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

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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 and transmission of human immunodeficiency virus. Proclivity to Neisseria gonorrhoeae5-7 and Chlamydia trachomatis6,7 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...
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. 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. 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. 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). 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).

**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*. 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.

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 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<sup>a</sup> | 2008-2010<sup>b</sup> | 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 | 80.4 | 83.7 | <0.0002 |

Abbreviation = TMA, transcription-mediated amplification

<sup>a</sup>n = 4838 patient encounters

<sup>b</sup>n = 8978 patient encounters
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).

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

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

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<sup>a</sup>Includes point-of-care wet mount assessments.  
<sup>b</sup>Includes wet mount assessments and/or T vaginalis ASR.  
<sup>c</sup>Point-of-care wet mount assessment for T vaginalis decreased 63% between 2 intervals.  
<sup>d</sup>Point-of-care wet mount assessment for T vaginalis decreased 28% between 2 intervals.
presence of yeast and leukocytes in vaginal collections also may contribute to false-positive *T vaginalis* wet mount analysis.\textsuperscript{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.

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**REFERENCES**
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