Rapid Diagnosis of Bacteremia[1]

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Early appropriate treatment of bacteremia is important in minimizing morbidity and mortality. Standard blood culture methods are not optimal since several days are often required for recovery and identification of organisms which may be present in the blood. The use of a membrane filter technique allows one to grow any organisms present in blood much more rapidly than by broth or pour plate culture. Furthermore, growth is in the form of typical colonies on the surface of solid media, and a series of rapid diagnostic tests may be used to provide speedy identification. Use of membrane filters also facilitates removal by washing of normal antibacterial factors and antimicrobial drugs which may be present in blood. Although the filter technique yielded the most rapid growth, broth culture and whole blood pour plates yielded more positive cultures and use of all three systems was necessary for maximal recovery of organisms in blood cultures. Data on quantitative aspects of bacteremia in the antimicrobial era are also presented. The number of low level bacteremias (10 colonies/ml or less) is surprisingly high. This is particularly true for gram-negative bacilli; antimicrobial therapy at the time of culture undoubtedly influenced these results greatly. Finally, suggestions are given for a much simpler and more efficient membrane filter blood culture technique.

Even with the availability of a number of potent antibacterial drugs, the mortality from bacteremia today is still 20 to 25% (8), varying with the underlying disease and the infecting organism. When shock accompanies sepsis, the mortality rate is 60 to 80%. The major factor which may lead to better recovery rates in bacteremia is early administration of appropriate antibacterial therapy. (1, 5, 6, 7, 10) Accordingly, it is imperative that one determine as rapidly as possible whether a patient has bacteremia, the identity of the infecting organism when bacteremia is present, and the susceptibility of the organism to antibacterial agents.

Standard methods for recovering bacteria from the blood are not optimal, since it usually requires several days for growth and identification of the infecting organism. There is also reason to believe that most currently used techniques fail to culture organisms from a proportion of patients with bacteremia. The problem has been aggravated in recent years by carry-over of antibacterial drugs into blood culture media; many of these agents are not known to be readily susceptible to inactivation.

It is surprising and disappointing, therefore,

that there have not been more intensive efforts to improve blood culture techniques. Relatively little work has recently been done in this field. There are many descriptions of methodology, but few definitive studies comparing various blood culture methods. The very fact that different laboratories and different authorities differ widely in the selection of media, additives, type of culture vessel, atmosphere of incubation, and quantitative vs. qualitative techniques indicates the need for comparative studies. There are a number of investigations which have evaluated and compared various procedures for isolation of a particular (usually fastidious) organism from the blood. These, however, are not entirely pertinent to the usual problem faced by the bacteriologist and clinician who ordinarily will not know which organism is responsible for a given bacteremia. In any case, it is necessary to use methods which insure the greatest possible recovery, with the greatest speed, of any of the organisms commonly encountered in bacteremia. Special media and techniques may still be indicated in the occasional situation where one may suspect an unusual pathogen.

Our group has been studying different approaches to more efficient methods of blood culturing for several years. The major innovation which we have introduced is a system which

makes it practical to filter blood plasma through a membrane filter.

The advantages or potential advantages of the membrane filter technique are the following. (i) Much more rapid growth of organisms is achieved; (ii) growth initially occurs in the form of characteristic colonies, which saves one subculture step and therefore an additional 12 to 24 hr; (iii) colonies on the filter may be used directly for rapid identification and antibacterial susceptibility tests; (iv) inhibitory substances may be washed away, allowing better evaluation of therapy; (v) differential or selective solid media, or both, may be used directly; (vi) rough quantitation of bacteria present is possible; (vii) distinction between extracellular and intracellular (in white blood cells) bacteremia is possible in the untreated patient; (viii) eventually it may be possible to develop techniques to allow direct recognition, and perhaps even identification, of organisms on the filter without prior incubation; (ix) and early recognition of multiple bacteremia is possible.

Since publication of the results of the first 100 differential membrane filter blood cultures [first phase of the study (11)], we have completed a second phase of this study which involved an additional 177 blood cultures utilizing identical techniques. This report presents the data from the second phase of the study, together with an overall analysis of results of the 277 cultures.

