Assessment of Screening Practices in a Subacute Clinical Setting Following Introduction of *Trichomonas vaginalis* Nucleic Acid Amplification Testing

Erik Munson, PhD; Cheryl Miller, BS; Maureen Napierala, BS; Timothy Kramme, BS; Robin Olson, BS; Kimber L. Munson, PhD; Sarah Olson, BS; Jeanne E. Hryciuk, MD; Ronald F. Schell, PhD

ABSTRACT

Objective: *Trichomonas vaginalis* analyte-specific reagent is a highly sensitive assay for *T vaginalis* detection. We report how this diagnostic innovation influenced the sexually transmitted infection ordering practice patterns of 20 subacute-care clinicians.

Methods: *T vaginalis, Neisseria gonorrhoeae,* and/or *Chlamydia trachomatis* screening data were audited on female swab submissions when only wet mount testing was available for detection of *T vaginalis* (2004-2007) and when *T vaginalis* detection options included analyte-specific reagent and wet mount (2008-2010).

Results: Analyte-specific reagent availability resulted in more screening and detection of *T vaginalis*, prompted less utilization of wet mount microscopy, and increased overall RNA-based screening for *N gonorrhoeae* and *C trachomatis* ($P < 0.0002$).

Conclusion: Clinician familiarity with *T vaginalis* analyte-specific reagent can benefit both clinical practice and public health.

BACKGROUND

Trichomonas vaginalis is considered a significant sexually transmitted infection (STI) etiology. It causes over 7 million infections in the United States annually and greater than 180 million cases of trichomoniasis worldwide.[1] An antecedent role for this protozoan has been reported in the acquisition[2,3] and transmission[4] of human immunodeficiency virus. Proclivity to *Neisseria gonorrhoeae*[5-7] and *Chlamydia trachomatis*[6,7] co-infection has been reported. The latter associations are important on a local level, in part, because the Milwaukee-Waukesha-West Allis (Wisconsin) Metropolitan Statistical Area (MSA) had a 2010 chlamydia incidence rate of 738.1 per 100,000 inhabitants. This rate was 63.1% higher than the national average and ranked number 2 in the country.[8] Similarly, the gonorrhea incidence rate of this MSA (219.6 per 100,000 population) was the 2nd highest in the United States and was nearly double that of the national average. In light of the widespread distribution of these 2 STIs throughout the community, our laboratory initiated live performance of *T vaginalis* analyte-specific reagent testing (ASR) in June 2007.

This introduction followed a 1086-specimen validation of the assay,[7] which demonstrated that 97.4% of positive vaginal saline suspension microscopy (wet mount) results (n = 76) yielded a positive ASR result. In addition, 82 wet mount-negative specimens generated a positive ASR result. These findings were confirmed by an alternative target molecular amplification assay.[7] The ASR utilizes an RNA amplification technology known as transcription-mediated amplification (TMA) and is performed on specimens treated with an oligonucleotide/magnetism-based target capture protocol. Target capture effectively removes inhibitors to nucleic acid amplification that can be endogenous to primary clinical specimens.[9] Products of TMA are detected by a secondary nucleic acid hybridization method. Enhanced performance characteristics derived from the *T vaginalis* ASR evaluation are supported by data generated from predicate wet mount and culture systems.[10-12]

Increased sensitivity of *T vaginalis* ASR has provided clinicians in a community-care setting with a reliable and convenient means of identifying patients with trichomoniasis.[13] In brief, a 3-year audit of *T vaginalis* ASR performance within a largely subacute care demographic (just 1.4% of requisitions

• • •

Author Affiliations: Wheaton Franciscan Laboratory, Wauwatosa, Wis (E. Munson, Miller, Napierala, Kramme, R. Olson, K. Munson, S. Olson, Hryciuk); College of Health Sciences, University of Wisconsin–Milwaukee, Milwaukee, Wis (E. Munson); Wisconsin State Laboratory of Hygiene, Madison, Wis (Schell); Departments of Bacteriology, Medical Microbiology and Immunology, University of Wisconsin–Madison, Madison, Wis (Schell).

