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NuSwab℠ VG: A new diagnostic approach to vaginitis

A nucleic acid amplification, single-specimen assay for detection of bacterial vaginosis, trichomoniasis, and vulvovaginal candidiasis

Vulvovaginitis is a major health problem and is the reason for at least 10 million gynecologic office visits each year in the United States.[1] The three most common infections identified at these visits? Bacterial vaginosis (BV), trichomoniasis, and vulvovaginal candidiasis (VVC).[1] Conditions that once were discussed in low voices behind closed doors are now the subject of national media attention, thanks to their broad prevalence and the desire of patients to keep informed about their health.

Just how prevalent are these infections? According to an analysis from the National Health and Nutrition Examination Survey (NHANES), BV affects approximately 29.2% of women.[2] And the American Congress of Obstetricians and Gynecologists (ACOG) estimates the prevalence of trichomoniasis at 4% to 35%, and VVC at 17% to 39%.[3]

Many infected women lack symptoms, however, or their symptoms overlap those of other vaginal complaints. As a result, as many as 72% of women who have vaginitis remain undiagnosed or misdiagnosed.[3] This is an important consideration, as effective treatment depends on accurate diagnosis.

IDENTIFYING CHARACTERISTICS

Bacterial vaginosis represents disruption of the vaginal flora, with overgrowth of anaerobic and facultative organisms such as *Gardnerella vaginalis, Mycoplasma hominis, Atopobium vaginae*, and other species. BV is the most common cause of abnormal vaginal discharge in women of reproductive age—but not all women who have BV exhibit abnormal discharge.[4] In fact, most women who have BV are asymptomatic. BV is associated with an increased risk of acquisition of HIV and herpes simplex virus-2 (HSV-2), as well as postoperative infection, preterm delivery (and other complications of pregnancy), and pelvic inflammatory disease.[3]

The precise cause, or causes, of BV remain to be elucidated. The role of sexual activity in its pathogenesis is unknown.[5]

Trichomoniasis develops as a consequence of sexually transmitted infection with a protozoan parasite, *Trichomonas vaginalis*. In the United States, approximately 7.4 million cases of trichomoniasis are diagnosed each year, but only about 30% of patients develop symptoms or signs.[6,7] Because trichomoniasis is not reportable to public health agencies or included in routine screening for sexually transmitted diseases, its prevalence is likely to be underestimated and may be as high as 32% in some populations.[7]

When trichomoniasis is present, a person may be more likely to acquire or transmit other sexually transmitted diseases, such as HIV and *Neisseria gonorrhoeae*.[6] Trichomoniasis is also associated with an increased risk of preterm delivery.[3]

As its name suggests, **candidiasis** is caused by species of the yeast genus *Candida*—usually *C albicans*, the species identified in most cases.[8] In recent years, however, other species have emerged in women who have VVC, including *C glabrata, C tropicalis,* and *C krusei.* Approximately 75% of women are affected by candidiasis during their lifetime—nearly 50% of them on more than one occasion.[8] Candidiasis is common in pregnancy.

THE CHALLENGE OF DIAGNOSING VAGINITIS

Bacterial vaginosis. Traditional clinical diagnosis of BV involves the use of **1)** Amsel's criteria or **2)** the Nugent score, based on analysis of a Gram stain.

A diagnosis of BV based on Amsel's criteria requires the presence of at least three of the following:

- **abnormal vaginal discharge** that is homogeneous, thin, and gray in color
- vaginal **pH level** above 4.5
- **a positive "whiff" or amine test**, i.e., a fishy odor before or

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after addition of 10% potassium hydroxide to a sample of vaginal secretions
- a wet mount revealing that at least 20% of epithelial cells are **clue cells**.[4]

The Nugent score, used primarily in research, is the gold standard diagnostic test. In determining the score, a value is assigned to the various bacterial morphotypes found on a Gram stain of vaginal secretions:
- A score of 0 to 3 is negative for BV
- A score of 7 or higher is positive
- A score of 4 to 6 signals intermediate risk

Compared with the Nugent score, Amsel's criteria exhibit sensitivity of 92% and specificity of 77%.[9]

Cultures are of no value in the diagnosis of BV because some organisms associated with the infection, such as *G vaginalis*, can also be found in normal vaginal flora. Moreover, cultures do not recover all organisms associated with BV.

