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Comparison of Different Medium Bases for the Semiquantitative Isolation of Anaerobes from Vaginal Secretions

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Two studies were performed to determine the best medium for the isolation of anaerobes from vaginal secretions. In the first, three different medium bases (brucella, Centers for Disease Control [CDC], and Schaedler) were compared semiquantitatively for ability to support the growth of gram-negative anaerobes from vaginal fluid. Media were supplemented with laked sheep blood, kanamycin, and vancomycin. The brucella base agar formulation supported the growth of anaerobic gram-negative bacilli better than either the CDC or Schaedler base agar formulation. In a second study, nonselective brucella and CDC base sheep blood agar were compared for ability to support the growth of anaerobic gram-positive cocci. Anaerobic gram-positive cocci grew in higher concentrations on CDC base agar than on brucella base agar. On the basis of these observations, we recommend that a CDC base sheep blood agar be used for the nonselective plate and a brucella base plate supplemented with laked sheep blood, kanamycin, and vancomycin be used for isolation of gram-negative bacilli in studies of the anaerobic flora of the female genital tract.

In 1984, we began a study of the effect of genitourinary tract microorganisms on pregnancy outcome. As part of this study, vaginal secretions were studied for the presence of aerobes, anaerobes, mycoplasmas, yeasts, and *Trichomonas vaginalis*. Three brucella agar base sheep blood media were used for the isolation of anaerobes, including a kanamycin and vancomycin (KV) selective plate with laked blood, a plate supplemented with phenylethyl alcohol (PEA), and a nonselective plate. Initially, all media were prepared in our laboratory, but midway through the study we changed to commercially prepared sheep blood medium, which used the Centers for Disease Control (CDC) agar base formulation (8) instead of the brucella agar base. Based on information contained in a standard text (1), there was little reason to believe that this medium change would have any significant effect on isolation rates. Tests of both media, using quality control strains of *Peptostreptococcus anaerobius*, *P. asaccharolyticus*, and *Bacteroides fragilis* as well as parallel inoculation of clinical specimens for 2 weeks, did not indicate a difference in organism growth between the CDC agar base medium and our in-house-produced brucella agar base medium. However, several months after initiating the medium change, we noted an apparent decline in the *Bacteroides* species isolation rate. At the same time, our isolation rate of anaerobic gram-positive cocci appeared to have increased. Therefore, we undertook two studies to determine the optimal medium for recovering anaerobes from vaginal secretions. The first study was designed to determine the optimal agar base for the isolation of gram-negative anaerobes on KV media, and the second was designed to compare brucella and CDC agar bases for the isolation of anaerobic gram-positive cocci. A review of the literature revealed only a few comparative studies of selective media for isolation of anaerobes (1, 2, 4). Even less information was available concerning differences in basal media (3, 4). Murray (4) compared five basal media, including brucella agar and Schaedler agar, and found no significant differences among them, but he studied stock organisms rather than clinical isolates.

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MATERIALS AND METHODS

Subjects. Normal pregnant women between weeks 23 and 26 of gestation were enrolled in the study at Charity Hospital in New Orleans, La., as part of a multicenter research protocol designed to determine the effect of various genitourinary tract microorganisms on pregnancy outcome. Specimens from 76 women were included in the study of anaerobic gram-negative rods (enrollment dates, 25 April 1988 to 16 May 1988), and specimens from 169 women were used in the study of gram-positive cocci (enrollment dates, 18 July 1988 to 22 August 1988).

Media. (i) Study 1. Brucella-based laked sheep blood KV agar was prepared in-house, using 43 g of brucella agar base (Difco Laboratories, Detroit, Mich.) with the addition of 5 g of hemin, 10 mg of vitamin K_1, 100 mg of kanamycin, 7.5 mg of vancomycin, and 50 ml of laked sheep blood per liter of distilled water. Preparation of this medium has been described in detail by Sutter et al. (8). Commercial brucella base KV medium was obtained from Scott Laboratories, Fiskeville, R.I. CDC agar base KV media were obtained from Scott Laboratories, from Remel, Lenexa, Kans., and from BBL Microbiology Systems, Cockeysville, Md. Schaedler agar base KV medium was obtained from BBL.

