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Blood Culture Technique Based on Centrifugation: Clinical Evaluation

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A total of 1,000 blood samples from patients suspected of having a bacteremia were analyzed concurrently, where possible, by three methods: (i) Trypticase soy broth with sodium polyanethol sulfonate and a CO_2 atmosphere; (ii) pour plates with either brain heart infusion agar or Sabouraud dextrose agar; and (iii) centrifugation of the suspected organism in a hypertonic solution. There were 176 positive cultures. The centrifugation technique recovered 73% of the positive cultures. The broth and pour plate techniques recovered 38 and 49%, respectively. The centrifugation technique showed an increased isolation rate for *Pseudomonas*, fungi, and gram-positive cocci. In general, for each organism the time required for the detection of a positive culture was shortest for the centrifugation technique.

For many years, isolation of microogranisms has been limited primarily to the use of broth culture techniques. Advances in blood culturing technology have largely been directed towards improvement of the broth technique. The filtration technique was introduced in an attempt to make blood culture methodology more flexible, faster, and quantitative (6). However, the filtration technique was plagued with various problems: it was cumbersome and time consuming, and the filters were prone to plugging. Attempts have been made to remedy these drawbacks, and the filtration system does have potential as a blood culture technique.

In our laboratories, an alternative blood culture method based on hemolysis of the blood sample and centrifugation of microorganisms present in that sample in a hypertonic solution was developed (4). Various studies showed that for most pathogens at least 60% of the microorganisms present in an artificially seeded blood sample were recovered by this technique (4). This clinical study was initiated to compare the centrifugation technique with two blood culture methods: the pour plate and broth techniques.

MATERIALS AND METHODS

From September 1972 until April 1974, all routine blood cultures at the Wadley Institutes of Molecular Medicine were performed as follows. The area of venipuncture was scrubbed with 2% aqueous iodine and washed with 70% isopropanol. A 30-ml amount of blood was drawn into a sterile syringe. At the bedside, 5 ml was inoculated into each of two blood culture bottles containing 50 ml of Trypticase soy broth (TSB), 0.05% sodium polyanethol sulfonate (SPS), and a 10% CO_2 atmosphere (BioQuest). Aliquots of the remaining blood were introduced aseptically into two Vacutainers (BBL) containing 0.35% SPS. The three blood samples were treated as follows. (i) One bottle was vented; the other remained anaerobic. These bottles were incubated at 35 C and examined daily for 10 days. Subcultures from the bottles were made at days 2 and 10 onto blood agar (BBL) (aerobic and anaerobic), chocolate agar (BBL) (10% CO_2), and Sabouraud dextrose agar (SDA), and incubated at 35 C. In addition, Gram stains were made from the bottles after 2 and 10 days of incubation. (ii) One of the two blood samples collected in Vacutainers was processed by the centrifugation technique described previously (4). Sucrose-gelatin was used as the supporting layer, and the sample was processed by method B (4). Since this clinical study was initiated before all of the developmental data were obtained, the conditions of centrifugation were arbitrarily established at 10 min and 6,000 $\times g$. After centrifugation, the processed sample was inoculated onto: blood agar (BBL) aerobic and anaerobic, 35 C; chocolate agar (BBL), 10% CO_2, 35 C; and SDA, 25 C and 35 C. (iii) The remaining Vacutainer of blood was used to prepare pour plates. Nine pour plates were made containing 0.5 ml of blood and 19 ml of cooled, melted brain heart infusion agar (BHIA). Three additional pour plates of 0.5 ml of blood and 19 ml of cooled, melted SDA were prepared. The pour plates were incubated as follows: three BHIA plates, aerobic, 35 C; three BHIA plates, anaerobic, 35 C; three BHIA plates in jars with 10% CO_2; and the three SDA plates at 35 C. A Thelco anaerobic incubator was used to promote growth of anaerobes.

Identification of bacterial and yeastlike isolates was done by methods described previously (2). Beginning in September 1973, the API-20 diagnostic

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test (Analytab Products, Inc.) was used to identify *Enterobacteriaceae* and some pseudomonads. Identification of *Aspergillus* species was confirmed by methods of Raper and Fennell (11). Other filamentous fungi were identified according to Bessey (1).

Records were maintained for each culture method. For the broth technique, the data and time of positivity, the observation of turbidity in one or both bottles, and/or growth upon subculture, whichever occurred first, were recorded. As colonies appeared on the agar plates used with the pour plate and centrifugation techniques, the date and time of their isolation were recorded, and the organisms were identified.

