Bacterial Vaginosis
(With Lactobacillus Profiling) qPCR Panel

The vaginal microflora is a dynamic ecosystem normally inhabited by lactobacilli. These bacteria support healthy vaginal conditions by maintaining an acidic environment that is inhospitable to other pathogenic microorganisms. *L. crispatus*, *L. gasseri*, *L. jensenii*, and *L. iners* are the four major vaginal *Lactobacillus* species[1]. Usually, the vaginal flora is dominated by one of these bacteria accompanied by less abundant and less frequently detected minor *Lactobacillus* species, including *L. acidophilus*, *L. johnsoni*, *L. vaginalis*, *L. fermentum*, and *L. reuteri* [2]. The numerical prevalence of lactobacilli in the vagina prevents its colonization by other pathogens. Many important aspects of women's sexual and reproductive health rely on the protective role of lactobacilli in the vaginal environment. *Lactobacillus* species protect the vagina and maintain microbial homeostasis through three main mechanisms. Firstly, they form biofilms that cover the epithelial cell receptors to prevent pathogenic microbes from binding. Secondly, they produce antimicrobial compounds, hydrogen peroxide, lactic acid, and bacteriocins, that suppress cell growth. Lastly, their co-accumulation with other pathogens increases epithelial barrier function, which can trigger innate immunity[3].

However, the composition of the vaginal microflora is not static but changes over time in response to various endogenous and exogenous influences. Bacterial vaginosis (BV) results from vaginal dysbiosis caused by the depletion of the normal *Lactobacillus* species and their replacement with high concentrations of anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, BV-associated bacteria (BVAB), Megasphaera, Prevotella, Mobiluncus, Mycoplasma, and *Ureaplasma* species, etc.[4–7] The exact mechanism(s) underlying the loss/reduction of the beneficial *Lactobacillus* species (notably *L. crispatus*, *L. jensenii*, *L. gasseri*) and permitting the accumulation of the pathogenic microbes in BV is still not fully understood, and neither is the implication of a single causative sexually transmitted pathogen known. However, BV is highly prevalent among women of reproductive age and is the most common condition in the vaginal microflora.[6,8] BV increases the risk of contracting HIV and other sexually transmitted infections (STIs) and pelvic inflammatory disease (PID).[9] It has been linked to the development of adverse pregnancy outcomes, including premature rupture of membranes, preterm birth, intra-amniotic infection, and postpartum endometritis in pregnant women.[10] Sexually active individuals, particularly those with multiple sexual partners and those having sexual intercourse with women only or with both women and men without protection, are at risk of developing BV. Other risk factors include vaginal douching, HSV-2 seropositivity, use of antibiotics, and hormonal fluctuations.[8]

As illustrated in Figure 1, BV is associated with an increase in vaginal pH from a healthy range (3.8 – 4.2) to > 4.5 in which an overgrowth of *Gardnerella vaginalis* (A), a modest increase in facultative anaerobes (B) and the depletion of lactobacilli (C) are observed. A dramatic increase of the obligate anaerobic bacteria (D) such as *Atopobium vaginae*, *Megasphaera species*, and BVAB2 is also observed during BV and is an important indicator of organisms associated with the BV state.[11] Muzny and colleagues recently showed that *Gardnerella vaginalis* initiates the pathogenesis of BV as an early colonizer, followed by *Prevotella bivia*.[12] However, these two species do not generate any inflammatory response. Secondary colonizers such as Sneathia *spp*., BVAB, and *Atopobium vaginae* are secondary colonizers that are more potent stimulators of the host immune response to BV. These late colonizers cause the symptoms of BV.[12] The classic clinical signs and symptoms of BV include:

- Increase in vaginal pH, usually >4.5
- Vaginal discharge, which can be thin, watery, white/grey
- Vaginal odor, which can be strong and fish-like, particularly after sex
- Vaginal itching or irritation
- Pain or burning sensation while urinating

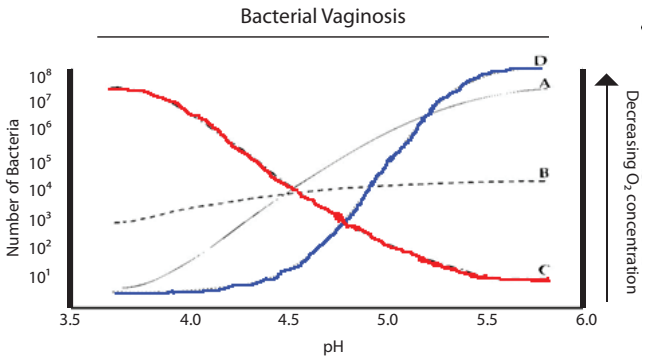


Figure 1. Key Changes in the vaginal microflora due to the development of bacterial vaginosis.[11]

Epidemiology

BV is the most common cause of abnormal vagina discharge in patients of reproductive age, with an estimated prevalence rate of 29% in reproductive women in North America.[6,8] This prevalence rate may be understated since it is based only on symptomatic women who seek gynecological care. While most BV patients are asymptomatic, the condition has been associated with the development or progression of common obstetric and gynecologic infectious complications worldwide. An estimated 10% - 30% of pregnant women in the US have BV, which, if left untreated, can increase the risk of complications, such as preterm birth and low birth weight.[10,13] It also occurs more frequently in Black, Hispanic, and Mexican American women than their white non-Hispanic counterparts.[11] The economic impact of BV is on the rise globally and is almost three times the economic costs in the US if BV-associated preterm births and HIV cases were included.[14] There is an ongoing debate over the classification of BV as a traditional STI. Many recent epidemiological and microbiological data seem to favor its inclusion, suggesting the central role of sexual transmission in its pathogenesis. The unacceptably high rates of recurrence that can probably be linked to re-infections from sexual partners also strongly support the condition being accorded the STI status.[15,16] Besides, BV may not just be important to women; the condition has also been linked to the development of *Gardnerella vaginalis* urinary tract infections in men.[17]