Although the Nugent score and Amsel's criteria are reliable tests, they aren't always practical for clinical use. Obtaining the Nugent score, for example, can be time-consuming and requires the presence of specially trained staff. Even diagnosis based on Amsel's criteria requires the use of microscopy and has the potential for variability in interpretation of specimens.

Trichomoniasis. Traditional diagnosis of trichomoniasis involves a culture or examination of a wet mount for trichomonads. However, these methods have low sensitivity, ranging from 43.0% to 83.3%.[7] In addition, examination of the wet mount for trichomonads must be performed within 10 to 20 minutes after taking the vaginal sample—or the organisms lose their viability.[6]

Moreover, as mentioned above, many women who have trichomoniasis are asymptomatic. Other diagnostic challenges include a risk that trichomoniasis may be misdiagnosed as BV, or may be part of a mixed infectious process.[7]

Candidiasis. VVC is traditionally identified by microscopic visualization of yeast-like cells or isolation of Candida species by culture.[3] However, microscopy lacks sensitivity and fails to identify the species, whereas cultures may have a long turnaround time. Also, because 10% to 20% of women harbor *Candida* species and other yeasts in the vagina with no adverse effects, a finding of *Candida* by itself (by means of culture) is not an indication for treatment unless symptoms are also present.[8]

Overall, the diagnosis of vaginitis using traditional methods tends to be subjective, time-consuming, or low in sensitivity (see the **Table**, page 3).

Advantages of NuSwab^{SM} VG test

Although the NuSwab^{SM} VG assay is not the first diagnostic test to identify three main causes of vaginitis, it has important distinctions, compared with other methodologies.

Bacterial vaginosis NuSwab^{SM} VG includes tests for three bacterial species quantitatively, unlike other DNA probe methodologies, which test only for *G vaginalis* as a single marker. Because *G vaginalis* may be present in up to 70% of women who do *not* have BV, tests that identify only *G vaginalis* are sometimes inaccurate.[20]

Trichomoniasis NuSwab^{SM} VG includes *T vaginalis* using transcription-mediated amplification technology. In a study of 766 patients, the NAA assay identified 36.6% more patients who were positive for *Trichomonas* than did a DNA probe assay.[21]

Vulvovaginal candidiasis The NuSwab^{SM} VG assay discerns *C albicans* and *C glabrata*, the species that constitute 93% to 97% of *Candida* species.[22,23] The DNA probe tests for total yeast species only and yields a result that is positive or negative for *Candida*. It does not identify the species involved.

BREAKTHROUGHS IN DIAGNOSTIC TESTING

DNA-based diagnostic tests, such as polymerase chain reaction (PCR), involve the amplification of a small fragment of DNA by several orders of magnitude, making it a more objective tool to detect and identify infectious organisms. Nucleic acid amplification (NAA) by PCR not only identifies BV-associated bacteria, but some PCR methods can also quantify their numbers. Information derived from PCR-based NAA has added to our understanding of the complexity of the microflora colonizing the vagina and aided in the development of more informative diagnostic tests.

Three markers of BV

An NAA test for BV was evaluated in a clinical trial involving 396 women.[10] In the trial, sponsored by LabCorp and conducted in association with Jane Schwebke, MD, of the University of Alabama at Birmingham, all vaginal specimens were assessed using Amsel criteria and the Nugent Gram stain. In addition, vaginal samples from the same 396 women were tested by quantitative PCR to detect the presence of five potential markers of BV. Analysis of the five markers and combinations of multiple markers led to development of an NAA test for BV based on PCR measurement of the three organisms found to be most predictive:
- *A vaginae*
- bacterial vaginosis–associated bacterium (BVAB)-2
- *megasphaera*-1.[10]

Somewhat surprisingly, although *G vaginalis* is commonly associated with BV, its presence in vaginal specimens did not add to the predictive value of the three-marker profile; nor was *G vaginalis* alone as informative as the three-marker profile. *Lactobacillus crispatus* was omitted from the final assay for the same reason. Moreover, the presence of *L crispatus* in normal vaginal microflora varies considerably, depending on the race and ethnicity of the individual; its inclusion in an assay for BV might, therefore, confound test results.