RESULTS

This study represents 1,000 blood cultures from 400 patients. Of these cultures, 883 from 362 patients were performed by all three blood culture methods. The remaining 117 were analyzed by the broth and centrifugation techniques. The patients observed in this study primarily had neoplastic diseases and were undergoing extensive chemotherapy. Our analysis of the positive blood cultures attempts to realistically evaluate a new blood culture procedure, the centrifugation technique.

Isolation of common laboratory contaminants, e.g., *Bacillus, Corynebacterium* (anaerobic, aerobic), *Staphylococcus epidermidis, Cladosporium, Rhizopus,* etc., by any technique was considered to produce a contaminated culture unless the clinical significance of the organism in question could be further documented. There were two isolates by the centrifugation technique, *Corynebacterium equi* and *Trichosporon cutaneum,* usually considered laboratory contaminants, that were thought to be clinically significant. The contamination rates for each method were: pour plate, 11%; centrifugation, 9.3%; and broth technique, 1.8%. For the pour plate and centrifugation techniques, 37 and 38%, respectively, of the contaminated cultures had only one colony on an entire set of plates. These cultures were considered negative, and the adjusted contamination rates were 6.9% for the pour plate and 5.8% for centrifugation. The tables presented in this paper are based on clinically significant isolates.

The organisms isolated by each technique and the time to positivity are listed in Table 1. There were 176 positive cultures, excluding the contaminants, representing 400 patients (Table 2). The centrifugation technique has a higher positive rate and isolates more gram-positive cocci, fungi, and pseudomonads than the other two techniques. For most of the organisms, the mean time to positivity by the centrifugation technique was shorter.

Of the 883 cultures compared by all three blood culture techniques, 161 were positive in one or more systems (Table 3). The centrifugation technique detected 73% (118) of these cultures, whereas the pour plate and broth tech-

TABLE 1. *Distribution of isolates and time interval to positivity for 176 cultures*

Organism	Centrifugation		Bottle		Pour plate	
	No.	Mean time (hr)	No.	Mean time (h)	No.	Mean time (h)
Clostridium	2	31	2	36	2	31
Corynebacterium equi	2	80	0	–[a]	0	–
Streptococcus pneumoniae	2	48	2	24	2	48
Peptostreptococcus	1	132	2	180	0	–
Staphylococcus aureus	10	53	1	120	3	36
Streptococcus	26	48	5	43	14	58
Citrobacter	1	24	0	–	2	24
Enterobacter	6	19	9	28	6	27
Escherichia coli	16	22	17	42	16	43
Haemophilus	0	–	0	–	1	64
Klebsiella	4	12	7	33	2	12
Acinetobacter calcoaceticus	2	36	1	24	1	48
Neisseria	1	24	0	–	0	–
Proteus	3	26	1	41	0	–
Pseudomonas	40	21	22	70	22	51
Aspergillus	5	50	0	–	7	55
Candida	4	44	1	72	4	72
Cryptococcus	9	73	0	–	1	72
Trichosporon cutaneum	2	58	0	–	0	–
Total cultures	1,000		1,000		833	
% Positive	13.6		7.0		9.4	

[a] –, Organism not recovered.

TABLE 2. *Number of patients positive by organism and method*

Organism	No. positive		
	Centrifugation	Bottle	Pour plate
Clostridium carnis	1	1	1
Corynebacterium equi	1	0	0
Streptococcus pneumoniae	1	1	1
Peptostreptococcus	1	2	0
Staphylococcus aureus	7	1	3
Alpha streptococcus	17	2	9
Streptococcus faecalis	1	0	1
Beta streptococcus, group A	2	2	2
Beta streptococcus, not group A	4	0	1
Citrobacter freundii	1	0	2
Enterobacter	5	8	5
Escherichia coli	13	16	14
Haemophilus	0	0	1
Klebsiella	4	7	2
Acinetobacter calcoaceticus	2	1	1
Neisseria	1	0	0
Proteus	3	1	0
Pseudomonas	35	21	20
Aspergillus flavus	1	0	0
A. fumigatus	1	0	1
A. niger	2	0	3
A. versicolor	0	0	1
A. glaucus	0	0	1
Candida albicans	3	1	2
C. guilliermondii	1	0	0
C. tropicalis	0	0	1
Cryptococcus albidus	2	0	0
C. laurentii	1	0	0
C. neoformans	5	0	1
C. terreus	1	0	0
Trichosporon cutaneum	1	0	0
Total	117	64	73
Total no. of patients	400		362

niques recovered 49% (80) and 38% (62), respectively. Included in these 161 positive cultures are 9 bacteremias from which 2 organisms were isolated. Of these nine cultures, six were detected only by the centrifugation method. Two were detected in all three systems, and one was detected by the broth and centrifugation methods. Close examination of Tables 1 and 2 reveals that some members of *Enterobacteriaceae,* namely, *Enterobacter, Escherichia coli,* and *Klebsiella,* were isolated at a slightly higher rate by the broth technique. These results may be due to low numbers of the infecting organism or to the low number of positive cultures with these organisms.

