Anthem

Clinical UM Guideline

Subject: Nucleic Acid Amplification Tests Using Algorithmic Analysis for the Diagnosis of Vaginitis
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Description

Vaginitis is the general term for infection or inflammation of the vagina. The spectrum of symptoms associated with vaginitis include vulvovaginal itching, burning, irritation, dyspareunia, abnormal vaginal discharge, and “fishy” vaginal odor (ACOG, 2020). The most common causes of vaginitis are bacterial vaginosis (BV), trichomoniasis and vulvovaginal candidiasis.

This document addresses the use of nucleic acid amplification tests using algorithmic analysis to detect vaginitis, including assays that detect bacterial vaginosis, Trichomonas vaginalis and/or Candida species. These multiplex assays use proprietary algorithms that are reported as either a positive or negative result (or high likelihood), or scoring system (for example, negative, positive, or intermediate) for the likelihood of bacterial vaginosis, Trichomonas vaginalis and/or candidiasis.

Note: This document does not address stand-alone testing for trichomoniasis or vulvovaginal candidiasis.

Clinical Indications

Medically Necessary:

Nucleic acid amplification testing for vaginitis (bacterial vaginosis-associated bacteria, Trichomonas vaginalis and/or Candida species) using algorithmic analysis is considered **medically necessary** when an individual meets the following criteria:

1. Symptomatic; **or**
2. All of the following:
    A. Pregnant; **and**
    B. Asymptomatic; **and**
    C. History of preterm birth.

Not Medically Necessary:

Nucleic acid amplification testing for vaginitis using algorithmic analysis is considered**not medically necessary** when the medically necessary criteria above are not met.

Coding

The following codes for treatments and procedures applicable to this guideline are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement policy. Please refer to the member's contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

When services may be Medically Necessary when criteria are met:

CPT	
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 Aptima® BV Assay, Hologic Inc
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 BD MAX™ Vaginal Panel, Becton Dickson and Company
81599	Unlisted multianalyte assay with algorithmic analysis [when specified as nucleic acid amplification testing for bacterial vaginosis using an algorithmic assay]
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 Xpert® Xpress MVP, Cepheid®

ICD-10 Diagnosis	
	All diagnoses

When services are Not Medically Necessary:
For the procedure codes listed above when criteria are not met.

Discussion/General Information

Vaginitis

According to the American College of Obstetricians and Gynecologists (ACOG, 2020):

> Vaginitis is defined as inflammation or infection of the vagina and is associated with a spectrum of symptoms, including vulvovaginal itching, burning, irritation, dyspareunia, “fishy” vaginal odor, and abnormal vaginal discharge. Vaginal symptoms are some of the most frequent reasons for patient visits to obstetrician–gynecologists and may have important consequences in terms of discomfort and pain, days lost from school or work, sexual functioning, and self-image.

Diagnosing the cause of vaginitis is based on a combination of symptoms, physical examination findings, and office-based or laboratory testing. Symptoms alone do not permit reliable differentiation of the causes of vaginitis. When a patient presents with vaginitis, bacterial vaginosis, vulvovaginal candidiasis (VVC) and Trichomonas vaginalis are all considered in the differential diagnosis. The diagnosis of the cause of vaginitis can also be complicated by coinfections (Schwebke, 2020; Sobel, 2013). Failure to accurately diagnosis the cause of vaginitis may result in the individual continuing to experience symptoms and/or sexual dysfunction as well as expose them to increased risk for STIs, candidiasis secondary to unnecessary antibiotic use, antibiotic resistance, and unnecessary health system encounters.

Bacterial Vaginosis

An optimum vaginal microbiome is comprised of more than 90 lactobacilli. Bacterial vaginosis (BV) occurs when there is a shift in vaginal flora to include a greater proportion of mixed anaerobic bacteria, such as the Gardnerella, Prevotella, and Atopobium species. Most often, BV does not produce symptoms (as much as 80% of individuals with BV are asymptomatic). However, when they do occur, symptoms typically include off-white, thin, homogenous discharge, a vaginal “fishy” odor, or both (Muzny, 2020; USPSTF, 2020).

