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Value of Routine Aerobic Subculturing of Unvented Blood Culture Bottles

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The value of performing routine aerobic subcultures of both vented and unvented blood culture bottles has not been evaluated critically. We studied 4,954 pairs of blood culture bottles consisting of one vented biphasic tryptic soy broth bottle (Roche Diagnostics) and one unvented Thiol broth bottle (Difco Laboratories). A total of 736 isolates were detected, of which 124 (17%) were in the Thiol broth bottle only. Some 15 isolates were detected only by subculturing the Thiol broth, and 13 of these isolates either were contaminants or were detected in previous positive cultures. Similar results were obtained when the unvented Thiol broth bottle was paired with a vented Difco tryptic soy broth bottle. Analysis of these pairs revealed a total of 360 isolates detected in 2,669 pairs of bottles, of which 83 isolates (23%) were in the Thiol broth bottle only. There were 11 isolates seen only in subcultures of the Thiol broth bottle, and 8 of these were probable contaminants. Thus, routine subculturing of unvented Thiol broth bottles had limited value. These results may differ with the use of other culture media or subculturing procedures. We recommend that each laboratory evaluate critically its experience with aerobic subcultures from unvented bottles.

The performance of routine blind subcultures of macroscopically negative blood culture bottles is a well-accepted technique for optimal detection of positive blood cultures (1, 3, 7, 8, 12, 15). Current recommendations state that aerobic subcultures should be performed on each bottle between 6 and 18 h after inoculation (1st day) and again after 48 h of incubation (3rd day) (12). Although numerous studies have examined the timing, number, and conditions required for the proper performance of subculturing (2, 3, 5-10, 13, 15), the necessity for routinely subculturing both the vented and unvented bottles of each two-bottle blood culture set has not been analyzed. Therefore, we studied the value of routine subculturing of an unvented Thiol broth bottle (Difco Laboratories) when paired with each of two different vented bottles, the Septi-Chek bottle (Roche Diagnostics) containing tryptic soy broth (TSB) and a Difco TSB bottle.

MATERIALS AND METHODS

The study was performed over two distinct time periods. Period 1 was from October 1981 to January 1982, and period 2 was from May 1982 to July 1982. During these two time periods, each blood culture set consisted of three bottles: period 1, TSB with 0.05% sodium polyanetholesulfonate (SPS) (Septi-Chek system), TSB with 0.025% SPS (Difco), and Thiol broth with 0.025% SPS (Difco); period 2, Roche TSB, Difco Thiol broth, and TSB with 0.05% SPS and 10% sucrose (Roche hypertonic broth). In this study we analyzed the value of subculturing routinely the unvented Thiol broth bottle when paired with the Roche TSB Septi-Chek system during periods 1 and 2 and when paired with the vented Difco TSB bottle during period 1. The hypertonic broth was not analyzed in this study.

Approximately 20 ml of blood was collected aseptically by venipuncture from patients with suspected bacteremia or fungemia, and an equal volume (5 to 7 ml) was placed into each bottle. Upon receipt in the laboratory, the Difco TSB bottle was vented chronically with a sterile cotton-plugged needle, and the Thiol broth bottle was not vented, to promote the recovery of anaerobes (16). Both the Difco TSB and the Thiol broth bottles were examined visually twice during the first 24 h and daily thereafter for evidence of microbial growth. Gram stains were performed on any bottles with macroscopic evidence of growth. In addition, aerobic blind subcultures to chocolate agar plates were made from all macroscopically negative TSB and Thiol broth bottles 6 to 18 h after inoculation (1st day) and after 48 h of incubation (3rd day). If a bottle was noted to be positive only on the blind subculture, no additional attempts were made to determine whether it became macroscopically positive at a later date.

