JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 1985, p. 481–485
0095-1137/85/040481-05$02.00/0

Vol. 21, No. 4

Determination of the Optimum Incubation Period of Blood Culture Broths for the Detection of Clinically Significant Septicemia

PATRICK R. MURRAY

Clinical Microbiology Laboratory, Barnes Hospital and Washington University School of Medicine, St. Louis, Missouri 63110

Received 2 November 1984/Accepted 13 December 1984

The value of incubating blood culture broths for more than 7 days was analyzed. A total of 20,155 blood cultures, consisting of a vented Roche tryptic soy broth (R-TSB) bottle and an unvented Difco thiol broth bottle, was processed; 2,509 organisms were recovered in the R-TSB bottles, and 1,865 organisms in the thiol bottles. Only 32 organisms isolated in the R-TSB bottles and 10 organisms isolated in the thiol bottles were detected after incubation for more than 7 days; 15 of the 32 isolates in the R-TSB bottles and all 10 of the isolates in the thiol bottles were either recovered in other blood cultures or were not considered to be clinically significant. Thus, incubation of the R-TSB bottles and unvented thiol bottles for more than 7 days is unnecessary for the detection of most clinically significant septicemias.

Numerous investigators have carefully analyzed the methods used for culturing blood, including the selection of culture media and the techniques used for detecting microbial growth (e.g., radiometry, microscopy, blind subcultures, biphasic systems, and lysis-centrifugation). However, one aspect of blood culturing is unresolved, namely, the value of incubating specimens for more than 1 week. Effersoe (4) reported in 1965 that he did not recover any organisms with incubation of the blood cultures for more than 7 days. In 1968 Ellner (5) analyzed approximately 40,000 cultures collected during the preceding 10 years and failed to find any cultures that were detected as positive after 5 days of incubation despite the fact that all cultures were incubated for a minimum of 14 days. Similar data were reported by Rosner in 1976 (15). However, other studies have demonstrated that prolonged incubation may be required to detect a small number of significant bacteremias. In 1973 Bartlett (1) reported that 5% of the clinically significant bacteremias in Hartford Hospital were detected after 7 days of incubation. Hall and co-workers (7) analyzed more than 8,600 blood cultures in 1974 and found that a small, but significant, number of isolates were detected between 7 and 14 days of incubation, including isolates of *Staphylococcus aureus*, *Bacteroidaceae*, *Streptococcus* spp., and *Enterobacteriaceae*. Weinstein reported that 5% of isolates were detected after 14 days of incubation (19). Other investigators have documented that prolonged incubation may be required for the detection of fastidious organisms such as *Actinobacillus* spp., *Cardiobacterium* spp., *Eikenella* spp., and *Haemophilus* spp., which can cause subacute bacterial endocarditis (6, 7, 17, 18). In addition, it is not known whether prolonged incubation is required for blood cultures processed with two newly introduced systems, the Septi-Chek biphasic slide system (Roche Diagnostics, Nutley, N.J.) and the Isolator lysis-centrifugation system (E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). Although the detection of significant isolates is more rapid with these systems in comparison with conventional systems (2, 8, 12), overall detection of isolates including fastidious organisms is also improved (2, 8, 9, 12, 16). Prolonged incubation may therefore be necessary for detecting previously unrecognized fastidious bacteria and fungi.

In the study reported herein, organisms isolated in blood cultures collected during a 12-month period were analyzed to determine (i) the frequency of detecting organisms after 7 days of incubation, (ii) the clinical significance of the slow-growing organisms, and (iii) the frequency of detecting slow-growing organisms in other clinical specimens. For this study the Roche Septi-Chek system and a conventional unvented bottle were used.

