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Update on Laboratory Diagnosis and Epidemiology of *Trichomonas vaginalis*: You Can Teach an "Old" Dog "New" Trichs

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Update on Laboratory Diagnosis and Epidemiology of Trichomonas vaginalis: You Can Teach an "Old" Dog "New" Trichs

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Abstract

Past viewpoints on *Trichomonas vaginalis* infection have characterized the associated clinical disease as a "nuisance" condition, with affected demographics largely being older African American females residing in urban centers. The advent of commercial molecular assays specific for *T. vaginalis*has offered a new outlook

on trichomoniasis. Within high-prevalence sexually transmitted infection populations, parasite distribution is not localized to specific population centers, and *T. vaginalis* prevalence is elevated among both younger and older age groups. Adaptation of these molecular assays can additionally facilitate male screening and subsequent epidemiologic characterization. These findings, combined with associations between *T. vaginalis* infection and human immunodeficiency virus (HIV) acquisition/transmission and persistent human papillomavirus infection, support consideration of the expansion of *T. vaginalis* screening efforts in the realms of clinical practice and public health.

Introduction

The French microscopist, clinician, and researcher A. F. Donné discovered the protozoan *T. vaginalis* 180 years ago. Trichomoniasis, the clinical entity ascribed to the pathogen, has become the most prevalent non-viral sexually transmitted infection (STI) in the United States (Fig. 1). A surveillance study conducted by the U.S. Centers for Disease Control and Prevention (CDC) in 2008 estimated trichomoniasis prevalence in this country at 4 million cases, with approximately 1 million new cases of *T. vaginalis* infection being diagnosed annually.[1] As will be posited and further described throughout this review, several factors impact the true characterization of trichomoniasis. As an introductory example, *T. vaginalis* prevalence rates are rather disparate on a worldwide basis. In contrast to reports of >20% prevalence in some U.S., African, and indigenous Australian population centers,[2,3,4,5] a number of European, Asian-Pacific, and non-indigenous Australian studies have documented rates lower than 5%.[6,7,8,9,10]

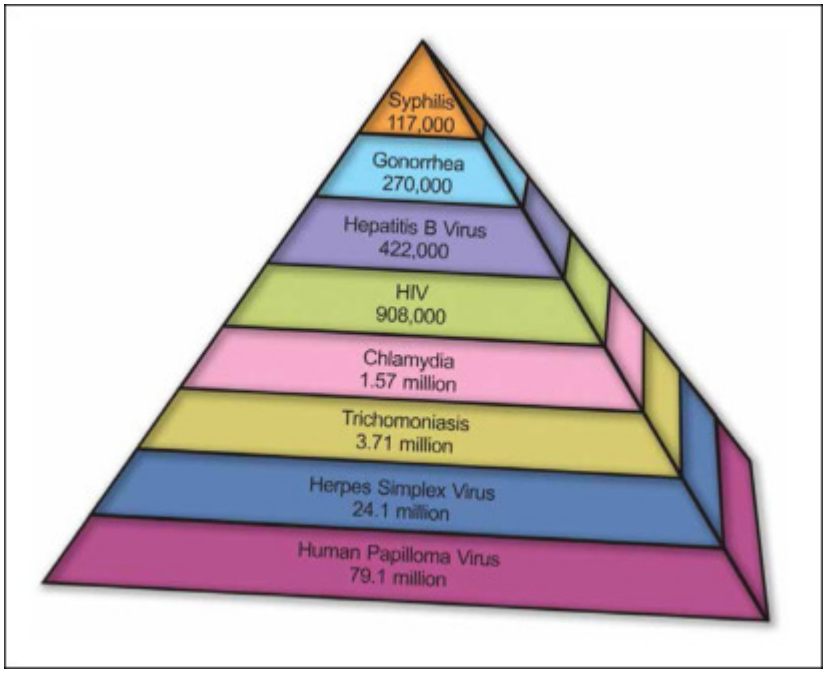

Figure 1. Estimated prevalence of STI in the United States (adapted from reference 75 with permission of the publisher).

The advent of molecular-based laboratory detection of *T. vaginalis* has broadened our knowledge of trichomoniasis, allowing new perspectives on disease epidemiology and opportunities for prevention. In addition, advancements related to diagnostic algorithms have garnered much attention in the clinical research setting. A PubMed primary literature search (U.S. National Library of Medicine and the National Institutes of Health) conducted in February 2016 using the search parameter "*Trichomonas vaginalis*" filtered by the possibility of "antigen," "molecular testing," "nucleic acid hybridization," "PCR," "nucleic acid amplification testing," "LAMP" (loop-mediated isothermal amplification), or "transcription-mediated amplification" selected 629 primary citations from the years 1964 to 2016, with over 25% published since the beginning of 2012. This brief commentary, with focus largely on literature published within the past 3 years, discusses updates on *T. vaginalis*epidemiology and laboratory testing.

