Genitourinary Pathogen Nucleic Acid Detection Panel Testing

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Instructions for Use

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Community Plan Policy

- Genitourinary Pathogen Nucleic Acid Detection Panel Testing

Coverage Rationale

The following are proven and medically necessary to evaluate symptomatic individuals for Vaginitis:

- Direct and amplified DNA probe testing for trichomoniasis vaginalis
- Direct probe testing for Candida sp

Due to insufficient evidence of efficacy, the following are unproven and not medically necessary:

- Amplified DNA probe testing for vulvovaginitis due to Candida sp
- Direct and amplified DNA probe testing for bacterial Vaginosis (i.e., Gardnerella vaginalis)
- Multiplex polymerase chain reaction (PCR) panel testing of genitourinary pathogens, including but not limited to pathogens commonly associated with Vaginitis
- Screening of asymptomatic individuals for Vaginitis

Note: This policy does not apply to tests for gonorrhea and chlamydia.

Definitions

Sexually Transmitted Infection (STI): An STI is an infection that is spread by sexual contact. Infections include chlamydia, gonorrhea, human papillomavirus (HPV), herpes, syphilis, and human immunodeficiency virus (HIV) (American College of Obstetricians and Gynecologists, 2019).

Vaginitis: Vaginitis is defined as inflammation or infection of the vagina. The most common causes of Vaginitis include vulvovaginal candidiasis, trichomoniasis, and bacterial Vaginosis (American College of Obstetricians and Gynecologists, 2020).

Vaginosis: Vaginosis is caused by the overgrowth of a number of organisms that are normally found in the vagina. It is a common cause of Vaginitis (American College of Obstetricians and Gynecologists, 2020).

Applicable Codes

The following list(s) of procedure and/or diagnosis codes is provided for reference purposes only and may not be all inclusive. Listing of a code in this policy does not imply that the service described by the code is a covered or non-covered health service. Benefit coverage for health services is determined by the member specific benefit plan document and applicable laws that may require coverage for a specific service. The inclusion of a code does not imply any right to reimbursement or guarantee claim payment. Other Policies and Guidelines may apply.

CPT Code	Description
0068U	Candida species panel (C. albicans, C. glabrata, C. parapsilosis, C. kruseii, C tropicalis, and C. auris), amplified probe technique with qualitative report of the presence or absence of each species
0330U	Infectious agent detection by nucleic acid (DNA or RNA), vaginal pathogen panel, identification of 27 organisms, amplified probe technique, vaginal swab
0352U	Infectious disease (bacterial vaginosis and vaginitis), multiplex amplified probe technique, for detection of bacterial vaginosis–associated bacteria (BVAB-2, Atopobium vaginae, and Megasphera type 1), algorithm reported as detected or not detected and separate detection of Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata/Candida krusei, and trichomonas vaginalis, vaginal-fluid specimen, each result reported as detected or not detected
81513	Infectious disease, bacterial vaginosis, quantitative real-time amplification of RNA markers for Atopobium vaginae, Gardnerella vaginalis, and Lactobacillus species, utilizing vaginal-fluid specimens, algorithm reported as a positive or negative result for bacterial vaginosis
81514	Infectious disease, bacterial vaginosis and vaginitis, quantitative real-time amplification of DNA markers for Gardnerella vaginalis, Atopobium vaginae, Megasphaera type 1, Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), and Lactobacillus species (L. crispatus and L. jensenii), utilizing vaginal-fluid specimens, algorithm reported as a positive or negative for high likelihood of bacterial vaginosis, includes separate detection of Trichomonas vaginalis and/or Candida species (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata, Candida krusei, when reported
87480	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, direct probe technique
87481	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, amplified probe technique
87482	Infectious agent detection by nucleic acid (DNA or RNA); Candida species, quantification
87510	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, direct probe technique
87511	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, amplified probe technique
87512	Infectious agent detection by nucleic acid (DNA or RNA); Gardnerella vaginalis, quantification
87660	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, direct probe technique
87661	Infectious agent detection by nucleic acid (DNA or RNA); Trichomonas vaginalis, amplified probe technique
87797	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; direct probe technique, each organism
87798	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; amplified probe technique, each organism
87799	Infectious agent detection by nucleic acid (DNA or RNA), not otherwise specified; quantification, each organism
87800	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; direct probe(s) technique
87801	Infectious agent detection by nucleic acid (DNA or RNA), multiple organisms; amplified probe(s) technique

Description of Services

Bacterial Vaginosis (BV), Trichomonas vaginalis (T. vaginalis) and Candida species cause the highest number of cases of acute vulvovaginal symptoms that lead a woman to seek medical care. The physician must assimilate information from the history and physical examination with information obtained from a vaginal swab to make a diagnosis for the appropriate treatment. Material from the swab can be used to make a determination of vaginal pH, to prepare slides for microscopy, to perform molecular tests and other rapid tests, and to culture organisms.