MATERIALS AND METHODS

The procedure utilized has been presented in detail in the earlier publication (11). The red blood cells are sedimented rapidly by addition of dextran and the supernatant fluid is aspirated through a 37-mm membrane filter in a field monitor. Bacteria present in the plasma are trapped on the surface of the membrane filter. The filter is then washed with 300 ml of solution to remove natural antibacterial factors and antibacterial drugs which may be present in the patient's blood. After this procedure, the filter is placed on the surface of appropriate culture media and these are incubated. White blood cells and the sedimented red blood cells are cultured separately, after washing to remove additional plasma from the red cell mass. The wash fluid is then passed through a second membrane filter. For comparative purposes, broth cultures (5 ml of blood added to each of two bottles containing 100 ml of Albimi Brucella broth plus 0.1% agar) and whole blood pour plates (0.5 ml of blood added to each of ten 20-ml portions of Albimi Brucella agar) were set up from the same blood specimen. Penicillinase was used when indicated.

RESULTS

Of the total of 277 cultures (first and second phase considered together), 74 were positive by at least one of the techniques used. Eight of these proved to be contaminants, reducing the number of significant positive blood cultures to 66. Distinction between true bacteremia and contamination was based upon the clinical picture, the nature of the organism isolated, whether growth was obtained in one, two, or several of the different culture setups, and on the basis of the number of colonies or amount of growth obtained. The incidence of contamination was similar in all techniques, but it was usually easier to suspect contamination with the filter and pour plate techniques, particularly because of the quantitation of growth. Disregarding contaminants, a total of 40 patients had 66 positive blood cultures with 72 different organisms isolated; several patients had more than one organism present in their blood cultures. In all, 16 different species were represented. The gram-positive organisms isolated were *Staphylococcus aureus*, coagulase-negative staphylococci, pneumococci, group A streptococci, microaerophilic streptococci, alpha-hemolytic streptococci, and *Streptococcus lactis*. The gram-negative organisms represented were *Escherichia coli*, Klebsiella-Enterobacter, *Pseudomonas aeruginosa*, *Proteus mirabilis*, Providence, *Salmonella typhi*, and *S. typhimurium*. Two fungi were also isolated, *Candida tropicalis* and *Cryptococcus neoformans*.

Table 1 indicates the number of positive blood cultures by type of organism and technique used for the overall study (277 cultures).

The single, most efficient technique in terms of yield of positive cultures was the broth culture. Whole blood pour plates yielded the second

TABLE 1. *Results of positive blood cultures for entire study*

Determination	Gram-positive organisms	Gram-negative organisms	Fungi	Total
Any technique......	30[a]	36[a]	4	70[a]
Whole blood pour plate.............	25[d]	25[d, e]	4	54[d]
Erythrocyte mass...	21	21[e]	2	44
Filter #1...........	23[b]	23[b]	3	49[b]
Filter #2...........	18[c]	16[c]	2	36[c]
Leukocyte culture..	23	17[e]	4	44
Parallel broth culture..............	23[a]	32[a]	4	59[a]

[a] Four cultures positive for both gram-positive and gram-negative organisms.

[b] Three cultures positive for both gram-positive and gram-negative organisms.

[c] Two cultures positive for both gram-positive and gram-negative organisms.

[d] One culture positive for both gram-positive and gram-negative organisms.
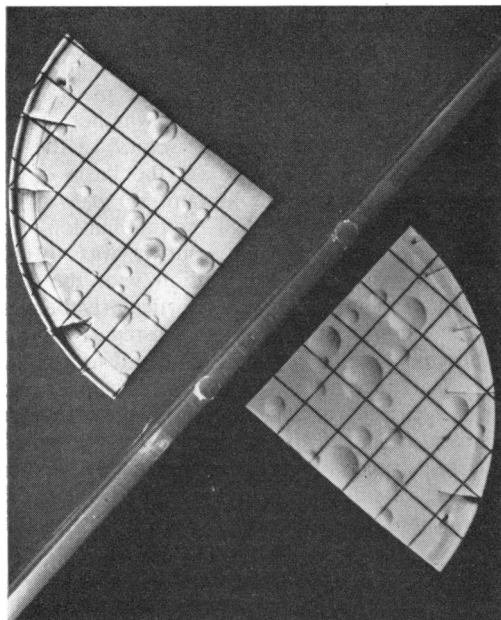
[e] Two to three cultures not performed or read.

FIG. 1. *Sectioned membrane filter; one piece on blood agar (darker background) and one piece on EMB agar. Note that on EMB agar the larger colonies (Klebsiella) have dark centers, indicating lactose fermentation. The other organism is S. aureus. Both colony types are larger on blood agar.*

highest number of positive cultures; the third highest number was yielded by filter no. 1. Figures 1 and 2 demonstrate the appearance of colonies on filters.

