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Comparison of a Slide Blood Culture System with a Supplemented Peptone Broth Culture Method

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A slide blood culture system (Roche Diagnostics, Div. Hoffman-La Roche, Inc., Montreal, Canada; Roche BCB) was compared with a supplemented peptone broth Vacutainer method (Becton, Dickinson & Co., Rutherford, N.J.) on blood samples taken from the same 1,209 patients. Significantly more clinically important isolates were isolated with the Roche BCB system, and technical processing time was reduced. However, significantly more contaminants were isolated with the Roche BCB system, and it could not readily be adapted for anaerobic culture. Contamination was reduced by careful tightening of the slide to the bottle top to prevent any leakage. Overall, the BCB system is a satisfactory method for aerobic blood culture, markedly reducing technical processing time relative to most other blood culture methods.

The blood culture system developed by Roche Diagnostics (Div. Hoffman-La Roche, Inc., Montreal, Canada) consists of a slide (BCB; blood culture bottle slide) that resembles the common dip-slide used for culture of urine and can be attached to a blood culture bottle. The slide contains chocolate agar on one side and separated MacConkey and malt agar media on the other side. It can be flooded by inversion of the blood culture bottle. The system contains the necessary growth media for culture of most clinically important bacteria and fungi. It has the apparent advantage of significantly reducing the technical time required for subculture. One of the major constraints placed on diagnostic laboratories in recent years has been the limitation of personnel budgets. Since the Roche BCB system seemed capable of significantly reducing technical processing time, it was evaluated and compared with the commonly used 18-ml Becton, Dickinson & Co. Rutherford, N.J.) (BD) Vacutainer blood culture system. The evaluation was carried out by using blood culture samples collected from 1,209 patients.

MATERIALS AND METHODS

Blood was collected after cleansing the venipuncture site with 2% betadine followed with 70% isopropyl alcohol. One blood culture system consisted of the approximately 18 ml of BD Vacutainer supplemented peptone broth capable of taking 2.2 ml of blood. Three such tubes were collected for each patient, using the Vacutainer collection system as recommended by the manufacturer. Blood was collected for the Roche BCB culture from each patient with either a double-ended collection unit or a syringe. The tops of both blood culture systems were cleansed with isopropyl alcohol before collection of blood. For the Roche system, approximately 8 ml of blood was collected into 70 ml of tryptic soy broth containing sodium polyanetholesulfonate (Roche Diagnostics). The total volume of blood collected into the BD tubes was about 6.6 ml.

Blood cultures were incubated within 30 min of collection. Two of the BD tubes were vented and one was not. The unvented tube was used for anaerobic culture. No separate sample for anaerobic cultures was taken for the Roche culture, and no attempt was made to keep that system anaerobic. Before incubation, the Roche BCB with chocolate, MacConkey, and malt agar media was attached to the aerobic blood culture bottle by removing the blood culture cap, flaming the neck of the bottle, and screwing the BCB onto the blood culture tube. The slide was tightened firmly to prevent leakage when the blood culture bottle was inverted or tipped to the horizontal.

Subculture procedures were based on generally recommended methods (2). Subculture from the BD aerobic cultures was carried out at 6 h to chocolate medium, at 24 h to blood agar plates and to choclate medium, at 72 h to blood agar plates incubated both anaerobically and aerobically, and at 14 days to blood agar plates incubated anaerobically and aerobically. All aerobic subculture incubations were carried out at 35°C in 5% CO_2. Subculture of the Roche cultures consisted of tipping the bottle to the horizontal, rotating the blood culture tube 360°, and returning the bottle to the vertical. Subculture was carried out either daily or every second day. Slide media were inspected daily for growth. Both Roche and BD Vacutainer bottles were incubated at 35°C in air.

Bacterial isolates were considered clinically significant if they were *Enterobacteriaceae*, *Haemophilus* species, *Staphylococcus aureus*, *Bacteroidaceae*,

Pseudomonas aeruginosa, yeasts, *Streptococcus pyogenes, Streptococcus pneumoniae, Listeria monocytogenes, Clostridium perfringens*, and *Peptostreptococcus intermedius*. Other bacteria were considered significant if isolated from two or more blood cultures or if isolated from a clinical situation in which that organism was recognized to be of pathogenic significance. The last situation involved *Staphylococcus epidermidis* isolates from patients with prosthetic valves and viridans and group D streptococci from patients with endocarditis.

