

Impact of *Trichomonas vaginalis* Transcription-Mediated Amplification-Based Analyte-Specific-Reagent Testing in a Metropolitan Setting of High Sexually Transmitted Disease Prevalence[∇]

Erik Munson,^{1,2,*} Maureen Napierala,¹ Robin Olson,¹ Tina Endes,^{1†} Timothy Block,¹ Jeanne E. Hryciuk,¹ and Ronald F. Schell^{3,4,5}

Wheaton Franciscan and Midwest Clinical Laboratories, Wauwatosa, Wisconsin 53226¹; College of Health Sciences, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53201²; and Wisconsin State Laboratory of Hygiene³ and Departments of Bacteriology⁴ and Medical Microbiology and Immunology,⁵ University of Wisconsin, Madison, Wisconsin 53706

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Trichomoniasis is a significant sexually transmitted disease (STD) in the spectrum of public health and primary care because of its association with agents such as human immunodeficiency virus and *Neisseria gonorrhoeae*. However, its true significance may be underestimated due to diagnostic modalities that exhibit poor sensitivity. A total of 1,086 genital specimens from two urban emergency departments, a suburban urgent-care facility, and a metropolitan outpatient physician group were subjected to transcription-mediated amplification-based *Trichomonas vaginalis* analyte-specific-reagent (ASR) testing (Gen-Probe, Inc.). The rate of positive molecular ASR results (14.5%) doubled that of direct saline preparation (7.0%; $P < 0.0002$). Analogous increases were observed at one emergency department and within the outpatient physician group ($P < 0.0002$). No significant increase in the rate of positive molecular ASR results was observed from the facilities that encountered a lower frequency of black/African American patients. While positive *T. vaginalis* findings via direct saline preparation did not have a significant association with concomitant *Chlamydia trachomatis* or *N. gonorrhoeae* infection overall, a positive *T. vaginalis* ASR result was a better predictor of concomitant *C. trachomatis* or *N. gonorrhoeae* infection (odds ratios of 2.34 and 4.46, respectively; $P < 0.0001$). The increased rate of positive *T. vaginalis* ASR results was observed in both point-of-care ($P = 0.02$ versus direct saline preparation) and laboratory ($P = 0.003$) testing. Highly sensitive *T. vaginalis* molecular ASR not only transcends issues of specimen integrity and microscopic acumen but also has an increased ability to predict the likelihood of additional STDs in defined populations.

In spite of the discovery of *Trichomonas vaginalis* nearly 175 years ago and documentation of its inhabitation of the female urogenital tract and the male urinary tract in the late 1800s, pathogenicity was not ascribed to this agent until the European literature of the 20th century (19). Reports have since shown the significance of antecedent *T. vaginalis* infection, especially in human immunodeficiency virus coinfection (22, 37), acquisition (21), and transmission (17, 23); in pregnancy-related complications (10, 43); and in associations with pelvic inflammatory disease (16) and *Neisseria gonorrhoeae* infection (16, 24). *T. vaginalis* is currently thought to be responsible for approximately 50% of all curable infections worldwide (5); worldwide estimates of annual trichomoniasis incidence have reached 180 million cases (42).

While the aforementioned data may be of tremendous significance, trichomoniasis prevalence rates, both worldwide and in the United States, are thought to be grossly underestimated. Schwebke and Burgess (32) hypothesize that the variable sen-

sitivity of *T. vaginalis* diagnostic testing contributes partially to these artificially low statistics. Direct examination of genital saline collections continues to serve as a common basis for laboratory detection of *T. vaginalis*, with assay sensitivity being inherently low (approximately 50 to 70%) due to its dependency upon live, motile flagellates. Nucleic acid hybridization (2), antigen detection (20), and culture (4, 12) modalities have enhanced analytical sensitivity rates of up to 50%, although such improvement has been offset by the advent of *T. vaginalis*-specific nucleic acid amplification testing (NAAT) (36; reviewed in reference 32).

