Tellurite Reduction Test to Aid in the Recognition of *Corynebacterium vaginale*

RODNEY F. SMITH,* JACK L. VOSS, AND ROBERT K. BAILEY

Public Health Laboratory, Contra Costa County Health Department, Martinez, California 94553

Received for publication 8 November 1976

Corynebacterium vaginale (*Haemophilus vaginalis*) does not reduce potassium tellurite. When a 1% aqueous solution of tellurite is added to starch agar plates previously inoculated with vaginal discharge material, other starch-fermenting and most non-starch-fermenting bacteria rapidly reduce tellurite to produce black or gray colonies. This test is a useful adjunct to methods for rapid presumptive identification of *C. vaginale*. *C. vaginale* is more susceptible to tellurite inhibition than a variety of other gram-positive bacteria.

Several media, including peptone-starch-dextrose agar (2), starch agar (8), and, most recently, Columbia colistin-nalidixic acid agar (5), have been reported to be successful in isolating *Corynebacterium vaginale* (*Haemophilus vaginalis*) from the vaginal tract. In our venereal disease clinics, *C. vaginale* is routinely isolated from women with a vaginal discharge on starch agar. Starch agar is a nonselective differential medium on which *C. vaginale* produces a strong acid reaction from starch fermentation (1, 8). Some species of streptococci also ferment starch on the medium, although these bacteria usually can be separated from *C. vaginale* by Gram stain. However, during the past 2 years, we have noted that the Gram stain may not provide an adequate means of presumptively identifying *C. vaginale*, particularly when a mixed bacterial flora is present on the starch plates. Dunkelberg et al. (3) determined that five strains of *C. vaginale* grew on media containing 0.01% potassium tellurite, but the tellurite was not reduced. In this report, we describe an application of the findings of Dunkelberg et al. (3) to aid in the recognition of *C. vaginale* on primary isolation media.

Patient examination, specimen collection, and use of starch agar to culture *C. vaginale* from the vaginal tract were previously described (1). Presumptive identification of *C. vaginale* on starch agar consisted of demonstrating starch fermentation, lack of catalase activity, and typical cellular morphology of the organism based on a Gram stain. A group of primary (clinic) starch plates was used in this study. Fifteen strains of *C. vaginale* previously isolated from the vaginal tract on starch agar and identified by the methods of Dunkelberg et al. (2) were also used. Various other named

species of bacteria or yeasts were used in experiments. The latter organisms originated from the Microbial Diseases Laboratory of the California State Health Department, Berkeley, as part of a voluntary intrastate proficiency test program for local health department laboratories. Pure cultures of *C. vaginale* and other organisms were streaked on starch agar and peptone-starch-dextrose agar and incubated at 37°C in 5% CO₂ for 24 to 72 h. The minimum inhibitory concentration of potassium tellurite against organisms was determined using two-fold serial dilutions of tellurite in broth. The broth medium contained, per liter: 10.0 g of proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.), 1.0 g of beef extract (Difco), 5.0 g of sodium chloride, 10.0 g of maltose, and 2.0 g of soluble starch, final pH 6.8 ± 0.1.

The 15 known strains of *C. vaginale* and various other species were grown on peptone-starch-dextrose and starch agars, and the plates were flooded with 1% tellurite. After the entire surface was wet, the excess reagent was decanted, and the plates were reincubated at 37°C for 60 min. Upon removal, none of the *C. vaginale* strains had reduced tellurite on either medium. By comparison, one strain each of *Streptococcus pyogenes*, *S. bovis*, *S. mitis*, *Staphylococcus aureus*, *S. epidermidis*, *Corynebacterium ulcerans*, *C. xerosis*, *C. diphtheriae*, *C. pseudodiphtheriticum*, and two strains of unidentified catalase-positive vaginal diphtheroids produced black to gray colonies on both media. Tellurite was not reduced by one strain each of *Lactobacillus acidophilus* and *Candida albicans*.

Ten strains of *C. vaginale* were inhibited by between 1.56 and 12.5 µg of tellurite in maltose-starch broth per ml. One strain each of *S. mitis*,

S. bovis, *S. pyogenes*, *S. aureus*, *S. epidermidis*, *C. xerosis*, *C. diphtheriae*, and *C. ulcerans* grew in broth containing 100 µg of tellurite per ml. One strain each of *Escherichia coli* and *Proteus vulgaris* was inhibited by less than 0.37 µg of tellurite per ml.

Seventy-five positive (acidic) clinic starch plates that had been incubated for 72 h were also flooded with tellurite solution. After reincubation for 1 h, these plates were examined with a binocular dissecting stereoscope at various magnifications, and non-tellurite-reducing colonies were Gram stained and tested for catalase. Organisms resembling *C. vaginale* were found on 46 of the plates. Most of these latter plates contained a mixed vaginal bacterial flora. Non-starch-fermenting, gram-positive, catalase-positive cocci and diphtheroids reduced tellurite, as did starch-fermenting and starch-negative streptococci. Yeasts and organisms resembling lactobacilli did not reduce tellurite. None of the organisms resembling *C. vaginale* on 72-h clinic plates were recovered viable on subculture from plates before or after adding tellurite. An experiment was then conducted using *C. vaginale* ATCC 14018. Starch agar plates were seeded with approximately 10⁸ colony-forming units. After incubation for 24 and 48 h, separate plates were flooded with sterile water and 1, 0.5, and 0.1% tellurite. At 30-, 60-, and 90-min intervals, a loopful of growth from each flooded plate was subcultured to fresh agar plates. Viable organisms were recovered on subculture from all of the flooded plates.

