

Validation of Performance of Plastic versus Glass Bottles for Culturing Anaerobes from Blood in BacT/ALERT SN Medium

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To validate performance, we compared the new plastic BacT/ALERT (bioMérieux, Durham, NC) SN bottle to the current glass SN bottle with samples of blood obtained for culture from adults and found them comparable for both recovery and speed of detection of microorganisms. We conclude that the safety advantage of plastic bottles can be achieved without compromising performance.

Both aerobic and anaerobic blood culture media are commonly used to detect microorganisms from the blood of adult patients with suspected bloodstream infection. bioMérieux, Inc. (Durham, NC) has reformulated its anaerobic standard culture medium in a clear plastic bottle for use in the BacT/ALERT blood culture instrument. The design of this plastic bottle is similar to that described by Snyder et al. for an aerobic standard SA medium (6) and Petti et al. for the adult aerobic FA (4) and pediatric PF (5) media. These plastic bottles with a repertoire of media were designed to eliminate breakage of glass bottles and thereby to minimize potential safety hazards.

The new plastic SN (PSN) bottle contains a casein-soy-based medium similar to that in glass SN (GSN) except for formula modifications that include addition of yeast extract, pyridoxine HCl, and sodium pyruvate. The bottle has a smaller stopper and contains a modified liquid emulsion sensor to reduce the risk of false-positive bottles. To assess the result of these changes, we compared the new PSN bottle to the existing GSN bottle when used in conjunction with an aerobic SA bottle for both the recovery of microorganisms and the time to detection of the growth in samples of blood obtained from adults with suspected bloodstream infection.

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Blood was collected from adults with suspected bloodstream infection who presented to Duke University Medical Center from April through December 2002. Institutional review board approval was obtained prior to the study, and all blood cultures were performed as part of standard patient care. Thirty milliliters of blood was obtained by venipuncture, and 10-ml aliquots were distributed into aerobic SA and anaerobic PSN and GSN bottles. Upon receipt in the laboratory, each bottle was visually compared to bottles of the same composition and medium marked at 8- and 12-ml added volumes in accord with laboratory protocols (4, 5). Only bottles containing 8 to 12 ml of blood were included in the analysis. Gram stains were done

only on bottles flagged positive by the instrument, and these bottles were subcultured to appropriate media for subsequent identification of microorganisms by standard laboratory methods (3). Gram stain-negative bottles were returned to the instrument for the remainder of the 5-day incubation period or until they were reflagged by the instrument. False positives were defined as bottles that were Gram stain and subculture negative after the instrument signaled positive.

An infectious disease physician reviewed each positive culture and coded it as a true positive, a contaminant, or an isolate of unknown clinical importance based on previously published criteria (7). Episodes of bloodstream infection were defined by growth of a clinically important blood culture isolate without recovery of a different microorganism during the subsequent 7-day period. If a different clinically important microorganism was recovered within 72 h, the two isolates were considered a polymicrobial episode and were not included in the analysis. If a different clinically significant microorganism was recovered after 72 h, the second isolate was considered a new episode. Statistical analysis of results was performed with the modified chi-square test described by McNemar (1).

Of 5,323 anaerobic bottles received, 3,652 (69%) met the adequacy criteria, which is comparable to previous reports (4, 5). There were 357 isolates positive in one or both adequately filled bottles. Of these 357 isolates, 240 clinically significant microorganisms were isolated from 205 patients. Of the 240 isolates, 30 were fungi and 14 were aerobic gram-negative rods (e.g., *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*) that one would expect to isolate preferentially from an aerobic bottle (2). Therefore, we omitted these groups of microorganisms from further consideration to emphasize the relative performances of PSN and GSN for anaerobic and facultative bacteria. Overall, clinically significant isolates, including strict anaerobes, were detected with equal frequencies in both study bottles (Table 1). The relative paucity of anaerobes is in accord with trends of recent decades at similar centers (2). There were 136 isolates recovered from both PSN and GSN bottles within 5 days, and the mean times to detection (Table 2) were similar in both bottles (for PSN, 16.4 h; for GSN, 17.9 h). Among the 3,652 adequate paired blood culture bottles, there were 27 false-positive bot-

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TABLE 1. Comparative yields of clinically important isolates in PSN versus GSN anaerobic blood culture bottles

Microorganism	No. of isolates recovered from:			P
	Both bottles	PSN only	GSN only	
<i>Staphylococcus aureus</i>	33	8	3	NS ^a
Coagulase-negative staphylococci	7	5	0	NS
<i>Streptococcus</i> spp. ^b	10	2	4	NS
<i>Enterococcus</i> spp. ^c	31	4	5	NS
<i>Enterobacteriaceae</i> ^d	51	8	10	NS
Anaerobic bacteria ^e	4	8	3	NS
All microorganisms	136	35	25	NS

^a NS, not significant.

