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New Centrifugation Blood Culture Device

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A single-tube blood culture device designed for centrifugation in a tabletop centrifuge is described. Reconstruction experiments using 21 different organisms and human donor blood indicate that excellent recovery can be obtained by centrifugation for 30 min at 3,000 × *g*.

Previous studies suggested that centrifugation can be used to provide the clinical laboratory with a rapid, quantitative, and versatile blood culture technique (1, 2). The original two-tube method was based upon the principle of centrifugation of microorganisms on or into a stabilizing density layer after effective lyses of the erythrocytes (2). This method showed considerable promise in that substantial increases in the number of positive cultures were obtained (13 versus 7%), and the time required to obtain a pure isolate was considerably shorter. Land et al. were able to demonstrate that the centrifuge technique was especially valuable in the detection of disseminated fungal infections (3). Nevertheless, this technique was cumbersome and yielded an undesirably high contamination rate (9.3 versus 1.8% for the bottle technique). In addition, excessively high centrifugation forces (6,000 × *g*) in a swinging-bucket rotor were required to achieve acceptable recovery for several microorganisms. In an effort to resolve these difficulties, a new vehicle and process has been developed that more readily lends itself to the clinical laboratory. This report describes the new methodology and provides data on the recovery of 21 different organisms from blood as a function of relative centrifugal force and time in an angle-rotor tabletop centrifuge (Sorval GLC-2, SP-X rotor; Ivan Sorvall, Inc., Norwalk, Conn.).

MATERIALS AND METHODS

A schematic of the new centrifugation vehicle is presented in Fig. 1. It is essentially a double-stopper, evacuated Pyrex tube that contains 0.3 ml of a high-density hydrophobic cushion (Fluorinert, 3-M Corp.) and 1.2 ml of an aqueous solution. This latter solution contains 0.08% Dow Corning Anti-Foam B, 10 mg of Solryth (Hoffman-La Roche, Inc., Nutley, N.J.), 5 mg of sodium polyanetholsulfonate (Hoffman-La Roche), and 15 μg of sodium thioglycolate (Nutritional Biochemicals Corp., Cleveland, Ohio). The bottom stopper has an angular plane complementary to the angle of the centrifuge rotor used. When this type of stopper is used, the high-density Fluorinert covers the entire stopper surface during centrifugation and thereby keeps the organism from becoming entrapped in the spaces between the glass wall and the stopper. The stopper was prepared by adding 1.1 ml of Sylgard 198 (Dow Corning Corp.) on top of a rubber stopper, and the tubes were held at an angle of 34° for 24 h at room temperature. After the angle stoppers were formed, the two solutions were added. The tubes were then evacuated to 35 mm of Hg, and the top stoppers were inserted. Sterility controls were done on a random selection of 5% of the tubes from each lot. Of the 4,000 tubes assembled to date, no contaminated tubes have been found.

To determine the optimal centrifugation force and time, 21 different organisms were seeded into 21-day-old human donor citrate-phosphate-dextrose-blood (Wadley Blood Bank, Dallas, Tex.). The rubber stopper surfaces of the tube were scrubbed with 2% aqueous iodine and 70% isopropanol before insertion of a sterile needle. Seven milliliters of seeded blood, containing between 100 and 2,000 colony-forming units, was aseptically added into the tube. The contents were vigorously mixed, and the tube was placed in the centrifuge, angle stopper down with the angular plane facing toward the center of the rotor. After centrifugation at the desired gravity and time, the vessel was removed, and a sterile cotton-plugged needle was inserted into the top of the stopper to serve as a vent. A 10-ml syringe was fitted with a 21-gauge, 1.5-inch (ca. 3.8-cm) needle. This needle was inserted through the bottom of the angle stopper to its upper limit, and the majority of the supernatant (approximately 7 ml) was slowly removed. The remaining 1.5 ml of solution was vigorously mixed and removed with a 2-ml syringe. The contents within this syringe were equally dispensed onto five plates containing suitable growth medium and incubated under the appropriate atmospheres (Tables 1 and 2). A 1-ml sample of the supernatant was also plated to determine the total count remaining in the supernatant. Control counts were made of each organism by preparing dilutions of the inoculum into an appropriate solution (Tables 1 and 2). From this data it was possible to obtain a mean ($\bar{x}$) and standard deviation (SD) of the relative recovery in the concentrate as compared with the total count in the centrifugation

vessel. In addition, a survival index (K value) was determined as follows: K = total count in supernatant + total count in concentrate/total count in the control (saline or broth). A $K = 1$ indicates that no microorganisms were lost through the killing action of the blood, the other components in the tube, or the manipulation process itself.

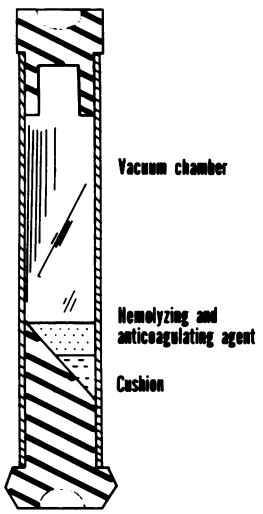

FIG. 1. *Cross section of the centrifugation vehicle.*

RESULTS

Tables 1 and 2 summarize the results obtained using three different combinations of gravity and time. The best overall average recovery (93%) was obtained at 3,000 × g for 30 min. With this combination only three organisms gave less than 80% recovery, namely, *Pseudomonas aeruginosa* (77%), *Bacteroides fragilis* (75%), and *Neisseria meningitidis* (59%). The other combinations gave lower overall recoveries (86 and 85%, respectively) and were generally less efficient with several of the other organisms tested, namely, *Brucella suis, Bacteroides melaninogenicus,* and *Salmonella typhi.* It should be emphasized that an average recovery of 93% does not mean that the organism was not detected 7% of the time. On the contrary, the data indicate that if 100 bacteria were present in the blood sample, on the average, 93 organisms would be recovered. With the exception of *Staphylococcus aureus,* the survival value (K) ranged from 0.8 to 1.1, indicating that no toxicity was observed with the present vehicle and proc-