Corresponding author: Erik Munson, PhD, Wheaton Franciscan Laboratory, St Francis Hospital, 3237 S 16th St, Milwaukee, WI 53215; phone 414.647.7589; fax 414.647.5504; e-mail erik.munson@wfhc.org.

Table 1. Comparison of Requisitions Placed on Female Genital Swab Specimens Submitted for Sexually Transmitted Infection Screening by 20 Clinicians in Subacute-Care Practice Before and After Introduction of Trichomonas vaginalis analyte-specific reagent testing (ASR)

Testing Modality	Percentage of Female Genital Swab Collections		
	2004-2007[a]	2008-2010[b]	*P* value
Any wet mount preparation	66.2	57.7	< 0.0002
Point-of-care wet mount preparation	27.8	22.4	< 0.0002
Any assessment for *Trichomonas vaginalis*	66.2	83.6	< 0.0002
Chlamydia trachomatis/Neisseria gonorrhoeae TMA[c]	80.4	83.7	< 0.0002

Abbreviation = TMA, transcription-mediated amplification
[a]n = 4838 patient encounters
[b]n = 8978 patient encounters

originating from emergent care facilities) revealed that the *T vaginalis* detection rate (9.1%) exceeded those generated by *C trachomatis* (5.9%) and *N gonorrhoeae* (1.5%) TMA-based screening.[13] Additional analyses from this 3-year audit form the basis for the current report. Herein we report that STI ordering practice patterns of clinicians in subacute care practice changed after the introduction of *T vaginalis* ASR screening.

METHODS

Setting

Wheaton Franciscan Laboratory serves an approximately 70-clinic physician group in subacute settings throughout the Milwaukee metropolitan area. The populace represents diverse racial and economic backgrounds and historically demonstrates a high rate of STIs.[8] In an institutional review board-approved protocol, clinician ordering practices were audited for separate 36-month intervals corresponding to before and after the introduction of *T vaginalis* ASR. Requisition parameters of interest included frequency of wet mount (including point-of-care wet mount), frequency of any assessment for *T vaginalis* (defined as wet mount and/or *T vaginalis* ASR), and frequency of *N gonorrhoeae/C trachomatis* TMA. To avoid introducing an element of bias, clinician commentary was not solicited pertaining to requisition decisions. Detection of *T vaginalis* was audited on the basis of results derived from wet mount analysis (including point-of-care) and a combined parameter of wet mount and/or *T vaginalis* ASR.

T vaginalis ASR requisition was completely elective (ie, testing was not automatically enacted as a result of requisitions for *N gonorrhoeae/C trachomatis* TMA or *T vaginalis* wet mount). Twenty-five clinicians were responsible for 87.4% of all *T vaginalis* ASR requisitions on female genital swabs. To prevent potential bias toward analysis of *T vaginalis* ASR data, clinicians who experienced a greater than 95% increase in overall STI patient encounters between the 2004-2007 and 2008-2010 intervals (n = 5) were excluded from analysis. The addition of new clinicians and practices reflected this change.

Diagnostic assays

Wet mounts were prepared by placing 1 drop of a vaginal saline suspension onto a glass slide, overlaid with a coverslip and examined by microscopy. *T vaginalis* was identified by characteristic morphology and motility when viewed at 100x total magnification.[14] Upon clinician requisition, primary genital specimens were subjected to *T vaginalis* ASR (Gen-Probe, Inc, San Diego, California) and TMA-based *C trachomatis* and *N gonorrhoeae* screening (APTIMA Combo 2; Gen-Probe).[13,15] For instances of negative wet mount results being reflexed to *T vaginalis* ASR performance, 200-µL aliquots of primary vaginal saline suspensions demonstrating no motile trichomonads were transferred into specimen lysis tubes (Gen-Probe).[11]

Statistics

The significance test of proportions was used to determine if changes in proportions of test requisitioning were significant. This analysis also determined if changes in *T vaginalis* detection rate via wet mount and/or *T vaginalis* ASR were significant. The alpha level was set at 0.05; all *P* values are 2-tailed.

