Diagnosis of trichomoniasis: Comparison of wet mount with nucleic acid amplification assays

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Trichomonas vaginalis (TV) is the most prevalent *treatable* sexually transmitted infection (STI) in the United States, with a prevalence recently estimated at 8.7% in women aged 18 to 81 from a diverse geographic distribution.[1,2] Unfortunately, a trichomoniasis diagnosis usually is only considered when a patient is symptomatic, despite the fact that approximately 50% of women who test positive for infection have no symptoms.[1–3] In addition, the most common diagnostic test used to identify this infection, a wet mount slide exam, is only about 50% sensitive, compared with about 98% sensitivity reported for amplification assays, including the newly FDA-approved amplification test, the APTIMA *Trichomonas vaginalis* assay (APTIMA TV assay; Hologic | Gen-Probe, San Diego, California).[2–5,9] Because multiple sequelae and adverse outcomes have been associated with Trichomonas infection (including increased development of pelvic inflammatory disease, posthysterectomy infections, low birth weight, and preterm birth, as well as increased transmission and acquisition of human immunodeficiency virus) and reliable treatment is available, it is critical to reassess how TV infection is diagnosed to limit adverse clinical repercussions.[3]

UNIQUE EPIDEMIOLOGY OF TRICHOMONAS

The estimated number of new TV infections is 7.2 million per year in the United States.[1] This estimate is likely to be low, however, since TV is not a reportable infection to public health agencies. Recent studies using amplified technology for diagnosis show the infection rates of TV to be equal to or greater than the infection rates of STIs typically screened for in the at-risk age group of 18- to 25-year-old adults, including *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT). TV infection also appears to have a uniquely high prevalence in patients older than age 40.[2,3,5,7] The reasons for this age distribution require further study.

DIAGNOSTIC DILEMMAS USING WET MOUNT

Microscopy of wet mount slide preparation is the most common method currently used to diagnose TV infection. To perform a wet mount, a vaginal swab is collected from a patient and placed into a tube of physiologic saline. A small drop of the saline is then placed on a slide, a coverslip is placed on top of the fluid and the slide is examined by light microscopy at the clinic site by the health care provider. A positive result requires visualization of the characteristic jerky motility of the trichomonas parasitic organism. While the wet mount test has the benefit of providing a rapid result at the point of care and is inexpensive, the specimen must be reviewed within minutes to detect the characteristic motility of the organisms while they are still viable. If the saline sample is sent to a laboratory, it is common that analysis of the specimen will not occur within the necessary time frame and a disclaimer such as, "Results of the test may be compromised secondary to delay in transport," will be added to the laboratory report for that test result.

One advantage of the wet mount is its high specificity (false positive results are minimal); however, health care providers can misinterpret clusters of white blood cells (WBCs) as positive for trichomonads since WBCs are similar in size and shape. Thus, expertise and continued competency in reading slides correctly need to be addressed for interpreting wet mount preparations. See **TABLE 1**.

SENSITIVITY OF WET MOUNT COMPARED WITH AMPLIFICATION

The performance of a lab-developed transcription-mediated amplification (TMA) nucleic acid amplification test (NAAT) for TV and wet mount for the diagnosis of TV infections has been compared side by side in several studies.[4–6,8] The performance of both the wet mount and the TMA-NAAT test have been consistent in all studies and highlight the poor sensitivity of wet mount and nearly double the detection of infections with the TMA-NAAT (**TABLES 2** and **3**). This is not surprising given that the viable organism required to visualize the pathogen by microscopy is 10,000 orgs/mL, while the analytical sensitivity of detection using the APTIMA TV test is less than 1 org/mL.[9,10]

Sensitivity of wet mount analysis also has been shown to vary widely depending on the presence or absence of symptoms, thus undermining the utility of the wet mount method for use as a screening test. In a study by Huppert and colleagues, the diagnostic sensitivity for wet mount in 330 patients was 50.8%, but when stratified by the presence or absence of symptoms, the sensitivity dropped to 38.1% in patients with no symptoms. The TMA-NAAT, however, showed overall sensitivity for detection of TV at 98.4% and stratification by presence or absence of symptoms showed no significant difference in performance (**TABLE 2**). Studies conducted by Nye and colleagues and Munson and colleagues evaluating wet

