JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 1992, p. 495–496
0095-1137/92/020495-02$02.00/0

Vol. 30, No. 2

NOTES

Nonvalue of Terminal Aerobic Subculture of Unvented Roche Columbia Broth Blood Culture Bottles

PAUL P. BOURBEAU,* BARBARA J. HEITER, AND DONNA W. NAUMOVITZ

Department of Laboratory Medicine, Geisinger Medical Center, Danville, Pennsylvania 17822

Received 10 June 1991/Accepted 30 October 1991

This study evaluated the need for a terminal aerobic blind subculture of unvented Roche Columbia broth. Only 2 of 2,871 bottles subcultured grew clinically significant organisms that were not also found in another blood culture. We conclude that in our tertiary care institution, blind subcultures of Roche Columbia broths are unwarranted.

The detection of bacteria from nonradiometric broth-based blood culture systems for aerobic or transiently vented bottles has traditionally been predicated on routine blind subcultures and macroscopic evaluation for evidence of growth (8). The Roche Septi-Chek system utilizes an agar paddle which is attached to the bottle. It is subcultured by tipping or inverting the bottle, obviating the necessity to perform the traditional blind subculture of the aerobic bottle by needle and syringe (6).

The need for blind subculture of unvented, anaerobic bottles of blood culture sets is not as clear. While it is generally agreed that routine anaerobic subculture of an unvented bottle is not necessary, conflicting recommendations exist regarding the necessity of an aerobic subculture of an unvented bottle (3, 4, 7, 8).

In general, those who have critically evaluated the benefits of routine subculture of unvented blood culture bottles have recommended that laboratories consider their specific patient population, the blood culture system in use, and the particular media utilized to determine whether to perform a subculture (3, 4, 7).

During an inspection by a regulatory agency, our laboratory was asked why routine aerobic subcultures of unvented Roche Columbia blood culture bottles were not performed. While it was our belief that such subcultures were unnecessary, no studies had been conducted which had specifically examined the benefits of a terminal, aerobic blind subculture of an unvented Columbia broth bottle when paired with an aerobic Septi-Chek tryptic soy broth (TSB) bottle. The purpose of this study was therefore to examine this issue in a large tertiary care medical center.

In our hospital, both the volume of blood collected and the type of blood culture bottles used are determined by the weight of the patient. Patients weighing 13 to 25 kg have 10 ml of blood collected, while those weighing over 25 kg have 20 ml collected. For both groups, the blood is evenly divided between a 70-ml Roche TSB bottle and a 70-ml Roche Columbia broth bottle. Because patients weighing less than 13 kg have only a single Roche TSB bottle inoculated, they were excluded from this study.

After receipt in the microbiology laboratory, a Septi-Chek paddle was attached to the TSB bottle in each set. This bottle was then immediately subcultured by tipping and placed on a shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) at 200 to 250 rpm for 24 h in a 35°C incubator (5). The unvented Columbia broth was incubated at 35°C without agitation.

Both the TSB bottle with the Septi-Chek paddle and the unvented Columbia broth bottle were visually examined for evidence of growth three times daily for the first 2 days of incubation, twice daily for the next 2 days, and then once daily through day 7. The TSB bottles were subcultured by tipping following each examination.

According to our routine laboratory protocol, if either bottle in a blood culture set was determined to be positive either by Gram stain or by growth on the Septi-Chek paddle, both bottles in the set were subcultured aerobically and anaerobically on appropriate media.

A 50-μl blind subculture on chocolate agar plates was performed for all Columbia broth bottles negative after 7 days of routine incubation. These plates were incubated for 48 h in 5 to 10% CO_2 at 35°C.

The determination of the clinical significance of all positive cultures, i.e., real or contaminant, was made by the hospital infection control staff after review of the patients' charts.

A total of 2,871 blood culture sets were included in this study. To more accurately compare the performance of the different broths and methodologies utilized in this study, isolates judged to be contaminants were excluded from analysis. As indicated in Table 1, 216 cultures yielded 231 clinically significant isolates, 8 of which were detected only by the blind, terminal subculture of the Columbia broth bottle. Of these eight isolates, three were previously detected in the TSB bottle of the same culture, while an additional three were detected in at least one other blood culture collected on the same day as the culture yielding the positive terminal subculture.

Only 2 of the 2,871 terminally subcultured Columbia broths drawn from two different patients contained significant isolates not found by routine examination in that or any other blood culture collected from these two patients. In both of these patients, cultures of urine samples collected on the same day as the samples which yielded positive blood cultures grew the same organism in pure culture (greater

* Corresponding author.

