Multitarget Polymerase Chain Reaction Testing for Diagnosis of Bacterial Vaginosis

Preauthorization is not required.

The following protocol contains medical necessity criteria that apply for this service. The criteria are also applicable to services provided in the local Medicare Advantage operating area for those members, unless separate Medicare Advantage criteria are indicated. If the criteria are not met, reimbursement will be denied and the patient cannot be billed. Please note that payment for covered services is subject to eligibility and the limitations noted in the patient’s contract at the time the services are rendered.

<table>
<thead>
<tr>
<th>Populations</th>
<th>Interventions</th>
<th>Comparators</th>
<th>Outcomes</th>
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<tbody>
<tr>
<td>Individuals:</td>
<td>Interventions of interest are:</td>
<td>Comparators of interest are:</td>
<td>Relevant outcomes include:</td>
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<td></td>
<td>• Multitarget polymerase chain reaction</td>
<td>• Clinical and microscopic evaluation,</td>
<td>• Test accuracy</td>
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<td></td>
<td>testing</td>
<td>including scoring systems (e.g.,</td>
<td>• Test validity</td>
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<td></td>
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<td>Nugent score)</td>
<td>• Symptoms</td>
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<td></td>
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<td>• Change in disease status</td>
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Description

Bacterial vaginosis (BV) is a common medical condition resulting from an imbalance in the normal vaginal flora. Although identification of Gardnerella vaginalis has traditionally been associated with BV, there is no single etiologic agent. Most cases are asymptomatic, and most symptomatic cases can be diagnosed using clinical and microscopic evaluation. Multitarget polymerase chain reaction (PCR) testing is proposed as an alternative to currently available laboratory tests to diagnosis BV. This test may improve outcomes if it is a more accurate and reliable method to diagnose BV, especially in symptomatic women with an indeterminate diagnosis.

Summary of Evidence

In individuals who have signs or symptoms of BV who receive multitarget PCR testing, the evidence includes several prospective studies on technical performance and diagnostic accuracy. Relevant outcomes are test accuracy and validity, symptoms, and change in disease status. Several studies have evaluated the diagnostic accuracy of multitarget PCR tests for BV, including two studies evaluating commercially available tests. The studies found sensitivities between 90% and 95% and specificities between 85% and 90% compared with standard methods of diagnosis. Most studies used a combination of the Amsel criteria and Nugent scoring as the reference standard. There is a lack of direct evidence on the clinical utility of PCR testing for BV (i.e., studies showing that testing leads to better patient management decisions and/or better health outcomes than current approaches). Moreover, a chain of evidence does not currently support multitarget testing because most symptomatic women can be diagnosed with a standard workup and/or a trial of empirical therapy, and it is not clear which subpopulations might benefit most from this test. Studies have not been conducted in the most clinically relevant target population: symptomatic women with indeterminate diagnoses after standard workup. The evidence is insufficient to determine the effects of the technology on health outcomes.
Policy
Multitarget polymerase chain reaction testing for diagnosis of bacterial vaginosis is considered investigational.

Policy Guidelines

Diagnostic Criteria

Amsel criteria

The most common diagnostic approach to BV is use of the Amsel criteria. The Amsel criteria require three of the following four to be present in order for a diagnosis of BV to be confirmed:

- vaginal discharge that is homogeneous, thin and whitish gray discharge;
- presence of clue cells on microscopic examination, which are squamous epithelial cells that normally have a sharply defined cell border but, in BV, have bacteria adherent to their surfaces and appear to be “peppered” with bacteria;
- pH of vaginal fluid greater than 4.5;
- a fishy odor of vaginal discharge before or after addition of 10% potassium hydroxide

For patients who cannot be diagnosed by the Amsel criteria, other scoring systems are used in conjunction with Gram stain for the laboratory diagnosis of BV: Nugent criteria and Ison and Hay criteria.

Nugent and Ison and Hay Criteria

For the Nugent criteria, levels of three types of bacteria-Lactobacillus, Gardnerella/Bacteroides, and Mobiluncus-in vaginal discharge samples are estimated. Levels of Lactobacillus and Gardnerella/Bacteroides are rated on a scale of zero to four based on the number of cells per field magnified at 100 times, and levels of Mobiluncus are rated on a scale from zero to two. A composite score is calculated by summing the three subscores, as follows:

Not consistent with BV:

- Score of zero to three; or
- Score of four to six with clue cells not present

Consistent with BV:

- Score of four to six with clue cells present; or
- Score of at least seven

Some clinicians include a third, middle category in Nugent scoring, with a total score of zero to three considered normal, four to six as intermediate/equivocal and seven to ten as definite BV.

