Marshfield Labs™
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# Laboratory News

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## NEW TEST ANNOUNCEMENT: HIGH RISK HUMAN PAPILLOMA VIRUS (HPV) GENOTYPING

*Timothy Uphoff, PhD, DABMG, MLS(ASCP)CM*

Effective February 9th, Marshfield Labs is offering genotyping of high risk human papillomavirus (HPV) for genotypes 16 and 18/45 (Test Code: HPVGENO). HPV genotyping should only be ordered for patients ≥30 years of age who have discordant co-testing results, i.e., normal cervical cytology and positive for high risk HPV. This test allows providers to make immediate treatment decisions in cases of discordant co-testing.

### INTRODUCTION

For decades, cervical cytology has been the mainstay of cervical cancer screening, but emerging evidence about the pathologic role of HPV in cervical cancer is changing the screening landscape for this disease. Significant evidence now exists to support a causal relationship between duration of infection with a high risk HPV genotype (HR-HPV) and development of cervical cancer (*1*).

### CERVICAL CANCER SCREENING GUIDELINES

2015 will mark the 40th anniversary of the American College of Obstetricians and Gynecologists (ACOG) having first published a technical bulletin as guidance for Pap testing (*2*). Screening guidelines have continually evolved since then, with various professional organizations often offering differing recommendations. By 2012 the American Cancer Society (ACS), American Society for Colposcopy and Cervical Pathology (ASCCP), and American Society for Clinical Pathology (ASCP) published joint consensus guidelines for the Prevention and Early Detection of Cervical Cancer (*3*), followed by ACOG, which later that year released new guidelines for cervical cancer (*4*).

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These guidelines are primarily in agreement with the U.S. Preventive Services Task Force's (USPSTF) current recommendations for cervical cancer screening (*5*). In general, the new guidelines extend the recommended screening intervals to every three years for women 21–29 years undergoing cytology alone, or every five years for those ages 30–65 who undergo both cytology and HPV testing. There is significant evidence that the use of co-testing in the latter group allows for an extended test interval and provides better sensitivity for ≥CIN 3 (cervical intra-epithelial neoplasia) than screening by cytology alone (*6-8*). The guidelines do not recommend co-testing in women 21–29 years because of the high prevalence of HPV in this age group; however, HPV testing can be useful for these patients if the cytology results identify atypical cells of undetermined significance (ASC-US) findings.

Screening more frequently than these recommendations not only offers no benefit but has significant risks. Both the USPSTF and ACS/ASCCP/ASCP documents state that screening more often than every three years causes significant harm in terms of potential short-term psychological stress, additional procedures and assessment and treatment of transient lesions, vaginal bleeding and infection, and potential adverse pregnancy outcomes.

## THE ROLE OF HPV GENOTYPING

The major guidelines published in 2012 also introduced an emerging role for definitively identifying HPV genotypes 16 and 18, specifically in women who have discordant co-testing results with normal cytology and a positive HR-HPV result. There is significant evidence to demonstrate that infections with HPV 16 and 18 are more likely to persist and can progress to cervical cancer much faster than other HR-HPV genotypes. Women with normal cytology and positive HR-HPV results can be managed by either repeat co-testing in one year or immediate HPV16/18 genotyping. If HPV genotyping reveals the presence of HPV 16 or 18, the patient should undergo colposcopy but if this result is negative she would undergo repeat co-testing in one year. The major guidelines do not denote a preference between immediate HPV genotyping and one year co-testing follow-up; however, genotyping can provide immediate information to direct testing and treatment rather than retesting in one year. The guidelines specify that women should not be tested for genotypes other than 16 and 18; however, since the guidelines were published in 2012, the FDA approved the Aptima HPV 16 18/45 Genotype Assay as a follow-up to positive HR-HPV results in ASC-US reflex and co-testing indications. Marshfield Labs has chosen this assay for HR-HPV genotyping which differentiates HPV 16 and 18 from other HR-HPV genotypes but does not differentiate HPV 18 from HPV 45. The assay was actually designed in this manner because of growing evidence that HPV 45 infections have similar oncogenicity to those of HPV 18 and should be treated in the same manner.