BV is the most common cause of vaginal symptoms and discharge in reproductive-age women in the United States. The overall prevalence of BV in North America in women of reproductive age is 27.4%, with an even higher prevalence in African American women (33.2%) and Hispanic women (30.7%) than in Caucasian (22.7%) or Asian women (11.1%). Nonpregnant women with BV are at an increased risk of various infections of the female reproductive tract, including pelvic inflammatory disease and postprocedural gynecologic infections, and have heightened susceptibility to sexually transmitted infections such as human immunodeficiency virus (HIV) and herpes simplex virus type 2. The prevalence of BV among pregnant women ranges from 5.8% to 19.3% but is higher in some races/ethnicities. BV during pregnancy has been associated with adverse obstetrical outcomes including early miscarriage, premature rupture of membranes, preterm labor, preterm delivery, low birth weight, and postpartum complications such as endometritis and wound infections (ACOG, 2020; Koumans, 2007; Muzny, 2020; USPSTF, 2020).

Characteristics associated with an increased risk of BV include multiple male sexual partners, female sexual partners, having concurrent sexual partners, lack of condom use, douching and being herpes simplex virus type 2 seropositive. Consistent condom usage is associated with a decreased risk (ACOG, 2020; Koumans, 2007; Peebles, 2019; Workowski, 2021).

BV can resolve spontaneously and recurs frequently, with or without treatment. Treatment is recommended for symptomatic women and generally involves a course of antibiotic therapy. Antibiotic treatment results in a high rate of remission of symptoms, but recurrences are common within the first year after treatment. Recurrent BV can have a substantial psychosocial impact on women, affecting sexual relationships and quality of life (ACOG, 2020; Koumans, 2007; Muzny, 2020; USPSTF, 2020; Workowski, 2021). The Society of Obstetricians and Gynaecologists of Canada reports that.

> Recurrence rates following treatment for bacterial vaginosis have been high in many studies, with up to one third of treated women recurring within 3 months, and with more women recurring the longer the length of follow-up. Before embarking on multiple courses of therapy, it is recommended to reconfirm the diagnosis (van Schalkwyk, 2015).

Diagnosis of BV

BV can be diagnosed using clinical criteria, point-of-care (POC) tests, laboratory tests and molecular assays. In the clinician’s office, if microscopy is available, the cause of BV can often be determined by utilizing the Amsel criteria. To fulfill the Amsel criteria, an individual must have at least three of the following:

- Homogeneous, thin, grayish-white discharge that smoothly covers the vaginal walls;
- Vaginal pH greater than 4.5;
- Positive whiff-amine test (the presence of a fishy odor when a drop of 10 percent potassium hydroxide (KOH) is added to a fresh sample of vaginal discharge);
- Clue cells on saline wet mount (ACOG, 2020; Amsel 1983).

Gram stain with Nugent scoring is conducted in the laboratory setting and is considered the gold-standard for the diagnosis of BV. The Nugent scoring system assigns a value to different bacterial morphotypes identified on Gram stain of vaginal secretions. Scores valued at 0–3 are interpreted as normal flora; scores ranging from 4–6 are intermediate flora; and scores reported from 7–10 are interpreted as bacterial vaginosis flora. If an intermediate score is obtained, then Amsel criteria are used to dispute or accept the diagnosis of BV (Nugent, 1991). The identification of clue cells on microscopy compares well with Gram stain findings and are the most reliable indicator of BV (ACOG, 2020; Powell, 2014; Workowski, 2021). According to the collaborative guidelines from the European International Union Against Sexually Transmitted Infections (IUSTI) and the World Health Organization (WHO), the Hay-Ison criteria is an alternative method of scoring based on the findings on a Gram-stained smear. The Hay-Ison criteria are quicker and easier to use in clinical practice and do include non-BV-associated bacteria. The Hay-Ison criteria classifies vaginal specimens as follows:

- Grade 0: Only epithelial cells present, no lactobacilli (indicates recent antibiotics);
- Grade 1: Lactobacillus morphotypes predominate, (normal flora);
- Grade 2: Mixed flora, Lactobacilli and Gardnerella or Mobiluncus morphotypes present (indeterminate);
- Grade 3: Predominantly Gardnerella and/or Mobiluncus morphotypes; few or absent Lactobacilli;
- Grade 4: Gram-positive cocci only, no lactobacilli (AV flora), not related to BV (Sherrard, 2018).

Although Gram staining using Nugent scoring is considered the diagnostic standard and demonstrated higher interobserver and intraobserver reproducibility than Amsel’s criteria, it is impractical (time consuming to perform), and its use is generally limited to the laboratory or research settings. Microscopy and the Amsel criteria are the preferred methods to diagnosis BV (ACOG, 2020; American Family Physician, 2018; Coleman, 2018). However, traditional microscopy-based diagnostics are declining in usage and are not always (or perhaps even infrequently) available in routine clinical practice. The CDC’s guidelines on Sexually Transmitted Infection (STIs) Treatment include NAATs as a diagnostic option “in settings where pH paper, KOH, and microscopy are unavailable” (Workowski 2021). The clinical value of nucleic acid amplification tests (NAATs) using algorithmic analysis to detect BV over standard

diagnostic testing (for example, Amsel criteria, Nugent score, and the Affirm VP III assay) has not been established.

