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Effect of Altered Headspace Atmosphere on Yield and Speed of Detection of the Oxoid Signal Blood Culture System versus the BACTEC Radiometric System

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The one-bottle Oxoid Signal blood culture system altered to provide a more aerobic bottle headspace was reassessed in a comparative study versus the two-bottle BACTEC radiometric system in 5,426 blood cultures. The BACTEC system detected more microorganisms ($P < 0.02$), particularly anaerobes ($P < 0.05$) and fungi ($P < 0.05$).

The Oxoid Signal blood culture system was initially devised as a potential one-bottle system for the detection of bacteremia and fungemia (3). In an initial comparative study versus the BACTEC radiometric system, the Oxoid system was processed without agitation and detected fewer microorganisms, most notably streptococci and aerobes including *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Haemophilus* spp., and fungi; and the speed of detecting positives was faster in the BACTEC system (5). In a second study, we agitated the Oxoid system for the first 24 to 48 h of incubation and performed terminal subcultures on negative Oxoid bottles on the seventh day of incubation. Performance of the Oxoid Signal system was improved, but the BACTEC system still detected more microorganisms, especially obligate aerobes such as *P. aeruginosa* and fungi; and the BACTEC speed advantage still was present (4). Based on these results, the manufacturer revised the headspace atmosphere such that it was no longer fully evacuated and devoid of oxygen. Murray et al. (2) compared this currently marketed version of the Oxoid Signal system with the Roche Septi-Chek system and found the aerobic Septi-Chek system superior for obligate aerobes and the Oxoid Signal system superior for anaerobes and viridans group streptococci. We report here our evaluation of this modification of the Oxoid Signal system versus the BACTEC radiometric system in 5,426 blood cultures at three university hospitals that used identical methods of obtaining and processing specimens.

The study materials and the methods used were identical to those described in our earlier report (4). Briefly, 20 ml of blood was obtained and distributed as follows: 10 ml to the Oxoid bottle, 5 ml to BACTEC 6B, and 5 ml to BACTEC 7D. Thus, the volume of blood inoculated into both systems was the same, and volume standards were used to ensure that the culture bottles actually received the specified amounts of blood. All patients with positive blood cultures were evaluated by an infectious disease specialist, who defined pathogens and contaminants by established criteria (6). Paired comparisons of the two blood culture systems were done only on adequately filled bottles that grew microorganisms causing true bacteremia and fungemia. Significance testing was done with the modified chi-square test of McNemar (1). When appropriate, the Yates correction for small numbers of observations was used.

A total of 5,426 adequately filled blood culture sets were studied. Of these, 722 (13.3%) were positive, including 482 (8.9%) that grew 519 microorganisms causing illness, 189 (3.5%) that grew one or more contaminants, 41 (0.7%) that grew one or more microorganisms that were indeterminate as a cause of sepsis, and 10 (0.2%) that grew a pathogen mixed with a contaminant or indeterminate isolate. Of the 519 clinically important microorganisms, 342 (65.9%) grew in both blood culture systems, 105 (20.2%) grew only in the BACTEC system, and 72 (13.9%) grew only in the Oxoid system.

Significantly more microorganisms ($P < 0.02$), especially anaerobes ($P < 0.05$) and fungi ($P < 0.05$), were detected by the BACTEC system (Table 1). Among the aerobic and facultative bacterial species or microorganism groups, there were no statistically significant differences.

Of the 342 microorganisms that grew in both systems, 223 (65.2%) were detected at the same time, 88 (25.7%) were detected earlier by the BACTEC system, and 32 (9.4%) were detected earlier by the Oxoid system (Table 2). In particular, staphylococci ($P < 0.05$) and *Acinetobacter* spp. ($P < 0.005$) were detected earlier by the BACTEC system.

The modified Oxoid Signal blood culture bottle used in this evaluation was an improvement over that used in our previous comparisons with the BACTEC radiometric two-bottle system. However, the BACTEC system still yielded more microroganisms and detected positive cultures earlier than did the Oxoid system. The change in headspace atmosphere of the Oxoid bottle, making it more aerobic, resulted in better detection of obligate aerobic bacteria, streptococci, and fungi but reduced detection of anaerobes and members of the family *Enterobacteriaceae* compared with our earlier studies (Table 3) (4, 5). In the current study, it was particularly noteworthy that statistical significance favoring the BACTEC system was achieved for the detection of both

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TABLE 1. Comparison of yields of clinically important bacteria and fungi from Oxoid Signal and BACTEC radiometric blood culture systems

Microorganism	No. of isolates from:			P
	Both systems	BACTEC only	Oxoid only	
Staphylococcus aureus	91	12	10	NS[a]
S. epidermidis	42	9	14	NS
Streptococci	61	11	12	NS
Other gram-positive bacteria[b]	3	1	1	NS
Escherichia coli	42	16	7	NS
Other *Enterobacteriaceae*	68	21	16	NS
Pseudomonas aeruginosa	8	7	2	NS
Other gram-negative bacteria[c]	14	4	3	NS
Anaerobic bacteria[d]	6	8	1	<0.05
Fungi[e]	7	16	6	<0.05
All microorganisms	342	105	72	<0.02