TABLE 1 Advantages and disadvantages of wet mount to diagnose *Trichomonas vaginalis*

Advantages of wet mount	Disadvantages of wet mount
Point of care	Requires immediate interpretation to visualize motile organisms; experienced microscopist
Inexpensive	Poor sensitivity (43% – 83.3%)[3]
High specificity	Only symptomatic patients; not appropriate for screening
	Only vaginal swab specimens

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TABLE 2 APTIMA TV sensitivity versus wet mount

Reference	Population	No. of patients	Wet mount (vaginal swab)	TMA-TV NAAT[a] (vaginal swab)
Nye 2009	Women attending a sexually transmitted diseases clinic	594	54.6%	96.6%[b]
Huppert 2007	Sexually active adolescent women attending a teen health center	330	50.8%[c]	98.4%[c]

[a] TMA-TV NAAT was developed using *Trichomonas vaginalis* analyte-specific reagents (ASRs) (Hologic | Gen-Probe).

[b] Based on the molecular-resolved algorithm, i.e., any positive test: culture, wet mount, polymerase chain reaction (PCR), or TMA-NAAT confirmed by Alt TMA-NAAT (an alternate research-use nucleic acid amplification assay for *Trichomonas vaginalis*).

[c] Based on a composite reference standard, i.e., any positive test: culture, wet mount, OSOM TV, or TMA-NAAT.

mount with a lab-developed TMA-NAAT also show similar results: increased detection of infections with amplification compared with wet mount.[4,6,8] These studies highlight two important diagnostic facts:

- Wet mount has very poor sensitivity compared with amplification and cannot be used for TV screening
- Amplification testing can be used for both diagnosing TV in symptomatic patients and screening for TV in patients at risk for STIs because of the test's very high sensitivity.

AMPLIFICATION REVOLUTIONIZES THE DIAGNOSIS OF TV

NAATs have revolutionized the diagnosis of chlamydia (CT) and gonorrhea (NG) due to their superior sensitivity over more traditional diagnostic standards, including culture. Not surprisingly, multiple studies now show TV amplification diagnostics will alter the clinical testing and diagnosis paradigm for TV, as was seen with NG and CT 10 years ago. Currently, the only amplification assay that is FDA-cleared for TV is the APTIMA TV assay. Diagnosis and screening can be performed using multiple specimen types, including patient-collected noninvasive urine specimens and provider-collected endocervical or vaginal swabs. In addition, the ability to use liquid cytology media with the APTIMA TV assay allows screening and testing for TV in an older patient population, in which a Pap smear may be the only sample collected. Liquid cytology sampling thus enables testing of women in an age group that is not typically encompassed in screening programs for CT and NG, which focus on women of younger age.

CONCLUSION

To achieve optimal sensitivity and detect all women infected with TV, a highly sensitive TV NAAT, such as the APTIMA TV assay, should be used instead of wet mount. ObGyn and primary care physicians can collect vaginal swabs, urine, or ThinPrep (Hologic, Inc) cervical samples from women at high-risk for STI or with vaginal symptoms, and send the specimens to a laboratory for analysis by the APTIMA TV assay, with results often available within 24 hours. Women with positive results should then be contacted to receive a prescription for an antibiotic and providers should address probable partner infection and/or treatment.

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TABLE 3 Comparison of wet mount and TMA-TV NAAT in determination of *Trichomonas vaginalis* prevalence

Reference	Population	No. of patients	Wet mount (vaginal swab)	TMA-TV NAAT[a] (vaginal swab)
Munson 2008	Women attending a suburban urgent-care facility and a metropolitan outpatient physician group	1086	7.0%	14.5%[b]
Munson 2010	Women attending a hospital emergency department	255	9.4%	20.0% [b]

[a] TMA-TV NAAT was developed using *Trichomonas vaginalis* ASR reagents (Hologic | Gen-Probe).

[b] All wet mount-positive samples were detected as positive by TMA-TV NAAT.