 in the 100-ml broth bottles; a dilution of 1:20 was felt to be preferable in terms of minimizing the effect of antibacterial agents and normal antibacterial factors. The Vacutainer tube which automatically takes in 8.3 ml of blood provided an excellent technique for drawing blood for subsequent processing by both the membrane filter technique and pour plates.

Nevertheless, the performance of the filter technique was outstanding in a way which was beyond what could be accounted for on the basis of the extra increment of blood. In an earlier study (4) the OS broth was distinctly better than the filter. This may be explained partly by the presence of a number of anaerobes (this is an anaerobic broth) and protoplasts picked up only by the OS broth in the earlier study and partly by a poorer OS broth utilized in the present study. The present OS broth was a pilot commerical batch with certain of the original ingredients deleted.

The speed of detection of bacteremia with the membrane filter system is one of its major attractions. There were eight instances in which this system detected the bacteremia first, compared to one for the next best system. It was not always possible to examine cultures at frequent intervals, particularly on weekends; had it been, the differences might have been even more striking.

One important advantage of the membrane filter procedure is that antimicrobial agents and other inhibitory substances in the blood are removed by this technique. Nineteen of the positive cultures were from patients on antimicrobials. Fourteen of the 19 were detected by the membrane filter (only seven by the next best system); seven were detected only by the filter system and five were detected first in this system.

A membrane filter setup comprised of three 47-mm filters (0.45-μm pore size) and a lysing solution of 0.08% Na_2CO_3-0.005% Triton X-100 plus 3 ml of a 20-ml dilution of SK-SD constitutes an excellent system for early, efficient detection of aerobic bacteremia. Since this system has not yet been tested with anaerobes, it is suggested that an appropriate broth for anaerobes be included for clinical blood cultures.

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