The filters offer a major advantage in terms of rapidity of diagnosis. Filter no. 1 grew out organisms in an average of 28 hr, compared to 46 hr for the whole blood pour plates, and 61 hr for the broth cultures. Furthermore, growth on the filters is in the form of surface colonies which have a typical morphology; in comparison with

broth cultures much time is saved, for a definitive identification of the organism can be quickly made.

The efficiency of the various culture techniques varied somewhat with the type of organism. For gram-negative organisms, the broth culture was distinctly more effective than the other techniques. For gram-positive organisms, whole blood pour plates were slightly better than broth cultures or filter no. 1. Use of the three best techniques (broth, whole blood pour plates, and filter no. 1) would have picked up all but two of the blood cultures positive by any technique. The other two were positive in the white blood cell culture (which we are presently combining with filter no. 1).

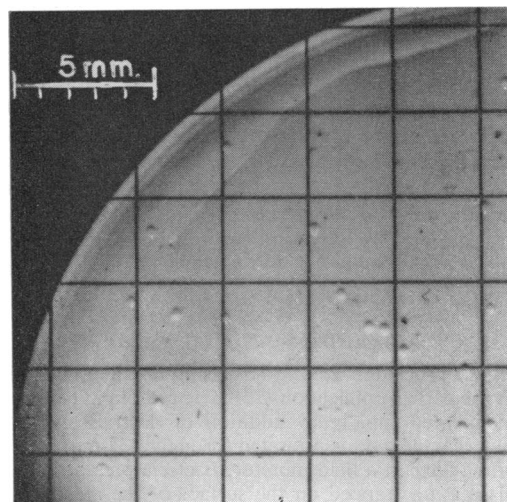Average and median colony counts per milliliter of blood (based on the whole blood

FIG. 2. *Early growth of S. aureus from clinical bacteremia on membrane filter. Note that even small colonies are readily distinguished from debris.*

TABLE 2. *Colony counts on whole blood pour plates for overall study*

Type of organism	Colony counts per ml of whole blood			No. of cultures with very low counts per ml of whole blood		
	No. of positive cultures	Avg colony count	Median colony count	No. of positive cultures	No. with counts of 1 or less per ml	No. with counts of 1–10/ml
Gram-positive.......	25	313	8	25	8 (6)[a]	5 (1)[a]
Gram-negative.......	25	17	1	25	13 (9)	9 (9)
Fungus..............	4	24	18	4	2 (0)	0
Total...............	54	155	7	54	23 (15)	14 (10)

[a] Number on antimicrobial therapy at time of culture indicated in parentheses.

TABLE 3. *Comparative effectiveness of different types of blood culture*

Parameter of evaluation	Broth (with 0.1% agar)	Pour plate	Membrane filter
Most rapid growth........			x
Best yield of positives			
Gram-positive cocci.....		x[a]	
Gram-negative bacilli....	x		
Overall.................	x		
Best for strict anaerobes...	x[b]	x[b]	
Best for strict aerobes.....			x
Best for patients on anti-bacterial agents[c]........			x
Best for quantitation......		x	
Best for low-level bacteremias[d].................	x		

[a] Advantage over other techniques was minor.

[b] Anaerobic incubation.

[c] Even with the use of inactivating agents such as penicillinase, there may be residual antibacterial activity.

[d] Measured as ≦10 organisms/ml of blood.

pour plates) varied widely according to the type of organism, as shown in Table 2. This table also shows the surprisingly large number of low-level bacteremias (arbitrarily defined as 10 or fewer colony-forming units per milliliter of whole blood), particularly with gram-negative organisms. The low counts of the organisms was perhaps partly related to therapy given prior to culture. Of 37 low-level bacteremias, as defined above, broth culture picked up 29, and filter no. 1 had 25 positives. Among 14 additional cultures negative by whole blood pour plate (implying even lower level bacteremia), eleven were positive in broth and three on filters. For the total of 51 low-level bacteremias, the broth culture was most efficient (40 positive) compared to the 37 positive by whole blood pour plate and 28 by filter.

Clear-cut differentiation of bacteremias as extracellular or intracellular (in the white blood cells) was usually not possible, perhaps because of the large number of patients on antibiotics at the time of culture.

Table 3 summarizes the advantages of each of the three most efficient blood culture techniques employed.