Molecular testing for diagnosis of vaginal infection is based on the detection of one or more specific nucleic acid sequences. In the United States, most molecular assays currently available for Vaginitis/Vaginosis are direct DNA probe tests and nucleic acid amplification tests (Coleman and Gaydos, 2018).

The potential use of nucleic acid probe technology for the diagnosis of Vaginitis/Vaginosis was explored in the mid-1990s with the development of a DNA probe assay. Several manufacturers have now developed nucleic acid amplification tests (NAAT) and panel assays using Polymerase Chain Reaction (PCR) which can detect multiple pathogens. For example, Affirm VPIII, a commercially available DNA probe test, utilizes hybridization of specific organismal sequences to specific labeled DNA probes to detect Candida species, Gardnerella vaginalis (as a marker for BV), and T. vaginalis.

Clinical Evidence

Common Causes of Vaginitis

The most common causes of vaginitis include trichomoniasis, bacterial vaginosis (BV), and vulvovaginal candidiasis (VVC). Table 1 describes the main features of these three causes.

Table 1. Features of Vaginitis/Vaginosis

Infection	Discharge	Whiff test	pH	Microscopy
Candida species	Thick	Negative	Normal (< 4.5)	Yeasts, hyphae
Bacterial vaginosis	Thin, homogeneous	Positive	Increased (> 4.5)	Clue cells, decreased Lactobacilli
Trichomonas vaginalis	Frothy, yellow-green	Positive	Increased (> 4.5)	Protozoa

Diagnosis of vaginitis/vaginosis typically hinges on the proper evaluation of a significant amount of data, including the information presented in the table above, and can be quite time-consuming. Despite the frequency with which women present to their doctors with complaints of vaginal symptoms, physicians do not always reliably carry out the diagnostic protocol (Schwiertz et al., 2006). Correct, timely identification of pathogens is critical for treatment, prevention of the spread of contagious disease, and reduction in the risks associated with vaginal infection.

Bacterial Vaginosis (BV)

BV is the most common documented cause of vulvovaginitis among women of reproductive age. In the United States, the prevalence of BV in the general population is estimated to be almost one in three women (Allsworth and Peipert, 2007). BV can produce vaginal discharge and a “fishy” odor, but the majority of women are asymptomatic (Koumans et al., 2007). BV is a polymicrobial infection that is characterized by a shift in vaginal microbiota from an acidic pH (< 4.5) with Lactobacillus species to a more alkaline pH heralded by the presence of Gardnerella vaginalis, a gram-variable coccobacillus, and marked by the presence of other species including Prevotella, Mobiluncus, Ureaplasma and Mycoplasma (Jones, 2019).

BV is of significant public health interest, not just because of its high prevalence, but because it is associated with an increased risk of other medical complications including preterm labor and pelvic inflammatory disease along with increased risk to acquire sexually transmitted infections (Paavonen and Brunham, 2018). Despite its association with adverse pregnancy outcomes, the United States Preventive Services Task Force does not currently recommend screening of asymptomatic pregnant women for bacterial vaginosis although workup of symptomatic women is recommended (USPSTF, 2008). BV can be successfully treated with antibiotics, though the recurrence rate is high. BV can be sexually transmitted and is one of the most commonly diagnosed infections in women following sexual assault. Treatment of sexual partners does not decrease the recurrence rate (CDC, 2021).

Diagnosis of bacterial vaginosis using clinical criteria may be performed by assessing a patient sample via wet prep microscopy for at least three of the four Amsel's criteria: thin and homogeneous vaginal discharge, pH > 4.5, positive whiff test, and presence of clue cells on microscopy (CDC, 2021). These criteria are indicative of the microbiota changes associated with bacterial vaginosis which allow overgrowth of species such as Gardnerella vaginalis. The Gram stain is the reference standard for BV diagnosis and evaluates the quantity of normal flora versus BV flora. Gram stains may be used in conjunction with Nugent's criteria to score the samples and categorize them as being normal flora (0-3), intermediate/mixed flora (4-6), or indicative of bacterial vaginosis (7-10) (Coleman and Gaydos, 2018).

A study designed to evaluate agreement among observers reviewing Gram stains for a diagnosis of bacterial vaginosis found complete agreement among reviewers in 76.2 percent of cases (Mohanty et al., 2010). Another study used κ chance-corrected agreement statistics to compare the microscopic diagnosis of Candida and bacterial vaginosis on wet prep by blinded pairs of observers; the study found agreement was moderate (κ= 0.45) for bacterial vaginosis and fair (κ= 0.3) for candidiasis in a ranking system with possible outcomes of almost perfect, substantial, moderate, fair and poor agreement (Whiteside et al., 2011).

