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Improved Blood Culture Technique Based on Centrifugation: Clinical Evaluation†

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A total of 3,335 blood samples from 1,180 patients suspected of having bacteremia were analyzed concurrently by two methods: (i) supplemented peptone broth with sodium polyanethanol sulfonate and a CO_2 atmosphere; and (ii) lysis centrifugation at 3,000 × *g* for 30 min onto a high-density, hydrophobic cushion. The centrifugation technique recovered 80% of the positive cultures as compared with 67% for the broth method. The centrifugation technique showed an apparent increase in the isolation of *Staphylococcus aureus*, *Pseudomonas*, and yeasts. In almost every instance, the time required for detection of a positive culture was shortest for the centrifugation method. Contamination rates for both systems were comparable (1.4%). Quantitation, offered only by the centrifugation method, proved useful on several occasions in discriminating between an opportunistic infection versus a skin contaminant and in judging efficacy of antimicrobial therapy.

A previous clinical study based on a system which required five entries suggested the potential value of centrifugation as an alternative blood culture technology (1). When compared with a conventional broth system, the centrifugation process showed an increased isolation rate and a decrease in the time required to obtain a pure isolate. However, the method had two major disadvantages: it required a swinging-bucket centrifuge capable of generating 6,000 × *g* and yielded a very high contamination rate (9.3% versus 1.8% for the bottle technique).

In an effort to remedy the inherent problems of the original centrifugation method, our laboratory developed a new vehicle and culturing process (2). The system is based on hemolysis of a blood sample and subsequent centrifugation in an angle rotor at 3,000 × *g* for 30 min, and the overall average recovery for 21 different pathogens was 93% (2).

This clinical study was initiated to compare the new centrifugation technique with a standard two-bottle broth system. The analysis of these culture techniques attempts to evaluate the new device realistically and to determine the most suitable means of processing a blood sample.

MATERIALS AND METHODS

From October 1976 through July 1978, all routine blood cultures at the Granville C. Morton Hospital were performed as follows. The area of venipuncture was scrubbed with 70% isopropanol followed by an iodophor. A 20-ml amount of blood was drawn into a sterile syringe, and the needle was replaced with a new one. At bedside, 5 ml was inoculated into each of two blood culture tubes containing 45 ml of supplemented peptone broth, 0.03% sodium polyanethanol sulfate, and a 10% CO_2 atmosphere (Becton-Dickinson, Rutherford, N.J.). A 7-ml sample of blood was introduced into the centrifugation device (2). The device was gently inverted four times to prevent coagulation and to initiate erythrocyte lysis. The two blood culture systems were treated in the following manner. (i) In the two-tube system, one culture tube was vented; the other remained anaerobic. These tubes were incubated at 35°C and examined daily for signs of turbidity through 10 days. Subcultures were made on days 1 (aerobic; between 8 and 24 h after collection), 2 (anaerobic), and 6 (aerobic and anaerobic) onto blood agar plates (BBL, Cockeysville, Md.), both aerobic and anaerobic, at 35°C; onto chocolate agar (BBL), 10% CO_2, at 35°C; and onto Sabouraud brain heart infusion agar (Difco Laboratories, Detroit, Mich.) at 25°C. Gram stains were prepared at the time of subculture. (ii) In the centrifugation system, the tube was spun at 3,000 × *g* for 30 min and processed as described previously (2). After processing, the 1.5 ml of concentrate was equally inoculated onto the following agar media plates: one aerobic blood, two anaerobic blood, one chocolate, and one Sabouraud brain heart infusion. Aerobic blood and chocolate agars were discarded at day 4; anaerobic plates were discarded at day 6, and Sabouraud brain heart infusion agar was discarded at day 8. For positive cultures, the number of organisms per milliliter of blood was approximately equal to 0.7 × (total number of colonies/total number of plates upon which the organism would be expected to grow).

† This paper is dedicated in memory of Sandra Winborn Chabot.