## WHEN TO ORDER THIS TEST AND HOW TO ACT ON FINDINGS

The only recommended indication for HPV genotyping is in cases when a woman has had a normal cervical cytology result in conjunction with a HR-HPV positive result. If HPV genotyping reveals the presence of HPV 16 or 18/45 the patient should be referred to colposcopy. If HPV genotyping does not reveal HPV 16 or 18/45 the patient should have cytology and HR-HPV testing performed again in 12 months.

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## HOW TO ORDER THIS TEST

| | |
|---|---|
| **Test Name:** | HPV-Human Papillomavirus Genotyping 16, 18/45(NAM) |
| **Test Code:** | HPVGENO |
| **Keywords:** | Human Papillomavirus, HPV, Genotyping, Nucleic Acid Test |
| **Ordering** | |
| **Clinic (Clinical Order Manager):** | HPV Genotyping 16, 18/45(NAM) |
| **Hospital (Centricity):** | HPV Genotyping 16, 18/45(NAM) |
| **Portal:** | HPV Genotyping 16, 18/45(NAM) |
| **Downtime:** | Write-In (Form I) |
| **Specimen Requirements** | |
| **Specimen Type:** | ThinPrep |
| **Preferred Container:** | ThinPrep Vial |
| **Acceptable Container:** | Green Label Aptima Tube |
| **Specimen Volume:** | 1.0 mL |
| **Specimen Minimum Volume:** | 1.0 mL (allows for one repeat) |
| **Rejection Criteria:** | Insufficient Amount (less than 1 mL) |
| **Storage:** | 2°-30° C |
| **Performing Lab:** | Marshfield Center |
| **Test Availability:** | Once per Week |
| **CPT Code:** | 87625 |
| **Qualitative Interpretation:** | Reported as **Negative**, **Positive** or **Indeterminate** for Human Papillomavirus Genotyping 16 and/or 18/45. Indeterminate results are inconclusive. Repeat testing with a new specimen is recommended. |
| **Results can be found in CMR under:** | • Lab By Date<br>• Lab By Panel (In the miscellaneous folder as "HPV Genotype" near other STDs such as Chlamydia and Gonorrhea.) |

## QUESTIONS

Test information is available in: Marshfield Labs' Test Reference Manual.
For Clinical & Technical information contact:
Timothy Uphoff, PhD, Molecular Pathology Lab at 800-222-5835.

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# NEW *TRICHOMONAS VAGINALIS* SCREENING TEST INTRODUCTION

*Timothy Uphoff, PhD, DABMG, MLS(ASCP)CM*

Beginning February 9, Marshfield Labs will offer screening for *Trichomonas vaginalis* using an FDA-cleared, fully automated, nucleic acid amplification test that has demonstrated better sensitivity and specificity compared to culture and probe based tests. Screening for *T. vaginalis* is useful to detect asymptomatic infections that can lead to serious complications and increase the risk of acquiring or spreading other sexually transmitted infections. Until recently, screening efforts have been limited due in part to the shortfalls of available laboratory options.

## BACKGROUND

**Prevalence:** *Trichomonas vaginalis* is estimated to be the most common curable sexually transmitted infection in the United States. The Centers for Disease Control and Prevention (CDC) estimates that 3.7 million people are infected with *T. vaginalis* each year (*1*). In recent studies, the prevalence of *T. vaginalis* is estimated to be greater than that of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* combined (*2,3*). In a recent study among 389 female samples submitted to Marshfield Labs for *C. trachomatis* and *N. gonorrhoeae* screening, we also found a slightly higher rate of positivity (2.6%) for *T. vaginalis* more than for *C. trachomatis* and *N. gonorrhoeae* combined (2.3%). A 2011 study by Napierala et al. demonstrated a positive detection rate of 9.1% for *T. vaginalis* in a sub-acute care population in southeastern Wisconsin between 2008 and 2010 (*4*). Recent studies also found that in contrast to *C. trachomatis* and *N. gonorrhoeae*, *T. vaginalis* is commonly detected in women over 40.

**Clinical Manifestations:** Humans are the only natural host of *T. vaginalis*, a sexually transmitted parasite that causes vaginitis, urethritis, and cervicitis in women. Untreated infections can lead to more serious complications such as atypical pelvic inflammatory disease and pre-term birth. More than half of all women with *T. vaginalis* are asymptomatic, but symptoms can include vaginal discharge, odor, pruritus and edema or erythema. In males, infection is usually asymptomatic, but it has been implicated as a cause of non-gonococcal urethritis.