There were 54 cultures in which the two quantitative techniques, the pour plate and centrifugation methods, recovered the same organism. In 46 (85%) of these matched samples, the number of organisms per milliliter of blood obtained by each technique was in agreement. Our data indicated that for the quantitative methods nearly 50% of the positive cultures for each system were low-level bacteremias, i.e., less than 1 organism per ml of blood (Table 4). Although it was not directly possible to know the number of organisms in the inoculum for the broth technique, there were 48 positive specimens for which a quantitative procedure could be compared with the broth technique. From the quantitative procedures, one could infer that 12 of these 48 cultures were started by less than 1 organism per ml of blood. In addition, there were 17 organisms isolated solely by the broth technique. Since the pour plate and centrifugation techniques were capable of recovering those organisms, these broth cultures were probably started by less than 1 organism per ml. Therefore, it can be inferred that 29 specimens (47%) were initiated by less than 1 organism per ml. These percentages of low bacteremias were in agreement with those of other investigators studying quantitative techniques for blood cultures (6, 15).

DISCUSSION

In this study, the centrifugation technique was compared to the pour plate and broth methods. The pour plate system was included as a control for the quantitative ability of the centrifugation process. When both quantitative techniques were positive, general agreement was observed in the quantitative data. The broth technique was included as a representative of the most commonly used blood culture methodologies.

The percentage of positive cultures, excluding contaminants, for the broth and pour plate techniques in this study is in close agreement with that reported by others (6, 8, 10, 13–15, 17, 18). One organism, *Bacteroides fragilis,* that has been found in as many as 11% of positive cultures (17) was not isolated in this study by any methodology. This absence of *B. fragilis* isolates is probably due to the type of patients included in this study, i.e., those having neoplastic diseases and undergoing extensive chemotherapy. Also, at the conclusion of this clinical study, it was found that the Thelco anaerobic incubator could not maintain sufficient anaerobic conditions to support the growth of *B. fragilis* on the agar plates. This might explain the lack of *Bacteroides* isolates when the pour plate and centrifugation methods were used. Additional studies will be re-

TABLE 3. *Number of isolates positive in one or more systems*[a]

Organism	No. positive							
	Total	Centrifugation only	Pour plate only	Bottle only	Bottle, centrifugation, and pour plate	Bottle centrifugation	Bottle and pour plate	Centrifugation and pour plate
Clostridium	2	0	0	0	2	0	0	0
Corynebacterium equi	2	2	0	0	0	0	0	0
Streptococcus pneumoniae	2	0	0	0	2	0	0	0
Peptostreptococcus	3	1	0	2	0	0	0	0
Staphylococcus aureus	9	5	2	1	0	0	0	1
Streptococcus	31	15	7	1	3	1	0	4
Citrobacter	2	0	1	0	0	0	0	1
Enterobacter	9	0	0	3	4	0	1	1
Escherichia coli	19	0	0	2	13	1	1	2
Haemophilus	1	0	1	0	0	0	0	0
Klebsiella	5	0	0	3	2	0	0	0
Acinetobacter calcoaceticus	2	1	1	0	0	0	0	0
Neisseria	1	1	0	0	0	0	0	0
Proteus	2	1	0	0	0	1	0	0
Pseudomonas	45	19	5	5	16	0	0	0
Aspergillus	11	4	6	0	0	0	0	1
Candida	6	3	2	0	1	0	0	0
Cryptococcus	7	6	0	0	0	0	0	1
Trichosporon	2	2	0	0	0	0	0	0
Total	161	60	25	17	43	3	2	11
% of total		38	16	10	27	2	1	7

[a] Data are based on cultures done by all three methods, i.e., 883 blood cultures.

quired to determine whether *B. fragilis* can be isolated by these techniques under more suitable clinical conditions.

When compared with the other two methods, the centrifugation technique appears to offer a potential alternative to blood culture technology. The technique offers several distinct advantages over the broth method. Atmospheric conditions and media are easily varied. Discrete colonies with their characteristic morphology are obtained, thereby enabling the technician to easily detect mixed bacteremias. Subculturing, required by the broth technique, is not necessary; therefore, the time required to identify the organism and determine its sensitivity pattern is reduced by 1 full day.