RESULTS

Recovery of clinically significant bacterial isolates. A total of 1,209 blood cultures were examined over a 7-month period by both systems. Sixty-one blood cultures were repeated for the same patient and were not included in the 1,209 total cultures. Repeat isolates from the same patient were not included in the results provided in Tables 1 or 2. By the criteria specified above, 126 isolates were considered to be clinically significant. The organisms isolated and the blood culture systems by which they were isolated are given in Table 1. Both systems gave positive results in 61 cases; the Roche BCB system was the only system giving positive results for 45 blood cultures, and the BD system was the only system giving positive results in 20 instances.

TABLE 1. *Specific organisms growing in Roche BCB only, BD only, or both blood culture systems*

Organism	No. of isolates growing in system		
	Both	Roche BCB only	BD only
A. Gram-positive cocci			
Staphylococcus aureus	12	9	3
Staphylococcus epidermidis	8	10	4
Streptococcus pyogenes	4	0	0
Viridans streptococci	3	3	1
Group D streptococci	1	1	0
Streptococcus pneumoniae	4	1	2
B. Gram-negative bacilli			
Escherichia coli	13	3	0
Klebsiella pneumoniae	3	3	0
Enterobacter cloacae	1	0	0
Acinetobacter anitratum	0	2	2
Morganella (Proteus) morganii	1	0	0
Pseudomonas aeruginosa	5	5	2
Pseudomonas species	0	2	0
Haemophilus influenzae	1	0	1
Haemophilus parainfluenzae	0	0	1
C. Anaerobic bacteria			
Peptostreptococcus intermedius	0	1	0
Bacteroides fragilis	0	0	1
Fusobacterium species	0	0	1
Clostridium perfringens	1	0	0
D. Yeast			
Torulopsis glabrata	1	0	0
Candida albicans	2	0	0
E. Other microorganisms			
Alcaligenes species	0	2	0
Listeria monocytogenes	0	1	1
Serratia liquefaciens	0	1	0
Salmonella enteritidis	1	1	0
Unidentified gram-negative bacillus	0	0	1

TABLE 2. *Bacterial contaminants isolated from Roche BCB and BD blood culture systems*

Organism	No. of isolates with contaminants	
	Roche BCB	BD
Bacillus species	19	5
Staphylococcus epidermidis	6	1
Micrococcus species	6	0
Propionibacterium species	8	3
Diphtheroid organisms	2	0
Gram-negative bacillus (unidentified)	1	0
Mixed organisms	1	0
Staphylococcus epidermidis (questionable significance)	9	1

During the period of this study, only four anaerobic isolates were encountered, excluding *Propionibacterium* species. The Roche BCB system detected two of these, whereas the BD system detected three. However, as noted above, no attempt was made to keep the Roche BCB system anaerobic because of space limitations.

The results shown in Table 1 demonstrate that the Roche BCB system had greater sensitivity than did the BD system, although, interestingly, even the Roche BCB system failed to grow some isolates.

Blood culture contamination. Table 2 shows the number of bacterial isolates that were considered contaminants for the Roche BCB system and the BD system. Many more contaminants were recovered with the Roche BCB bottles. An early problem was failure to adequately tighten the slide system onto the blood culture bottles. Two batches of Roche BCB blood cultures were used in the study. The second batch had a sealing ring different from that of the first batch which allowed the slide to be more firmly tightened to the bottle. Of 730 blood cultures handled with the second batch of bottles, only 3 showed contamination with aerobic sporeforming bacilli, whereas 16 of the first 479 cultures with the original sealing ring grew them.

Time of recognition of initial growth. The two culture systems were generally equivalent for time of initial growth when the isolate grew

in both systems. However, all of the five *P. aeruginosa* isolates grown in both systems were detected 24 h earlier in the Roche BCB culture. On one occasion, a *Candida albicans* isolate grew in 48 h in the Roche system but required 14 days to be detected in the BD system. Other than these observations, no significant differences were noted, although there was a trend for earlier detection of growth with the BCB system.

