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Comparative Evaluation of the Oxoid Signal and Roche Septi-Chek Blood Culture Systems

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The Oxoid Signal blood culture system (Oxoid USA, Inc., Columbia, Md.) was compared with the Roche Septi-Chek system (Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.), with the latter consisting of a tryptic soy broth (R-TSB) bottle with an attached agar slide unit and a Columbia broth bottle. A total of 5,034 cultures with equal volumes of blood in each bottle were processed. Overall, more organisms were recovered in the R-TSB bottle than in the Signal bottle, with significantly more aerobic organisms (*Pseudomonas* spp., *Acinetobacter* spp., and yeasts) recovered in the R-TSB bottles and anaerobes and viridans group streptococci recovered in Signal bottles. Approximately equivalent numbers of organisms were recovered in the Signal and Columbia broth bottles. The times of detection were essentially identical with the three blood culture broth systems. During the study, 30.6% of the Signal bottles had a positive indicator of growth, of which 1,103 (71.7%) were false-positive cultures. Additionally, nonviable organisms resembling streptococci were observed in 13.7% of the Signal bottles that were Gram stained and in uninoculated blood culture bottles. With appropriate modifications of the preparation of the media, the latter problem can be eliminated.

One of the most important specimens received in the clinical microbiology laboratory is blood for the detection of septicemia. During the last 20 years, a number of blood culture systems have been developed. The initial standard was the conventional two-bottle system developed for the isolation of both aerobic and anaerobic organisms (8). The major limitation of this system was the need for carefully timed blind subcultures and microscopic stains for the detection of fastidious or slow-growing organisms (10). The BACTEC system (Johnston Laboratories, Inc., Towson, Md.) was developed to circumvent these problems (2, 5, 15). CO_2 production by growing organisms can be detected in the system by either radiometry or infrared spectroscopy. Although use of this system has been widely accepted in the United States, expensive instrumentation is required. Two other approaches have been used to detect growth in blood cultures: biphasic systems (e.g., Septi-Chek; Roche Diagnostics, Div. Hoffmann-La Roche Inc., Nutley, N.J.) and lysis-centrifugation (e.g., Isolator; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.). The Septi-Chek and Isolator systems provide the benefits of standardized detection of growth (4, 7) without the need for additional instrumentation. Recently, Oxoid (Oxoid USA, Inc., Columbia, Md.) has introduced another blood culturing system, the Signal system, that detects CO_2 production by microorganisms (9, 12, 13). Unlike the BACTEC system, however, the gas production is not detected by an instrument. Gas is detected by the positive pressure that transfers the blood-broth mixture from the main bottle to the Signal growth chamber. In the study reported herein, we compared the recovery of bacteria and fungi from blood cultured in the Septi-Chek biphasic and Signal systems.

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MATERIALS AND METHODS

Clinical specimens. During a 3-month period, a total of 20 ml of blood was collected for each culture and divided equally into three bottles: Oxoid Signal bottle, Roche tryptic soy broth (R-TSB) bottle, and Roche Columbia broth (R-COL) bottle. Cultures with underfilled bottles were not included in the study.

Culture procedures. Processing of the two Septi-Chek bottles has been described previously (7). Briefly, at the time that the bottles were received in the laboratory, the Septi-Chek slide unit was attached to the R-TSB bottle, the bottle was inverted to inoculate the agar surfaces, and then the bottle was placed in a 35°C incubator on a mechanical mixer (150 rpm; New Brunswick Scientific Co., Inc., Edison, N.J.) for the first 24 h of incubation. The R-COL bottle was placed into a 35°C incubator without the use of the slide unit or mechanical agitation. For the duration of this study, both bottles were examined twice on day 1 and then daily for a total of 7 days.

