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Poor Performance of BACTEC NR 730 Blood Culture System in Early Detection of *Neisseria meningitidis*

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During an 8-month period at Children's Hospital, Oakland, Calif., a 9% rate for positive blood culture for children with *Neisseria meningitidis* meningitis was identified. The blood culture system used in each case was the BACTEC NR 730. This rate seemed significantly lower than previous rates (33 to 55%) (P. R. Dodge and M. N. Swartz, N. Engl. J. Med. 272:1003–1010, 1965; A. L. Hoyne and R. H. Brown, Ann. Intern. Med. 28:248–259, 1948; S. Levin and M. B. Painter, Ann. Intern. Med. 64:1049–1057, 1966). The low rate prompted our study. With 14 test strains, anaerobic and aerobic BACTEC bottles were evaluated for their ability to support and detect the growth of *N. meningitidis*. Sodium polyanetholesufonate (SPS) and inoculum size, two factors thought to affect the growth of *N. meningitidis*, were controlled for by use of bottles with and without SPS and by inoculum sizes simulating the magnitudes of bacteremia previously described for children infected with *N. meningitidis* (L. J. La Scolea, Jr., D. Dryja, T. D. Sullivan, L. Mosovich, N. Ellerstein, and E. Neter, J. Clin. Microbiol. 13:478–482, 1981). BACTEC failed to detect growth in aerobic bottles after 6 h of incubation, while 76 of 80 bottles (95%) showed growth when subcultured. At 24 h, BACTEC detected growth in only 29 of 80 bottles (36%); when subcultured, all 80 cultures grew confluently. At 48 h, BACTEC detected growth in the remaining 53 bottles. BACTEC failed to detect growth in anaerobic bottles at 6 h and at 1, 2, 4, and 5 days of incubation despite growth in subculture. Subcultures from bottles with tryptic soy broth with and without SPS showed growth in 63 of 76 bottles in 6 h and in all bottles after 24 h. The presence of SPS in BACTEC bottles had no effect on growth detection. On the basis of these studies and our clinical experience, we find the BACTEC NR 730 system to be insensitive and unsuitable for detection of *N. meningitidis* in ≤24 h. However, all test strains were detected after 48 h.

The following study was undertaken after 10 of 11 blood cultures of children with meningitis due to *Neisseria meningitidis* of various serogroups remained negative despite growth in simultaneously obtained cerebrospinal fluid samples, yielding a positive blood culture rate of 9%. The serogroups included B (two samples), C (seven samples), and Y (one sample). One sample was not grouped. All the children were diagnosed and treated at Children's Hospital, Oakland, Calif., between October 1987 and May 1988. The blood culture system used in each case was the BACTEC NR 730 system, a nonradiometric system which detects CO_2 production by infrared analysis. An increase in CO_2 concentration indicates the presence of growing organisms.

Our rate of 9% positivity for blood cultures from children with *N. meningitidis* meningitis seemed significantly lower than that observed in previous studies. Hoyne and Brown (4) found a 51.4% rate for positive blood cultures in a study of 400 patients with meningococcal meningitis. Dodge and Swartz (3) and Levin and Painter (7), with smaller sample sizes, reported rates of 33 and 53%, respectively. This disparity in findings prompted the following evaluation of the BACTEC NR 730 system for ability to support the growth and provide early detection of *N. meningitidis*.

MATERIALS AND METHODS

Bacterial isolates. Fourteen frozen (−70°C) isolates of *N. meningitidis*, recovered previously from blood or cerebrospinal fluid of children, were thawed and inoculated on chocolate agar plates and checked for purity. Identification was confirmed by colonial morphology, Gram stain, oxidase, and serological testing. Ten isolates were group B, and four were group C; the group of one isolate was unknown.

Preparation of inocula. Normal saline suspensions of an overnight growth equivalent to a 0.5 McFarland standard (1) were prepared for each of the 14 test isolates, yielding concentrations of 10^8 CFU/ml. A 0.01-ml portion of each suspension was transferred to 9.5 ml of normal saline supplemented with 0.5 ml of fresh human blood to yield a concentration of 10^5 CFU/ml. From the 10^5-CFU/ml suspension, 1 ml was transferred to 8.5 ml of normal saline supplemented with 0.5 ml of human blood to yield a concentration of 10^4 CFU/ml. In the same manner, additional dilutions were made to achieve concentrations of 10^3, 10^2, and 10^1 CFU/ml. These were the concentrations used for inoculation in this study.