To assess the effect of sample distribution and education of laboratory staff on results obtained with the centrifugation technique, the study was divided into three phases. (i) From October 1976 through April 1977, personnel were instructed to place the concentrate on the upper portion of the plate and to streak through the material with a 0.01-ml sterile disposable loop (A/S Nunc, Roskilde, Denmark) (drop-plus-streak technique). A different loop was used for each plate. (ii) From May 1977 through November 1977, they were instructed to place the concentrate on the center of the dish and omit the streaking process (drop technique). (iii) From December 1977 through July 1978, personnel were given an in-depth lecture on sterile procedure with regard to the handling and inspection of the plates. The drop-plus-streak technique was reestablished.

Identification of bacterial and yeastlike isolates was done by methods described previously (6, 7). The API-20E (Analytab Products, Inc., Plainview, N.Y.) was used to identify *Enterobacteriaceae* and most nonfermentative gram-negative bacilli. The Minitek anaerobic system (BBL) with gas chromatography profiles was used to identify most of the anaerobic bacteria. Identification of *Aspergillus* species was confirmed by the methods of Raper and Fennell (8). Other filamentous fungi were identified according to Gilman (4).

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 or growth upon subculture, whichever occurred first) were recorded. As colonies first appeared on the agar plates inoculated with the centrifuged concentrate, the data, the time of their isolation, and the number of colonies on each plate were recorded.

RESULTS

This study describes the results obtained from 3,335 blood samples processed concomitantly by the broth and the centrifugation method. Of the centrifugation cultures, 1,012 were in phase I (drop-plus-streak technique), 982 in phase II (drop technique), and 1,341 in phase III (drop-plus-streak technique). The majority of the patients cultured in this series had neoplastic diseases and were undergoing extensive chemotherapy.

The appearance of a positive culture when either the drop or the drop-plus-streak technique was used can be seen in Fig. 1. The drop technique required less manipulation, but it had certain inherent limitations. When there were more than 30 colonies per plate, counting became difficult, and if a second type of organism was present, it was difficult to obtain a pure isolate directly from the plate. Furthermore, the residual Flourinert (3M Corp., St. Paul, Minn.) was concentrated in the drop and caused some distortion of normal colonial morphology.

Recovery of common laboratory contaminants, e.g., *Bacillus*, *Corynebacterium*, *Propionibacterium*, *Staphylococcus epidermidis*, *Hormodendrum*, etc., was considered a contaminated culture unless the clinical significance of the organism could be documented by our pathologists. There were seven isolates (three patients) of *S. epidermidis* in phase III that were thought to be clinically significant. The unadjusted contamination rates for the centrifugation method were as follows: phase I, 5.7%; phase II, 2.6%; phase III, 2.2%. It was noted that 66% of the contaminated cultures done by the centrifugation method had only one colony on one of the five plates. Analysis of patient data indicated that this type of positive culture was never associated with an infection. If these cultures are treated as obvious laboratory contaminants, the adjusted contamination rates are: phase I, 3.8%; phase II, 1.7%; and phase III, 1.4%. The overall contamination for the broth method during this study was 1.4%.

The organisms isolated and the time to positivity for each method are listed in Table 1. There were 545 positive cultures, excluding contaminants, representing 1,180 patients (Table 2).

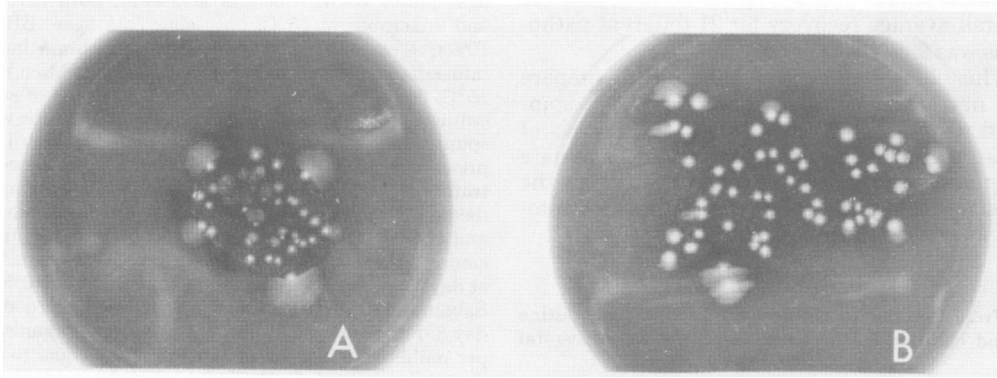

FIG. 1. *Appearance of positive centrifugation cultures when the drop technique (A) or drop-plus-streak technique (B) was employed.*

