New testing options for *Trichomonas vaginalis* respond to growing awareness

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Despite decades of epidemiologic research demonstrating that *Trichomonas vaginalis* remains the most prevalent non-viral sexually transmitted infection (STI) in the United States and globally, public health efforts to control this infection remain limited. Infection with *T. vaginalis* is not directly monitored by any public health agency, and in the U.S., the recommendations for screening are passive at best. While there are diagnostic recommendations for men with recurring urethritis, there are no general screening recommendations for men. Screening recommendations for women are focused on either women with high-risk behaviors, which may be difficult to define or operationalize, or women living in high-prevalence populations. Unfortunately, this may be circular logic; given that prevalence estimation requires screening data. Many practitioners and laboratorians continue to question the relevance of trichomonal infections despite strong epidemiologic data showing a relationship between trichomonas infections and HIV acquisition and transmission, and adverse outcomes of pregnancy. As a result of this somewhat lukewarm interest in controlling or reducing trichomonas infection at the population level, uptake of available diagnostics and reimbursement by third-party payers remains limited.

**Trichomonas myths**

The development of trichomonas control efforts has been both similar to and different from the story of chlamydia control efforts. With chlamydia, it took decades of data collection to show the importance of detection and treatment of this often- unnoticed STI, and control efforts still did not fully evolve until improved diagnostic tools supported broad screening activities. While trichomonas has been recognized as a pathogen longer than chlamydia, infections have often been considered to be only a nuisance; not present if a woman does not exhibit signs or symptoms; always detectable by microscopy; and a disease that affects only women. Therefore, the STI community has spent decades establishing the relevance of diagnosing and treating this infection in order to improve women’s sexual and reproductive health. As the myths related to trichomonas have been debunked, and now that improved diagnostics are becoming widely available, improved understanding of the epidemiology of the disease will hopefully follow and result in more active control efforts, as we saw with chlamydia.

The good news? There have been many exciting technological advances in the diagnosis of *T. vaginalis* over the last five years. New nucleic acid amplification test (NAAT) options, some laboratory-based and others not, continue to be developed in this rapidly changing arena of molecular diagnostics. Several new assays are now available in the U.S., and the list is ever changing and evolving. Below are brief descriptions of recent additions to the NAAT toolbox that can be used for detection of *T. vaginalis*.

**Diagnostic options**

The diagnostic tests commercially available in the U.S. all have excellent sensitivity and specificity for detection of *T. vaginalis* DNA or RNA, so the descriptions here focus on other features that offer laboratory or patient management efficiencies. Starting with high-throughput platforms, there are now two RNA-based transcription mediation amplification assays that can detect trichomonas using similar chemistries on different instruments. One platform utilizes female urine, endocervical samples, and vaginal swabs, while the other can be performed only using endocervical samples and vaginal swabs. Several hundred samples can be tested per day, and testing can include any combination of chlamydia, gonorrhea, and trichomonas. This is important given the high frequency of co-infections, which suggests that treating for chlamydia or gonorrhea alone may not resolve infections and that trichomonas testing would provide important information for clinical management.

The trichomonas assay is distinct from the chlamydia/gonorrhea assays and thus requires multiple sample processing steps and separate reagents on the instrument. However, the high-throughput automation mitigates these potential concerns.

Another mid- to high-throughput system, available since 2012, utilizes strand displacement amplification. Sample types include female urine, endocervical samples, and vaginal swabs. This assay can be performed alone or in combination with a chlamydia/gonorrhea assay as well. When run to get a single diagnosis, the patient sample is used for DNA extraction, and the extracted material is used for all of the tests ordered. Adding the trichomonas assay does reduce the total number of samples that can be tested in a single run; however, the system can perform multiple runs during a typical single-shift work day. This system, and those described above, offer solutions for trichomonas detection that are semi- or fully automated and offer time-saving lab efficiencies for central or reference laboratories that have medium to high demand and test volume.

Of potential interest to smaller, local laboratories is an assay recently cleared by the U.S. Food and Drug Administration (FDA), which is the first true triplex assay for detection of chlamydia, gonorrhea, and trichomonas to be commercially available in the U.S. This means that a single sample, a single extraction process, and a single set of reagents are used to generate all three results. Sample types include female urine, endocervical specimens, and vaginal swabs. This PCR assay is run on a bench-top platform that can run one to 24 samples and takes approximately 3.5 hours to generate results. The broad menu of the platform also includes various infectious (non-STI) pathogens, making this platform suitable for use in small- to medium-throughput settings that need to minimize the number of platforms in the laboratory. The components are packaged in individual strips, stable at ambient temperature and disposable following completion of the test, in an effort to minimize both waste products and the potential for environmental contamination.

