Viability of *Trichomonas vaginalis* In Vitro at Four Temperatures

RODNEY F. SMITH

Public Health Division Laboratory, Department of Health Services, Martinez, California 94553

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The effect of temperature as a possible factor on the survival of *Trichomonas vaginalis* for shipment or routine laboratory maintenance was studied. Ten strains of *T. vaginalis*, ATCC 30001, ATCC 30238, and eight clinical isolates, were examined for viability when kept incubated at 37°C or removed from this temperature and held at 42, 22, or 5°C for increasing lengths of time without subculture or reincubation at 37°C. The order in which the strains remained viable without subculture was: 5°C, 8 to 10 days; 22°C, 4 to 8 days; 37°C, 4 to 6 days; 42°C, less than 2 days. Vials of medium with cells were also held at 22 and 5°C and then reincubated at 37°C. Cultures held at 22°C remained viable 6 to 8 days, whereas those stored at 5°C remained viable 10 to 14 days. These data show that *T. vaginalis* withstands a wide range of temperatures, particularly below normal growth temperatures without subcultures, beyond what would be expected in mailing cultures.

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**MATERIALS AND METHODS**

**Organisms.** *T. vaginalis* ATCC 30001 and ATCC 30238 were obtained directly from the American Type Culture Collection, Rockville, Md. Eight additional strains were isolated from women attending a clinic for sexually transmitted diseases and were axenized by methods previously described (15).

**Medium.** A modification of Hollander agar (5) was used for this study and contained the following ingredients per 950 ml of deionized water: Trypticase peptone (BBL Microbiology Systems, Cockeysville, Md.), 20.0 g; yeast extract (Difco Laboratories, Detroit, Mich.), 10.0 g; maltose, 5.0 g; ascorbic acid, 1.0 g; KCl, 1.0 g; KHCO₃, 1.0 g; KH₂PO₄, 1.0 g; K₂HPO₄, 0.5 g; FeSO₄, 0.1 g; agar, 1 g. The medium was designated Hollander fluid (HF) medium. Heat-inactivated pooled human serum (5%) was used in place of 10% horse serum, and 100 μg of chloramphenicol per ml replaced penicillin and streptomycin to prevent bacterial contaminants (16). The final medium had a pH of 6.0 and was dispensed in screw-cap tubes in 9.5-ml amounts.

**Viability studies.** Proliferation of *T. vaginalis* measured by hemocytometer cell counts had been found to be equal to cell counts based on agar plate dilution methods until approximately midway through the stationary growth phase of the organism (2). In this study, all cell counts were made with a Neubauer hemocytometer (A. H. Thomas Co., Philadelphia, Pa.). Each strain was tested in duplicate. A Bausch & Lomb binocular light microscope with a zoom lens was set at 200 magnifications so that each field visualized 1 mm². The final cell count per milliliter of medium was based on the average number of cells counted in 36 fields examined, 18 per culture.

Axenic strains of *T. vaginalis* in HF medium were

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Methods for the successful preservation of *Trichomonas vaginalis* require freezing in stablites (6, 7, 10, 12). Most investigators who study *T. vaginalis* in vitro maintain viability of the organisms by frequent serial transfers in culture media held at 37°C. This procedure may possibly attenuate the virulence of *T. vaginalis* or alter antigenic properties of the organism (6, 10). However, serial transfers, subcultures, and shipment of *T. vaginalis* under various conditions have not been found to affect the susceptibility or resistance of the organism to zinc (8) or metronidazole (1, 9, 13–15).

This laboratory has begun to receive and test cultures of *T. vaginalis* strains isolated from women when clinicians suspect that the patient is infected by a metronidazole-resistant strain of the organism (15). These cultures are sent entirely through the mail, and the question of *T. vaginalis* survival at different temperatures is important.

Studies have shown that *T. vaginalis* can remain viable in urine for several days or in tap water for a few hours at 5 to 7°C. There is little information available on the viability and survival of *T. vaginalis* at different temperatures for the lengths of time that might be encountered in attempts to send a strain through the mail. The objective of this study was to determine whether and to what extent *T. vaginalis* could be maintained in vitro by prolonged holding of cultures at different temperatures without subculture.
incubated at 37°C and subcultured at intervals of 2 or 3 days. A growth curve was established for the 10 *T. vaginalis* strains in HF medium with a starting inoculum of 40,000 cells per ml. The average cell counts per milliliter from three separate determinations were: at 24 h, 225,000, range 100,000 to 450,000; at 48 h, average 1,428,000, range 1,000,000 to 2,430,000; at 72 h, average 2,400,000, range 1,750,000 to 2,775,000.

Since the growth rates of the strains were not synchronous, there was variation in the time each strain reached a given cell count. Usually, an incubation between 40 and 58 h with an average of 48 h produced a minimum of 1,000,000 cells per ml with a maximum number of motile cells (95 to 100%) in any field. Motility was defined as either cellular movement (which included ameboid-like activity), flagellar action, or both. Estimates of the percentage of motile cells out of the total cells in the inoculum were made with a hemocytometer as described for making cell counts.

**Method 1.** Each strain of *T. vaginalis* was inoculated into 60-ml screw-cap bottles containing 40 ml of HF medium with a starting inoculum of 40,000 actively motile cells per ml and incubated at 37°C. When cell counts for each strain reached 1,000,000/ml, the contents of each bottle were split equally into four 10-ml amounts, each in a screw-cap tube. One tube each was placed in incubators at 42, 37, or 22°C or the refrigerator at 5°C. At 2-day intervals, the tubes were mixed several times by inversion, and 0.5 ml of HF medium from each tube was transferred to 9.5 ml of fresh HF medium. Viability of the cells was defined as the ability of the transferred cells to proliferate at 37°C in the freshly inoculated HF medium within 10 days. This method was repeated twice.