Pathogenesis

The exact underlying cause and mechanisms of BV pathogenesis are still poorly understood as multiple factors involving the intricate interplay between the vaginal microbiota, host immune responses, and external factors which may be responsible for its development.[4] It is also a multi-pathogenic condition, which makes it more complex to investigate. Nevertheless, the key phenomena in BV are the depletion of lactobacilli from the vaginal flora and the consequent overgrowth of Gram-negative and facultative anaerobes. The development of culture-independent molecular diagnostics has greatly increased the number of bacterial species associated with BV including *Gardnerella vaginalis*, *Atopobium vaginae*, *Prevotella bivia*, BVAB, *Megasphaera*, *Mobiluncus*, *Mycoplasma* species, *Sneathia sanguinegens*, *Streptococcus anginosus*, *Ureaplasma urealyticum*, *Bifidobacterium breve*, and *Bacteriodes fragilis*.[2,4,18–21] These bacteria thrive in environments with reduced oxygen levels and can produce substances such as amines, including trimethylamine, putrescine, and cadaverine, which contribute to the characteristic fishy odor in BV. The production of biofilms by these anaerobic bacteria (notably *G. vaginalis*) enables them to strongly adhere to the vaginal epithelium and evade the host immune responses, as well as enhance their survival and persistence. Biofilms have been purported to be instrumental in the high rate of BV recurrence in reproductive women.[15] BV is also associated with elevated pro-inflammatory cytokines e.g., interleukin (IL)-1β, IL-6), tumor necrosis factor-alpha (TNF-α), etc., and an altered immune response in the vaginal tissues that are induced by the overgrowth of pathogens and their metabolic byproducts.[22] The precise mechanism underlying the depletion of lactobacilli and the subsequent replacement by BV-associated bacteria during BV is also still unknown. One possibility is that a change in vaginal pH is the cause of BV. Lactobacillus produces lactic acid, which maintains the vaginal pH between 3.8 and 4.2. This environment favors the growth of Lactobacillus and inhibits the growth of pathogenic bacteria. BV risk factors include douching, menstruation, and unprotected sexual contact, all of which raise the vaginal pH above the optimum for Lactobacillus growth.[8] As microorganisms associated with BV are shown to have their natural habitat in the gastrointestinal tract, BV might be an endogenous infection.[23]

Metabolic signatures, through the application of multi-platform metabolomic approaches, have been utilized to better understand the biochemical disturbances in the vaginal microflora resulting from BV. The nature of the temporal vaginal microbial load largely determines the composition of the vaginal metabolites. The levels of the estrogen hormone, which controls the glycogen-rich vaginal ecosystem, must be maintained to sustain the normal healthy lactobacilli-dominated state.[2] *Lactobacillus* species trigger the fermentation of glycogen to produce organic acids (majorly lactic acid) that ensures the protective acidic pH of the vagina. The lactic acid bacteria presence in the vagina has been associated with an increased proportion of maltose and kynurenine, L-tryptophan catabolic products, and the accumulation of nicotinamide adenine dinucleotide (NAD+).2,24 The replacement of healthy vaginal lactobacilli by an overgrowth of anaerobic pathogens results in the anaerobic fermentation of glycogen to form metabolites such as short-chain fatty acid (SCFA), organic acids like acetate, malonate, propionate, butyrate, etc., which in turn can activate the host's pro-inflammatory cytokines and inflammation in the vagina.[25,26] The anaerobic bacteria are also responsible for the decarboxylation of the host's vaginal proteins to polyamines which increases the pH and develops the characteristic 'fishy' odor seen in BV.

Characteristics of microorganisms associated with normal, transitional, and abnormal vaginal microflora (BV)

The exact pathogenic mechanisms of BV remain a subject of scientific debate; however, it has been established that the condition is multi-pathogenic, involving multifactorial etiology. The occurrence of normal, transitional, and abnormal vaginal microflora (BV) at any given time is

dependent on the composition of the lactobacilli species and the Gram-negative and facultative anaerobes. In this section, the key contributions of *Lactobacillus crispatus*, *L. gasseri*, *L. jensenii*, *L. iners*, *L. acidophilus*, *Gardnerella vaginalis*, *Atopium vaginae*, *Prevotella bivia*, BVAB 1-3, Megasphaera, Mobiluncus, *Mycoplasma* species, *Sneathia sanguinegens*, *Streptococcus anginosus*, *Ureaplasma urealyticum*, *Bifidobacterium breve*, *Bacteriodes fragilis* to the vaginal microflora states are summarized.

***Lactobacillus* species** – *Lactobacillus crispatus*, *L. gasseri*, and *L. jensenii* are Gram-positive, rod-shaped, facultative anaerobic non-spore-forming bacteria and common members of healthy vaginal bacterial microflora. Along with *L. iners*, they are classified as the four major vaginal *Lactobacillus* species. *L. crispatus*, *L. gasseri*, and *L. jensenii* promote a healthy vaginal microenvironment by supporting an acidic pH, producing hydrogen peroxide, and preventing colonization by other microbial pathogens.[1,27] Vaginal microflora dominated by any of these species is considered normal. The amount of *L. crispatus*, *L. gasseri*, and/or *L. jensenii* bacteria is reduced in certain vaginal conditions, including BV. A negative association between the presence of these *Lactobacillus* species and BV has been frequently demonstrated.[2] *L. acidophilus*, though less common, also produces lactic acid and bacteriocins through the fermentation of sugars, which helps to maintain an acidic environment in the vagina, inhibiting pathogenic overgrowth.[28] The Gram-positive, rod-shaped, facultative anaerobe is a major component of many probiotics explored as a potential therapeutic approach for BV.[29,30]

Lactobacillus iners is a rod-shaped facultative anaerobic, non-spore-forming Gram-positive bacteria. It is a common member of the human-associated bacterial microflora. Physiologically, *L. iners* is different from other vaginal lactobacilli as it is less prone to hydrogen peroxide production and is the most fastidious microorganism compared with the other *Lactobacillus* species.[31] *L. iners* can be detected in both healthy and disturbed vaginal microflora, including BV.[31,32] *L. iners'* dominance, along with the depletion of other *Lactobacillus* species, indicates that the vaginal microflora may be in a transitional stage or intermediate state between abnormal and normal.[2] The medical relevance of transitional or intermediate vaginal microflora is currently an issue of debate in the literature.