Blood cultures. Prior to inoculation of the test organism, 2 ml of fresh human blood was introduced into each blood culture bottle. For each isolate, 1 ml of each of the three concentrations of the test organism was inoculated into a set of aerobic and anaerobic blood culture bottles. A set of aerobic bottles consisted of a BACTEC NR6A bottle (nonradiometric aerobic with sodium polyanetholesulfonate [SPS]) and a BACTEC NR6AX bottle (nonradiometric aerobic without SPS). An anaerobic set consisted of a BACTEC NR7A bottle (nonradiometric anaerobic with SPS) and a BACTEC NR7AX bottle (without SPS). Tryptic soy broth (TSB) (Difco Laboratories, Detroit, Mich.) with and without SPS was treated in the same manner and used as a control for each set of BACTEC bottles. The BACTEC bottles contained 30 ml of broth, and the TSB contained 50 ml of broth. Thus, final volumes were 33 ml for the BACTEC system and 53 ml for the TSB bottles. Fourteen isolates were

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inoculated into aerobic sets, and five were inoculated into anaerobic sets. TSB bottles were not vented.

Colony counts. Colony counts on the inocula were done by culturing 1 ml of each inoculum on a chocolate agar plate (150 by 15 mm) which subsequently was incubated under 4 to 10% CO_2 at 36°C overnight without agitation.

Incubation and processing. After 6 h of incubation at 36°C, 1 ml of broth from each bottle was cultured on three chocolate agar plates (100 by 15 mm) to determine CFU per milliliter. Subcultures, colony counts, and BACTEC readings on all bottles were obtained at 6, 24, and 48 h until confluent growth and a positive reading were obtained. Readings were interpreted by using the guidelines of the manufacturer: a growth value of ≥30 or an increase of 10 over a previous reading was considered positive. All BACTEC bottles which did not give positive readings were reincubated. Specimens with negative subcultures were subjected to repeat subcultures and BACTEC readings for up to 5 to 7 days.

RESULTS

Growth versus no growth. Only 3 of 42 (7.1%) inoculated BACTEC aerobic bottles with SPS and 1 of 42 (2.4%) inoculated aerobic BACTEC bottles without SPS did not show *N. meningitidis* growth at all. In the anaerobic BACTEC system, 6 of 15 (40%) with SPS versus 4 out of 15 (27%) without SPS did not show growth. The most striking number of negative bottles (no growth) was in the TSB bottles with SPS (17 of 51 [33%] of inoculated bottles exhibited no growth). This was in contrast to the TSB bottles without SPS, of which only 5 of 51 (10%) of inoculated bottles did not show growth.

Colony counts of inocula. Sizes of colony counts ranging from 1 to 900 had no significant effect on the rate of detection by BACTEC or on the rate of growth as determined by positive subcultures (Table 1). At 24 h, 32% (11 of 34) of the high inocula, 43% (13 of 30) of the mid-level inocula, and 25% (5 of 20) of the low inocula gave positive BACTEC readings. By 48 h, 94% (32 of 34) of the high, 100% (30 of 30) of the mid-level, and 90% (18 of 20) of the low inocula had positive BACTEC readings. By contrast, 94% (32 of 34) of the high, 97% (29 of 30) of the mid-level, and 75% (15 of 20) of the low inocula showed growth when subcultured at 6 h. By 24 h, 94% of the high, 100% of the mid-level, and 90% of the low inocula were showing confluent growth (Fig. 1).

BACTEC aerobic cultures. At 6 h, all BACTEC readings were negative, although 95% (76 of 80) of bottles with and without SPS showed growth when subcultured (Table 1). Of these, 69 demonstrated an increase in CFU over the inoculum (not shown). At 24 h, 36% (29 of 80) of the bottles had positive readings, while all subcultures (excluding those with no growth) showed confluent growth. The highest positive growth value reading at 24 h was 24, and the average reading was 14. At 48 h, the remaining 53 aerobic bottles had positive readings. These readings ranged between 13 and 67, with an average positive reading of 36.

BACTEC anaerobic cultures. BACTEC failed to detect growth in and did not consistently support the growth of the anaerobic cultures (Table 1). Readings taken at 6 h and at 1, 2, 4, and 5 days were all consistently negative. Of 30 inoculated bottles, 20 (67%) of subcultures of anaerobic bottles with and without SPS were positive at 6 h, although only 1 subculture demonstrated an increase in CFU over the inoculum (data not shown). On subsequent subculture, 10 of these 20 failed to grow, whereas the other 10 showed confluent growth when subcultured at 5 days. Therefore, only 33% (10 of the original 30) of anaerobic bottles consistently supported growth as indicated by subculture, and none of these yielded positive BACTEC readings.

