Controlled Clinical Comparison of BacT/ALERT Standard Aerobic and Standard Anaerobic Blood Culture Bottles Inoculated Directly or after Transport in Sodium Polyanethol Sulfonate Tubes[▽]

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To assess relative performances in the BacT/ALERT blood culture system, we compared results from the direct inoculation of standard media and inoculation after the transport of blood samples in Vacutainer tubes with sodium polyanethol sulfonate. No significant differences in yields or times to detection were found for 387 clinically important isolates from 4,306 blood culture sets.

Sodium polyanethol sulfonate (SPS) is a common component of blood culture media, owing to its anticoagulant, anti-complement, and antiphagocytic properties (2, 3, 17, 18). It also inactivates lysozyme, aminoglycosides, and polymyxins (1). Although SPS has been shown to improve the recovery of commonly isolated microorganisms in older medium formulations (3, 5, 8, 12, 13, 16), it inhibits the growth of *Peptostreptococcus anaerobius*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Streptobacillus moniliformis*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, and *Capnocytophaga* spp. (4, 7, 11, 14).

For four decades, Vacutainer SPS tubes (BD Diagnostic Systems, Franklin Lakes, NJ) have been used at the Durham Veterans Affairs Medical Center, Durham, NC, and other institutions to transport whole blood until it can be inoculated into culture media. The historical advantages of this approach include being able to inoculate a variety of culture media and having plasma for serological testing (15). The effect that SPS transport tubes have on the yields from currently used blood culture media, however, has not been reported. Furthermore, the transfer of tube contents poses a percutaneous injury risk to laboratory staff. Consequently, we performed a controlled comparison of direct inoculation into standard BacT/ALERT (BA; bioMérieux, Inc., Durham, NC) blood culture bottles and inoculation after transport in SPS tubes.

(This work was presented in part at the 104th General Meeting of the American Society for Microbiology in 2004 [B.C. Pien, S. Mirrett, B. Crews, L. B. Reller, and C. W. Woods, abstr. C080].)

Blood cultures were collected from adult patients at the Durham Veterans Affairs Medical Center between January 2003 and March 2005. Institutional Review Board approval was obtained, and all blood cultures were performed as part of routine care. Four-bottle kits were provided with instructions to obtain 30 ml of blood per set and to distribute 7.5 ml sequentially into a standard aerobic (SA) bottle, an SPS tube,

a standard anaerobic (SN) bottle, and a second SPS tube without changing needles. Uninoculated SPS tubes contained 1.7 ml of 0.35% SPS in 0.85% sodium chloride. The SA and SN bottles each contained 40 ml of supplemented tryptic soy broth with 0.035% SPS.

The filling adequacy (adequate, underfilled, or overfilled) of each bottle was measured against marked volumetric standards; 6 to 9 ml of blood was considered adequate. All bottles were processed regardless of the volume received. The contents of each SPS tube were transferred into additional SA and SN bottles in a biological safety cabinet. All four bottles were loaded onto the BA instrument and incubated for 5 days or until they were flagged as positive. The contents from flagged bottles were subcultured, and isolates were identified according to standard techniques (10).

An infectious disease physician reviewed the record of each patient with a positive blood culture to determine whether an isolate represented true infection, contamination, or unknown clinical importance. These assessments were made in accordance with published criteria (19). An episode of bacteremia or fungemia was defined as a period beginning with the first positive blood culture and ending when 7 days had passed without another blood culture positive for the same microorganism.

We compared rates of recovery from sets of adequately filled bottles with the chi-square test of McNemar by using Stata 9 (StataCorp, College Station, TX) and reported results as a two-sided *P* value. A prespecified α significance level of 0.05 was used. Mean times to detection were compared using the Wilcoxon rank sum test for nonparametric data. Assuming 100% specificity for either blood culture method when a clinically significant isolate was obtained, test sensitivities with 95% confidence intervals (95% CI) were calculated for each blood culture method.

Of the original 8,788 sets of blood cultures, 937 (11%) were positive with one or more isolates. Among the 4,306 (49%) adequately filled four-bottle sets, there were 387 (9.0%) with clinically significant isolates, representing 293 episodes, and 164 (3.8%) with one or more contaminants. No needle stick injuries occurred in the microbiology laboratory during the study period.