MATERIALS AND METHODS

A total of 20,155 blood cultures were processed in the Barnes Hospital Microbiology Laboratory during 1983. Each blood culture consisted of two bottles, one Roche bottle containing 70 ml of tryptic soy broth (R-TSB) with 0.05% sodium polyanethol-sulfonate and one bottle (Difco Laboratories, Detroit, Mich.) containing 100 ml of thiol broth with 0.025% sodium polyanetholesulfonate. Approximately 20 ml of blood was collected aseptically by venipuncture from patients with suspected bacteremia or fungemia and was divided equally into the two bottles. Upon receipt of the bottles in the laboratory, the Roche agar-coated slide Septi-Chek system was attached to the R-TSB bottle, and the thiol bottle was not vented to promote the growth of anaerobes. Both bottles were incubated at 35°C for a total of 14 days. The bottles were examined twice during the first 24 h, daily for the next 6 days, and one more time on day 14. In addition, blood cultures received for the recovery of fungi were incubated for a total of 4 weeks and were examined as described above and after 3 and 4 weeks of incubation. The R-TSB bottles were routinely subcultured after each visual inspection onto the agar slide system as described previously (12). The unvented thiol bottles were not blindly subcultured (11, 13). A record of which bottle was positive and the initial time of detection was maintained for each positive culture.

TABLE 1. Time required for detection of organisms in R-TSB and thiol broth

Organism	R-TSB				Thiol broth			
	No. of isolates	No. (%) detected on days:			No. of isolates	No. (%) detected on days:		
		1 to 5	6 to 7	8 to 14		1 to 5	6 to 7	8 to 14
Staphylococcus aureus	201	198 (99)	3 (1)		178	171 (96)	6 (3)	1 (1)
Staphylococcus epidermidis	624	566 (91)	40 (6)	18 (3)	439	408 (93)	27 (6)	4 (1)
Streptococcus spp.								
Group D enterococci	80	78 (98)	2 (2)		82	81 (99)	1 (1)	
S. pneumoniae	54	54 (100)			45	45 (100)		
Other species	126	125 (99)	1 (1)		120	118 (98)	2 (2)	
Corynebacterium spp.	59	36 (61)	14 (24)	9 (15)	24	14 (58)	3 (13)	7 (29)
Bacillus spp.	31	25 (81)	5 (16)	1 (3)	18	17 (94)	1 (6)	
Listeria spp.	8	8 (100)			10	10 (100)		
Enterobacteriaceae								
Escherichia spp.	286	282 (99)	4 (1)		266	260 (98)	6 (2)	
Klebsiella spp.	236	228 (97)	5 (2)	3 (1)	220	218 (98)	1 (1)	1 (1)
Enterobacter spp.	83	82 (99)		1 (1)	84	83 (99)	1 (1)	
Serratia spp.	47	46 (98)	1 (2)		38	38 (100)		
Proteus spp.	32	30 (94)	2 (6)		32	32 (100)		
Citrobacter spp.	22	22 (100)			20	20 (100)		
Salmonella spp.	7	7 (100)			7	7 (100)		
Morganella spp.	7	5 (71)	2 (29)		5	4 (80)	1 (20)	
Providencia spp.	6	6 (100)			6	6 (100)		
Pseudomonas aeruginosa	134	132 (98)	1 (1)	1 (1)	54	53 (98)	1 (2)	
Other *Pseudomonas* spp.	25	16 (64)	6 (24)	3 (12)	11	9 (82)	1 (6)	1 (6)
Acinetobacter spp.	51	50 (98)	1 (2)		15	15 (100)		
Aeromonas spp.	11	11 (100)			12	12 (100)		
Haemophilus spp.	8	6 (75)	1 (12)	1 (12)	3	2 (67)		1 (33)
Flavobacterium spp.	6	6 (100)						
Neisseria spp.	5	4 (80)	1 (20)		5	4 (80)	1 (20)	
Alcaligenes spp.	3	3 (100)			3	3 (100)		
Erysipelothrix spp.	2	2 (100)			6	6 (100)		
Kluyrera spp.	2	2 (100)			2	2 (100)		
Moraxella spp.	2	2 (100)						
Actinobacillus spp.	2			2 (100)				
Francisella spp.	1			2 (100)				
Anaerobes								
Bacteroides spp.	23	22 (96)		1 (4)	44	41 (93)	2 (5)	1 (2)
Clostridium spp.	26	26 (100)			38	36 (95)	2 (5)	
Propionibacterium spp.	87	4 (5)	13 (15)	70 (80)	33	1 (3)	7 (21)	25 (76)
Peptococcus spp.	7	6 (86)	1 (14)		14	12 (86)	1 (7)	1 (7)
Peptostreptococcus spp.	6	4 (67)		2 (33)	5	5 (100)		
Veillonella spp.	4	4 (100)			6	5 (83)		1 (17)
Actinomyces spp.	4	2 (50)	2 (50)		1			1 (100)
Eubacterium spp.	3	3 (100)			3	3 (100)		
Lactobacillus spp.	2	2 (100)						
Bifidobacterium spp.	1	1 (100)			1	1 (100)		
Fusobacterium spp.					4	2 (50)	1 (25)	1 (25)
Fungi								
Candida spp.	135	118 (87)	13 (10)	4 (3)	26	24 (92)	1 (4)	1 (4)
Torulopsis spp.	29	27 (93)	2 (7)		6	5 (83)	1 (17)	
Histoplasma spp.	11			11 (100)				
Cryptococcus spp.	6	2 (33)	3 (50)	1 (17)				
Other	4	2 (50)	2 (50)		3		1 (33)	2 (67)