Epidemiology

Females

Trichomoniasis can be characterized by a diffuse, malodorous, yellow-green vaginal discharge with vulvar irritation.[11] While pruritis and dysuria may be present, a majority of *T. vaginalis* infections are actually thought to be asymptomatic. This has long been a source of consternation for clinicians and researchers in the context of proper laboratory diagnostic strategies and clinical management. Data published nearly 25 years ago stated that approximately one-third of patients with asymptomatic *T. vaginalis* infection become symptomatic within 6 months,[12] perhaps necessitating additional encounters with health care. Mathematic modeling suggests that the average duration of *T. vaginalis* infection in women approaches 3 to 5 years.[13]

Such data imply indolent *T. vaginalis* infection in a significant percentage of women, yet the organism has been associated with pregnancy complications, including preterm delivery, low-birth-weight delivery, and delivery of offspring later diagnosed with intellectual disability or attention deficit-hyperactivity disorder.[reviewed in reference 14] From a retrospective audit of 108,346 Medicaid recipients [15] it was concluded that females diagnosed with trichomoniasis within the first 7 months of pregnancy were more likely to deliver at ≤33 weeks of gestation (hazard ratio, 1.22 [95% confidence interval, 1.02 to 1.46]), while those diagnosed within the first 8 months of pregnancy were more likely to deliver late pre-term (33 to 36 weeks; hazard ratio, 1.59 [95% confidence interval, 1.18 to 2.14]). In single-site studies of South African and U.S. females,[reviewed in reference 14] *T. vaginalis* associations with pelvic inflammatory disease in HIV-positive patients, acute endometritis, and tubal infertility have been reported.

T. vaginalis has also achieved a status of importance in the public health community. The natural course of human papillomavirus (HPV) infection is largely self-limiting, with 60 to 80% of infections becoming undetectable in 8 to 10 months.[16] Prolonged or persistent HPV infections, particularly those involving high-risk HPV genotypes, contribute to ultimate development of cervical neoplasia. From an adolescent cohort, Shew et al.[16] demonstrated that median time to clearance of primary HPV infection was 172 days in patients without concomitant *T. vaginalis* infection. The median value increased to 436 days in patients with concurrent *T. vaginalis* infection. Donders et al.[8] performed multiplex *T. vaginalis*/HPV real-time PCR on 63,251 liquid-based cytology collections from Belgian women. In patients with detectable high-risk HPV DNA, the likelihood of having concomitant cytology findings of atypical squamous cells of uncertain significance or greater (ASC-US+) was higher when *T. vaginalis* was present than when *T. vaginalis* was absent (odds ratio, 1.98 [95% confidence interval, 1.04 to 3.50]; $P = 0.049$). Moreover, 17.8% of patients with detectable *T. vaginalis* DNA had cytology findings of ASC-US+, while only 8.7% of *T. vaginalis*-negative specimens had such findings (odds ratio, 2.3 [95% confidence interval, 1.6 to 3.2]; $P < 0.0001$). In an investigation of commercial sex workers in Kenya using RNA amplification techniques specific to both STI agents and high-risk HPV, Ting et al.[17] described higher rates of HPV positivity in women also positive for *T. vaginalis*.

The public health significance of *T. vaginalis* detection also extends to HIV. From a study of adolescents with behavioral risk factors for HIV infection, Mullins et al.[18] reported a higher *T. vaginalis* incidence rate in females who were HIV positive (1.3/100 person months) than in those who were not (0.6/100 person months; $P = 0.002$). Several studies have characterized a role for *T. vaginalis* in HIV acquisition by females. One prospective study of 4,948 sexually active African females[19] reported that *T. vaginalis*-infected women were more likely to test positive for HIV at a subsequent health care encounter (adjusted hazard ratio, 2.05 [95% confidence interval, 1.05 to 4.02]). Hughes et al.[20] prospectively studied 3,297 African HIV-serodiscordant couples and found that *T. vaginalis* infection of the female partner was an independent risk factor for increased probability of HIV acquisition per coital act (odds ratio, 2.57 [95% confidence interval 1.42 to 4.65]).

In similar fashion, studies have elucidated a role for *T. vaginalis* in HIV transmission. Co-incubation of *T. vaginalis* with acutely infected peripheral blood mononuclear cells *in vitro* has been shown to increase the HIV replication rate.[21] A Louisiana cohort with documented infection by both HIV and *T. vaginalis* were prospectively observed following a course of metronidazole therapy.[22] Successful anti-trichomonad therapy (determined by *T. vaginalis* culture) decreased the likelihood of HIV shedding at 3 months post-therapy compared to baseline shedding (relative risk, 0.34 [95% confidence interval, 0.12 to 0.92]). This finding was not duplicated in HIV-positive women without antecedent *T. vaginalis* infection. Mathematical modeling of an HIV-infected population in North Carolina (with a

presumed 22% *T. vaginalis* prevalence) predicted the occurrence of 0.076 HIV transmission events per 100 HIV-positive females who test positive for *T. vaginalis*.[23] This datum contrasts with the rate of 0.062 HIV transmission events in HIV-positive women who are *T. vaginalis* negative. In the former scenario, 23% of HIV transmission events were attributable to *T. vaginalis*infection.

Compared to commonly assessed STI agents, such as *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, the prevalence of *T. vaginalis* among older females is especially significant. Napierala et al.[24] summarized clinical laboratory testing of a subacute care population within a high-prevalence STI community using commercially available molecular assays for *C. trachomatis*, N. gonorrhoeae, and *T. vaginalis*. The *T. vaginalis* detection rate (9.3%) within a subset of 7,277 females exceeded those of *C. trachomatis* (5.7%) and *N. gonorrhoeae* (1.4%; both $P < 0.0002$). When detection rates were delineated by age decade,[25] the predictable finding of increased *T. vaginalis* detection versus *C. trachomatis* detection in patients aged 31 to 60 years was observed (Fig. 2). A *T. vaginalis* detection rate of greater than 11% was realized for females over the age of 60; however, the small subset did not allow statistical comparison versus *C. trachomatis* detection. Novel findings included elevated *T. vaginalis* detection rates in females under the age of 21 (Fig. 2) and significantly increased *T. vaginalis* detection in years 21 to 30 over that of *C. trachomatis*.