Hardick et al. (15) reported that transcription-mediated amplification (TMA) exhibited >96% sensitivity and specificity in the detection of *T. vaginalis* in a sexually transmitted disease (STD) clinic setting. Similar levels of TMA sensitivity and specificity were demonstrated in a study of adolescent women recruited during presentation at an emergency department or teen health center (18). We report findings of a retrospective study that examined the potential utility of TMA on routine *T. vaginalis* detection within a regional health care system (having substantive basis in emergent care and family practice) that is centered in a metropolitan area of high STD prevalence.

(Results of this work were previously presented, in part, at the 108th General Meeting of the American Society for Microbiology, Boston, MA, 1 to 5 June 2008 [27]).

* Corresponding author. Mailing address: Wheaton Franciscan Laboratory, 11020 West Plank Court, Suite 100, Wauwatosa, WI 53226. Phone: (414) 256-1479. Fax: (414) 256-5566. E-mail: Erik.Munson@wfhc.org.

† Present address: Wheaton Franciscan Medical Group, Franklin, WI 53132.

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MATERIALS AND METHODS

Setting. A 2006 analysis of data from United States metropolitan statistical areas (MSAs) (7) reported that the Milwaukee-Waukesha-West Allis MSA had a chlamydia rate of 693.9 cases per 100,000 people. This figure ranked the second highest in the United States and was 89.4% higher than the national cumulative MSA rate of 366.4 cases per 100,000 people. The same MSA had the second-highest gonorrhea rate among United States MSAs (330.9 cases per 100,000 people; more than double the national MSA total rate of 131.1 cases per 100,000 people). Wheaton Franciscan Laboratory serves four Milwaukee metropolitan hospitals (with one major urgent-care facility) and an approximately 55-clinic metropolitan outpatient physician group in a three-county region of southeastern Wisconsin.

Direct saline preparation for *T. vaginalis*. Collection and direct microscopic examination of vaginal specimens for *T. vaginalis* followed previously established guidelines (3). A 6-month audit of *T. vaginalis* testing via direct saline preparation revealed that of the 3,948 collections within the health care system, 41.8% occurred at one urban emergency department (emergency department A), while 6.6% occurred at a second urban emergency department (emergency department B). Eight percent of the specimens were collected at a suburban urgent-care facility, and the remaining 43.6% were collected by entities of the outpatient physician group. A total of 23.6% of all direct saline preparations encountered in the audit were examined in a point-of-care setting, with provider-performed microscopy undertaken only within the auspices of the outpatient physician group (54.2% of all outpatient physician group specimens). Laboratorian-performed microscopy for specimens obtained from the urgent-care facility and both emergency departments occurred at an in-facility laboratory, while the non-provider-performed microscopy from the outpatient physician group was completed at an off-site laboratory. Both laboratorians and direct health care providers examining direct saline preparations were subjected to clinical microscopy proficiency testing administered by laboratory specialists.

Specimen selection for evaluation of *T. vaginalis* molecular assay. A secondary laboratory information system audit identified 3,250 patient encounters within the aforementioned audit, during which screening for *Neisseria gonorrhoeae* and *Chlamydia trachomatis* via NAAT was also performed. Frequencies of specimens yielding the eight permutations of *N. gonorrhoeae* NAAT, *C. trachomatis* NAAT, and *T. vaginalis* direct saline preparation test results were computed for the four health care entities. These data, in tandem with overall detection rates for the three agents and frequencies of vaginal saline collection within each of the four health care entities, were used to retrospectively select specimens for *T. vaginalis* NAAT. *C. trachomatis* and *N. gonorrhoeae* NAAT results, *T. vaginalis* direct saline preparation results, health care location, and site of direct saline preparation performance (point-of-care versus laboratory) were documented for 1,086 females prior to removal of all patient identifiers and subsequent cryptic encoding of primary clinical material and data.

Primary molecular screening assays. The APTIMA Combo 2 assay (Gen-Probe, Inc., San Diego, CA) screened primary clinical endocervical specimens submitted in APTIMA swab specimen transport tubes for *C. trachomatis* and *N. gonorrhoeae*. Aliquots (400 μ l) were subsequently subjected to Gen-Probe *T. vaginalis* molecular analyte-specific-reagent (ASR) testing. The assay targeted organism-specific 16S rRNA and was executed in a fashion analogous to other APTIMA-based protocols (14) utilizing the principles of target capture, TMA, and chemiluminescent hybridization protection. A relative light unit value of 60,000 was utilized as the cutoff for a positive result (15).

