Comparison of Isolation of *Haemophilus vaginalis* (Corynebacterium vaginale) from Peptone-Starch-Dextrose Agar and Columbia Colistin-Nalidixic Acid Agar

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A total of 447 cervical or vaginal specimens were inoculated in parallel onto peptone-starch-dextrose (PSD) and Columbia colistin (10 mg/ml)-nalidixic acid (15 μg/ml) (CNA) agar and were incubated for 48 h at 35°C in an atmosphere with 2 to 10% CO₂. One hundred (22.4%) of the cultures were positive for *Haemophilus vaginalis*. Forty-eight of the isolates were recovered from both PSD and Columbia CNA agar, five from PSD only, and 47 from Columbia CNA agar only (*P* < 0.001). On Columbia CNA agar, 76 of the isolates were detected after 24 h of incubation, and the remainder were detected within 4 days of incubation.

Since its original description by Gardner and Dukes in 1955 (10) as an etiological agent in nonspecific vaginitis, both the taxonomic position and the clinical significance of *Haemophilus vaginalis* have been questioned. Zinnever and Turner (20) suggested in 1963 that the species belongs to *Corynebacterium*; however, ultrastructural and biochemical studies by Criswell et al. (3) have demonstrated characteristics more commonly associated with those of gram-negative rather than of gram-positive organisms. While most workers agree that the organism does not belong in the genus *Haemophilus*, it remains listed as *H. vaginalis* (species incertae sedis) in the latest edition of *Bergey's Manual* until such a time as its taxonomic position is settled (12).

In their original article (10) and in a later one with their co-workers (2), Gardner and Dukes have presented a considerable amount of data regarding this organism’s pathogenicity, including its ability to infect normal volunteers. Lewis et al. (13, 14) in a study of 1,008 pregnant and nonpregnant women found that the incidence of cultures positive for *H. vaginalis* was significantly greater (*P* < 0.001) in patients with vaginitis than in those without vaginitis. In their entire study population, 88.6% of patients with vaginitis and 10.4% of patients without vaginitis had positive cultures. Studies by Frampton and Lee (9) and by Mendel and Haberman (15), however, have resulted in substantially higher rates of isolation of *H. vaginalis* from vaginal cultures of asymptomatic women and have failed to demonstrate a statistically significant difference between controls and patients with vulvovaginitis. In a study of the vaginal flora in 100 asymptomatic women by Tashjian et al. (18) at the Mayo Clinic, *H. vaginalis* was isolated from 21. Recently, McCormack et al. (Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 229, 1975) reported that *H. vaginalis* was more prevalent in women with documented vaginal discharge and that its presence in vaginal cultures was also related to sexual experience.

Reported differences in recovery rates of *H. vaginalis* from vaginal cultures and in its prevalence in the presence or absence of vulvovaginitis may be related to such variables as the populations studied, criteria used for the diagnosis of vulvovaginitis, specimen collection and transport, the media used for cultures, and the methods and criteria used for identification of the organism. The present study was prompted by our observation that organisms resembling *H. vaginalis* were more frequently isolated from Columbia CNA agar than from chocolate blood agar or from soybean-casein digest agar with 5% sheep blood.

**MATERIALS AND METHODS**

Swabs (Culturette, Marion Scientific Corp., Rockford, Ill.) of material from the cervix and vagina submitted for bacteriological culture were inoculated in parallel onto soybean-casein digest agar (tryptic soy, Difco Laboratories, Detroit, Mich.) containing 5% sheep blood, Columbia CNA (colistin, 10 μg/ml; nalidixic acid, 15 μg/ml) agar (BioQuest, Cockeysville, Md.), and proteose peptone-starch-dextrose agar (PSD). Plates were incubated at 35°C in an atmosphere with 2 to 10% CO₂. PSD agar
plates were prepared and were examined with a dissecting microscope after 48 h of incubation, as
recommended by Dunkelberg et al. (6). The other plates were examined macroscopically both after 24
and 48 h of incubation.

Colonies suspected of representing H. vaginalis
on the basis of characteristic Gram stain morphol-
ogy and negative catalase reaction were identified
as follows. After their isolation on CNA, several
colonies were inoculated into carbohydrate fermenta-
tion media (19) with Andrade indicator, supple-
mented with 10% sterile horse serum and contain-
ing dextrose, maltose, levulose, and sucrose. These
tests were incubated at 35°C in room air for a maxi-
imum of 7 days. Other tests included those for nitrate
reduction, indole production, and oxidase (19).

