It’s likely that ObGyns don’t need to see the statistics to know that vulvovaginitis is a major health problem. In fact, you probably devote a significant percentage of your clinical practice to the diagnosis and treatment of vulvovaginal infections—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.⁹ 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
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 stan-
dard 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 sensi-
tivity 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 po-
tential for variability in interpretation of specimens.
**Trichomoniasis.** Traditional diagnosis of trichomoniasis in-
volves a culture or examination of a wet mount for trichomo-
nads. 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.5 However, microscopy lacks sensitivity and fails to
develop an antigen that confers potential for variability in interpretation of specimens. Alternatively, the presence of clue cells may be misdiagnosed as BV, or may be part of a mixed infectious process.7

### Advantages of NuSwab™ VG test

Although the NuSwab™ VG assay is not the first diagnostic
test to identify three main causes of vaginitis, it has impor-
tant distinctions, compared with other methodologies.

**Bacterial vaginosis** NuSwab™ 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™ VG includes T vaginalis using
transcription-mediated amplification technology. In a study of
766 patients, the NAA assay identified 36.6% more pa-
patients who were positive for Trichomonas than did a DNA
probe assay.21

**Vulvovaginal candidiasis** The NuSwab™ 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 nega-
tive 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 Al-
abama 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. Analy-
ysis of the five markers and combinations of multiple markers led
to development of an NAA test for BV based on PCR measure-
ment of the three organisms found to be most predictive:
• A vaginae
• bacterial vaginosis–associated bacterium (BVAB)-2
• megalophaga -1.19

Somewhat surprisingly, although G vaginalis is commonly asso-
ciated 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™ 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 per-
form and offer impressive sensitivity.
Diagnosing vaginitis: How 4 approaches stack up

<table>
<thead>
<tr>
<th>Cause of vaginitis</th>
<th>Clinical findings and/or microscopy</th>
<th>Diagnostic method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis (BV)</td>
<td>Amsel criteria4,9</td>
<td>DNA probe assay</td>
</tr>
<tr>
<td></td>
<td>• Abnormal vaginal discharge</td>
<td>DNA probe for G vaginalis15, 21</td>
</tr>
<tr>
<td></td>
<td>• Vaginal pH level &gt;4.5</td>
<td>Objective test for G vaginalis15, 21</td>
</tr>
<tr>
<td></td>
<td>• Positive “whiff,” or amine, test</td>
<td>T vaginalis culture1, 2, 5</td>
</tr>
<tr>
<td></td>
<td>• Wet mount showing clue cells</td>
<td>DNA probe for T vaginalis1, 2, 5</td>
</tr>
<tr>
<td></td>
<td>Quick, inexpensive methods that are performed at the point of care. Subjective; some symptoms are nonspecific.</td>
<td>Objective test. Shown to be 36% less sensitive than T vaginalis NAA testing in a head-to-head study.</td>
</tr>
<tr>
<td></td>
<td>Sensitivity of 92% and specificity of 77%, compared with Nugent score.</td>
<td>Tests for T vaginalis by NAA15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Objective test. Differentiates two most prevalent species of Candida. Highly concordant with culture results.</td>
</tr>
<tr>
<td>Trichomoniasis</td>
<td>Microscopy3, 6</td>
<td>Yeast culture3</td>
</tr>
<tr>
<td></td>
<td>Quick, inexpensive methods that are performed at the point of care. Only about 50%–60% sensitive.</td>
<td>Usually considered the diagnostic standard. Can discern predominant species. Long turnaround time may impact follow-up and treatment.</td>
</tr>
<tr>
<td></td>
<td>Time from specimen collection to test impacts sensitivity.</td>
<td>DNA probe for Candida species24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Objective test. Does not identify species.</td>
</tr>
<tr>
<td>Vulvovaginal candidiasis</td>
<td>Microscopy3, 6</td>
<td>Tests for C albicans and C glabrata by NAA16</td>
</tr>
<tr>
<td></td>
<td>Quick, inexpensive methods that are performed at the point of care. Only about 50% sensitive. Unable to identify the species of Candida.</td>
<td>Objective test. Differentiates two most prevalent species of Candida. Highly concordant with culture results.</td>
</tr>
</tbody>
</table>

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 1-4 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 NAA®s 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)
References

15. Caldicot and Cglandwa by NAA. LABgpdata. LabCorp.

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 NuSwabSM."

NuSwabSM 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 NuSwabSM 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. NuSwabSM 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. NuSwabSM 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. NuSwabSM provides that reassurance. It’s a highly sensitive and specific test—but also a flexible test, offering many options.”