[a] NS, $P \geq 0.05$.
[b] Includes three *Corynebacterium* spp. and two *Corynebacterium* sp. group JK.
[c] Includes 14 *Acinetobacter calcoaceticus* subsp. *anitratus*, 3 *Haemophilus parainfluenzae*, and 1 each of *Campylobacter jejuni*, *Aeromonas* sp., *Capnocytophaga* sp., and *H. influenzae* type b.
[d] Includes two *Clostridium* spp., one *Peptostreptococcus prevotii*, one *Peptococcus* sp., seven *Bacteroides fragilis*, three *B. melaninogenicus*, and 1 *B. ureolyticus*.
[e] Includes 12 *Candida albicans*, 2 *C. parapsilosis*, 1 *C. tropicalis*, 1 *Candida* sp., 10 *Cryptococcus neoformans*, 2 *Torulopsis glabrata*, and 1 unidentified yeast species.

obligate anaerobes and fungi, which are obligate aerobes. Thus, it seems clear that the one-bottle Oxoid Signal system (and perhaps any one-bottle system) cannot provide an environment for growth that is optimal for detecting pathogens with requirements at either extreme of the atmospheric spectrum and that two-bottle systems may be inherently superior.

TABLE 2. Comparison of speeds of detection of clinically important bacteria and fungi from Oxoid Signal and BACTEC radiometric blood culture systems

Microorganism	No. of isolates from:			P
	Both at same time	BACTEC earlier	Oxoid earlier	
S. aureus	65	19	7	<0.05
S. epidermidis	17	17	8	NS[a]
Streptococci	49	9	3	NS
Other gram-positive bacteria[b]	0	3	0	NS
E. coli	32	6	5	NS
Other *Enterobacteriaceae*	50	12	6	NS
P. aeruginosa	4	3	1	NS
Other gram-negative bacteria[c]	3	11	0	<0.005
Anaerobic bacteria[d]	3	2	1	NS
Fungi[e]	0	6	1	NS
All microorganisms	223	88	32	<0.001

[a] NS $P \geq 0.05$.
[b] Includes two *Corynebacterium* sp. group JK and one *Corynebacterium* sp.
[c] Includes 11 *A. calcoaceticus* subsp. *anitratus*, 1 *Aeromonas* sp., 1 *Haemophilus influenzae*, and 1 *H. parainfluenzae*.
[d] Includes three *B. fragilis*, two *B. melaninogenicus*, and 1 *Peptococcus* sp.
[e] Includes two *C. albicans*, one *C. parapsilosis*, one *C. tropicalis*, and three *Cryptococcus neoformans*.

TABLE 3. Comparative yield of clinically important bacteria and fungi in three sequential evaluations of the Oxoid Signal and BACTEC radiometric blood culture systems[a]

Microorganism	Significance (P) of the following change in Oxoid Signal processing:		
	Static incubation (5)	Agitation and terminal subculture (4)	Current study
Staphylococci	NS	NS	NS
Streptococci	<0.05	NS	NS
Other gram-positive bacteria	NS	NS	NS
E. coli	NS	NS	NS
Other *Enterobacteriaceae*	NS	NS	NS
P. aeruginosa	NS	<0.05	NS
A. calcoaceticus	<0.001	NS	NS
Other gram-negative bacteria	NS	NS	NS
Anaerobic bacteria	NS	NS	<0.05
Fungi	<0.001	<0.005	<0.025
All isolates	<0.001	<0.005	<0.025

[a] NS, $P > 0.05$. All significant differences favor the BACTEC radiometric system.

As noted by Murray et al. (2), we experienced two additional technical problems with the Oxoid system during this study. We, too, noted false-positive signals in the upper chambers of the system, virtually always less than 5 mm above the outer green plastic sleeve that anchored the signal device to the neck of the bottle. In only 5 of 962 systematically monitored bottles was a false-positive signal >3 mm above the top of the sleeve, suggesting that the 3-mm elongation of the sleeve already implemented and marketed by the manufacturer will alleviate this problem. The second problem was the presence of nonviable organisms appearing as gram-positive cocci by staining. The manufacturer investigated this problem and subsequently modified its medium preparation. There was no recurrence of this problem during the remainder of the study. Leakage around the bottle stopper and pushing of the rubber stopper into the bottles by the Signal device, both noted by Murray and colleagues (2), were not encountered in our laboratories.

In summary, although this study has documented further improvement in the performance of the Oxoid system overall, its yield and speed of detecting positive blood cultures still were not equivalent to those of the two-bottle BACTEC radiometric system. The adjustment of headspace atmosphere to improve detection of aerobic microorganisms resulted in decreased detection of anaerobes, leading to the conclusion that, even at its optimum, this one-bottle system will not perform as well as a two-bottle system. Used without agitation or with a fully evacuated headspace, the Oxoid Signal system might complement the aerobic Roche Septi-Chek and decrease the technical effort associated with conventional processing of an anaerobic Septi-Chek bottle. Alternatively, the Oxoid system could be used to supplement another system in laboratories wishing to improve the detection of bacteremia by increasing the volume of blood obtained per culture. Development of a two-bottle Oxoid Signal system, one bottle designed for improved detection of

aerobes and the other primarily for anaerobes, could enhance the potential value of this system.

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