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

Organism	Centrifugation				Bottle	
	Drop plus streak		Drop			
	No.	Mean time (h)	No.	Mean time (h)	No.	Mean time (h)
Listeria monocytogenes	1	42	0	0	4	40
Micrococcus	3	40	0	0	0	0
Staphylococcus aureus	26	37	10	48	30	70
Streptococcus viridans	11	52	4	43	14	53
Streptococcus (group D)	8	35	4	56	14	36
β-Streptococcus	1	19	1	46	2	30
Streptococcus pneumoniae	4	30	2	48	5	26
Streptomyces	2	108	0	0	0	0
Aerococcus	0	0	1	22	1	22
Aeromonas hydrophila	0	0	1	71	1	71
Citrobacter	1	29	1	99	0	0
Enterobacter	9	36	3	46	9	60
Escherichia coli A-D	1	20	1	36	2	27
Escherichia coli	35	35	11	41	46	35
Haemophilus influenzae	1	71	0	0	0	0
Acinetobacter anitratus	1	24	3	91	1	48
Klebsiella pneumoniae	25	27	9	55	35	44
Moraxella	2	57	1	61	4	171
Proteus	3	32	1	24	5	44
Pseudomonas aeruginosa	35	37	8	33	31	55
Pseudomonas	7	70	0	0	1	91
Salmonella typhi	0	0	1	96	1	143
Alcaligenes	0	0	1	91	0	0
Bacteroides	15	69	0	0	14	126
Fusobacterium nucleatum	1	91	0	0	0	0
Clostridium	7	86	0	0	9	67
Eubacterium lentum	1	141	1	100	1	74
Peptostreptococcus	2	100	0	0	3	182
Peptococcus	2	119	0	0	0	0
Candida	20	44	1	24	14	89
Cryptococcus neoformans	7	62	0	0	0	0
Rhodotorula glutinis	1	50	0	0	0	0
Torulopsis glabrata	0	0	0	0	1	92
Total positive (percent)	232 (9.9)		65 (6.6)		248 (7.4)	
Total cultures	1,012		982		1,341	
Average time to positive		44.7		50.1		59.4

The combined centrifugation data indicated a higher positive rate (20% more than the broth method) and a higher isolation rate for *Staphylococcus aureus*, pseudomonads, and fungi (Table 3).

For almost every organism, the mean time to positivity was shortest with the centrifugation method (drop-plus-streak technique). The overall time to positivity was 2.5 days for the broth method versus 1.9 days for the drop-plus-streak centrifugation method and 2.1 days for the drop technique. The use of this last technique caused a substantial delay to positivity for many clinically significant isolates (Table 1). For this and the other reasons discussed previously, the drop-plus-streak centrifugation technique appears to be the method of choice.

Of the 3,335 paired specimens compared by the broth and centrifugation techniques (Table 3), 370 were positive in one or both systems. The centrifugation method detected 80% of these cultures, whereas the broth method only detected 67%. Included in these 370 positive cultures are 63 bacteremias from which two or more clinically significant microorganisms were isolated. Of these 63 cultures, 29 were detected by the centrifugation method only, 13 were detected by both systems, and 11 were detected solely by the broth method.

A closer examination of the data in Tables 1 and 2 reveals that only one organism appeared to be more readily isolated by the broth tech-

TABLE 2. *Number of patients (of 1,180) positive by organism and method*

Organism	No. positive	
	Centrifugation	Bottle
Listeria monocytogenes	1	2
Micrococcus	3	0
Staphylococcus aureus	17	16
Streptococcus viridans	14	13
Streptococcus (group D)	8	10
β-Streptococcus	2	2
Streptococcus pneumoniae	5	4
Streptomyces	2	0
Aerococcus	1	1
Aeromonas hydrophila	1	1
Citrobacter	2	0
Enterobacter	12	9
Escherichia coli A-D	2	2
Escherichia coli	25	27
Haemophilus influenzae	1	0
Acinetobacter anitratus	4	1
Klebsiella pneumoniae	26	25
Moraxella	3	4
Proteus	4	4
Pseudomonas aeruginosa	27	19
Pseudomonas	7	1
Salmonella typhi	1	1
Alcaligenes	1	0
Bacteroides	11	12
Fusobacterium nucleatum	1	0
Clostridium	5	8
Eubacterium lentum	2	1
Peptostreptococcus	2	3
Peptococcus	2	0
Candida	14	10
Cryptococcus neoformans	7	0
Rhodotorula glutinis	1	0
Torulopsis glabrata	0	1
Total	214	177