TABLE 1. *Percent recovery of various microorganisms from blood by centrifugation*

Organism	Avg CFU[b]/tube	Recovery[a]		
		3,000 RCF[c] (min)		1,500 RCF min
		30	15	30
		$\bar{x}$ ± SD (%)	$\bar{x}$ ± SD (%)	$\bar{x}$ ± SD (%)
Aspergillus nidulans	1,433	97 ± 2	99 ± 1	98 ± 1
Candida albicans	1,085	100	99 ± 2	99 ± 1
Cryptococcus neoformans	669	100	99 ± 1	100
Brucella suis (ATCC 4312)	600	82 ± 2	68 ± 4	72 ± 5
Citrobacter freundii	700	95 ± 2	97 ± 2	75 ± 10
Enterobacter cloacae	250	94 ± 5	72 ± 7	92 ± 4
Escherichia coli	742	95 ± 2	81 ± 3	88 ± 3
Haemophilus influenzae (ATCC 19418)	300	98 ± 3	86 ± 5	82 ± 6
Klebsiella pneumoniae	200	98 ± 4	98 ± 3	98 ± 2
Proteus vulgaris (ATCC 6380)	100	95 ± 5	94 ± 11	91 ± 7
Pseudomonas aeruginosa	950	77 ± 8	92 ± 14	68 ± 7
Salmonella typhi (ATCC 6539)	600	96 ± 5	78 ± 19	77 ± 14
Neisseria meningitidis (ATCC 13077)	1,568	59 ± 13	45 ± 4	47 ± 3
Staphylococcus aureus	982	100	100 ± 1	99 ± 1
Streptococcus pneumoniae (ATCC 6301)	500	90 ± 6	87 ± 7	92 ± 5
Streptococcus pyogenes (ATCC 19616)	300	100 ± 1	93 ± 10	99 ± 2
Bacteroides fragilis (ATCC 23745)	1,351	75 ± 2	63 ± 4	57 ± 4
Bacteroides melaninogenicus (ATCC 25845)	1,980	98 ± 2	86 ± 2	65 ± 3
Clostridium sporogenes (ATCC 500310)	750	98 ± 1	90 ± 9	94 ± 3
Fusobacterium nucleatum (ATCC 25534)	2,000	100 ± 1	99 ± 1	99 ± 1
Listeria monocytogenes (ATCC 984)	850	97 ± 6	90 ± 6	90 ± 5
Avg recovery	—	93 ± 4	86 ± 6	85 ± 4

[a] Percent recovery given as $\bar{x}$ ± SD. Six tubes were processed for each unique combination of RCF, time, and organism.

[b] CFU, Colony-forming unit.

[c] RCF, Relative centrifugal force.

TABLE 2. *Conditions for recovery of microorganisms from blood by centrifugation*

Organism	Growth medium	Dilutant	Plate medium	Atmospheric conditions
Aspergillus nidulans	Sabouraud agar (Difco)	Normal saline	Sabouraud agar	36°C, aerobic, 24 h
Candida albicans *Cryptococcus neoformans* *Citrobacter freundii* *Enterobacter cloacae* *Escherichia coli* *Klebsiella pneumoniae* *Proteus vulgaris* *Pseudomonas aeruginosa* *Salmonella typhi* *Staphylococcus aureus*	1% Dextrose, 1% peptone, and nutrient broth (Difco)	Brain heart infusion broth (Difco)	Blood agar (BBL)	36°C, aerobic, 24 h
Brucella suis *Neisseria meningitidis* *Listeria monocytogenes*	Brain heart infusion	Brain heart infusion	Chocolate agar	36°C, 5% CO_2, 48 h
Streptococcus pneumoniae *Streptococcus pyogenes*	Todd-Hewitt (Difco)	Todd-Hewitt	Blood agar	36°C, 5% CO_2, 48 h
Bacteroides fragilis *Bacteroides melaninogenicus* *Clostridium sporogenes* *Fusobacterium nucleatum*	Chopped meat (Difco)	Prereduced brain heart infusion	Chocolate agar	36°C, BBL anaerobic jar, 48 h
Haemophilus influenzae	Brain heart infusion and supplement B (Difco)	Brain heart infusion and supplement B	Chocolate agar	36°C, 5% CO_2, 48 h

ess for the organisms tested. One possible explanation for the 1.4 K value for *S. aureus* is that the inoculum contained aggregates of several colony-forming units that were dispersed during the centrifugation procedure. No contamination by a second organism was observed during this study. However, these experiments were done under carefully controlled research conditions.

DISCUSSION

The single-tube vehicle described here required fewer entries and less manipulation than its two-tube predecessor. Furthermore, it is adaptable to a nonrefrigerated tabletop centrifuge (Sorval GLC or Beckman TJ-6) fitted with a multiple-sample angle rotor. The reconstruction data indicated that, at 3,000 × g for 30 min, the efficiency of the new vehicle is far superior to the earlier two-tube system that required a swinging-bucket rotor (2). A clinical trial is currently in progress to ascertain the relative efficiency and contamination rate of this system in a clinical environment.

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