JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1988, p. 2262–2265
0095-1137/88/112262-04$02.00/0

Vol. 26, No. 11

Rapid Detection of Positive Blood Cultures with the BACTEC NR-660 Does Not Require First-Day Subculturing

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An analysis of blood culture data was performed to determine whether subculturing within the first 24 h of incubation decreased the time to detection of positive blood cultures when compared with the routine use of the BACTEC NR-660 system (Johnston Laboratories, Inc., Towson, Md.). During a 9-month period (June 1985 to February 1986), 17,913 blood cultures were received in our laboratory, of which 1,463 (8.2%) became positive. Of the positive cultures, 97% were detected with equal or greater rapidity by the NR-660 system than by visual inspection and first-day blind subculturing. There were 37 delayed positive cultures from which only one isolate (0.07%) was not eventually detected by the NR-660 system. Coagulase-negative staphylococcus was the most frequent isolate among the delayed positive cultures, but only 3 of 15 isolates were known to be clinically significant isolates. The longest delay in detection by the NR-660 system was 6 days for one isolate of *Cryptococcus neoformans* and one isolate of *Klebsiella pneumoniae*. Although subculturing may decrease the time to detection of a few cultures, the majority of positive blood cultures were detected faster or with equal speed by the NR-660 system. When the data were evaluated, routine use of the NR-660 system was sufficient for the detection of positive blood cultures and was cost-effective.

The BACTEC NR-660 system (Johnston Laboratories, Inc., Towson, Md.) is a blood culture system that is based on the infrared detection of carbon dioxide in blood culture vials. Its predecessor, the BACTEC 460 system, detects ^{14}C-labeled carbon dioxide and has proven to be an accurate and rapid means of detecting microbial growth in blood culture vials (6, 10). Results of initial studies indicate that the NR-660 system is equal to, if not superior to, the 460 system (3, 7). Some microbiology texts state that for the rapid detection of positive blood cultures, laboratories should routinely subculture the primary blood cultures within the first 24 h of incubation (5, 6). Other investigators have demonstrated that there is no clinical advantage to this procedure (9). The purpose of this study was to determine whether subculturing of blood culture bottles within the first 24 h of incubation, in addition to the routine use of the NR-660 system, reduces the time to detection of positive cultures.

MATERIALS AND METHODS

Routine blood culture vials, consisting of BACTEC NR-6A (aerobic) and NR-7A (anaerobic) vials (Johnston Laboratories), were transported to the microbiology laboratory, entered into the BACTEC NR-660 system data base, and incubated at 35°C for up to 7 days. In-house staff members were instructed to inoculate each blood culture bottle with 3 to 5 ml of blood. The NR-6A vials were read twice on days 1 through 3 and once on days 4 through 7. The NR-7A vials were read daily on days 1 through 7. Blood cultures received by the laboratory by 11:59 a.m. were entered into the NR-660 testing protocol for that working day, while blood cultures received after 12:00 p.m. were entered into the NR-660 testing protocol for the next working day. All blood cultures were subcultured each day of the week at approximately 4:00 p.m. By using this entry format, blood cultures were tested for microbial growth after 4 to 16 h of incubation. The criteria for NR-660-positive vials were based on visual inspection of the vial, first-day blind subculturing, a growth index of ≥ 30, or a change in the growth index of ≥ 15 between any two readings (no units given by Johnston Laboratories). The presence of microorganisms in these bottles was then confirmed by Gram staining and culturing. Chocolate agar subculture plates were incubated at 35°C in 5% CO_2 or anaerobically, depending on which bottles were being subcultured. Centrifuged bacterial pellets from Gram stain-positive blood culture bottles and standard methods were used to obtain preliminary identification and susceptibility test results. The first-day blind subcultures were observed at 24-h intervals for 3 days (equivalent to day 4 of the BACTEC protocol). If any of the subcultures demonstrated bacterial growth before it was detected by the NR-660 system, it was considered to be a delayed positive culture. The delayed positive cultures were recorded in a data book, as were the results of the 7-day NR-660 system testing. Bacteria and yeast isolated from blood culture vials were identified by standard methods.