RESULTS AND DISCUSSION

Overall requisitions for *T vaginalis* assessment increased significantly in the interval following introduction of molecular ASR screening. Concurrently, there was a significant decrease in wet mount requisitions (both $P < 0.0002$; Table 1). These findings, together with an overall increase in *N gonorrhoeae/C trachomatis* TMA requisitions, demonstrated a shift in ordering practices to identify more STIs in subacute clinical practice. Recent assessments of community-wide TMA-based screening for these 3 agents revealed that up to 64% of patient encounters yielding at least 1 STI etiology harbored *T vaginalis*.[16,17] Therefore, increased utilization of newly FDA-approved *T vaginalis* TMA-based screening has future potential to affect diagnosis and treatment of STIs in both symptomatic and asymptomatic females.[18]

On an individual clinician basis, 4 major paradigm shifts in ordering practices were observed. These ordering paradigms are demonstrated in Table 2, with representative clinician examples. A number of clinicians increased all assessments for *N gonorrhoeae, C trachomatis,* and *T vaginalis* and decreased

Table 2. Clinician-specific Representations of the 4 Most Common Paradigms Observed Within *Neisseria gonorrhoeae, Chlamydia trachomatis*, and *Trichomonas vaginalis* Ordering Variances Before and After Introduction of *T vaginalis* analyte-specific reagent testing (ASR)

Ordering Paradigm[a,b]	Percentage of Female Genital Swab Collections Submitted for:								
	Chlamydia trachomatis/ Neisseria gonorrhoeae TMA			Any Wet Mount Preparation[c]			Any Assessment for *Trichomonas vaginalis*[d]		
	2004-2007	2008-2010	*P* value	2004-2007	2008-2010	*P* value	2004-2007	2008-2010	*P* value
I	92.1	99.9	<0.0002	15.6	0.01	<0.0002	15.6	87.9	<0.0002
II	89.3	91.3	0.26	98.6	99.3	0.22	98.6	100.0	0.003
III	25.8	66.2	<0.0002	99.1	98.1	0.32	99.1	99.2	0.91
IV	88.3	93.4	0.01	98.5	99.8	0.03	98.5	100.0	0.0006

Abbreviations = TMA, transcription-mediated amplification
[a]Sample data for each ordering paradigm are from 1 representative clinician.
[b]Ordering paradigm I characterized the ordering variances of 20% of audited clinicians; paradigm II characterized 15%; paradigm III characterized 20%; paradigm IV characterized 35%.
[c]Includes point-of-care wet mount preparations.
[d]Includes wet mount preparations and/or *T. vaginalis* ASR.

reliance on wet mounts. A 2nd paradigm involved no change in *N gonorrhoeae/C trachomatis* TMA-based screening or wet mount utilization, but an increase in overall *T vaginalis* assessment. Other clinicians increased *N gonorrhoeae/C trachomatis* screening, with no change in *T vaginalis* assessment. Finally, a number of clinicians increased both *N gonorrhoeae/C trachomatis* screening and overall *T vaginalis* assessment. Of particular interest, the clinician representative of paradigm I (Table 2) nearly completely eliminated wet mount testing by shifting to *T vaginalis* ASR requisitions. Two representative clinicians added *T vaginalis* ASR to all assessments for *T vaginalis* (paradigms II and IV). Requisitions for *N gonorrhoeae/C trachomatis* TMA-based screening increased significantly for 30% of sampled clinicians (data not shown).

Table 3. Representations of Variances Observed With *Trichomonas vaginalis* Detection Rates Before and After Introduction of *T vaginalis* analyte-specific reagent testing (ASR)

Paradigm	Representative Clinician	*Trichomonas vaginalis* Detection Rate (%) via:					
		Any Wet Mount Preparation[a]			Any Assessment for *T vaginalis*[b]		
		2004-2007	2008-2010	*P* value	2004-2007	2008-2010	*P* value
1	A	2.4	4.8	0.02	2.4	6.1	0.0008
2	B	3.4	3.0	0.83	3.4	6.0	0.19
	C	4.6	5.8	0.39	4.6	10.1	0.0008
3	D[c]	19.7	9.4	0.03	19.7	9.1	0.003
	E[d]	13.3	3.9	0.02	13.3	8.8	0.36
	F	6.8	2.2	0.03	6.8	14.0	0.02

[a]Includes point-of-care wet mount assessments.
[b]Includes wet mount assessments and/or *T vaginalis* ASR.
[c]Point-of-care wet mount assessment for *T vaginalis* decreased 63% between 2 intervals.
[d]Point-of-care wet mount assessment for *T vaginalis* decreased 28% between 2 intervals.