The simplified Ison and Hay criteria are as follows:

- Grade 1 (Normal): Lactobacillus morphotypes predominate;
- Grade 2 (Intermediate): Flora are mixed with some Lactobacillus morphotypes and some Gardnerella or Mobiluncus morphotypes present;
- Grade 3 (BV): Gardnerella and/or Mobiluncus morphotypes predominate. Lactobacilli morphotypes are few or absent.
Background

Bacterial Vaginosis

BV is a condition caused by an imbalance in the normal bacteria vaginal flora. It is common, especially in women of reproductive age. While there is no single known etiologic agent, there is a shift in vaginal flora that involves a depletion of Lactobacillus species and overgrowth of other bacteria, including Gardnerella vaginalis, Mycoplasma hominis, Peptostreptococcus, Mobiluncus species, and other anaerobic gram-negative rods. Prevalence of the condition is high, and it is asymptomatic in most cases. According to data from a nationally representative sample of women surveyed in 2001 to 2004, the prevalence of BV among women ages 14 to 49 in the United States was 29%. Additionally, BV is often confused with nonbacterial causes of vaginitis, including Candida (i.e., yeast infection, caused by a fungus) and Trichomonas (caused by a parasite).

When symptomatic, BV is associated with characteristic signs and symptoms. The most common sign of BV is an abnormal grayish-white vaginal discharge, generally with an unpleasant, often “fishy” smell. Some women experience mild itching. Additionally, BV may be a risk factor for conditions such as preterm delivery and spontaneous abortion in pregnant women, pelvic inflammatory disease, as well as acquisition of HIV and other sexually transmitted infections. Because of potential risks during pregnancy, treatment of BV is indicated for symptomatic pregnant women. However, national organizations do not recommend routine screening for BV among pregnant women, and national guidelines do not address screening of nonpregnant women.

Treatment

Though BV resolves spontaneously in a high percentage of women, treatment for symptomatic BV is usually a course of oral antibiotics, either metronidazole or clindamycin. Antibiotic treatment results in a high rate of remission of symptoms, but recurrences are common within the first year after treatment. Probiotics, alone or in conjunction with antibiotics, are also used, but their efficacy in improving cure rates or preventing recurrences is not well-characterized.

Laboratory- and Examination-Based Methods of Diagnosis

Often BV can be diagnosed in the primary care setting based on patient-reported symptoms, clinical findings during vaginal examination, and analysis of vaginal discharge. Office-based analysis of vaginal discharge includes a wet mount preparation using saline, an odor (“whiff”) test to detect amines before or after the addition of 10% potassium hydroxide, and a test of the pH level. Clinical diagnosis generally involves applying the Amsel criteria, which require three of the following four criteria to be present for a diagnosis of BV to be confirmed:

- vaginal discharge that is homogeneous, thin, and whitish-gray;
- presence of clue cells on microscopic examination, which are squamous epithelial cells that normally have a sharply defined cell border but in BV, have bacteria adherent to their surfaces and appear to be “peppered” with bacteria;
- pH of vaginal fluid greater than 4.5;
- a “fishy” odor of vaginal discharge before or after addition of 10% potassium hydroxide

In most cases of uncomplicated BV, clinical and microscopic examination of the discharge is sufficient to make a presumptive diagnosis using the Amsel criteria. For patients with a moderate to high probability of BV following the clinical and microscopic exam, an empirical treatment trial can be prescribed. Patients who respond to empirical treatment do not require further workup.

A subset of women may require more definitive tests to determine whether BV is present. They include women with unusual or unexpected signs and symptoms and those in whom it is not possible to exclude other etiologies with certainty. In these cases, laboratory tests can assist in making a definitive diagnosis. Gram staining of
vaginal discharge samples is the conventional laboratory method of BV diagnosis, and what many experts consider to be the criterion standard for diagnosing BV. Samples are analyzed using the Nugent criteria or a modified version by Ison and Hay.

A limitation of both diagnostic methods (i.e., clinical diagnosis using the Amsel criteria and laboratory diagnosis using Nugent or Ison and Hay criteria) is that they have subjective components and, therefore, may be imprecise. Moreover, Gram stain examination is time-consuming, requires substantial training, and it is difficult to determine an appropriate clinical response for intermediate scores. The two methods of diagnosis can also be used in combination to increase diagnostic accuracy.

Various commercial tests provide rapid and accurate pH evaluation and amine detection. For example, automated devices that measure the volatile gases produced from vaginal samples and a colorimetric pH test are commercially available.

Vaginal culture is not an appropriate diagnostic method to identify BV because BV is not caused by the presence of a particular bacterial species.