Gardnerella vaginalis is a Gram-variable facultative anaerobic bacterium and was one of the first organisms to be associated with BV. The abilities of *G. vaginalis* to form a biofilm and produce prolidase, sialidase, β-galactosidase, and vaginolysin may play a role in the pathogenesis of this condition.[33–35] In recent years, the application of culture-independent techniques has revealed the ubiquitous nature of *G. vaginalis*. Owing to the common occurrence of this microorganism in healthy women, the role of *G. vaginalis* as a BV diagnostic marker has been challenged.[5,19,36–38] Even though the presence of *G. vaginalis* bacteria in the vaginal milieu signifies an occurring disturbance, the concurrent detection of *G. vaginalis* with other BV-associated microorganisms is more indicative of BV.[5,36,38]

Atopobium vaginae (now called *Fannyhessea vaginae*) is a Gram-positive anaerobic bacterium that has more recently become associated with BV. Like *G. vaginalis*, the presence of the organism at a high concentration is highly sensitive and specific for the diagnosis of BV.[5,36,39] In addition, the presence of *G. vaginalis* and *A. vaginae* together is associated with disease recurrence.[34,40,41] Some *A. vaginae* isolates exhibit reduced susceptibility to metronidazole *in vitro*, which could be a contributing factor to disease recurrence.[42,43] *A. vaginae* triggers an inflammatory response from vaginal epithelial cells, which may contribute to the pathogenesis of BV.[12,44]

***Megasphaera* species** are Gram-negative, fastidious, anaerobic organisms that cannot be cultivated in the laboratory. A recent study has identified 16S rDNA present in BV patient samples as belonging to a *Megasphaera* species that has not yet been cultured.[19] Detection of *Megasphaera* species DNA provides high sensitivity and specificity compared to Amsel criteria and Nugent score.[45,46] Successful antibiotic treatment of BV reduces the vaginal concentration of *Megasphaera* species[47], and the persistence of this organism is associated with chronic BV.[48] From our analyses, *Megasphaera* species type 1 and type 2 also contain several distinct strains that are phylogenetically distinct from each other suggesting that the actual *Megasphaera* species involved in BV are yet unknown.

Bacterial Vaginosis – Associated Bacterium (BVAB)s were identified through the molecular characterization of 16S rDNA sequences of the vaginal flora of women suffering from BV, which revealed three uncultured species belonging to the order Clostridiales associated with the disease, named BVAB 1, 2, and 3.[49,50] The role of these BVABs and the detection of their 16S rDNA sequences provided high sensitivity and specificity for the diagnosis of BV[47] similar to *Megasphaera* species. In addition, the 3- to 4-log reductions in median bacterial loads of BVAB 1-3 by antibiotic (metronidazole) therapy are associated with disease resolution[47], and their persistence was associated with chronic disease.[48] BVAB 1, 2, and 3 are not closely related as initially thought. A recent phylogenetic analysis identified them to belong to different species with wider evolutionary distances.[51] The actual species of BVAB 1, 2, and 3 were found to be *Clostridiales* genomosp. BVAB 1, *Oscillospiraceae* bacterium strain CHIC02 (BVAB 2), and *Mageeibacillus indolicus* (BVAB 3), respectively. A positive association between BV and prevalent high-risk HPV genotypes has been reported due to the occurrence of BVAB 1 & 3 and other BV-associated- bacteria in

women co-infected with HIV and HPV.[52] The same study also showed that the presence of BVAB 1 & 3 had an elevated likelihood of increasing the severity of cervical neoplasia in this population. We recently reported that the relative abundance and concentrations of BVAB 1 & 3 are more than that of BVAB 2 in a cohort of 946 vaginal swabs from a heterogenous population of adult women tested for BV.[53]

Prevotella bivia is an unpigmented Gram-negative bacterium that is a member of the commensal flora in humans, mostly in the vaginal mucosa. Its growth and pathogenicity are favored by an excess of estrogens or the synergistic cooperation of other aerobic microorganisms. *P. bivia*, which thrives in the vaginal tract, is found in high concentrations in women with BV and is associated symbiotically with BV, PID, and perianal abscesses.[54] *P. bivia* has been strongly associated with *G. vaginalis* in BV, with the former being shown to quickly follow the latter during the colonization of the vaginal mucosa during BV pathogenesis. Machado *et al.*,[55] similarly noted a symbiotic relationship between *G. vaginalis* and *P. bivia*, demonstrating that the presence of a *G. vaginalis* biofilm stimulates the growth of *P. bivia in vitro*. Notably, *P. bivia* was the first BV-associated species to increase above baseline before incident BV in daily swab samples, consistent with its potential role as a driver of this condition.[12,56,57]

***Mobiluncus* species** are curved, anaerobic bacteria usually isolated from the vagina of women with BV. *Mobilincus* species are motile, rod-shaped bacteria that have multiple subpolar flagella and multilayered Gram-variable cell walls, which produce succinate and acetate, are stimulated by rabbit serum, and have guanine-plus-cytosine contents of 49 to 52 mol%. *Mobiluncus curtisii* strains are small (length, 1.7 µm) and gram variable, are stimulated by arginine, and produce ornithine, citrulline, and ammonia from arginine. Further research has suggested that although it is mainly associated with genital infections, it may be a colonizer of the gastrointestinal tract.[58] Most isolates of *Mobiluncus curtisii* are resistant to metronidazole and its hydroxy metabolite, while *Mobiluncus mulieris* is often sensitive.