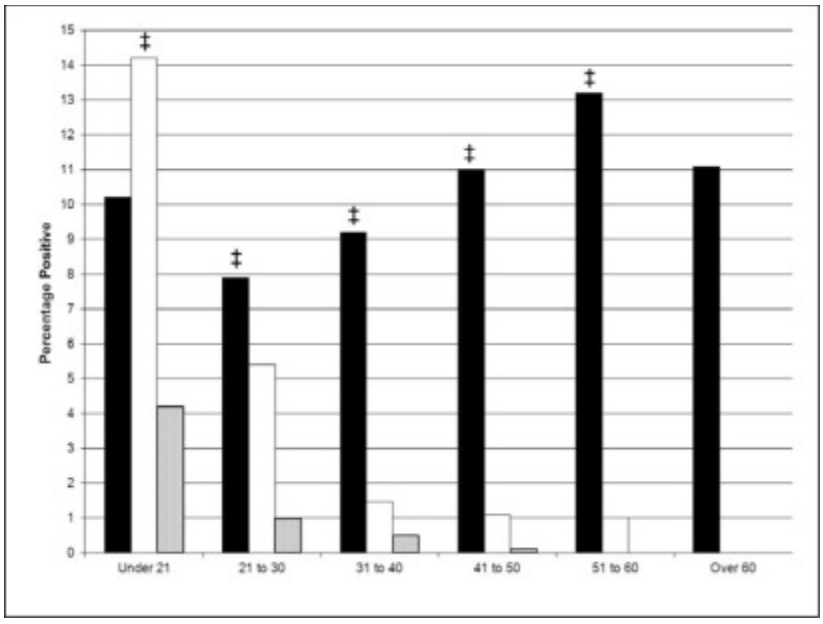

Figure 2. Prevalence of *Trichomonas vaginalis* (solid bars), *Chlamydia trachomatis*(open bars), and *Neisseria gonorrhoeae* (shaded bars) among 7277 females screened by commercial transcription-mediated amplification, delineated by age. ‡ represents comparisons between *C. trachomatis* and *T. vaginalis* detection rates in which $P < 0.05$.
Adapted from data published in,[25] with permission from the publisher.

Recent investigations have attempted to elucidate the *T. vaginalis* proclivity for older populations. Hearn et al.[26] stratified 264 Baltimore women into age groups of 18 to 44 years and ≥45 years. The prevalence of *T. vaginalis* did not differ between the groups, yet older adults who tested positive for *T. vaginalis*were more likely to have used marijuana (adjusted odds ratio, 3.1; $P = 0.036$) and crack cocaine (adjusted odds ratio, 3.8; $P = 0.010$) in the previous 6 months. In addition to general immunity decline during the aging process, the authors inferred a link between illicit drug use and increased susceptibility to infection. *In vitro T. vaginalis* infection models[27] demonstrated suppression of secretory leukocyte protease inhibitor (SLPI), an agent produced by female reproductive tract mucosal epithelia that protects against HIV acquisition. Huppert et al.[28] correlated *T. vaginalis*-SLPI interaction in a clinical setting, as a >50% reduction of this marker was consistent with recurrent *T. vaginalis*infection. The *T. vaginalis*-SLPI paradigm provides an additional perspective on the necessity for diagnosis of low-level or asymptomatic trichomoniasis. Finally, Fichorova et al.[29] concluded that hormonal contraceptives are associated with alterations in cervical immunity that are dependent upon the presence of genital infections, such as trichomoniasis. In a broad sense, these immune alterations may increase susceptibility to HIV acquisition. The authors posit the potential impact of undiagnosed, asymptomatic genital tract infection on this paradigm.

Past epidemiologic data, often derived from studies utilizing less sensitive diagnostic assays, have suggested that trichomoniasis is largely an urban disease. When considering the dataset in Fig. 2, it is important to note that <2% of those data were gathered from females in acute care.[25] By way of STI phenotype calculation (defined as permutations of *T. vaginalis, C. trachomatis*, and *N. gonorrhoeae* detection from any health care encounter that resulted in detection of at least one STI) and comparison of such data[25] with those from acute care[30] and from a combined subacute and acute care cohort,[31] one can ascertain parasite distribution across an entire metropolitan setting. The STI phenotype from subacute care demonstrated the greatest proclivity for sole detection of *T. vaginalis* (54.2% of health care encounters) (Table 1). In contrast, sole detection of *T. vaginalis* from acute care was 40.7%. Moreover, phenotypes that involved any detection of *T. vaginalis* were 63.5% and 55.8% in the subacute care and acute care populations, respectively. The above-mentioned data flank *T. vaginalis*phenotypes derived from an overall community setting.[31] These separate large-scale data collections implicate *T. vaginalis* distribution as being widespread throughout a metropolitan setting and signal the necessity for accurate laboratory diagnostics and clinician utilization of those modalities.