Discrepancy resolution. Specimens yielding *T. vaginalis* molecular ASR results that were discordant with the previously documented direct saline preparation result were confirmed by repeat molecular ASR testing. Furthermore, when sufficient specimen volume allowed, specimens were subjected to a research-use-only, TMA-based alternative target assay using proprietary primers, probes, and target capture oligomers generously supplied by Gen-Probe. A relative light unit value of 60,000 was arbitrarily utilized as the cutoff for a positive result.

Extrapolation of race/ethnicity data. Fiscal year 2007 patient encounter data relative to the outpatient physician group, both emergency departments, and the urgent-care facility were obtained and sorted into rank order by ZIP code. The least number of ZIP codes that comprised a cumulative 70% of patient encounters per health care location was determined. The U.S. Census Bureau Census 2000 five-digit ZIP code tabulation areas were accessed (<http://factfinder.census.gov>) to provide racial/ethnicity distribution by ZIP code. Tabulated racial and ethnicity categories included Asian, black/African American, Caucasian, Hispanic/Latino, and other race. ZIP code tabulation areas that comprised the 70th percentile were summed to provide a capsule of general demographics served by health care location.

TABLE 1. Rates of sexually transmitted agent detection for 1,086 primary genital specimens included in a retrospective evaluation of a *Trichomonas vaginalis* molecular ASR

Healthcare entity	n	% Positive results		
		Microscopic detection of <i>T. vaginalis</i>	Molecular detection of <i>C. trachomatis</i>	Molecular detection of <i>N. gonorrhoeae</i>
Outpatient physician group	508	4.3	5.1	1.4
Emergency department A	431	10.4	15.8	12.3
Urgent-care facility	77	5.2	3.9	5.2
Emergency department B	70	7.1	8.6	2.9
Total	1,086	7.0	9.5	6.1

Statistical analysis. The significance test of proportions (41) was used to determine if differences in the rate of positive test results were significant. Odds ratios for presence of a concomitant sexually transmitted agent in the context of direct saline preparation and molecular ASR were computed by a chi-square test of association and Fisher's exact probability test. The alpha level was set at 0.05 before the investigations commenced, and all *P* values are two-tailed.

RESULTS

Characterization of specimens selected for evaluation of *T. vaginalis* molecular ASR. Of the 1,086 specimens selected for retrospective evaluation of the *T. vaginalis* molecular ASR, the proportion collected within the outpatient physician group (46.8%) was similar to the percentage of direct saline preparations in the audit originating from that entity ($P = 0.06$). A proportional rate of specimens from emergency department A (39.7%), the urgent-care facility (7.1%), and emergency department B (6.4%) were selected for the evaluation (P values ≥ 0.22 versus corresponding audit data).

The frequency distributions of provider-performed and laboratory-performed direct saline preparations within the outpatient physician group were not significantly different between the evaluation specimens and those captured by the audit ($P = 0.52$). Rates of *T. vaginalis* detection derived from point-of-care-based and laboratory-based examination of direct saline preparations were not significantly different between data collected in the audit and those associated with the evaluation specimens ($P \geq 0.66$).

The overall rate of positive direct saline preparation results in evaluation specimens (7.0%) (Table 1) did not significantly differ from the rate documented in the audit (6.8%; $P = 0.84$). When delineated by health care entity, rates did not significantly differ ($P \geq 0.23$). In a similar fashion, a comparison of audit and evaluation data revealed no significant differences between entity-specific rates of *C. trachomatis* ($P \geq 0.55$) and *N. gonorrhoeae* ($P \geq 0.58$) detection. Overall rates of *C. trachomatis* and *N. gonorrhoeae* nucleic acid detection were 9.5% and 6.1%, respectively.

Of all evaluation patients testing positive for at least one sexually transmitted agent, 27.7% had solely a positive direct saline preparation for *T. vaginalis*. A total of 32.5% had solely detectable *C. trachomatis* nucleic acid, and 20.4% tested positive only for *N. gonorrhoeae*. Testing phenotype frequencies documented in the audit and in retrospective specimen selection did not differ significantly ($P \geq 0.16$).