RESULTS

A total of 2,509 organisms were recovered in the vented R-TSB bottles, and 1,865 organisms were recovered in the unvented thiol bottles. The times the organisms were detected initially in the R-TSB and thiol bottles are summarized in Table 1. Greater than 90% of the organisms in most major groups of bacteria and fungi were detected within the first 7 days of incubation. Of the genera with at least 10 isolates, the only groups with fewer than 90% of the organisms detected by day 7 were *Corynebacterium* spp., *Propionibacterium* spp., *Pseudomonas* spp. other than *Pseudomonas aeruginosa*, and *Histoplasma* spp. in the R-TSB bottles and *Corynebacterium* spp. and *Propionibacterium* spp. in the thiol bottles.

If organisms commonly considered to be contaminants (e.g., *Staphylococcus epidermidis*, *Corynebacterium* spp., *Propionibacterium* spp., and *Bacillus* spp.) were disregarded, then 98.1 and 99.3% of the isolates in the R-TSB and thiol bottles, respectively, were detected within 7 days (Fig.

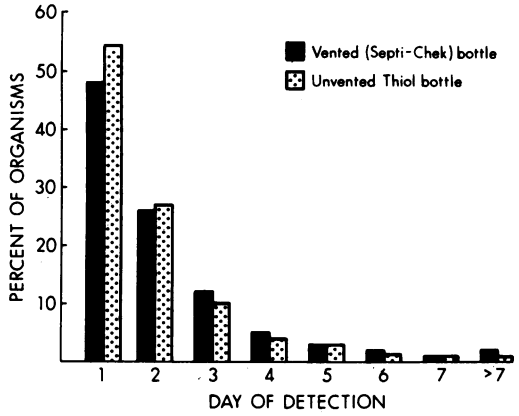

FIG. 1. Time of detection of blood culture isolates in Septi-Chek and thiol broths.

TABLE 3. Recovery of organisms in thiol broth after incubation for more than 7 days

Organism detected after 7 days	Detection of organism in less than 7 days in:	
	Companion bottle	Other blood cultures
Klebsiella pneumoniae	Yes	No
Haemophilus influenzae	Yes	Yes
Staphylococcus aureus	No	Yes
Bacteroides melaninogenicus	No	Yes
Bacteroides distasonis	No	Yes
Fusobacterium nucleatum	No	No
Peptococcus sp.	No	No
Veillonella sp.	No	Yes
Actinomyces sp.	No	Yes
Candida tropicalis	Yes	Yes

1). Only 32 (1.9%) organisms in the R-TSB bottles and 10 (0.7%) organisms in the thiol bottles were detected after incubation for more than 7 days. The 32 organisms recovered in the R-TSB bottles during the second week of incubation are listed in Table 2. Two of the 32 organisms were detected in the companion (thiol) blood culture bottle during the first 7 days of incubation. In addition, other positive blood cultures (collected within 2 days of the positive delayed culture) were associated with nine isolates, including one organism with a positive companion bottle. Therefore, 10 (31%) of the 32 organisms detected in R-TSB bottles during the second week of incubation were detected during the first week in the companion bottle or in another blood culture. Similar data for organisms detected during the second week of incubation in the thiol bottles are summarized in Table 3. Eight of the ten organisms were detected in either the companion bottle or another blood culture.

