JOURNAL OF CLINICAL MICROBIOLOGY, May 1997, p. 1290–1292 Vol. 35, No. 5
0095-1137/97/$04.00+0
Copyright © 1997, American Society for Microbiology

Four-Day Incubation Period for Blood Culture Bottles Processed with the Difco ESP Blood Culture System

GARY V. DOERN,[1]* ANGELA B. BRUEGGEMANN,[1] W. MICHAEL DUNNE,[2] STEPHEN G. JENKINS,[3] DIANE C. HALSTEAD,[4] AND JAMES C. MCLAUGHLIN[5]

University of Massachusetts Medical Center, Worcester, Massachusetts 01655;[1] Henry Ford Hospital, Detroit, Michigan 48202;[2] University of Florida Health Science Center, Jacksonville, Florida 32209;[3] Baptist Medical Center/Wolfson Children's Hospital, Jacksonville, Florida 32207;[4] and University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87106[5]

Received 18 November 1996/Returned for modification 13 January 1997/Accepted 3 February 1997

Blood culture records from 1994 to 1995 from five U.S. medical centers all using the Difco ESP continuous monitoring blood culture system were reviewed retrospectively. Among a total of 7,362 isolates of bacteria and yeasts, only 0.1% of possibly significant isolates would have been missed had blood cultures been routinely incubated for 4 days instead of the 5 days recommended by the manufacturer. Conversely, numerous contaminants, detected only on day 5, would have been eliminated by a 4-day incubation period.

Three different continuous monitoring blood culture systems have achieved widespread application in the United States: the BAC TEC 9240 system (Becton Dickinson Microbiology Systems, Cockeysville, Md.), the ESP system (Difco Laboratories, Detroit, Mich.), and the BacT/Alert system (Organon Teknika, Durham, N.C.) (3–9, 11). Each manufacturer recommends a 5-day incubation period for blood culture bottles before they are discarded as negative, and there are studies to support this length of incubation for the BacT/Alert instrument (1, 10). To our knowledge, however, there are no published data specifically addressing the optimum duration of incubation of blood culture bottles with the Difco ESP system. The goal of this study was to systematically examine this issue.

Archived blood culture records covering 7- to 12-month periods between 1994 and 1995 from five medical centers were examined (Table 1). Three hospitals were in academic medical centers. All five hospitals provided tertiary care in a referral setting but also offered many ambulatory-care services. Primary and tertiary care in pediatrics were provided by all five participating hospitals, as were transplantation and trauma services and care to large human immunodeficiency virus-infected patient populations. Each of the centers used the Difco ESP system as the primary method of blood culture analysis. When sufficient blood was available for culture, up to 20 ml was routinely collected and distributed equally into one aerobic (40A or 80A medium) and one anaerobic (40N or 80N medium) blood culture bottle. In circumstances where ≤10 ml of blood could be obtained, the aerobic bottle was usually the only bottle inoculated. All blood cultures were processed precisely according to the manufacturer's instructions and incubated for 5 days before being reported as negative.

For the purpose of data analysis, non-*Staphylococcus aureus* staphylococci, *Corynebacterium* spp. other than *Corynebacterium jeikeium*, *Micrococcus* spp., *Bacillus* spp., and *Propionibacterium* spp. were considered probable contaminants under the following circumstances: (i) when the isolate was recovered from only one of two blood culture bottles of a set when only a single set was drawn or (ii) when the isolate was recovered from both bottles of a blood culture set when additional blood culture or intravascular catheter tip cultures performed on the same patient within a 24 h period of the index-positive culture were negative. All other blood culture isolates were considered likely to have clinical significance.

A summary of the length of time required to detect individual blood culture isolates at all study sites is shown in Table 2. The observations of the individual laboratories were comparable to the combined results. A total of 7,362 blood culture isolates were recovered. As can be seen in Table 2, 63.2% of all isolates were detected during the first 24 h of incubation, while an additional 25.4% of blood culture isolates were recognized on day 2 and 6.5% were recognized on day 3. Only 2.7 and 2.2% of isolates were recovered on days 4 and 5, respectively. Among individual organism groups, 96.1% of all enterococci, 96% of all members of the family *Enterobacteriaceae*, 96.4% of all *Pseudomonas aeruginosa* strains, 89.4% of all *S. aureus* strains, and 100% of all pneumococci and group A and B beta-hemolytic streptococci were detected within 48 h of incubation. Ninety-seven percent of the latter three categories of organisms were detected within the first 24 h of incubation.

