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Optimal timing for *Trichomonas vaginalis* test of cure using nucleic acid amplification testing

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Abstract

Background ––The optimal timing for nucleic acid amplification testing (NAAT) post-treatment for *Trichomonas vaginalis* has not been fully established. Testing too soon post-treatment may detect remnant nucleic acid that is not from viable organisms, falsely misclassifying person as infected. The purpose of this study was to examine how long *T. vaginalis* nucleic acid is detectable post metronidazole (MTZ) treatment.

Methods ––Women diagnosed with *T. vaginalis* treated with MTZ (2 g single-dose or 500 mg twice daily for 7 days multi-dose) self-collected a vaginal swab for NAAT at baseline and each week post completion of treatment through test of cure (TOC) at week 4, when a culture was also performed. Women who reported interim sexual exposure or who were culture positive at 4 weeks were excluded. Time to first negative NAAT was examined using Kaplan Meier analysis.

Results ––All women receiving multi-dose metronidazole were NAAT negative by 21 days and those receiving single-dose by 28 days post completion of treatment. Though over half (60.7%) of the cohort reinitiated sex during follow-up¸ all reported using condoms during sex or that they and their partner were treated prior to sex. Six of 89 (6.7%) had a positive NAAT following their first negative NAAT.

Conclusions ––The optimal timing for *T. vaginalis* retesting after completion of treatment is three weeks for those receiving multi-dose MTZ and four weeks for those receiving single-dose, though sexual re-exposure and false negatives should be considered.

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Conflict of Interest:

All investigators except Dr. Van der Pol received a portion of their salary, via their institutions from the NIH/NIAID 1R01AI097080–01A1. Patricia Kissinger, Charleigh Granade, Megan Clare Craig-Kuhn, Norine Schmidt, Rebecca A. Lillis have no other disclosures. . Stephanie N. Taylor has received research support from Becton-Dickinson, Hologic, Cepheid, Beckman-Coulter, Roche, ELITech, GlaxoSmithKline, Melinta and Entasis Consulting and peer educator honoraria, and advisory board honoraria from Hologic and GlaxoSmithKline. David H. Martin has served as a consultant for BioFire Diagnostics and GlaxoSmithKline. Barbara Van Der Pol receives research support, honorarium or consulting fees from Abbott Molecular, Becton Dickinson, BioFire Diagnostics, Hologic, Roche Molecular, Rheonix and SpeeDx. Christina Muzny has served as a consultant for Lupin Pharmaceuticals.

Summary :

Women with trichomoniasis who were culture negative at 4 weeks post-treatment-completion and denied follow-up sexual exposure were retested weekly by NAAT. No detectable trichomonal rRNA was found post-treatment with metronidazole at 3 weeks for multi-dose and 4 weeks for single-dose.

Keywords

sexually transmitted infections; *Trichomonas vaginalis*; trichomoniasis; nucleic acid amplification test; rRNA clearance; metronidazole

Introduction

Trichomonas vaginalis, the most common curable sexually transmitted infection (STI) among women worldwide[1], is associated with vaginitis, cervicitis, urethritis, low birth weight, preterm delivery, endometritis and may increase the risk of herpes simplex virus (HSV) and human immunodeficiency virus (HIV) acquisition and transmission.[1–4] Repeat infections are common, ranging from 5%−31%, and share similar sequelae to primary infections.[5-9]

While wet prep has been used for decades to detect *T. vaginalis*, it has been found to have low sensitivity ranging from 40%−60%.[10] Culture has been considered the gold standard and has a sensitivity of 75%–96% and a specificity of up to 100%.[11] Nucleic acid amplification tests (NAAT) for *T. vaginalis* are more sensitive than culture (92–97% sensitivity)[11] and have been commercially available since 2011. Several highly sensitive *T. vaginalis* NAATs have been approved by the US and Food and Drug Administration (FDA) for use in women on urine, vaginal swab (including self-collected), and endocervical specimens.[12–14] These include the Aptima TV (Hologic Gen-Probe), the BD ProbeTec Qx (BD Viper System; Becton Dickinson), and GeneXpert TV (Cepheid) assay. Other FDA approved NAAT tests that are close to becoming point of care assays include the Solana® Trichomonas Assay[15] and the AmpliVue® Trichomonas Assay.[16] NAAT technology based on DNA or rRNA extraction from specimens, followed by amplification of captured nucleic acids.