An intense inflammatory response associated with *T. vaginalis* infection is thought to play a role in both acquisition and transmission of other sexually transmitted viruses. Gottlieb et al. demonstrated that women with newly diagnosed trichomoniasis were almost four times as likely as non-infected

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controls to acquire herpes simplex virus type 2 (HSV-2) during the study period (*5*). The risk for infection with and transmission of the human immunodeficiency virus (HIV) is also increased for women infected with *T. vaginalis* (*6*). A recent study of HIV-positive women showed that infection with *T. vaginalis* increases the vaginal shedding of HIV infection and that treatment of trichomoniasis resulted in less HIV shedding (*7*).

**Pregnancy:** Vaginal trichomoniasis has been associated with adverse pregnancy outcomes, particularly premature rupture of membranes, preterm delivery, and low birth weight. Treatment of *T. vaginalis* may relieve symptoms of vaginal discharge in pregnant women and prevent respiratory or genital infection of the newborn and further sexual transmission. While some trials suggest the possibility of increased prematurity or low birth weight after metronidazole treatment, more recent studies have not confirmed these findings (*8,9*). Providers should counsel patients regarding the potential risks and benefits of treatment and communicate the option of therapy deferral in asymptomatic pregnant women until after 37 weeks gestation (*10*). All symptomatic pregnant women should be considered for treatment regardless of pregnancy stage and counseled regarding the continued risk of sexual transmission. Women can be treated with 2 g metronidazole in a single dose at any stage of pregnancy. Multiple studies and meta-analyses have not demonstrated an association between metronidazole use during pregnancy and teratogenic or mutagenic effects in infants.

**Follow-Up:** Because of the high rate of reinfection among patients in whom trichomoniasis was diagnosed (17% were re-infected within three months in one study), rescreening for *T. vaginalis* at three months following initial infection can be considered for sexually active women with trichomoniasis; the benefit of this approach, however, has not been fully evaluated (*11*). While most recurrent *T. vaginalis* infections are thought to result from having sex with an untreated partner (i.e., reinfection), some recurrent cases can be attributed to diminished susceptibility to metronidazole. Sex partners of patients with *T. vaginalis* should also be treated. Patients should be instructed to abstain from sex until they and their sex partners have completed treatment.

## SCREENING GUIDELINES

Currently, the CDC sexually transmitted disease treatment guidelines recommend *T. vaginalis* screening for all HIV–infected women when care is initiated, then at least annually, and testing for all symptomatic women presenting with vaginal discharge (*12*). The CDC guidelines also recommend that screening be considered for asymptomatic women at high risk for infection, such as those with new or multiple sex partners, those with a history of STDs, or women in high-prevalence settings. There is significant debate in the public health community regarding the utility of implementing more widespread screening for *T. vaginalis* in the U.S. considering the high sensitivity of nucleic acid amplification methods now available (*13*).

## TESTING OPTIONS

The gold standard for the diagnosis of *T. vaginalis* infection is considered culture; however, the sensitivity of commercially available culture has been reported to be 75% to 89% compared to amplified methods (*14,15*). The time required for results of the culture typically range from two to five days. Many clinicians resort to “wet prep” tests which while rapid, have a sensitivity in the range of 50% (*15*). The Aptima *Trichomonas* assay was also shown to be more sensitive than the BD Affirm assay (Marshfield Labs Test Code: BVDNA) identifying 36.6% more positive patients

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and demonstrating 100% versus 63.4%, (P < 0.0001) sensitivity respectively (*2*). The Aptima *Trichomonas* detection method now offered by Marshfield Labs has published sensitivity claims of 100% based on FDA clinical trial data using vaginal, endocervical or cytology specimens (Table 1).

