New Differential Medium for the Isolation of *Corynebacterium vaginale*

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A new differential medium for isolation of *Corynebacterium vaginale* is described. This opaque medium containing 1% corn starch allows detection of *C. vaginale* by the zones of clearing developing around the colonies.

Corynebacterium vaginale (*Haemophilus vaginalis*) is a venereally transmitted agent that has been implicated as a cause of vaginitis. The frequency with which this organism has been isolated from genital cultures generally ranges from 10 to 40%, depending upon the population sampled and the methods used by different investigators (1, 4, 5, 7, 8).

Although *C. vaginale* grows on blood and chocolate agars (6), colonies that develop after 48 h of incubation are usually of pin-point size and do not demonstrate a unique appearance. For these reasons, the presence of this organism in culture may often go undetected. For isolation of *C. vaginale*, Dunkelberg et al. have recommended peptone-starch-dextrose (PSD) medium, on which this organism may be recognized by its microscopic colonial morphology (2). Smith modified PSD by incorporating a pH indicator in the medium, allowing detection of *C. vaginale* by the production of acid from starch (10). This report describes an alternative differential medium for isolation of *C. vaginale*. On this medium, which is opaque due to the presence of 1% corn starch, *C. vaginale* may be recognized by the zones of clearing appearing around colonies. This is presumably due to the ability of this organism to solubilize large aggregates of corn starch.

The medium is prepared by dissolving 36 g of GC agar base (BBL) and 10 g of corn starch (Argo) in a liter of distilled water. After boiling for 1 to 2 min, 2.0 ml each of colistin (5 mg/ml) and nalidixic acid (7.5 mg/ml) solutions are added to minimize the growth of enteric flora. After sterilization by autoclaving, 10 ml of Iso-VitaleX (BBL) is added to the cooled medium, and 15- to 18-ml volumes are distributed to 100-mm petri plates.

Zones of clearing surrounding colonies of *C. vaginale* on corn starch medium are best observed by using indirect light (Fig. 1). Colonies are white and convex and possess entire borders. Preliminary testing of this medium re-

vealed that strains of alpha- and nonhemolytic streptococci may also hydrolyze corn starch. *C. vaginale* may be identified by its appearance on Gram stain, a negative catalase test, β -hemolysis on rabbit blood agar, and the fermentation of dextrose, maltose, and starch (2, 3, 9).

In the development of the corn starch medium, eight agar bases were evaluated for their ability to support the growth of *C. vaginale* and to demonstrate zones of clearing. Brain heart infusion (BBL), Casman (Difco), Columbia (BBL), Trypticase soy (BBL), and Mueller-Hinton (BBL) agar bases and GC medium base (Difco) were prepared with 1% corn starch. PSD medium of Dunkelberg et al. (2), prepared with 1% corn starch rather than soluble starch, and the modified PSD medium described by Smith (10) also were examined. Twenty strains of *C. vaginale* were used to evaluate each medium. These included four reference cultures from R. E. Weaver (Special Bacteriology Unit, Communicable Disease Center, Atlanta, Ga.), seven reference cultures from W. E. Dunkelberg (U.S. Army Medical Laboratory, Fort McPherson, Ga.), and nine isolates from genital tract cultures submitted to our laboratory. Media were inoculated with a standardized suspension of organisms and were examined after incubation in GasPak jars (BBL) for 48 h at 35°C.

PSD medium (2) and media prepared with GC and Casman agar bases performed best in supporting the growth of *C. vaginale*. The growth of a few strains of *C. vaginale* was poor on all media tested. The addition of 0.1% lysed rabbit blood or 1% supplement C enhanced the growth of most strains of *C. vaginale* on PSD, Casman, and GC media, with supplement C being the more stimulatory additive. Although there was strain variation as to which of these three supplemented media best supported the growth of *C. vaginale*, the medium prepared with the GC medium base generally performed better than the PSD or Casman base. Zones of

FIG. 1. Colonies of *C. vaginale* surrounded by zones of clearing after 48 h of anaerobic incubation on corn starch medium.

clearing were most easily detected using Casman base and were least outstanding on PSD. Because the GC base was the more supportive medium for most strains of *C. vaginale* and evidenced readily detectable zones of clearing, we have selected it for use.

As previously observed by Malone et al. (9), we have found that most strains of *C. vaginale* grow better under anaerobic conditions than in a 5 to 10% CO₂ environment. We therefore have used anaerobic incubation for our media evaluation as well as for isolation of *C. vaginale* from clinical specimens.

Using our corn starch medium, we are currently isolating *C. vaginale* from approximately 30% of female genital tract cultures. The successful detection of *C. vaginale* will allow us to better evaluate the role of this organism in genital tract disease.

ADDENDUM

Since our preliminary studies with this medium, we have experienced significant lot variation with both supplement C (Difco) and GC medium base (Difco). We have found that more reliable and reproducible results are obtained if the medium is prepared with GC agar base (BBL) and supplemented with 1% IsoVitaleX (BBL) rather than supplement C.

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