Reference strains A2508 and A7057 of H. vaginalis
were obtained from R. E. Weaver, Center for Dis-
ease Control, Atlanta, Ga., and were used for pur-
poses of quality control of the media and their reac-
tions.

Additional studies with H. vaginalis CDC-A2508
were carried out to determine relative growth rates
on 5% sheep blood in Columbia agar base, on 5% 
sheep blood in soybean-casein digest agar base, and
on CNA (BioQuest, Cockeysville, Md.). A broth sus-
pension of the organism was prepared so as to con-
tain 10⁴ colony-forming units/ml. 0.1/ml was then
spread with a sterile glass rod over the agar surface.
Plates were incubated for 48 h at 35°C in 2 to 10% 
CO₂, and then examined macroscopically.

RESULTS

All catalase-negative and oxidase-negative, gram-
negative bacilli which exhibited colonial and microscopical morphology consistent with
that of H. vaginalis and which fermented dex-
trose (100%), maltose (100%), and levulose (92%)
but not sucrose (3%) were considered to be H. vaginalis, according to criteria published
by King (11). Other characteristics included
their failure to reduce nitrates or to produce
indole. Early in the study several isolates re-
sembling H. vaginalis were submitted to the
Center for Disease Control, Atlanta, Ga., and
their identity was confirmed by R. E. Weaver.
All subsequent strains’ reactions were identical
to those of both the confirmed and the reference
strains.

Of 447 cultures of cervical and vaginal mate-
rial, H. vaginalis was recovered from 100.
Forty-seven isolates were recovered from CNA
only, 5 from PSD only, and 48 from both CNA
and PSD. The greater recovery of H. vaginalis
on CNA than on PSD was statistically signifi-
cant (P < 0.001). Colonies were initially de-
tected on CNA after 1 day of incubation in 76
instances, and the remainder were detected
within 4 days of incubation. Colonies were in-
distinct and often overgrown by other bacteria
on sheep blood agar.

Examination of the agar plates inoculated
with the suspension of H. vaginalis CDC-A2508
demonstrated colonies on 5% sheep blood in
Columbia agar base and on CNA which were at
least twice the diameter of those on 5% sheep
blood in soybean-casein digest agar base.

DISCUSSION

A variety of media have been utilized for the
cultivation of H. vaginalis including proteose
no. 3 agar (Difco) containing 10% sheep blood
(10), Casman agar with rabbit blood (1), blood
agar with 1% maltose (7), peptone-maltose-dex-
trose agar (4), dextrose-starch agar (16), pro-
teose PSD agar (6), and starch-dextrose agar
with purple broth base (17). Whereas proteose
peptone no. 3 (Difco) has been frequently used
in media for the isolation of H. vaginalis, the
other components included in such media have
varied. Moreover, there have been few studies
comparing isolation rates of the organisms in
different media. Although Smith recovered
more strains of H. vaginalis on chocolate blood
agar with GC medium base (Difco) and on 10%
sheep blood agar (tryptose base) than on PSD
and more strains on starch agar than on choco-
late blood agar or 10% sheep blood agar, he
never compared isolation rates on PSD and
starch agar concurrently (17). Nonetheless, it
would appear from his data that H. vaginalis
was more readily and frequently isolated from
starch agar than from PSD. Smith speculated
that the absence of dextrose in starch agar may
have reduced overgrowth of H. vaginalis by
other bacteria and, therefore, facilitated the
recognition of this organism (17).

In our study CNA yielded more positive cul-
tures with H. vaginalis than did PSD. The
reasons for our findings are not completely evi-
dent; however, the composition of the Columbia
agar base (8) used in Columbia CNA agar gen-
erally resembles that of Casman agar (1) and
appears to contain all of the growth require-
ments for H. vaginalis described by Dunkel-
berg and McVeigh (5). Moreover, Columbia
CNA agar does not contain dextrose but does
contain antimicrobial agents to which H. va-
ginalis is resistant (16) but which do suppress
overgrowth of other bacteria, the presence of
which on noninhibitory media interferes with
the recognition of colonies of H. vaginalis.

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