Also noteworthy is a more rapid, potentially point-of-care (POC) assay, requiring approximately 90 minutes for test completion, using a compact bench-top instrument. The assay is classified as CLIA moderate complexity. The system is available in single, double, four-slot, and higher instrument sizes in order to meet a variety of demand and volume situations. The platform is cartridge-based and requires minimal hands-on time. The test for *T. vaginalis* is performed in a separate cartridge, and running the trichomonas and the chlamydia/gonorrhea (in a single cartridge) assay requires two instrument slots per patient. Most interestingly, this system is the first in the U.S. to be FDA-cleared for male urine in addition to female urine and vaginal swabs. This affords an exciting opportunity to test men who are at risk (e.g., as sexual contacts to trichomonas or because of unresolved symptoms of urethritis following routine treatment) and could have a substantial impact on male-to-female transmission. This platform also has a broad infectious diseases menu that provides rapid results which may be useful in emergency departments and urgent care settings.

Finally, another POC, rapid NAAT is now available for trichomonas, with results available in about 45 minutes. This assay is isothermal and thus requires only basic equipment (heating continued on page 26
blocks) and has a lateral flow readout that is performed in a hand-held instrument. While this assay does not have CLIA-waived status and does require (minimal) instrumentation, the test is suitable for use in settings where immediate results can positively impact patient care and management. Laboratory savings resulting from the lack of capital equipment costs may make this a useful tool in many resource-constrained settings. There is no comparable chlamydia/gonorrhoea testing that could be performed from the same patient sample.

An improving diagnostic landscape

The assays mentioned above offer a wide variety of solutions that make it possible to provide testing for trichomomas in almost any setting. While the POC options do not have CLIA waivers and require more time than patients might be willing to wait in many settings, there are applications for these assays. Single-test options, such as those offered by any of the last three tests described above, may be useful in emergency departments when patients may, first, have an extended wait prior to the conclusion of a visit and, second, may benefit from immediate treatment. Women presenting with discharge and pelvic pain would likely fall into this category. Beyond the POC applications, assays that can be run in small batch sizes, particularly those that can be combined with chlamydia/gonorrhoea testing on platforms that offer a broad testing menu, may be able to perform clinical testing at smaller laboratories, obviating the need for referral to centralized or reference laboratories. Finally, the larger platforms all offer combined trichomomas and chlamydia/gonorrhoea testing and support higher test volumes. Adoption of the solution best suited to each particular laboratory may facilitate clinical adoption of trichomomas testing in many settings. In addition to improved patient care and management, additional testing may improve our understanding of the population most at risk for this highly prevalent disease. In populations where HIV risk and high rates of trichomomas intersect, such data could lead to improved public health efforts targeting at reducing risk of both of these diseases.16,17

REFERENCES


INTENDED USE: The BioPlex® 2200 HIV Ag-Ab assay is a multiplex flow immunoassay intended for the simultaneous qualitative detection and differentiation of the individual analytes HIV-1 p24 antigen, HIV-1 (groups M and O) antibodies, and HIV-2 antibodies in human serum or plasma (fresh or frozen K2 EDTA, K3 EDTA, lithium heparin, sodium heparin; fresh citrate). This assay is intended as an aid in the diagnosis of infection with HIV-1 and/or HIV-2, including acute (primary) HIV-1 infection. The assay may also be used as an aid in the diagnosis of infection with HIV-1 and/or HIV-2 in pediatric subjects as young as two years of age, and pregnant women.

The BioPlex® 2200 HIV Ag-Ab assay is intended for use in testing plasma specimens to screen organ donors when specimens are obtained while the donor’s heart is still beating.

The BioPlex® 2200 HIV Ag-Ab assay is not intended for use in screening blood or plasma donors, as the effectiveness of this test for use in the screening of these donors has not been established. However, in urgent situations where traditional licensed blood donor screening tests are unavailable or their use is impractical, this assay can be used as a blood donor screening assay.

WARNING: FDA has approved this test for use with serum and plasma specimens only. Use of this test kit with specimens other than those specifically approved for use with this test kit may result in inaccurate test results. This test is not intended for use in children younger than 2 years of age.

CAUTION: United States federal law restricts this device to sale by or on the order of a physician, or to a clinical laboratory.