Streptococcus anginosus is one of three species (including *Streptococcus constellatus* and *Streptococcus intermedius*) forming the Streptococcus anginosus group (SAG) or the *S. milleri* group. *Streptococcus anginosus* may be beta-hemolytic or nonhemolytic and is part of the human bacteria flora but can cause diseases, including brain and liver abscesses, under certain circumstances. They are Gram-positive, catalase-negative facultative anaerobic cocci that form small colonies on agar media. It is a denizen of a wide variety of sites inside the human body: the mouth, sinuses, throat, feces, and vagina, yielding both hemolytic (mouth) and nonhemolytic (fecal and vaginal) strains.[59,60] Most *S. milleri* strains are resistant to bacitracin and nitrofurazone, and sulfonamides are ineffective.[61] Besides BV, *Streptococcus anginosus* is also implicated in aerobic vaginitis (AV), a dysbiosis of the vaginal microbiota caused by aerobic bacterial pathogens such as *Escherichia coli*, Group B Streptococcus, *Staphylococcus aureus*, and *Enterococcus faecalis*.[62]

Sneathia sanguinensis. *Sneathia* is a genus of Gram-negative, rod-shaped, anaerobic, non-motile bacteria recently identified as an important contributor to common obstetric, neonatal, and gynecologic pathologies. Although scarce, emerging data suggest that vaginally residing *Sneathia* becomes pathogenic following its ascension into the upper urogenital tract, amniotic fluid, placenta, and fetal membranes. The role of *Sneathia* in women's health and disease is generally underappreciated because the cultivation of these bacteria is limited by their complex nutritional requirements, slow growth patterns, and anaerobic nature. For this reason, molecular methods are typically required for the detection and differential diagnosis of *Sneathia* infections. *Sneathia* species are more often present in the vaginal samples of women with symptoms of bacterial vaginosis than in those from asymptomatic women. Indeed, the presence of *Sneathia* is positively associated with the diagnostic criteria of bacterial vaginosis. A recent study further indicated that Sneathia had among the greatest relative abundance and effect size of the vaginal bacteria associated with preterm birth (delivery at <37 weeks of gestation).[63]

***Bifidobacterium* species**. *Bifidobacterium breve* is a non-motile Gram-positive anaerobe. It is a commensal that inhabits the gut, oral cavity, and vagina. Owing to their ability to produce lactic acid and hydrogen peroxide, which contribute to vaginal and gut microbial homeostasis, they are used as a probiotic. Indeed, studies have shown little to no differences between *Bifidobacterium* species in the gut and vagina.[64] Giordani and colleagues[65] proved the ability of *B. breve* encapsulated in mucoadhesive tablets to exert a strong antimicrobial activity against urogenital and enteric pathogens. The same study also showed the ability of *B. breve* to easily colonize the vagina and gut, making them excellent probiotic agents that can help prevent urogenital infections.[65] This indicates that *B. breve* is an important and beneficial commensal that augments the positive effects of *Lactobacillus* species.[66]

Bacteroides fragilis group (BFG) is the most frequently recovered species of Bacteroidaceae in clinical specimens. BFG are resistant to penicillins, mostly through the production of β-lactamase. They include several members, the most commonly isolated of which is *B. fragilis*. *B. fragilis* exists both as a part of normal commensal flora and as a pathogenic bacterium expressing a zinc metalloprotease called *Bacteroides fragilis* toxin or fragilysin. The human colon has the greatest population

of bacteria in the body (over ten organisms per gram of wet weight), and the largest part of these organisms are anaerobes. Of these, approximately 25% are species of *Bacteroides*. *B. fragilis* is part of the normal microbiota of the human colon. Disruption of the mucosal surface either by inflammation, trauma, or surgery and the spread of *B. fragilis* to the bloodstream or surrounding tissues results in clinically significant infection.[67] Despite their dominance in the colon, *B. fragilis* has been implicated in bacterial vaginosis.[68]

Diagnosis

There are two major approaches in conventional BV diagnostics: clinical and laboratory. Clinical BV diagnosis is based on the fulfillment of at least 3 out of 4 criteria described by Amsel and others[69]:

1. Increased vaginal pH > 4.5
2. The presence of clue cells (exfoliated vaginal epithelial cells with attached bacteria) by wet-mount microscope
3. Positive "whiff test" or amine test (fishy odor after addition of 10% KOH)
4. The presence of a thin, non-clumping gray-white adherent vaginal discharge

Laboratory BV testing involves Gram staining of vaginal smears, microscopic evaluation, and scoring numbers of bacterial morphotypes and clue cells, according to Nugent and colleagues[70] or Ison and Hay.[71] As most bacterial species associated with BV are fastidious anaerobic microorganisms that are either difficult to culture or, in some cases, have yet to be cultured, conventional microbiological techniques other than Gram staining are not appropriate for BV diagnostic purposes.[72] The Amsel criteria and Nugent scoring approaches suffer from subjective interpretation and, as shown in multiple studies, do not perfectly agree with one another.[72,73] Up to half of all women who meet the diagnostic criteria for BV might not exhibit clinical symptoms.[72]

