Nucleic Acid Testing for *Trichomonas vaginalis*

Also in This Issue

Cystatin C: A useful marker for glomerular filtration rate (GFR)
Choose Wisely: Preserving the Value of Laboratory Data

“Quality in Laboratory Diagnosis”
Trichomoniasis is a persistent disease of the genitourinary tract caused by the flagellated protozoan *Trichomonas vaginalis*. Trichomoniasis is the most common nonviral sexually transmitted disease (STD) in the United States, affecting an estimated 3.7 million people (1).

The overall prevalence of *T. vaginalis* infection is >11% in women aged ≥40 years (2). In symptomatic women who visit STD clinics, the reported prevalence is 26% (3) and in one study of incarcerated individuals, the infection rates were 9%-32% for women and 2-9% for men.

**Symptoms**

Seventy to eighty-five percent of *T. vaginalis*-infected individuals have minimal or no symptoms and when left untreated, these infections can last for months to years. (4-7)

Symptomatic women can present with vaginitis with small petechial or sometimes punctate red “strawberry” spots and profuse, thin, foamy, greenish-yellow discharge with foul odor. The disease may also cause cystitis or urethritis. Vulvar involvement is variable.

In men, *T. vaginalis* can cause urethritis, epididymitis, or prostatitis. These infections cause 5-10% of non-gonococcal urethritis in men. (8)

**Complications**

*Trichomonas vaginalis* infection is associated with adverse pregnancy outcomes including premature rupture of membranes, preterm delivery, and delivery of a low birthweight infant. (9-12) Infection is also associated with two- to threefold increased risk for HIV acquisition. (13-16) Among
women with HIV, *T. vaginalis* infection is associated with increased risk for pelvic inflammatory disease [17-19] and routine screening of asymptomatic women with HIV infection is recommended because of the adverse events associated with trichomoniasis and HIV infection.

**Transmission/Prevention**

Although partners might be unaware of their infection, organisms are readily passed between sex partners during penile-vaginal sex. [8] Partners of men who have been circumcised might have a somewhat reduced risk of *T. vaginalis* infection acquisition. [20, 21]

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**The most common STD - Trichomonas vaginalis affects an estimated 3.7 million people in the United States.**

There is no vaccine for trichomoniasis, and for sexually active individuals, the best way to prevent trichomoniasis is through consistent and correct use of condoms during all penile-vaginal sexual encounters. [22]

**Persistent or Recurrent Infection**

While most recurrent *T. vaginalis* infections are thought to result from reinfection, some infections can be attributed to antimicrobial resistance. Metronidazole resistance occurs in 4%-10% of cases of vaginal trichomoniasis [23, 24] and tinidazole resistance in 1%. [24]

Emerging nitroimidazole-resistant trichomoniasis is concerning because few alternatives to standard therapy exist. The Centers for Disease Control and Infection (CDC) has experience with susceptibility testing for nitroimidazole-resistant *T. vaginalis* and can provide treatment assistance in these cases (telephone: 404-718-4141; website: [http://www.cdc.gov/std](http://www.cdc.gov/std))

**Who Should be Tested?**

As mentioned previously, the CDC recommends routine screening of asymptomatic women with HIV infection because of the adverse events associated with trichomoniasis and HIV infection. Diagnostic testing should also be performed in women seeking care for vaginal discharge.

Screening might be considered for persons receiving care in high-prevalence settings (e.g., STD clinics and correctional facilities) and for asymptomatic persons at high risk for infection (e.g., persons with multiple sex partners, exchanging sex for payment, illicit drug use, or a history of STD).

However, data are lacking on whether screening and treatment for asymptomatic trichomoniasis in high prevalence settings or persons at high risk can reduce any adverse health events and health disparities or reduce community burden of infection. [8] Rectal and oral testing for *T. vaginalis* is not recommended. [8]
Table 1. Sensitivity of diagnostic methods for detecting *Trichomonas vaginalis* in vaginal specimens.

