Stability of *Trichomonas vaginalis* DNA in Urine Specimens[▽]

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***Trichomonas vaginalis* is an important pathogen in both men and women. Culture is considered the diagnostic gold standard, although studies have shown that PCR is more sensitive than either culture or wet mount for the diagnosis of *T. vaginalis* infections. We sought to identify a simple method for stabilizing *T. vaginalis* DNA in urine samples that could be easily applied to molecular testing. The stability of *T. vaginalis* DNA in 40 urine samples was assessed by storage for various times at either 4°C or room temperature with or without the Becton Dickinson urine preservative transport (UPT) kit. Overall, there was better stability of *T. vaginalis* DNA when specimens were stored at 4°C than when they were stored at 20 to 22°C and when the UPT system was used. *T. vaginalis* DNA was stable in specimens stored without using the UPT at 4°C for about 3 days and at room temperature for only 1 day. For specimens placed in the UPT within 24 h (times of 1, 6, and 24 h) of collection, the DNA was stable for up to 30 days when stored at 4°C. For specimens stored at room temperature, the urine should be added to the UPT ideally within 1 hour of collection, and in this case the DNA remained stable for up to 30 days. When storing specimens at room temperature, a delay of 24 h prior to adding to UPT led to an unacceptably high loss of assay sensitivity.**

Trichomonas vaginalis is a common sexually transmitted disease, with an estimated 7.4 million new cases annually in the United States and as many as 180 million cases worldwide (13). *T. vaginalis* can cause symptoms in both men and women and can cause severe complications in pregnant women. Symptoms in women include vaginal discharge and vulval irritation; if untreated, more-severe complications may occur, including endometritis, cervical erosion, and infertility (3, 9). *T. vaginalis* infections in pregnant women can lead to premature rupture of membranes, low-birth-weight infants, and preterm deliveries (2). In men, infection with *T. vaginalis* may cause nongonococcal urethritis, with untreated infections causing chronic prostatitis, epididymitis, infertility, or urethral strictures (3, 9). Moreover, *T. vaginalis* infection can lead to an increased risk of acquiring human immunodeficiency virus type 1 infection due to the local inflammation caused by *T. vaginalis* infections in the genital tract (5, 7, 8, 11).

There are numerous reports supporting the role of PCR in the diagnosis of *T. vaginalis* infections from genital specimens; these reports have shown that the detection of *T. vaginalis* DNA by PCR is significantly more sensitive than both culture and wet mount examination (1, 4, 6, 12, 14, 15). We have previously reported that vaginal swabs collected with the BDProbeTec dry swab system were a reliable specimen for PCR testing and allowed the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *T. vaginalis* from a single specimen (1). For clinical studies using urine specimens, our procedure for stabilizing *T. vaginalis* was cumbersome and involved adding an aliquot of urine

to Fuji medium (Remel, Lenexa, KS), to prevent autolysing of the *T. vaginalis* organisms. In this report, we describe a simple method for stabilizing *T. vaginalis* DNA in urine samples by use of the Becton Dickinson urine preservative transport (UPT) kit, which is routinely used for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* testing in clinical laboratories. The use of UPT for *T. vaginalis* testing would be easy for clinics and laboratories and would still allow testing for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* DNA from a single specimen.

MATERIALS AND METHODS

Samples. Urine samples were collected from 40 volunteers following an institutional review board-approved protocol (#IRB00001614). Fifty to 100 ml of first-voided urine was collected from each volunteer in a sterile cup. An aliquot of each sample was tested for the presence of *T. vaginalis* DNA by use of a real-time PCR assay (1). All samples tested negative for *T. vaginalis* DNA. The urine specimens were then spiked with *T. vaginalis* organisms. For this purpose, *T. vaginalis* was cultured using the InPouch TV system (BioMed Diagnostics, San Jose, CA) in the clinical microbiology laboratory. An aliquot of this suspension was used to spike each of the 40 urine samples. The goal was to create specimens with a low to moderate concentration of *T. vaginalis* DNA; the amount of suspension used to spike the urine samples was estimated to give a positive signal with a cycle threshold (C_T) value of 30 to 35. This range of C_T values was chosen based on clinical experience with the assay; culture-negative PCR-positive specimens tend to have a higher C_T value, indicating a lower concentration of DNA in the sample. This high range of C_T values is near the limit of detection of the assay and should allow the detection of subtle changes in DNA degradation.

***Trichomonas vaginalis* real-time PCR assay.** Urine samples were processed either directly or with the BD UPT kit (Becton Dickinson, Sparks, MD) (see below for details of study design). The UPT tubes contain 50 μ l of a <742.5 mM solution of potassium-EDTA. For samples processed with the UPT, 3 ml of urine was added to the UPT and, after storage for the designated period of time, the specimen was processed following the manufacturer's recommendations. Briefly, the sample was heated to 114°C for 10 min, cooled for 15 min, and centrifuged at 3,000 $\times$ g for 30 min. The pellet was resuspended in 1.5 ml of the Becton Dickinson dimethyl sulfoxide buffer. A 5- μ l aliquot was used for PCR testing. For samples processed without the UPT, 3 ml of urine was heated directly and the above-described procedure was followed. The *T. vaginalis* real-time PCR assay was performed as previously described (1).