The centrifugation method also had more positive cultures than the broth technique. This high rate was due in part to the increased isolation of fungi, gram-positive cocci, and *Pseudomonas*. Each of these classes of microorganisms represents a threat to the compromised patient (3, 5, 16). In the immunosuppressed patient, systemic fungal infection is a definite possibility. Of 3,335 cultures in a study comparing a filtration and a broth method, Komorowski and Farmer (9) reported 29 (0.9%) and 17 (0.5%) to be *Candida* isolates, respectively. Roberts and Washington suggested a vented biphasic media for fungi (12) and reported 119 (1.6%) positive cultures from the biphasic media and 29 (0.4%) fungal isolates from a TSB medium. In our study of 1,000 cultures, 20 fungal isolates (2%) were recovered by the centrifugation technique, whereas only 1 fungal isolate was recovered from TSB.

There are two possible explanations for the increased isolation of gram-positive cocci by the centrifugation technique over the broth method. The bacteria are sedimented into a hypertonic solution which could protect organisms with damaged cell walls. Similar increases in the isolation of gram-positive cocci were observed when sucrose was incorporated into broth media (13, 14), perhaps due to a similar protective mechanism. Secondly, there is a 1:400 dilution of the antimicrobial factors in blood, e.g., antibiotics, complement, etc., in addition to the effect SPS has on such factors. This dilution is greater than the 1:10 dilution obtained from the broth technique or the 1:40 dilution obtained from the pour plate technique.

In the leukemic patient, colonization by *Pseudomonas* has been reported (3). Since a majority of the *Pseudomonas* isolates contain

TABLE 4. *Number of organisms isolated per milliliter of blood in positive cultures*

Organism	Centrifugation method					Pour plate method				
	Total no.	No. of isolates/ml				Total no.	No. of isolates/ml			
		<1	1-10	11-100	>100		<1	1-10	11-100	>100
Clostridium	2	0	0	1	1	2	0	0	1	1
Corynebacterium equi	2	0	0	2	0	0	0	0	0	0
Streptococcus pneumoniae	2	1	0	0	1	2	1	0	0	1
Peptostreptococcus	1	1	0	0	0	0	0	0	0	0
Staphylococcus aureus	10	5	5	0	0	3	1	2	0	0
Streptococcus	26	13	7	5	1	14	10	3	0	1
Citrobacter	1	0	0	1	0	2	1	0	1	0
Enterobacter	6	1	0	4	1	6	2	1	2	1
Escherichia coli	16	4	4	6	2	16	4	8	2	2
Haemophilus	0	0	0	0	0	1	1	0	0	0
Klebsiella	4	1	1	2	0	2	1	0	1	0
Acinetobacter calcoaceticus	2	1	0	0	1	1	1	0	0	0
Neisseria	1	0	1	0	0	0	0	0	0	0
Proteus	3	1	1	1	0	0	0	0	0	0
Pseudomonas	40	22	11	5	2	23	9	9	2	3
Aspergillus	5	5	0	0	0	7	6	0	1	0
Candida	4	2	0	2	0	4	4	0	0	0
Cryptococcus	9	7	2	0	0	1	1	0	0	0
Trichosporon	2	0	1	1	0	0	0	0	0	0
Total	136	64	33	30	9	84	42	23	10	9
% of total		47	24	22	7		49	28	12	11

less than 1 organism per ml of blood, the question might be raised as to whether these low-level isolates are due to contamination. As indicated earlier, for any given technique, approximately 50% of the positive cultures are initiated by less than 1 organism per ml. In this study, examination of either of the two quantitative techniques reveals two interesting observations: (i) an equal number of cultures demonstrated less than 1 *S. epidermidis* or *Pseudomonas* per ml of blood; (ii) in one instance where both the pour plate and centrifugation technique indicated 40 *S. epidermidis* per ml of blood, the blood broth was negative. Herein lies the potential value of quantitation for blood culturing. Repeat cultures, perhaps one every 6 h, using different areas for venipuncture, could demonstrate the following: a persistent low-level bacteremia, perhaps associated with endocarditis; a prelude to a severe septic crisis, evidenced by increasing numbers of bacteria in the bloodstream; an opportunistic infection; or a contaminated culture. The centrifugation technique is ideal for such studies.

One potential drawback to the centrifugation technique is the contamination rate: 9.3%. Although the centrifugation technique used in this study is essentially a closed system until the last step (plating of the sample), it does require five separate entries with a syringe and needle: (i) introduction of the sample into the SPS Vacutainer, (ii) addition of the saponin, (iii) removal of 8 ml of lysed, anticoagulated blood, (iv) introduction of the blood into a special centrifuge tube, and (v) removal of the sucrose-gelatin layer. With each step, the possibility of contamination increases. The incorporation of saponin and SPS into the sample tube would eliminate one entry. Hence, the system can be reduced readily to a four-entry system. A prototype single-tube, two-entry system has recently been developed. Upon assembly of a sufficient number of units, a clinical program will be undertaken to resolve the issues raised by this feasibility study.

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