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# Retrospective Assessment of Transcription-Mediated Amplification-Based Screening for *Trichomonas vaginalis* in Male Sexually Transmitted Infection Clinic Patients

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**Transcription-mediated amplification (TMA) enhances detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* from rectal and pharyngeal sources. The utility of TMA for detection of *Trichomonas vaginalis* has recently been described. We report on the performance of TMA for detection of sexually transmitted infection (STI) agents from extraurogenital sources, with a focus on *T. vaginalis*. Within a 21-month interval, 1,314 consecutive male patient encounters at an STI clinic resulted in collection of 2,408 specimens for *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* TMA screening. A total of 471 encounters were managed with a single specimen collection (94.9% urine), with 12.7% positive for at least one STI agent. This detection percentage increased to 14.4% with collection of specimens from two sources and to 20.3% with collection from three sources ($P = 0.03$ versus single-source sampling). A total of 44.4% of encounters were managed by collection of urine and pharyngeal specimens and 19.1% by the addition of a third (rectal) collection. While procurement of urine and rectal specimens resulted in greater detection of *C. trachomatis* (6.1% and 11.3% rates, respectively) than of other STI agents, 858 pharyngeal specimens yielded a 2.9% *T. vaginalis* detection rate compared with 2.1% for *N. gonorrhoeae* and 1.6% for *C. trachomatis*. All *T. vaginalis* pharyngeal detections were confirmed by TMA-based alternative target testing. A total of 38.1% of *T. vaginalis*-positive pharyngeal specimens were derived from symptomatic patient encounters. A total of 85.7% of males with *T. vaginalis*-positive pharyngeal collections indicated strictly heterosexual preference. Additional specimen source sampling is necessary to make STI screening comprehensive. Incorporation of extraurogenital sources into assessment for *T. vaginalis* detection may identify additional symptomatic and asymptomatic male STI carriers.**

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). Typically, these data were derived from females using *Trichomonas vaginalis* diagnostics such as vaginal saline suspension microscopy (wet mount) and culture. Molecular techniques have greatly improved the detection of *T. vaginalis* in females (2–5). However, there is a paucity of data obtained from males. On a largely experimental and point-prevalence basis, PCR testing has revealed increased detection of *T. vaginalis* in males compared to culture techniques (6, 7). Application of transcription-mediated amplification (TMA) (8) to a clinical laboratory format has now facilitated *T. vaginalis* diagnostics in males (5, 9, 10). TMA-based clinical testing has provided an important tool for assessment of trichomoniasis epidemiology in males (11).

Nucleic acid amplification testing (NAAT) has demonstrated efficacy in the detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* from extraurogenital sources, including the rectum (12–16) and oropharynx (13–18). A number of studies have reported increased pharyngeal and rectal detection rates of these agents via TMA compared to DNA-based amplification modalities (12, 14–16, 18). This improved performance is hypothesized to be, in part, the result of efficient removal of endogenous specimen inhibitors of nucleic acid amplification (19, 20).

In this report, we take advantage of highly sensitive TMA, with its inherent capacity for endogenous inhibitor removal, to perform a comprehensive assessment of *T. vaginalis* incidence in males presenting to a sexually transmitted infection (STI) clinic. Our data document both symptomatic and asymptomatic extraurogenital carriage of this STI agent and ultimately advocate multispecimen source analysis for identification of male carriers of STI agents.

(Results of this work were previously presented, in part, at the 112th General Meeting of the American Society for Microbiology, San Francisco, CA, 16 to 19 June 2012.)

## MATERIALS AND METHODS

**Setting.** A recent analysis of data from United States metropolitan statistical areas (MSAs) documents a long-standing trend of high STI prevalence in the Milwaukee (Wisconsin) metropolitan area (21). These data reveal a Milwaukee-Waukesha-West Allis MSA chlamydial infection rate of 752.7 per 100,000 population in 2011. This figure ranked the third highest in the United States and was 54.7% higher than the national cumulative MSA rate of 486.5 per 100,000 population. The same MSA had the third-highest gonorrhea rate among United States MSAs (215.2 per

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100,000 population, nearly double the national MSA rate of 117.9 per 100,000 population).

