

# Screening of Male Patients for *Trichomonas vaginalis* with Transcription-Mediated Amplification in a Community with a High Prevalence of Sexually Transmitted Infection

Kimber L. Munson,<sup>a</sup> Maureen Napierala,<sup>a</sup> Erik Munson,<sup>a,b</sup> Ronald F. Schell,<sup>c,d,e</sup> Timothy Kramme,<sup>a</sup> Cheryl Miller,<sup>a</sup> Jeanne E. Hryciuk<sup>a</sup>

Wheaton Franciscan Laboratory, Wauwatosa, Wisconsin, USA<sup>a</sup>; College of Health Sciences, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin, USA<sup>b</sup>; Wisconsin State Laboratory of Hygiene<sup>c</sup> and Departments of Microbiology<sup>d</sup> and Medical Microbiology and Immunology,<sup>e</sup> University of Wisconsin, Madison, Wisconsin, USA

*Trichomonas vaginalis* infection in males has been largely uncharacterized. Past reports indicated increased susceptibility to other sexually transmitted infection (STI) agents such as human immunodeficiency virus and *Neisseria gonorrhoeae* with concurrent *T. vaginalis* infection. This warrants a more thorough review of male *T. vaginalis* incidence. A retrospective 3-year investigation of transcription-mediated amplification (TMA)-based urethral swab and first-void urine screening for *T. vaginalis* within a regional health care system was performed to address *T. vaginalis* prevalence in males. Of 622 total samples tested, 6.6% were positive for *T. vaginalis*. Delimitation of all specimens by ZIP code of patient residence revealed 11 predominant ZIP codes with respect to testing volume and detection rates. Within these 11 ZIP codes, representing 78.3% of total testing volume, urine was the preferred specimen source compared to urethral swabs. Seven of these 11 ZIP codes contained majority African American populations. The aggregate *T. vaginalis* detection rate trended higher than that of the remaining four ZIP codes, which were comprised primarily of Caucasian populations (8.9% versus 5.0%, respectively;  $P = 0.15$ ). The average age of a *T. vaginalis*-infected male (39.9 years) was significantly greater than those for *Chlamydia trachomatis* or *N. gonorrhoeae* (27.6 and 25.9 years, respectively;  $P < 0.001$ ). Given the significant rate of *T. vaginalis* detection, with age distribution analogous to that reported in females, TMA-based detection of *T. vaginalis* can be a routine constituent within a comprehensive STI screening panel for males in high-prevalence STI communities.

Past literature has estimated the worldwide incidence of trichomoniasis at 180 million cases, with 3 to 5 million cases occurring annually in the United States (1). *Trichomonas vaginalis* infection also plays an important role in the acquisition (2, 3) and transmission (4, 5) of human immunodeficiency virus. Moreover, codetection of *Neisseria gonorrhoeae* with *T. vaginalis* is a common occurrence (6). These data were largely generated using *T. vaginalis* diagnostic modalities (typically, vaginal saline suspension microscopy [wet mount] and culture) which have proven to be less sensitive than nucleic acid amplification testing (7–9).

Little clinical information is available about asymptomatic trichomoniasis in males (10). Molecular diagnostic techniques may enhance our ability to detect trichomoniasis. The advent of noncommercial PCR testing has increased *T. vaginalis* detection in males compared to culture techniques (11, 12). Prevalence rates in males presenting to sexually transmitted infection (STI) clinics have exceeded 10% (11, 12), with limited data suggesting increased distribution in older males (13). Furthermore, studies in females utilizing transcription-mediated amplification (TMA) demonstrated significant codetection of *N. gonorrhoeae* with *T. vaginalis* and further association with *Chlamydia trachomatis* (8, 14). These methods lend themselves to a true assessment of *T. vaginalis* in male populations. We hereby present data on the prevalence of trichomoniasis from a large male population presenting for nonemergent care within a metropolitan area of high STI prevalence.

(Results of this work were previously presented, in part, at the 111th General Meeting of the American Society for Microbiology, New Orleans, LA, 21 to 24 May 2011.)

## MATERIALS AND METHODS

**Setting.** U.S. metropolitan statistical area (MSA) data document a long-standing trend of high STI prevalence in the Milwaukee (Wisconsin) met-

ropolitan area (15). The Milwaukee-Waukesha-West Allis MSA gonorrhea rate was 219.6 per 100,000 population in 2010. This was the second highest in the United States and 92.8% higher than the national cumulative MSA rate of 113.9 per 100,000 population. This MSA also had the second-highest chlamydia rate (738.1 per 100,000 population). The national MSA chlamydia rate was 452.6 per 100,000 population. Testing was performed at Wheaton Franciscan Laboratory, a centralized laboratory serving five Milwaukee metropolitan hospitals and an approximately 70-clinic outpatient physician group. The TMA-based *T. vaginalis* analyte-specific reagent (ASR) was offered to clinicians as a stand-alone assay or in conjunction with *C. trachomatis* and *N. gonorrhoeae* molecular screening.