At the time that the Oxoid Signal bottle was received in the laboratory, the rubber stopper was disinfected and the Signal chamber was attached. The Signal chamber consists of a long needle and a clear plastic chamber with a narrow cylinder at the bottom and a larger upper reservoir. The needle of the Signal chamber was inserted through the rubber stopper and positioned below the surface of the culture medium. The chamber was secured with a plastic locking sleeve that was attached to the top of the bottle. As organisms grew in the Signal bottle and utilized the nutrients, gas was produced. The increased pressure in the air space above the broth forced the broth into the chamber, signaling a positive culture. After the Signal chambers were attached, the bottles were placed in a 35°C incubator on a mechanical agitator. All bottles were agitated at 150 rpm for 24 h and were then incubated for an additional 6 days without agitation. All bottles were inspected twice on day 1 and then daily thereafter. Terminal subcultures were per-

TABLE 1. Comparison of number of organisms recovered in the three blood culture bottles

Blood culture bottle	No. of organisms	
	Significant	Contaminants[a]
Signal	382	87
R-TSB	413	151
R-COL	356	86

[a] Defined as single isolates of *Staphylococcus epidermidis*, *Corynebacterium* spp., *Propionibacterium* spp., and *Bacillus* spp.

formed with the Signal bottles for the last 3,000 cultures processed in this study.

Statistical analysis. The Signal system is reported to be a one-bottle system. That is, both strictly aerobic and anaerobic organisms should be recovered in this system. Because each bottle was inoculated with the same volume of blood, a comparison of the single Oxoid Signal bottle with two Roche bottles would be invalid. For that reason, paired comparisons were performed for all three bottles used in this study (i.e., Signal versus R-TSB, Signal versus R-COL, and R-TSB versus R-COL). The statistical significance of observed differences was measured with the modified chi-square test with Yates correction (1).

RESULTS

A total of 5,034 properly filled blood cultures were processed during the study period. Organisms were recovered in 693 (13.8%) cultures from 376 patients. The number of clinically significant isolates and contaminants recovered in each bottle is summarized in Table 1. The most isolates (significant and contaminants) were recovered in the R-TSB bottles. Paired comparisons of the recovery of isolates in the three bottles are summarized in Tables 2 (Signal versus R-TSB), 3 (Signal versus R-COL), and 4 (R-TSB versus R-COL). Overall, more organisms were recovered in the R-TSB bottle (413 isolates) than in the Signal bottle (382 isolates), with significantly more strictly aerobic organisms (*Pseudomonas* spp., *Acinetobacter* spp., and yeasts) recovered in R-TSB bottles and anaerobes and viridans group streptococci recovered in Signal bottles (Table 2). In the comparison of Signal with R-COL bottles (Table 3), more organisms were recovered in the Signal bottle (382 isolates) than in the R-COL bottle (356 isolates), although this difference was not statistically significant. The difference between these two bottles was the increased recovery of *Staphylococcus epidermidis* and yeasts in the Signal bottle. In the comparison of the R-TSB and R-COL bottles (Table 4), significantly more isolates of *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp., and yeasts were recovered in the R-TSB bottle; and more anaerobes were recovered in the R-COL bottle. Overall, significantly more organisms were recovered in the R-TSB bottle than in the R-COL bottle.

A comparison of the time required to detect isolates in each set of blood culture bottles is summarized in Tables 5 to 7. The mean time of detection for isolates recovered in both Signal and R-TSB bottles (Table 5) was not significantly different except for yeasts, which were recovered earlier in the R-TSB bottle (3.1 days) than in the Signal bottle (4.3 days). The same time period was required to detect growth in the Signal and R-COL bottles for all groups of organisms except isolates of *Staphylococcus* spp. (Table 6). In the comparison of organisms recovered in both the R-TSB and R-COL bottles, a significant difference in detection time was observed only for yeasts (Table 7).

