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Evaluation of the Sterifil Lysis-Filtration Blood Culture System[1]

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This paper describes the comparison of the Sterifil lysis-filtration (SLF) blood culture procedure with a standard Trypticase soy broth (TSB) technique. The lysing solutions employed in the SLF system, Triton X-100 (alkyl phenoxy polyethoxy ethanol) and sodium carbonate, were deleterious to most bacteria commonly encountered in bacteremia except staphylococci and enterococci. *Candida* was not adversely affected. There was a positive correlation between the tolerance of the microbial isolants to the lysing solutions and their recovery by the SLF technique. A total of 3,554 cultures were run in parallel and 398 isolants were obtained. Of 201 gram-positive isolants, 135 were recovered by both techniques, 43 were detected by the TSB technique only, and 23 were recovered only with the SLF method. In sharp contrast, of 168 gram-negative isolants, 28 were recovered in common, 130 were isolated only by TSB, and 10 were recovered only with the SLF method. The SLF method detected all cases of candidemia detected by the TSB method plus an additional 12 for a total of 29 cases. The SLF method, as currently described, is generally too toxic to bacteria for routine use in a clinical laboratory.

Conventional methodologies used for bacteremia detection have several obvious shortcomings which cause the interval of time between collection of the blood specimen and the identification of the bacterial isolant to be too long for optimum diagnosis and treatment. This delay is caused in part by (i) a delay in the appearance of visible indications of microbial growth in the broth substrate or a total lack of it altogether; (ii) the necessity of subculturing from the initial growth flask to appropriate solid media for characterization and identification; and (iii) the occurrence of growth-inhibiting substances in some blood such as antibodies, complement, properdin, β-lysin, lysozyme, and the carry-over of antibiotics, which may be partly responsible for growth retardation or inhibition.

Several investigators (1-4; S. M. Finegold et al., Bacteriol. Proc., p. 106, 1969) have recently demonstrated the advantages of the use of membrane techniques to overcome most of the shortcomings listed above. However, these membrane techniques vary in complexity and ease of use in the clinical laboratory setting. In an attempt to facilitate the handling of large numbers of specimens with a minimum outlay for equipment, the Millipore Corp. (Bedford, Mass.) marketed a complete unit called the Sterifil system which employs the lysis of erythrocytes with Triton X-100 (TX; alkyl phenoxy polyethoxy ethanol, Rohm and Haas, Philadelphia, Pa.) and sodium carbonate (SC) before membrane filtration.

The authors are aware that significant dissimilarities exist between the Sterifil system, (e.g., anticoagulated blood, lysing solutions, choice of media, and atmospheric conditions during cultivation) and the conventional broth methodology. However, the purpose of this study was to compare the sensitivity of this complete system with the conventional broth flask methodology for detecting aerobic bacteria in the blood of patients suspected of being bacteremic. Further, the toxicity of the lysing agents was tested against typical microbial isolants.

MATERIALS AND METHODS

Collection of blood sample. Approximately 15 ml of blood was collected aseptically by venipuncture in a syringe. A 5-ml amount was first added to Trypticase soy broth for the reference broth technique de-

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scribed below, and then 8.3 ml was added to a vacutainer tube (Becton-Dickenson, Rutherford, N.J.) containing 1.7 ml of Liquoid (0.35% sodium polyanethal sulfonate; Hoffmann-La Roche, Nutley, N.J.). The contents of the tube were completely mixed to prevent microclot formation.