**Nucleic Acid Probes**

DNA probes are available to detect and quantify the bacteria in vaginal fluid samples directly. Bacterial DNA is extracted and amplified by PCR methods, using either universal or specific primers. Bacteria are then identified by characterizing their ribosomal DNA sequences. The specific target is typically the ribosomal subunit of the 16SrRNA gene, which is present in all bacteria. The 16SrRNA genes can be amplified by PCR using universal and/or specific primers. The amplified product is then quantified to assess how many microorganisms are present. In addition to diagnosing health conditions more accurately, use of these new techniques can identify previously unrecognized cultivation-resistant organisms in vaginal fluid.

**PROPOSED MULTITARGET PCR TEST**

Several commercially available tests measure multiple organisms using PCR technology for the diagnosis of BV. The tests and the organisms in the panels are shown in Table 1.

<table>
<thead>
<tr>
<th>Organism</th>
<th>SureSwab</th>
<th>BD Max</th>
<th>MDL Panel</th>
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<tbody>
<tr>
<td>Atopobium vaginae</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Lactobacillus species</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Megasphaera (type 1, type 2, and/or species)</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>BVAB (type 1 and/or type 2)</td>
<td>X</td>
<td>X</td>
<td></td>
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BVAB: bacterial vaginosis-associated bacteria; MDL: Medical Diagnostics Laboratory; PCR: polymerase chain reaction.

SureSwab (Quest Diagnostics) tests for Lactobacillus species, G. vaginalis, Atopobium vaginae, and Megasphaera species. A. vaginae is a bacterium species which, using molecular-based techniques, has been found to be more common in women with BV than women with normal flora.²

The SureSwab Total test involves obtaining vaginal swab specimens and extracting total DNA. Next, real-time PCR is used to quantitate the four types of bacteria. Results are reported as log cells per milliliter for each organism (concentrations of all Lactobacilli species are reported together). In addition, the company provides summary interpretive information based on the findings from all tests. Interpretive information accompanying test results classify findings into one of the following three categories: not supportive, equivocal, and supportive.

A classification of not supportive of BV diagnosis is based on:
• The presence of Lactobacillus species, G. vaginalis levels < 6.0 log cells/mL, and absence of A. vaginae and Megasphaera species; or
• The absence of Lactobacillus species, G. vaginalis levels < 6.0 log cells/mL, and absence of A. vaginae and Megasphaera species; or
• The absence of all targeted organisms.

A classification of equivocal is based on:
• The presence of Lactobacillus species, plus G. vaginalis at least 6.0 log cells/mL, and/or presence of A. vaginae and/or Megasphaera species.

A classification of supportive of BV diagnosis is based on the presence of Lactobacillus species, G. vaginalis levels at least 6.0 log cells/mL, and presence of A. vaginae and/or Megasphaera species.

Quest Diagnostics also offers a SureSwab® bacterial vaginosis/vaginitis test that includes the bacterial vaginosis test, previously described, and tests for Trichomonas vaginalis and four Candidiasis species.

Another product, the BD Max, tests for markers of BV and vaginitis. The test uses a similar process to that described for SureSwab. Vaginal swab specimens are collected, DNA is extracted, and real-time PCR is used to quantitate targeted organisms. Results of BV marker tests are not reported for individual organisms. Instead, qualitative BV results are reported based on the relative quantity of the various organisms. In addition to the BV markers, the BD Max also tests for the vaginitis markers Candida glabrata, Candida krusei, other Candida species, and Trichomonas vaginalis.

Medical Diagnostics Laboratory offers a Bacterial Vaginosis Panel. Four markers (shown above in Table 1) are assessed using real-time PCR and Lactobacillus is profiled using quantitative PCR.

Regulatory Status

In October 2016, the Food and Drug Administration completed a review of a de novo request for classification of the BD Max™ Vaginal Panel (Becton, Dickinson, Franklin Lakes NJ). The test was granted class II designation, marketing authorization, and is indicated for the direct detection of DNA targets from bacteria associated with bacterial vaginosis (DEN160001).

Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratory-developed tests (LDTs) must meet the general regulatory standards of the Clinical Laboratory Improvement Act (CLIA). No multitarget quantitative polymerase chain reaction tests for bacterial vaginosis are available under the auspices of CLIA. Laboratories that offer LDTs must be licensed by CLIA for high-complexity testing. CLIA-approved tests (e.g., SureSwab®; Quest Diagnostics, Madison, NJ; Bacterial Vaginosis Panel; Medical Diagnostics Laboratory) are also commercially available.

Services that are the subject of a clinical trial do not meet our Technology Assessment Protocol criteria and are considered investigational. For explanation of experimental and investigational, please refer to the Technology Assessment Protocol.

It is expected that only appropriate and medically necessary services will be rendered. We reserve the right to conduct prepayment and postpayment reviews to assess the medical appropriateness of the above-referenced
procedures. Some of this protocol may not pertain to the patients you provide care to, as it may relate to products that are not available in your geographic area.

References

We are not responsible for the continuing viability of web site addresses that may be listed in any references below.