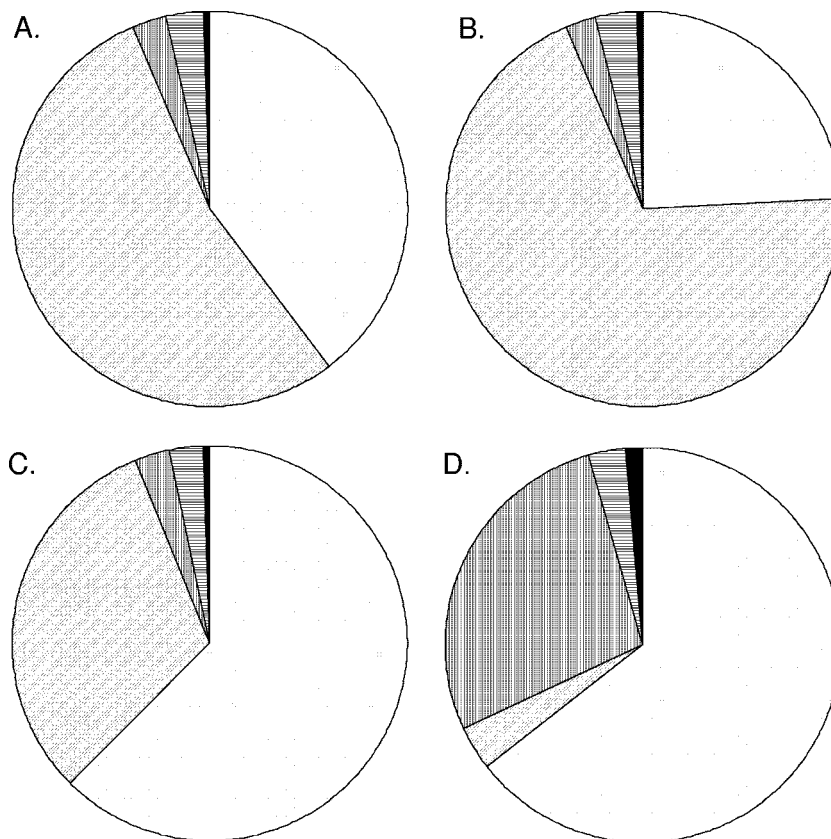


FIG. 1. Race and ethnicity distribution in rank order five-digit ZIP code tabulation areas constituting the 70th percentile of health care encounters within the metropolitan outpatient physician group (A), urban emergency department A (B), suburban urgent-care facility (C), and urban emergency department B (D). Diagonally striped sections represent blacks/African Americans; vertically striped sections represent Hispanics/Latinos; horizontally striped sections represent Asians; dotted sections represent Caucasians; and solid sections represent other races or ethnicities.

Racial and ethnic background of patients served by health care entities. Compilation of data from 17 rank order five-digit ZIP code tabulation areas was required to reach the 70th percentile of health care encounters at the urgent-care facility. In contrast, emergency departments A and B utilized data from only six and five ZIP codes, respectively, to achieve this percentile. Twelve rank order ZIP codes comprised the 70th percentile of health care encounters within the outpatient physician group. Regions most frequently served by emergency department A and the outpatient physician group were populated by a black/African American racial majority (69.2% and 53.7%, respectively) (Fig. 1A and B). In contrast, Caucasians constituted 62.5% and 64.5% of the areas predominantly served by the urgent-care facility and emergency department B, respectively. A nearly 8:1 ratio of Hispanic/Latino ethnicity to black/African American race in the population served by emergency department B was documented (Fig. 1D).