DISCUSSION

It is apparent from this study that the use of multiple techniques for culturing blood increased the yield of positive cultures obtained over any one technique. Whether this is really related to the techniques per se or simply to the fact that

more blood was cultured is uncertain from the data obtained to date, but the different techniques offer various advantages, as outlined in Table 3. Broth culture yielded the highest number of positives. We diluted the blood 1:20 in the broth. Less dilution of the blood (i.e., 10 ml of blood in 50 to 100 ml of broth, as commonly used in blood culture work) very likely would have given a lower yield.

Surprisingly, no advantage (in terms of the number of positive cultures) could be demonstrated for the filter in our clinical study for patients receiving an antibiotic at the time the culture was taken. However, filter no. 1 frequently became plugged so that it was usually only possible to filter the plasma from 3 ml of blood; also, it was often not possible to wash the filter adequately. Our unpublished in vitro experiments have clearly shown that washing the filters is efficacious in removing natural serum factors inhibitory for group A streptococcus and in removing as much as 100 times the usual blood levels of penicillin and chloramphenicol, when the filters did not plug.

Filters do offer a very important advantage in terms of speed of diagnosis, saving 18 hr compared to pour plates and 33 hr plus subculture time compared to broth cultures.

Having demonstrated that much more rapid diagnosis of bacteremia was possible with a membrane filter technique and that the use of broth culture and whole blood pour plates with filters increased the total number of positive cultures, we have recently modified our procedures so as to simplify and improve them.

The original procedure (used in the studies reported in this paper) was very cumbersome and time-consuming. It took approximately 2 hr to process a blood culture through the filtration setup, although the bacteriologist was free to do other things during much of this time. The results clearly indicated that culture of the red blood cell mass and use of filter no. 2 could be dispensed with, without sacrificing any benefits. Similarly, for ordinary clinical purposes, separate culture of the white blood cells would be unnecessary. Our unpublished experience with artificial intracellular *Staphylococcus aureus* bacteremia indicates that quantitative colonial recovery of viable phagocytized bacteria can probably be obtained on the surface of membrane filters.

We have recently been using a 90-mm membrane filter (BioQuest, Cockeysville, Md., and Millipore Corp., Bedford, Mass.) in place of the 37-mm filter (Millipore Corp.), in a specially designed field monitor. Plasma is much more readily filtered through this larger area filter.

We now sediment the blood directly in a syringe (2). This allows one to express the plasma-dextran supernatant fluid directly without vacuum aspiration. Aspiration was not only more cumbersome, but led to either loss of some of the supernatant fluid or stirring up of previously sedimented red blood cells, thus making subsequent filtration more difficult.

Another significant improvement is the use of filters with larger pore sizes (S. Finegold, V. Sutter, and W. Carter, Bacteriol. Proc., 1969, p. 106). The optimal pore size is 0.8 μm because filters with larger pores yield atypical and small colonies.

Using the 90-mm diam, 0.8-μ pore size membrane filter, we are now consistently able to pass the plasma from 10 ml of blood through the filter and wash it with 500 ml of wash solution, usually in less than 5 min. The filter is cut into four pieces and placed on the surface of various media (blood agar, eosin methylene blue agar, and chocolate agar are used) under various atmospheres (aerobic, 10% CO_2 in air, and anaerobic with CO_2). We are presently accumulating data from cases of clinical bacteremia using the modified technique with larger filters.

Our present membrane filter blood culture procedure is as follows. (i) Draw 10 ml of blood aseptically into a 30-ml, disposable polypropylene syringe. (Becton-Dickinson & Co., Rutherford, N.J.) Clotting must be avoided. (ii) Promptly draw into the same syringe 20 ml of citrated dextran solution [autoclaved 3% solution of dextran, average molecular weight between 100,000 and 200,000 (Pharmachem Corp., Bethlehem, Pa.), in distilled water containing 0.6% sodium chloride and 1% sodium citrate]. Mix thoroughly. (iii) Insert the syringe into the center of a plastic housing with a snug rubber collar so that the syringe can be stood upright with plunger down and the barrel (with the blood-dextran mixture) surrounded by an ice-water bath. (iv) Allow blood-dextran mixture to sediment in syringe for 30 min. (v) The tip of the syringe which normally holds the needle is fitted into one of the valves of a V-5530 Add-A-Flo 4-way valve setup with 20-inch extension tubing attached (Don Baxter, Inc., Glendale, Calif., and McGaw Laboratories, Evanston, Ill.). The end of the extension tubing is fitted to the top surface of a special 90-mm field monitor (BioQuest) previously assembled with a 90-mm membrane filter of 0.8 μm pore size and autoclaved (15 lb. pressure for 5 min with a drying cycle of 3 min.) A 500-ml bottle of isotonic wash solution (0.60% NaCl + 1.0% sodium citrate, pH 7.4) is hooked into the system by attaching a V1450 Vacoset I.V. set