The Roche Septi-Chek bottles were processed as described previously (11). Briefly, upon receipt in the laboratory, the agar-coated slide system was attached to the bottle, and the bottle was transiently inverted. The bottles were examined twice during the first 24 h and daily thereafter, as was done with the TSB and Thiol broth bottles. Daily subcultures were performed by inverting the bottle at the time of each macroscopic

examination. The agar surfaces of the slide were inspected daily for evidence of growth. No additional blind subcultures were performed. Gram stains were performed on bottles showing signs of turbidity, hemolysis, or gas production.

RESULTS

During the two periods of study, a total of 4,954 sets of blood cultures were collected, each containing a Roche Septi-Chek bottle (vented) and a Difco Thiol broth bottle (unvented). Microorganisms were detected in one or both of these bottles in 670 cultures. A total of 736 isolates were recovered in these cultures, including 276 in the Roche bottles only, 124 in the Thiol broth bottles only, and 336 in both Roche and Thiol broth bottles. We analyzed initially the method of detecting organisms recovered only in the Thiol broth. Of these 124 isolates, 15 were detected by subculturing, 67 were detected by macroscopic examination, and 42 were detected simultaneously by both subculturing and macroscopic examination (Table 1). Of the 15 isolates detected only by subculturing, 10 were *Staphylococcus epidermidis*, of which 8 were single isolates and presumed to be contaminants. The remaining five isolates detected by subculturing alone were *Staphylococcus aureus* (three isolates) and *Streptococcus* spp. (two isolates). The three *S. aureus* isolates were all from patients with previous cultures positive for this organism.

To determine whether isolates are detected more rapidly by subculturing the unvented Thiol broth, we examined the method of detecting organisms recovered in both bottles. A total of 336 isolates were detected in both the Roche TSB bottle and the Thiol broth bottle, of which 62 were detected first in the Roche bottle, 40 were detected first in the Thiol broth bottle, and 234 were detected simultaneously in both bottles. Only one of the 40 isolates detected initially in the Thiol broth bottle was detected by subculturing alone. This organism was an isolate of *S. aureus* that had been detected previously in multiple cultures.

We also examined the value of subculturing the unvented Thiol broth bottle when the companion bottle was a vented Difco TSB bottle. A total of 2,669 sets of blood cultures consisting of a vented Difco TSB bottle and an unvented Difco Thiol broth were collected, of which 324 were positive. A total of 360 isolates were recovered in these cultures, including 88 only in the Difco TSB bottle, 83 only in the Difco Thiol broth bottle, and 189 in both the TSB and Thiol broth bottles. Of the 83 isolates detected exclusively in the Thiol broth bottle, 11 were detected by subculturing alone, 35 were detected by macroscopic examination alone, and 37 were detected simultaneously by both macroscopic examination and subculturing (Table 2). Of the 11 isolates detected only by subculturing, 6 were *S. epidermidis*, all of which were single isolates and presumed to be contaminants. The remaining five isolates were *Streptococcus* spp., of which three were enterococci from a single patient and the remaining two were single isolates of viridans-group streptococci from two separate patients.

TABLE 1. Isolates detected only in the Thiol broth bottle of the Roche TSB-Difco Thiol broth pair

Organism	No. of isolates detected by:		
	Subculture	Macroscopic examination	Both
Gram-negative:			
Enterobacteriaceae		19	15
Pseudomonas spp.			1
Other		2	
Gram-positive:			
S. epidermidis	10	19	12
S. aureus	3	1	5
Streptococcus spp.	2	13	9
Other		13	

A total of 189 isolates were detected in both the Difco TSB bottle and the Difco Thiol broth bottle, of which 10 were detected first in the TSB bottle, 12 were detected first in the Thiol broth bottle, and 154 were detected simultaneously in both bottles. Of the 12 isolates detected initially in the Thiol broth bottle, 5 were detected exclusively by subculturing. Four of these isolates (three isolates of *S. epidermidis* and one *Corynebacterium* sp.) were presumed to be contaminants. The remaining isolate was a *S. aureus* that had been detected previously in multiple cultures.