**Specimen collection.** Primary urethral specimens were deposited into Aptima swab specimen transport tubes per the Aptima Combo 2 assay (Gen-Probe, Incorporated, San Diego, CA) package insert protocol. Alternatively, approximate 2-ml aliquots of first-void male urine were added to Aptima urine specimen transport tubes within 24 h of specimen procurement.

**Primary molecular screening assays.** Nonautomated *T. vaginalis* ASR, detecting organism-specific 16S rRNA via target capture, TMA, and chemiluminescent hybridization protection, was previously validated in-house on male urethral and urine specimens in conjunction with a proprietary alternative target TMA-based confirmatory assay (Gen-Probe). Hardick et al. (16) reported >96% sensitivity and specificity of a TMA-based assay for detection of *T. vaginalis* from males. Relative light unit values (RLU) of  $\geq 50,000$ , as generated by stand-alone luminometry, were

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Address correspondence to Erik Munson, Erik.Munson@wfhc.org.

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**TABLE 1** STI phenotype, determined by TMA-based assays specific for *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* among screened males positive for at least one STI

STI phenotype <sup>a</sup>			% of patient encounters by specimen source		
<i>Trichomonas vaginalis</i>	<i>Chlamydia trachomatis</i>	<i>Neisseria gonorrhoeae</i>	First-void urine <sup>b</sup>	Urethra <sup>c</sup>	Total <sup>d</sup>
+	-	-	39.7	28.0	36.7
+	+	-	2.7	4.0	3.1
+	+	+	1.4	0.0	1.0
+	-	+	1.4	0.0	1.0
-	+	-	34.2	56.0	39.8
-	-	+	6.8	8.0	7.1
-	+	+	13.7	4.0	11.2

<sup>a</sup> +, positive TMA screen; -, negative TMA screen.

<sup>b</sup> Seventy-three (15.5%) encounters had at least one STI.

<sup>c</sup> Twenty-five (17.2%) encounters had at least one STI.

<sup>d</sup> Ninety-eight (15.9%) encounters had at least one STI.

interpreted as positive. A similar method, the Aptima Combo 2 assay, was used for detection of *N. gonorrhoeae*-specific 16S rRNA and *Chlamydia trachomatis*-specific 23S rRNA (17).

**Study.** The Wheaton Franciscan Healthcare institutional review board approved this 3-year study of all male primary clinical specimens subjected to *T. vaginalis* ASR. Greater than 99% of all specimens were also screened for *C. trachomatis* and *N. gonorrhoeae*. Specimen source, screening result, age of patient, race/ethnicity of patient (when available), and ZIP code of patient residence were collected. Five-digit ZIP code tabulation areas were accessed through the 2000 United States Census database to provide racial/ethnicity distribution. The STI phenotype (permutations of *T. vaginalis*, *N. gonorrhoeae*, and/or *C. trachomatis* TMA-based detection) was computed from any health care encounter that resulted in detection of at least one STI.

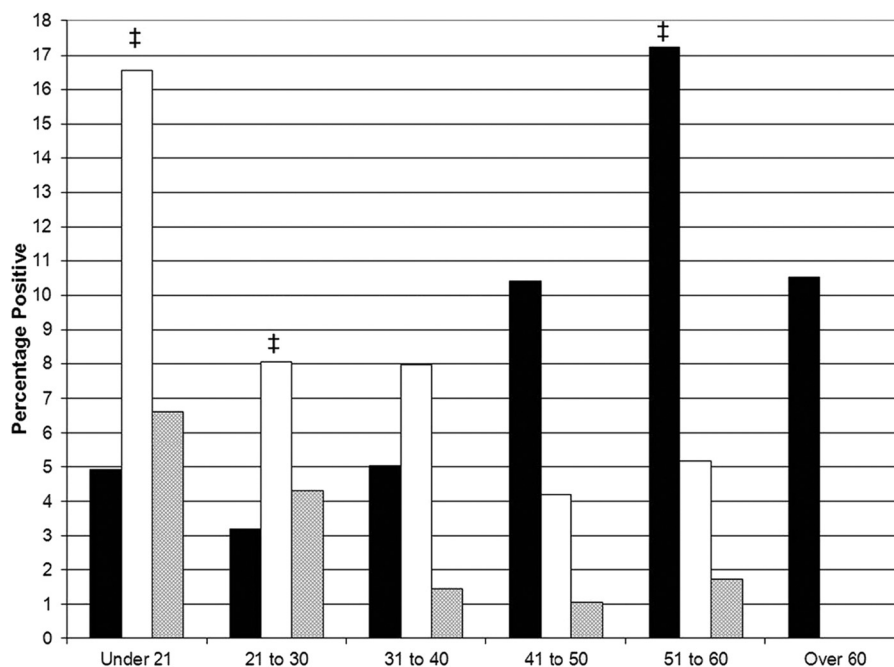
**Statistical analysis.** The significance test of proportions (two-proportion Z-ratio) determined if differences in either rates of positive screening results, proportion of specimen source, or STI phenotype were significant. The *t* test for independent samples determined if differences in mean patient age associated with positive results were significant between STIs. The alpha level was set at 0.05 before the investigations commenced, and all *P* values are two-tailed.