One issue in retesting after treatment is that remnant nucleic acid can still exist in vaginal fluids even if no viable organism persists, resulting in a positive test that may not have clinical meaning. Given high *T. vaginalis* repeat infection rates, the Centers for Disease Control and Prevention (CDC) recommends rescreening women for *T. vaginalis* 3 months after completing treatment.[17] However, there is some indication that most repeat infections occur early[7] and that they may be due to treatment failure.[18] There may be, therefore, a need to test sooner than 3 months. The CDC STD Treatment Guidelines[17] suggests that NAAT testing can occur as early as 2 weeks after treatment based on two studies among adolescent women who were mostly asymptomatic.[19,20] Those studies did not take sexual exposure or treatment failure into consideration. The purpose of this present study was to determine when *T. vaginalis* nucleic acid clears among women who were successfully treated with

metronidazole for trichomoniasis and who were not sexually exposed between diagnosis and rescreening.

Methods:

This study was a sub-study of a multi-centered randomized trial of two different doses of metronidazole for the treatment of trichomoniasis. Methods have been described elsewhere,[21] but briefly, study participants were recruited from three clinics: Delgado Personal Health Clinic, Crescent Care Health and Wellness Center in New Orleans, LA and the Jefferson County Department of Health (JCDH) - STD Specialty Clinic in Birmingham, AL. These clinics serve mostly African American, low income women, the demographic group most highly affected by *T. vaginalis* infections.[16]

Women were eligible to enroll into the study if they attended any of the clinics, were HIV-uninfected, tested positive for *T. vaginalis* by clinical testing (wet prep or NAAT testing) with confirmation by another study test (culture or NAAT), and were willing to return to the clinic for follow up visits. At enrollment, subjects' clinical diagnosis was confirmed with InPouch™ Culture or Aptima *T. vaginalis* (ATV) NAAT. Eligible participants were treated with oral metronidazole (MTZ) in either a 2 g single-dose (4 × 500mg tablets) or a 7-day 500 mg twice-daily regimen.

Women were asked to complete a total of 5 study visits: the enrollment visit and 4 weekly follow up visits. The first weekly follow-up visit was scheduled 7 days after the completion of the metronidazole treatment. For women taking the 2 g single dose this was 7 days after the enrollment visit and for women on the 7-day 500 mg twice-daily regimen this was 14 days after the enrollment visit

At each of the four follow up visits, subjects self-collected a vaginal swab for *T. vaginalis* NAAT testing. At visit five (test of cure visit), subjects also self-collected a vaginal swab for *T. vaginalis* testing using culture. In addition to biological specimens, study participants were either interviewed by study staff or asked to complete a brief computer-assisted survey at each visit eliciting information on medication adherence, sexual exposure and other clinical and behavioral factors.

NAAT specimens were tested in batches at either the LSUHSC or UAB laboratories using ATV. Results for NAAT tests were not available to provider or subjects during the study period and did not affect their study participation or treatment.

To be included in the analysis, subjects needed to: be adherent to treatment, self-report no sexual exposure during follow-up, not miss more than two consecutive follow-up visits, and have a *T. vaginalis* negative culture at their TOC visit. Sexual exposure was defined as vaginal sex without the use of a condom before both partners were treated.

Kaplan-Meier survival analysis was used to assess time to first NAAT negative test after treatment completion. The log-rank test was used to test for differences in survival functions between groups to favor larger time values. The 95% Hall-Wellner confidence bands are

shown in Figures 1 and 2 to provide an estimate of confidence across the entire survival curve. SAS 9.4 was used for this analysis.