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TABLE 1. Comparative yields of clinically important isolates after SPS transport and after direct inoculation into BacT/ALERT SA and SN bottles

Microorganism(s)	No. of isolates detected by:			<i>P</i> value
	Both direct and SPS methods	Direct SA-SN method only	SPS SA-SN method only	
<i>Staphylococcus aureus</i>	83	9	13	0.39
Coagulase-negative staphylococci	11	3	0	0.25
<i>Streptococcus</i> spp.	16	2	2	1.00
<i>Enterococcus</i> spp.	23	4	2	0.69
Gram-positive bacilli	1	0	2	0.50
<i>Enterobacteriaceae</i>	76	15	12	0.56
Other gram-negative bacilli	14	5	5	1.00
Anaerobic bacteria	8	6	3	0.51
Yeasts	56	8	8	1.00
All microorganisms	288	52	47	0.62

We did not find any significant differences between the methods in rates of recovery of staphylococci, *Enterobacteriaceae*, nonenteric gram-negative bacilli, anaerobes, *Candida* spp. and *Cryptococcus* spp., and all clinically significant microorganisms combined (Table 1). Both methods also had similar levels of detection of clinically important episodes, with mean overall sensitivities of 91% (95% CI, 88 to 94%) and 92% (95% CI, 88 to 95%) for direct inoculation and the SPS tube method, respectively (Table 2). The mean times to detection and the comparative yields of clinically significant isolates obtained while patients received effective antimicrobial therapy also did not differ significantly (data not shown); however, the SPS tubes yielded fewer contaminating microorganisms ($P = 0.03$; Table 3).

The SPS transport tube was designed to allow microbiologists to inoculate different media in the laboratory without stocking the more expensive blood culture bottles in hospital units or clinics. Although the laboratory time required to transfer tube contents into blood culture media and the finite needle stick injury risk may increase net expenses, about 7 million SPS tubes are purchased annually (B. McLaughlin, personal communication). Furthermore, the SPS tube may be useful for direct PCR detection of bloodstream pathogens. Although SPS tends to copurify with DNA and inhibit PCR, a

TABLE 3. Comparative yields of contaminant isolates after SPS transport and after direct inoculation into BacT/ALERT SA and SN bottles

Microorganisms	No. of isolates detected by:			<i>P</i> value
	Both methods	Direct method only	SPS method only	
Coagulase-negative staphylococci	44	51	29	0.02
Other gram-positive cocci	7	3	4	1.00
Gram-positive bacilli	6	11	9	0.82
All microorganisms	57	65	42	0.03

benzyl alcohol organic extraction procedure has been described previously (6).

BA bottles inoculated with blood from the SPS transport system have a final SPS concentration slightly higher than those inoculated directly (0.041% compared to 0.030%). However, blood may remain for several hours in the transport tubes containing a concentration of SPS (0.065%) twice that of directly inoculated BA bottles. This difference could affect the recovery of clinically important isolates and contaminants in laboratories that are located offsite or that are not operated 24 h daily.

It was unexpected to find that the SPS transport tubes had fewer contaminants, since the use of the tubes requires an additional specimen transfer step which may increase the risk of contamination. The reason for this observation, however, is not clear in that SPS tubes do not appear to inhibit the detection of clinically significant staphylococci. Two previous studies showed that the addition of SPS was associated with increased contamination; however, transport tubes were not used and the comparison media did not contain SPS (3, 9).

In conclusion, no significant differences in the rates of recovery of clinically important isolates after transport in SPS tubes and after direct inoculation into standard BA bottles were detected. Although fewer contaminating microorganisms may be recovered, transport in SPS tubes requires additional processing in the laboratory, including the transfer of blood.

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TABLE 2. Comparative yields of clinically important episodes detected after SPS transport and after direct inoculation into BacT/ALERT SA and SN bottles

Microorganism(s)	No. of episodes detected by:			% Sensitivity (95% CI) of:		<i>P</i> value
	Both methods	Direct method only	SPS method only	Direct method	SPS method	
<i>Staphylococcus aureus</i>	78	5	5	94 (87–98)	94 (87–98)	1.00
Other gram-positive bacteria	40	2	4	91 (79–98)	96 (85–99)	0.67
<i>Enterobacteriaceae</i>	75	7	8	91 (83–96)	92 (85–97)	0.80
Other gram-negative bacilli	13	6	2	86 (64–97)	71 (48–89)	0.29
Anaerobic bacteria	4	2	2	75 (35–97)	75 (35–97)	1.00
Yeasts	34	2	4	90 (76–97)	95 (83–99)	0.69
All microorganisms	244	24	25	91 (88–94)	92 (88–95)	0.89

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