Identifying the exact cause of vaginitis may also be impacted by coinfection (the identification of two or more vaginal pathogens and a potential pathogen may be present but may not be causing the existing vaginal symptoms). Sobel and colleagues evaluated the epidemiology of vaginitis in the presence of two or more potential vaginal pathogens and reported that pathogen coinfection often occurs in women with vaginitis. The authors estimated that "approximately 20%–30% of women with bacterial vaginosis (BV) are coinfected with Candida species. Coexistence of BV pathogens and *T. vaginalis* is even more common, with coinfection rates of 60%–80%" (Sobel, 2013; Schwebke, 2020).

Several professional, medical societies or governmental organizations have published guidelines on the diagnosis, treatment, and screening of BV. At the time of this review, no professional or medical societies recommend the use of NAAT to diagnose BV above standard diagnostic testing (Amsel clinical criteria or Gram stain with Nugent scoring).

In their discussion on DNA testing for the diagnosis of BV, the American Family Physician concluded:

> Some data show that newer laboratory tests such as DNA and antigen testing for bacterial vaginosis and vulvovaginal candidiasis, or vaginal fluid sialidase testing for bacterial vaginosis, may have similar or better sensitivity and specificity compared with office-based testing. However, more comparisons with diagnostic standard testing (i.e., Gram stain for bacterial vaginosis and culture for vulvovaginal candidiasis) are needed (American Family Physician, 2018).

The IUSTI/WHO Guidelines Group recommends that the current best method to diagnose BV in women is microscopy using the Hay-Ison Criteria (Sherrard, 2018).

The American College of Obstetricians and Gynecologists (ACOG) recommends that Amsel clinical criteria or Gram stain with Nugent scoring be used for the diagnosis of BV and clarifies the most appropriate setting for each:

> In research settings, Gram stain with Nugent scoring is considered the criterion standard for diagnosing bacterial vaginosis; however, it is impractical for most clinical practitioners and, therefore, Amsel criteria typically are used for the diagnosis of bacterial vaginosis. Overdiagnosis of bacterial vaginosis is common and clinical correlation is necessary to avoid overtreatment of a condition that is usually asymptomatic (ACOG, 2020).

ACOG also states:

> Polymerase chain reaction (PCR) has been used in research settings for the detection of G vaginalis as well as a variety of organisms associated with bacterial vaginosis; however, until recently, its use as a clinical diagnostic test for bacterial vaginosis was still investigational. An advanced single-swab panel test that combines multiplex PCR and DNA probe technology can diagnose bacterial vaginosis by determining the ratio of lactobacilli species ("good bacteria") to several bacterial vaginosis-associated bacterial species ("bad bacteria") in a patient-collected or physician-collected single-swab sample and has demonstrated comparable diagnostic sensitivity and specificity to Nugent scoring and Amsel criteria. This multiplex PCR panel also can detect other common causes of vaginitis, such as trichomoniasis and candidiasis. Although the clinical utility of PCR testing for the diagnosis of bacterial vaginosis is still being evaluated, this single-swab multiplex test may be a promising alternative to microscopy (ACOG, 2020).

In their guidelines on Sexually Transmitted Infections Treatment Guidelines, the Centers for Disease Control and Prevention (CDC) state:

> BV NAATs should be used among symptomatic women only (e.g., women with vaginal discharge, odor, or itch) because their accuracy is not well defined for asymptomatic women. 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" (Workowski 2021).

Clinicians routinely evaluate and treat individuals (both pregnant and nonpregnant) when they are symptomatic for BV. Treatment of BV is recommended for all symptomatic pregnant women because symptomatic BV has been linked to adverse pregnancy outcomes, including premature rupture of membranes, preterm birth, intra-amniotic infection, and postpartum endometritis. Recognized benefits of therapy among affected women are relief of vaginal symptoms and signs of infection. Other potential benefits of treatment include reduction in the risk for transmitting and acquiring N. gonorrhoeae, C. trachomatis, Trichomonas vaginalis (*T. vaginalis*), M. genitalium, HIV, HPV, and HSV-2 (ACOG, 2020; USPSTF, 2020; Workowski, 2021).