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Sensitivity</th>
<th>Time to Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet Mount</td>
<td>51-65%</td>
<td>&lt;1 hour</td>
</tr>
<tr>
<td>Culture</td>
<td>75-96%</td>
<td>1-4 days</td>
</tr>
<tr>
<td>Nucleic Acids</td>
<td>95-100%</td>
<td>2-3 days</td>
</tr>
</tbody>
</table>

**Testing Methods**

**MICROSCOPIC EVALUATION**

Microscopic evaluation of wet mount preparations is the most common method for the diagnosis of *T. vaginalis* genital infections due to the convenience of the procedure and its relatively low cost. Unfortunately, the sensitivity of the wet mount procedure is low (51-65%) for vaginal specimens (Table 1) and lower still in specimens from men (urethral specimens, urine sediments, and semen).  

Evaluation of wet mount preps must be done promptly after collection because the already low sensitivity of the procedure declines by to 20% within one hour after collection.

While *T. vaginalis* may be an incidental finding in a Pap test, microscopic examination of conventional or liquid-based Pap specimens should not be used as the primary means for detecting *T. vaginalis* because false positive and false negative results can occur.

**CULTURE**

Culture was considered the gold standard method for diagnosing *T. vaginalis* infection before molecular detection methods became available. Culture has a sensitivity of 75%-96% and a specificity of up to 100%. In women, vaginal secretions are the preferred specimen type for culture, as urine culture is less sensitive. In men, culture specimens require a urethral swab, urine sediment, and/or semen. To improve yield, multiple specimens from men can be used to inoculate a single culture.

**NUCLEIC ACID AMPLIFICATION TESTING (NAAT)**

Nucleic acid amplification testing is rapidly replacing culture as the gold-standard for *T. vaginalis* testing. In the 2015 STD treatment guidelines, the CDC recommends the use of highly sensitive NAAT testing for the detection of *T. vaginalis*.

Among women, NAAT detects three to five times more *T. vaginalis* infections than wet-mount microscopy. When NAAT testing on specimens is not feasible, a testing algorithm (e.g., wet mount first, followed by NAAT if negative) can improve diagnostic sensitivity in persons with an initial negative result by wet mount.
**Trichomonas vaginalis testing at Warde**

Warde Medical Laboratory, uses the highly sensitive and specific APTIMA Trichomonas vaginalis Assay for the detection of *T. vaginalis* ribosomal RNA in clinician-collected endocervical swabs, clinician-collected vaginal swabs, female first-void urine specimens, and specimens in PreservCyt Solution. Specimens can be collected from symptomatic and asymptomatic patients. We have also validated the use of male first-void urine specimens and male urethral specimens with this test. The overall sensitivity of the assay is 95.2% and the specificity is 98.0%. The analytical sensitivity of the APTIMA procedure is 0.1 organism/mL.

Clinical testing is performed Monday - Friday and the time to result is 1-3 days. More information about specimen stability, test codes, and LOINC Codes can be found at the Warde Medical Laboratory website (wardelab.com).

**Literature Cited**


Cystatin C

Richard S. Bak, Ph.D.,
Director of Laboratory Operations,
Warde Medical Laboratory

Cystatin C is a low molecular weight protein synthesized by all nucleated cells. It is an inhibitor of cysteine protease.

Cystatin C has a constant rate of endogenous production, is freely filtered by the glomerulus, and has no extrarenal excretion making it a very useful marker for glomerular filtration rate (GFR). In patients with impaired GFR, the serum level of cystatin C increases.

A number of studies have compared creatinine versus serum cystatin C as an indicator of renal function, and the general consensus is that cystatin C is better than creatinine in correlating with the true GFR.[1]. In a group of patients with a range of GFRs, the cystatin C concentration increased sooner than the creatinine as GFR declined.