**Specimen collection.** Approximate 2-ml aliquots of first-void male urine were added in clinic to APTIMA urine specimen transport tubes per the APTIMA Combo 2 assay (Gen-Probe, Incorporated, San Diego, CA) package insert protocol following specimen procurement. Off-label male pharyngeal and rectal specimens were obtained using APTIMA unisex collection swabs and then deposited into APTIMA swab specimen transport tubes (Gen-Probe). All swabs were stored at 2 to 30°C and tested within 60 days of specimen collection; urine aliquots were stored at 2 to 30°C and tested within 30 days of primary collection.

**Molecular screening assays.** The APTIMA Combo 2 assay detected *N. gonorrhoeae*-specific 16S rRNA and *C. trachomatis*-specific 23S rRNA (22) from all urine aliquots. The assay was also performed on primary pharyngeal and rectal swab specimens on the basis of an off-label validation executed in-house by the Wheaton Franciscan Laboratory. In addition, all urine aliquots, along with pharyngeal and rectal collections, were subjected to analysis using *T. vaginalis* analyte-specific reagent (ASR; Gen-Probe). The assay was previously validated in-house on male urethral and urine specimens (10). Hardick et al. (9) demonstrated >96% sensitivity of the assay for detection of *T. vaginalis* from male urine. This nonautomated assay detected organism-specific 16S rRNA by utilizing the principles of target capture, TMA, and chemiluminescent hybridization protection. Target molecules were isolated via oligomer-specific capture onto the surface of magnetic particles. Unique primers, in tandem with reverse transcriptase, catalyzed construction of a double-stranded DNA binding site for RNA polymerase. Following isothermal transcription, product detection was achieved by stand-alone luminometry via kinetic assessment employing a single-stranded DNA probe labeled with an acridinium ester molecule.

Using the same principle, a subset of rectal and pharyngeal specimens was subjected to a proprietary research-use-only confirmatory assay (23) supplied by Gen-Probe. This TMA-based assay utilized an alternative rRNA target and was performed with nonautomated methodology and stand-alone luminometry.

**Interpretation of results.** *T. vaginalis* ASR relative light unit values (RLU) ≥ 50,000 derived from first-void urine testing were interpreted as positive, as previously validated (10). Pharyngeal specimens generating RLU ≥ 20,000 were subject to repeat ASR analysis and the alternative target assay. On the basis of frequency distribution data, *T. vaginalis* ASR RLU output > 20,000, combined with *T. vaginalis* alternative target RLU output > 100,000, denoted a positive pharyngeal result.

**Audit.** The Wheaton Franciscan Healthcare institutional review board approved this July 2010 through March 2012 retrospective audit of *T. vaginalis* ASR screening of male STI clinic patients. Data pertinent to patient race/ethnicity, sexual preferences, and site-specific symptomatology at time of visit were collected.

**Statistical analysis.** The basis for STI phenotype computation was any health care encounter that resulted in TMA-based detection of at least one STI agent. The significance test of proportions determined if differences in rates of positive screening result, proportions of given specimen source, or STI phenotypes were significant. The *t* test for independent samples determined if differences in mean patient age associated with positive results were significant between STI etiologies. This analysis also determined if RLU differences derived from rectal and pharyngeal specificity assessments were significant. The alpha level was set at 0.05 before the investigations commenced, and all *P* values are two tailed.

## RESULTS

**Male patient encounters at STI clinic.** Within the retrospective 21-month study interval, 471 patient encounters (35.8% of all encounters) resulted in single-source specimen collection (Fig. 1), of which 447 (34.0% of all encounters) involved first-void urine. A combination of pharyngeal and first-void urine collection accounted for the greatest proportion (44.4%) of all patient encounters. In total, 592 patient encounters (45.1% of all encounters) resulted in collection of two specimens. A total of 251 (19.1%) patient encounters were managed with collection of first-void urine, pharyngeal, and rectal specimens.