Five of the 22 organisms detected only during the second week of incubation in the R-TSB bottles appeared to be clinically insignificant. *Pseudomonas mesophilica* was isolated in only one of multiple blood cultures collected from a man with documented *Escherichia coli* bacteremia and was not present in cultures of sputum or urine. *Pseudomonas vesicularis* was isolated in one of seven blood cultures collected over a 3-day period from a patient with Raynaud's phenomenon. The patient's polyarthralgias and vasculitis were not considered to be due to infection, and he was not treated with antibiotics during the hospitalization. *Pseudomonas paucimobilis* was isolated in a single blood culture collected from a patient with Graves' disease who was hospitalized for a subtotal thyroidectomy. The organism was not present in other cultures, and the patient was not treated with antibiotics. *Fusobacterium nucleatum*, together with *S. epidermidis*, *Corynebacterium*, and alpha-hemolytic streptococci, were isolated in a single culture from a patient with no evidence of infection. These organisms were thought to be contaminants introduced into the culture either at the time of collection or during processing in the laboratory. Last, an isolate of *Bacteroides melaninogenicus* was recovered from a diabetic patient who was hospitalized for control of a hypoglycemic episode. There was no identifiable source for this organism, and the patient was not treated with antibiotics during the hospitalization.

TABLE 2. Recovery of organisms in R-TSB after incubation for more than 7 days

Organism detected after 7 days (no. of isolates)	Detection or organism in less than 7 days in:	
	Companion bottle	Other blood cultures
Klebsiella pneumoniae	Yes	Yes
Klebsiella pneumoniae (2)	No	Yes
Klebsiella pneumoniae	No	No
Pseudomonas aeruginosa	No	Yes
Pseudomonas mesophilica	No	No
Pseudomonas vesicularis	No	No
Pseudomonas paucimobilis	No	No
Francisella tularensis	No	No
Haemophilus influenzae	No	No
Actinobacillus sp. (2)	No	No
Bacteroides melaninogenicus	No	No
Fusobacterium nucleatum	No	No
Peptostreptococcus sp.	Yes	No
Peptostreptococcus sp.	No	Yes
Candida albicans	No	Yes
Candida parapsilosis	No	No
Candida parapsilosis (2)	No	Yes
Cryptococcus neoformans	No	Yes
Histoplasma capsulatum (11)	No	No

The remaining 17 organisms detected in the R-TSB bottles were from six patients and were clinically significant. *Klebsiella pneumoniae* was recovered in one of two blood cultures collected from a diabetic patient who was admitted for drainage of a perirectal abscess. *K. pneumoniae*, together with *Proteus mirabilis* and a nonhemolytic *Streptococcus* sp., was cultured from the abscess material. The patient was receiving effective antibiotics (ampicillin, tobramycin, and metronidazole) at the time the blood was cultured, which probably was responsible for the slow growth of the isolate in the blood culture. Recovery of the organism from blood did not affect management of the patient.

Francisella tularensis was recovered in one of two blood cultures collected from a patient who was gravely ill at the time of transfer from a rural hospital. Sepsis due to *Neisseria meningitidis*, *Streptococcus pneumoniae*, or Rocky Mountain spotted fever was considered, and the patient was started on antimicrobial therapy after the blood was collected for culture. No record of therapy before admission was available from the referring hospital. Despite medical therapy the patient continued to deteriorate and died 2 days after admission and before the positive culture was detected. Fluorescent microscopic examination of postmortem lung

tissue was positive for *F. tularensis*. However, this test was performed because the blood culture was positive. It was likely that the diagnosis would not have been made without the positive blood culture.