The protocol was reviewed and received ethical approval by Institutional Review Boards at Tulane University, Louisiana State University Health Sciences Center (LSUHSC), the University of Alabama at Birmingham (UAB) and the JCDH Research Review Committee.

Results:

Of 98 women participating in the study, 9 were excluded due to the following reasons: missed more than two consecutive visits (n=4), positive or unknown culture result at TOC (n=4), and sexual exposure to untreated partner during follow-up (n=1)

The 89 women included in the analyses were predominantly African American (95.5%) and younger than 30 years (mean 26, range 19–70). Most (60.7%) received single dose metronidazole and most (60.7%) were enrolled at the JCDH clinic. Additional demographics, behavioral risk factors, bacterial vaginosis, past TV infection, and birth control are described in Table 1.

Median time to first ATV negative test was 8 days (range 6–28 days) post completion of treatment. Among all women, the percentage testing negative at 7 days was 41.6% (95% CI, 32.1%−52.5%), 91.0% (95% CI, 84.0%−95.8%) at 14 days, and 96.6% (95% CI, 91.3% −99.1%) at 21 days. By 28 days all women had at least one negative test result by NAAT. By one week after completion of treatment, 19.8% had engaged in sexual intercourse, which increased to 60.7% by 4 weeks. All reported that they always used condoms during vaginal sexual intercourse or both the participant and sexual partner(s) had completed treatment. (Table 2).

The median time to clearance for women receiving the single dose versus multi-dose was 9 days (range 6–28 days) versus 7 days (range 7–20 days), (Log-Rank p-value=0.04). By day 21 post completion of treatment, all women on the multi-dose had tested NAAT negative and by day 28 all women on the single-dose tested NAAT negative (Figure 2).

Time to first negative NAAT is defined as time between treatment completion and first ATV negative test. Time to negative NAAT was shorter for those receiving multi-dose compared to single-dose (p=0.04) but were similar by baseline vaginal douching status, baseline symptoms, history of *T. vaginalis*, and BV at enrollment. (Table 3).

Six of the 89 (6.7%) women, after having a negative NAAT result, had at least one positive NAAT result at a subsequent visit. Two (2.2%) of these women were NAAT positive at TOC (Table 4). These two women were retested 8 weeks later and one woman was culture and NAAT negative and the other women was culture positive but NAAT negative.

Discussion:

We found that trichomonal nucleic acid was no longer detectable for all women receiving multi-dose MTZ by 3 weeks and all women receiving single-dose MTZ by 4 weeks post

treatment completion indicating that *T. vaginalis* NAAT retesting should not occur before those times. Other studies where only single-dose MTZ was used found that at 3 weeks, clearance rates are between 85%[20] (which was significantly lower than our study) and 93%[19] (which was within the same range we found). The lower rates found in these studies could have been attributed to small sample sizes, misclassification due to treatment failure or re-exposure, or the higher organism load needed for DNA based testing.

Unlike the other studies, we removed women who were either potentially re-exposed or failed treatment (as detected by a positive culture at 4 weeks), thus reducing the potential for misclassification. By three weeks post treatment completion, nearly half of the women had engaged in vaginal sex, though all stated they used condoms or had condomless sex only after index and partner(s) were treated. While it is possible that self-reported sexual behavior is not accurate, weekly interviews should have reduced recall bias. The majority of the participants were interviewed using computer-assisted self-administered interviews (CASI) which has been shown to reduce social desirability bias.[22,23] It should be noted that sexual activity increased each week and by 3 weeks nearly half of the cohort had reinitiated vaginal intercourse. Waiting to test at 3 weeks does have some potential to mix treatment failure with reinfection.

Whereas other studies had smaller sample sizes, our study had a power of >0.95 to detect the clearance rates at 2, 3, and 4 weeks. While our sample size was largely symptomatic, analysis stratified by symptoms did not indicate a trend by symptom status.