Table 1. High-prevalence community STI phenotypes, determined by commercial transcription-mediated amplification specific for *Trichomonas vaginalis*, *Chlamydia trachomatis*, and *Neisseria gonorrhoeae* within acute care, communitywide, and subacute care samplings of females positive at least one STI

STI phenotype[a]			Percent patient encounters by specimen source		
T. vaginalis	***C. trachomatis***	***N. gonorrhoeae***	**Acute care[b]**	**Communitywide[c]**	**Subacute care[d]**
+	−	−	40.7	45.2	54.2
+	+	−	8.1	9.6	6.6
+	−	+	3.5	2.6	1.5
+	+	+	3.5	1.5	1.2
−	+	−	22.2	21.0	29.1
−	+	+	4.7	5.9	2.2
−	−	+	17.4	14.3	4.7

[a]+, positive TMA screen; −, negative TMA screen.
[b]Eighty-six (33.7%) encounters had at least one STI. (Data adapted from reference 30with permission of the publisher.)
[c]Two hundred seventy-three (35.0%) encounters had at least one STI. (Data adapted from reference 31 with permission of the publisher.)
[d]One thousand thirty-six (14.5%) encounters had at least one STI. (Data adapted from reference 25 with permission of the publisher.)

Males

Trichomoniasis had been largely uncharacterized in males due to a perceived lack of testing options; however, recent data have further elucidated clinically significant disease in males. Strockij et al.[32] studied prostatic secretions from 203 males with chronic recurrent urethroprostatitis and no detectable bacterial microflora. *T. vaginalis* was detected in 75.5% of specimens; amoeboid, spherical, and flagellated forms of the parasite were observed via electron microscopy. Sviben et al.[33] reported a *T. vaginalis* PCR detection rate of 8.2% in 500 men with urethritis, while the rate in 200 men without symptoms was 2.2%. *T. vaginalis* can influence hypogonadism, orchitis, and male infertility.[34]

Few data exist regarding the role of *T. vaginalis* in male HIV acquisition and transmission. Hobbs et al.[35] reported that the median seminal HIV RNA load in Malawi males was increased approximately 2 $\log_{10}$ units in *T. vaginalis*urethritis. Paz-Bailey et al.[36] investigated 173 HIV-positive males in South Africa with detectable HIV-1 RNA in genital ulcers. Those who were also infected with *T. vaginalis* exhibited increased crude viral loads over those who were not infected (mean difference, 0.62 $\log_{10}$ [95% confidence interval, 0.07 to 1.2; $P = 0.027$]). The role of the male sexual partner with respect to trichomoniasis and HIV infection should not be overlooked. Chesson et al.[37] estimated that 750 instances of new HIV infections in U.S. females were facilitated by concomitant *T. vaginalis* infection; the cost of treating *T. vaginalis*-attributable HIV infection is $167 million.

Over the past 10 years, investigators have attempted to establish an association between prostate carcinoma and antecedent *T. vaginalis*exposure. Studies based on *T. vaginalis* seroprevalence have demonstrated modest associations with nocturia, large prostate volume, and benign prostatic hyperplasia/lower urinary tract symptom-related outcomes (with prevalence ratios ranging from 1.21 to 1.36)[38] and with overall prostate cancer risk (odds ratio, 1.25).[39] Conversely, Mitteregger et al.[40] detected *T. vaginalis* by PCR in 34% of tissue specimens with benign prostatic hyperplasia. Stark et al.[39] described significant association between documented *T. vaginalis* infection and lethal prostate cancer (odds ratio, 6.4 [95% confidence interval, 1.5 to 27.9]) compared to controls with no demonstrable *T. vaginalis* infection. Akin to HPV triage in cervical carcinoma, studies may be warranted to elucidate a potential role for molecular *T. vaginalis* triage in prostate carcinoma.

Within a high-prevalence STI community, 622 males were screened for *C. trachomatis, N. gonorrhoeae*, and *T. vaginalis* by RNA amplification;[41] 6.6% of the patients yielded a positive result for *T. vaginalis*, with a mean age 39.9 years ($P < 0.0001$ versus the mean ages for *C. trachomatis* and *N. gonorrhoeae*). STI agent distribution by age decade (Fig. 3) mimicked data observed in females. The authors also demonstrated no difference in *T. vaginalis* distribution within majority African American geographic areas of the metropolitan setting (8.9% detection rate) versus that observed in highly predominantly Caucasian geographic areas of the same metropolitan setting (5.0% detection rate; $P = 0.15$). These data further show the value of *T. vaginalis* screening with a broad demographic and geographic basis.