TABLE 2. Comparison of microbial recovery in Signal and R-TSB bottles

Organism	No. of isolates from:			*P* value
	Signal only	R-TSB only	Both bottles	
Staphylococcus spp.	46	55	126	NS[a]
Staphylococcus aureus	17	13	59	NS
Staphylococcus epidermidis	29	42	67	NS
Streptococcus spp.	18	11	43	NS
Viridans group streptococci	13	4	6	<0.05
Streptococcus groups A, B, and G	0	1	8	NS
Streptococcus pneumoniae	0	0	4	NS
Streptococcus bovis	0	1	6	NS
Enterococcus spp.	5	5	19	NS
Listeria sp.	1	1	2	NS
Enterobacteriaceae	14	18	72	NS
Escherichia spp.	9	8	34	NS
Klebsiella spp.	4	4	11	NS
Enterobacter spp.	0	2	15	NS
Proteus spp.	1	4	8	NS
Citrobacter spp.	0	0	2	NS
Serratia sp.	0	0	2	NS
Pseudomonas spp.	1	10	18	<0.01
Acinetobacter spp.	0	8	1	<0.01
Moraxella sp.	1	0	0	NS
Haemophilus sp.	1	0	0	NS
Capnocytophaga sp.	0	0	1	NS
Neisseria sp.	0	1	0	NS
Branhamella sp.	0	0	1	NS
Anaerobes	11	2	3	<0.05
Peptostreptococcus sp.	1	0	1	NS
Actinomyces sp.	0	0	1	NS
Clostridium sp.	3	1	0	NS
Bifidobacterium sp.	0	1	0	NS
Bacteroides spp.	5	0	0	<0.05
Veillonella sp.	2	0	1	NS
Fungi	0	18	22	<0.001
Candida spp.	0	10	4	<0.01
Torulopsis sp.	0	7	18	<0.01
Cryptococcus sp.	0	1	0	NS
All organisms	93	124	289	<0.05

[a] NS, Not significant.

Microbial growth was detected in the Signal system by the presence of broth in the Signal chamber or macroscopic evidence of growth in the broth (e.g., turbidity, hemolysis, or discrete colonies). Of the 5,034 Signal bottles received during this study, 1,539 (30.6%) had a positive indicator for growth, including 392 bottles with only macroscopic evidence of growth in the broth. A total of 322 (82.1%) of the 392 bottles were false-positive bottles. We also observed false-positives with 92.7% of the 744 bottles with <5 mm of broth in the Signal chamber, 66.7% of the 36 bottles with 5

TABLE 3. Comparison of microbial recovery in Signal and R-COL bottles

Organism	No. of isolates from:			P value
	Signal only	R-COL only	Both bottles	
Staphylococcus spp.	58	36	114	<0.05
Staphylococcus aureus	15	11	61	NS[a]
Staphylococcus epidermidis	43	25	53	<0.05
Streptococcus spp.	16	21	45	NS
Viridans group streptococci	7	6	12	NS
Streptococcus groups A, B, and G	0	3	8	NS
Streptococcus pneumoniae	2	2	2	NS
Streptococcus bovis	0	0	6	NS
Enterococcus spp.	7	10	17	NS
Listeria sp.	1	1	2	NS
Enterobacteriaceae	13	13	73	NS
Escherichia spp.	6	8	37	NS
Klebsiella spp.	4	3	11	NS
Enterobacter spp.	0	1	15	NS
Proteus spp.	2	1	7	NS
Citrobacter spp.	0	0	2	NS
Serratia sp.	1	0	1	NS
Pseudomonas spp.	4	2	15	NS
Acinetobacter spp.	0	3	1	NS
Moraxella sp.	1	0	0	NS
Haemophilus sp.	1	0	0	NS
Capnocytophaga sp.	0	0	1	NS
Branhamella sp.	0	0	1	NS
Anaerobes	7	6	7	NS
Peptostreptococcus spp.	0	3	2	NS
Actinomyces sp.	0	1	1	NS
Clostridium sp.	3	1	0	NS
Bacteroides spp.	2	0	3	NS
Veillonella sp.	2	1	1	NS
Fungi	10	3	12	<0.05
Candida spp.	3	1	1	NS
Torulopsis sp.	7	2	11	NS
All organisms	111	85	271	NS

[a] NS, Not significant.