## RESULTS

**STI profile in males.** The *T. vaginalis* detection rate (6.6%) from 622 specimens showed no significant difference versus that of *C. trachomatis* (8.8%;  $P = 0.15$ ). Less *N. gonorrhoeae* (1.9%) was detected than *T. vaginalis* ( $P < 0.0002$ ). Six hundred seventeen of the specimens came from health care encounters that resulted in TMA-based screening for all three STIs; 98 (15.9%) health care encounters resulted in detection of at least one STI. Instances of sole *T. vaginalis* detection (36.7% in a combined first-void urine and urethral swab data set; Table 1) nearly equaled those of sole *C. trachomatis* detection (39.8%). In terms of individual STI agents, Table 1 reveals that STI phenotype differences between first-void urine and urethral collections were minimal ( $P \geq 0.05$ ).

**Demographics of males with *T. vaginalis*.** Mean and median ages of males tested were 33 and 31, respectively (range, 13 to 87 years). Detection of *T. vaginalis* was more common in older men (Fig. 1) and exceeded detection rates of the other STIs in this demographic. The age range of males with *T. vaginalis* was 14 to 67 years (median, 45); the mean age of 39.9 was greater than those associated with *C. trachomatis* and *N. gonorrhoeae* (27.6 and 25.9, respectively;  $P < 0.0001$ ). *T. vaginalis* was the only STI agent detected in men over 60 years.

For males with detectable *T. vaginalis* from whom race/ethnicity data were available ( $n = 29$ ), 89.7% were African American. Eleven Milwaukee County ZIP code tabulation areas yielded more



**FIG 1** Prevalence of *Trichomonas vaginalis* (solid bars), *Chlamydia trachomatis* (open bars), and *Neisseria gonorrhoeae* (shaded bars) among 622 males screened by transcription-mediated amplification-based methods, delineated by age (x axis). ‡, comparisons between *T. vaginalis* and *C. trachomatis* detection rates in which *P* values are  $< 0.05$ .

**TABLE 2** Delineation of 11 Milwaukee County ZIP code tabulation areas responsible for significant *T. vaginalis* male screening into geographical areas with majority African American or Caucasian populations, with characterization of the two subgroups

Parameter	Geographical region race majority		P value
	African American <sup>a</sup>	Caucasian <sup>b</sup>	
Percentage of urine submissions <sup>c</sup>	78.4	72.3	0.16
Mean no. of screenings per ZIP code	49.7	34.8	0.26
Percentage detection of <sup>d</sup> :			
<i>Trichomonas vaginalis</i>	8.9	5.0	0.15
<i>Chlamydia trachomatis</i>	8.4	9.4	0.74
<i>Neisseria gonorrhoeae</i>	3.7	4.3	0.78

<sup>a</sup> The mean difference between African American and Caucasian populations was 43.9% per ZIP code tabulation area ( $n = 7$ ).

<sup>b</sup> The mean difference between Caucasian and African American populations was 32.6% per ZIP code tabulation area ( $n = 4$ ).

<sup>c</sup> Percentage of all submissions.

<sup>d</sup> Combined urethral and male urine submissions.

than 78% of all male *T. vaginalis* ASR screens. Characteristics of this data subset, in terms of STI phenotype and specimen submission ratio, were representative of the overall data set described in this report (data not illustrated). In spite of seven ZIP code tabulation areas having an African American majority (Table 2), the *T. vaginalis* detection rate was not significantly higher in this aggregate than in a four-ZIP code compilation that exhibited a Caucasian majority ( $P = 0.15$ ).