Cystatin C concentrations started to increase as GFR fell below about 80 mls/min/1.73m² compared with about 40 mls /min/1.73m² for serum creatinine[2]. Cystatin C is therefore especially useful when trying to determine mild to moderate impairment of kidney function[3, 4].

The serum concentration of cystatin C remains unchanged with infections, inflammatory or neoplastic states, and is not affected by body mass, diet, or gender[5]. Its concentration is also not influenced by analytical interferences like bilirubin and hemoglobin, as is creatinine. These are common challenges in pediatric samples due to neonatal jaundice and in vitro hemolysis occurring during pediatric sample collection.

Other conditions where cystatin C may provide more accurate assessment of GFR than creatinine include the very obese, elderly, or malnourished patients. However, several publications suggest an influence of thyroid hormone[6,7,8].

With the emergence of GFR as the primary criterion in the classification of chronic kidney disease[9], there is an increased interest among nephrologists, and other clinicians, for a more accurate estimate of GFR. Cystatin C, although not a perfect marker, has proven to be superior to serum creatinine or creatinine clearance.

Cystatin C is now performed at Warde Medical Laboratory Sunday through Friday with a turnaround time of one to two days. Order test code CYSTC.
References


In 2012, the American Board of Internal Medicine (ABIM) Foundation introduced the Choosing Wisely initiative, with “the goal of advancing a national dialogue on avoiding wasteful or unnecessary medical tests, treatments and procedures.”¹ I have had the privilege of working closely in this effort with the American Society for Clinical Pathology (ASCP), the organization that represents the specialty of Pathology and Laboratory Medicine to the Choosing Wisely initiative.

Since its inception, the Choosing Wisely project has produced a large series of specific recommendations, each released as 5-item lists, from each of the sponsoring societies spanning a diverse array of medical specialties. ASCP has released a total of 10 specific recommendations, with the third list of 5 coming soon in 2016, and other lists to follow. In addition, many of the recommendations made by other clinical medical societies are centered on clinical laboratory ordering practices, further emphasizing the central role the clinical laboratory plays in the effective practice of medicine and the judicious use of medical resources.

The Choosing Wisely lists are generated from recommendations drawn from the individual contributing societies’ members, and are then subject to peer review of available evidence. After a thorough vetting process they are released in the form of the above-mentioned lists. The specific lists of recommendations—from ASCP and from many other medical organizations—are available at www.chosingwisely.org.

For the clinical laboratory, recommendations range from guidance on the prudent use of routine testing (“Avoid routine preoperative testing for low risk surgeries without a clinical indication”) to guidance on more esoteric laboratory testing (“Only order Methylated Septin 9 (WEPT9) to screen for colon cancer on patients for whom conventional...
diagnostics are not possible”), and recommendations to end certain practices entirely (“Don’t use bleeding time test to guide patient care.” “Don’t perform population based screening for 25-OH-Vitamin D deficiency.” “Don’t perform low-risk HPV testing.”)

**Sunsetting Obsolete Tests**

In my experience it can be more difficult to sunset an obsolete clinical laboratory test than it is to onboard a new test. Many of us remember the persistent demand for “LE cell preps” for years after the discovery that the root cause of the formation of “LE cells” in vitro was the presence of anti-nuclear antibodies that could be evaluated directly with a targeted, more accurate, more precise ANA assay.[2]

Likewise, the subjective and labor-intensive leukocyte alkaline phosphatase (LAP) score for the prediction of chronic myelogenous leukemia (CML) was replaced by the highly sensitive and specific BCR-ABL fusion study;[3] but demand for the LAP score persisted, and many physicians in training were counseled on its use long after it became obsolete. The Choosing Wisely initiative gives us a toolbox for the data-driven, peer-reviewed discontinuation of tests that may have persistent champions in medicine, but that are simply not indicated in modern practice.