(Don Baxter, Inc., and McGaw Laboratories) to the bottle and to another of the valves of the Add-A-Flo 4-way valve setup, as diagrammed in the paper by Winn et al. (11). The field monitor is hooked up to a vacuum source by means of a P-892 Thiopental Sodium Aspirating Set (plastic tubing, American Hospital Supply Corp., Evanston, Ill.), as pictured in the above reference, and the field monitor is primed (filled to the top) with wash solution. (vi) Gentle, steady pressure on the plunger of the syringe is used to push all of the plasma-dextran supernatant fluid through the valve and tubing into the field monitor. This is filtered, and then washed with all of the wash solution, aided by the vacuum pump. The filter is pulled dry. (vii) The apparatus is disconnected and the field monitor is opened. A sterile scalpel blade is used to cut the filter into four pieces. These are aseptically placed (the surface which has been directly exposed to the plasma facing upward) on the surface of two blood agar plates (one incubated aerobically and one anaerobically), one EMB or MacConkey's agar plate (incubated aerobically; Difco), and one chocolate agar plate (incubated under 10% CO_2). (viii) The use of a broth culture (preferably anaerobic) and, if possible, pour plate cultures of additional amounts of blood is recommended in addition to the filter culture.

Another improvement in our technique involves the use of polyanethol sulfonate (Liquoid) in broth cultures (4). Liquoid has been shown to reduce effectively the normal bactericidal power of blood and the phagocytic activity of leukocytes (which may persist for 24 hr in broth culture); it compares favorably with other agents used for these purposes or as anticoagulants such as oxalate, citrate, ethylenediaminetetraacetate, heparin, and trypsin (3, 9; G. Evans et al, Bacteriol. Proc., 1967, p. 97). Our studies have shown that this compound is very useful in broth culture, but it is of no use in pour plates; we have also shown that it is detrimental in the case of membrane filter cultures, at least in a concentration of 0.5% (4).

Finally, it should be pointed out that the membrane filter technique developed for blood cultures has also worked well with spinal fluids and might prove suitable for recovery of small numbers of organisms from other body fluids, e.g., tubercle bacilli from serous cavity fluids or from urine.

LITERATURE CITED

1. Austrian, R., and J. Gold. 1964. Pneumococcal bacteremia with special reference to bacteremic pneumococcal pneumonia. Ann. Intern. Med. 60:759–776.
2. Christlieb, A. R., A. J. Sbarra, and W. A. Bardawil. 1962. Isolation of highly purified, viable leukocytes from blood. Amer. J. Clin. Pathol. 37:257–262.
3. Ellner, P. D., and C. J. Stoessel. 1966. The role of temperature and anticoagulant on the in vitro survival of bacteria in blood. J. Infec. Dis. 116:238–242.
4. Finegold, S. M., I. Ziment, M. L. White, W. R. Winn, and W. T. Carter. 1968. Evaluation of polyanethol sulfonate (Liquoid) in blood cultures. Antimicrob. Agents Chemother.—1967. p. 692–696.
5. Hall, W. H., and D. Gold. 1955. Shock associated with

6. bacteremia: review of 35 cases. Arch. Intern. Med. 96: 403–412.
6. Hodgin, U. G., and J. P. Sanford. 1965. Gram-negative rod bacteremia. An analysis of 100 patients. Amer. J. Med. 39:952–960.
7. McCabe, W. R., and G. G. Jackson. 1962. Gram-negative bacteremia. II. Clinical, laboratory and therapeutic observations. Arch. Intern. Med. 110:856–864.
8. Merrill, S. L., A. Davis, B. Smolens, and S. M. Finegold. 1966. Cephalothin in Serious Bacterial Infection. Ann. Intern. Med. 64:1–12.
9. von Haebler, T., and A. A. Miles. 1938. The action of sodium polyanethol sulphonate ("Liquoid") on blood cultures. J. Pathol. Bacteriol. 46:245–252.
10. Weil, M. H., and W. W. Spink. 1958. The shock syndrome associated with bacteremia due to Gram-negative bacilli. Arch. Int. Med. 101:184–193.
11. Winn, W. R., M. L. White, W. T. Carter, A. B. Miller, and S. M. Finegold. 1966. Rapid diagnosis of bacteremia with quantitative differential-membrane filtration culture. J. Amer. Med. Ass. 197:539–548.