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TABLE 1. Study design^a

Arm	Times (h) prior to UPT	Storage times (days)		Storage temp
		UPT	No-UPT	
Arm 1	1, 6, 24	1, 3, 7, 30	NA	4°C
Arm 2	1, 6, 24	1, 3, 7, 30	NA	20–22°C
Arm 3	NA	NA	1, 3, 7, 30	4°C
Arm 4	NA	NA	1, 3, 7, 30	20–22°C

^a NA, not applicable.

Study design. The study was designed to determine how long the *T. vaginalis* DNA would remain stable at 4°C or room temperature before it was added to the UPT and then to determine how long the DNA was stable once in the UPT (Table 1). There were four arms to the study, with each containing 10 different urine samples. An aliquot of each urine sample was processed within 5 min of spiking with *T. vaginalis* and served as the time zero specimen. For arm 1, each of the 10 urine samples was stored at 4°C and an aliquot of each was added to UPT 1 h, 6 h, and 24 h after spiking; once in the UPT, an aliquot was processed 1 day, 3 days, 7 days, and 30 days later. For arm 2, each of the 10 urine samples was stored at room temperature (20 to 22°C) and an aliquot of each was added to UPT 1 h, 6 h, and 24 h after spiking; once in the UPT, an aliquot was processed 1 day, 3 days, 7 days, and 30 days later. For arm 3, the 10 urine samples were stored at 4°C without UPT and an aliquot was processed 1 day, 3 days, 7 days, and 30 days later. For arm 4, the 10 urine samples were stored at room temperature (20 to 22°C) without UPT and an aliquot was processed 1 day, 3 days, 7 days, and 30 days later. All specimens from a given time point were processed together and tested in the same run. All runs included positive and negative controls; the positive control C_T values fell within a range of 30.6 to 31.7.

RESULTS

Thirty-eight of the time zero specimens tested positive for *T. vaginalis* DNA, with C_T values ranging from 30.8 to 39.3. Two of the samples were undetectable at time zero but were detectable at subsequent time points. This likely relates to sampling error in our goal to create specimens with a low amount of DNA.

The results of the four arms were initially analyzed based on the qualitative result of positive or negative results of tests for *T. vaginalis* DNA. In comparing arms 3 and 4 (Table 2), storage at room temperature without the use of UPT led to a high number of aliquots negative for *T. vaginalis* DNA compared to storage at 4°C; the dropout of positive results increased with longer storage time. When stored at 4°C, only 1 of the 40 aliquots was negative at the four time points tested; however, when stored at 20 to 22°C, a total of 10 of the 40 aliquots were negative. For arms 1 and 2, with the use of UPT, all aliquots remained positive even with delays of up to 24 h prior to putting samples into UPT and after storing in the UPT for up to 30 days.

TABLE 2. Results for urine samples stored at 4°C or room temperature without addition to the UPT

Time in storage (days)	No. of samples positive for <i>T. vaginalis</i> DNA/total no. of samples tested	
	Storage at 4°C (arm 3)	Storage at room temp (arm 4)
1	10/10	9/10
3	10/10	8/10
7	9/10	6/10
30	10/10	7/10

TABLE 3. Results for arm 1 (4°C)

Time in UPT (days)	Cycle number for indicated time (h) prior to UPT ^a		
	1	6	24
1	0	0.7	0.6
3	0.2	0	1.1
7	1.5	1.3	0 ^b
30	0.9	0	0.3

^a Results shown are the difference in the mean C_T values for each time point compared to the mean C_T for time zero. Data are expressed as cycle numbers.^b Mean C_T was less than mean C_T at time zero.

The C_T values obtained from the PCR testing were also analyzed, as this provides a semiquantitative measure of the amount of DNA in the sample. For arm 1 (Table 3), under the various storage conditions, the rise in C_T for the aliquots ranged from 0 to 1.5 cycles, and there was no consistent increase in C_T for aliquots that were maintained for up to 24 h at 4°C prior to placing the sample in UPT. Moreover, once in UPT, the DNA remained stable for up to 30 days. For storage at 20 to 22°C (arm 2) (Table 4), there was a consistent increase in the C_T values for aliquots held for 24 h prior to the addition of the sample to the UPT; a similar but less pronounced effect was seen for samples maintained at room temperature for 6 h prior to the addition of UPT. A drop off in sensitivity of $\geq 1 \log_{10}$ (≥ 3.3 cycles) was seen for three different time points when aliquots were stored with UPT at 20 to 22°C, compared to none for samples held at 4°C. For specimens stored without the use of UPT (arms 3 and 4) (Table 5), there was a 0.6-cycle increase in C_T for aliquots held for 1 and 3 days at 4°C; this increased to 3.6 and 2.3 cycles for aliquots held 7 and 30 days, respectively. An even more pronounced increase in C_T value was seen for specimens stored at room temperature without the use of UPT, with the most dramatic increases being observed at 7 and 30 days. The rise in C_T value observed for aliquots held 7 and 30 days at room temperature would correlate with a drop in sensitivity of 1.9 to 2.0 $\log_{10}$.