nique, namely, *Listeria monocytogenes*. Of the four positive broth cultures listed, three came from one patient. The centrifugation method was positive once for this patient and was able to recover only a single *Listeria* bacterium. Of three cultures taken on the second patient, only one broth culture went positive.

The data presented in Table 4 reconfirm our earlier observation (1) and those of others (3) that a large percentage (37%) of the positive cultures represent low-level bacteremias, i.e., less than one organism per milliliter of blood.

DISCUSSION

In this study, a new centrifugation device and culturing process were compared against a conventional broth system for the purpose of determining if the new system would eliminate problems inherent in the earlier protocol and still maintain comparable results in the percent positive cultures and time to positivity. The new centrifugation vehicle requires four entries within a single tube as opposed to six entries for the two-bottle broth system employing two subcultures. Once centrifuged, the preferred method for processing the sample is to place 0.3 ml in the upper quadrant of the plate and then to streak through the material for maximum distribution. This drop-and-streak technique prevented the changes in colonial morphology seen with the drop technique and permitted improved quantitation and better separation of polymicrobial bacteremias. The new centrifugation device is designed for use in a nonrefrigerated table-top centrifuge fitted with a multiple-sample angle rotor. The following centrifuges can be adapted to the centrifugation pro-

TABLE 3. *Number of isolates positive in one or both systems*

Organism	Bottle only	Centrifugation only	Bottle and centrifugation
Listeria monocytogenes	3	0	1
Micrococcus	0	3	0
Staphylococcus aureus	5	11	25
Streptococcus viridans	8	9	6
Streptococcus (group D)	6	4	8
β-Streptococcus	1	1	1
Streptococcus pneumoniae	1	2	4
Streptomyces	0	2	0
Aerococcus	0	0	1
Aeromonas hydrophila	0	0	1
Citrobacter	0	2	0
Enterobacter	3	6	6
Escherichia coli A-D	0	0	2
Escherichia coli	11	11	35
Haemophilus influenzae	0	1	0
Acinetobacter anitratus	0	3	1
Klebsiella pneumoniae	7	6	28
Moraxella	3	2	1
Proteus	2	1	3
Pseudomonas aeruginosa	4	16	27
Pseudomonas	1	7	0
Salmonella typhi	1	1	0
Alcaligenes	0	1	0
Bacteroides	6	7	8
Fusobacterium nucleatum	0	1	0
Clostridium	4	0	6
Eubacterium lentum	0	1	1
Peptostreptococcus	3	2	0
Peptococcus	0	2	0
Candida	4	11	10
Cryptococcus neoformans	0	7	0
Rhodotorula glutinis	0	1	0
Torulopsis glabrata	1	0	0
Total	74	121	175
Percent of total	20%	32.7%	47.3%

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

Organism	No. of isolates per ml			
	<1	1-10	11-100	>100
Listeria monocytogenes	1	0	0	0
Micrococcus	3	0	0	0
Staphylococcus aureus	11	7	7	11
Streptococcus viridans	8	5	2	0
Streptococcus (group D)	4	4	0	4
β-Streptococcus	1	0	1	0
Streptococcus pneumoniae	0	1	1	4
Streptomyces	1	1	0	0
Aerococcus	0	0	1	0
Aeromonas hydrophila	0	0	0	1
Citrobacter	2	0	0	0
Enterobacter	3	4	3	2
Escherichia coli A-D	0	1	0	1
Escherichia coli	16	18	8	4
Haemophilus influenzae	1	0	0	0
Acinetobacter anitratus	2	2	0	0
Klebsiella pneumoniae	5	11	4	14
Moraxella	2	1	0	0
Proteus	1	0	2	1
Pseudomonas aeruginosa	11	16	9	7
Pseudomonas	5	2	0	0
Salmonella typhi	1	0	0	0
Alcaligenes	0	0	0	1
Bacteroides	15	0	0	0
Fusobacterium nucleatum	1	0	0	0
Clostridium	7	0	0	0
Eubacterium lentum	0	0	0	2
Peptostreptococcus	2	0	0	0
Peptococcus	2	0	0	0
Candida	4	3	1	13
Cryptococcus neoformans	1	5	1	0
Rhodotorula glutinis	1	0	0	0
Torulopsis glabrata	0	0	0	0
Total	111	81	40	65
Percent of total	37.4%	27.3%	13.5%	21.9%