Evaluation of *T. vaginalis* molecular ASR. Seventy-six genital specimens with a positive *T. vaginalis* direct saline preparation result generated a positive molecular ASR result. In addition, 82 direct saline preparation-negative specimens yielded a positive result with the molecular ASR, establishing an overall molecular detection rate of 14.5%. Molecular ASR detection rates ranged from 6.5% at the urgent-care facility to 21.6% at

emergency department A (Fig. 2). Increased rates of *T. vaginalis* detection occurred with the molecular ASR over the direct saline preparation within the outpatient physician group, emergency department A, and the entire study set ($P < 0.0002$). The percentage of STD patients testing positive solely for *T. vaginalis* increased from 27.7% to 45.2%, following the performance of the molecular ASR ($P < 0.0002$). This increase was offset by reductions in *C. trachomatis*-alone and *N. gonorrhoeae*-alone testing phenotype frequencies following molecular ASR testing (data not shown).

Confirmatory testing of *T. vaginalis* molecular ASR. All *T. vaginalis* direct saline preparation-negative/molecular ASR-positive specimens yielded a positive result upon repeat molecular ASR performance. A subset of these specimens ($n = 76$) was subjected to alternative target TMA; all generated a positive result (Fig. 3). Two *T. vaginalis* direct saline preparation-positive/molecular ASR-negative specimens yielded a negative result upon both repeat molecular ASR and alternative target TMA performances. Specificity of alternative target TMA was further demonstrated by the generation of negative results from 95.4% of 65 randomly selected specimens which yielded a negative result when initially screened by the molecular ASR (data not shown).

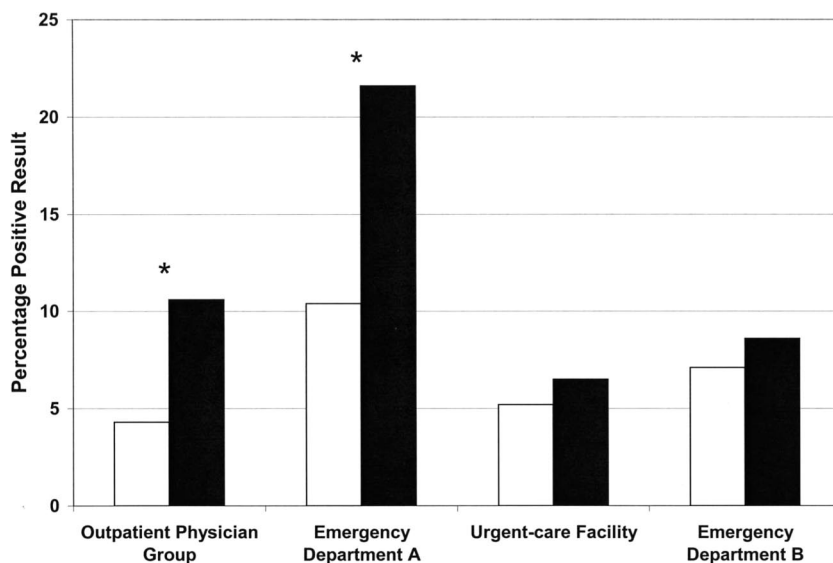


FIG. 2. Percentage of positive *Trichomonas vaginalis* results determined by direct saline preparation (open bars) and molecular ASR (solid bars) and stratified by health care entity for 1,086 female genital specimens. Asterisks denote *P* values of <0.0002.

Concomitant *C. trachomatis* or *N. gonorrhoeae* nucleic acid detection. Specimens testing positive for *T. vaginalis* via direct saline preparation did not have a significant likelihood for simultaneous detection of *C. trachomatis* or *N. gonorrhoeae* nucleic acid (Table 2). However, utilization of the molecular ASR resulted in odds ratios of 2.34 and 4.46 for a concomitant positive *C. trachomatis* and *N. gonorrhoeae* screen, respectively (*P* < 0.0001). This phenomenon was also observed for each sexually transmitted agent within the outpatient physician group and emergency department A (*P* ≤ 0.006).

The factor by which odds ratios for the simultaneous presence of *C. trachomatis* nucleic acid increased from direct saline preparation to *T. vaginalis* molecular ASR was 5.65 within the outpatient physician group and 3.65 at emergency department A (data not shown). The analogous factors for concomitant *N. gonorrhoeae* detection were 4.39 (outpatient physician group) and 3.43 (emergency department A). Odds ratios were not significant at the urgent-care facility or emergency department B for codetection of *C. trachomatis* or *N. gonorrhoeae* nucleic acid by either testing modality (*P* ≥ 0.65).