While bacterial vaginosis is a condition that can be identified on Pap test, it is a diagnosis that is often missed. Conventional Pap smear techniques have higher diagnostic utility than liquid-based thin-layer prep (Takei et al., 2006), but due to low sensitivity and specificity, the CDC does not recommend the use of Pap smear for the diagnosis of bacterial vaginosis. Bacterial culture is also not recommended as it is nonspecific (CDC, 2021).

Trichomonas Vaginalis

Trichomoniasis is caused by a microscopic organism called Trichomonas vaginalis (T. vaginalis or TV). T. vaginalis is a sexually-transmitted motile protozoan that causes vaginal discharge and pruritus although the majority of cases are believed to be asymptomatic. The characteristic appearance of the cervix associated with this infection, strawberry cervix, only occurs in a small number of cases and therefore is an inconsistent diagnostic feature (Huppert, 2009).

T. vaginalis is considered a sexually transmitted disease, and concurrent treatment is important for the index case and all sexual partners to eradicate infection. Like BV, T. vaginalis is one of the most common infections following sexual assault. Due to the high rate of reinfection with T. vaginalis, the CDC recommends retesting for T. vaginalis infection within 3 months following initial treatment for all sexually active women (CDC, 2021).

Successful treatment of T. vaginalis infection is important because it has been associated with infertility and adverse pregnancy outcomes. Further, because T. vaginalis has been associated with increased vaginal shedding of HIV, screening of all HIV-positive women entering care is recommended by the Centers for Disease Control and Prevention. T. vaginalis can also cause cervicitis, leading to vaginal discharge, and the CDC recommends women with cervicitis who are symptomatic for infection should have additional testing if trichomonads are not identified by microscopy (CDC, 2021).

Although the characteristic flagellated organisms can be visualized moving about on wet prep, the sensitivity and specificity for the diagnosis of T. vaginalis on wet prep is low compared to culture. In a study comparing diagnostic modalities for the diagnosis of T. vaginalis, wet mount detected 56% of infections and rapid test plus wet mount increased detection to 86% (Pattullo et al., 2008). And while culture is a reliable diagnostic modality, it takes as many as five days for results (Huppert, 2009), and is no longer the gold standard for T. vaginalis diagnosis since the advent of valid molecular diagnostic methods (CDC, 2021).

Vulvovaginal Candidiasis (VVC)

In the United States, Candida albicans is responsible for most cases of VVC followed by Candida glabrata. C. albicans is a fungus that is part of the normal flora of the oral cavity, gastrointestinal tract, and female genital tract. Morphologically, it grows as yeast and a hyphal form in contrast to Candida glabrata, which lacks hyphal elements. VVC symptoms are nonspecific and typically include vulvar pruritus, vulvovaginal irritation, and a thick curdy discharge (Achkar and Fries, 2010).

Candida is usually not sexually transmitted, and VVC can occur spontaneously or as a result of a clinical risk factor such as antibiotic therapy. The true prevalence of VVC is somewhat obfuscated due to the availability of over-the-counter therapies (Sobel, 2007) which allow self-diagnosis and treatment but can also result in delay of correct diagnosis and treatment due to erroneous self-diagnosis (Ferris et al., 2002). It is estimated that 75% of women will have at least one instance of VVC in their

lifetime. Treatment of uncomplicated cases is usually by topical azoles or oral fluconazole. Long-term fluconazole therapy is used for patients with recurrent VVC, defined as 3 or more cases < 1 year (CDC, 2021).

Diagnosis of VVC may be made when a woman presenting with symptoms of vaginitis has either 1) a Gram stain or wet prep of vaginal discharge that demonstrates budding yeasts, hyphae, or pseudohyphae or 2) culture or other test is positive for Candida. While KOH preps and Gram stains demonstrate budding yeasts, Candida glabrata does not form hyphae or pseudohyphae and thus may escape microscopic diagnosis (CDC, 2021). Pap tests are even less sensitive than wet prep for Candida species. Patients often treat themselves with over-the-counter antimycotics based on empiric diagnosis of Candida, but a study that offered clinical testing to women purchasing antimycotics found that only 33.7% of them actually had Candida (Ferris et al., 2002).

DNA Probe Testing

DNA probe testing for Trichomoniasis vaginalis or Candida sp may be beneficial for evaluating symptomatic women for vaginitis. There is limited evidence to demonstrate the clinical utility of direct and amplified DNA probe tests for bacterial vaginosis and amplified DNA probe tests for vulvovaginitis due to Candida sp.

DNA probe-based tests hybridize nucleic acid probes to unamplified pathogen DNA in vaginal samples and may be particularly useful for physicians who are less skilled in office laboratory diagnostic techniques for vaginitis.

