Controlled Clinical Comparison of BacT/ALERT SA and SN Blood Culture Bottles

Inoculated Directly or after Transport in Sodium Polyanethol Sulfonate Tubes

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ABSTRACT (49 WORDS)

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

TEXT (1040 WORDS)

Sodium polyanethol sulfonate (SPS) is a common component of blood culture media, owing to its anticoagulant, anticomplement, 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 media formulations (3,6,8,12,13,16), it inhibits the growth of *Peptostreptococcus anaerobius*, *Neisseria gonorrhoeae*, *N. meningitidis*, *Streptobacillus moniliformis*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, and *Capnocytophaga* spp.(4,7,11,14).

For four decades a Vacutainer SPS tube (BD Diagnostic Systems, Franklin Lakes, N.J.) has been used by the Durham VA Medical Center (DVAMC) 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 yield of current 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, N.C.) blood culture bottles versus inoculation after transport in SPS tubes.

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

Blood cultures were collected from adult patients at DVAMC 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 supplemented tryptic soy broth with 0.035\% SPS.

Filling adequacy (adequate, underfilled, or overfilled) of each bottle was measured against marked volumetric standards; 6 to 9 mL of blood were 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 flagged positive. 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 positive blood culture with the same microorganism.

We compared recovery rates from sets of adequately filled bottles with the chi-square test of McNemar using Stata 9 (StataCorp, College Station, TX) and reported results as a two-sided p-value. A pre-specified $\alpha$ significance level of 0.05 was used. Mean time-to-detection was compared using the Wilcoxon rank sum test for non-parametric data. Assuming 100% specificity for each clinically significant blood culture arm, test sensitivities with 95% confidence intervals (95%CI) were calculated.

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 $\geq$1 contaminants. No needlestick injuries occurred in the microbiology laboratory during the study period.

In the comparative yield in isolate recovery, we did not find any significant differences for staphylococci, \textit{Enterobacteriaceae}, non-enteric gram-negative bacilli, anaerobes, yeasts, and all clinically significant microorganisms combined (Table 1). Both methods also had similar detection of clinically important episodes with mean overall sensitivities of 91% (95%CI 88-94%) and 92% (95%CI 88-95%) for direct inoculation and the SPS tube, respectively (Table 2). The mean time-to-detection and the comparative yield of clinically significant isolates obtained
while patients received effective antimicrobial therapy also did not differ significantly (data not shown), however, the SPS tube 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 transport tube contents into blood culture media and finite needlestick injury risk may increase net expenses, about 2.8 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 benzyl alcohol organic extraction procedure has been described (5).

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

It was unexpected to find that the SPS transport tube had fewer contaminants, since its use requires an additional specimen transfer which may increase contamination risk. 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 recovery of clinically important isolates
were detected after transport in SPS tubes compared to direct inoculation into standard BA
bottles. Although fewer contaminating microorganisms may be recovered, transport in SPS
tubes requires additional processing in the laboratory, including transfer of blood.

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Institute of Allergy and Infectious Diseases (CWW). Media was provided in part by bioMérieux,
Inc., Durham, N.C.
References


Table 1. Comparative yield of clinically important isolates in SPS transport versus direct inoculation into BacT/ALERT SA and SN bottles

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

Table 2. Comparative yield of clinically important episodes in SPS transport versus direct inoculation into BacT/ALERT SA and SN bottles

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Both Direct only</th>
<th>SPS only</th>
<th>Percent sensitivity (95% CI)</th>
<th>Percent sensitivity (95% CI)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>78</td>
<td>5</td>
<td>94 (87-98)</td>
<td>5</td>
<td>94 (87-98)</td>
</tr>
<tr>
<td>Other gram-positive bacteria</td>
<td>40</td>
<td>2</td>
<td>91 (79-98)</td>
<td>4</td>
<td>96 (85-99)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>75</td>
<td>7</td>
<td>91 (83-96)</td>
<td>8</td>
<td>92 (85-97)</td>
</tr>
<tr>
<td>Other gram-negative bacilli</td>
<td>13</td>
<td>6</td>
<td>86 (64-97)</td>
<td>2</td>
<td>71 (48-89)</td>
</tr>
<tr>
<td>Anaerobic bacteria</td>
<td>4</td>
<td>2</td>
<td>75 (35-97)</td>
<td>2</td>
<td>75 (35-97)</td>
</tr>
<tr>
<td>Yeasts</td>
<td>34</td>
<td>2</td>
<td>90 (76-97)</td>
<td>4</td>
<td>95 (83-99)</td>
</tr>
<tr>
<td>All microorganisms</td>
<td>244</td>
<td>24</td>
<td>91 (88-94)</td>
<td>25</td>
<td>92 (88-95)</td>
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</tbody>
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Table 3. Comparative yield of contaminant isolates in SPS transport versus direct inoculation into BacT/ALERT SA and SN bottles

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Both</th>
<th>Direct only</th>
<th>SPS only</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci</td>
<td>44</td>
<td>51</td>
<td>29</td>
<td>0.02</td>
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<tr>
<td>Other gram-positive cocci</td>
<td>7</td>
<td>3</td>
<td>4</td>
<td>1.00</td>
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<tr>
<td>Gram-positive bacilli</td>
<td>6</td>
<td>11</td>
<td>9</td>
<td>0.82</td>
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<tr>
<td>All microorganisms</td>
<td>57</td>
<td>65</td>
<td>42</td>
<td>0.03</td>
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