Haemophilus influenzae was recovered in one blood culture collected from a patient with hypogammaglobulinemia. The patient had an inflamed, swollen right knee, from which *H. influenzae* was cultured. It is not known whether the delayed detection of the isolate in blood was due to fastidious growth requirements of the organism or partial inhibition of growth by previous treatment with ampicillin. Recovery of the isolate in blood did not influence the course of antimicrobial therapy.

Actinobacillus actinomycetemcomitans was recovered in two blood cultures collected from an elderly patient who was subsequently diagnosed to have subacute bacterial endocarditis. This patient had not received antibiotics before the cultures were collected, and endocarditis was not suspected until after the positive cultures were reported.

Candida parapsilosis was isolated in two blood cultures from a patient who had a craniotomy. After surgery a tracheostomy had to be performed, and 1 week later the patient developed an aspiration pneumonia. Cultures of respiratory secretions for bacteria were nondiagnostic, and the patient continued to have a low-grade fever despite aggressive therapy with antibacterial agents. Unfortunately, the patient was transferred to another hospital and was lost to follow-up. However, it is likely that the *C. parapsilosis* was responsible for the persistent low-grade fever and respiratory symptoms.

The last patient had disseminated histoplasmosis with *Histoplasma capsulatum* isolated in 11 blood cultures and a bone marrow biopsy. This patient died before the positive cultures were detected. However, a disseminated fungal infection was suspected, which was the reason for incubating the cultures for 4 weeks.

Of the 10 organisms detected in the thiol bottles after incubation for more than 7 days, only two organisms were not isolated in the companion bottle or in another blood culture. Both organisms, *Fusobacterium nucleatum* and *Peptococcus* sp., were isolated from patients with urosepsis that responded to intravenous cephalothin therapy. In one patient *K. pneumoniae* was isolated from a urine culture and two blood cultures, and in the second patient *E. coli* was isolated from urine and blood cultures. Thus, the two anaerobic isolates did not appear to be clinically significant.

DISCUSSION

Recent surveys (3, 10) have documented that 73 to 90% of clinical microbiology laboratories routinely incubate blood cultures for 7 days or less. Together with Reller and MacLowry, I stated empirically in *Cumitech 1A* (14) that incubation beyond 7 days is unnecessary, except for patients receiving antibiotics or infected with fastidious bacteria or yeast. Unfortunately, many patients are receiving antibiotics at the time the blood is collected, and infections with fastidious bacteria or yeast cannot be reliably predicted. Thus, the microbiologist must decide how long blood cultures should be routinely incubated.

In this study only 0.7 and 1.9% of the organisms in the thiol and R-TSB bottles, respectively, were detected after 1 week of incubation. Eight of the 10 organisms detected in the thiol bottle during the second week of incubation had either been detected in the companion bottle or in another blood culture. The other two isolates appeared to be clinically insignificant. Fifteen of the 32 isolates in the R-TSB bottle were either recovered in other blood cultures or were not considered to be clinically significant. Of the remaining 17 isolates from six patients, only the recovery of *Actinobacillus* sp. in two blood cultures from one patient significantly affected clinical management. Two clinically significant organisms (*F. tularensis* in 1 culture and *H. capsulatum* in 10 cultures) were isolated after the patients died. Three organisms (*K. pneumoniae* in one culture, *H. influenzae* in one culture, and *C. parapsilosis* in two cultures) were clinically important, but their recovery did not affect the management of the patients. Thus, in this investigator's experience with more than 20,000 blood cultures, there appears to be little clinical value in incubating unvented thiol or R-TSB bottles for more than 7 days. Although incubation of blood culture bottles for 7 days will not detect some fastidious bacteria and fungi, routine incubation for a longer period is not justified even in a tertiary care center such as Barnes Hospital.