The potential value of DNA probe tests for aiding in the diagnosis of vaginosis was demonstrated by Ferris and colleagues (1995) in a study that compared the performance of routine primary care physician-performed office laboratory diagnostic techniques for women with abnormal vaginal symptoms to the results obtained by a DNA probe test for *T. vaginalis, Gardnerella vaginalis, and Candida species* (Affirm VIP III). The clinical microscopic results for sensitivity and specificity were vulvovaginal candidiasis (VVC) 39.6 % and 94 %, trichomoniasis 75.0 % and 96.6 %, and bacterial vaginosis (BV) 76.5 % and 70.8 %. By comparison, the sensitivity and specificity of the DNA probe test for VVC was 75.0 % and 95.7 %, trichomoniasis was 86.5 % and 98.5 %, and BV was 95.4 % and 60.7 %. The researchers concluded that primary care physicians demonstrated a high specificity but low sensitivity when identifying trichomoniasis and VVC by microscopic techniques, and that the DNA probe test was more accurate. However, each pathogen associated with common genitourinary pathogens has its own diagnostic and clinical considerations (Table 1) that in turn influences the clinical utility of the DNA probe tests.

In a comparison of Affirm VPIII to liquid-based Pap test, Levi et al. (2011) reviewed 431 cases where material for Pap test and Affirm testing were simultaneously obtained. Affirm VPIII identified more cases of infection with all three etiologic agents than did Pap test. Using κ statistics, there was poor agreement between Pap test and Affirm VPIII for diagnosis of bacterial vaginosis and T. vaginalis. Of note, Affirm VPIII identified 30 cases of coinfection by two or more organisms whereas Pap test only identified coinfection in 5 cases. This study demonstrates that Affirm VPIII may be useful for detecting mixed infection. According to the authors, this study was limited because they were not able to estimate the sensitivity and specificity of the Affirm VPIII assay and Pap tests due to not comparing their results with the gold standards such as microbial cultures or Gram stain.

In a study of 535 military women presenting with symptoms of acute vulvovaginitis, vaginal specimens were collected for DNA probe analysis by Affirm VPIII. The patients were treated based on the results of wet prep microscopy, whiff test, and pH determination only and not on the basis of the molecular tests. Follow-up telephone calls were made to assess resolution of symptoms. Of 64 cases that were negative by clinical exam, DNA probe analysis detected 4 cases of Candida, 21 cases of BV, and 3 cases of mixed BV and Candida. Eight of twenty-eight women complaining of symptoms not resolved after the clinic visit represented missed cases of BV (Lowe et al., 2009). This study highlights that Affirm VPIII has the potential to decrease the number of repeat patient visits to establish a definitive diagnosis. Study limitations include its observational nature and small subgroup size for trichomoniasis vaginalis.

Amplified Probe and Polymerase Chain Reaction (PCR) Panel Testing

There is a lack of studies that demonstrate clinical utility of panel testing for multiple genitourinary pathogens. Each of the clinical presentations of these infections is different for the various pathogens and there are unique single tests available. Nucleic acid amplification testing has limitations when applied to organisms that potentially form part of the normal human flora (Bursle and Robson, 2016) as it may lead to overdiagnosis. In an evaluation of a PCR assay for BV, van der Veer and colleagues

(2018) concluded that while the test was sensitive, positive results need to be interpreted with clinical symptoms due to asymptomatic vaginal dysbiosis.

While the clinical presentations and diagnostic criteria are different from the different pathogens associated with vaginitis (Table 1), panels that screen for multiple pathogens simultaneously have been developed. Examples of commercially available multitarget PCR tests include BD MAX vaginal panel, MDL BV panel, SureSwab, and NuSwab. All tests are designed to detect bacterial species whose presence or absence is informative in the diagnosis of BV, but differ somewhat in which indicator organisms were selected for the panel, as well as in sensitivity and specificity metrics.