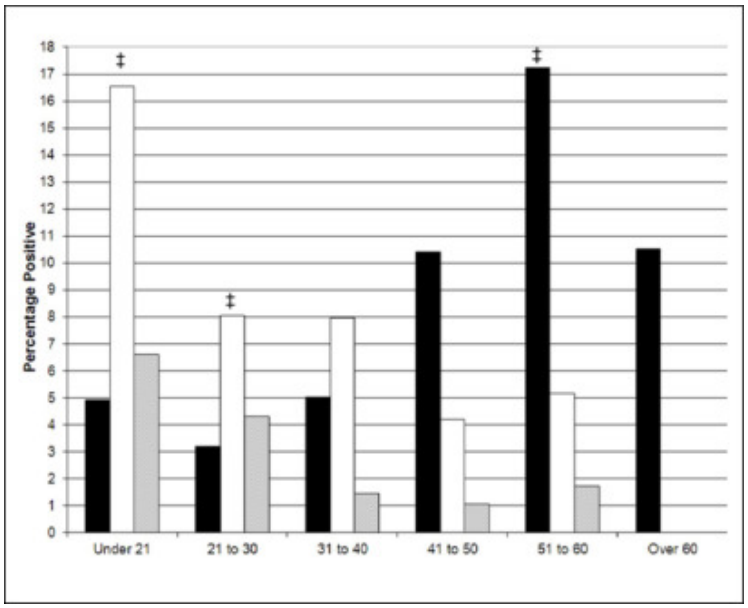

Figure 3. Prevalence of *Trichomonas vaginalis* (solid bars), *Chlamydia trachomatis*(open bars), and *Neisseria gonorrhoeae* (shaded bars) among 622 males screened by commercial transcription-mediated amplification-

based methods, delineated by age. ‡ represents comparisons between *T. vaginalis* and *C. trachomatis* detection rates in which $P < 0.05$.
Adapted from data published in [41], with permission from the publisher.

Extra-urogenital detection

Studies have characterized *T. vaginalis* in non-genitourinary sites. An internet-based STI surveillance program reported a 6.3% *T. vaginalis* molecular detection rate from self-collected female rectal swabs.[42] Within a subset of these positive patients who also submitted a self-collected vaginal swab, 100% of the vaginal swab collections were also positive for *T. vaginalis* (C. Gaydos, personal communication). Munson et al.[43] reported a 2.9% *T. vaginalis* pharyngeal detection rate from male STI clinic attendees. Positive results were confirmed by a second molecular assay targeting an alternative *T. vaginalis* sequence; 85.7% of patients testing positive indicated strictly heterosexual preference, and 38.1% of *T. vaginalis*-positive pharyngeal specimens were derived from symptomatic-patient encounters.

Laboratory Diagnosis

Non-molecular modalities

The success of non-molecular means of *T. vaginalis* detection is largely depenent on the organism burden or disease prevalence. Wet-mount analysis involves collection of a vaginal swab, placement of the swab into physiologic saline (vaginal saline suspension), followed by microscopic observation of the suspension for motile flagellates using a high-power objective. This technique may have its best application in remote and underserved locales that may also experience financial constraints. In a study of Sudanese women attending a gynecologic clinic and experiencing a mean 14.4-day duration of symptoms, Saleh et al.[3] reported 99.2% wet-mount sensitivity compared to the performance of in-house *T. vaginalis* PCR. In contrast, from a London STI clinic with 9.8% *T. vaginalis* prevalence via a composite reference standard, Nathan et al. [44] reported wet-mount sensitivity of 38%. In a U.S. population with nearly 30% *T. vaginalis* prevalence, Nye et al.[45] documented 54.6% wet-mount sensitivity compared to a composite molecular reference. Decreased sensitivity of wet mount may, in part, be related to the insufficient collection or missed detection of pleomorphic *T. vaginalis* developmental stages.[46]

T. vaginalis culture is a past standard for parasite detection, with performance also predicated on the burden of infection or prevalence. Inoculation of vaginal saline suspensions from a population with approximately 85% *T. vaginalis* prevalence[3] into Diamond's medium extrapolated to 100% analytical sensitivity of culture. In contrast, Nye et al.[45] demonstrated 75% sensitivity of InPouch TV culture (BioMed Diagnostics, White City, OR) compared to a composite molecular reference. InPouch TV

evaluation is made by direct microscopy of the culture pouch itself (without aliquoting of culture contents), which may facilitate testing in satellite settings. One advantage inherent to *T. vaginalis* culture is cultivation of viable organisms for nitroimidazole susceptibility testing,[11] particularly in patients who experience treatment failure.

The performance of a second non-molecular adjunct to microscopy, *T. vaginalis* antigen detection, is also dependent on geographic prevalence. In the cohort with approximately 85% *T. vaginalis* prevalence, 99.6% sensitivity of the Kalon TV latex agglutination test (Kalon Biological, Surrey, UK) was reported compared to PCR.[3] Abu-Sarkodie et al.[47] documented a kappa index of 0.93 for result agreement between this latex agglutination assay and InPouch TV; molecular diagnostics were not incorporated into the study. Jones et al.[48] assessed the OSOM Trichomonas rapid test (Sekisui Diagnostics, San Diego, CA) using self-collected vaginal swabs from centers in South Africa and Brazil with *T. vaginalis* prevalence of 10% and 3%, respectively. Compared to a PCR reference, the OSOM Trichomonas rapid test demonstrated 83.3% and 68.4% sensitivity, respectively. Nathan et al.[44] documented 92% sensitivity of the OSOM Trichomonas rapid test at an STI clinic with 9.8% *T. vaginalis* prevalence. A recent investigation[49] of a U.S. health care system encompassing both low- and high-prevalence STI locales (4.0% and 21.4% *T. vaginalis* detection rates, respectively) documented 35.1% and 85.7% sensitivity of the OSOM Trichomonas rapid test in these areas, respectively. Clinician-collected vaginal swabs submitted for antigen testing were compared to a molecular reference collection. In addition to the potential caveats inherent to antigen-based *T. vaginalis*detection, it should be noted that FDA clearance has not been granted to these modalities for urine screening or for assessment of male specimens.