## DISCUSSION

Past diagnostic modalities for male STI screening, including those based on molecular diagnostics, have largely been limited to assays specific for *C. trachomatis* and *N. gonorrhoeae*. Introduction of the *T. vaginalis* ASR has enhanced screening for *T. vaginalis*, especially in females (18). However, *T. vaginalis* screening in males has been largely uncharacterized. Wet mount and culture diagnostics, traditionally implemented in female populations, were not utilized in our study set. One study with a limited number of positive patients (16) reported >96% sensitivity of TMA for detection of *T. vaginalis* in male urine. In our investigation, 6.6% of males within a subacute care population were positive for *T. vaginalis*. We also show that older men may be a reservoir for transmission of the protozoan. In addition, the *T. vaginalis* detection rate did not differ from that of *C. trachomatis* ( $P = 0.15$ ) but exceeded that of *N. gonorrhoeae* ( $P < 0.0002$ ).

Our study provides a more accurate description of STI in the male community. In contrast to female populations that exhibit increased *T. vaginalis* detection versus *C. trachomatis* (19, 20), *T. vaginalis* detection in our male population was not significantly less than *C. trachomatis*. This finding is relatively novel due to a perceived lack of laboratory diagnostic options for *T. vaginalis* in males. The male STI phenotype for *T. vaginalis*, *C. trachomatis*, and *N. gonorrhoeae*, although exhibiting no significant difference between first-void urine and urethral specimen sources (Table 1), differed markedly from traditional female STI phenotype data (20–22). In general, a 17 to 24% increase in sole detection of *T. vaginalis* over *C. trachomatis* was observed in female populations with STI versus the ~5% difference noted in this male population.

Sole detection of *C. trachomatis* in female populations with STI was ~10% less than that observed in this male population.

The age distribution of males with *T. vaginalis* mimics those previously presented for females (19, 20). To summarize, *T. vaginalis* detection rates increased with patient age (Fig. 1). These data included an increased rate of *T. vaginalis* detection over that of *C. trachomatis* for the 51- to 60-year-old population within this high STI prevalence region ( $P = 0.04$ ). In addition, a 10.5% detection rate was observed in men over age 60. Such findings now extend to both genders (20) and support the paradigm of *T. vaginalis* being a more-relevant marker of risky sexual behavior among multiple age groups, including older men. Krashin et al. (23) reported on a risk factor for *T. vaginalis* infection in a female adolescent population, i.e., having a male sexual partner  $\geq 5$  years older. The authors raise the possibility of older males being a conduit between an older female reservoir of trichomoniasis and female adolescents. *T. vaginalis* ASR presents a viable option for screening these male populations.

A great majority of males with detectable *T. vaginalis* in this study were African American. One inherent limitation was that not all laboratory requisitions provided race/ethnicity data (data were available for 97% of patients yielding a positive *T. vaginalis* result). Subsequent translation of patient residence ZIP code into general race/ethnicity representations of local environments via census data suggested widespread organism distribution among both majority Caucasian and majority African American geographical regions (Table 2). While secondary limitations (residence migration during census intervals or sexual partners in other ZIP code tabulation areas) can confound this approach, the apparent community-wide distribution of *T. vaginalis* itself may result from enhanced analytical sensitivity of the *T. vaginalis* ASR. Moreover, this finding may have significant clinical and public health ramifications. Additional studies are needed to determine the prevalence of trichomoniasis in the general population.

Our findings warrant additional large-scale investigations. Studies addressing prevalence of *T. vaginalis* in Caucasian men have documented 11 to 23% *T. vaginalis* seroprevalence rates (24, 25). However, these studies were conducted in health professional cohorts. Second, when our group first evaluated the *T. vaginalis* ASR in a metropolitan female population (8), the patients were predominately African American. We had limited access to a Caucasian population. Finally, preliminary data attempting to portray an increased risk of prostate cancer with antecedent *T. vaginalis* infection (24–26) were inconclusive. However, the report from Stark et al. (24) stated that *T. vaginalis*-seropositive patients with lethal prostate cancer were 6.4 times more likely to have a documented history of *T. vaginalis* infection than case controls. Highly sensitive *T. vaginalis* ASR could provide additional data on the association of *T. vaginalis* with prostate cancer in all populations.

In conclusion, evidence is provided for another utility of TMA-based testing. In a general sense, *T. vaginalis* ASR presents the opportunity, in a commercialized format (27), to satisfy a largely unmet clinical and public health need. Increased analytical sensitivity of TMA versus those of other nucleic acid amplification methodologies (28–30) can augment this benefit. This enhanced diagnostic option may ultimately provide epidemiologists the ability to define a more accurate picture of trichomoniasis in the male community. In turn, this may influence screening practices in the realm of targeted populations and chronic disease prevention.



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