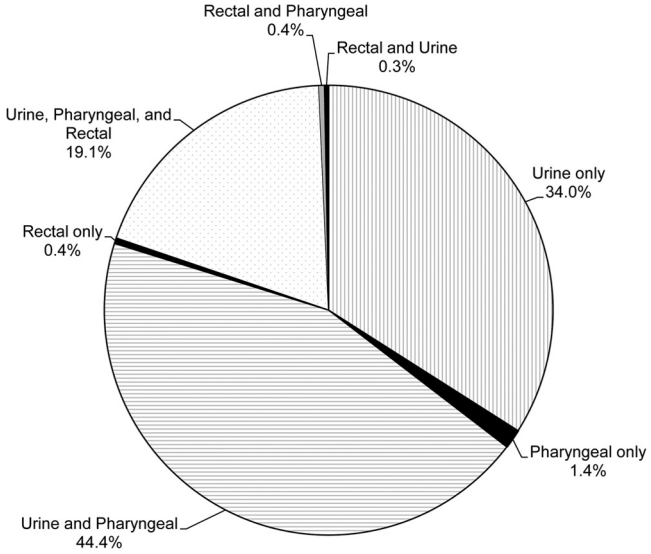


**FIG 1** Sexually transmitted infection (STI) screening of 1,314 males from a Milwaukee STI clinic, delineated by proportions of urine, rectal, and pharyngeal specimen collection.

**Detection of STI agents.** Of 1,285 first-void urine specimens screened for all three agents, detection of *C. trachomatis* (6.1% rate; Fig. 2) exceeded the rates of detection of *N. gonorrhoeae* (3.3%) and *T. vaginalis* (1.4%; $P \leq 0.0008$). Predominance of *C. trachomatis* (11.3% detection rate) was also demonstrated for a set of 265 rectal specimens ($P = 0.03$ versus *N. gonorrhoeae* detection rate). No *T. vaginalis* was detected from rectal swabs.

In contrast, the *T. vaginalis* detection rate from pharyngeal specimens (2.9%; Fig. 2) exceeded the rates for *N. gonorrhoeae* and *C. trachomatis* (2.1% and 1.6%, respectively). All pharyngeal detections of *T. vaginalis* were confirmed by repeat ASR testing and by generation of significant luminescence data from alternative target testing (mean RLU, 1,030,995; range, 130,099 to 1,288,550).

**Pharyngeal detection of *T. vaginalis*.** A total of 6.4% of pharyngeal collections resulted in detection of at least one STI agent (Table 1). Within this subset, 45.4% of positive screens involved detection of *T. vaginalis* (43.6% involved sole detection of *T. vaginalis*). These detection data exceeded those associated with *T. vaginalis* detection from first-void urine. A total of 14.4% of positive first-void urine screens involved *T. vaginalis* detection (Table 1), with sole *T. vaginalis* detection in 12.8% ($P < 0.0002$ versus analogous pharyngeal detection rates).

Demographic data were gathered from 21 males with detectable pharyngeal *T. vaginalis*. The mean age of these patients was 31.9 (median, 31; range, 22 to 57). These men had had on average 5.19 sexual partners within the previous 12 months (median, 3; range, 1 to 15), and 90.5% claimed heterosexual practices. Clinical presentation of eight patients (38.1%) was characterized as symptomatic for pharyngeal disease.

***T. vaginalis* TMA specificity assessment.** A total of 34 rectal specimens, previously screened as negative by *T. vaginalis* ASR, generated minimal RLU (mean, 339; range, 246 to 581) (Fig. 3)