Determining the exact cause of vaginitis can be challenging. The diagnosis of vaginitis is made based on a combination of symptoms, physical findings, and office-based or laboratory testing. Because there is significant overlap in the symptoms associated with BV, VC, and Trichomoniasis, reliable differentiation of the causes of vaginitis is generally not possible based on symptoms alone. Historically, microscopy was considered a key diagnostic tool in determining the cause of vaginitis, but more recently, the use of traditional microscopy-based diagnostics is declining and may not be available in routine clinical practice. The identification of the exact cause of vaginitis can be complicated by pathogenic coinfection, frequently involving BV and VVC or BV and trichomoniasis. Additionally, recurrent or persistent BV is relatively common in treated individuals. Nucleic acid amplification testing using an algorithmic analysis may be considered an acceptable diagnostic option to identify the cause of vaginitis in symptomatic individuals with bacterial vaginosis-associated bacteria, Candida species, and/or Trichomonas vaginalis.

Screening Asymptomatic Pregnant Women at Risk for Preterm Labor/Delivery

With regards to testing for BV in asymptomatic pregnant women, multiple studies have reported an association between preterm labor/delivery and BV (Hillier, 1995; Laxmi, 2012; Koumans, 2001; Nelson, 2009). However, the cause of preterm delivery is likely multifactorial. According to USPSTF:

> Numerous risk factors are associated with an increased risk for preterm birth. History of a prior preterm delivery is associated with a 2.5-fold higher odds for preterm delivery in subsequent pregnancies. While bacterial vaginosis during pregnancy is associated with a 2-fold higher odds for preterm delivery, it is not clear that bacterial vaginosis is a cause of preterm delivery. Other additional risk factors for preterm delivery include, but are not limited to, cervical insufficiency, multifetal gestation, young or advanced maternal age, low maternal body mass index (<20, calculated as weight in kilograms divided by height in meters squared), genitourinary infections, HIV infection, and other maternal medical conditions.
>
> The association of these additional risk factors with preterm delivery is small to moderate, and factors can act in isolation or in combination. Preterm birth rates also vary by race/ethnicity in the US; recent data report preterm birth rates of 8.6% among Asian women, 11.8% among Native Hawaiian/Other Pacific Islander women, 9.7%

> among Hispanic women, 11.5% among American Indian/Alaska Native women, 14.1% among black women, and 9.1% among white women. Among women with a prior preterm delivery, the rate of recurrent preterm delivery in African American women is 4 times higher than the rate of recurrent preterm delivery in white women. Even when these risk factors are present, it is unclear whether screening and treating asymptomatic bacterial vaginosis in pregnant persons at increased risk for preterm delivery prevents preterm delivery (USPSTF, 2020).

At the time of this review, no professional medical organization was identified that supported routine screening for BV in asymptomatic pregnant persons (both those at high risk and those at low risk) to reduce the likelihood of preterm birth. In their 2021 guidelines, (Workowski, 2021), the CDC reported that the treatment of asymptomatic BV among pregnant women at high risk for preterm delivery has been evaluated by several studies and produced mixed results: one demonstrated harm (Odendaal, 2002), two reported no benefit (Carey, 2000, Vermeulen, 1999), and four revealed benefit (Hauth, 1995; Morales, 1994; McDonald, 1997; Ugwumadu, 2003). In a similar fashion, the USPSTF recommends against screening for BV in pregnant persons who are not at increased risk for preterm delivery (pregnant persons with no history of previous preterm delivery or other risk factors for preterm delivery). Additionally, the USPSTF concluded that the current evidence is insufficient to assess the balance of benefits and harms of screening for BV in pregnant persons who are at increased risk for preterm delivery (USPSTF, 2020).

Although the routine screening and treating of asymptomatic pregnant women for BV remains controversial because the available data do not show a consistent benefit to this approach, screening for BV in asymptomatic pregnant individuals who have a history of a previous preterm delivery may result in improved health outcomes for this population of individuals. NAAT provides the most sensitive and medically appropriate modality for BV screening in this population, especially when standard diagnostic testing is not available, or the results of the latter are indeterminate.

Vulvovaginal Candidiasis

Candida is a normal vaginal flora, however, when it overgrows and penetrates the superficial epithelial cells, vulvovaginitis occurs. VVC signifies inflammation and infection of the vagina with Candida albicans or some other Candida species. Some of the most common symptoms of uncomplicated VVC include intense vulvar itching, burning and soreness, dysuria, and dyspareunia. The vulva and vagina may appear erythematous. Excoriation and fissures in the vulvar region may also be present. Frequently there is little or no vaginal discharge; and when present, it is classically white, thick, sticky, and clumpy (curd) with no or minimal odor. Distinguishing vaginal from vulvar symptoms is important to direct evaluation and provide appropriate treatment (ACOG, 2020).