In an effort to compare the performance of clinical assessment with molecular detection using a vaginal panel assay, Broache et al. (2021) evaluated 489 participants in a prospective, cross-sectional, multi-center study. Clinical diagnosis occurred at the time of the visit with no knowledge of results of the vaginal panel assay and was based on signs and symptoms and wet mount microscopy. Positive percent agreement between clinical diagnosis and vaginal panel assay was 59.9% for bacterial vaginosis (BV), 53.5% for vulvovaginal candidiasis (VVC) and 28.0% for *T vaginalis*. Negative percent agreement was 80.2% for BV, 77.0% for VVC and 99.8% for *T vaginalis*. Sixty-five percent of participants with BV, 44% of participants with VVC and 56% of participants with *T vaginalis* by panel assay were not treated for vaginitis based on clinical assessment and diagnosis. False-positive rates of 19.8% for BV, 23.0% for VVC and 0.2% for *T vaginalis* were found in the comparison between clinical diagnosis and assay results, leading to potential overtreatment. The study also showed a significant difference in paired proportions between the panel test and clinical diagnosis specific to BV, suggesting that diagnosis of BV may vary depending on the type of in-clinic testing available and subjectivity when using Amsel's criteria. The researchers point out that while Gram stain with Nugent scoring is the reference standard for BV and culture is the reference standard for VVC, challenges exist with these modalities, including the lack of availability of Gram stain at some clinics and the potentially lengthy turnaround time for culture. As such, the authors concluded that use of vaginal panel assay could improve accuracy in diagnosis of vaginitis and help facilitate more timely and appropriate treatment. They recommend future studies to determine whether utilization of vaginal panel assay reduces overall rate of vaginitis return visits. Of note, the study was sponsored by Becton, Dickinson and Company, makers of the BD Max Vaginal Panel test. Of the six authors of the study, 3 are employed by Becton Dickinson and 2 reported potential conflicts of interest related to grants and consulting fees from Becton Dickinson, creating potential for bias.

Hillier et al. (2021) conducted an observational study on women seeking routine care for vaginitis. The study included 303 symptomatic women from 8 clinics. Participants were assessed and treated according to the discretion of the clinician provider and the practice algorithms in their clinical setting. The researchers note that standard point-of-care tests including wet mount microscopy, vaginal pH, and potassium hydroxide/whiff were rarely performed (17%, 15% and 21%, respectively.) As part of the study, five vaginal swabs (one of which was cryopreserved) were collected for FDA approved nucleic acid amplification testing (NAAT) for vaginosis/vaginitis with the BD Max Vaginal Panel (MAX VP), Nugent scoring for bacterial vaginosis (BV), yeast culture for vulvovaginal candidiasis (VVC) and NAAT for *trichomonas vaginalis* (TV). Results of this laboratory testing were not provided to either the evaluating clinician or the study enrollees. Of the 303 women, 290 women had samples that could be evaluated. Results of standard laboratory-based testing were compared with MAX VP results. For BV, there was 88% concordance between the two tests (Nugent Gram stain score n = 104 and MAX VP n = 107) and 30% of all women were positive for BV by both tests. Cultures for yeast found more *Candida* than NAAT (124 vs. 99, respectively) and 32% of the women tested had one of the *Candida* species group (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. dubliniensis*) by both culture and NAAT. Culture and NAAT testing were in agreement 90% of the time for the Candida species group (112 positive with culture, 92 positive with MAX VP). TV results showed 100% concordance between the two NAAT tests. Of note, laboratory-confirmed testing revealed that 10% of the women evaluated had mixed infections and 41% had no vaginal infections detected. Overall, 170 women had a laboratory-diagnosed cause for vaginitis. Of these, 47% received at least one inappropriate prescription. Antibiotics or antifungals were prescribed in 34% of women who did not have BV, TV, or VVC. Women without infectious vaginitis who were treated empirically were more likely to return for vaginitis symptoms than those who did not receive treatment (9/41 vs 5/79, p =.02). Ultimately, the researchers found that most assessments for vaginitis in these community practice settings did not include the use of recommended point-of-care tests and 42% of women with vaginitis symptoms received inappropriate treatment. Based on these findings, the authors concluded that different models of care may be needed for woman with symptoms of vaginitis, including sensitive and specific laboratory testing and careful patient evaluation to reach an accurate diagnosis.

Kim et al. (2020) performed a two-year, retrospective cohort study examining the utility of testing for Trichomonas vaginalis by wet mount or NAAT in the routine prenatal setting. Of a total of 3,265 pregnant women, 2,489 patients were tested for T vaginalis; 1,808 (55%), 1,661 (51%) and 980 (30%) were testing by wet mount, NAAT, or both methods, respectively.

Microscopy yielded a sensitivity of 26% compared to NAAT, and a specificity of 99%. The overall prevalence of trichomoniasis was 15% by either testing method. The researchers also determined that the risk factors for trichomoniasis included younger age (aRR 0.97, P < .01), being of black race (aRR 2.62, P < .01), abnormal vaginal discharge (aRR 1.45, P < .01), and chlamydia during the current pregnancy (aRR 1.70, P <.01).