**Method 2.** This consisted of the inoculation of 100 ml of HF medium contained in 120-ml screw-cap bottles. The *T. vaginalis* strains between 24 and 36 h incubation at 37°C were utilized when cell counts reached 500,000 cells per ml. After the cells were mixed in the bottles, the entire contents were divided into screw-cap vials filled to the top with 8 ml of medium. The turbidity of these vials was 0.5 by the McFarland nephelometer standard. The vials were placed at 22 and 5°C for storage and at intervals were placed again at 37°C to measure resumed multiplication. The vials were reincubated at 37°C for 10 days. Cells that were viable and proliferated produced a turbidity greater than 3.0 by the McFarland standard within the 10-day reincubation period. This method was repeated twice. For cells stored at 22 and 5°C, 1-ml samples of medium were placed in small sterile tubes and incubated at 37°C for 1 h before motile cell counts were made. In some cases, cells stored at 22°C were examined for motility before and after warming at 37°C, and motility of cells stored at 5°C was estimated after first warming to 25°C, then to 37°C.

**RESULTS**

Table 1 summarizes the results of two tests on 10 strains of *T. vaginalis* examined for viability by method 1, the subculture technique, after being held at four temperatures. None of the strains kept at 37°C for 8 days or at 42°C for 6 days was viable. Viability of most strains was lost when cultures were kept at 37°C for 6 days or held at 42°C for 4 days. By comparison, 4 to 7 strains remained viable when held at 22°C for 6 days, and 9 to 10 strains were viable after being held for 8 days at 5°C.

With the second method, vials with 8 ml of medium and 500,000 cells of each strain of *T. vaginalis* per ml were held at 22 and 5°C and were reincubated at 37°C at 2-day intervals. The ranges of survival times in two tests done by this method were as follows. From the group held at 22°C, the strains remained viable from 6 to 8 days; in the group held at 5°C, strains remained viable 10 to 14 days.

It was found that some strains of *T. vaginalis* 8 days old and held at 22°C for 6 days were still motile without it being necessary to warm the cultures at 37°C first. Fewer than 5% of the cells in any field viewed exhibited motility. This was also observed in some strains 10 days old and held at 5°C for 8 days, but only after the cultures were warmed at 37°C.

There were no differences in viability between the reference and clinical isolates of *T. vaginalis*.

**DISCUSSION**

Under the test conditions employed, *T. vaginalis* remained viable without subculture for reasonably long periods at 22 or 5°C. These results differ from those of Whittington (17), who found that *T. vaginalis* survived in vaginal exudates or cultures only 2 to 3 days in a temperature range between 6 and 16°C. However, in Whittington's study the temperatures fluctuated. This might be more injurious to *T. vaginalis*, at least in the temperature range that was used. The results of this study show that axenic *T. vaginalis* withstands a wide range of constant temperatures.
particularly below normal growth temperatures and for periods beyond what would be expected for receiving a mailed culture.

Thus far (unpublished data), we have successfully received and recovered in viable condition 10 of 20 cultures of T. vaginalis inoculated in HF medium and sent through the mail without knowledge of what temperatures or variations in temperatures the cultures were exposed to. Six negative cultures were grossly contaminated, and four had no growth. The latter four were reported to be positive from examinations of wet mounts of vaginal discharge material, but the cultures inoculated with discharge material were mailed before being incubated at 37°C. The 10 viable strains had first been incubated at 37°C for 24 to 48 h before shipment. Five of these 10 strains were found to be resistant to metronidazole in vitro by currently accepted methods and criteria to detect T. vaginalis resistance to the compound (9, 14, 15). Thus far, there have not been any reported alterations in metronidazole susceptibility or resistance patterns in vitro among T. vaginalis strains subject to temperature variations and other conditions of shipment, maintenance, and frequent or delayed serial transfers (1, 9, 13–15).

Clinicians who submit newly isolated strains of T. vaginalis through the mail are not likely to have a means of determining viability of the strain at the time of shipment except by observing motility from a simple wet mount of an incubated culture. From this, an estimate of the multiplication of the strain in the medium can be made before shipment. It appears that newly isolated strains that have been given the opportunity to multiply in the medium and are then shipped as actively growing 24- to 48-h-old cultures have the best chance of surviving.

It has been determined that fewer than 10 cells of newly isolated T. vaginalis per ml initiate growth in media (4). The survival of minimal numbers of cells of T. vaginalis in a vaginal discharge held at different temperatures is not well known and requires further study.

The results of this study may benefit those who serially transfer T. vaginalis in vitro by reducing the time and media required to maintain the organism for various studies. Diamond (3) determined that between 50,000 and 200,000 cells of T. vaginalis were required to maintain axenic cultures. However, twice this number were required to maintain cultures at room temperature for 5 days followed by incubation at 35.5°C for 2 additional days to reduce transfers to once per week. The present study utilized what appeared to be the maximum number of motile cells incubated 48 h at 37°C and from which no fewer than 500,000 cells per ml were subcultured in a 0.5-ml inoculum (method 1).

The greater survivability of 500,000 cells per ml in 8-ml vials (method 2) would be expected because there were simply many more viable cells available to reinitiate growth at 37°C. Variations in viability of T. vaginalis strains held at different temperatures were not related to whether the cells were 24 or 48 h old.

The results of this study and our experience thus far with mailed cultures indicate that actively growing strains of T. vaginalis can survive various temperature- and time-related maintenance and transport situations in viable condition.

LITERATURE CITED