LITERATURE CITED

1. **Bartlett, R. C.** 1973. Contemporary blood culture practices, p. 15–35. *In* A. Balows and A. C. Sonnenwirth (ed.), Bacteremia—laboratory and clinical aspects. Charles C Thomas, Publisher, Springfield, Ill.
2. **Bille, J., L. Stockman, G. D. Roberts, C. D. Horstmeier, and D. M. Ilstrup.** 1983. Evaluation of a lysis-centrifugation system for recovery of yeasts and filamentous fungi from blood. J. Clin. Microbiol. **18**:469–471.
3. **Blazevic, D. J., L. R. McCarthy, and J. A. Morello.** 1981. Results of survey of blood culture methods. Clin. Microbiol. Newsl. **3**:80.
4. **Effersoe, P.** 1965. The importance of the duration of incubation in the investigation of blood cultures. Acta Pathol. Microbiol. Scand. **65**:129.
5. **Ellner, P. D.** 1968. System for inoculation of blood in the laboratory. Appl. Microbiol. **16**:1892—1894.
6. **Geraci, J. E., W. R. Wilson, and J. A. Washington II.** 1980. Infective endocarditis caused by Actinobacillus actinomycetemcomitans: report of four cases. Mayo Clin. Proc. **55**:415.
7. **Hall, M., E. Warren, and J. A. Washington II.** 1974. Detection of bacteremia with liquid media containing sodium polyanetholesulfonate. Appl. Microbiol. **27**:187–191.
8. **Henry, N. K., C. M. Grewell, C. A. McLimans, and J. A. Washington II.** 1984. Comparison of the Roche Septi-Chek blood culture bottle with a brain heart infusion biphasic medium bottle and with a tryptic soy broth bottle. J. Clin. Microbiol. **19**:315–317.
9. **Henry, N. K., C. A. McLimans, A. J. Wright, R. L. Thompson, W. R. Wilson, and J. A. Washington II.** 1983. Microbiological and clinical evaluation of the Isolator lysis-centrifugation blood culture tube. J. Clin. Microbiol. **17**:864–869.
10. **Morello, J. A.** 1983. Blood culture practices currently in use, p. 61–95. *In* A. Balows and A. C. Sonnenwirth (ed.), Bacteremia—laboratory and clinical aspects. Charles C Thomas, Publisher, Springfield, Ill.
11. **Murray, P. R., and J. E. Sondag.** 1978. Evaluation of routine subcultures of macroscopically negative blood cultures for detection of anaerobes. J. Clin. Microbiol. **8**:427–430.
12. **Pfaller, M. A., T. K. Sibley, L. M. Westfall, J. E. Hoppe-Bauer, M. A. Keating, and P. R. Murray.** 1982. Clinical laboratory comparison of a slide blood culture system with a conventional broth system. J. Clin. Microbiol. **16**:525–530.
13. **Pfaller, M. A., L. M. Westfall, and P. R. Murray.** 1983. Value of routine aerobic subculturing or unvented blood culture bottles. J. Clin. Microbiol. **17**:601–604.
14. **Reller, L. B., P. R. Murray, and J. D. MacLowry.** 1982. Cumitech 1A, Blood cultures. Coordinating ed., J. A. Washington II. American Society for Microbiology, Washington, D.C.
15. **Rosner, R.** 1976. Growth patterns of a wide spectrum of organisms encountered in clinical blood cultures using both

hypertonic and isotonic media. Am. J. Clin. Pathol. **65**:706.
16. **Smith, J. A., F. J. Roberts, and J. Ngui-Yen.** 1983. Comparison of a radiometric and a broth-slide system for aerobic blood culture. J. Clin. Microbiol. **18**:217–218.
17. **Washington, J. A. II.** 1978. Conventional approaches to blood culture, p. 41–87. *In* J. A. Washington II (ed.), The detection of septicemia. CRC Press, West Palm Beach, Fla.
18. **Washington, J. A. II.** 1982. The role of the microbiology laboratory in the diagnosis and antimicrobial treatment of infective endocarditis. Mayo Clin. Proc. **57**:22.
19. **Weinstein, M. P.** 1982. Detection of bacteremia: technical aspects of the blood culture (letter). Infect. Control **3**:284–286.