Influence of the test performance site in detection of *T. vaginalis*. Of the 508 specimens evaluated from the outpatient physician group, 267 (52.5%) originated from a point-of-care setting. The rate of *T. vaginalis* detection by molecular ASR was more than double that of the direct saline preparation (*P* = 0.02 versus provider-performed microscopy) (Table 3). Similarly, a 12.0% rate of detection by molecular ASR was observed in 241 specimens originally subjected to direct saline preparation in the laboratory (*P* = 0.002 versus microscopy).

Outpatient Physician Group		Molecular ASR Result	
		Positive	Negative
Direct Saline Preparation Result	Positive	22	1 ^a
	Negative	32 ^b	453

Emergency Department A		Molecular ASR Result	
		Positive	Negative
Direct Saline Preparation Result	Positive	45	1 ^a
	Negative	48 ^c	337

Urgent-care Facility		Molecular ASR Result	
		Positive	Negative
Direct Saline Preparation Result	Positive	4	0
	Negative	1 ^d	72

Emergency Department B		Molecular ASR Result	
		Positive	Negative
Direct Saline Preparation Result	Positive	5	0
	Negative	1 ^d	64

^aSpecimen yielded negative result by alternative target TMA
^b100% of 27 specimens tested yielded positive result by alternative target TMA
^c100% of 47 specimens tested yielded positive result by alternative target TMA
^dSpecimen yielded positive result by alternative target TMA

FIG. 3. Direct comparisons of *Trichomonas vaginalis* direct saline preparation and molecular ASR results, delineated by health care entity, in a retrospective evaluation of 1,086 primary genital specimens.

TABLE 2. Odds ratios for concomitant molecular detection of *Chlamydia trachomatis*- or *Neisseria gonorrhoeae*-specific nucleic acid on the basis of *Trichomonas vaginalis* detection by direct saline preparation or molecular ASR

Agent codetected	Modality of <i>T. vaginalis</i> detection ^a			
	Direct saline preparation		Molecular ASR	
	Odds ratio (95% CI)	<i>P</i>	Odds ratio (95% CI)	<i>P</i>
<i>Chlamydia trachomatis</i>	0.61 (0.41–0.90)	0.02	2.34 (1.66–3.31)	<0.0001
<i>Neisseria gonorrhoeae</i>	1.29 (0.86–1.94)	0.26	4.46 (3.09–6.44)	<0.0001

^a CI, confidence interval.

TABLE 3. Rates of *Trichomonas vaginalis* detection by direct saline preparation and subsequent molecular ASR from 508 primary genital specimens collected in an outpatient physician group setting, delineated by laboratory-based and point-of-care-based microscopy

Site of test performance	n	Rate of <i>T. vaginalis</i> detection (%)		P
		Direct saline preparation	Molecular ASR	
Laboratory	241	4.1	12.0	0.002
Point-of-care	267	4.5	9.7	0.02

DISCUSSION

In contrast to previously published studies of TMA-based detection of *T. vaginalis* that involved focused populations (15, 18), our study assessed laboratory diagnosis of this agent in an entire health care system. This approach was justified for a number of reasons. The Milwaukee-Waukesha-West Allis MSA is ranked second nationally in gonorrhea incidence, with a 2006 rate that is 58.6% higher than that of the Baltimore-Towson, MD, MSA and 66.8% higher than that of the Cincinnati-Middletown, OH/KY/IN, MSA. Chlamydia incidence in the Milwaukee metropolitan area in 2006 was 77.4% and 88.9% higher than rates reported for Cincinnati and Baltimore, respectively (7). Because other sexually transmitted agents, especially *N. gonorrhoeae* (16, 24), are frequently codetected with *T. vaginalis*, a potentially high *T. vaginalis* prevalence throughout this entire community setting was deduced, providing a multifaceted demographic to investigate the impact of molecular ASR. Furthermore, analysis of an in toto health care population is pertinent, compared to the focused study of adolescent women (18), because trichomoniasis is observed with significant frequency over an expansive range of age groups (24, 25).