Sensitivity and specificity of the NAA test for BV were 96.2% and 92.1%, respectively, compared with Amsel diagnosis and Nugent Gram stain.[11] The NAA test, which evolved to become the BV component of NuSwab^{SM} VG, had a positive predictive value of 94.0% and a negative predictive value of 95.0%.[11]

One clear advantage of the NAA test is the quick availability of test results. PCR NAA tests also omit the need for invasive collection of test specimens and can be performed on a patient-collected vaginal swab. Although the cost of an NAA test is slightly higher than traditional diagnostic methods, NAA tests might be preferred by patients for the ease of sampling and quick results.[12–14] In a clinical setting, they are easy to collect and perform and offer impressive sensitivity.

Diagnosing vaginitis: How 4 approaches stack up

Cause of vaginitis	Diagnostic method			
	Clinical findings and/or microscopy	Culture	DNA probe assay	NuSwab℠ VG by nucleic acid amplification (NAA)
Bacterial vaginosis (BV)	Amsel criteria[4,9] • Abnormal vaginal discharge • Vaginal pH level >4.5 • Positive "whiff," or amine, test • Wet mount showing clue cells Quick, inexpensive methods that are performed at the point of care. Subjective; some symptoms are nonspecific. Sensitivity of 92% and specificity of 77%, compared with Nugent score.	Genital culture[3] Cultures are not diagnostic for BV because they do not differentiate abnormal levels of bacteria from normal flora.	DNA probe for *G vaginalis*[3,24] Objective test for an indicator organism for BV, but *G vaginalis* is also part of normal vaginal flora. Test is not diagnostic for BV (see package insert).	Combination NAA test for BV using *A vaginae*, BV-associated bacterium (BVAB)-2, and *megasphaera*-1[11] Objective, quantitative tests for 3 BV-associated organisms. Combination yields a result that is 96% sensitive and 92% specific, compared with Nugent score and Amsel criteria.
Trichomoniasis	Microscopy[3,6] Quick, inexpensive methods that are performed at the point of care. Only about 50%–60% sensitive. Time from specimen collection to test impacts sensitivity.	*T vaginalis* culture[7] Low sensitivity (70%–80%). Long turnaround time may impact follow-up and treatment.	DNA probe for *T vaginalis*[21] Objective test. Shown to be 36% less sensitive than *T vaginalis* NAA testing in a head-to-head study.	Tests for *T vaginalis* by NAA[15] Objective test. 100% sensitive; 99% specific (see APTIMA® package insert).
Vulvovaginal candidiasis	Microscopy[3,8] Quick, inexpensive methods that are performed at the point of care. Only about 50% sensitive. Unable to identify the species of *Candida*.	Yeast culture[3] Usually considered the diagnostic standard. Can discern predominant species. Long turnaround time may impact follow-up and treatment.	DNA probe for *Candida* species[24] Objective test. Does not identify species.	Tests for *C albicans* and *C glabrata* by NAA[16] Objective test. Differentiates two most prevalent species of *Candida*. Highly concordant with culture results.

NAA for trichomoniasis and VVC

When NAA was applied to the diagnosis of trichomoniasis, sensitivity improved considerably, compared with traditional diagnostic methods. An NAA test (the automated APTIMA® *Trichomonas vaginalis* assay [Gen-Probe Incorporated]) was tested in 1,025 asymptomatic and symptomatic women using vaginal swabs and other collection methods. Clinical sensitivity and specificity of the APTIMA® assay were 100.0% and 99.0%, respectively, for the vaginal swab. The assay performed similarly in asymptomatic and symptomatic women.[15]

An NAA assay also performed well in the diagnosis of VVC. When candidiasis was identified by NAA assay, results were concordant with those of culture in 89.8% of specimens tested (230 of 256 specimens).[16] (*C glabrata* is the predominant non-albicans *Candida* species associated with VVC in the United States, Europe, and Australia.[17] According to the findings of two large studies in the United States, *C albicans* and *C glabrata* constitute approximately 93% to 97% of all *Candida* species.[18,19] Other species are comparatively rare.)