(ii) Study 2. Study 2 was designed to compare brucella base with CDC base sheep blood agar for the isolation of gram-positive anaerobes. Media used included CDC agar base anaerobic medium with 5% sheep blood with and without PEA (CDC-BA, CDC-PEA), obtained from BBL. Anaerobic brucella base sheep blood agar (brucella-BA) was obtained from Scott Laboratories. Brucella base PEA blood agar was made in-house as described previously (1).

Semiquantitative culture technique. Vaginal wash fluid was obtained by injection of 3 ml of prereduced sterile saline into the posterior vagina after insertion of a plastic speculum. The vaginal pool was then aspirated into a sterile syringe and transferred to a Port-a-Cul vial (BBL). Specimens were transported to the laboratory and plated to media within 4 h.

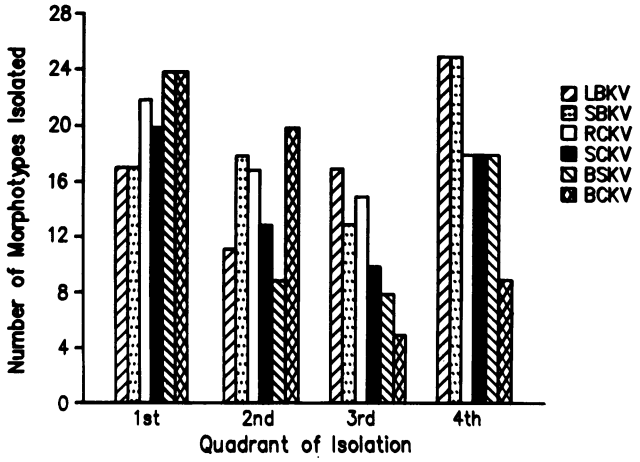

FIG. 1. Highest growth quadrant of anaerobic organisms isolated after 96 h of incubation from 76 vaginal secretion samples in each of six different laked sheep blood KV plates. Media used were as follows: LBKV, in-house brucella base KV agar plate; SBKV, brucella base KV agar plate, Scott Laboratories; RCKV, CDC base KV agar plate, Remel; SCKV, CDC base KV agar plate, Scott; BSKV, Schaedler base KV agar plate, BBL; BCKV, CDC base KV agar plate, BBL.

All media used in both studies were held in an anaerobic atmosphere for at least 4 h prior to inoculation. Each test plate was inoculated with 0.05 ml of vaginal wash and streaked to four quadrants for isolation. Semiquantitative growth of organisms was measured as 1+ to 4+ depending on the highest plate quadrant in which organisms were detected. Plates were incubated at 35°C in an anaerobic chamber (Forma Scientific, Marietta, Ohio), and all plates were examined for growth at 48 and 96 h. In study 1, all bacterial colonial morphotypes observed on each plate were noted and recorded, but purification and identification were carried out only for organisms growing in the third and fourth quadrants. In study 2, only organisms growing in the third and fourth streak areas of the nonselective plates under study were isolated and identified. One of the goals of the clinical study was the diagnosis of bacterial vaginosis, and the protocol we were following called for identification of only those anaerobes present in highest concentrations.

Organism identification. Organisms were first confirmed as anaerobes by inoculation to chocolate agar and incubation at 35°C in 5% CO_2 for 48 h. Isolates not growing on chocolate agar were Gram stained and tested for catalase production and fluorescence under long-wave UV. Pure anaerobic isolates were identified to the species level, using gas-liquid chromatographic patterns and Minitek (BBL) biochemical profiles (7, 8).

RESULTS

Study 1: comparison of medium bases used in laked sheep blood agar KV plates for isolation of gram-negative anaerobes. Of 76 specimens evaluated, growth of anaerobic organisms was detected on at least one of the six KV plates in 57 (75%) of the specimens. Figure 1 shows the recovery of gram-negative morphotypes from the six KV plates by the highest quadrant in which growth was detected at 96 h. Growth in the third and fourth quadrants was observed more commonly on both brucella/KV plates than in any of the other four plates. Of the 76 patients studied, 32 had ≥3+ growth of anaerobic gram-negative rods on at least one of the six KV plates tested. Of these 32, 22 patients yielded one isolate, 8 yielded two isolates, and 2 yielded three or more isolates for a total of 45 organisms isolated at the ≥3+ level. All of these 32 women were diagnosed by Gram stain criteria as having bacterial vaginosis. The most common organism isolated was *B. bivius* (64%), followed by *B. disiens* (11%) (Table 1). Figure 2 compares the recovery of different *Bacteroides* species in the third and fourth plate quadrants after 96 h of incubation on each of the KV plates tested. *B. bivius* was recovered more often in high concentration on both brucella base media tested than on any of the CDC or Schaedler base medium formulations. The numbers of *B. disiens* and other *Bacteroides* spp. isolated were small, and there was not a clear difference between the medium tested and its ability to support growth of these organisms in high concentrations, though the two brucella base plates clearly performed at least as well as any of the other four plates.