While there are different diagnostic possibilities for BV diagnosis targeting the various macromolecules and metabolic pathways in the vaginal microenvironment (Figure 2), the use of molecular techniques is fast becoming the mainstay in addition to the conventional microscopic Nugent and Amsel's criteria. This is primarily due to the high sensitivity and specificity for the detection of the microbes responsible for the loss of the vaginal microflora homeostasis seen in BV.[36] Recently, the utility of molecular techniques, such as quantitative PCR (qPCR), for the quantitation of major bacterial species inhabiting the vaginal environment of healthy women and BV carriers has been demonstrated.[36,74–76] Quantitative PCR assessment of BV-related bacteria correlates significantly with high sensitivity and specificity to the Nugent score and, to a lesser extent, with Amsel criteria in the diagnosis of BV.[77] The application of molecular methods, especially PCR, for the characterization of vaginal microflora in BV

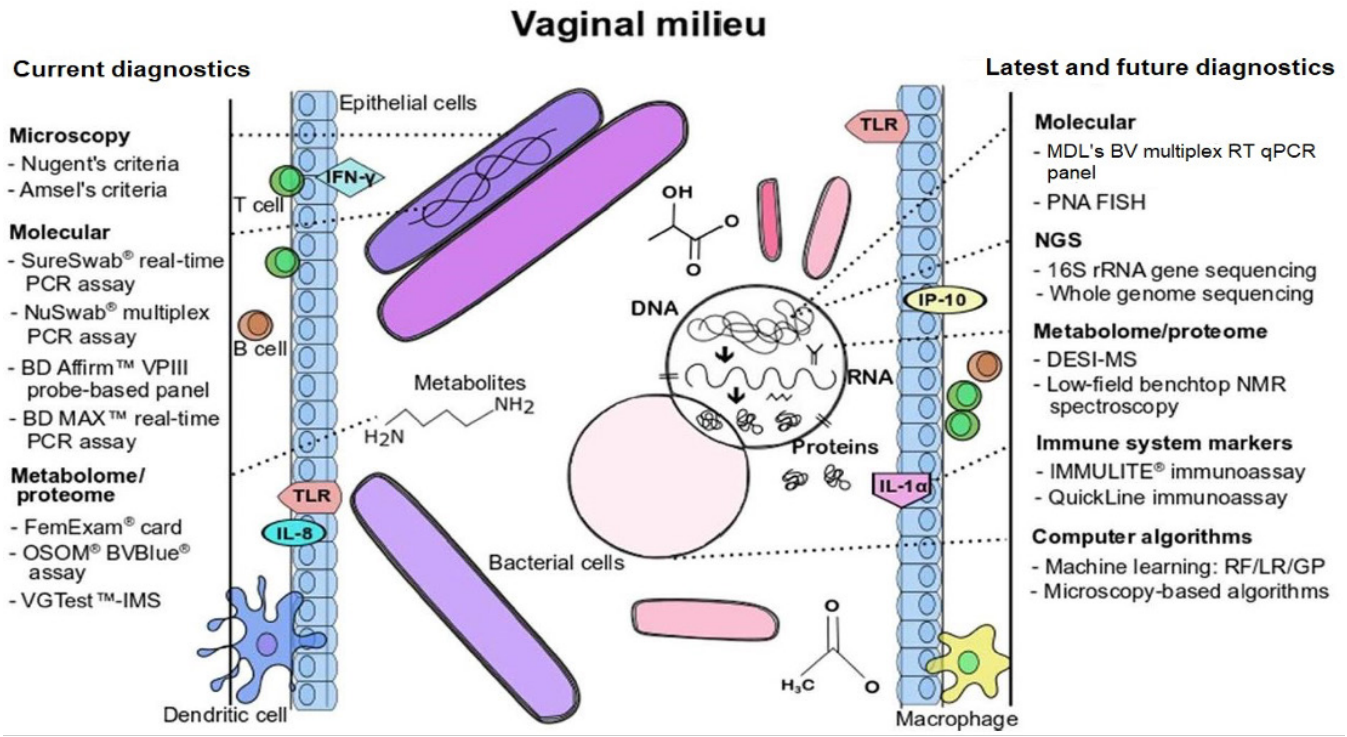


Figure 2. Current diagnostics and possibilities for BV detection and/or pathogenesis characterization. Adapted from Redelinghuys *et al* [36]. BV, Bacterial Vaginosis; DESI-MS, desorption electrospray ionization-mass spectrometry; GP, genetic programming; IFN, interferon; IL, interleukin; LR, logistic regression; NMR, nuclear magnetic resonance; PCR, polymerase chain reaction; PNA-FISH, peptide nucleic acid fluorescence in situ hybridization; RF, random forest; TLR, toll-like receptor; VGTest-IMS, VGTest-ion mobility spectrometry.

patients has become an accepted practice and a major trend in laboratory diagnostics. Comparative diagnostic studies have shown that PCR assays effectively detected the BV status of subjects whose vaginal microflora had been characterized and defined using the Nugent criteria.[36,78] PCR was also employed as a tool in identifying patients at high risk for recurrent BV based on the higher concentrations of Megasphaera Type 2 & BVAB2 at initial diagnosis and greater *G. vaginalis* amounts, vaginal pH, as well as higher Nugent score following treatment with metronidazole.[45]

The use of the PCR method in BV diagnosis can also assist in detecting BV in asymptomatic pregnant women, which is crucial in preventing the potential gynecological complications often associated with untreated BV. In 456 asymptomatic pregnant women with BV, *Atopobium vaginae*, BVAB2, *Gardnerella* species, *Mobiluncus curtisii*, *Mobiluncus mulieris*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Prevotella bivia*, Megasphaera 1, and Megasphaera 2 were detected in their vaginal samples by qPCR thus overcoming the limit of the Nugent's scoring.[77] In an earlier study involving 499 pregnant women with BV, PCR-identified Mycoplasma, Mobilincus, and *Atopobium* species were positively correlated with an increased risk of preterm births.[13]