The first negative NAAT test was sooner for women on multi-dose therapy compared to single-dose therapy. It is not clear why this occurred, but it should be noted that women who were on the multi-dose treatment had 7 days of treatment compared to 1 day of treatment for single-dose before follow-up started, thus this difference could have been attributed to lead time bias. It is important evaluate the difference in time to negative NAAT for women receiving multi-dose MTZ since, while single-dose is presently the first line of treatment, there is mounting evidence that multi-dose is superior to single-dose.[18,21,24]

It is also interesting that 6/89 women had a negative ATV result and then had a subsequent positive result, despite a negative 4 week culture. Some of this could have been false negative testing from the test itself. Another possibility is that sampling error could have occurred since these were self-collected vaginal swabs. It is also possible that *T. vaginalis* had not been fully cured and was in a persistent undetectable state, as has been found by others.[25,26] With the widespread use of NAAT for *T. vaginalis* testing, it is essential to understand the optimal timing for testing so that unnecessary re-treatment can be avoided. Re-testing using *T. vaginalis* NAAT should occur at three weeks or greater after treatment completion for those on multi-dose and 4 week for those on single-dose. Clinical application of this time estimate must account for the time needed to complete the prescribed treatment regimen, suspected adherence, and sexual activity.

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Study data were collected and managed using REDCap electronic data capture tools hosted at Tulane University. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

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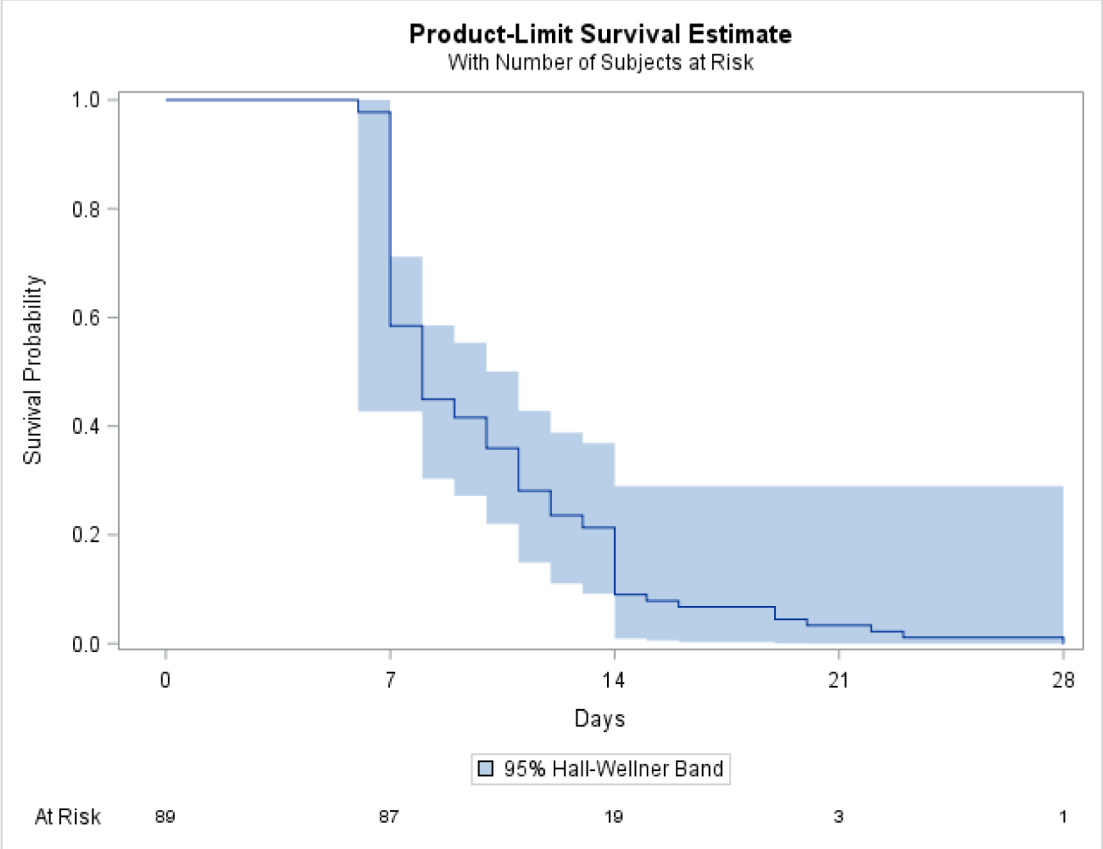