TABLE 1. Organisms recovered from vaginal secretions by using KV selective agar plates

Organisms (n = 45)	No. recovered	% of total
B. bivius	29	64
B. disiens	5	11
B. melaninogenicus	2	4
Bacteroides sp.	2	4
B. capillosus	3	6
B. asaccharolyticus	1	2
B. thetaiotaomicron	1	2
B. intermedius	1	2
Fusobacterium sp.	1	2

The total number of organisms growing in the third and fourth quadrants of either of the two brucella/KV plates was compared with the total number of organisms growing on all three possible combinations of two CDC/KV plates. Of the possible total of 45, 42 grew on at least one of the brucella/KV plates, while the highest number growing on at least one of any two CDC/KV plates was 33 ($P < 0.01$ by McNemar's test for correlated proportions).

Study 2: comparison of CDC agar base media with brucella agar base media for isolation of gram-positive anaerobes. CDC base nonselective medium provided an advantage for

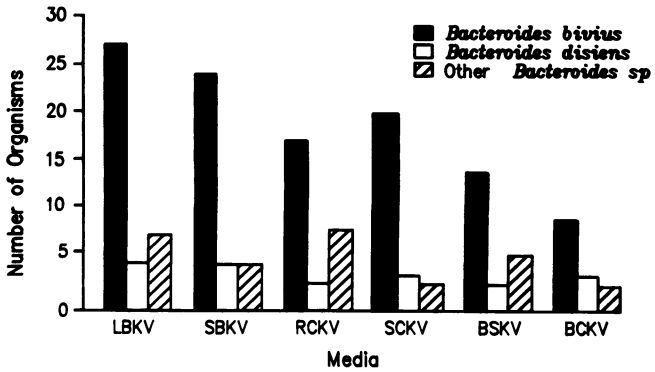

FIG. 2. Comparison of isolation rates of anaerobic gram-negative bacilli from 76 vaginal secretion specimens identified in the third and fourth growth quadrants of six different laked blood KV plates after 96 h of incubation. Media used were as follows: LBKV, in-house brucella base KV agar plate; SBKV, brucella base KV agar plate, Scott Laboratories; RCKV, CDC base KV agar plate, Remel; SCKV, CDC base KV agar plate, Scott; BSKV, Schaedler base KV agar plate, BBL; BCKV, CDC base KV agar plate, BBL.

TABLE 2. Gram-positive cocci recovered from vaginal secretions by using two different base media for BA and PEA plates

Organisms	Total no. recovered	No. (%) recovered			
		CDC base		Brucella base	
		BA	PEA	BA	PEA
Peptostreptococcus tetradius	12	7 (58)	9 (75)	1 (8)	5 (41)
Peptostreptococcus anaerobius	5	4 (80)	3 (60)	1 (20)	1 (20)
Peptostreptococcus asaccharolyticus	12	9 (75)	7 (58)	6 (50)	4 (33)
Peptostreptococcus sp.	10	3 (30)	3 (30)	4 (40)	2 (20)
Total	39	23 (59)	22 (56)	12 (31)	12 (31)

the isolation of high concentrations of gram-positive cocci (Table 2). Fifty-nine percent of all gram-positive cocci isolated in the third and fourth quadrants on any of the plates studied were isolated on CDC-BA medium compared with only 31% on brucella-BA medium. Recovery of *Proteus* sp. from vaginal wash specimens in this study was rare, and there was little difference in gram-positive anaerobe recovery between BA and PEA plates. Only three gram-positive anaerobes were identified in third and fourth quadrants of the CDC-PEA plate that were not present at the ≥3+ level on the CDC-BA plate. Another observation of interest was that, of the six *Veillonella* strains isolated in the third and fourth quadrants in this study, none grew on brucella-BA medium.