How NAA testing can guide treatment decisions

The ability of the NAA assay to identify the *Candida* species as *C albicans* versus *C glabrata* is an important distinction. *C albicans* infection typically responds to standard azole antifungal therapy. Women who have infection with a non-*albicans* species require more aggressive treatment, however. Among the treatment options for women who have "complicated" VVC (i.e., non-*albicans* infection) is a standard course of topical imidazole, which may be effective in as many as 50% of cases. When this approach fails to eliminate infection, vaginal boric acid for a minimum of 14 days is an option. Refractory cases should be referred to a specialist.[3]

NAA tests are also useful when microscopy or clinical findings, or both, are inconclusive, or when infection involves more than one pathogen. For example, a patient who has both BV and VVC may require individualized azole therapy and, in some cases, extended antibiotic treatment as well.[3]

In addition, NAA tests eliminate the long turnaround time (which can delay treatment) associated with cultures and are particularly useful when there is a likelihood that the patient would be lost to follow-up during the wait for culture results.

THE BENEFITS OF MULTIPLE NAATs ON A SINGLE SWAB

The trials mentioned above led to development of the NuSwab℠ VG assay (LabCorp), which identifies BV, trichomoniasis, and candidiasis (two species: *C albicans* and *C glabrata*)

in a single test with high sensitivity and specificity. Tests to identify *Chlamydia trachomatis, N gonorrhea,* HSV-1, and/or HSV-2 with high sensitivity are also available on the same collection device.

This quick test helps clinicians determine whether the cause of vaginitis is bacterial, parasitic, or fungal, and enables them to tailor treatment accordingly. It also simplifies management of test supplies and can reduce errors associated with use of the "wrong swab."

See "NuSwab[SM] VG test in practice," below, for discussion of its use in a clinical setting. ●

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High sensitivity and specificity, plus ease of use, make for a valuable diagnostic tool

In her single-specialty group practice in Orlando, Florida, Leigh White, MD, PhD, sees approximately 50 patients a month whose main complaint is vaginitis. Dr. White practices obstetrics and gynecology, with an emphasis on vaginitis, at Women's Care Florida, Partners in Women's Healthcare.

"Most patients who are referred to me have been unsuccessfully treated multiple times," Dr. White reports. "I start with a complete vaginitis work-up, which includes NuSwab[SM]."

NuSwab[SM] has proved valuable to Dr. White, including the following cases:

- "K. M.," 33 years old, complained of urinary symptoms. She was certain (and correct) that she had a urinary tract infection, but also wondered whether some of her symptoms might be vaginal in nature. A NuSwab[SM] test was negative for yeast, trichomoniasis, and bacterial vaginosis (BV), which reassured her that treatment for the urinary tract infection would clear her symptoms completely.

- "G. D.," 36, had been treated for yeast infections in 2008, 2009, and 2010. In 2011, she reported the onset of malodor during the third week of her cycle, with increased discharge and odor after intercourse. A wet mount was prepared, revealing clue cells; a whiff test was positive. NuSwab[SM] confirmed BV. The patient was reassured that no yeast was present. Infection cleared after treatment with oral metronidazole.

- "A. M.," 27, reported for her annual well-woman exam despite the start of menses earlier in the day. She complained of vaginal itching, but a wet mount was impractical because of heavy bleeding. NuSwab[SM] identified her as having *C albicans* infection and made it possible to initiate treatment immediately rather than wait for her menses to end.

"When patients come to me, even those who have been treated elsewhere, I generally try to start with a complete initial workup," Dr. White reports. "Some women will have *Candida* at one point but go on to develop BV. Sometimes the vaginal flora can be disrupted, even when they just have yeast. Patients tend to think it's all yeast; sometimes it is—but it's always helpful to have definitive confirmation. NuSwab[SM] provides that reassurance. It's a highly sensitive and specific test—but also a flexible test, offering many options."