0095-1137/78/0007-0059$02.00/0
JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1978, p. 59-62

Vol. 7, No. 1
Printed in U.S.A.

Quantitative Evaluation of Three Commercial Blood Culture Media for Growth of Anaerobic Organisms

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Received for publication 25 July 1977

The ability of three different commercial blood culture media—brain heart infusion broth (Pfizer), thiol broth (Difco), and PRS-peptone broth (Becton, Dickinson & Co.)—to support the growth of five different anaerobes is described. Inocula of 100 and 1,000 colony-forming units per ml were used to evaluate potential differences in survival, lag time, growth rate, and doubling times of each anaerobe in each medium. In addition, each medium was evaluated for its ability to neutralize the antibacterial effects of whole blood. The results of this study indicate that the PRS-peptone broth is superior to brain heart infusion and thiol broths. Shorter lag times and accelerated generation times and growth rates were noted for more different anaerobes in the PRS-peptone broth. Neither the size of inoculum nor the addition of normal whole blood appeared to alter the survival or growth characteristics of the anaerobes in any medium. However, the addition of normal whole blood did extend the lag time of each anaerobe by approximately 1 to 2 h in each medium.

Studies to determine the efficiency of blood culture media to maintain the viability and to support the growth of bacteria can be performed by at least two methods. The most widely employed method compares various media under direct clinical conditions, e.g., blood from patients suspected of bacteremia (8, 13, 17-19). The medium that recovers the most isolates in the shortest period of time is considered to be optimal under the test conditions.

Another type of evaluation is to inoculate a predetermined number of organisms into each medium and, after a prescribed period of time, determine whether the organisms survived and replicated. The lag time, growth rate, and doubling time of the test organism in each medium can then be determined (11, 12). Although this method does not take into account the adverse effects of the whole blood of patients on the test organisms (6, 7, 21), it does provide data that eliminate some variables associated with direct clinical studies.

The purpose of this quantitative growth study was to compare three different commercial blood culture media for their ability to support the growth of five different anaerobes and to determine the lag times, growth rates, and doubling times of each organism in each medium. Also, each medium was evaluated for its ability to neutralize the effects of normal whole blood. This information may help elucidate conditions that allow for optimal growth and recovery of anaerobic bacteria from blood specimens.

(This work was submitted in part by J.I.M. to San Jose State University in partial fulfillment of the M.A. degree, May 1976.)

MATERIALS AND METHODS

Media. The following commercial blood culture media were used: 50 ml of brain heart infusion (BHI) broth under vacuum and with CO_2 (Pfizer Inc., New York, N.Y.); 50 ml of thiol broth under vacuum and with CO_2 (Difco Laboratories, Detroit, Mich.); and 50 ml of PRS-peptone broth in a Vacutainer bottle under vacuum and with added CO_2 (Becton, Dickinson & Co., Rutherford, N.J.). All blood culture media contained between 0.025 and 0.05% sodium polyanetholsulfonate.

Organisms. Pure cultures of *Bacteroides fragilis, Bacteroides melaninogenicus, Fusobacterium nucleatum, Peptostreptococcus anaerobius,* and *Eubacterium limosum* were selected because they are among the most common anaerobes associated with bacteremia (19, 20) and because of their diverse physiological traits (14). These five anaerobes were isolated from patients previously seen at Stanford Medical Center and were identified by the methods of Holdeman and Moore (5). Stock cultures were maintained in prereduced chopped-meat carbohydrate (CMC) broth (Scott Laboratories, Inc., Fiskeville, R.I.).

Inoculum. Seed cultures were prepared by subculturing each stock culture into CMC broth. After 24 h at 35°C, the turbidity of each CMC broth culture was

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adjusted to a McFarland nephelometer standard no. 1 with CMC broth, and smears were prepared for Gram staining. To obtain a final inoculum size of 100 or 1,000 colony-forming units (CFU) per ml, serial dilutions were made by aseptically transferring 0.1 ml of each standardized seed culture to 9.9 ml of PRS-BHI broth (Scott Laboratories, Inc.). All subcultures and dilutions were made using a VPI anaerobe inoculator (Bellco Glass, Inc., Vineland, N.J.). The colony counts were determined by spreading 0.1 ml of each dilution over the surface of freshly prepared Trypticase soy agar base (Baltimore Biological Laboratory, Cockeysville, Md.) enriched with 5% defibrinated sheep blood, 10.0 μg of vitamin K_1 (ICN Pharmaceuticals, Cleveland, Ohio), and 5.0 μg of hemin (Eastman Kodak Co., Rochester, N.Y.) per ml. All plates were placed in vented GasPak jars (Baltimore Biological Laboratory); the atmosphere of the jars was evacuated and replaced three times with a gaseous mixture of 80% nitrogen, 10% carbon dioxide, and 10% hydrogen (Liquid Carbonic Co., San Carlos, Calif.). The jars were sealed and incubated at 35°C for 48 h. Before each test run, the number of CFU of the inoculum per milliliter was rechecked by inoculating 0.1 of the final dilution onto a freshly prepared 5% sheep blood agar plate supplemented and incubated as previously described. These plates also serve as pure culture controls for each inoculum.