Sterifil lysis-filtration (SLF) technique. A 50-ml amount of TX (alkyl phenoxy polyethoxy ethanol; 0.05%) was added to a Sterifil unit containing an HA membrane (Millipore Corp., Bedford, Mass.) with a hydrophobic periphery and a pore size of 0.45 μm. The unit was mounted onto a six-place manifold. A 3-ml amount of Liquoid anticoagulated blood was added slowly to minimize bubble formation. The blood was mixed with the TX by carefully removing the unit from the manifold and gently swirling in a circular pattern. A 50-ml amount of SC (0.08%) was added to the unit and the unit was again mixed gently. The mixture was allowed to set for 3 min or until clearing was apparent. The lysed blood was drawn by negative pressure through the membrane filter unit and manifold and into a waste collection flask. The membrane was rinsed with 50 ml of sterile saline (0.85%) and again pulled dry by negative pressure filtration. The membrane filter was transferred aseptically with sterile forceps onto the surface of antibiotic free Thayer-Martin chocolate-agar with Isovitalex enrichment (Bioquest, Cockeysville, Md.) in cluster dishes (Falcon Plastics) and incubated at 35 C in an atmosphere of 5% CO_2. Plates were examined daily for 3 days for evidence of colony formation. A hand lens facilitated the observation.

Reference broth technique. A 5-ml amount of blood obtained by venipuncture was added to a flask containing 50 ml of sterile Trypticase soy broth (BBL) with 0.1% agar. The flasks were incubated at 35 C for 9 days or until evidence of growth was obtained, i.e., turbidity, early hemolysis, or colony formation. Subcultures were made to appropriate media as suggested by the Gram-stained smear of the positive flask. If no evidence of growth was apparent after 9 days of incubation, a blind subculture was made onto the surface of sheep blood-agar and incubated overnight at 35 C. If no growth was observed, the culture was considered negative and discarded.

Toxicity of TX-SC solutions. To test the toxicity of the lysing agent upon the various genera and species of microorganisms, sufficient organisms were added to give a concentration of approximately 10^4/ml in a mixture of TX and SC, having final concentrations of 0.025 and 0.04%, respectively.

A sample was removed from the TX-SC mixture at 0.5-, 3-, 6-, and 20-min intervals and added to a Sterifil unit containing 10 ml of sterile saline. This mixture was promptly pulled through the unit with negative pressure, and the membrane was washed with 50 ml of sterile saline to remove trace quantities of the TX-SC solution. For assay of samples containing high counts, serial decimal dilutions were made in sterile saline before filtration. The potential total exposure time was approximately 1 min longer than the listed exposure periods because of the time required to filter and rinse away the residual TX-SC solution.

The membranes were aseptically removed with sterile forceps and placed onto the surface of indicated growth agars, i.e., Trypticase soy agar, 5% sheep blood-agar, chocolate-agar, etc. The conditions for incubation were the same as outlined above.

RESULTS

Of a total of 3,554 blood cultures compared, 398 were positive with at least one of the two techniques employed. The clinical significance of 67 isolants was not ascertained, reducing the number of known significant positive blood cultures to 331. The contamination rate with *Staphylococcus epidermidis* (1.4%) was similar for the two systems. Diphtheroids were found as contaminants in the reference broth technique (0.3%) but were not encountered with the SLF procedure.

One is impressed by the failure of the SLF system to detect a large number of bacteremias (Table 1). It is obvious that there are significantly more isolations with the Trypticase broth technique. The SLF procedure failed to detect 82% (130/158) of the significant gram-negative isolants. The difference is statistically significant for *Escherichia coli*, *Klebsiella*, *Pseudomonas*, *Proteus*, and *Enterobacter*. Numbers are too small for statistical comparisons of *Acinetobacter*, *Brucella*, *Haemophilus*, Providence group, *Salmonella*, *Serratia*, *Alcali-*

TABLE 1. *Recovery of 168 aerobic gram-negative bacterial isolants from 3,554 blood cultures employing a standard broth technique and the Sterifil lysis-filtration (SLF) technique*[a]

Organism	No. of isolations			
	Trypticase soy broth		SLF	
Escherichia coli	65	(55)	12	(2)
Klebsiella sp.	36	(24)	13	(1)
Pseudomonas sp.	19	(17)	7	(5)
Proteus sp.	16	(16)	0	
Enterobacter sp.	12	(9)	3	
Acinetobacter sp.	2	(2)	1	(1)
Brucella sp.	2	(2)	0	
Haemophilus influenzae	2	(2)	0	
Providencia	2	(2)	0	
Salmonella sp.	1	(1)	0	
Serratia	1	(1)	0	
Alcaligenes sp.	0		1	(1)
Neisseria meningitidis	1	(1)	0	
Totals	158	(130)	36	(10)

[a] Numbers in parentheses indicate the number of isolants recovered by one system which were not recovered by the other.

genes, and *Neisseria* on an individual basis. However, collectively, a significant difference is observed between the two systems.