With this investigation being retrospective in nature, the study set was validated using three approaches. First, raw percentages of specimens selected from each of the four health care entities did not differ ($P \geq 0.06$) from analogous percentages realized during the 6-month audit of *T. vaginalis* diagnostic practices. Secondly, entity-specific incidence of *N. gonorrhoeae*, *C. trachomatis*, or *T. vaginalis* detection within specimens selected for the evaluation showed no difference over rates encountered in the audit ($P \geq 0.23$). Finally, the encounter-specific STD profile, delineated for each health care entity, did not differ between evaluation and audit data. Within this community-wide population, the *T. vaginalis* molecular ASR detection rate (14.5%) more than doubled that yielded by direct saline preparation (7.0%). These data extend the findings of Huppert et al. (18), who reported a nearly twofold increase of *T. vaginalis* detection (9.4% to 18.1%) upon utilization of TMA.

In our study, alternative target TMA was performed on a subset of the direct saline preparation-negative/molecular ASR-positive specimens and generated 100% concordance for presence of *T. vaginalis*. This alternative target assay was performed in the context of a Centers for Disease Control and Prevention (CDC) recommendation for confirmation of *C. trachomatis*- and *N. gonorrhoeae*-specific NAAT (6). In a subset

of 65 molecular ASR-negative specimens, the *T. vaginalis*-specific alternative target TMA showed a slight proclivity for generating positive results ($P = 0.08$). These data mirror those previously published for *C. trachomatis*-specific (26, 31) and *N. gonorrhoeae*-specific (26) alternative target TMA and likely reflect the high analytical sensitivity of the method (8). Repeat molecular ASR testing was also used in our study to confirm the presence of *T. vaginalis* within specimens yielding discordant results. Huppert et al. (18) repeated TMA analysis on all positive specimens. The fact that repeat TMA performance on primary specimens has been espoused for *C. trachomatis*-specific (26, 31) and *N. gonorrhoeae*-specific NAAT (26) suggests the need for additional studies to further determine a role for confirmatory *T. vaginalis* molecular testing.

Of the two urban emergency departments studied, only emergency department A showed a significant increase in *T. vaginalis* detection by molecular ASR over that observed by direct saline preparation. For a variety of cultural, socioeconomic, and intrinsic reasons, an elevated rate of trichomoniasis has been reported in black/African American women (38). In our study, black/African American individuals comprised a higher proportion of the emergency department A demographic than those of emergency department B ($P < 0.0002$) (Fig. 1). The 21.6% *T. vaginalis* incidence rate derived by molecular ASR testing of patients from emergency department A was similar to the 18.1% incidence rate from a TMA-based analysis of an emergent care population that was 82% black/African American (18). The outpatient physician group, a second entity that experienced a significant increase in *T. vaginalis* molecular ASR detection rate, also served a predominately black/African American demographic. With percentages of Caucasian encounters nearly the same at the urgent-care facility and emergency department B, the increased Hispanic/Latino population encountered at emergency department B provided a means for assessing potential benefit of *T. vaginalis* molecular ASR within this ethnicity. However, no increase in *T. vaginalis* detection via molecular ASR was observed at either entity compared to initial direct saline preparation results. Other studies (10, 34, 35) observed a similar trichomoniasis incidence pattern in Caucasians and in Hispanics/Latinos, with rates 15 to 20% lower than those observed in black/African American populations.

Past findings have documented *N. gonorrhoeae* as the sexually transmitted agent most commonly codetected with *T. vaginalis* (16, 24). Our study corroborates these data to a certain extent by demonstrating an elevated odds ratio for concomitant *N. gonorrhoeae* detection in genital specimens yielding *T. vaginalis* upon direct saline preparation (Table 2). However, performance of *T. vaginalis* molecular ASR on the same study set revealed significant odds ratios for simultaneous *C. trachomatis* or *N. gonorrhoeae* detection ($P < 0.0001$). Huppert et al. (18), when using a composite reference standard for *T. vaginalis* (including NAAT), reported significant rates of individual *C. trachomatis* and *N. gonorrhoeae* codetection with *T. vaginalis*. Taken together, these data suggest that *T. vaginalis* molecular ASR has a greater potential to predict a simultaneous sexually transmitted agent and may even benefit clinicians with respect to follow-up ordering practices.