DISCUSSION

Most microbiology textbooks recommend the use of a selective medium in addition to a nonselective medium for isolating anaerobic organisms from clinical specimens (1, 8). These sources present several choices of agar base for use in these media without indicating which might be optimal for the recovery of specific organisms. Murray (4) evaluated five media (brucella, brain heart infusion, Columbia, Schaedler, and tryptic soy agar) for quantitative growth and rate of growth of anaerobic isolates and found that quantitative growth was similar on all media. Anaerobic tryptic soy agar is basically the same medium as the CDC base medium we used (5). However, Murray studied laboratory-passed and -purified organisms rather than organisms direct from clinical specimens as reported here. The difference we found in the growth of organisms isolated directly from clinical specimens in the third and fourth streak areas implies that there is a difference in the ability of brucella, CDC, and Schaedler base media to support growth of some anaerobic species in primary isolation. Our studies have shown that brucella base agar supports the growth of anaerobic gram-negative rods on primary isolation from vaginal secretions consistently better than either CDC or Schaedler base agar media. The difference was most apparent with *B. bivius*, the most common gram-negative anaerobe in the female genital tract. If one takes into account growth in the first and second quadrants, the differences in overall anaerobe recovery among the various agar bases were not as apparent (Fig. 1), suggesting that the advantage of brucella base agar medium in supporting the growth of anaerobic gram-negative rods is quantitative rather than qualitative.

On the other hand, anaerobic cocci were more likely to grow in the third and fourth quadrants of CDC agar base medium than brucella agar base medium. *Proteus* was not a common organism in vaginal secretions in this study, so PEA selective agar did not improve gram-positive isolation rates compared with nonselective sheep blood agar. There are a few references in the literature to a new selective agar for anaerobic cocci, using nalidixic acid-Tween 80, which may provide better recovery of these organisms (2, 10). These studies were done with a brucella base. It would be of interest to study this medium formulated with a CDC base which, as suggested by our observations, might be an even better medium for isolating anaerobic cocci.

On the basis of the observations reported here, we think that a CDC-BA plate with or without a CDC-PEA plate and a brucella/KV plate constitutes an optimal combination of nonselective and selective media for the recovery of anaerobes from vaginal secretions. This recommendation also should apply to studies of the bacterial flora of pelvic infections in women, as the primary source of these organisms appears to be the vagina (9).

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LITERATURE CITED

1. **Allen, S. D., J. A. Siders, and L. M. Marler.** 1985. Isolation and examination of anaerobic bacteria, p. 413–433. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
2. **Downes, J., L. Stern, and J. H. Andrew.** 1986. A comparison of selective media for the isolation of anaerobic bacteria from clinical material. Pathology **18**:141–144.
3. **Hanson, C. W., and W. J. Martin.** 1976. Evaluation of enrichment, storage, and age of blood agar medium in relation to its ability to support growth of anaerobic bacteria. J. Clin. Microbiol. **4**:394–399.
4. **Murray, P. R.** 1978. Growth of clinical isolates of anaerobic bacteria on agar media: effects of medium composition, storage conditions, and reduction under anaerobic conditions. J. Clin. Microbiol. **8**:708–714.
5. **Phillips, E., and P. Nash.** 1985. Culture media, p. 1051–1092. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. **Sondag, J. E., M. Ali, and P. Murray.** 1979. Relative recovery of anaerobes from different isolation media. J. Clin. Microbiol. **10**:756–757.
7. **Stargel, M. D., F. S. Thompson, S. E. Phillips, G. L. Lombard, and V. R. Dowell, Jr.** 1976. Modification of the Minitek miniaturized differentiation system for characterization of anaerobic bacteria. J. Clin. Microbiol. **3**:291–301.
8. **Sutter, V., D. Citron, and S. Finegold.** 1980. Wadsworth anaerobic bacteriology manual, 3rd ed. C. V. Mosby Co., St. Louis.
9. **Sweet, R. L., and R. S. Gibbs.** 1985. Mixed anaerobic-aerobic pelvic infections, p. 127–141. *In* R. L. Sweet and R. S. Gibbs (ed.), Infectious diseases of the female genital tract. The Williams & Wilkins, Co. Baltimore.
10. **Wren, M. W. D.** 1989. Multiple selective media for the isolation of anaerobic bacteria from clinical specimens. J. Clin. Pathol. **33**:61–65.