TABLE 4. Comparison of microbial recovery in R-TSB and R-COL bottles

Organism	No. of isolates from:			P value
	R-TSB only	R-COL only	Both bottles	
Staphylococcus spp.	65	34	116	<0.01
Staphylococcus aureus	14	14	58	NS[a]
Staphylococcus epidermidis	51	20	58	<0.001
Streptococcus spp.	13	25	41	NS
Viridans group streptococci	5	13	5	NS
Streptococcus groups A, B, and G	0	2	9	NS
Streptococcus pneumoniae	1	1	3	NS
Streptococcus bovis	1	0	6	NS
Enterococcus spp.	6	9	18	NS
Listeria sp.	1	1	2	NS
Enterobacteriaceae	15	11	75	NS
Escherichia spp.	4	7	38	NS
Klebsiella spp.	4	3	11	NS
Enterobacter spp.	2	1	15	NS
Proteus spp.	4	0	8	NS
Citrobacter spp.	0	0	2	NS
Serratia sp.	1	0	1	NS
Pseudomonas spp.	12	1	16	<0.01
Acinetobacter spp.	5	0	4	<0.05
Capnocytophaga sp.	0	0	1	NS
Neisseria sp.	1	0	0	NS
Branhamella sp.	0	0	1	NS
Anaerobes	2	10	3	<0.05
Peptostreptococcus spp.	0	4	1	NS
Actinomyces sp.	0	1	1	NS
Clostridium sp.	1	1	0	NS
Bifidobacterium sp.	1	0	0	NS
Bacteroides spp.	0	3	0	NS
Veillonella sp.	0	1	1	NS
Fungi	26	1	14	<0.001
Candida spp.	12	0	2	<0.001
Torulopsis sp.	13	1	12	<0.01
Cryptococcus sp.	1	0	0	NS
All organisms	140	83	273	<0.001

[a] NS, Not significant.

mm of broth, 13.4% of the 292 bottles with >5 mm of broth in the chamber, and 37.3% of the 75 bottles in which the amount of broth in the chamber was not recorded. Thus, a significant proportion of the false-positive Signal bottles were either turbid or the broth was in less than half of the chamber. In contrast with the Signal bottles, 31.4% of the R-TSB bottles and 46.1% of the R-COL bottles that appeared to be macroscopically positive were false-positive cultures.

Nonviable, gram-positive cocci resembling streptococci were observed in 153 (13.7%) of the culture-negative Signal bottles that were Gram stained. These organisms were also observed in uninoculated Signal blood culture bottles and in five of eight lots of media received during the study.

The frequency of positive Signal bottles with a negative indicator in the Signal chamber was also examined. A total

TABLE 5. Time of detection for growth in Signal and R-TSB bottles

Organism (no.)	Mean time (day) of detection		P value
	Signal	R-TSB	
Staphylococcus spp. (126)	1.8	1.9	NS[a]
Streptococcus spp. (43)	1.1	1.4	NS
Enterobacteriaceae (72)	1.3	1.3	NS
Pseudomonas spp. (18)	2.9	2.6	NS
Fungi (22)	4.3	3.1	<0.05

[a] NS, Not significant.

TABLE 6. Time of detection for growth in Signal and R-COL bottles

Organism (no.)	Mean time (day) of detection		P value
	Signal	R-COL	
Staphylococcus spp. (114)	1.9	2.4	<0.05
Streptococcus spp. (45)	1.1	1.3	NS[a]
Enterobacteriaceae (73)	1.2	1.2	NS
Pseudomonas spp. (15)	2.8	3.0	NS
Fungi (12)	3.5	3.5	NS

[a] NS, Not significant.

of 70 (16.1%) of the 436 positive bottles (note that there were 403 monomicrobic and 33 polymicrobic bottles) were positive by macroscopic examination only. The most common isolates detected macroscopically were yeasts (10 isolates), *Staphylococcus aureus* (8 isolates), anaerobes (4 isolates), and *Pseudomonas aeruginosa* (4 isolates).

The value of terminal subcultures was examined with the last 3,000 cultures that were processed in this study. A total of 11 isolates were recovered: 3 *Staphylococcus epidermidis*; 3 *Propionibacterium* spp.; 2 *Torulopsis glabrata*; and one isolate each of *Staphylococcus aureus*, viridans group streptococci, and *Escherichia coli*. The value of these terminal subcultures was limited and did not significantly increase the detection of clinically significant pathogens.

DISCUSSION

The Oxoid Signal blood culture system is a novel approach to nonautomated detection of positive blood cultures. Gas production during microbial metabolism of nutrients in the culture medium is detected easily by the transfer of broth from the culture bottle into the Signal chamber. The data presented herein demonstrated that recovery of organisms in the Oxoid Signal bottles was equivalent to that in the R-COL bottles but less than that in R-TSB bottles. Previous reports (11, 14) have documented that gelatin interferes with the recovery of *Staphylococcus* spp. and members of the family *Enterobacteriaceae*. Differences in the recovery of these organisms were not observed in the comparison of the gelatin-supplemented medium (Signal) and the unsupplemented medium (R-TSB). The time of detection was essentially identical with all three blood culture broths. Insertion of the Signal chamber was not associated with an increased incidence of contamination, in contrast with the use of the Roche Septi-Chek slide unit. However, despite the simplicity of the Signal system, some problems were encountered during this evaluation.