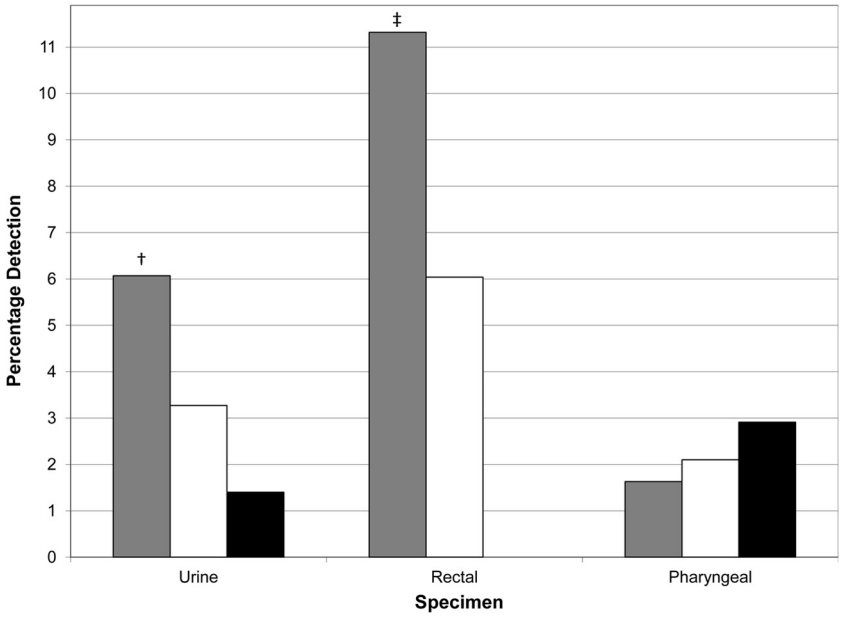


**FIG 2** Transcription-mediated amplification-based detection rates of *Chlamydia trachomatis* (gray bars), *Neisseria gonorrhoeae* (white bars), and *Trichomonas vaginalis* (black bars) in male urine, rectal, and pharyngeal specimens. † indicates $P \leq 0.0008$ versus detection of other etiologies; ‡ indicates $P \leq 0.03$.

upon alternative target TMA. Just 3 of 159 (1.9%) negative pharyngeal specimens yielded RLU > 100,000 (range, 652,364 to 1,161,727) upon alternative target testing, suggesting a high degree of specificity inherent in alternative target TMA. Within the remaining 156 pharyngeal specimens was a subset of 124 specimens that generated RLU (mean, 364; range, 260 to 567) similar to those produced by rectal specimen alternative target testing ($P = 0.62$; Fig. 3). The final 32 pharyngeal specimens yielded a third modal distribution, with a mean RLU of 50,148 (range, 31,677 to 64,010; Fig. 3).

**Additional extraurogenital collection for detection of STI.** The specimen source that yielded the highest proportion of positive STI screens was rectal swab (Table 1), as 15.5% of specimens yielded at least one STI agent ($P = 0.006$ versus the rate from urine

**TABLE 1** Sexually transmitted infection phenotype, determined by transcription-mediated amplification-based assays specific for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*, within screened males positive for at least one STI[a]

| Sexually-transmitted infection phenotype[b] | | | % patient encounters delineated by specimen source | | |
|---|---|---|---|---|---|
| *Chlamydia trachomatis* | *Neisseria gonorrhoeae* | *Trichomonas vaginalis* | Urine[c] | Rectal[d] | Pharyngeal[e] |
| + | − | − | 52.8 | 61.0 | 23.6 |
| + | + | − | 8.8 | 12.2 | 0.0 |
| + | + | + | 0.0 | 0.0 | 0.0 |
| + | − | + | 0.8 | 0.0 | 1.8 |
| − | + | − | 24.0 | 26.8 | 30.9 |
| − | − | + | 12.8 | 0.0 | 43.6 |
| − | + | + | 0.8 | 0.0 | 0.0 |

[a] STI, sexually transmitted infection.
[b] +, positive TMA screen result; −, negative TMA screen result.
[c] A total of 9.7% of encounters yielded at least one STI.
[d] A total of 15.5% of encounters yielded at least one STI.
[e] A total of 6.4% of encounters yielded at least one STI.