Bacterial Vaginosis (with Lactobacillus profiling) qPCR Panel

Medical Diagnostic Laboratories (MDL) has developed a new state-of-the-art qPCR molecular assay targeting a set of bacteria commonly found in normal healthy vaginal microflora and microflora associated with BV. MDL's new Bacterial Vaginosis (with Lactobacillus profiling) qPCR Panel includes tests for five (5) major *Lactobacillus* species (*L. crispatus*, *L. jensenii*, *L. gasseri*, *L. iners*, and *L. acidophilus*) whose depletion has been demonstrated in BV in addition to seventeen (14) main pathogens whose overgrowth was implicated in BV pathogenesis. The pathogens include *Gardnerella vaginalis*, *Atopobium vaginae*, Bacterial Vaginosis Associated Bacterium (BVAB) 1, 2 & 3, *Megasphaera* species 1 & 2, *Prevotella bivia*, *Mobilincus mulieris*, *Mobilincus curtisii*, *Sneathia sanguinegens*, *Streptococcus anginosus*, *Bifidobacterium breve*, and *Bacteriodes fragilis*. The panel is one of the most comprehensive molecular-based BV tests currently available, greatly strengthening the capacity to accurately and promptly diagnose the condition in the laboratory and assist in informing effective treatment to ensure patient wellness. Although many of the qPCR tests in the panel can be ordered and performed separately, only the combination of the assays in a single panel allows for the accurate relative quantitative evaluation of the bacterial species composition in each clinical sample. Using the qPCR panel and employing machine learning techniques, we developed a novel diagnostic model, the *MDL-BV index*, utilizing the vaginal microbiome dataset from 946 women to diagnose BV-positive, BV-negative, and transitional BV based on the species and their relative abundance, organized into four biomarkers.[53] The integration of this innovative two-tier, four-biomarker diagnostic approach promises to greatly enhance the accuracy and efficiency of BV diagnosis.

MDL's Bacterial Vaginosis (with Lactobacillus profiling) qPCR Panel results are reported in two formats: text-based and graphical. The text format has a standard layout of diagnostic qualitative test reporting. The graphic format is a representation of the results of all the quantitative tests included in the panel. The relative DNA ratio of species in each sample in proportion to one another reflects the relative concentrations of different bacteria in vaginal specimens. This user-friendly test report simplifies data interpretation and analysis. The single slider chart provides the physician with a snapshot of the vaginal bacterial microflora accompanied by a summary suggestive of the vaginal microflora state: either normal, transitional, or affected by BV (Figure 3).

Generally, molecular diagnostic tests for BV are focused on the detection of recognized pathogenic markers of the disease. The incorporation of the Lactobacillus qPCR assays makes our test considerably more comprehensive and greatly extends the diagnostics available for the assessment of vaginal health. MDL's new Bacterial Vaginosis (with Lactobacillus profiling) qPCR Panel is a significant advancement beyond the qualitative identification of BV-associated microorganisms since it now covers microbial markers of the normal vaginal environment. It can be used successfully for the determination of relative vaginal microflora composition and bacterial loads, which might facilitate monitoring of the response to antibiotic therapy.

Treatment

In 2021, the Centers for Disease Control and Prevention (CDC) updated their treatment recommendations for all patients exhibiting symptomatic BV as defined by Amsel criteria and/or Nugent score.[8] In addition, asymptomatic pregnant BV patients at risk for preterm labor and before invasive procedures such as abortion or hysterectomy to reduce the possibility of complicating infections are advised to be treated. The following treatments are recommended:

- Metronidazole, 500 mg orally 2x daily for 7 days OR
- Metronidazole gel, 0.75% one full applicator (5 g) intravaginally, once daily for 5 days OR
- Clindamycin cream, 2% one full applicator (5 g) intravaginally at bedtime for 7 days

Alternatively,

- Clindamycin, 300 mg orally 2x daily for 7 days OR
- Clindamycin ovules, 100 mg intravaginally once at

bedtime for 3 days OR

- Secnidazole, 2 g oral granules in a single dose OR
- Tinidazole, 2 g orally once daily for 2 days or 1 g orally once daily for 5 days

The restoration of normal microflora is the final result physicians strive to achieve with antimicrobial therapy for BV. Successful treatment of BV with antimicrobial results in a three- to four-log decrease in the vaginal concentrations of BV-associated microorganisms followed by a rise in lactobacilli concentration of about the same magnitude.47,79 Eradication of BV-related bacteria and their replacement with Lactobacillus species suggests a complete BV cure; conversely, the failure of BV antibiotic therapy is associated with only minor changes in the composition of the vaginal bacteria.[47,79]

MDL's Bacterial Vaginosis (with *Lactobacillus profiling*) qPCR Panel offers an opportunity for physicians to monitor the efficacy of antimicrobial therapy. Dynamic changes in bacterial composition during the course of treatment and post-treatment may be observed to help physicians assess treatment success with the return of Lactobacilli or treatment failure with persistence or recurrence of the BV-associated organisms. Treatment regimens can, therefore, be appropriately adjusted to achieve an efficient cure.