cedure: Sorvall GLC-2B (Ivan Sorvall, Inc., Newtown, Conn.), Beckman TJ-6 (Beckman Instruments, Inc., Irvine, Calif.), and International Centra-7 (International Equipment Co., Needham Heights, Mass.).

Once again, it was observed that the centrifugation technique offers several distinct advantages over the broth technique. Media and atmospheric conditions can be readily changed. Discrete colonies are obtained, which in turn enables the technologist to detect mixed bacteremias easily. In addition, further purification or processing prior to identification or sensitivity testing is not necessary. In general, the time required to obtain a pure isolate was appreciably shorter with the centrifugation process than the conventional system. In many cases, the difference was striking, i.e., 24 to 48 h for the tube versus ≥5 days for broth.

As observed previously, the centrifugation method obtained more positive cultures. The method appears especially sensitive for the recovery of *S. aureus*, pseudomonads, and yeasts. It has been previously reported that the centrifugation technique might be useful for the isolation of the dimorphic pathogenic fungi (5). Although none were isolated in this series, we have recently recovered, on three separate occasions, *Histoplasma capsulatum* from a single patient.

The apparent failure to recover anaerobes in the earlier study has been rectified. In this study, the same percentage of anaerobes (0.8%) was recovered by both the centrifugation and the broth techniques. The improved recovery by the new device may be due to the fact that a single tube was required, which in turn led to fewer manipulations. Furthermore, all anaerobic plates were placed into the BBL GasPak jar system, as opposed to the Thelco anaerobic incubator used in the previous study (1).

The data presented in this study add additional support to the view that a high percentage of positive blood cultures are initiated with a low-number inoculum. An example of the effect of low numbers, perhaps, is illustrated by the centrifugation method's relative difficulty with *L. monocytogenes*. In this case, we feel that the paucity of results with tubes merely reflects a statistical artifact of low numbers in the inoculum. This concept correlates well with the other investigators who have observed that the percentage of positive cultures is related to the volume of blood analyzed (9).

Quantitation has, on several occasions, proved helpful in distinguishing between an obvious skin or laboratory contaminant (0.1 colony-forming unit of *S. epidermidis* per ml) and a clinical infection with a common contaminant (35 colony-forming units of *S. epidermidis* per ml). When the colony count is initially high, subsequent quantitative cultures have proven valuable in monitoring the efficacy of the selected antimicrobial agent(s). It is suggested that a more detailed clinical analysis be made to establish the potential clinical value of quantitation as an aid to the diagnosis and management of septicemia.

A major problem with the original two-tube centrifugation system was the high contamination rate of 9.3%. This study illustrates that if personnel are instructed on the proper selection and handling of plates, it is possible to achieve a contamination rate comparable to the two-bottle broth system, namely, 1.4%. To accom-

plish this, one must use predried plates of good quality, and incubator or jars must be kept clean. Technologists should also be discouraged from needless opening of plates. When the plates are streaked, one should avoid the outer edges of the dish since this area is more prone to airborne contamination.

Another feature of the centrifugation procedure which may prove problematic to the routine clinical laboratory is the need for essentially time-zero subculture. If this is not done, the time to a positive result will be delayed and quantitation becomes meaningless. Since septicemia is a critical condition demanding rapid therapy, we feel that blood cultures should be added to "stat" laboratory procedures. With the introduction of several rapid microbiology procedures for identification and sensitivity testing, it is suggested that the antiquated views regarding the role of clinical microbiology in patient care deserve critical review and revision.

If the above conditions are acceptable, the single-tube centrifugation method and drop-plus-streak inoculation satisfy the requirements of a rapid, quantitative, and versatile alternative blood culture technique.

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