In this vein, the *T. vaginalis* molecular ASR was evaluated using the same endocervical specimen utilized for *C. tracho-*

matis- and *N. gonorrhoeae*-specific TMA. A total of 97.4% of 78 patients with a positive direct saline preparation of a vaginal collection yielded a positive molecular ASR result from the contents of the APTIMA swab specimen transport tube. The two *T. vaginalis* direct saline preparation-positive/molecular ASR-negative results (confirmed to be negative by both repeat testing and alternative target TMA) suggest a false-positive direct saline preparation, a scenario that has been previously described (4, 13, 30). *T. vaginalis* detection from vaginal saline collections by molecular ASR has correlated with TMA analysis of a separate endocervical collection (28). To further support the validity of this specimen source, Papanicolaou-stained smears have reasonable success in the detection of *T. vaginalis* (44). Other studies (29, 30), incorporating various reference standards, reported an approximately 10% *T. vaginalis* recovery rate from culture of endocervical specimens.

As a result, a single genital collection can be appropriate for molecular screening of *C. trachomatis*, *N. gonorrhoeae*, and *T. vaginalis* by using highly sensitive NAAT. Yet the direct saline preparation of vaginal collections may not be completely discounted in the assessment of female lower-genital disease. Our data ascribe excellent predictive value to a positive microscopic result (Fig. 3), suggesting a role for the direct saline preparation as a rapid screening assay within a molecular reflex testing algorithm. While NAAT had previously been considered non-advantageous for *T. vaginalis* testing in females because of purported reliability of culture (32), the converse now appears to hold true. This likely represents a corollary of studies in multiple disease agents (8, 9) that have documented greater analytical sensitivity of TMA than that of PCR.

Viability of the trophozoite following specimen transport may be a significant factor in (non-office) laboratory-based microscopic detection of *T. vaginalis* (13). Direct saline preparation sensitivity rates may also be impacted by the acumen of non-laboratory microscopists, some of whom may have experienced inadequate training (11) or a lack of participation in proficiency testing programs and competency assessments (39, 40). Data from the outpatient physician group (Table 3) suggest that neither specimen transport nor microscopy skill was exclusively responsible for the dramatic increase in the rate of positive molecular ASR results. Non-planktonic amoeboid morphotypes of *T. vaginalis*, in close association with vaginal epithelial cells (1), may be contributory to the decreased sensitivity of the direct saline preparation versus that exhibited by the molecular ASR.

Highly sensitive *T. vaginalis* molecular ASR could provide significant public health benefits. Fouts and Kraus (12) reported that nearly 50% of women infected with *T. vaginalis* are asymptomatic; one-third of these individuals progress to a symptomatic state within 6 months (16). Schwebke and Hook (33) utilized PCR to demonstrate a 17.3% prevalence rate of *T. vaginalis* in men attending an STD clinic. In addition, *T. vaginalis* was detected in a greater proportion of asymptomatic males (51.4%) than in those with symptoms (23%; $P = 0.009$). Hardick et al. (15) reported 100% sensitivity and specificity of TMA in the detection of *T. vaginalis* from male urine specimens. Taken together, these data solidify a role for *T. vaginalis* molecular ASR to obviate either a return clinic visit or a potential loss to follow-up encounters in a health care setting.

In conclusion, molecular ASR testing demonstrated en-

hanced sensitivity over that of direct saline preparation for the detection of *T. vaginalis*. This difference appeared to be related to patient demographics and was independent of direct saline preparation confounders, such as specimen transport and microscopic acumen. *T. vaginalis* molecular ASR is a more reliable predictor of concomitant *N. gonorrhoeae* and *C. trachomatis* nucleic acid detection, with all detections potentially being facilitated by a single genital collection. Strengthened by previous data describing the utility of TMA in the detection of *T. vaginalis* from male urine specimens, *T. vaginalis* molecular ASR should be an essential tool for STD prevention both clinically and in the public health sector.

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