VVC accounts for approximately one-third of vaginitis cases and is the second most common cause of vaginitis symptoms. It has been estimated that 75% of women will have at least one episode of VVC, and 40%–45% will have two or more episodes. VVC is the second most common cause of vaginitis with 29–49% of females reporting at least one lifetime episode (ACOG, 2020; Workowski, 2021).

Based on clinical presentation, microbiology, host factors, and response to therapy, VVC can be categorized as either uncomplicated VVC and complicated VVC. Uncomplicated VVC includes all the following criteria:

- Infrequent, sporadic episodes (3 or fewer episodes per/year);
- Mild to moderate signs and symptoms;
- Likely infection with Candida albicans;
- Healthy, nonpregnant individual;
- Individual is not immunocompromised.

Characteristics of complicated VVC infections include one or more of the following criteria:

- Severe signs and symptoms; or
- Species other than C. albicans, especially C. glabrata; or
- Immunosuppression, debilitation, pregnancy, poorly controlled diabetes mellitus; or
- History of recurrent (three or more per year) culture-verified VVC.

VVC is treated based on the clinical presentation, microbiology, host factors, and response to therapy. Asymptomatic individuals do not require treatment. Treatment of uncomplicated VVC is indicated to relieve symptoms and typically includes oral and topical antimycotic drugs. Individuals with complicated VVC are generally treated with oral and topical antimycotic drugs but may require higher doses of medication and longer courses of therapy.

Diagnosis of Vulvovaginal Candidiasis

The diagnosis of VVC is based upon the presence of Candida on wet mount (preferred), Gram stain, culture, or molecular testing of vaginal discharge in a woman with characteristic clinical findings (for example, vulvovaginal pruritus, erythema, burning, edema, and/or curd-like discharge on the vaginal wall) and no other pathogens to account for her symptoms. Importantly, although vulvar pruritus is a cardinal symptom of the VVC, less than 50 percent of women with genital pruritus have the disorder.

Several organizations have published guidelines on the diagnosis of VVC. According to ACOG, VVC “cannot be reliably diagnosed based on clinical symptoms alone”. In symptomatic individuals, the diagnosis of VVC requires either of the following two findings:

- Visualization of spores, pseudohyphae, or hyphae on wet-mount microscopy; or
- Vaginal fungal culture or commercial diagnostic test results positive for Candida species (ACOG, 2020).

While Gram stains and KOH preps reveal budding yeasts, Candida glabrata does not form hyphae and may thereby escape microscopic detection. Culture is not necessary if microscopy reveals yeast, but should be obtained in:

- Individuals with clinical features of VVC, who have normal vaginal pH and negative microscopy;
- Individuals with complicated disease (persistent or recurrent symptoms) because these women may have a nonalbicans strain of Candida that is resistant to azoles (Workowski, 2021).

According to IUSTI/WHO, the diagnosis of candidiasis is based on clinical symptoms and signs supported by laboratory test findings. The IUSTI/WHO Guidelines Group concluded that, microscopy is currently the best test to diagnose candida (strength of recommendation: Grade 1, quality of evidence: Grade B) (Sherrard, 2018).

In their discussion of an NAAT panel with algorithm assay that can be used to detect BV, trichomoniasis and candidiasis (Gaydos, 2017), ACOG concluded:

> Polymerase chain reaction testing for Candida species offers results within a few hours compared with culture and has comparable sensitivity and specificity (97.7% and 93.2%, respectively). However, these PCR tests often are considerably more expensive than fungal culture (ACOG, 2020).

Trichomonas Vaginalis

Trichomoniasis is a genitourinary infection caused by the protozoan parasite *Trichomonas vaginalis (T. vaginalis)* and is one of the three common infectious causes of vaginal complaints among reproductive-aged females in the United States. More than 50% of individuals with trichomoniasis are asymptomatic or have minimal symptoms. A symptomatic woman with trichomoniasis may report an abnormal (yellow-to-green frothy) vaginal discharge, abnormal vaginal odor, burning, pruritus, irritation, dysuria or postcoital bleeding. Men with trichomoniasis may have symptoms of prostatitis, urethritis, or epididymitis. Although individuals may be unaware of their infection, it is readily passed between sex partners during penile-vaginal sex or through transmission of infected vaginal fluids or fomites among women who have sex with women. Coinfection of BV and *T. vaginalis* is common with rates of coinfection ranging from 20-60, and sometimes as high as 80%. Trichomoniasis causes reproductive morbidity and has been reported to be associated with a 1.4-times greater likelihood of preterm birth, premature rupture of membranes, and infants who are small for gestational age. In addition, the infection also increases the risk of transmission of human immunodeficiency virus (HIV) in both men and women (ACOG, 2020; Gaydos, 2017; Sherrard, 2018; Sobel, 2013; Stat Pearls, 2022; Workowski, 2021).