TABLE 1. Numbers and types of organisms isolated with different media by routine methods and blind subculture of Columbia broth

Organism	No. of organisms isolated from			Total no. of positive cultures
	TSB	Columbia		
		Routine	Blind subculture	
Gram negative				
Citrobacter freundii	4	2	0	4
Escherichia coli	35	35	2	44
Enterobacter aerogenes	1	1	0	2
Enterobacter agglomerans	1	0	0	1
Enterobacter cloacae	8	1	1	8
Enterobacter spp.	1	2	0	2
Klebsiella pneumoniae	9	10	0	11
Proteus mirabilis	4	3	0	5
Pseudomonas aeruginosa	7	5	1	8
Serratia marcescens	2	2	0	2
Bacteroides fragilis	0	1	0	1
Bacteroides fragilis group	0	2	0	2
Gram positive				
Staphylococcus aureus	46	38	1	49
Coagulase-negative staphylococci	42	31	2	47
Streptococcus spp.				
Group A	4	4	0	4
Group B	1	2	0	2
Group F	2	2	0	2
Group G	2	3	0	3
Streptococcus pneumoniae	7	5	0	7
Viridans streptococci	7	5	0	7
Enterococcus faecalis	3	0	0	3
Bifidiobacterium spp.	0	2	0	2
Clostridium perfringens	2	2	0	2
Yeasts				
Candida albicans	4	0	0	4
Candida lusitaniae	1	0	1	1
Candida parapsilosis	5	1	0	5
Cryptococcus neoformans	3	2	0	3

than 100,000 CFU/ml). One was *Escherichia coli* and the other was *Pseudomonas aeruginosa*.

There is no clear consensus regarding the necessity or benefit of aerobic blind subcultures of unvented blood culture bottles (3, 4, 7, 8). This lack of consensus can be attributed to differences in patient populations, the types of isolates recovered in a particular institution, the type of broth media, the volume of blood cultured, and the blood culture method.

The Septi-Chek system has demonstrated superior recovery of certain groups of organisms when compared with routine broth culture (2, 6). Therefore, caution must be exercised when comparing studies that utilized Septi-Chek with studies that utilized routine broth culture. On the basis of this study, we conclude that aerobic subculture of unvented Columbia broth is not necessary when it has been paired with a Septi-Chek TSB bottle. Pfaller et al. reached a similar conclusion from their evaluation of the benefit of aerobic subculture of unvented thiol broth when paired with a Septi-Chek TSB broth (6).

A legitimate question which can be raised is whether sufficient numbers of different bacterial genera were recovered to demonstrate that for some specific genus or genera, a blind subculture might be beneficial. For example, would a significant number of *Haemophilus influenzae* isolates be missed without a blind subculture of the unvented bottle? The recovery of *H. influenzae* is specifically mentioned because Henry and Washington concluded that blind subculture of an unvented TSB bottle should be considered when infection with *H. influenzae* is possible (3). Although the conclusions of Henry and Washington were based upon a comparison of unvented and vented TSB bottles, no published studies have included a sufficient number of *Haemophilus* isolates to adequately assess the recovery of *Haemophilus* spp. by the Septi-Chek method (2, 6, 7). Of the 3,112 blood cultures included in our study, none grew *H. influenzae*; however, unpublished data from over 100,000 blood cultures drawn over several years at the Mayo Clinic and inoculated into Isolator lysis centrifugation tubes, Roche Septi-Chek bottles with TSB, and unvented TSB bottles showed that the Septi-Chek bottles with TSB detected 46 of 49 *Haemophilus* isolates while the unvented TSB bottles detected only 14 of 49 (1). These data demonstrate the superior recovery of *Haemophilus* spp. from the Roche Septi-Chek bottles with TSB when compared with the unvented TSB bottles, thus obviating the necessity to subculture the unvented bottles for this organism.

In summary, the data from this study clearly demonstrate that there is no benefit to blind aerobic subculture of an unvented Roche Columbia blood culture bottle when it is paired with the Roche Septi-Check bottle with TSB under the conditions described in this paper. No evidence has been presented which contradicts this conclusion, either in terms of overall recovery or in the recovery of specific organisms.

We thank John Anhalt for critical review of the manuscript.

REFERENCES

1. **Anhalt, J. P. (Mayo Clinic).** 1990. Personal communication.
2. **Henry, N., 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.
3. **Henry, N., and J. A. Washington II.** 1984. Initial detection of bacteremia by subculture of unvented tryptic soy broth blood culture bottles. Diagn. Microbiol. Infect. Dis. **2**:107–111.
4. **Kiehn, T. E., B. Wong, F. F. Edwards, and D. Armstrong.** 1983. Routine aerobic terminal subculturing of blood cultures in a cancer hospital. J. Clin. Microbiol. **18**:885–889.
5. **Kim, M. J., R. L. Gottschall, L. D. Schwabe, and E. L. Randall.** 1987. Effect of agitation and frequent subculturing on recovery of aerobic and facultative pathogens by Roche Septi-Chek and BACTEC blood culture systems. J. Clin. Microbiol. **25**:312–315.
6. **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.
7. **Pfaller, M. A., L. M. Westfall, and P. R. Murray.** 1983. Value of routine aerobic subculturing of unvented blood culture bottles. J. Clin. Microbiol. **17**:601–604.
8. **Reller, L. B., P. R. Murray, and J. D. MacLowry.** 1982. Cumitech 1A, Blood cultures II. J. A. Washington II (ed.) American Society for Microbiology, Washington, D.C.