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NOTES

Comparison of Two Media for Isolation of *Haemophilus vaginalis*

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Starch agar and V agar were comparable in the isolation of *Haemophilus vaginalis* from vaginal discharge specimens. Each medium had certain advantages over the other, which are described.

Greenwood et al. (4) recently developed a human blood medium, vaginalis (V) agar, which was successfully used to isolate *Haemophilus vaginalis* from vaginal discharges. This report describes the results of a study to compare V agar and starch agar (6) for *H. vaginalis* isolation. On V agar, *H. vaginalis* produces a diffuse form of beta-hemolysis not produced by other vaginal bacteria. During the growth of *H. vaginalis* on starch agar, bromocresol purple becomes yellow, indicating the production of acid.

Vaginal discharge material (5) was collected with a swab during routine pelvic examinations of women attending a venereal disease clinic. The two isolation media were swabbed in alternating fashion so that the same medium was not inoculated first each time. Both media were incubated at 37°C in 5 to 10% CO_2 and observed daily for 3 days. Isolates were subcultured to Dunkelberg peptone-starch-dextrose agar (3). The isolates were identified on the basis of Gram stain and colonial morphology on peptone-starch-dextrose agar, lack of catalase, failure to reduce tellurite (7), reactions on V and starch agars, and susceptibility or resistance to *Streptococcus pneumoniae*, thionin, bacitracin (Taxo A, BBL), metronidazole, and sulfonamides (1). A total of 98 strains of *H. vaginalis* were isolated from 224 patients. Seventy-two strains were isolated on both V agar and starch agar, 10 strains were isolated on starch agar only, and 18 strains were isolated on V agar only (P, not significant at 0.05 level).

Each medium had certain advantages over the other. It was noted that *H. vaginalis* strains produced more coccobacillary or short straight bacillus shapes on V agar, which were gram negative. On starch agar, the cells were more pleomorphic, clumped, and gram variable and exhibited more beading. Individual bacilli also varied more in length on starch agar. Viability of *H. vaginalis* was longer on V agar possibly because less acid was being produced. Fewer numbers of *H. vaginalis* colonies were detected on V agar when a mixed primarily nonhemolytic flora was also present. On starch agar, starch-fermenting streptococci and some lactobacilli obscure *H. vaginalis*, or a mixed flora of non-starch-fermenting bacteria will alkalinize the starch medium so that small acid areas are not evident. Starch agar can be used directly for a spot catalase test and either medium may be used for the tellurite test (7). Some strains of *H. vaginalis* isolated in this study on subculture produced weak beta-hemolysis or what would be considered alpha-hemolysis on V agar. However, this was in part a variable caused by certain lots of media. Some strains of lactobacilli and streptococci produced forms of hemolysis on V agar that could be confused with that of *H. vaginalis*, but Gram stains and other simple tests eliminated these organisms. Starch agar was more suited for examining the colonial morphology of *H. vaginalis* than was V agar (3). Finally, 65 strains previously identified as *H. vaginalis* (courtesy of Janet Bissell and William M. McCormack) were tested on V agar, and 57 (88%) produced diffuse beta-hemolysis, indicating that this reaction is a variable characteristic.

Brashear et al. (2) reported that starch agar was comparable to selective media for isolation of *Candida albicans* from vaginal specimens. In the present study, *C. albicans* was isolated from 25 of the 224 specimens and occurred equally on V and starch agars.

In conclusion, although both V agar and starch agar have advantages and drawbacks, they appear to be comparable for their purposes.

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