Several points regarding the tellurite test must be emphasized. One percent tellurite has a pH of 11.0 and may change acidified areas of starch agar alkaline without reversion to the acidic state. This will most likely occur if the original acidic area is small. This is less likely to happen using 0.1% tellurite. If hydrogen peroxide is applied to selected colonies for the catalase test, the peroxide will reoxidize tellurite-positive (black or gray) colonies to a colorless form. Confluent growth will interfere with distinguishing tellurite- and catalase-positive colonies from those that are negative for one or both tests. Lactobacilli do not appear to reduce tellurite and seldom ferment starch (7). Plates heavily populated by starch-fermenting streptococci rapidly reduce tellurite, but a diffuse blackish sediment derived from streptococcal colonies may settle upon the agar surface. Although rarely isolated from the vaginal tract, some species of the genus *Bacillus* ferment starch (4). These bacteria are catalase positive and form spores. Species of the genus *Bacillus*

were not tested for tellurite reduction. We have encountered strains of fastidious unclassified diphtheroids from the genital tract that do not reduce tellurite and may be catalase negative or weakly positive. Such colonies appear white on starch agar, but starch is not fermented. One percent tellurite will bleach the normally yellow colonies of *C. vaginale* on starch plates to a white color. Tellurite-flooded plates can remain incubated for longer than 1 h or left overnight at room temperature without any change occurring on plates containing *C. vaginale*. Observation of colonies under a dissecting microscope is essential.

The major value of the tellurite reduction test is that it helps to distinguish *C. vaginale* from most other vaginal bacteria capable of growing on starch agar. *C. vaginale* appears to be more sensitive to tellurite inhibition than was previously reported (3). Viability, using a reference strain of *C. vaginale*, did not appear to be affected by exposure to tellurite. We have noted that viability of *C. vaginale* is generally unpredictable and seldom likely on starch plates 72 h old. Lapage (6) reported that viability of the organism on solid media may cease after 24 h of incubation. We also find that *C. vaginale* is easily overgrown on subculture when primary isolation plates contain a mixed flora. The contaminants usually consist of streptococci and staphylococci; only rarely are they enteric gram-negative bacilli.

The acidic reaction produced by *C. vaginale* on starch agar may occur after 18 h of incubation or require several days, depending upon initial concentration of viable organisms in the inoculum and its size. Recognition of *C. vaginale* on clinic plates after 48 h of incubation is generally good and may not require a tellurite test when *C. vaginale* is the predominant organism. However, recovery of viable *C. vaginale* from starch plates may be affected by several factors, and the influence of tellurite on viability of newly isolated strains of *C. vaginale* requires additional study.

In mass public health venereal disease clinics where *C. vaginale* is routinely isolated, time and cost may prevent extensive efforts to subculture and purify *C. vaginale* for confirmation. In such instances, the tellurite test used with the catalase test, Gram stain, and demonstration of starch fermentation can support a presumptive identification of *C. vaginale*. A negative tellurite reduction test is not specific for *C. vaginale*.

LITERATURE CITED

1. Brashear, M. D., J. Wong, P. Hines, and R. F. Smith.

1976. Comparative isolation of vaginal yeasts on selective and nonselective media. *J. Clin. Microbiol.* 3:640-642.
2. Dunkelberg, W. E., Jr., R. Skaggs, R. and D. S. Kellogg, Jr. 1970. Method for isolation and identification of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *Appl. Microbiol.* 19:47-52.
 3. Dunkelberg, W. E., Jr., R. Skaggs, and D. S. Kellogg, Jr. 1970. A study and new description of *Corynebacterium vaginale* (*Haemophilus vaginalis*). *Am. J. Clin. Pathol.* 53:370-377.
 4. Gibson, T., and R. E. Gordon. 1974. Genus I. *Bacillus*, p. 529-550. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
 5. Golberg, R. L., and J. A. Washington II. 1976. Comparison of isolation of *Haemophilus vaginalis* (*Corynebacterium vaginale*) from peptone-starch-dextrose agar and Columbia colistin-nalidixic acid agar. *J. Clin. Microbiol.* 4:245-247.
 6. Lapage, S. P. 1974. *Haemophilus vaginalis*, p. 368-370. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
 7. Rogosa, M. 1974. Genus I. *Lactobacillus*, p. 576-593. In R. E. Buchanan and N. E. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams and Wilkins Co., Baltimore.
 8. Smith, R. F. 1975. New medium for isolation of *Corynebacterium vaginale*. *Health Lab. Sci.* 12:219-224.