^b Includes viridans group streptococci ($n = 7$), *Streptococcus agalactiae* ($n = 5$), *Streptococcus pneumoniae* ($n = 3$), and *Streptococcus bovis* ($n = 1$).

^c Includes *Enterococcus faecium* ($n = 21$), *Enterococcus faecalis* ($n = 13$), *Enterococcus avium* ($n = 4$), and *Enterococcus gallinarum* ($n = 2$).

^d Includes *Klebsiella pneumoniae* ($n = 28$), *Escherichia coli* ($n = 16$), *Serratia marcescens* ($n = 8$), *Klebsiella oxytoca* ($n = 7$), *Enterobacter cloacae* ($n = 5$), *Enterobacter aerogenes* ($n = 2$), *Citrobacter freundii* ($n = 2$), and *Pantoea agglomerans* ($n = 1$).

^e Includes *Bacteroides thetaiotaomicron/ovatus* group ($n = 4$), *Bacteroides fragilis* ($n = 3$), *Clostridium clostridioforme* ($n = 1$), *Clostridium perfringens* ($n = 1$), *Clostridium ramosum* ($n = 1$), *Eubacterium lentum* ($n = 1$), *Peptostreptococcus* species ($n = 1$), *Bacteroides distasonis* ($n = 1$), *Bacteroides vulgatus* ($n = 1$), and *Prevotella bivia* ($n = 1$).

ties in PSN (0.7%) and 30 in GSN (0.8%). Contaminants were isolated with equal frequencies from PSN bottles and GSN bottles (both, 20; PSN, 31; GSN, 21; P , not significant).

An anaerobic medium is always used with a companion aerobic medium; therefore, we compared the detection of episodes of bacteremia or fungemia using all three bottles. A total of 5,308 blood culture sets that contained blood in all three bottles were received, including 3,408 (64%) with an adequate volume of blood in all three bottles. The episode analysis showed no statistically significant difference between episodes detected with SA/PSN and those detected with SA/GSN.

In this study, the overall performance of the anaerobic plastic bottle was similar to that of the glass bottle for both the recovery of clinically significant microorganisms and the time to detection of growth of anaerobic and facultative bacteria from adult patients with suspected bloodstream infection and is in accord with previously published reports with plastic PF medium for children and SA and FA media for adults (4–6). The plastic bottle is lighter than glass, resists breakage, and reduces the risk of exposure of healthcare personnel to infectious substances. Therefore, we recommend the use of PSN bottles in the BacT/ALERT blood culture system.

TABLE 2. Comparative times to positivity of clinically important bacteria and fungi when both PSN and GSN culture bottles were positive within 5 days

Microorganism	No. of isolates	Mean time to positivity (hours)	
		PSN	GSN
<i>Staphylococcus aureus</i>	33	17.5	24.1
Coagulase-negative staphylococci	7	13.4	14.8
<i>Streptococcus</i> spp. ^a	10	11.1	12.8
<i>Enterococcus</i> spp. ^b	31	20.9	18.5
<i>Enterobacteriaceae</i> ^c	51	12.1	12.2
Anaerobic bacteria ^d	4	44.1	54.9
All microorganisms	136	16.4	17.9

^a Includes *Streptococcus agalactiae* ($n = 4$), viridans group streptococci ($n = 3$), *Streptococcus pneumoniae* ($n = 2$), and *Streptococcus bovis* ($n = 1$).

^b Includes *Enterococcus faecium* ($n = 16$), *Enterococcus faecalis* ($n = 9$), *Enterococcus avium* ($n = 4$), and *Enterococcus gallinarum* ($n = 2$).

^c Includes *Klebsiella pneumoniae* ($n = 22$), *Escherichia coli* ($n = 10$), *Klebsiella oxytoca* ($n = 7$), *Enterobacter cloacae* ($n = 4$), *Serratia marcescens* ($n = 4$), *Citrobacter freundii* ($n = 2$), and *Enterobacter aerogenes* ($n = 2$).

^d Includes *Bacteroides fragilis* ($n = 2$), *Bacteroides thetaiotaomicron/ovatus* group ($n = 1$), and *Clostridium perfringens* ($n = 1$).

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