Time required to process blood cultures. I recorded the time to process 100 blood cultures by both Roche BCB and Vacutainer systems and determined that additional time was required to process the Vacutainer system. This time included that required to subculture and to prepare the subculture media. Under my conditions of subculture, the additional time required to process one blood culture completely was a minimum of 3 and a maximum of 6 min. I also recorded the time required to process the Roche BCB system as compared with two blood culture systems consisting, respectively, of 50- and 100-ml bottles. Compared with the 50- and 100-ml bottles, the time saving was a minimum of 2 and a maximum of 5 min per blood culture when the Roche BCB system was used.

DISCUSSION

In my view, the Roche BCB system had several advantages as well as some disadvantages when compared with the BD Vacutainer blood culture system. The type of comparison undertaken in this study is, I believe, of considerable value since it represents bacteria isolated under normal clinical laboratory circumstances rather than by the use of seeded blood cultures.

A major advantage of the BCB system was that it greatly reduced the technical processing time required for subculture. The advantage was apparent upon comparison with the BD Vacutainer system but was also found when time required for subculture was compared with that required for 50- and 100-ml blood cultures, one of which required removal of samples with a syringe for subculture. A second advantage was the greater sensitivity of the Roche BCB system over the Vacutainer system, against which it was compared. The greater sensitivity might be accounted for by the differences in blood volume taken. This is particularly true for strictly aerobic bacteria like *P. aeruginosa*. One BD tube was incubated anaerobically. Thus, the volume of blood for *P. aeruginosa* growth was reduced to 4.4 ml. The BD system isolated two anaerobic organisms which the Roche system did not isolate. The microorganisms isolated throughout the 7-month period of this study were similar to those isolated over the previous 7-month period. They were also similar to those reported recently in a survey of several large American hospitals (1) and in a study at a large Canadian hospital (3).

An apparent disadvantage of the Roche BCB system was the increased rate of contamination. However, with careful attention to sterile attachment of the slide and bottle, to flaming the bottle top, and to tightening the slide to the bottle as well as care in skin and bottle top antisepsis, contamination could be significantly reduced. Some of these concerns detracted from the time saving with the blood culture system, although a substantial time saving was still retained. If the slide were securely tightened onto the bottle, an early problem of leakage of the blood culture medium upon inversion of the bottles could be prevented.

Another disadvantage was that the Roche BCB method was not readily adaptable to anaerobic culture. My hospital possesses a small anaerobic chamber as well as anaerobic jars. These were inadequate to accomodate the space required by the blood culture bottle and top. However, it would be possible to combine the BCB system with a sealed anaerobic blood culture bottle. The technical time required to perform the anaerobic blood culture is usually less in most laboratories since primary subculture does not normally occur until 48 or 72 h because of the slow growth of these organisms. Thus, the combination of the Roche BCB system with any of several other anaerobic blood culture systems would be an adequate method of blood culture.

I did not experience any major difficulties with the slide system. With the method of flooding the slide media described above, I experienced neither loss of media from the slide nor loss of colonies from the slide media. I also did not experience drying or other problems with the slide media. There was no evidence of contamination of unflooded slides. In a few instances, as noted, the slides did allow the opportunity to detect earlier growth.

A previous evaluation of the Roche slide system comparing it with a 100-ml tryptic soy broth system with sodium polyanetholesulfonate (Difco Laboratories, Detroit, Mich.) has been reported (J. W. Keathley and C. N. Walker, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, C7, p. 311). These authors found similar isolation rates for the two systems and also found that most species were isolated more rapidly with the BCB method. In addition, they noted a significantly higher contamination rate and increased convenience in subculture for the BCB cultures.

This study suggests that the recovery of bacteria from the blood is dependent on the blood

culture system. An interesting observation made during this study was that, whereas the Roche BCB system detected 45 organisms that the BD system did not, the reverse situation was also encountered. The BD system detected 20 isolates not detected by the Roche system. Two of these organisms were *S. pneumoniae* and one was *Haemophilus influenzae*. However, it is obvious from my results that the Roche BCB system will grow *S. pneumoniae* since it did so in five cases, and it grew *H. influenzae* in one case.

In summary, I found the Roche BCB system to be a satisfactory blood culture method for aerobic bacteria, providing that marked care was taken in handling cultures to avoid contamination. The system allows a significant saving in technical processing time.

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