Prevalence disparities have been observed with this vaginal condition. African American women are 10 times more frequently affected compared with non-Hispanic white women. Other risk factors associated with Trichomoniasis include increased number of sex partners, low socioeconomic status, and treatment of Trichomonas vaginalis infections typically involve a course of antiprotozoal-antibacterial therapy douching (ACOG, 2020; Sherrard, 2018; Workowski, 2021).

Diagnosis of Trichomonas Vaginalis

Tests to diagnose Trichomonas vaginalis include vaginal pH, wet mount microscopy, culture and NAATs. Prior to the introduction of molecular detection methods, Trichomonas culture was considered the most sensitive and the preferred method for detecting Trichomonas vaginalis in individuals. However, culture is inconvenient, takes at least 5 days to grow in culture samples, and often requires preemptive discussion with a local microbiology laboratory with special media (ACOG, 2020). NAATs are preferred for diagnosing Trichomonas vaginalis infection due to the high sensitivity and specificity (both approaching 100 percent) compared with wet mount microscopy or culture. NAATs may be used selectively for those with concerning symptoms and/or vaginal discharge but negative microscopy results. Limitations of NAATs when compared with microscopy, include longer turn-around time and higher cost. Culture is not as sensitive as NAATs but may be performed if neither NAATs nor microscopy are available (Sherrard, 2018; Workowski, 2021). Wet mount microscopy and vaginal pH testing are only applicable to vaginal discharge and is not performed on anal or urethral specimens.

Several professional, medical societies or governmental organizations provide guidance on the use of NAATs as the preferred diagnostic test to detect *T. vaginalis*.

The IUSTI/WHO, indicate that NAATs provide the highest sensitivity for the detection of Trichomonas vaginalis in comparison to both microscopy and culture. The IUSTI/WHO Guidelines Group consider NAATs to be the best tests to diagnose Trichomonas vaginalis in women and suggests that they be the test of choice where resources allow (Sherrard, 2018).

The collaborative guidelines released by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM), indicate that “NAATs “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” (Miller, 2018).

ACOG has issued a Level A recommendation (based on good and consistent scientific evidence) in support of the use of NAAT for the diagnosis of trichomoniasis. In their discussion of diagnostic testing for Trichomonas vaginalis, ACOG states the following:

> Nucleic acid amplification testing (NAAT) is recommended for the diagnosis of trichomoniasis. Nucleic acid amplification testing is highly sensitive compared with microscopy and is the recommended diagnostic method for trichomoniasis. Nucleic acid amplification testing can be performed on vaginal, cervical, or urine specimens with equal sensitivity (95.3–100%) and specificity (95.2–100%) (ACOG, 2020).

In a similar manner, the CDC considers NAAT to be the preferred diagnostic method to detect*T. vaginalis*. According to the CDC:

> “Wet-mount microscopy traditionally has been used as the preferred diagnostic test for T. vaginalis among women because it is inexpensive and can be performed at the POC; however, it has low sensitivity (44%–68%) compared with culture… NAATs are highly sensitive, detecting more T. vaginalis infections than wet-mount microscopy among women” (Workowski, 2021)

Because most strains of *T. vaginalis* are highly susceptible to them, treatment of Trichomonas vaginalis may involve a course of the 5-nitroimidazole agents (metronidazole, tinidazole, or secnidazole).

Nucleic Acid Amplification Tests (NAATs) Using Algorithmic Analysis

Nucleic acid amplification tests (NAATs), such as polymerase chain reaction (PCR) are being investigated as an alternative means to detect of Gardnerella vaginalis as well as a variety of organisms associated with BV in the clinical setting and when microscopy is unavailable. The limit of detection (LOD) of a NAAT ranges from 10 organisms to $3x10^4$ organisms per ml, depending on the target being examined. These assays are based on identification of specific bacterial nucleic acids and have high sensitivity and specificity for BV (i.e., G. vaginalis, BVAB2, A. vaginae, or Megasphaera type 1) and certain lactobacilli (i.e, Lactobacillus crispatus, Lactobacillus gasseri and Lactobacillus jensenii). Because DNA amplification can be observed in real-time, the need for postamplification analysis is eliminated and chances for sample contamination are diminished. These tests can be performed on either clinician- or self-collected vaginal specimens with results available in less than 24 hours, depending on the availability of the molecular diagnostic platform (Coleman, 2018; Workowski, 2021).