Figure 1. Time to first negative NAAT for all participants (N=89)
The highlights represent the 95% confidence bands

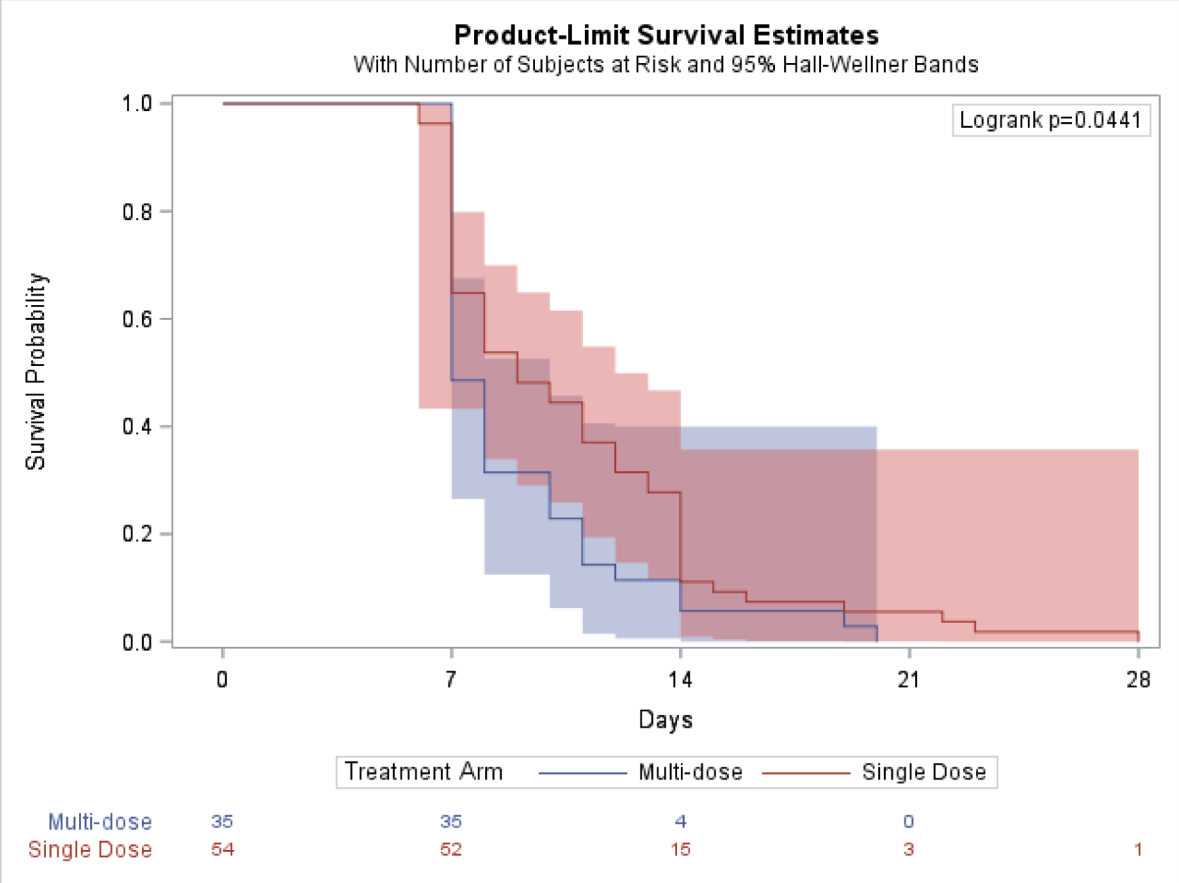

Figure 2. Time to first negative NAAT by treatment arm (N=89)

The highlights represent the 95% confidence bands

Table 1.

