Evaluation of the Sterifil Lysis-Filtration Blood Culture System

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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 isolate 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-lactam, 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-
scribed below, and then 8.3 ml was added to a vacu-
tainer tube (Becton-Dickenson, Rutherford, N.J.)
containing 1.7 ml of Liquoid (0.35% sodium polyane-
thal 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 contain-
ing 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 forma-
tion. 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 mem-
brane 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 choc-
olate-agar with Isovitalex enrichment (Bioquest,
Cockeysville, Md.) in cluster dishes (Falcon Plastics)
and incubated at 35 C in an atmosphere of 5% CO2.
Plates were examined daily for 3 days for evidence of
colony formation. A hand lens facilitated the obser-
vation.

**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 ob-
tained, 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 ap-
parent after 9 days of incubation, a blind subculture
was made onto the surface of sheep agar and
incubated overnight at 35 C. If no growth was
observed, the culture was considered negative and dis-
carded.

**Toxicity of TX-SC solutions.** To test the toxicity
of the lysis agent upon the various genera and spe-
cies of microorganisms, sufficient organisms were
added to give a concentration of approximately
10⁷/ml in a mixture of TX and SC, having final con-
centrations 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 quanti-
ties 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 indi-
cated 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 tech-
nique (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 bactere-
mias (Table 1). It is obvious that there are sig-
ificant 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 compari-
sions 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**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Trypticase soy broth</th>
<th>SLF</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>65 (55)</td>
<td>12 (2)</td>
</tr>
<tr>
<td><em>Klebsiella</em> sp.</td>
<td>36 (24)</td>
<td>13 (1)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>19 (17)</td>
<td>7 (5)</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>16 (16)</td>
<td>0</td>
</tr>
<tr>
<td><em>Enterobacter</em> sp.</td>
<td>12 (9)</td>
<td>3</td>
</tr>
<tr>
<td><em>Acinetobacter</em> sp.</td>
<td>2 (2)</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Brucella</em> sp.</td>
<td>2 (2)</td>
<td>0</td>
</tr>
</tbody>
</table>
| *Haemophilus influen-
zae*                    | 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)** |

*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 comparison 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>
<thead>
<tr>
<th>Organism</th>
<th>No. of isolations</th>
<th>Trypticase soy broth</th>
<th>SLF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus epidermidis</td>
<td>53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58&lt;sup&gt;a&lt;/sup&gt; (21)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>50 (13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>35 (21)</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Streptococcus (Viridans type)</td>
<td>13 (9)</td>
<td>6 (2)</td>
<td></td>
</tr>
<tr>
<td>Diphtheroids</td>
<td>13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>S. pyogenes (group A)</td>
<td>10</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>S. pyogenes (not group A)</td>
<td>7</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enterococcus</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Bacillus subtilis</td>
<td>0</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Totals</td>
<td>178 (43)</td>
<td>134 (23)</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> Clinical significance of these isolants is unknown and they are excluded 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.
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 prefiter 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.

LITERATURE CITED