RESULTS

During the 9-month study period (June 1985 to February 1986), 17,913 blood culture sets were processed in our laboratory. A total of 1,463 positive blood cultures were detected (8.2% of the total blood cultures), and 1,622 organisms were identified from these positive cultures (Table 1). All anaerobic organisms detected by the NR-660 system were also recovered by subculturing onto chocolate and blood agar. A total of 22 of the isolates were from 22 patients with suspected subacute bacterial endocarditis and were tested and detected by subculturing only between laboratory days 7 and 14. These cultures were not considered in further data analyses.

The NR-660 system detected 1,404 of 1,441 (97%) positive blood cultures with equal or greater rapidity than visual

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TABLE 1. Blood culture isolates

Isolate	Total no. detected (no. of patients)[a]	% of total
Actinomyces israelii	1 (1)	0.06
Acinetobacter calcoaceticus	31 (18)	2.0
Arachnia propionica	1 (1)	0.06
Bacillus cereus	12 (8)	0.70
Bacteroides fragilis	8 (7)	0.50
Bacteroides fragilis group	8 (5)	0.50
Bacteroides melaninogenicus	1 (1)	0.06
Campylobacter jejuni	1 (1)	0.06
Candida albicans	57 (27)	3.50
Candida parapsilosis	2 (2)	0.10
Candida tropicalis	15 (8)	0.90
Citrobacter diversus	2 (2)	0.10
Citrobacter freundii	6 (4)	0.40
Clostridium perfringens	7 (5)	0.40
Clostridium ramosum	1 (1)	0.06
Corynebacterium xerosis	17 (9)	1.00
Corynebacterium sp. strain JK	3 (3)	0.20
Corynebacterium sp.	22 (21)	1.40
Cryptococcus neoformans	6 (1)	0.40
Eikenella corrodens	10 (1)	0.60
Enterobacter aerogenes	8 (4)	0.50
Enterobacter agglomerans	3 (1)	0.20
Enterobacter cloacae	26 (12)	1.50
Escherichia coli	185 (105)	11.50
Eubacterium sp.	4 (4)	0.25
Fusobacterium necrophorum	4 (3)	0.25
Haemophilus aphrophilus	2 (2)	0.10
Haemophilus influenzae	8 (7)	0.50
Klebsiella oxytoca	14 (4)	0.80
Klebsiella pneumoniae	90 (48)	5.60
Kluyvera sp.	2 (2)	0.10
Lactobacillus sp.	7 (6)	0.40
Micrococcus sp.	9 (8)	0.50
Morganella morganii	4 (2)	0.25
Neisseria meningitidis	1 (1)	0.06
Neisseria mucosa	1 (1)	0.06
Neisseria subflava	1 (1)	0.06
Neisseria sp.	1 (1)	0.06
Peptococcus sp.	4 (4)	0.25
Propionibacterium acnes	24 (23)	1.50
Propionibacterium sp.	8 (4)	0.50
Proteus mirabilis	35 (26)	2.20
Proteus vulgaris	1 (1)	0.06
Proteus sp.	1 (1)	0.06
Providencia stuartii	2 (2)	0.10
Pseudomonas aeruginosa	45 (28)	2.80
Pseudomonas cepacia	4 (3)	0.25
Pseudomonas maltophilia	3 (2)	0.20
Pseudomonas sp.	2 (1)	0.10
Saccharomyces cerevisiae	1 (1)	0.06
Salmonella sp.		
Group B	11 (4)	0.70
Groups C_1 and C_2	8 (2)	0.40
Group D	16 (5)	0.85
Unknown	1 (1)	0.06
Serratia marcescens	13 (8)	0.80
Staphylococcus aureus	228 (110)	14.00
Coagulase-negative staphylococci	306 (196)	19.00
Streptococcus bovis	2 (2)	0.10
Streptococcus faecalis	61 (33)	3.80
Streptococcus faecium	6 (5)	0.40
Streptococcus MG-*intermedius*	21 (17)	1.30
Streptococcus mitis	18 (17)	1.00
Streptococcus mutans	4 (1)	0.25
Streptococcus pneumoniae	86 (57)	5.30
Streptococcus sanguis types I and II	42 (20)	2.60
Beta-hemolytic streptococci		
Group A	1 (1)	0.06
Group B	19 (10)	1.00
Group F	2 (2)	0.10
Group G	3 (2)	0.20
Unknown	6 (5)	0.40
Torulopsis glabrata	16 (6)	1.00
Yeasts	2 (2)	0.10
Unidentified		
Gram positive	6 (5)	0.40
Gram negative	6 (6)	0.40
Mixed flora	15 (10)	0.90
Identification unavailable	6 (6)	0.40
Total	1,622 (967)	

