

## Effect of Sodium Polyanetholesulfonate and Gelatin on the Recovery of *Gardnerella vaginalis* from Blood Culture Media

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**Sodium polyanetholesulfonate (SPS) is used as a routine supplement to blood culture media to enhance recovery of microorganisms, but it inhibits the growth of *Peptostreptococcus anaerobius*, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and *Streptobacillus moniliformis*. Comparative clinical blood culture studies at the University of Colorado Hospital suggested that SPS also inhibits the growth of *Gardnerella vaginalis*. We inoculated 16 blood culture isolates of *G. vaginalis* into 11 blood culture media containing SPS or sodium amylosulfate, with and without gelatin. In the absence of gelatin, only brain heart infusion and thiol broths with SPS supported the growth of more than five strains of *G. vaginalis*, whereas all media except Bactec 6B and 7C and brucella broths recovered most isolates with SPS and gelatin or with sodium amylosulfate alone. We conclude that SPS inhibits the growth of *G. vaginalis* in blood culture media but that this inhibition is medium dependent and can be overcome by supplementation of most media with gelatin.**

Sodium polyanetholesulfonate (SPS) is now routinely added to most commercial blood culture media as an anticoagulant (16). SPS also inhibits phagocytosis and complement; precipitates lipoprotein, fibrinogen, C3, C4, and immunoglobulin G; and inactivates aminoglycosides and polymyxins (2, 20). The recovery of most microorganisms from blood cultures is improved by the presence of SPS. However, SPS has been shown to inhibit the growth of *Peptostreptococcus anaerobius* (8), *Neisseria gonorrhoeae* (18), *Neisseria meningitidis* (6), and *Streptobacillus moniliformis* (9). The adverse effect of SPS on each of these organisms has been reduced by the addition of gelatin to SPS-containing media (7, 12, 17, 23).

In ongoing blood culture studies at the University of Colorado Hospital, strains of *Gardnerella vaginalis* were recovered only in blood culture media that contained the anticoagulant sodium amylosulfate (SAS) instead of SPS or in supplemented peptone broth that contained SPS and 1.2% gelatin. To determine if *G. vaginalis* is also inhibited by SPS, we inoculated clinical strains of *G. vaginalis* into 11 blood culture media with SAS or SPS and with and without gelatin.

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### MATERIALS AND METHODS

**Bacteria.** We tested 16 blood culture isolates of *G. vaginalis* from the University of Colorado Hospital (confirmed by the Centers for Disease Control) in all media, and 37 cervical isolates (19 from the University of Colorado Hospital and 18 provided by K. K. Holmes of the University of Washington) in Trypticase soy broth (TSB) (BBL Microbiology Systems, Cockeysville, Md.) only.

**Media.** We inoculated the *G. vaginalis* strains into the following commercially prepared broths: BACTEC 6B and 7C (Johnston Laboratories, Inc., Towson, Md.); brain heart

infusion, brucella, tryptose phosphate, and thiol (Difco Laboratories, Detroit, Mich.); and Columbia, supplemented peptone, thioglycolate, TSB, and TSB modified by addition of gelatin and 1% yeast extract (Becton Dickinson Vacutainer Systems, Rutherford, N.J.). Each medium contained either 0.03% SPS or 0.05% SAS. All media containing SPS were tested before and after addition of enough gelatin to make a final concentration of 1.2%. Gelatin was prepared by making a solution of dehydrated gelatin (Difco) and water. The solution was filter sterilized before being dispensed into culture bottles. Special lots of Columbia, supplemental peptone, thioglycolate, thiol, and tryptose phosphate broths with SAS or without gelatin were provided by Difco and Becton Dickinson Vacutainer Systems. Since initial trials showed that only sporadic growth of *G. vaginalis* occurred without human blood, all broths were tested with 5% outdated human blood. Preliminary studies also showed that outdated blood performed as well as freshly drawn blood in supporting the growth of *G. vaginalis*.

**Inocula.** Inocula were prepared from stock cultures that had been stored at  $-70^{\circ}\text{C}$  in 50% TSB and 50% fetal calf serum with 1% yeast extract. Stock strains were grown overnight on brucella blood agar plates (brucella blood agar base [Difco]) with 5% defibrinated sheep blood, 5 g of hemin (Eastman Kodak Co., Rochester, N.Y.) per ml and 10 g of vitamin K (ICN Pharmaceuticals Inc., Cleveland, Ohio) per ml. Organisms were suspended and diluted in saline to final inocula of  $10^2$  and  $10^4$  organisms per ml as confirmed by a drop count method (10).

Inoculum (1 ml) was then added to each test broth. Cultures were vented, incubated at  $35^{\circ}\text{C}$  in air, and subcultured when visually positive or at 7 and 14 days to brucella blood agar plates. Work with *G. vaginalis* in our laboratory has shown this schedule for blind subcultures to be adequate for recovery. Recovery of *G. vaginalis* was confirmed by Gram-stained smear, typical colony morphology, and growth inhibition by alpha-hemolytic streptococci (4).

Some strains lost viability upon storage. Since all experiments were not done at the same time, some broths could not be tested with all of the original strains.