Among the 164 isolates recovered on day 5, all but 8 isolates were either classified as probable contaminants (n = 120;

TABLE 1. Participating centers and study characteristics

Medical center	Study period[a]	No. of blood cultures performed
Baptist Medical Center, Jacksonville, Fla.	26 Jan. 1995 to 31 Aug. 1995	ca. 8,000
University of Florida Health Science Center, Jacksonville, Fla.	24 Jan. 1995 to 12 Aug. 1995	5,760
University of New Mexico Medical Center, Albuquerque, N. Mex.	1 Jan. 1995 to 31 Oct. 1995	23,167
Henry Ford Hospital, Detroit, Mich.	1 Nov. 1994 to 1 Oct. 1995	ca. 40,000
University of Massachusetts Medical Center, Worcester, Mass.	1 July 1994 to 30 June 1995	14,337

[a] Jan., January; Aug., August; Oct., October; Nov., November.

* Corresponding author. Mailing address: Clinical Microbiology Laboratories, University of Massachusetts Medical Center, 55 Lake Ave. North, Worcester, MA 01655. Phone: (508) 856-6417. Fax: (508) 856-1537.

Downloaded from jcm.asm.org at UNIV OF MASS MED SCH on July 15, 2008

TABLE 2. Recovery of blood culture isolates by day of incubation

Organism	No. (%) of isolates recovered on day:				
	1	2	3	4	5
Staphylococcus aureus	666 (63.4)	273 (26.0)	63 (6.0)	31 (3.0)	16 (1.5)
Non-*Staphylococcus aureus* staphylococci	1,467 (51.3)	978 (34.2)	258 (9.0)	94 (3.3)	61 (2.1)
Micrococcus spp.	6 (20.7)	19 (65.5)	4 (13.8)	0	0
Streptococcus pneumoniae	298 (97.1)	9 (2.9)	0	0	0
Group A streptococci	53 (94.6)	3 (5.4)	0	0	0
Group B streptococci	75 (98.7)	1 (1.3)	0	0	0
Group C, G, or F streptococci	21 (87.5)	3 (12.5)	0	0	0
Enterococcus spp.	331 (80.1)	66 (16.0)	10 (2.4)	5 (1.2)	1 (0.2)
Viridans group streptococci	312 (74.8)	79 (18.9)	17 (4.1)	8 (1.9)	1 (0.2)
Streptococcus bovis	15 (100)	0	0	0	0
Nutritionally variant streptococci	1 (33.3)	1 (33.3)	1 (33.3)	0	0
Moraxella catarrhalis	2 (100)	0	0	0	0
Moraxella spp.	1 (50)	1 (50)	0	0	0
Neisseria meningitidis	5 (71.4)	2 (28.6)	0	0	0
Neisseria spp.	4 (57.1)	3 (42.9)	0	0	0
Listeria monocytogenes	8 (100)	0	0	0	0
Corynebacterium spp.	30 (15.5)	89 (46.1)	38 (20.0)	16 (8.3)	20 (10.4)
Lactobacillus spp.	2 (14.3)	5 (35.7)	3 (21.4)	3 (21.4)	1 (7.1)
Bacillus spp.	94 (70.7)	24 (18.0)	12 (9.0)	1 (0.8)	2 (1.5)
Escherichia coli	504 (87.0)	66 (11.4)	3 (0.5)	3 (0.5)	3 (0.5)
Klebsiella pneumoniae	170 (85.4)	17 (8.5)	7 (3.5)	1 (0.5)	4 (2.0)
Klebsiella spp.	23 (79.3)	4 (13.8)	0	1 (3.4)	1 (3.4)
Enterobacter cloacae	116 (84.7)	13 (9.5)	2 (1.5)	4 (2.9)	2 (1.5)
Enterobacter spp.	32 (76.2)	5 (11.9)	3 (7.1)	1 (2.4)	1 (2.4)
Citrobacter freundii	14 (93.3)	1 (6.7)	0	0	0
Citrobacter diversus	7 (100)	0	0	0	0
Serratia marcescens	38 (67.9)	14 (25.0)	0	2 (3.6)	2 (3.6)
Proteus mirabilis	46 (90.2)	3 (5.9)	1 (2.0)	1 (2.0)	0
Proteus vulgaris	0	0	0	0	0
Providencia spp.	3 (100)	0	0	0	0
Morganella morganii	8 (66.7)	0	3 (25.0)	1 (8.3)	0
Hafnia alvei	1 (25.0)	3 (75.0)	0	0	0
Salmonella spp.	9 (100)	0	0	0	0
Other enteric gram-negative bacilli	4 (80.0)	1 (20.0)	0	0	0
Pseudomonas aeruginosa	150 (77.3)	37 (19.1)	2 (1.0)	5 (2.6)	0
Acinetobacter spp.	61 (89.7)	4 (5.9)	0	3 (4.4)	0
Stenotrophomonas maltophilia	28 (82.4)	4 (11.8)	2 (5.9)	0	0
Alcaligenes spp.	9 (64.3)	3 (21.4)	2 (14.3)	0	0
Campylobacter spp.	0	4 (100)	0	0	0
Haemophilus influenzae	6 (75.0)	2 (25.0)	0	0	0
Miscellaneous gram-negative bacilli	32 (72.7)	7 (15.9)	4 (9.1)	1 (2.3)	0
Peptostreptococcus spp.	5 (25.0)	8 (40.0)	4 (20.0)	0	3 (15.0)
Clostridium perfringens	20 (90.9)	1 (4.5)	0	1 (4.5)	0
Clostridium spp.	16 (66.7)	5 (20.8)	1 (4.2)	1 (4.2)	1 (4.2)
Propionibacterium spp.	1 (2.1)	4 (8.3)	2 (4.2)	0	41 (85.4)
Actinomyces spp.	0	4 (57.1)	3 (42.9)	0	0
Other anaerobic NSF gram-positive bacilli[a]	0	1 (25.0)	0	3 (75.0)	0
Bacteroides fragilis group	17 (28.8)	30 (50.8)	9 (15.3)	3 (5.1)	0
Other *Bacteroides* spp.	3 (37.5)	3 (37.5)	2 (25.0)	0	0
Prevotella spp.	4 (44.4)	2 (22.2)	3 (33.3)	0	0
Fusobacterium spp.	6 (31.6)	4 (21.1)	4 (21.1)	4 (21.1)	1 (5.3)
Candida albicans	15 (15.6)	53 (55.2)	19 (19.8)	9 (9.4)	0
Non-*Candida albicans* candidas	12 (18.8)	38 (59.4)	6 (9.4)	0	8 (12.5)
Torulopsis glabrata	6 (27.3)	13 (59.1)	2 (9.1)	1 (4.5)	0
Total	4,757 (63.2)	1,910 (25.4)	490 (6.5)	204 (2.7)	169 (2.2)