The recovery of gram-positive organisms varied greatly among the various isolants (Table 2). No significant difference was observed between the two methods with *Staphylococcus aureus*. However, pneumococcal isolations were significantly lower with the SLF procedure. There is a trend toward better isolation of other gram-positive organisms with the reference broth technique. However, small numbers did not permit statistical comparison.

The SLF technique was statistically superior to the reference broth procedure for the detection of candidemia. Twenty-nine cases of candidiasis were detected with the SLF methodology as compared to 17 with the TSB technique. The details of this aspect of the comparisons will comprise a separate report.

Most of the bacterial isolants exhibited a decrease of viability after exposure to TX and SC (Fig. 1, 2, and 3). Only two bacteria, staphylococci and enterococci, failed to be adversely affected by the agents during the period of observation. At the other end of the spectrum, *H. influenzae*, *N. meningitidis*, and *Streptococcus pyogenes* were exquisitely sensitive, losing 4 logs of activity after 0.5 to 1.5 min of exposure. Other organisms demonstrated intermediate sensitivity. Viability of pneumococci could no longer be demonstrated after 6 min of exposure. *Proteus*, *Enterobacter*, and *Streptococcus* (Viridans type) were inactivated

TABLE 2. *Recovery of 201 aerobic gram-positive bacterial isolants from 3,554 blood cultures employing a standard broth technique and the Sterifil lysis-filtration (SLF) technique*

Organism	No. of isolations			
	Trypticase soy broth		SLF	
Staphylococcus epidermidis	53[a]		50[a]	
S. aureus	50	(13)	58	(21)
Streptococcus pneumoniae	35	(21)	14	
Streptococcus (Viridans type)	13	(9)	6	(2)
Diphtheroids	13[a]		0	
S. pyogenes (group A)	10		3	
S. pyogenes (not group A)	7		2	
Enterococcus	2		2	
Bacillus subtilis	0		1[a]	
Totals	178	(43)	134	(23)

[a] Clinical significance of these isolants is unknown and they are exluded from the comparison of the two procedures. Numbers in parentheses indicate the number of isolants recovered by one system which were not recovered by the other.

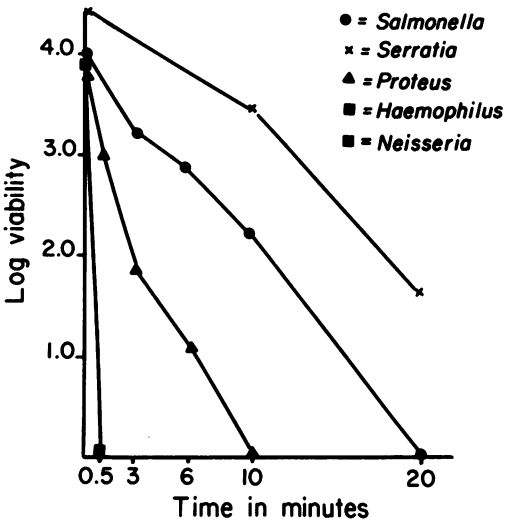

FIG. 1. *Effect of 0.025% Triton X-100 (alkyl phenoxy polyethoxy ethanol) and 0.04% sodium carbonate on the viability of selected gram-negative bacteria.*