[a] Numbers in parentheses are the numbers of patients with the indicated organism.

inspection and first-day blind subculturing of the blood culture vials. Of the 37 cultures with a delayed positive result, 30 were first detected by blind subculturing and 7 were detected by visual inspection of the vials (Table 2). Only 1 of the 37 (0.07%) subcultured organisms, a coagulase-negative staphylococcus, was not detected by the NR-660 system by the end of the 7-day protocol.

The 37 blood culture isolates with delayed positive results were from 29 patients (Table 2). Of these 29 patients, 16 had blood cultures with coagulase-negative staphylococci, *Micrococcus* sp., or corynebacteria. Of these 16 patients, 4 had clinically significant infections, as indicated by the infectious disease service; blood cultures from 7 patients either were known to contain skin contaminants, as indicated by the infectious disease service, or were presumed to contain skin contaminants because isolates were obtained from only a single blood culture within 1 week of detection of a delayed positive isolate in the patients; 5 patients had isolates of unknown clinical significance. During the study period, 291 isolates of coagulase-negative staphylococci from blood were detected by the NR-660 system in less than or equal to the amount of time it took to detect these isolates by subculturing. An estimate of the average time delay was calculated for the delayed isolates of coagulase-negative staphylococci at 1.1 days (range, 1 to 2 days; $n = 9$). Seven of the 29 patients with delayed positive cultures had one or

TABLE 2. Delayed detection of isolates in positive cultures by the NR-660 system

Isolate	No. of cultures	No. of patients	Mean delay time (days)
Coagulase-negative staphylococci	15	12	1.1
Staphylococcus aureus	4	3	1
Micrococcus sp.	2	2	ND[a]
Alpha-hemolytic streptococci	3	2	ND
Streptococcus pneumoniae	1	1	ND[b]
Corynebacterium sp.	3	2	2.5
Pseudomonas aeruginosa	2	2	ND
Pseudomonas maltophilia	1	1	ND
Klebsiella pneumoniae	1	1	6
Candida albicans	3	2	1
Cryptococcus neoformans	2	1	6

[a] ND, Not done.
[b] See text for explanation of the mean delay time for this isolate.

more positive blood cultures detected by the NR-660 system on the same day with the same organism. Three other patients had previous positive cultures with the same organism that now gave a delayed positive culture.

The longest delays were recorded for one isolate of *Klebsiella pneumoniae* and one isolate of *Cryptococcus neoformans*, which were detected by first-day blind subculturing after 48 h of incubation but were not detected by the NR-660 system until day 7. One isolate of *Streptococcus pneumoniae* was erroneously placed on the delayed positive list by the blood culture technologist. This isolate was actually detected by the NR-660 system, but it did not grow when it was subcultured. The bacteria in the blood culture were confirmed by Gram staining.