[a] NSF, non-spore-forming.

73.2%) or had been recovered within 4 days of incubation from another blood culture taken from the same patient ($n = 36$; 22.0%). The eight exceptions included four isolates of *Klebsiella pneumoniae*, two isolates of *S. aureus*, and one isolate each of *Serratia marcescens* and *Enterobacter aerogenes*. Therefore, only 8 presumed clinically significant blood culture isolates from a total of 7,362 isolates (0.1%) were detected exclusively on the fifth day of incubation.

The present study was marked by two shortcomings. First, the definitions used to classify blood culture isolates as contaminants did not take into account clinical information and, as a result, were not strictly inclusive. The criteria employed were chosen so as to permit consistent classification of contaminants among all five participating laboratories. Undoubtedly, certain contaminated blood cultures were misclassified as possibly significant. Such errors, however, would not have altered our

Downloaded from jcm.asm.org at UNIV OF MASS MED SCH on July 15, 2008

findings since only 8 possibly significant isolates were recovered exclusively on day 5. Second, the incubation period in all laboratories was terminated after day 5 with no blind subcultures performed on instrument-negative cultures. The question of what would have been detected either by prolonging the incubation period beyond day 5 or by performing blind subcultures can be asked, but obviously it cannot be answered with certainty. In general, blind subcultures of negative bottles from instrument-based blood culture systems have been shown to be unnecessary (1, 2). Furthermore, examination of rates of detection by day of incubation in the current study suggested that there would have been little if any additional yield beyond the 5-day incubation period. Recovery rates fell off conspicuously after day 2 of incubation, 72 h prior to the end of the 5-day incubation period.