Description of the study population at baseline (N=89)

Characteristics	N	%
Metronidazole treatment		
1 g single-dose	54/89	(60.7)
500 mg twice daily for 7 days	35/89	(39.3)
Study Site		
New Orleans	35/89	(39.9)
Birmingham	54/89	(60.7)
Race		
African American	84/88	(95.5)
Other	4/88	(4.5)
Age		
< 30 years	53/88	(60.2)
≥ 30 years	35/88	(39.8)
Employment		
Unemployment	35/85	(41.2)
Employed	50/85	(58.8)
Education		
High school or less	50/88	(56.8)
Vocational or College	38/88	(43.2)
Douching		
No	55/88	(62.5)
Yes	33/88	(37.5)
Smoking in last month		
No	49/88	(55.7)
Yes	39/88	(44.3)
Drinking in last month		
No	25/71	(47.7)
Yes	46/71	(52.3)
Bacterial Vaginosis per Nugent score		
No	48/89	(53.9)
Yes	41/89	(46.1)
History of *T vaginalis* infection		
No	42/88	(47.7)
Yes	46/88	(52.3)
***T vaginalis* Symptoms**		
Vaginal discharge	50/88	(56.8)
Vaginal odor	9/88	(10.2)
Vaginal itching	39/88	(44.3)
Pain while urinating	9/88	(10.2)
Pelvic pain	13/88	(14.8)

Characteristics	N	%
Other	2/88	(2.3)
None	18/88	(20.5)
Birth control method		
Condoms	14/88	(15.9)
Hormones	15/88	(17.0)
Tubal ligation	12/88	(13.6)
Abstinence/withdrawal/none	49/88	(55.7)
Other	4/88	(4.5)
Number of male sex partners in past 3 months		
0	3/88	(3.4)
1	51/88	(58.0)
2 or more	34/88	(38.6)
Reported female sex in past 3 months		
Any	7/88	(8.0)
None	81/88	(92.0)

Table 2.

Cumulative vaginal sex during follow-up, by week and dose *

Day	Cumulative vaginal sexual intercourse since treatment
Total cohort	
7	17/86 (19.8%)
14	35/89 (39.3%)
21	44/89 (49.4%)
28	54/89 (60.7%)
Single dose	
7	11/52 (21.2%)
14	25/54 (46.3%)
21	34/54 (63.0%)
28	40/54 (74.1%)
7-day dose	
7	6/34 (17.6%)
14	10/35 (28.6%)
21	10/35 (28.6%)
28	14/35 (40.0%)

* Women self-reported either condom use or that both she and her partner(s) had been treated before sexual intercourse.

Table 3.

Time to first negative *T. vaginalis* NAAT test by selected variables (n=89)

	Median (min-max range) [IQR]	Log-Rank (for comparison)
Metronidazole Dose		
Single	9(6-28) [7-14]	0.04
Multi	7(7-20) [7-10]	
Douching*		
Yes	8 (6, 22) [7-14]	0.87
No	8 (7, 28) [7-12]	
Baseline Symptoms**		
Yes	8 (6-28) [7-12]	0.81
No	8 (6-19) [7-13]	
History of *T. vaginalis*		
Yes	8 (6-28) [7-11]	0.58
No	8 (6-23) [7-14]	
Bacterial Vaginosis		
Yes	8 (6-23) [7-12]	0.30
No	8 (6-34) [7-14]	

*Douching defined as any douching in the 30 months prior to the baseline visit.

**Baseline symptom is defined as having any one (or more) of the following: vaginal discharge, vaginal odor, vaginal itching or irritation, pain while urinating, pelvic pain, or other vaginal complaints.

Table 4.

NAAT Result patterns among women with intermittent test negatives (n=6)

	Week 0 (baseline)*	Week 1	Week 2	Week 3	Week 4 (TOC)**
2018	+	−	+	−	−
4385	+	+	−	+	−
4418	+	+	−	+	+
4422	+	−	−	−	+
4424	+	−	+	−	−
4440	+	−	***	+	−

* All subjects had positive InPouch™ at baseline visit.

** All subjects had negative InPouch™ at TOC.

*** missed visit