Molecular modalities

The BD Affirm VPIII Microbial Identification Test (Becton Dickinson, Sparks, MD) provides a nucleic acid hybridization format for detection of *T. vaginalis*, in addition to simultaneous assessments for *Gardnerella vaginalis* and *Candida albicans*. Recent studies have assessed the *T. vaginalis* component of the assay in the context of a nucleic acid amplification test (NAAT) standard. Within a U.S. population of females with vaginal complaints or history suggestive of STI (5% *T. vaginalis* prevalence), Andrea and Chapin[50] reported 63.4% sensitivity of the BD Affirm VPIII Microbial Identification Test compared to a commercial NAAT. The same reference standard was employed by Cartwright et al.[51] in evaluation of a symptomatic 320-female U.S. cohort. The BD Affirm VPIII Microbial Identification Test yielded 46.3% sensitivity in the population exhibiting 16.9% *T. vaginalis* prevalence. Subsequent to analytic assessments of DNA and RNA amplification efficiency, it was postulated that analogous differences exist between commercialized formats of these technologies.[52] This concept was

validated with an elegant set of *in vitro* data published by Ikeda-Dantsuji et al.[53] Standardized quantities of *C. trachomatis* elementary bodies were distributed into mock clinical specimens; subsequent dilution series demonstrated commercial transcription-mediated amplification (TMA) sensitivity that was 1,000-fold greater than that of commercial PCR. This difference in sensitivity may also lie in the multiplicity of the target nucleic acid (in particular, rRNA targeted by commercial TMA) and in the removal of endogenous inhibitory agents by an oligonucleotide-based target capture system.

Experimental differences between RNA and DNA amplification have been extrapolated to clinical practice. Schachter et al.[54] demonstrated 44.4% sensitivity of commercial PCR for detection of *N. gonorrhoeae* from rectal specimens compared to commercial TMA. The authors reported just 60.0% sensitivity of commercial PCR for detection of the agent from pharyngeal swabs. Commercial TMA was also 12.4% and 14.8% more sensitive than strand displacement amplification (an alternative form of DNA amplification) in detection of *N. gonorrhoeae* from pharyngeal and rectal sites, respectively. Nye et al.[45] noted a similar paradigm when assessing commercial TMA and an in-house PCR for *T. vaginalis*. Using a molecularly resolved algorithm, the sensitivities of commercial *T. vaginalis* TMA on 296 first-void female urine, endocervical, and vaginal specimens were 87.5%, 89.8%, and 96.6%, respectively. The analogous values derived from PCR were 76.1%, 80.9%, and 83.0%. From 298 first-void male urine and urethral swab specimens, the sensitivity of commercial TMA ranged from 73.8% (urine) to 95.2% (urethral swab) when utilizing a molecularly resolved algorithm. The corresponding sensitivity data from PCR were 47.6% and 54.8%, respectively. The sensitivity of *T. vaginalis* culture from male specimens was 28.6%.

The first FDA-cleared NAAT for *T. vaginalis* is the TMA-based Aptima *Trichomonas vaginalis* Assay (Hologic, San Diego, CA),[55] with indications for clinician-collected vaginal swabs, endocervical swabs, and liquid-based cytology collections (PreservCyt solution; Cytyc Corporation, Marlborough, MA). Because the assay can be performed on the same collection tube as *C. trachomatis*/*N. gonorrhoeae* TMA, a number of laboratories have performed off-label verification of the assay on male first-void urine and urethral swab specimens using laboratory-defined luminescent cutoff values. Napierala et al.[24] reported that the *T. vaginalis* detection rate from males did not differ significantly from *C. trachomatis* detection ($P = 0.17$) and was increased over *N. gonorrhoeae* ($P = 0.006$). The luminescent output from positive specimens did not differ significantly from the values derived from female testing ($P \geq 0.29$). Increased clinician utilization of a laboratory-verified first-void female urine specimen was also noted during a 3-year testing interval.[24]

A second FDA-cleared NAAT for *T. vaginalis* is the strand displacement amplification BD ProbeTec *Trichomonas vaginalis* Qx Amplified DNA Assay (TV Qx) (BD Diagnostics, Sparks, MD). The indicated specimen sources include female urine, self-collected vaginal swabs, and clinician-collected endocervical swabs. Van Der Pol et al.[56] assessed 838 self-collected vaginal swabs using TV Qx in a multi-center U.S. clinical trial cohort with 12.5% *T. vaginalis* prevalence. The kappa value between the TV Qx results and the data from the Aptima *Trichomonas vaginalis* Assay analysis of clinician-collected swabs from the same participants was 0.938 ($P = 0.09$). The sensitivity and specificity of TV Qx were 98.3% and 99.6%, respectively. Ten specimens yielded Aptima *Trichomonas vaginalis* Assay-positive/TV Qx-negative results; the converse was true for three specimens.