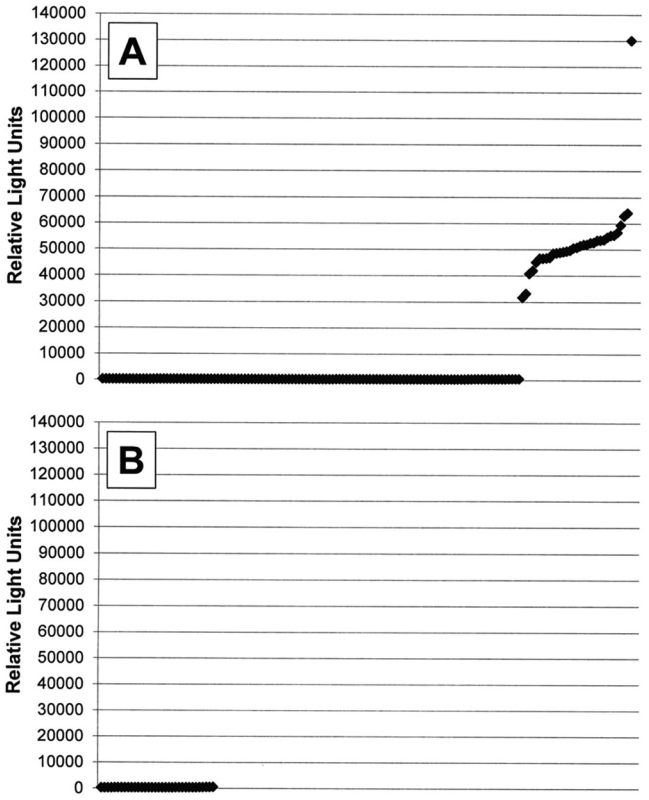


**FIG 3** Scattergram of rank-order relative light unit values derived from alternative target TMA of 159 pharyngeal specimens (A) and 34 rectal specimens (B) previously negative by *T. vaginalis* ASR. All ASR-positive pharyngeal specimens yielded a positive alternative target test result; as a point of reference, the lowest alternative target TMA RLU (130,099) from an ASR-positive pharyngeal specimen is illustrated in panel A (black diamond).

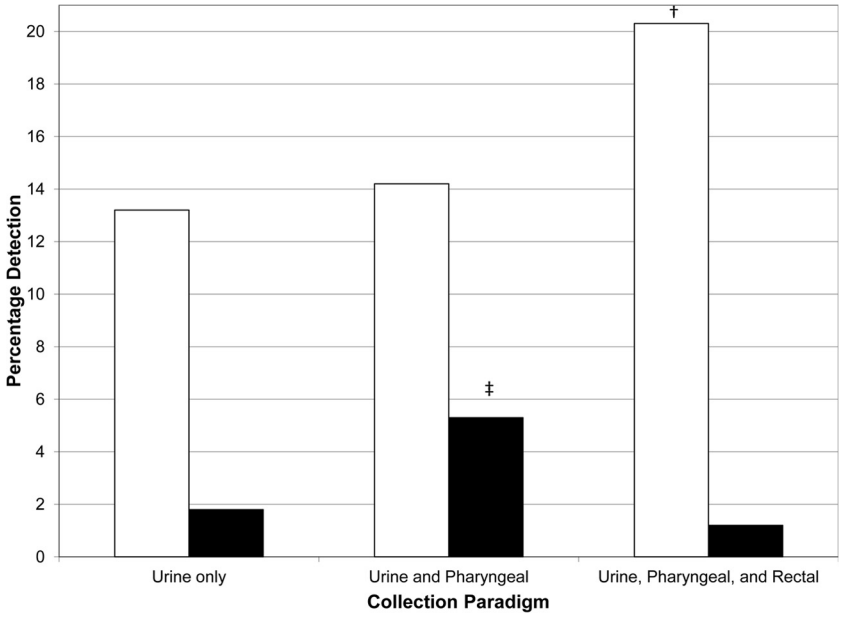


**FIG 4** Transcription-mediated amplification-based *Trichomonas vaginalis* detection rate (black bars) and sexually transmitted infection agent detection rate (white bars) as a function of specimen source collections per health care encounter. † indicates $P \leq 0.03$ versus other collection paradigms; ‡ indicates $P \leq 0.01$.

screening). Approximately 60% and 25% of positive rectal screenings involved sole detection of *C. trachomatis* and *N. gonorrhoeae*, respectively. Screening of male patients for all three STI using urine, pharyngeal, and rectal specimens identified an STI rate (20.3%; Fig. 4) that was higher than that seen with urine and pharyngeal specimens (14.2%; $P = 0.03$). Addition of a pharyngeal collection to a first-void urine specimen did not significantly increase detection of STI agents (13.2% detection rate for any STI agent from first-void urine; $P = 0.63$ versus combined urine and pharyngeal detection rate). However, overall detection of *T. vaginalis* was enhanced by combined pharyngeal and first-void urine screening (5.3% detection rate) compared to first-void urine (1.8% rate; $P = 0.01$).