**Utilization Management**

The increased attention in recent years to utilization management in clinical laboratory ordering practices has been a very positive development toward the “triple aim” of improving the patient experience, improving the health of populations, and reducing the per capita cost of health care.[4] Such efforts are moving beyond the ineffective gatekeeping that traditionally took place at the level of the clinical laboratory after a given test had been ordered, and are now moving toward more effective institutional utilization management committees, laboratory formularies, and other institution-level (and in some larger health systems, enterprise-wide) control models.[5] But effective laboratory test utilization transcends the recommendation of specific ordering practices.

In his book *The Seven Habits of Highly Effective People*, author Steven Covey famously advised us to “begin with the end in mind.”[6] The same is true for the ordering of clinical laboratory tests. Ordering large panels or disparate arrays of numerous laboratory tests is very likely to yield one or two abnormal results even in healthy individuals, and those abnormal results may have very little medical value in the absence of pre-test suspicion for disease.

In an editorial in a previous *Warde Report*, I outlined the perils of “shotgun testing,” and emphasized that the ability of an abnormal laboratory test to predict the presence of disease goes down measurably in proportion to the decrease in the pre-test probability of disease.[7]

There does not exist a test that is exempt from the possibility of false positive results—each of us has the potential to harbor a cross-reactive antibody, a normal concentration of a given analyte that is out of sync with most of our fellow beings, or a transient but clinically insignificant abnormality that over time will normalize.

For some analytes, even the most cutting-edge methodology may lack precision and yield high coefficients of variation (CVs). For these reasons, as the pre-test probability of disease decreases, the relative proportion of false positives as a percentage of total positives increases, reducing the predictive value of the test. At the extreme, a positive test for a disease that no longer exists has essentially a 100% probability of being a false-positive, and a predictive value of zero. Likewise, the predictive value of a positive test ordered for a patient who is extremely unlikely to have a given disease
(say, the ordering of an ovarian cancer marker in a male), is likely to be essentially zero.

In essence, clinical laboratory results should be viewed as tools by which to provide likelihood ratios for adjusting pre-test probability. If a patient’s likelihood of having a particular medical condition (based on history, physical examination, perhaps radiographic information, etc) is deemed to be, say, 5%, then a clinical laboratory result that increases that likelihood by 400% still only yields a 20% probability for that particular condition.

**Clinical laboratory results should be viewed as tools to provide likelihood ratios for adjusting pre-test probability.**

This explains the surprisingly low predictive values of some of the best-performing laboratory tests when ordered in low prevalence populations. Modern HIV screening methods, for example, have stellar performance characteristics when measured over entire populations (typically with sensitivity and specificity exceeding 99%), but may have low positive predictive values (perhaps dipping below 50%) for individual results within very low prevalence populations. This in turn explains the apparent paradox of the need to perform additional confirmatory testing for certain conditions for which screening methods demonstrate outstanding performance.

We need to evolve to a culture in which tests that simply do not perform to modern standards of precision and accuracy quickly yield to more current and predictive tests. But beyond that, every test that is ordered should be ordered with a clear view of how any particular result will affect clinical decision-making and diagnostic probabilities. Otherwise the value of laboratory data is measurably diminished. It is the solemn obligation of each member of the medical community to choose wisely.

**References**


Now you have a choice. You can work with a laboratory whose mission is mandated by the need for ever-increasing quarterly earnings. Or, you can work with Warde Medical Laboratory, where the physician-owners believe that principles are just as important as profits.

Exceptional People.
Exceptional Quality.
Exceptional Service.

Our purpose to provide critical information to healthcare practitioners is supported by our team of distinguished medical professionals. Working under the guidance of internationally known pathologists with impeccable reputations, our clinical specialists bring their particular expertise to test selection and interpretation. The result is an exceptional level of quality and outstanding customer service.

For more information about how Warde Medical Laboratory can provide exceptional quality and service while lowering the cost of your reference testing, please contact:

Jon T. Sanford, Business Development Manager
Office: 800-876-6522 | Mobile: 517-394-3222
sanfordjt@trinity-health.org | www.wardelab.com