The Oxoid Signal system cannot be considered a one-bottle system. Although recovery of strictly anaerobic organisms was best in the Signal bottles, the growth of some aerobic organisms (e.g., *Pseudomonas* spp., *Acinetobacter* spp., and yeasts) was significantly decreased compared with that in the R-TSB bottles. This was most likely due to the presence of reducing agents in the Signal medium (i.e., sodium pyruvate, sodium thioglycolate, cysteine hydrochloride, and dithiothreitol). Previous studies (3, 6) suggested that the recovery of aerobic organisms could be improved with the use of agitation and terminal subcultures. However, we were unable to confirm this with the Signal system.

TABLE 7. Time of detection for growth in R-TSB and R-COL bottles

Organism (no.)	Mean time (day) of detection		P value
	R-TSB	R-COL	
Staphylococcus spp. (116)	2.0	2.2	NS[a]
Streptococcus spp. (41)	1.3	1.4	NS
Enterobacteriaceae (75)	1.3	1.5	NS
Pseudomonas spp. (16)	2.7	3.2	NS
Fungi (14)	3.0	3.9	<0.05

[a] NS, Not significant.

A large number of false-positive Signal bottles was observed during this study, with the largest proportion in bottles with <5 mm of broth in the Signal chamber. Elevated incubation temperatures can cause this effect. However, the temperatures were maintained at 35°C and were closely monitored throughout the study. We believe that the problem with false-positive Signal broths was related to the medium preparation. Throughout the course of this study, false-positive bottles were encountered. However, we had particular problems with one lot of medium in which virtually all bottles were positive after 1 to 2 days of incubation. During an evaluation of the Oxoid system at St. Louis Childrens Hospital, the same lot of medium was associated with a high incidence of false-positive bottles (G. Storch, personal communications). Our observation is consistent with that previously reported (E. B. Kaczmarski and G. J. Roberts, Letter, J. Clin. Microbiol. **26**:1434, 1988). In an effort to correct this problem, the manufacturer is carefully reexamining the process of media preparation and has also increased the length of the locking sleeve by 3 mm. Our experience with the Signal system indicates that this latter change will eliminate approximately half of the false-positive bottles. However, this will also increase the number of true-positive bottles that can only be detected by either macroscopic examination of the bottles or blind, terminal subcultures.

The presence of nonviable organisms also caused problems with the Signal system. These organisms resembled streptococci and were observed in five of the eight lots of media used during this study. We were able to demonstrate that the organisms were in the broth and were not introduced during laboratory processing by removing broth from uninoculated bottles, concentrating the broth by cytocentrifugation, and Gram staining the sediment. The same organisms were observed in all the stained broths. Because of this problem, the manufacturer has modified the preparation of the medium. It is anticipated that this step should eliminate the nonviable organisms.

Additional problems were encountered with the Signal system during the study. When the Signal chamber was attached to a small number of bottles, the rubber stopper was pushed into the broth. Oxoid reports that this problem has now been corrected by degreasing the stoppers during manufacturing and before they are inserted into the bottle. We also encountered problems with leakage at the point that the chamber assembly is inserted into the rubber stopper. This occurred most commonly when the needle was not inserted vertically into the bottle. When the locking sleeve was attached, a gap was created between the needle and the rubber stopper, with resulting leakage. In addition to the problem with leakage, gas could escape through this gap, potentially delaying or preventing the detection of positive cultures until macroscopic growth was observed. Many of these problems can be circumvented by carefully inserting the chamber assembly onto the bottle.

In summary, the Signal system is an ingenious approach to

the detection of pathogens in blood cultures that attempts to provide many of the advantages of standardized detection without the need for expensive instrumentation. This evaluation demonstrated that the system cannot be considered a single-bottle system because of the poor recovery of strictly aerobic organisms. However, excellent recovery of anaerobes was observed and justifies consideration the use of this system as a supplement to existing systems. Before this can be pursued, however, the problems associated with media preparation and system design will have to be resolved.

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