Viability of *Trichomonas vaginalis* in Urine: Epidemiologic and Clinical Implications

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The impact of the viability of *Trichomonas vaginalis* in urine on wet mount, culture, and PCR methods was assessed. To minimize the chance of false-negative results, urine specimens should be processed within 30 min of specimen collection and maintained at 37°C, since temperature appears to affect the viability of *Trichomonas*.

*Trichomonas vaginalis* is the most prevalent nonviral sexually transmitted infection worldwide (3). The estimated global incidence of *T. vaginalis* infections is over 170 million cases per year, with over 8 million new cases per year in the United States alone. Moreover, infection with *T. vaginalis* has been shown to be a risk factor for transmission and infection with human immunodeficiency virus as well as for adverse birth outcomes (4, 5, 10, 11).

Despite the importance of *T. vaginalis*, few data exist on the viability of this organism and optimal methods for specimen handling. Current procedures, which allow transport of urine specimens, cite a review article which referenced a 1960 publication indicating that *T. vaginalis* could survive in urine for several hours. However, the 1960 report apparently misinterpreted results from a study published in 1957 which presented data on survival of *T. vaginalis* in vaginal secretions, not from urine (1, 8, 14).

Given the growing importance of *T. vaginalis*, information on the viability of this organism in diagnostic specimens is important. Because current diagnostic methods rely on the continued viability of the organism, this study attempted to determine the parameters of viability of *T. vaginalis* in urine and the impact of processing delays on diagnostic techniques.

The strain of *Trichomonas vaginalis* used throughout this study, T1, originated from a clinical isolate from a patient in Taiwan and was maintained in modified Diamond’s medium at 37°C in aerobic conditions (2).

One hundred milliliters of clean-catch urine was collected from 10 healthy male volunteers. Immediately following collection, each urine sample was inoculated to a concentration of \(1.23 \times 10^5\) organisms/ml with a stock culture of the live T1 strain of *T. vaginalis*. Fifty milliliters, half of the urine from each volunteer, was kept at room temperature; the other half was incubated at 37°C.

Every 30 min following inoculation for a total of 4 h, 1 to 2 drops of urine was examined by direct microscopy under oil immersion at a magnification of \(\times1,000\) for the presence of motile trichomonads. Additionally, 1 to 2 drops of urine was inoculated into the InPouch TV culture medium (BioMed Diagnostics, White City, OR). The InPouches were examined microscopically by a trained microscopist for a minimum of 1 minute each for the presence of motile trichomonads at 24 h, 48 h, and 5 days. The presence of motile parasites with morphology and motility characteristic of *T. vaginalis* would have indicated that trichomonads were viable at the inoculation time point.

At each time point, an aliquot of urine was also frozen for subsequent testing via PCR. These samples were subsequently thawed, and DNA was extracted using the previously described Chelex method (7, 13). Extracted specimens were stored at \(-70\)°C. The presence of extracted DNA in positive controls processed by this method was verified using a spectrophotometer.

PCR was run as previously described including the BTUB 9/2, AP 65 A/B, and TVA 5-1/6 primers (6, 7, 9, 12). Positive and negative controls were included with every run. Twenty microliters of amplification product was electrophoresed on 4% agarose (E-gel; Invitrogen, Carlsbad, CA). The size of the amplified product was assessed by comparison with a commercial 50-bp weight marker (exACTGene; Fisher Scientific International, St. Paul, MN).

When the specimens were examined via wet mount, at each time point and temperature, 5 to 10 motile trichomonads were visualized. The majority of the organisms that were observed had rounded up, were nonmotile, and were no longer recognizable as *T. vaginalis*.

When TV InPouches were inoculated within 30 min after the trichomonads were added to the urine sample, all InPouches were positive within 5 days (Table 1). No differences were observed between holding temperatures. When the InPouches were inoculated within 60 min and held at \(37\)°C, 50% were positive within 5 days, while for those held at room temperature, only 20% were positive within 5 days. No samples were positive if the urine was held for 180 or 240 min.

All samples were positive by PCR for urine held at both \(37\)°C and room temperature for 30 min following inoculation (Table 2). All samples held at \(37\)°C were PCR positive at 60 min following inoculation, but only 8 of 10 (80%) samples were...
positive when held at room temperature. Seven of 10 (70%) samples were positive at 120 min when held at 37°C, compared with only 3 of 10 (30%) samples held at room temperature. No samples were positive by PCR if they were processed and frozen 3 h or longer after the urine was inoculated with *T. vaginalis*.

Our results indicate that the time of processing of urine specimens and the temperature at which the specimens are held before processing can affect the sensitivity of both culture and molecular techniques in the diagnosis of *T. vaginalis*. In order to minimize the chance of false-negative results, urine specimens for culture should be processed within 30 min of specimen collection. Additionally, whenever possible, the specimen should be kept at 37°C.

If PCR is used, urine should be processed and frozen within 2 hours of collection if it is being held at 37°C and within 1 hour if held at room temperature to minimize false negatives. *T. vaginalis* possesses highly active endonucleases that could be responsible for the degradation of DNA, resulting in false-negative test results (6).

Further experiments need to be undertaken to identify the component of urine that results in loss of viability, which would allow for the development of techniques or substances to prolong the viability.

These findings have implications for interpreting studies of prevalence and incidence of trichomoniasis. If more than 30 min elapsed between collection and testing of urine in previously published studies, then it is likely that the reported prevalence values represent an underestimate of the true prevalence values in the studied populations.

The results from the viability experiments should also influence future study design and practices in doctor’s offices and clinics. Studies in which urine is collected and then transported to another location for testing may not be advisable since the sensitivity of the tests will be drastically decreased due to reduced viability of the parasite. Practitioners should be encouraged either to test urine on site immediately following collection or to attempt to collect other diagnostic specimens such as semen or prostatic secretions.

The generalizability of these results is potentially limited. Different strains of *T. vaginalis* may have various survival times, and laboratory-adapted strains may vary from natural infections. Therefore, our experimental methods may not reflect actual circumstances of natural infection and clinic circumstances. Further studies of the viability of *T. vaginalis* in urine in clinical settings would be of value, as well as evaluation of rapid immunochromatographic techniques for the diagnosis of *T. vaginalis* in urine.

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**REFERENCES**


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**TABLE 1. Effect of processing time delay on culture (TV InPouch)**

<table>
<thead>
<tr>
<th>Specimen no.</th>
<th>Result for time (min) and temp*</th>
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* +, positive; −, negative.

**TABLE 2. Effect of processing time delay on PCR results from urine inoculated with *T. vaginalis* and then held at 37°C or room temperature (RT)**

<table>
<thead>
<tr>
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