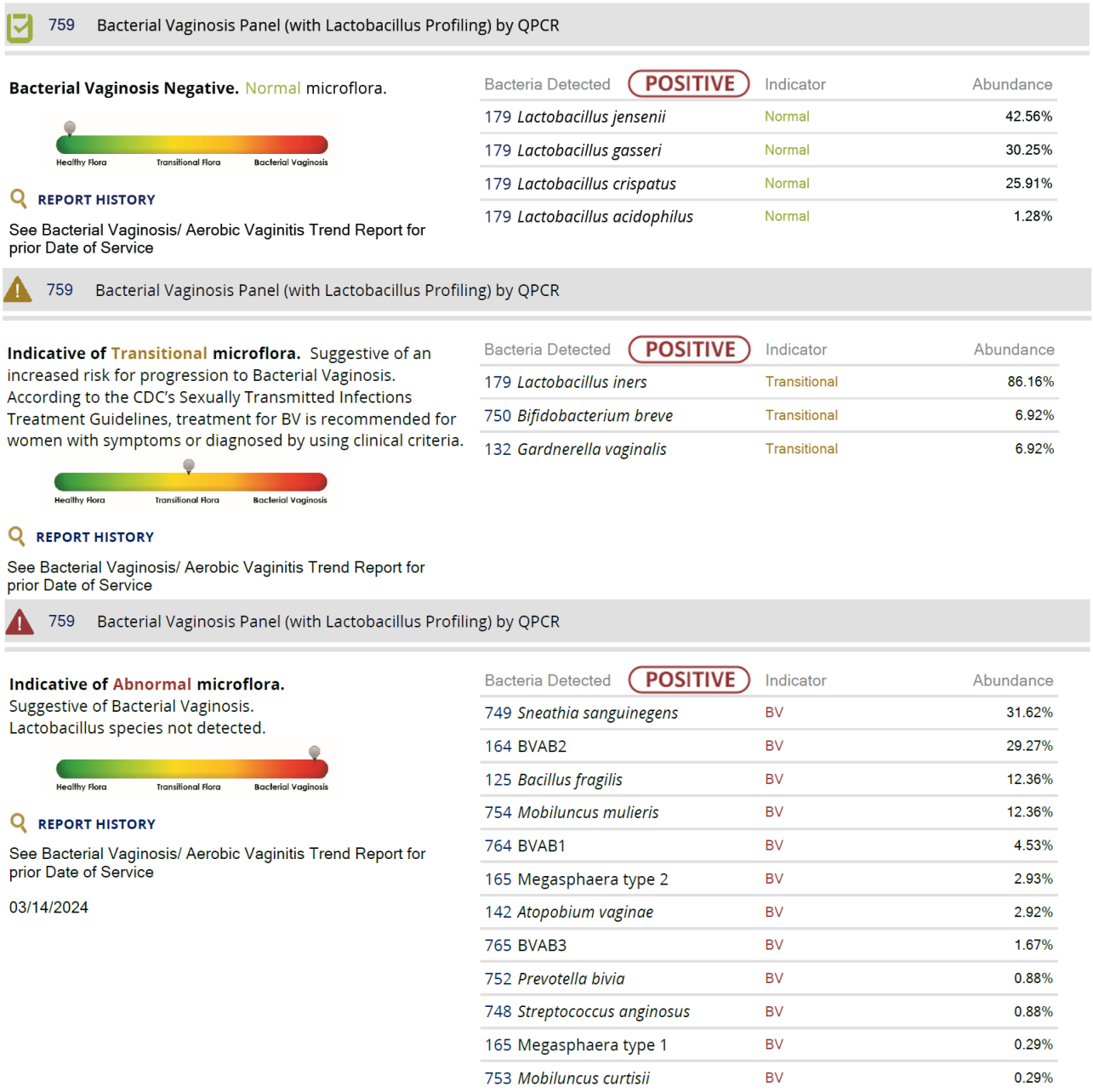
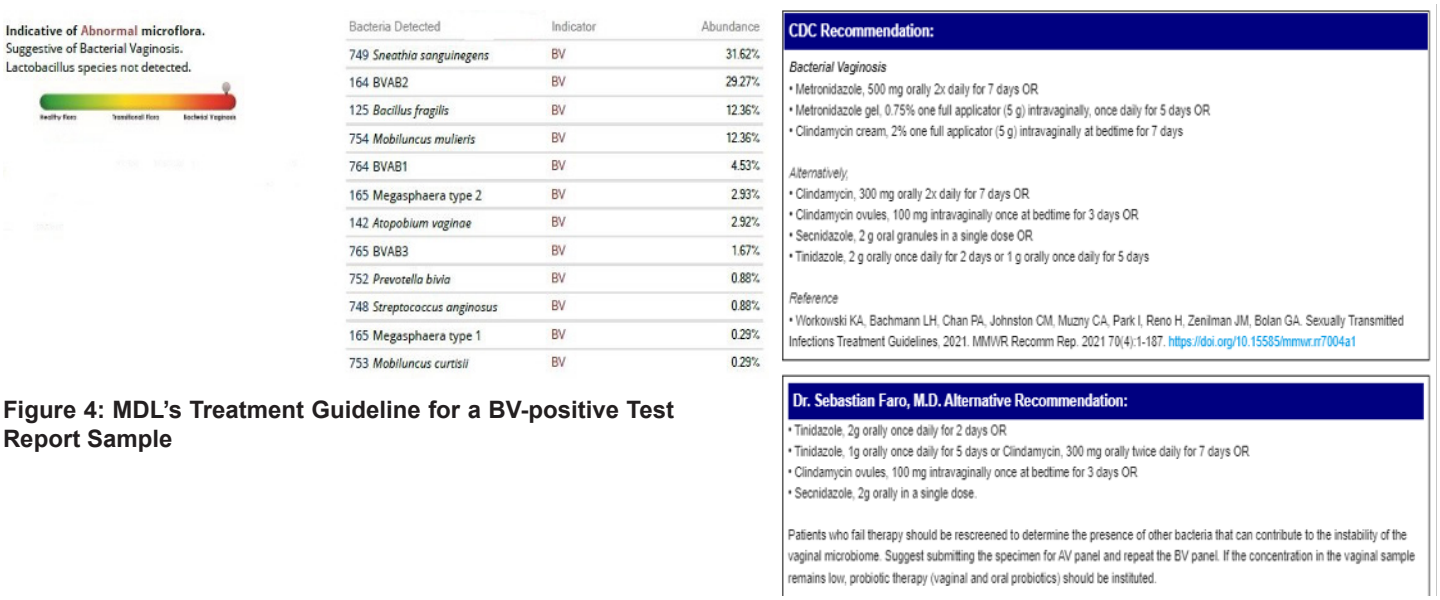


Figure 3: Graphic representations of MDL's Bacterial Vaginosis (with Lactobacillus profiling) qPCR Panel test results for normal and abnormal vaginal microflora.