DISCUSSION

The practice of performing routine blind subcultures of macroscopically negative blood culture bottles has been the subject of much investigation in recent years (2, 3, 5–10, 13, 15). Based on these studies, recommendations about the timing, frequency, and incubation atmosphere of the subcultures have undergone significant revision since 1974 (1, 12). The thrust of many of these studies has been to limit the number of subcultures performed to the minimum necessary to provide useful information. Thus, we have seen elimination of both very early (less than 6 h postinoculation) and late (days 5 to 7 and 14) aerobic subculturing and of all routine anaerobic subculturing (2, 5, 6, 9, 10, 13). Despite these efforts, several questions remain concerning the optimal subculturing protocol.

TABLE 2. Isolates detected only in the Thiol broth bottle of the Difco TSB-Difco Thiol broth pair

Organism	No. of isolates detected by:		
	Subculture	Macroscopic examination	Both
Gram-negative:			
Enterobacteriaceae		12	15
Gram-positive:			
S. epidermidis	6	9	11
S. aureus		2	3
Streptococcus spp.	5	8	8
Other		4	

Specifically, the necessity for routine aerobic subculturing of the unvented blood culture bottles has not been examined. Since routine blind subculturing has the greatest value in enhancing the detection of fastidious aerobes such as *Neisseria* spp., *Pseudomonas aeruginosa*, and yeast, all of which are detected primarily in vented bottles (4, 8, 14), it would seem that routine aerobic subculturing of the unvented bottles would be of little value. A recent study by McLaughlin et al. demonstrated the lack of requirement for blind aerobic subculturing of both aerobic and anaerobic BACTEC blood culture bottles (9). However, it is unclear whether these same recommendations can be applied to other blood culture systems.

The present study was performed to evaluate the necessity of routinely subculturing macroscopically negative, unvented Thiol broth blood culture bottles when these bottles are paired with vented TSB bottles. Two different types of TSB bottles were used, and a different subculturing protocol was used for each. The Roche Septi-Chek system has been described previously (11) and allows the performance of daily subcultures by simple inversion of the bottles at the time of macroscopic examination. The Difco TSB bottle is processed in a more conventional manner, with routine subcultures performed on days 1 and 3 of incubation, as was done with the Thiol broth bottle.

Analysis of the results obtained with the Roche TSB-Difco Thiol broth pairs of blood culture bottles revealed that only 15 isolates were recovered exclusively by subculturing the Difco Thiol broth bottle (Table 1), and 13 of these isolates either were contaminants or were detected in previous positive cultures. In addition, isolates were not detected significantly faster by subculturing the Thiol broth bottle.

Similar results were seen with the Difco TSB-Difco Thiol broth pairs. Only 11 of 360 total isolates were detected exclusively by subculturing the Thiol broth bottle (Table 2), including single isolates of *S. epidermidis* or *Streptococcus* spp. from eight patients. Likewise, subculturing the Thiol broth bottle did not result in faster detection of isolates. Only one significant isolate was detected initially in the Thiol broth bottle. However, this organism had been detected in many earlier cultures. Thus, it appears that routinely subculturing the unvented Thiol broth bottle is unnecessary when the companion bottle is either a vented Difco or a Roche TSB bottle.

Our conclusions and recommendations based on the data obtained in this study are that the routine aerobic subculturing of macroscopically negative unvented Thiol broth bottles is unnecessary when the vented companion bottle contains TSB and is subcultured on a regular schedule. These conclusions should not be extrapolated to blood culture sets containing different media formulations or to those processed by different subculturing protocols. In addition, we encountered very few isolates of *Pseudomonas* spp., *Neisseria* spp., or *Haemophilus* spp. in this study. Although we feel that detection of these fastidious organisms would not be compromised by the protocol described, we cannot make a definitive statement about the feasibility of our subculturing protocol in hospitals where a large number of these isolates are seen. Finally, we recommend that each laboratory critically evaluate its own subculturing procedures to ensure that the most accurate, cost-effective, and time-efficient protocol is being used that also provides optimal detection of positive blood cultures.

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