TABLE 1. Growth of *N. meningitidis* as detected by subculture and affected by inoculum size and SPS

Inoculum	Range[a]	No. of bottles:			
		Inoculated	Positive at 6 h	Positive at 24 h	With no growth[b]
Aerobic BACTEC with SPS	High	17	16	16	1
	Middle	15	14	15	0
	Low	10	6	8	2
Aerobic BACTEC without SPS	High	17	16	16	1
	Middle	15	15	15	0
	Low	10	9	10	0
Anaerobic BACTEC with SPS	High	8	7	7	1
	Middle	6	2	2	4
	Low	1	0	0	1
Anaerobic BACTEC without SPS	High	9	9	9	0
	Middle	4	2	2	2
	Low	2	0	0	2
TSB with SPS	High	21	18	19	2
	Middle	20	8	10	10
	Low	10	2	5	5
TSB without SPS	High	22	22	22	0
	Middle	18	14	16	2
	Low	11	4	8	3

[a] Inoculum CFU per milliliter ranges were as follows: for aerobic BACTEC, 101 to 912 (high), 12 to 51 (middle), and 0 to 6 (low); for anaerobic BACTEC, 101 to 506 (high), 15 to 29 (middle), and 4 (low); and for TSB, 101 to 912 (high), 12 to 51 (middle), and 0 to 6 (low).

[b] No growth after subculturing for up to 5 days.

TSB bottles. Subcultures from TSB bottles showed growth in 67% (68 of 102 inoculated bottles) in 6 h and confluent growth in 78% (80 of 102 inoculated bottles) after 24 h of incubation.

DISCUSSION

N. meningitidis is an important pediatric pathogen. In addition to our own findings, previous studies have also indicated difficulty in the growth and early detection of *N. meningitidis* (2, 9, 10). Two of the factors thought to contribute to these problems are the inhibitory effect of SPS, an anticoagulant added to almost all blood culture systems, and inoculum size.

The most striking finding in our study was the insensitivity of the BACTEC NR 730 blood culture system in the early detection of *N. meningitidis*. At 6 h, while 95% of the subcultures showed growth, all aerobic BACTEC readings were negative. Even more striking, at 24 h, despite confluent growth in subcultures of 80 aerobic bottles, the BACTEC system detected only 36% (29 of 80) as positive, with the remaining 53 bottles detected as positive at 48 h. These findings in the nonradiometric BACTEC system are supported by previous studies done with the radiometric BACTEC system, which is basically equivalent (5). In their study, Meadow and Schwartz (8) found that only 12% of 33 isolates of *N. meningitidis* were detected at 24 h, 61% were detected at 48 h, and 100% were detected at 72 h. In a recent article by Carey (2), in which the Isolator 1.5 system

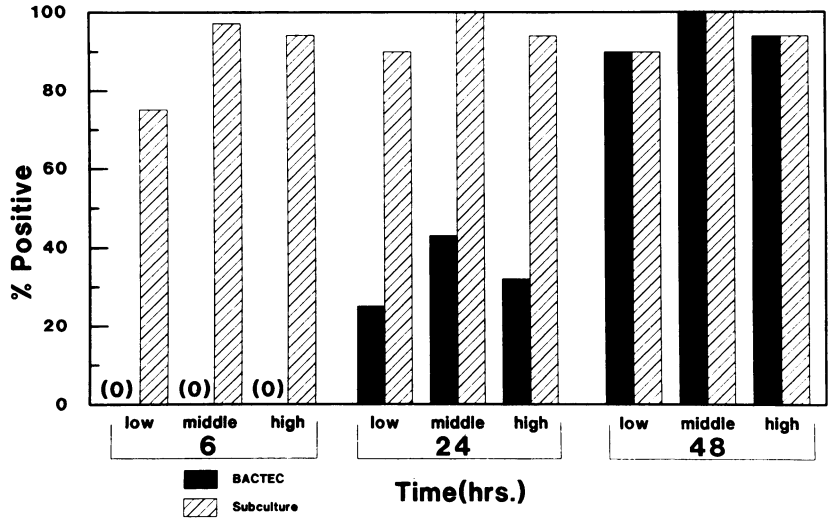

FIG. 1. Percent positivity of the BACTEC system with aerobic bottles as a function of time and inoculum size compared with simultaneous subcultures. Inoculum concentration (CFU per milliliter): low, 0 to 6; middle, 12 to 51; high, 101 to 912.

was compared with the radiometric BACTEC system, the BACTEC system again demonstrated delayed detection, although the sample size was small.