## DISCUSSION

TMA has provided an additional tool for the investigation of STI in males. From a diagnostic perspective, Napierala et al. (10) reported 6.6% *T. vaginalis* detection from 622 male urogenital specimens in a largely subacute, community care setting. This rate was significantly greater than the rate seen with *N. gonorrhoeae* (3.2% detection; $P = 0.006$) but not significantly different from the *C. trachomatis* detection rate (8.8%; $P = 0.17$) in this metropolitan area of high STI prevalence. The urine detection rate in males did not vary from that yielded by urethral collections ($P = 0.46$). From an epidemiological perspective, the TMA assay in men portrays some similarities to findings in women (11, 24). Older males have a greater propensity to harbor *T. vaginalis* than other STI agents. Furthermore, Munson et al. (11) suggested that distribution of the *T. vaginalis* protozoan in predominately Caucasian geographical areas rivals that in African American-majority areas.

From a technical standpoint, the clinical laboratory TMA format has been augmented by the addition of target capture oligonucleotides with concomitant washing and aspiration of inhibitory substances. Prior to incorporation of target capture, Mahony et al. (25) reported an 11.9% inhibition rate of *C. trachomatis* detection via first-generation TMA. Chernesky et al. (26) reported that a second-generation TMA assay yielded a 0.3% inhibition rate for *C. trachomatis* detection from urine specimens. In an effort to model the inhibitory effect of excess erythrocytes, *ex vivo* introduction of phosphate and iron to mock specimens containing *C. trachomatis* near the lower limit of detection generated no false-negative results upon TMA analysis (27). In our previous report on *T. vaginalis* male testing, second-generation TMA provided an unequivocal test result in 99.8% of instances (10).

Data derived from the aforementioned studies potentiate analysis of STI agents from extraurogenital sources. Baseline studies determined the culture sensitivity range for *C. trachomatis* detection from pharyngeal (14) and rectal (12, 14, 15) specimens to be 21.1% to 45.7% using a NAAT reference standard. The analogous range for culture-based detection of *Neisseria gonorrhoeae* was 34.3% to 47.4% (13, 16, 17). Within the realm of NAAT, Ota et al. (15) demonstrated 64.7% sensitivity of commercial PCR for detection of *C. trachomatis* from rectal specimens compared to TMA. Bachmann et al. (12) calculated sensitivity ranges of commercial PCR, DNA amplification in the format of strand displacement amplification (SDA), and TMA at 80.7% to 95.5%, 92.2% to 100.0%, and 100%, respectively, for detection of rectal chlamydia using a rotating infected patient status. Sensitivity of TMA was 30% and 33% greater than that of commercial SDA for detection of rectal and pharyngeal chlamydia, respectively, in specimens from a MSM demographic (15). Moreover, Bachmann and colleagues (12, 18) determined that the performance of PCR for detection of *N. gonorrhoeae* from pharyngeal and rectal sources was inferior to those of SDA and TMA.

Our retrospective data also reflect efficient detection of STI agents from extraurogenital sources. Increased *C. trachomatis* detection from rectal specimens (11.3%) was observed in comparison to that from urine (6.1%; $P = 0.0002$). In a similar fashion, the rectal *N. gonorrhoeae* detection rate (6.0%) was increased over that

from urine (3.3%; $P = 0.006$). STI phenotype data suggested that 15.5% of rectal samplings revealed at least one STI (Table 1), a value higher than STI agent detection from pharyngeal or urine collections ($P \leq 0.0016$). These data contributed to the generalized finding that multiple specimen source sampling resulted in additional identification of STI agents in male STI clinic patients (Fig. 4). Despite the fact that combined urine, pharyngeal, and rectal sampling was performed in just 19.1% of patient encounters (Fig. 1), this sampling paradigm resulted in greatest yield of any STI agent (Fig. 4).