The AmpliVue Trichomonas Assay (Quidel, San Diego, CA) received FDA clearance in March 2015 for clinician-collected vaginal swabs. Following isothermal helicase-dependent amplification, *T. vaginalis* DNA is detected via lateral-flow colorimetric readout in a self-contained disposable device, with a turnaround time of approximately 1 hour. Unpublished data (C. Gaydos, personal communication) have described the performance of the AmpliVue Trichomonas Assay versus culture, wet mount, and the Aptima *Trichomonas vaginalis* Assay for 1,132 vaginal swabs. The rapid assay detected *T. vaginalis* from all specimens that were also positive by wet mount or culture analysis. Positive agreement with the Aptima *Trichomonas vaginalis* Assay data was 88.5%.
The Xpert TV is performed on the GeneXpert system (Cepheid, Sunnyvale, CA). This DNA amplification assay received FDA clearance in October 2015 for female urine, self-collected vaginal swabs, and clinician-collected endocervical swabs. In a proof-of-concept study by Badman et al.,[57] 60 consecutive urine specimens yielding positive and negative results by an in-house *T. vaginalis* PCR (acquired from 42 males and 72 females) were subjected to automated processing, cell lysis, purification, DNA amplification, and target detection; 97.4% overall agreement was observed, with 95% positive agreement.

One commercialized assay for *T. vaginalis* molecular detection has received Conformité Européene marking. The AmpliSens *N. gonorrhoeae*/*C. trachomatis*/*M. genitalium*/*T. vaginalis*-Multiprime-FRT PCR kit (InterLabScience, Moscow, Russia) is a multiplex real-time PCR assay that requires an off-line DNA extraction step. Rumyantseva et al.[58] investigated its performance using first-void urine from 554 males, first-void urine from 498 females, and 209 vaginal swabs. *T. vaginalis* assessment was difficult on the basis of just one positive reference specimen in the evaluation. The assay was also limited in its capacity to detect *Mycoplasma* genitalium from both male and female specimens.

Additional modalities under development include a point-of-care cartridge (Atlas Genetics, Wiltshire, UK) that manages DNA extraction, amplification, and detection within 30 minutes using molded

subcircuits. The final output is an electrochemical signal obtained using differential pulse voltammetry. Pearce et al.[59] assessed a single-analyte *T. vaginalis* assay with 90 self-collected vaginal swabs previously tested by the Aptima *Trichomonas vaginalis* Assay. Sensitivity and specificity indices of 95.5% and 95.7%, respectively, were reported. The detection limit of the assay was five *T. vaginalis* organisms. As part of an investigational FilmArray nested-PCR panel for the identification of nine STI agents (BioFire Diagnostics, Salt Lake City, UT), Kriesel et al.[60] identified *T. vaginalis* DNA from 9 of 295 clinical specimens. However, not all the specimens were assessed with a predicate device. Finally, recent proof-of-concept studies have discussed detection of *T. vaginalis* by loop-mediated isothermal amplification[61] and PCR-based microarray.[62]

Value of *T. vaginalis* screening

The CDC recommendations for *T. vaginalis* diagnostic testing, using highly sensitive and specific modalities, apply to females seeking care for vaginal discharge.[11] The screening recommendations extend to persons receiving care in high-prevalence settings (correctional facilities and STI clinics) and to individuals with high-risk STI behavior (commercial sex workers, illicit drug use, and persons with multiple sexual partners or a history of STI). Moreover, local *T. vaginalis* epidemiology may influence screening decisions.[11]

The scope of trichomoniasis may extend beyond these demographics, as Tomas et al.[63] described scenarios in acute care in which STI (including *T. vaginalis* infection) was underdiagnosed in females in favor of empiric treatment for urinary tract infection. Retrospective chart reviews of 234 symptomatic females receiving *C. trachomatis* and *N. gonorrhoeae* screening[64] revealed that only 31 were screened for *T. vaginalis*. While a laboratory diagnosis of *T. vaginalis* was rendered in just 1.3% (3/234) of eligible females, 17 patients were administered anti-trichomonad therapy (82.3% without significant laboratory data). Following commencement of an algorithm that automatically added *T. vaginalis* laboratory assessment to those for *C. trachomatis* and *N. gonorrhoeae, T. vaginalis* was detected in 18.3% (39/213) of eligible females; 95.8% of *T. vaginalis* laboratory diagnoses resulted in therapeutic regimens, while only 25% of overall anti-trichomonad regimens were administered on an empiric basis. Data from a high-prevalence STI community[65] revealed that introduction of *T. vaginalis* TMA to the testing options resulted in a significant increase in laboratory assessments for *T. vaginalis* compared to an interval when only wet-mount testing was offered. In addition, significant reductions in both clinician requisition of wet-mount analysis and clinician performance of point-of-care wet-mount microscopy was observed. Although NAAT yielded 38% more *T. vaginalis* detection than culture in one study, the prospect of universal screening may be somewhat tempered in lower-prevalence settings.[7]

Asymptomatic patients

One concern about *T. vaginalis* molecular screening is the unnecessary laboratory diagnosis of asymptomatic patients,[66] with a corollary being that less sensitive assays that detect higher organism burdens are more clinically relevant. However, Muzny et al.[2] demonstrated that 58.5% of 301 females with wet-mount-negative/TMA-positive results reported urogenital symptoms of vaginal discharge, vaginal odor, and/or dysuria. This datum was similar to the 65.7% symptomatic rate of 720 females with wet-mount-positive/TMA-positive results (adjusted odds ratio, 1.00; $P = 0.98$). Munson et al.[49] reported that 56.8% of antigen assay-negative/TMA-positive patients from a low-prevalence STI community had symptoms of urogenital infection, while 75.0% of patients from the same setting with antigen assay-positive/TMA-positive results were symptomatic ($P = 0.17$). In addition, symptomatic status did not impact concordant and discordant antigen assay/TMA frequencies in a higher-prevalence STI population ($P = 0.54$). These data support the hypothesis that the performance of non-molecular *T. vaginalis* assays is more accurately predicated on disease prevalence than on symptomatic status.