Based on the results of this survey, we conclude that shortening the length of incubation of aerobic and anaerobic blood culture bottles inoculated with ca. 10 ml of blood and processed with the Difco ESP blood culture system from 5 to 4 days would not significantly diminish the utility of this system as a means of detecting significant bacteremia and candidemia. A favorable ramification of such a change would be the reduction of the number of contaminants recovered. The only organism recovered frequently as a significant blood culture isolate for which a 4-day incubation cycle with the ESP blood culture system might be problematic was *K. pneumoniae*. In circumstances where blood cultures are performed on patients likely to have *K. pneumoniae* bacteremia, e.g., intensive care unit outbreak situations and cases associated with isolation of this organism from other body sites, it may be prudent to incubate ESP blood cultures for at least 5 days. It should be emphasized that these observations are applicable only to the Difco ESP blood culture system. Surveys like this one must be conducted with other continuous-monitoring blood culture methods before similar recommendations can be made for these systems.

We are indebted to the laboratory staffs of their respective laboratories for excellent technical assistance. We also thank Deborah McQuaid for secretarial support.

This survey was supported by a grant from Difco Laboratories.

REFERENCES

1. **Hardy, D. J., B. B. Hulbert, and P. C. Migneault.** 1992. Time to detection of positive BacT/Alert blood cultures and lack of need for routine subculture of 5- to 7-day negative cultures. J. Clin. Microbiol. **30:**2743–2745.
2. **Harrell, L. J., S. Mirrett, and L. B. Reller.** 1994. Subcultures of BACTEC-positive but Gram or acridine orange stain-negative NR 6A and 7A blood culture bottles are unnecessary. Diagn. Microbiol. Infect. Dis. **20:**121–125.
3. **Morello, J. A., C. Leitch, S. Nitz, J. W. Dyke, M. Andruszewski, G. Maier, W. Landau, and M. A. Beard.** 1994. Detection of bacteremia by Difco ESP blood culture system. J. Clin. Microbiol. **32:**811–818.
4. **Nolte, F. S., J. M. Williams, R. C. Jerris, J. A. Morello, C. D. Leitch, S. Matushek, L. D. Schwabe, F. Dorigan, and F. E. Kocka.** 1993. Multicenter clinical evaluation of a continuous monitoring blood culture system using fluorescent-sensor technology (BACTEC 9240). J. Clin. Microbiol. **31:**552–557.
5. **Pohlman, J. K., B. A. Kirkley, K. A. Easley, B. A. Basille, and J. A. Washington.** 1995. Controlled clinical evaluation of BACTEC Plus Aerobic/F and BacT/Alert aerobic FAN bottles for detection of blood stream infections. J. Clin. Microbiol. **33:**2856–2858.
6. **Schwabe, L. D., R. B. Thomson, Jr., K. K. Flint, and F. P. Koontz.** 1995. Evaluation of BACTEC 9240 blood culture system by using high-volume aerobic resin media. J. Clin. Microbiol. **33:**2451–2453.
7. **Smith, J. A., E. A. Bryce, J. H. Ngui-Yen, and F. J. Roberts.** 1995. Comparison of BACTEC 9240 and BacT/Alert blood culture systems in an adult hospital. J. Clin. Microbiol. **33:**1905–1908.
8. **Thorpe, T. C., M. L. Wilson, J. E. Turner, J. L. DiGuiseppi, M. Willert, S. Mirrett, and L. B. Reller.** 1990. BacT/Alert: an automated colorimetric microbial detection system. J. Clin. Microbiol. **28:**1608–1612.
9. **Wilson, M. L., M. P. Weinstein, S. Mirrett, L. G. Reimer, R. J. Feldman, C. R. Chuard, and L. B. Reller.** 1995. Controlled evaluation of BacT/Alert standard anaerobic and FAN anaerobic blood culture bottles for the detection of bacteremia and fungemia. J. Clin. Microbiol. **33:**2265–2270.
10. **Wilson, M. L., S. Mirrett, L. B. Reller, M. P. Weinstein, and L. G. Reimer.** 1993. Recovery of clinically important microorganisms from the BacT/Alert blood culture system does not require testing for seven days. Diagn. Microbiol. Infect. Dis. **16:**31–34.
11. **Zwadyk, P., Jr., C. L. Pierson, and C. Young.** 1994. Comparison of Difco ESP and Organon Teknika BacT/Alert continuous-monitoring blood culture systems. J. Clin. Microbiol. **32:**1273–1279.

Downloaded from jcm.asm.org at UNIV OF MASS MED SCH on July 15, 2008