Extraurogenital detection of *T. vaginalis* contributed to this phenomenon but only in the context of pharyngeal specimen collection. STI phenotype data revealed that sole detection of *T. vaginalis* was responsible for nearly 44% of all STI detection from pharyngeal specimens (Table 1). While our demographic assessment was limited to a degree by the small number of men harboring pharyngeal *T. vaginalis*, data did reveal an older age distribution of males harboring the organism. Mean age was greater than in males with detectable pharyngeal *C. trachomatis* ($P = 0.03$), mimicking past data (11) with respect to urogenital trichomoniasis. While less than 40% of males were symptomatic for pharyngeal disease (*C. trachomatis* codetection was observed in one patient), the preponderance of heterosexual practices among these patients suggests a pharyngeal reservoir-like role for transmission of *T. vaginalis*. Taken together, these findings may contribute to further risk factor delineation in trichomoniasis. Of note, Krashin et al. (28) reported that a male sexual partner at least 5 years older was a risk factor for trichomoniasis in adolescent females.

*T. vaginalis* alternative target TMA specificity was previously assessed by our laboratory in female populations. When alternative target testing was performed on 65 *T. vaginalis* ASR-negative endocervical specimens, three (4.6%) yielded a positive result (4). When analogous testing was performed on 62 *T. vaginalis* ASR-negative vaginal saline suspension aliquots, one (1.6%) yielded a positive result (23). Similar to these studies, the current data set exhibited 0% and 1.9% alternative target positivity rates in ASR-negative rectal and pharyngeal specimens, respectively. However, approximately 20% of an ASR-negative pharyngeal sampling yielded low-level luminescence via alternative target TMA. *Trichomonas tenax*, a largely commensal protozoan of the human oral cavity, has documented prevalence rates of 4% to 53% (29). Both this protozoan and *T. vaginalis* have been associated with bronchopulmonary infections following endogenous and acquired oral cavity colonization, respectively. Kucknoor et al. (30) recently determined that these two protozoa have significant genetic identity, with *T. vaginalis* exhibiting higher levels of gene expression than *T. tenax*. While it is possible that *T. tenax* may have exhibited a low degree of cross-reactivity with the alternative target TMA, this likely did not factor into specific *T. vaginalis* detection from pharyngeal specimens via *T. vaginalis* ASR. In support, package insert data from the U.S. Food and Drug Administration-approved *T. vaginalis* TMA assay (31) state that mock introduction of *T. tenax* into female urogenital matrices containing *T. vaginalis* had no effect on the specificity of the assay. Finally, none of the ASR-positive pharyngeal specimens in our study yielded RLU in the range of 30,000 to 60,000 (Fig. 3) upon alternative target TMA. The range of such data was 130,099 to 1,288,550.

Reports of *T. vaginalis* detection in extraurogenital specimens are rare. In an investigation of 949 female pharyngeal swabs concomitantly assessed to be free of amplification inhibitors, the *T. vaginalis* detection rate was 0.4% by PCR (32). The same study revealed a 1.5% *T. vaginalis* detection rate from rectal swabs collected from a group of 952 women. An initial assessment of *T. vaginalis* extraurogenital detection in men (33) revealed that 3 of 70 human immunodeficiency virus-positive individuals had detectable pharyngeal *T. vaginalis* by PCR. Two of the men were asymptomatic at the time of specimen collection, and all acknowledged a history of orogenital sexual activity. In a study of 225 males, with 12.9% claiming symptoms of rectal disease, Cosentino et al. (34) reported a 0.9% *T. vaginalis* detection rate by TMA. Our data revealing increased pharyngeal detection of *T. vaginalis* over that recovered from the rectum extend these findings. It was of further interest that 27.9% of the rectal swabs collected from females in the study by Crucitti et al. (32) revealed evidence of amplification inhibition, resulting in a nonvalid *T. vaginalis* PCR result. Cosentino et al. (34), with the benefit of target capture-enhanced TMA, was able to report an 8.8% rate of *T. vaginalis* detection from female rectal swabs.

In conclusion, target capture-based TMA modalities effectively detected *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* RNA from extraurogenital specimens collected from male STI clinic attendees. *T. vaginalis* was the STI etiology most commonly detected from pharyngeal specimens. Procurement of rectal, pharyngeal, and urine specimens significantly increased overall detection of male STI carrier status. Application of *T. vaginalis* TMA-based detection to pharyngeal specimens presents an opportunity for further study of the role of pharyngeal *T. vaginalis* carriage with respect to trichomoniasis epidemiology. In total, further utility of this technology can facilitate additional intervention and prevention opportunities for clinical and public health professionals.

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