Schwebke et al. (2020) performed a prospective, multicenter clinical study to validate the performance of an in vitro diagnostic transcription-mediated amplification nucleic acid test (NAATs) for the diagnosis of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC), and trichomonas. Clinician and patient obtained swab samples were collected from symptomatic women and were tested using the Aptima BV and Aptima Candida/Trichomonas vaginitis (CV/TV) assays. Results were compared to Nugent (plus Amsel for intermediate Nugent) scores for BV, Candida and DNA sequencing for VVC, and a composite of NAAT and culture for T. vaginalis. There were 1,519 subjects enrolled. Clinician collected samples for the investigational tests revealed a 95.0% sensitivity and 89.6% specificity for BV; a 91.7% sensitivity and 94.9% specificity for Candida; 84.7% sensitivity and 99.1 % specificity for C. glabrata; and a 96.5% sensitivity and 95.1% specificity for T. vaginalis. Similar results were observed from the patient collected samples. Clinician diagnosis, in-clinic assessments and investigational assay results were compared with gold standard reference methods in a secondary assessment. This secondary assessment for BV resulted in a sensitivity of ≥ 96.2% and specificity of ≥ 92.4% for the investigational-assay samples, compared to 83.4% and 85.5% for clinicians' diagnoses, 75.9% and 94.4% for original Amsel criteria, 81.1% and 90.1% for modified Amsel criteria, and ≤ 82.8% and ≤ 91.1% for any of the individual Amsel criterion components (vaginal pH, clue cells, and whiff test). For VVC due to the Candida species group or C. glabrata, sensitivity and specificity were ≥ 91.2% and ≥ 98.9%, respectively, for the investigational-assay samples compared to ≤ 27.9% and ≤ 56.4% for potassium hydroxide testing and ≤ 54.9% and ≤ 85.5% for clinicians' diagnoses. For trichomoniasis, sensitivity was ≥ 96.4% for the investigational-assay samples compared to 78.8% for culture and 38.1% for clinicians' diagnoses; specificity estimates were greater than 95% for all trichomoniasis detection methods. The authors reported that overall, the investigational tests revealed a higher sensitivity and specificity for detecting and diagnosing the causes of vaginitis compared to traditional methodologies for diagnosis. Study limitations included lack of diversity with regard to ethnic groups and high specificity of molecular testing, impacting sensitivity to disease attributable to minor species (e.g., Prevotella, candida krusei), which were not included in assay design.

The clinical validity of a PCR-based assay for BV detection was conducted by Cartwright et al. (2018) during a multicenter investigational study. PCR results from 1,579 patients were compared to Nugent Gram stain samples and a clinical evaluation following utilization of the Amsel criteria; next-generation sequencing was used to confirm conflicting results. Nugent Gram stain with Amsel criteria (used to resolve intermediate samples), yielded a prevalence of BV in the study population 34.1%. Of the 1579 samples tested, 579 (36.7%) were determined to be BV positive, 905 (57.3%) BV negative, and 95 (6.0%) BV indeterminate by PCR. Overall agreement between BV-PCR and the Nugent/ Amsel algorithm, after exclusion of BV-PCR indeterminate samples, was 92.2% (1368/1484). Using the Nugent/Amsel algorithm as the reference standard, the BV-PCR assay had a sensitivity of 96.0%, a specificity of 90.2%, a positive predictive value of 83.4%, and a negative predictive value of 97.8%. Following the resolution of conflicting results, the BV-PCR assay had a reported sensitivity of 98.7%, a specificity of 95.9%, a positive predictive value of 92.9% and a negative predictive value of 96.9%. The limitations of current methods for diagnosing BV were a confounder in this and other studies conducted on nucleic acid amplification-based assays. Researchers leading this study attempted to address this issue using an alternate molecular approach to resolve differences between the Nugent/Amsel algorithm and BV-PCR. They state that adoption of a standardized scoring system to define the microflora consistent with BV would be a logical step forward to improve accuracy of reference methods. Another limitation is that all authors of this study were employees of the study sponsor.

Schwebke and colleagues (2018) analyzed the BD MAX vaginal panel compared to reference, for detection of BV, Candida spp., and T. vaginalis. Specimens from 1,740 women were analyzed using the BD MAX panel. Clinician diagnosis (Amsel's test, KOH preparation, and wet mount) were also performed. All testing methods were compared to the respective reference methods. The BD MAX panel resulted in significantly higher sensitivity and negative predictive value than clinician diagnosis. In addition, this test showed a statistically higher overall percent agreement with each of the 3 reference methods than did clinician diagnosis. The authors concluded that findings from the current study supported the potential utility of the BD MAX vaginal panel in the differential diagnosis of vaginitis. The authors indicated that future studies are required to establish the benefits regarding the application of this investigational test in a practical setting.