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TABLE 1. Recovery of *G. vaginalis* in blood culture media with SAS, SPS, or SPS and gelatin

Medium (no. tested)	No. of strains recovered with:		
	SAS	SPS	SPS + gelatin
BACTEC 6B (14)	— <sup>a</sup>	2	5
BACTEC 7C (14)	—	6	5
Brain heart infusion (11)	—	11	11
Brucella (16)	—	3	3
Columbia (16)	15	2	13
Supplemented peptone (16)	16	0	16
Thioglycolate (16)	16	3	13
Thiol (7)	6	5	6
TSB (16)	16	0	16
TSB modified <sup>b</sup> (16)	—	—	16
Tryptose phosphate (7)	—	3	5

<sup>a</sup> —, Not tested.

<sup>b</sup> TSB with 1.2% gelatin and 1% yeast extract.

## RESULTS

The number of blood culture strains of *G. vaginalis* recovered in each medium with an inoculum of  $10^2$  CFU is shown in Table 1. The majority of strains were recovered in most media with either SAS or SPS and gelatin. However, fewer than half the strains grew in BACTEC 6B and 7C and brucella broths with SPS, even with added gelatin. Most broths with SPS but without gelatin did not support growth of *G. vaginalis*. The exceptions were brain heart infusion broth, in which all strains grew, and thiol broth, in which five of seven strains grew even without gelatin.

By using an inoculation of  $10^4$  organisms, we recovered two additional strains in brucella broth with SPS, one in brucella broth with SPS and gelatin, three in supplemented peptone broth with SPS, and one in thiol broth with SAS.

Of 37 cervical isolates tested, only 4 grew in TSB with SPS; 17 grew in TSB with SPS and gelatin; 35 grew in TSB with SPS, gelatin, and yeast extract; and all 37 grew in TSB with SAS.

## DISCUSSION

The value of SPS as a supplement to blood culture media is well recognized (16). However, its adverse effect on a few species of bacteria, in particular *P. anaerobius*, *N. gonorrhoeae*, *N. meningitidis*, and *S. moniliformis*, is also known (6, 8, 9, 18). In fact, inhibition of *P. anaerobius* by SPS is used for identification of this species (22).

Wilkins and West (23) showed that inhibition of *P. anaerobius* by SPS was medium dependent and suggested that inhibition was overcome by the presence of gelatin or proteose peptone in the media. Eng and Holten (7) showed that inhibition of *N. meningitidis* by SPS could be eliminated by 1.2% gelatin in laboratory-inoculated cultures, and Pai and Sorger (12) confirmed both the inhibition of *N. meningitidis* by SPS and the neutralization of this effect by 1% gelatin in clinical blood cultures. In simulated blood cultures, Staneck and Vincent (18) demonstrated inhibition of *N. gonorrhoeae* that could be overcome by the addition of 1% gelatin. In contrast, Rintala and Pollock (17) showed that when yeast extract was present in media with SPS and gelatin, the recovery of *N. meningitidis* was less than in media with SPS and gelatin alone.

The present study suggests that *G. vaginalis* should be included among the bacteria inhibited by SPS. Gelatin appears to protect *G. vaginalis* in SPS-containing media, since most or all of our strains were recovered in media

containing this combination, and five or fewer were recovered without gelatin. Notable exceptions were brain heart infusion broth, in which all strains grew, and BACTEC and brucella media, in which fewer than half the strains grew with or without gelatin. The results for BACTEC media are interesting in comparison with those for TSB, since both are tryptic digest broths. The difference again demonstrates the variability of blood culture products from different manufacturers (16). Whereas yeast extract may enhance the inhibition of *N. meningitidis* by SPS, there was no adverse effect against *G. vaginalis* when yeast extract was added to TSB with SPS and gelatin.

Recently, Edberg and Edberg (5) suggested that the effect of SPS is also neutralized by hemoglobin. The concentration of free hemoglobin in the outdated blood we used was 0.7 g/100 ml. After dilution in the media its concentration was 0.035 g/100 ml, a concentration much lower than that suggested to have an effect (5). We do not believe our results were affected by the addition of blood to the media.

Although commonly associated with bacterial vaginosis, *B. vaginalis* is among the normal colonizing flora of young women (13). Bacteremia with *G. vaginalis* has been reported rarely, and almost exclusively in women at the time of parturition or abortion. Since bacteremia with *G. vaginalis* usually has a benign course (1, 3, 11, 14, 15, 21), the failure of blood culture media to grow *G. vaginalis* could easily be overlooked clinically. Our work suggests that *G. vaginalis* sepsis is more common than earlier studies indicate. The largest series of patients previously described included 29 patients collected over 14 years (21). We have reported 30 episodes of *G. vaginalis* sepsis over a 4-year period in which supplemented peptone broth with SPS and gelatin was used (15).

The replacement of SPS with SAS as the anticoagulant of choice in blood culture media may be considered; in this study, media with SAS were as good as or better than media with SPS and gelatin for the strains tested. However, other studies from our institution have shown that SAS inhibits the growth of gram-positive cocci, especially *Staphylococcus aureus* (19). Since aerobic gram-positive cocci cause a much larger number of bacteremias, we would not recommend changing from media containing SPS. A reasonable solution to ensure recovery of *G. vaginalis* as well as *P. anaerobius*, *N. gonorrhoeae*, *N. meningitidis*, and *S. moniliformis* would be to include a medium which contains gelatin in addition to SPS. The brucella and BACTEC media tested in this study, however, should not be relied on for recovery of *G. vaginalis*.

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