DISCUSSION

T. vaginalis remains an important pathogen in both men and women. Traditionally, the diagnosis has been made by either wet mount or culture. However, several recent reports have shown that the detection of *T. vaginalis* DNA by PCR is more sensitive than either culture or wet mount (1, 4, 6, 12, 14, 15). Despite the superior performance of PCR, few laboratories have adopted molecular testing for the routine detection of

TABLE 4. Results for arm 2 (20 to 22°C)

Time in UPT (days)	Cycle number for indicated time (h) prior to UPT ^a		
	1	6	24
1	0.1	2.9	1.8
3	0.1	0.4	2.9
7	0.8	1.5	3.7
30	0.9	3.6	4.9

^a Results shown are the difference in the mean C_T values for each time point compared to the mean C_T for time zero. Data are expressed as cycle numbers.

TABLE 5. Results for arms 3 and 4

Time in storage (days)	Cycle number for ^a :	
	Arm 3 (4°C)	Arm 4 (20–22°C)
1	0.6	0.1
3	0.6	1.9
7	3.6	4.4
30	2.3	4.6

^a Results shown are the difference in the mean C_T values for each time point compared to the mean C_T for time zero. Data are expressed as cycle numbers.

T. vaginalis infections. The ability to use the same specimen, transport device, and nucleic acid extraction method that is used for *C. trachomatis* and *N. gonorrhoeae* testing would be helpful to laboratories considering implementing *T. vaginalis* PCR testing. We have previously described the use of the BD swab system as an appropriate collection device for *T. vaginalis* PCR testing (1). This report describes the stability of *T. vaginalis* DNA in urine stored at both 4°C and room temperature, with and without the use of UPT.

Overall, there was better stability of *T. vaginalis* DNA when urine specimens were stored at 4°C than when they were stored at 20 to 22°C and when the UPT system was used. Based on qualitative results (positive or negative for *T. vaginalis* DNA), it appeared that *T. vaginalis* DNA in urine was stable at 4°C for 30 days without the use of UPT, as 39 of the 40 aliquots remained positive over that time period. However, when assessing the rise in C_T value when specimens were stored at 4°C, it appears that there was a loss of sensitivity of the PCR assay when aliquots were held for more than 3 days without the use of UPT, with a rise in the mean C_T value of 2.3 to 3.6 cycles. For specimens stored at room temperature without the use of UPT, the rise in C_T value for urine aliquots held for more than 3 days was even more pronounced, at 4.4 to 4.6 cycles. To better assess what rise in C_T value was important, we reviewed data from our previously published study (1), in which urine samples were collected from women and tested for *T. vaginalis* DNA. A total of 52 specimens were positive for *T. vaginalis* DNA. The mean C_T observed for specimens that were both PCR and culture positive for *T. vaginalis* was 26.2, with a range of 18.3 to 39.1, while specimens that were culture negative and PCR positive had a mean C_T value of 33.7, with a range of 23.7 to 39.8 (1). In that study, there were no data available on whether the women were asymptomatic or symptomatic. Seven of the 52 (13.5%) positive specimens had a C_T value of >36. Based on these data, a significant number of clinical specimens would be expected to have a high C_T value, so a rise in C_T value of greater than 2 cycles may lead to a loss in clinical sensitivity. Using this 2-cycle rise in C_T value as a general guide, *T. vaginalis* DNA in urine was stable when stored without UPT at 4°C for about 3 days and at room temperature for only 1 day. For specimens placed in UPT within 24 h (times of 1, 6, and 24 h) of collection, the DNA was stable for up to 30 days when stored at 4°C. For specimens stored at room temperature, ideally the urine should be added to the UPT within 1 hour of collection, which can be done at the site of collection. DNA then remained stable for up to 30 days. When storing specimens at room temperature, a delay of 24 h prior to adding the aliquot to UPT led to an unacceptably high loss of assay sensitivity.

Shafir et al. (10) recently studied the effect of processing

time delay on culture and PCR testing for *T. vaginalis*. Samples were held for various times at either room temperature or 37°C. The sensitivity of culture dropped off dramatically after 30 min for samples held at either 37°C or room temperature. For PCR testing, the sensitivity of the test began to drop off after 60 min of storage at either 37°C or room temperature. Our results were similar in that the sensitivity of PCR testing began to drop off after 1 day of storage at room temperature without the use of UPT.

In summary, the use of the UPT is an effective method to stabilize *T. vaginalis* DNA in urine for PCR testing. Ideally, urine should be added to the UPT within 1 hour of collection or stored at 4°C for up to 3 days before adding the urine to the UPT. Under these conditions, once in the UPT, the DNA is stable for up to 30 days. The use of the UPT allows laboratories to easily test for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* DNA from a single specimen.

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