BD MAX vaginal panel is capable of detecting several Candida species and T. vaginalis in addition to diagnosing bacterial vaginosis via a proprietary algorithm which performs a quantitative assessment of G. vaginalis, Megasphaera type 1, A. vaginae, Lactobacillus spp., and BVAB2. In a cross-sectional study by Gaydos et al. (2017) the BD MAX assay results were compared to

reference methods for the diagnosis of bacterial vaginosis (Nugent's and Amsel's criteria), Candida infection (culture), and trichomoniasis (wet mount and culture) for samples collected from 1,740 symptomatic women. BD MAX test sensitivity was 90.5% (95% CI 88.3-92.2%) and specificity was 85.8% (95% CI 83.0-88.3%) for bacterial vaginosis. Candida group test sensitivity was 90.9% (95% CI 88.1-93.1%) and specificity was 94.1% (95% CI 92.6-95.4%), with lower sensitivity for Candida glabrata (75.9% (95% CI 57.9-87.8%)) but a high specificity (99.7% (95% CI 99.3-99.9%)). BD MAX vaginal panel test sensitivity was 93.1% (95% CI 87.4-96.3%) and specificity was 99.3% (95% CI 98.7-99.6%) for the presence of T. vaginalis. According to the authors, this investigational test appears to be a promising molecular assay for detection of vaginitis using molecular amplification of vaginal microbiome organisms, indicating a one-assay platform could potentially aid clinicians in diagnosing vaginitis. Research will be required to demonstrate performance and outcomes in various populations such as pregnant women, hypoestrogenic women, and asymptomatic women.

APTIMA T. vaginalis is a nucleic acid amplification test that has been reported to be more sensitive than culture for the diagnosis of T. vaginalis (Nye et al., 2009). In a large, multi-center trial sponsored by Gen-Probe, APTIMA T. vaginalis sensitivity was found to be 100% in vaginal swab, endocervical swab, and liquid cytology cervical specimens. The test also had high sensitivity for urine specimens. Specificities for the various specimen sources ranged from 98.9% to 99.6% (Schwebke et al., 2011). Another Gen-Probe sponsored study reported a sensitivity and specificity of 100% when using an in-house second transcription-mediated amplification test with a different primer and probe set as a confirmatory test (Andrea and Chapin, 2011).

Clinical Practice Guidelines

American College of Obstetricians and Gynecologists (ACOG)

ACOG published a recent Clinical Management Guideline to describe the diagnosis and treatment of the common causes of vaginitis in nonpregnant women (ACOG 2020). In the summary of recommendations, ACOG gives the following recommendations a Level A rating (based on good and consistent scientific evidence):

- Use of Amsel clinical criteria or Gram stain with Nugent scoring for the diagnosis of BV
- Nucleic acid amplification testing (NAAT) for the diagnosis of trichomoniasis
- In a symptomatic patient, diagnosis of VVC requires one of the following two findings: 1) spores, pseudohyphae, or hyphae on wet-mount microscopy or 2) positive vaginal fungal culture or commercial diagnostic test

Level B recommendations (based on limited of inconsistent scientific evidence) include:

- Pap tests are not reliable for the diagnosis of vaginitis. Diagnostic confirmation is recommended for incidental findings of VVC, BV or TV on a Pap test.

Infectious Diseases Society of America/American Society for Microbiology

The Infectious Diseases Society of America and the American Society for Microbiology released a joint guide (Miller et al., 2018) that contains the following recommendations for the diagnosis of vaginosis/vaginitis:

- Nucleic acid amplification tests are recommended for suspected diagnosis of T. vaginalis infection due to the wide variation in sensitivity and ability to detect T. vaginalis between observers using microscopy.
- For the diagnosis of bacterial vaginosis, the use of Amsel's clinical criteria or scored Gram stain of vaginal discharge are preferred over probe hybridization or culture for only G. vaginalis due to the lower specificity of probe and culture testing for BV.
- For candidiasis diagnosis, wet prep, culture, or DNA probe are the recommended methods, with culture being preferred in cases of recurrent candidiasis.

Centers for Disease Control and Prevention (CDC)

Guidelines from the Centers for Disease Control and Prevention (2021) state, "Despite the availability of BV NAATs, traditional methods of BV diagnosis, including the Amsel criteria, Nugent score, and the Affirm VP III assay, remain useful for diagnosing symptomatic BV because of their lower cost and ability to provide a rapid diagnosis." For trichomonas, the guidelines indicate that wet-mount microscopy has historically been the preferred diagnostic test for T. vaginalis because it is inexpensive and can be performed at POC, however sensitivity is low (44%–68%) compared with culture. NAATs detect more T. vaginalis infections than wet-mount microscopy due to their high sensitivity. Regarding the use of PCR testing for diagnosis of uncomplicated VVC, the guidelines state: "The majority of PCR tests for yeast are not FDA cleared, and providers who use these tests should be

familiar with the performance characteristics of the specific test used. Yeast culture, which can identify a broad group of pathogenic yeasts, remains the reference standard for diagnosis."