Several CLIA-certified laboratories provide PCR assays including, but not limited to NAATs, to identify bacteria associated with BV. However, at the time of this review, at least three NAATs that employ algorithmic analysis for the diagnosis of BV in symptomatic women had received marketing clearance from the United States Food and Drug Administration (FDA): Aptima® BV Assay, BD Max™ Vaginal Panel, and the Cepheid Xpert® Xpress MVP.

Aptima BV Assay

The Aptima BV assay (Hologic, San Diego CA) is an in vitro NAAT that utilizes real time transcription-mediated amplification (TMA) for identification and quantitation of ribosomal RNA from bacteria associated with BV, including Lactobacillus (L. gasseri, L. crispatus, and L. jensenii), Gardnerella vaginalis, and Atopobium vaginae. The assay provides a qualitative result for BV and does not report results for individual organisms. The assay is intended to aid in the diagnosis of BV on the automated Panther system using patient-collected or clinician-collected vaginal swab specimens from females with a clinical presentation consistent with vaginitis and/or vaginosis. The Aptima BV Assay was cleared for marketing by the U.S. Food and Drug Administration (K190452) with the BD Max as

the predicate device. The Aptima BV assay reported sensitivity and specificity ranging from 95.0% to 97.3% and 85.8% to 89.6%, respectively (using either clinician-collected or patient-collected vaginal swabs) (US FDA 510[K]a); Schwebke 2020).

BD Max Vaginal Panel

In October 2016, the FDA granted class II designation and marketing authorization for the BD Max Vaginal Panel (Becton, Dickinson, Sparks, MD). The BD MAX Vaginal Panel is carried out on the BD MAX system (a bench-top molecular diagnostics workstation). The panel is an automated assay that utilizes real-time PCR for the amplification of specific DNA targets from bacteria associated with BV including Lactobacillus (L. crispatus, and L. jensenii), Lactobacillus (L. gasseri, L. crispatus, and L. jensenii), Atopobium vaginae, Bacterial Vaginosis Associated Bacteria-2 (BVAB-2), Megasphaera-1, Candida (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis), Candida glabrata, Candida krusei, and Trichomonas vaginalis. The panel reports a positive or negative result for BV based on a quantitative algorithm that ascertains the ratio of vaginal bacteria (i.e., the assay detects BV as a syndrome, rather than identification based on the presence or absence of specific bacterial species alone). The BD MAX received FDA marketing clearance (K191957) for the diagnosis of vaginitis in symptomatic women. According to information provided in the FDA Substantial Equivalence Determination Decision Summary for the BD MAX Vaginal Panel, when compared to the reference of a combined Nugent score and Amsel's criteria, the test demonstrated 90.5% sensitivity (95% confidence interval [CI], 88.3% to 92.2%), 85.8% specificity (95% CI, 83% to 88.3%), 89% PPV (95% CI, 87.1 to 90.7), and 87.7% NPV (95% CI, 85.4 to 89.8) for BV (US FDA 510[K]b).

Cepheid Xpert Xpress MVP

According to the FDA Substantial Equivalence Determination Decision Summary (K212213), the Xpert Xpress MVP (Cepheid, Sunnyvale, CA) test is to be used to aid in the diagnosis of vaginal infections in women with a clinical presentation consistent with bacterial vaginosis, trichomoniasis or vulvovaginal candidiasis. The Xpert Xpress MVP test is conducted using the GeneXpert Instrument Systems, an automated qualitative in vitro diagnostic test for the identification of DNA targets from anaerobic bacteria associated with BV, Candida species associated with vulvovaginal candidiasis, and T. vaginalis. The Xpert Xpress MVP test utilizes clinician-collected and self-collected vaginal swabs (collected in a clinical setting) from patients who exhibiting symptoms of vaginitis/vaginosis. The Xpert Xpress MVP test uses real-PCR for the amplification of specific DNA targets and utilizes fluorogenic target-specific hybridization probes to detect and differentiate DNA from the following:

- Organisms associated with bacterial vaginosis (detected organisms not reported individually)
    - Atopobium spp. (Atopobium vaginae, Atopobium novel species CCUG 55226)
    - Bacterial Vaginosis-Associated Bacterium 2 (BVAB2)
    - Megasphaera-1
- Candida spp. (C. albicans, C. tropicalis, C. parapsilosis, C. dubliniensis, species not differentiated)
- Candida glabrata/Candida krusei (species not differentiated)
- Trichomonas vaginalis (FDA 510[K]c).