Our study did indicate that the aerobic BACTEC NR 730 system is able to support the growth of *N. meningitidis*. Contrary to our expectations and the results of others (9, 10), the presence or absence of SPS did not appear to significantly alter the growth pattern (i.e., the onset of growth) or to markedly affect the ability of the BACTEC aerobic and anaerobic systems to sustain or detect growth. SPS did appear to have an inhibitory effect on the growth of *N. meningitidis* in TSB. However, the small sample sizes preclude definitive conclusions. The concentrations of SPS in the BACTEC and TSB systems were the same (0.025%).

The anaerobic system did not consistently support the growth of *N. meningitidis*, a finding consistent with those of previous studies (8). However, when growth did occur, as indicated by confluent growth in subculture, the BACTEC system consistently failed to detect it. Inoculum size, the second factor evaluated in our study, appeared to have no effect on the rate of growth or the detection of *N. meningitidis* in the aerobic bottles. Quantitative direct plating studies have indicated that children infected with *N. meningitidis* have a range of 10 to 1,000 organisms per ml of blood, confirming the clinical appropriateness of the inoculum concentration that we used (6, 11, 12).

In summary, the aerobic BACTEC system was able to support the growth of *N. meningitidis*, while the anaerobic system was unable to consistently do so. In neither BACTEC system did SPS or inoculum size affect the growth or detection of *N. meningitidis*. The BACTEC system consistently failed to detect the growth of *N. meningitidis* in any of the anaerobic bottles, despite confluent growth on subculture. The aerobic system showed unacceptable delayed detection of positive blood cultures. Institution of a routine 24-h subculture policy for all BACTEC bottles might circumvent some of the problems of delayed detection. However, a prime feature of the BACTEC system is its automation and the consequent reduction in manual labor. Therefore, routine subculturing would seem self-defeating. On the basis of our results, we find the BACTEC NR 730 system to be insensitive and unsuitable for detection of *N. meningitidis* in ≤24 h. However, all strains tested were detected after 48 h of incubation.

LITERATURE CITED

1. **Barry, A. L.** 1976. The antimicrobic susceptibility test; principles and practices, p. 99. Lea & Febiger, Philadelphia.
2. **Carey, R.** 1984. Clinical comparison of the Isolator 1.5 microbial tube and the BACTEC radiometric system for detection of bacteremia in children. J. Clin. Microbiol. **19**:634–638.
3. **Dodge, P. R., and M. N. Swartz.** 1965. Bacterial meningitis. A review of selected aspects. II. Special neurologic problems, postmeningitic complications and clinicopathologic correlations. N. Engl. J. Med. **272**:1003–1010.
4. **Hoyne, A. L., and R. H. Brown.** 1948. 727 Meningococcic cases: an analysis. Ann. Intern. Med. **28**:248–259.
5. **Jungkind, D., J. Millan, S. Allen, J. Dyke, and E. Hill.** 1986. Clinical comparison of a new automated infrared blood culture system with the BACTEC 460 system. J. Clin. Microbiol. **23**:262–266.
6. **La Scolea, L. J., Jr., D. Dryja, T. D. Sullivan, L. Mosovich, N. Ellerstein, and E. Neter.** 1981. Diagnosis of bacteremia in children by quantitative direct plating and a radiometric procedure. J. Clin. Microbiol. **13**:478–482.
7. **Levin, S., and M. B. Painter.** 1966. The treatment of acute meningococcal infection in adults. Ann. Intern. Med. **64**:1049–1057.
8. **Meadow, W., and I. Schwartz.** 1986. Time course of radiometric detection of positive blood cultures in childhood. Pediatr. Infect. Dis. **5**:333–336.
9. **Pai, C., and S. Sorger.** 1981. Enhancement of recovery of *Neisseria meningitidis* by gelatin in blood culture media. J. Clin. Microbiol. **14**:20–23.
10. **Scribner, R., and D. Welch.** 1984. Neutralization of the inhibitory effect of sodium polyanetholesulfonate on *Neisseria meningitidis* in blood cultures processed with the Du Pont Isolator system. J. Clin. Microbiol. **20**:40–42.
11. **Sullivan, T. D., and L. J. La Scolea.** 1987. *Neisseria meningitidis* bacteremia in children: quantitation of bacteremia and spontaneous clinical recovery without antibiotic therapy. Pediatrics **80**:63–67.
12. **Sullivan, T. D., L. J. La Scolea, and E. Neter.** 1982. Relationship between the magnitude of bacteremia in children and the clinical disease. Pediatrics **69**:699–702.