The CDC recommends the following diagnostic modalities for the treatment of bacterial vaginosis, Trichomonas vaginalis and Candida in the Sexually Transmitted Treatment Guidelines published in 2015 (CDC, 2015):

- For the diagnosis of bacterial vaginosis, the following tests are recommended: Amsel's clinical criteria or Gram stain. While DNA probe tests have been shown to have acceptable performance characteristics compared with Gram stain, the Gram stain remains the gold standard laboratory method. Other enzymatic activity tests, such as the proline aminopeptidase card test, are not recommended due to low sensitivity and specificity. Culture is not recommended, and the Pap test is not useful for this purpose.
- For the diagnosis of Trichomonas vaginalis in women, the following tests are recommended: wet prep microscopy, DNA probe testing, rapid antigen tests, nucleic acid amplification tests, and culture. Due to its low sensitivity for detecting T. vaginalis in vaginal specimens, it is recommended that this method be used in conjunction with a highly sensitive test, such as nucleic acid amplification.
- For the diagnosis of Candida, a diagnosis can be made when wet prep, Gram stain, culture or other test is positive for a yeast species.

The CDC Quality STD Clinical Services (STD QCS) report provides the following clinical services recommendations for sexually transmitted diseases as a complement to its 2015 Sexually Transmitted Diseases Treatment Guideline (Barrow et al., 2020):

- In primary care settings, basic STD services can include testing for trichomoniasis, bacterial vaginosis, and vulvovaginal candidiasis. Laboratory testing should include urogenital/extragenital NAAT for gonorrhea and chlamydia and oncogenic HPV NAAT testing. NAAT for trichomoniasis may be available in some laboratories.
- In STD specialty care settings, testing for trichomoniasis, bacterial vaginosis and vulvovaginal candidiasis should be available. Clinical laboratory testing should include urogenital/extragenital NAAT for gonorrhea, chlamydia, and trichomoniasis, and oncogenic HPV NAAT.

United States Preventive Services Task Force (USPSTF)

The USPSTF recommends against screening for bacterial vaginosis in pregnant women who are not at increased risk for preterm delivery. Additionally, it states that there is currently insufficient evidence to assess the benefits and risks of screening for bacterial vaginosis in pregnant women who are at risk for preterm delivery (U.S. Preventive Services Task Force, 2008).

Kahwati et al. (2020) published an updated 2020 recommendation statement from the USPSTF that advises against screening for bacterial vaginitis (BV) in pregnant women who do not have signs or symptoms of BV and who are not an increased risk for premature delivery (grade D). The task force recommends additional research to determine if BV screening for patients at risk for pre-term delivery is beneficial (insufficient evidence statement).

British Association for Sexual Health and HIV

The British Association for Sexual Health and HIV recommends the following diagnostic tests in women presenting with signs and symptoms of vaginal infection (British Association for Sexual Health and HIV, 2019; Sherrard et al., 2014):

- For suspected yeast infection, microscopy examination of wet prep slide is recommended; culture is only recommended in cases of recurrent infection.
- For diagnosis of trichomoniasis, nucleic acid amplification tests are recommended over microscopy or culture as it has higher sensitivity and is becoming the gold standard for T. vaginalis diagnosis.

U.S. Food and Drug Administration (FDA)

This section is to be used for informational purposes only. FDA approval alone is not a basis for coverage.

There are several commercial multiplex polymerase chain reaction (PCR) kits that have been cleared through the FDA 510(k) clearance process. For more information regarding specific tests and FDA approval status may be found on the FDA website at: https://www.fda.gov/medical-devices/vitro-diagnostics/nucleic-acid-based-tests. (Accessed Jan 6, 2022)

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Policy History/Revision Information

Date	Summary of Changes
10/01/2022	Applicable Codes • Updated list of CPT codes to reflect quarterly edits; added 0352U Supporting Information • Archived previous policy version 2022T0608F

Instructions for Use

This Medical Policy provides assistance in interpreting UnitedHealthcare standard benefit plans. When deciding coverage, the member specific benefit plan document must be referenced as the terms of the member specific benefit plan may differ from the standard plan. In the event of a conflict, the member specific benefit plan document governs. Before using this policy, please check the member specific benefit plan document and any applicable federal or state mandates. UnitedHealthcare reserves the right to modify its Policies and Guidelines as necessary. This Medical Policy is provided for informational purposes. It does not constitute medical advice.

This Medical Policy may also be applied to Medicare Advantage plans in certain instances. In the absence of a Medicare National Coverage Determination (NCD), Local Coverage Determination (LCD), or other Medicare coverage guidance, CMS allows a Medicare Advantage Organization (MAO) to create its own coverage determinations, using objective evidence-based rationale relying on authoritative evidence (Medicare IOM Pub. No. 100-16, Ch. 4, §90.5).

UnitedHealthcare may also use tools developed by third parties, such as the InterQual® criteria, to assist us in administering health benefits. UnitedHealthcare Medical Policies are intended to be used in connection with the independent professional medical judgment of a qualified health care provider and do not constitute the practice of medicine or medical advice.