**Table 1.**

| Specimen Type | Sensitivity % (95% CI) | Specificity % (95% CI) |
|---|---|---|
| Vaginal Swab | 100 (94.7-100) | 98.2 (96.7-100) |
| Endocervical Swab | 100 (94.6-100) | 98.1 (96.7-100) |
| PreservCyt Solution | 100 (95.6-100) | 98.6 (97.4-100) |

http://www.hologic.com/products/clinical-diagnostics-blood-screening/assays-and-tests/aptima-trichomonas-vaginalis-assay#sthash.EzWR72zT.dpuf

## HOW TO ORDER THIS TEST

**Test Name:** *Trichomonas vaginalis,* Nucleic Acid Method (NAM)
**Test Code:** TRICNAM
**Keywords:** *Trichomonas vaginalis*, Nucleic Acid Test
**Ordering**
**Clinic (Clinical Order Manager):** Trichomonas vaginalis (NAM)
**Hospital (Centricity):** Trichomonas vaginalis (NAM)
**Portal:** Trichomonas vaginalis (NAM)
**Downtime:** Write-In (Form I)

**Specimen Requirements:**

| Body Site | Specimen Type | Swab Description | Tube Description | Minimum Volume |
|---|---|---|---|---|
| Vaginal | Aptima – Vaginal Swab | Pink shaft collection swab | Orange labeled tube | 1 Swab |
| Cervical | Aptima - Unisex Swab | Blue shaft collection swab | White labeled tube | 1 Swab |
| Urethral – Males only | Aptima – Unisex Swab | Blue shaft collection swab | White labeled tube | 1 Swab |
| Thin Prep | Aptima – Thin Prep Tube | Not applicable | Green labeled tube | 2.0 mL |
| Urine – first catch | Aptima – Urine | Not applicable | Yellow labeled tube | 2.0 mL |

**Collection Instructions:**
Please reference the Collection Procedure Guides available through the links below or in the Marshfield Labs' Test Reference Manual under TRICNAM.

- Urine Collection
- Clinician Collected Vaginal Swab
- Patient Collected Vaginal Swab
- Unisex Swab Collection

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**Rejection Criteria:**

- Vaginal specimens collected with blue swab in unisex (white label) tube.
- Cervical specimens collected with pink swab in vaginal (orange label) tube.
- Transported improperly.
- Improper specimen source (rectal, eye, throat, etc.).
- Pre-pubescent children or medico-legal cases.
- Female urethral swabs.

| | |
|---|---|
| **Storage:** | 2°-30° C |
| **Performing Lab:** | Marshfield Center |
| **Test Availability:** | Monday - Friday |
| **CPT Code:** | 87661 |
| **Qualitative Interpretation:** | Reported as **Negative**, **Positive** or **Indeterminate** for *Trichomonas vaginalis* RNA by TMA. Indeterminate results are inconclusive. Repeat testing with a new specimen is recommended. |

## QUESTIONS

Test information is available in: Marshfield Labs' Test Reference Manual.
For Clinical & Technical information contact:
Timothy Uphoff, PhD, Molecular Pathology Lab at 800-222-5835.

## REFERENCES


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## BLEEDING TIME ASSAY DISCONTINUED

*Gene Shaw, MD and Kajal Sitwala, MD*

Starting February 20, 2015, Marshfield Labs will discontinue the Bleeding Time assay at all sites. Multiple studies have shown that the Bleeding Time test is not predictive of surgical bleeding and is insensitive to von Willebrand's disease. In the *Choosing Wisely* campaign conducted by the American Board of Internal Medicine (ABIM), the American College of Clinical Pathologists identified Bleeding Time in their top five list of questionable practices.

If, based on a thorough bleeding history, a qualitative platelet functional abnormality is suspected, consultation with a hematologist is generally advised. The PFA-100 platelet function test (PFT) offers greater standardization than Bleeding Time, though it suffers from the same non-specificity and relatively poor sensitivity. Similarly to Bleeding Time, PFT is a poor predictor of surgical bleeding and is not recommended for that purpose.

PFA-100 instruments are available in Marshfield and Weston (DTC). The PFT needs to be performed within four hours of collection on a specimen kept at room temperature. Thus, the PFT will only be an orderable test at these two centers. Although it may be logistically possible to transport a specimen from other centers within this time frame, this is *not* generally recommended due to the sensitivity of platelets to mechanical agitation and temperature changes. Screening for von Willebrand disease (the most common inherited qualitative disorder of platelet function) can be accomplished with testing for von Willebrand factor (vWF) activity, vWF antigen, and factor VIII; these assays can be ordered from any location for performance at Marshfield Center. A platelet aggregation study may be appropriate in rare patients to screen for other disorders; this assay is

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only available if blood is drawn at Marshfield Center.

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