The Xpert Xpress MVP is considered substantially equivalent to the predicate device (BD MAX Vaginal Panel).

Summary

Vaginitis is an infection or inflammation of the vagina which is typically accompanied by symptoms of abnormal vaginal discharge, odor, irritation, burning or pruritus. The most common causes of vaginitis are BV, VVC, and trichomoniasis.

Untreated and inappropriately treated vaginitis can result in negative health outcomes. As examples, untreated BV can result in early miscarriage, premature rupture of membranes, preterm labor, preterm delivery, and postpartum complications such as endometritis and wound infections. Untreated trichomoniasis is associated with adverse pregnancy outcomes and increases the risk of HIV transmission. Misdiagnosed vaginitis may also cause the patient to continue to experience vaginal symptoms, make multiple visits to the healthcare provider for treatment of persistent symptoms, and undergo ineffective courses of treatment which may then result in secondary vaginal infections caused by the inappropriate use of antibiotics.

Determining the exact cause of vaginitis can be challenging. The diagnosis of vaginitis is made based on a combination of symptoms, physical findings, and office-based or laboratory testing. Because there is significant overlap in the symptoms associated with BV, VC, and Trichomoniasis, reliable differentiation of the causes of vaginitis is generally not possible based on symptoms alone. Historically, microscopy was considered a key diagnostic tool in determining the cause of vaginitis, but more recently, the use of traditional microscopy-based diagnostics is declining and may not be available in routine clinical practice. Additionally, the identification of the cause of vaginitis can be complicated by pathogen coinfection, for the most part involving BV and VVC as well as BV and trichomoniasis. Recurrent or persistent BV is not uncommon in treated individuals.

Several peer-reviewed studies have reported an association between preterm labor/delivery and BV. However, routine screening of asymptomatic pregnant women for BV remains controversial because the available data do not consistently demonstrate benefits with this approach. However, there may be benefits to early screening for BV in asymptomatic pregnant individuals who have a history of a previous preterm delivery. NAAT provides the most sensitive and medically appropriate modality for BV screening in this population, especially when standard diagnostic testing is not available, or the results of the latter are indeterminate. Identifying and characterizing the specific features of the subgroup of individuals who might respond favorably to screening protocols is an active area of investigation.

The diagnosis of VVC is typically made based on visualization of spores, pseudohyphae, or hyphae on wet-mount microscopy or vaginal fungal culture or commercial diagnostic test results positive for Candida species. However, candida glabrata does not form hyphae and may be undetectable on microscopic examination. Culture is not done if microscopy reveals yeast but is typically obtained in individuals with clinical features of VVC, who have normal vaginal pH and negative microscopy and in individuals with complicated disease (persistent or recurrent symptoms) because these individuals may have a nonalbicans strain of Candida that is resistant to treatment with azoles. NAATs may be a diagnostic option to identify other Candida species (including but not limited to nonalbicans Candida) in symptomatic individuals.

NAATs are considered the first-tier diagnostic test for Trichomonas.

For the reasons discussed above, nucleic acid amplification testing using an algorithmic analysis may be considered an acceptable diagnostic option to identify the cause of vaginitis (bacterial vaginosis-associated bacteria, Trichomonas vaginalis and/or Candida species) in symptomatic individuals and in asymptomatic pregnant women with a history of preterm birth.

Definitions

Morphotype: A group of bacterial strains within a single species that can be distinguished from other such strains because of morphological characteristics.

Vaginitis coinfection: The presence of at least two distinct vaginal pathogens, but the pathogens may or may not be the cause of the vaginitis symptoms.

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Index

The use of specific product names is illustrative only. It is not intended to be a recommendation of one product over another, and is not intended to represent a complete listing of all products available.

History

Status	Date	Action
	12/06/2023	Revised References section.
Revised	05/11/2023	Medical Policy & Technology Assessment Committee (MPTAC) review. Title of document changed to “Nucleic Acid Amplification Tests Using Algorithmic Analysis for the Diagnosis of Vaginitis”. Expanded scope of document to address vaginitis (including bacterial vaginosis-associated bacteria, Trichomonas vaginalis and/or Candida species). Revised criteria to consider NAAT using algorithmic analysis for the dx of vaginitis is MN in symptomatic individuals and in pregnant individuals who are asymptomatic and have a history of preterm birth. Updated Discussion, Definitions, Coding, References and History sections.
New	08/11/2022	MPTAC review. Initial document development.