DISCUSSION

Our data demonstrate that 97% of positive blood cultures were detected by the NR-660 system without subculturing in less than or equal to the amount of time it took to use the NR-660 system with subculturing. The organisms that gave delayed positive cultures tended to be common blood culture contaminants or organisms that produce indolent infections. The isolation of *Pseudomonas* spp. (two of *Pseudomonas aeruginosa* and one of *Pseudomonas maltophilia*) was delayed by use of the NR-660 system without subculturing for three patients. One might predict delayed pseudomonal growth in the anaerobic bottles because of their nonfermentative metabolism, and in this study detection of *P. aeruginosa* was delayed when cultures were grown in anaerobic bottles. One of the *P. aeruginosa* isolates did not become positive in the anaerobic bottle for 72 h, but the *P. aeruginosa* isolate in the aerobic bottle was positive after 24 h. The *P. maltophilia* isolate with delayed growth was grown in the aerobic bottle. A second set of blood cultures from the patient from which the *P. maltophilia* isolate with delayed growth was obtained was taken on the same day. The same organism grew in these cultures, and no delay in detection was demonstrated by the NR-660 system. In a detailed study in which conventional biphasic blood culture bottles were compared with the NR-660 system, it was shown that the latter detected *P. aeruginosa* more rapidly (3). Although the delayed detection of positive blood cultures can have serious clinical ramifications, factors such as the volume of blood inoculated into the bottle and the use of broad-spectrum antibiotics may be more important in causing delays in detection than the use of a conventional or an automated blood culture detection system (11).

This study was done during the months of June through February, covering the seasons in which community-acquired diseases caused by pneumococci and meningococci occur most frequently. Our data for pneumococci agree with those of Courcol et al. (3), who have reported that the NR-660 system is superior to the conventional system (3). During the study period, we had 86 blood cultures with pneumococcus, all of which were detected by the NR-660 system in the same or less time than it took to subculture within the first 24 h of incubation. In our laboratory the detection of *Streptococcus pneumoniae* in blood cultures has been improved by the NR-660 system, because in strains that are susceptible to autolysis, the NR-660 system can detect organisms prior to cell death. *Haemophilus influenzae* in eight blood cultures was recognized by the NR-660 system without any need for subculturing, but the possibility of missing this organism in routine blood culture vials has been recognized (4, 11). If a laboratory has a patient population which is highly susceptible to *H. influenzae*, it would be prudent to use subculturing or hypertonic medium. No conclusions can be drawn about *Neisseria meningitidis* because of the small numbers of organisms seen in most studies. It is known that sodium polyanetholsulfonate, a component of the BACTEC blood culture bottles, inhibits the growth of meningococci (8), but the need for subculturing to detect this organism rapidly is a factor that needs to be addressed.

Early reports on the routine use of the BACTEC 460 system demonstrated an 80% sensitivity compared with that of blind subculturing within the first day of incubation, but in a later report by Strand (9) it was concluded that first-day subculturing does not increase the number of significant positive blood cultures that are detected compared with the number detected by the BACTEC 460 system (1). Our data demonstrate that the NR-660 system enables clinical laboratories to report positive blood cultures in a time period which is equal to or shorter than that gained from subculturing within the first 24 h. Courcol et al. (3) have shown that the NR-660 system detects growth in blood cultures in about 32 h, compared with 56 h for a conventional blood culture system. This difference was statistically significant. Even if we assume that the 37 delayed positive cultures in our study would not have been detected by the NR-660 system, the sensitivity of the NR-660 system was 97%. False-positive results still occur with the NR-660 system, but they are easily eliminated by Gram staining.

The use of the NR-660 system without routine subculturing produced a financial savings in our laboratory. Based on the College of American Pathology time allotment for subculturing of BACTEC vials (1.5 min per bottle) (2), a personnel cost of $16/h, and the subculturing of 4,000 blood vials per month, our laboratory savings were on the order of $20,000 per year and 1,200 work-hours per year. The savings in materials for subculturing were $4,000 per year. Although we realize that cost savings in technologist time do not always convert to laboratory savings, we believe that the use of the NR-660 system without subculturing does produce an increase in productivity.

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