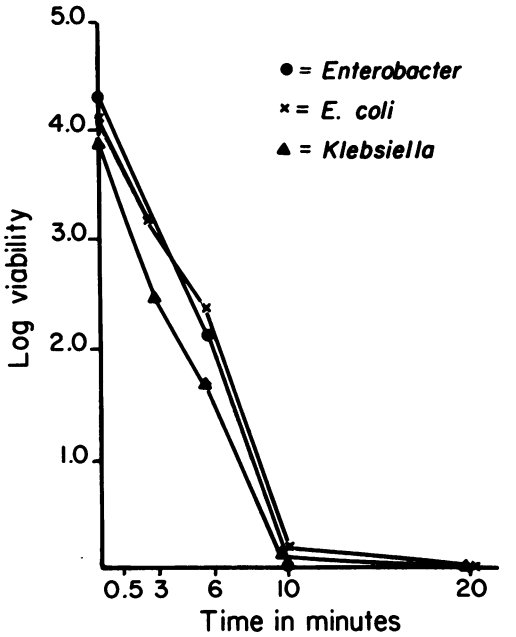

FIG. 2. *Effect of 0.025% Triton X-100 (alkyl phenoxy polyethoxy ethanol) and 0.04% sodium carbonate on the viability of selected gram-negative bacilli.*

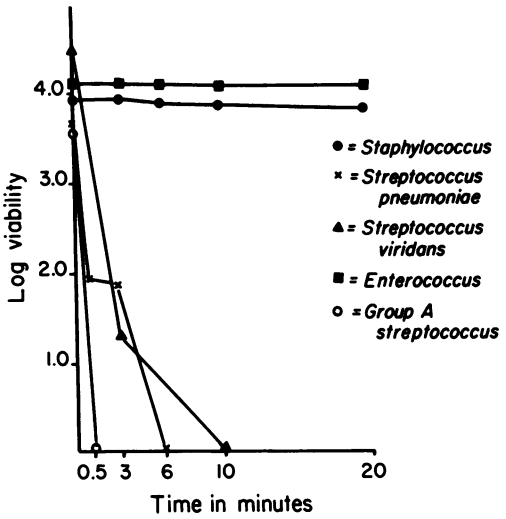

FIG. 3. *Effect of 0.025% Triton X-100 (alkyl phenoxy polyethoxy ethanol) and 0.04% sodium carbonate on the viability of selected gram-positive cocci.*

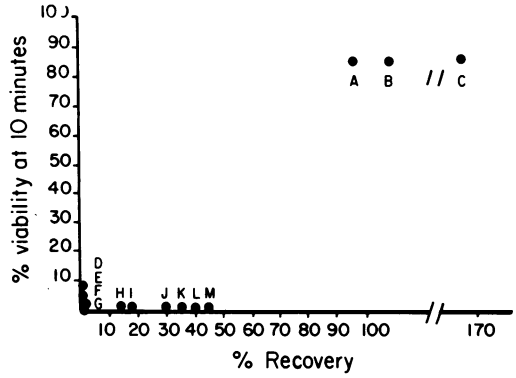

FIG. 4. *Relationship between per cent recovery of microorganisms with Sterifil lysis-filtration blood culture technique as compared to a standard broth procedure and the per cent viability of that genus/species after 10 min of exposure to a solution of 0.025% Triton X-100 (alkyl phenoxy polyethoxy ethanol) and 0.04% sodium carbonate. A, Enterococcus; B, Staphylococcus; C, Candida; D, Serratia; E, Salmonella; F, Haemophilus or Neisseria; G, Proteus; H, E. coli; I, Enterobacter; J, Streptococcus pyogenes; K, Klebsiella; L, Streptococcus pneumoniae; M, Streptococcus, Viridans type.*

after 10 min of exposure to the TX-SC mixture.

It was suggested, from the record of isolations, that the success in isolating microorganisms employing the SLF technique might bear some relationship to the tolerance of the microorganism to the lysing agents. Figure 4 shows the per cent viability after 10 min of exposure to the TX-SC mixture plotted against the per cent recovery of the organism by the SLF procedure as compared to the reference broth technique. It is obvious that those organisms which are the most tolerant of the TX-SC mixture, i.e., staphylococci and enterococci, have higher recovery rates. Conversely, those adversely affected are recovered less frequently with the SLF procedure.