Most importantly, detection rate for *T vaginalis* increased from 5.5% to 7.9% in this study set following the advent of *T vaginalis* ASR ($P<0.0002$; data not shown). Moreover, no significant change in wet mount-based *T vaginalis* detection occurred between the intervals before (5.5%) and after (4.5%) the introduction of *T vaginalis* ASR ($P=0.054$). Taken together, these data suggest that the increased detection was largely due to sensitivity of the molecular assay, rather than substantial changes in patient populations. Three paradigms in *T vaginalis* detection rate variance are highlighted by clinician-specific examples in Table 3. Paradigms 1 and 2 trended to an overall increase in detection rate, in spite of nominal increases in wet mount detection rates. Paradigm 3 illustrated decreased wet mount detection of *T vaginalis* that appeared to be supplemented in 1 instance by increased detection via *T vaginalis* ASR (clinician F). Within paradigm 3, clinicians D and E differed from clinician F on the basis of a downward trend in overall *T vaginalis* detection from 2004-2007 to 2008-2010. Because these 2 clinicians utilized point-of-care wet mount far less in the latter interval than the former interval, it can be inferred that the elevated *T vaginalis* detection rates of 19.7% and 13.3% may be the result of false-positive wet mount observations. While literature has spoken to inaccuracy of office-performed clinical microscopy on the basis of insufficient training, competency, and proficiency,[19-21] the

presence of yeast and leukocytes in vaginal collections also may contribute to false-positive *T vaginalis* wet mount analysis.[22,23]

CONCLUSION

Clinicians in subacute care clinical practice altered STI diagnostic practice patterns through incorporation of *T vaginalis* ASR. In this setting of completely elective STI screen requisitioning, decreased reliance on wet mount for detection of *T vaginalis* was observed. Introduction of *T vaginalis* ASR resulted in an overall increase in molecular screening for *C trachomatis* and *N gonorrhoeae.* A single genital swab collection is appropriate for performance of all 3 of these molecular assays; this factor may have contributed to the overall increase in screening frequency. Taken together, these modalities provide a comprehensive screen for STIs in a community setting.

Acknowledgement: The authors express their sincere gratitude to Deb Hamer for expert technical assistance.

Financial Disclosures: Gen-Probe, Inc provided travel expenses and a speaker honorarium to Dr Erik Munson, and travel expenses to Dr Kimber Munson, Ms Miller, and Mr Kramme for their presentation of these study results, at the 111th General Meeting of the American Society for Microbiology, New Orleans, LA, May 21-24, 2011.

Funding/Support: None declared.

REFERENCES

1. Weinstock H, Berman S, Cates W Jr. Sexually transmitted diseases among American youth: incidence and prevalence estimates. *Perspect Sex Reprod Health.* 2004;36(1):6-10.

2. Chesson HW, Blandford JM, Pinkerton SD. Estimates of the annual number and cost of new HIV infections among women attributable to trichomoniasis in the United States. *Sex Transm Dis.* 2004;31(9):547-551.

3. Guenthner PC, Secor WE, Dezzutti CS. *Trichomonas vaginalis*-induced epithelial monolayer disruption and human immunodeficiency virus type 1 (HIV-1) replication: implications for the sexual transmission of HIV-1. *Infect Immun.* 2005;73(7):4155-4160.

4. Kissinger P, Amedee A, Clark RA, et al. *Trichomonas vaginalis* treatment reduces vaginal HIV-1 shedding. *Sex Transm Dis.* 2009;36(1):11-16.

5. Heine P, McGregor JA. *Trichomonas vaginalis*: a reemerging pathogen. *Clin Obstet Gynecol.* 1993;36(1):137-144.

6. Huppert JS, Mortensen JE, Reed JL, et al. Rapid antigen testing compares favorably with transcription-mediated amplification assay for the detection of *Trichomosas vaginalis* in young women. *Clin Infect Dis.* 2007;45(2):194-198.