Growth curve studies. A 1-ml amount of seed culture (100 or 1,000 CFU/ml) was inoculated into each test medium and incubated at 35°C. At various time intervals (0, 2, 4, 6, 8, 10, 12, 18, 24, and 36 h), a 0.5-ml sample was withdrawn from each test medium and placed into 4.5 ml of PRS-BHI broth. Serial dilutions (10^{-2} through 10^{-8}) were made in PRS-BHI broth. All subcultures and dilutions were made with a VPI anaerobe inoculator. A 0.1-ml amount of each dilution was spread over the surface of freshly prepared 5% defibrinated sheep blood agar plates supplemented as previously described, and each dilution was sampled in duplicate. The plates were placed in vented GasPak jars, evacuated and replaced three times with the aforementioned gaseous mixture, and sealed and incubated at 35°C for 48 h. The average number of colonies per dilution was calculated. This procedure was repeated twice with each of the five anaerobes.

To determine the effect of whole blood, the aforementioned procedures, using 100 and 1,000 CFU/ml, were repeated after 5 ml of whole blood from a single normal, healthy donor was added to each test medium. All blood samples were collected just before seeding each test medium. This procedure was repeated twice with each inoculum size.

Growth curve data from the four different experiments were compiled, and the averages were plotted on semilog paper. From these data, the lag times, growth rates, and doubling times were determined (9, 10).

RESULTS

The survival, lag times, growth rates, and doubling times with an inoculum size of 100 CFU/ml are shown in Table 1. *P. anaerobius* and *B. melaninogenicus* did not grow in BHI broth, and *B. melaninogenicus* did not grow in thiol broth. All test organisms grew in the PRS-peptone broth. The duration of the lag phase for those organisms that survived was shorter in the PRS-peptone broth. The fastest growth rates and the shortest doubling times for each of the five anaerobes occurred in the PRS-peptone broth.

Effect of inoculum size. An increased inoculum size (1,000 CFU/ml) neither affected the growth pattern of any organism nor altered the lag times, growth rates, or doubling times (Table 2). The organisms that failed to survive in a particular blood culture medium at an inoculum size of 100 CFU/ml also failed to survive when the inoculum size was 1,000 CFU/ml.

Effect of whole blood. The addition of 5 ml of whole blood (Table 3) prolonged the lag time for those anaerobes that survived in each medium by 1 to 2 h. However, once the organisms entered the logarithmic phase, the growth rates and doubling times were approximately the same as previously obtained. No change was noted in the survival, lag time, growth rate, or doubling time as a result of a larger inoculum size (Table 4). *P. anerobius* failed to survive in BHI, whereas *B. melaninogenicus* survived in PRS-peptone only.

DISCUSSION

The need for blood culture media that support the viability and enhance cell replication is essential for early recovery and identification of anaerobes in bacteremia. Because the metabolic activity and the growth rate of a bacterial cell are dependent in part upon the composition of the medium in which the cell replicated (1, 10), an ideal blood culture medium must provide factors for a short lag phase and a rapid growth rate. Organisms in a nutritionally poor medium will require a longer lag phase and will exhibit a slower growth rate and a longer doubling time (10, 15), therefore prolonging the time necessary for detecting the presence of the organism in the blood culture medium.

Data from this study show that a more diverse group of anaerobes grew in PRS-peptone broth within a shorter period of time and at a faster growth rate than in BHI or thiol broth (Tables 1 and 2).

B. melaninogenicus did not grow in BHI or thiol broth (Tables 1 through 4), but grew in the PRS-peptone broth. *B. melaninogenicus* is known to require vitamin K_1 and/or hemin (3, 14); possibly the concentration of vitamin K_1 and/or hemin is insufficient in BHI and thiol broths or the concentration of preformed organic peroxides is greater in BHI and thiol broths

TABLE 1. *Comparison of lag times, growth rates, and doubling times after inoculation of 100 CFU/ml*

Organism	BHI			Thiol			PRS-peptone		
	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)
B. fragilis	5	1.28	46.9	6	1.41	42.6	4	1.56	38.5
F. nucleatum	7	1.12	53.6	6	1.10	54.5	4	1.33	45.1
E. limosum	8.5	0.57	105.3	8	0.81	74.0	6	0.94	63.8
P. anaerobius	NG[a]	NG	NG	9	0.59	101.7	7	0.67	89.5
B. melaninogenicus	NG	NG	NG	NG	NG	NG	9	0.49	122.4

[a] NG, No growth in medium after 36 h.