DISCUSSION

It is obvious from our work and that of others that no one procedure suffices for the isolation of all microorganisms producing bacteremia. On a statistical basis, the SLF system under evaluation was significantly inferior to a standard broth technique. Nevertheless, 10 gram-negative bacteria, 23 gram-positive bacteria, and 12 fungi were isolated only with the SLF technique. If one excludes the 21 staphylococci that survived well in the presence of the TX-SC lysing agents, there were still 12 somewhat sensitive isolants that survived exposure to these agents and grew on the membrane from blood yielding negative broth cultures. This would suggest that there are inhibiting substances in the blood samples that are carried over into the culture flask and restrict microbial growth.

However, Finegold et al. (1), in their limited series, were unable to demonstrate any greater isolation on the membrane filter from patients receiving antibiotic therapy. Nonetheless, they were able to demonstrate that washing of filters was efficacious in removing natural serum factors inhibitory to group A streptococci and the antibiotics penicillin and chloramphenicol in concentrations 100-fold the normal serum levels. Kozub et al. (2) did not wash their filters inasmuch as they believed that all potential inhibitors were adequately diluted initially and did not selectively bind onto the membrane.

It is interesting to note that, although Kozub et al. (2) obtained three more isolations with their dilution-filtration technique than with their parallel broth, Finegold et al. (1) did not increase their total number of isolants. Even so, one can only conclude that both of those procedures were far less detrimental to the microbial population than the procedure reported herein. The SLF method failed to detect greater than 80% of the isolants obtained by the parallel broth procedure.

One problem encountered with membrane filtration techniques is plugging of the membrane pores. We encountered this problem

most frequently with blood improperly mixed with the anticoagulant and with blood which had been refrigerated before processing. To circumvent this problem, three approaches have been made: (i) use a prefilter of larger porosity (3 μm) proximal to the secondary filter of smaller porosity (0.45 μm; reference 2), (ii) use one filter of larger porosity (0.8 μm; S. M. Finegold et al., Bacteriol. Proc., p. 106, 1969), and (iii) use of 90-mm rather than a 47-mm diameter filter (1). Theoretically, by prior lysis of the erythrocytes, one could circumvent these considerations and use only one filter membrane of 0.45 μm pore size and still obtain reasonable filtration rates without plugging. However, in practice, it was not completely operational. Many specimens received from the wards plugged the filters; many more required excessive filtration times. No positive membrane cultures were obtained from specimens that did not filter promptly, due to the bactericidal effect of the lysing agents.

The comparisons made in this study were only with the aerobic phase of the system. The small membrane (47 mm) did not lend itself to being cut into several sections, each for a different growth condition, as did the 90-mm one employed by Finegold et al. (1). Processing duplicate or triplicate samples was not feasible.

It is well recognized that the volume of blood cultured can have a bearing upon the success of microbial isolation. Kozub et al. (2) could process a sample size of as large as 25 ml. The method of Finegold et al. (1) normally processed 10 ml. The manufacturer of the Sterifil system suggested a 5-ml sample, but it was virtually impossible to process that amount unless the specimen was processed immediately after collection. A 3-ml amount was finally established as the routine sample size. It is possible that some of the observed differences in recovery rates were due, in part, to the fact that only 3 rather than 5 ml of blood was used in the SLF technique. However, in a separate experiment in which over 700 specimens were processed with identical measured volumes of blood, the recovery rate for the SLF system was still only about 20% that of the standard broth technique.

One salient advantage of the filtration techniques is the shorter interval of time required for the isolation and identification of the microbial isolants. Although all membrane filtration procedures are somewhat time-consuming, the authors believe that the early growth on the filter membranes would compensate for the extra initial processing time, providing the system was not deleterious to the microbial population. In contrast to other membrane filter systems which employ nontoxic dextran (1) or glucose (2) solutions, the Sterifil system employed TX and SC. The surfactant effect of the TX and the marked alkalinity of the resulting mixture proved too toxic for all but a few of the organisms associated with bacteremia. It is our conclusion that the lysis-filtration approach does not hold much promise for the routine clinical laboratory as an effective tool for the evaluation of bacteremic patients. Those agents which are capable of lysing erythrocytes also adversely affect the viability of the microbial population.

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