7. Munson E, Napierala M, Olson R, et al. Impact of *Trichomonas vaginal*is transcription-mediated amplification-based analyte-specific-reagent testing in a metropolitan setting of high sexually transmitted disease prevalence. *J Clin Microbiol.* 2008;46(10):3368-3374.

8. Centers for Disease Control and Prevention. *2010 Sexually Transmitted Disease Surveillance.* Atlanta, GA: U.S. Department of Health and Human Services; 2011.

9. Chernesky M, Jang D, Luinstra K, et al. High analytic sensitivity and low rates of inhibition may contribute to detection of *Chlamydia trachomatis* in significantly more women by the APTIMA Combo 2 assay. *J Clin Microbiol.* 2006;44(2):400-405.

10. Huppert JS, Hesse E, Kim G, et al. Adolescent women can perform a point-of-care test for trichomoniasis as accurately as clinicians. *Sex Transm Infect.* 2010;86(7):514-519.

11. Munson E, Napierala M, Basile J, et al. *Trichomonas vaginalis* transcription-mediated amplification-based analyte-specific reagent and alternative target testing of primary clinical vaginal saline suspensions. *Diagn Microbiol Infect Dis.* 2010;68(1):66-72.

12. Nye MB, Schwebke JR, Body BA. Comparison of APTIMA *Trichomonas vaginalis* transcription-mediated amplification to wet mount microscopy, culture, and polymerase chain reaction for diagnosis of trichomoniasis in men and women. *Am J Obstet Gynecol.* 2009;200(2):188.e1-7.

13. Napierala M, Munson E, Munson KL, et al. Three-year history of transcription-mediated amplification-based *Trichomonas vaginalis* analyte-specific reagent testing in a subacute care patient population. *J Clin Microbiol.* 2011;49(12):4190-4194.

14. Bruckner DA. Urogenital specimens, direct saline mount. In: Isenberg HD, ed. *Clinical Microbiology Procedures Handbook*, 2nd ed. Washington DC: ASM Press; 2004: 9.6.6.1-9.6.6.4.

15. Gaydos CA, Quinn TC, Willis D, et al. Performance of the APTIMA Combo 2 assay for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in female urine and endocervical swab specimens. *J Clin Microbiol.* 2003;41(1):304-309.

16. Kramme T, Munson K, Miller C, Napierala M, Olson R, Munson E. Live *Trichomonas vaginalis* ASR testing in a high-prevalence STI metropolitan area: female epidemiology, abstract 2120. 111th General Meeting of the American Society for Microbiology. New Orleans, LA; 2011.

17. Munson E, Firmani M. Molecular diagnosis of *Neisseria gonorrhoeae* infection in the United States. *Expert Opin Med Diagn.* 2009;3(3):327-343.

18. Schwebke JR, Hobbs MM, Taylor SN, et al. Molecular testing for *Trichomonas vaginalis* in women; results from a prospective US clinical trial. *J Clin Microbiol.* 2011;49(12):4106-4111.

19. Ferris DG, Hamrick HJ, Pollock PG, et al. Physician office laboratory education and training in primary care residency programs. *Arch Fam Med.* 1995;4(1):34-39.

20. Steindel SJ, Granade S, Lee J, et al. Practice patterns of testing waived under the clinical laboratory improvement amendments. *Arch Pathol Lab Med.* 2002;126(12):1471-1479.

21. Stull TM, Hearn TL, Hancock JS, Handsfield JH, Collins C. Variation in proficiency testing performance by testing site. *JAMA.* 1998;279(6):463-467.

22. Garber GE. The laboratory diagnosis of *Trichomonas vaginalis*. *Can J Infect Dis Med Microbiol.* 2005;16(1):35-38.

23. Rein MF, Chapel TA. Trichomoniasis, candidiasis, and the minor venereal diseases. *Clin Obstet Gynecol.* 1975;18(1):73-88.

advancing the art & science of medicine in the midwest

WMJ

WMJ (ISSN 1098-1861) is published through a collaboration between The Medical College of Wisconsin and The University of Wisconsin School of Medicine and Public Health. The mission of *WMJ* is to provide an opportunity to publish original research, case reports, review articles, and essays about current medical and public health issues.

Visit www.wmjonline.org to learn more.