Detection of asymptomatic trichomoniasis is also important, because studies[67,68] have documented persistent indolent *T. vaginalis* infection based on laboratory detection of the organism following previously negative test results in the face of sexual abstinence. Munson et al.[49] stated that 27% of patients in a low-prevalence STI setting with antigen assay-negative/(retrospective) TMA-positive results returned for clinical STI evaluation.

Two papers discuss this paradigm in the context of prevalent/incident *T. vaginalis* infection. A nine-center study[69] characterized the initial *T. vaginalis* prevalence as 14.6%. The cumulative 6-month incidence of *T. vaginalis* infection (defined as *T. vaginalis* detection in patients with either a negative *T. vaginalis* result at baseline or a positive result at baseline and receiving anti-trichomonad therapy) was 7.5%. Patients more likely to have incident *T. vaginalis* infections included those with baseline HIV infection (relative risk, 1.59) and baseline *T. vaginalis* infection (relative risk, 3.37). Because nearly 50% of patients positive for *T. vaginalis* at baseline received therapy, the authors speculate that incident *T. vaginalis* was related to re-infection or treatment failure. Gaydos et al.[70] studied 304 female repeat participants in an internet STI screening program. Within this cohort, the *T. vaginalis* TMA detection rate was 7.9% during the first assessment, with 9.2% detection upon repeat testing (60% reporting symptoms). *T. vaginalis*detection among repeat participants was also associated with previous detection of *T. vaginalis* ($P < 0.05$).

Final notes on specimen collection

Recent data from the CDC[71] support the collection of vaginal swabs for laboratory detection of *C. trachomatis* and *N. gonorrhoeae*. Such collections are preferred for assessment of *T. vaginalis* infection; this preference is substantiated by data from Nye et al.[45] Other studies in the literature espouse first-void urine as a viable specimen option for detection of *T. vaginalis* in females. Napierala et al.[24] reported a 12.6% *T. vaginalis*detection rate from first-void urine in contrast to 8.9% and 8.6% rates from endocervical and vaginal specimens, respectively. A follow-up assessment of 2,478 females from a large metropolitan cohort (including both high- and low-prevalence locales)[72] reported increased *T. vaginalis* detection from first-void urine (11.3%) compared to endocervical specimens (8.2%; $P = 0.04$) and a trend toward increased detection from first-void urine versus a combined endocervical/vaginal specimen data set ($P = 0.06$). The above-mentioned studies are limited because data analysis was based on the specimens collected by clinicians during routine medical practice; multiple specimens were not collected from the same patient. However, Andrea and Chapin[50] reported 11.5% and 3.2% *T. vaginalis* detection rates from female first-void urine and endocervical collection, respectively ($P < 0.0002$).

When *C. trachomatis* and *T. vaginalis* detection rates were compared in a demographic (under age 21) in which *T. vaginalis* is not typically considered within an STI differential diagnosis (Fig. 2), the *C. trachomatis* detection rate among approximately 1,500 females was higher than that of *T. vaginalis* ($P = 0.001$). However, when the detection rates were stratified by specimen source,[25] the *T. vaginalis* detection rate (11.2%) among nearly 300 females who were screened with first-void urine rivaled the rate for *C. trachomatis* (10.9%; $P = 0.92$). First-void urine screening seemingly eliminated a disparity between African American and Caucasian female demographics with respect to *T. vaginalis* detection. Overall, TMA detection of the parasite in African American majority geographic locales across a large metropolitan area was 11.7% compared to 9.0% in predominantly Caucasian locales ($P = 0.004$). When stratified by first-void urine collection, *T. vaginalis* detection rates exhibited minimal difference (14.4% and 12.5% in African American and Caucasian majority locales, respectively; $P = 0.54$).[25]

Gaydos et al.[73] described 3.7% detection of *T. vaginalis* RNA from 1,699 males using self-collected penile-meatal swabs in the context of an Internet screening program. Similar to previous studies of trichomoniasis epidemiology in males, the associated risk factors included age 30 to 39 years (odds ratio, 6.63) and age >40 years (odds ratio, 5.31). In a second cohort, Dize et al.[74] investigated concomitant submission of self-collected penile-meatal swabs and first-void urine from 634 males. From 56 patients with detectable *T. vaginalis* infection via TMA, the swab sensitivity was 80.4% and the

first-void urine sensitivity was 39.3%; similar disparities in sensitivity were observed for *C. trachomatis* and *N. gonorrhoeae*.

Conclusion

Evaluation of reports regarding past modalities for diagnosis of trichomoniasis is compromised by an evolving molecular reference standard. This is further confounded by analytical sensitivity differences between DNA and RNA amplification methods demonstrated both *in vitro* and in the clinical setting. However, these recently commercialized, highly accurate diagnostic modalities, particularly those based on TMA, are facilitating "new" outlooks on the "old" epidemiology of trichomoniasis. This clinical entity is now widely distributed in a geographic setting, in both genders, and in wide range of age demographics. Evidence increasingly supports the value of *T. vaginalis* molecular diagnostics in the assessment of both symptomatic and asymptomatic populations. In conclusion, molecular assays for trichomoniasis, including those adapted to laboratory-modified testing formats, have the potential to impact public health and clinical practice.

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