MDL Treatment Guidelines

Besides the user-friendly and easy-to-read BV panel test report, MDL has also designed an innovative compilation of the CDC's treatment recommendations together with an experienced physician's alternative recommendation to accompany each test report for easy access. MDL's treatment guidelines are specifically tailored to assist doctors and clinicians in accurately interpreting laboratory test results and making prompt diagnoses to ensure patient wellness. Some of the test report scenarios and corresponding treatment guidelines are provided in Figures 4-6.



Indicative of Abnormal microflora.
Suggestive of Bacterial Vaginosis.
Lactobacillus species not detected.

Bacteria Detected	Indicator	Abundance
749 *Sneathia sanguinegens*	BV	31.62%
164 BVAB2	BV	29.27%
125 *Bacillus fragilis*	BV	12.36%
754 *Mobiluncus mulieris*	BV	12.36%
764 BVAB1	BV	4.53%
165 Megasphaera type 2	BV	2.93%
142 *Atopobium vaginae*	BV	2.92%
765 BVAB3	BV	1.67%
752 *Prevotella bivia*	BV	0.88%
748 *Streptococcus anginosus*	BV	0.88%
165 Megasphaera type 1	BV	0.29%
753 *Mobiluncus curtisii*	BV	0.29%

CDC Recommendation:

Bacterial Vaginosis
- Metronidazole, 500 mg orally 2x daily for 7 days OR
- Metronidazole gel, 0.75% one full applicator (5 g) intravaginally, once daily for 5 days OR
- Clindamycin cream, 2% one full applicator (5 g) intravaginally at bedtime for 7 days

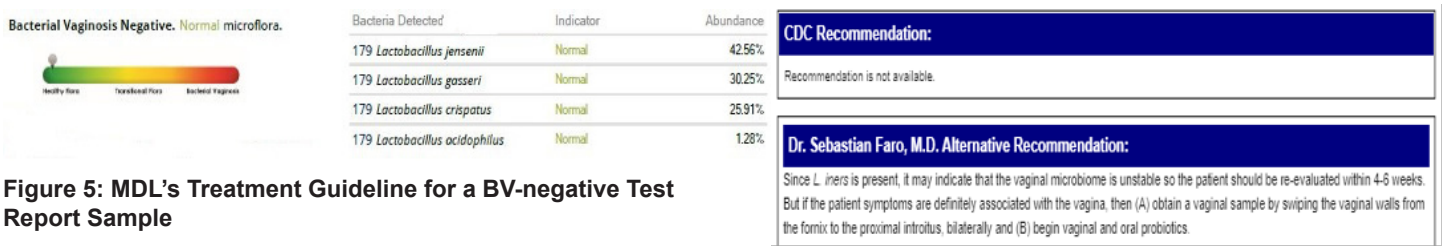
Alternatively,
- Clindamycin, 300 mg orally 2x daily for 7 days OR
- Clindamycin ovules, 100 mg intravaginally once at bedtime for 3 days OR
- Secnidazole, 2 g oral granules in a single dose OR
- Tinidazole, 2 g orally once daily for 2 days or 1 g orally once daily for 5 days

Reference
- Workowski KA, Bachmann LH, Chan PA, Johnston CM, Muzny CA, Park I, Reno H, Zenilman JM, Bolan GA. Sexually Transmitted Infections Treatment Guidelines, 2021. MMWR Recomm Rep. 2021 70(4):1-187. https://doi.org/10.15585/mmwr.rr7004a1

Dr. Sebastian Faro, M.D. Alternative Recommendation:
- Tinidazole, 2g orally once daily for 2 days OR
- Tinidazole, 1g orally once daily for 5 days or Clindamycin, 300 mg orally twice daily for 7 days OR
- Clindamycin ovules, 100 mg intravaginally once at bedtime for 3 days OR
- Secnidazole, 2g orally in a single dose.

Patients who fail therapy should be rescreened to determine the presence of other bacteria that can contribute to the instability of the vaginal microbiome. Suggest submitting the specimen for AV panel and repeat the BV panel. If the concentration in the vaginal sample remains low, probiotic therapy (vaginal and oral probiotics) should be instituted.

Figure 4: MDL's Treatment Guideline for a BV-positive Test Report Sample



Bacterial Vaginosis Negative. Normal microflora.

Bacteria Detected	Indicator	Abundance
179 *Lactobacillus jensenii*	Normal	42.56%
179 *Lactobacillus gasseri*	Normal	30.25%
179 *Lactobacillus crispatus*	Normal	25.91%
179 *Lactobacillus acidophilus*	Normal	1.28%

CDC Recommendation:

Recommendation is not available.

Dr. Sebastian Faro, M.D. Alternative Recommendation:

Since *L. iners* is present, it may indicate that the vaginal microbiome is unstable so the patient should be re-evaluated within 4-6 weeks. But if the patient symptoms are definitely associated with the vagina, then (A) obtain a vaginal sample by swiping the vaginal walls from the fornix to the proximal introitus, bilaterally and (B) begin vaginal and oral probiotics.

Figure 5: MDL's Treatment Guideline for a BV-negative Test Report Sample

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