TABLE 2. *Comparison of lag times, growth rates, and doubling times after inoculation of 1,000 CFU/ml*

Organism	BHI			Thiol			PRS-peptone		
	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)
B. fragilis	5	1.31	53.1	5	1.40	42.9	4	1.59	37.7
F. nucleatum	7	1.10	54.5	6	1.15	52.2	4	1.39	43.2
E. limosum	8	0.59	101.7	8	0.75	80.0	6	0.90	66.7
P. anaerobius	NG[a]	NG	NG	9	0.52	115.4	7	0.65	92.3
B. melaninogenicus	NG	NG	NG	NG	NG	NG	9	0.51	117.6

[a] NG, No growth in medium after 36 h.

TABLE 3. *Comparison of lag times, growth rates, and doubling times after the addition of 5 ml of whole blood and 100 CFU per ml*

Organism	BHI			Thiol			PRS-peptone		
	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)
B. fragilis	7	1.20	50.0	6	1.34	45.0	5	1.55	38.7
F. nucleatum	7.5	1.08	55.6	7.5	1.28	46.9	6	1.43	42.0
E. limosum	9.5	0.50	120.0	9	0.75	80.0	7	1.03	58.2
P. anaerobius	NG[a]	NG	NG	10	0.46	130.4	9	0.59	101.7
B. melaninogenicus	NG	NG	NG	NG	NG	NG	10	0.55	109.1

[a] NG, No growth in medium after 36 h.

TABLE 4. *Comparison of lag times, growth rates, and doubling times after the addition of 5 ml of whole blood and 1,000 CFU per ml*

Organism	BHI			Thiol			PRS-peptone		
	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)	Lag time (h)	Growth rate (per h)	Doubling time (min)
B. fragilis	6	1.18	51.0	6.5	1.31	46.0	5	1.52	39.5
F. nucleatum	7.5	1.12	53.6	7	1.23	49.0	6.5	1.48	40.5
E. limosum	9	0.48	125.0	9.5	0.72	83.3	7	1.11	54.1
P. anaerobius	NG[a]	NG	NG	10	0.50	120.0	9	0.57	105.3
B. melaninogenicus	NG	NG	NG	NG	NG	NG	10	0.53	113.2

[a] NG, No growth in medium after 36 h.

because these media are not prereduced. This strain of *B. melaninogenicus* was initially recovered from thiol and PRS-peptone broths but not from BHI broth during clinical blood culture studies at Stanford (8). It is possible, because the blood culture bottles were not sampled beyond 36 h, that *B. melaninogenicus* would have grown sufficiently to be detected by blind subculturing. It should be noted that this strain, upon original isolation, was not detected until

after day 14 in BHI and after day 3 in thiol (8).

P. anaerobius did not grow in BHI and thiol broths. Possibly the concentrations of sodium polyanetholsulfonate (4) or dissolved oxygen and/or organic peroxides were too great in these media (14). This strain of *P. anaerobius* was initially recovered in PRS-peptone broth after 4 days of incubation (8). No growth was detected in BHI and thiol broths after 14 days.

Tables 1 and 2 show that increasing the inoculum size had no effect on survival, lag time, or growth rate pattern. It has been shown, however, that in general bacteremia exhibits fewer organisms per milliliter than were used in this study (2, 16). Within these limits, it appears that the lag time may not be a function of the number of organisms present but rather of the ability of the organism(s) to accommodate to a new environment and/or repair defective forms (10, 21). The addition of whole blood to the culture media should have created a growth pattern quite different from that of the culture sets containing no blood because normal human blood may adversely affect the growth of bacteria (6, 7, 12). It is apparent that each medium had the capacity to neutralize equally any bactericidal and/or bacteriostatic effects of whole blood. The only significant difference was an extended lag phase by about 1 to 2 h.

It is highly probable that the pure cultures used in these studies differed considerably from those generally found in the blood stream. Organisms recovered from bacteremic patients probably are more susceptible to inhibitory substances and less capable of growth as a result of prior stresses and trauma due to the antibacterial action of the blood of the patient (6, 7). Extensions of the lag time or the complete absence of growth under clinical conditions might be related to prior injury of the organism rather than to an inadequate blood culture medium. This present study did not investigate the physical condition of bacteria in the blood stream of the patient or the capacity of the test medium to support the growth of these damaged organisms.

In conclusion, the quantitative evaluation used in this study showed the effects of nutritional and/or environmental composition of growth rate, the influences of inoculum size on growth rate, and the effects of normal whole blood on the growth rate. Because the inoculum size, volume of blood introduced, and type of organism are controlled, the differences in recovery rate as a result of these variables and chance distribution are reduced. Of the three media, the PRS-peptone broth was superior. This might be because it contained the best combination of nutrients and environmental conditions to support the widest range of anaerobes.

ACKNOWLEDGMENTS

This work was supported by a student research fellowship, grant-in-aid, NSF 02-01-1644, from San Jose State University, San Jose, Calif., and by a grant-in-aid from Becton, Dickinson & Co., Rutherford, N.J.

We thank R. D. Haight of San Jose State University for his helpful comments and critical review of this manuscript.

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