

BiomeFx™

INTERPRETATION GUIDE

The results from this test kit are for informational purposes only and are not intended to be a substitute for professional medical advice, diagnosis, or treatment. Always seek the advice of your physician or qualified health provider with any questions you may have regarding a medical condition.



TABLE OF CONTENTS

- 3 General Overview of the Vaginal Microbiome
- 4 General Healthy Guidelines for Vaginal Health
- 5-6 Probiotics and Supplements
- 7-8 Community State Types
- 9-10 References

General Overview of the Vaginal Microbiome

The vaginal microbiome (VMB) is a dynamic microecosystem consisting of bacteria, yeast and fungi.¹ These microbes protect the vagina against infection, support vaginal mucosal health and may also support healthy pregnancy outcomes. Unlike the gut microbiome, a healthy VMB thrives on low microbial diversity and is typically dominated by one of the following *Lactobacillus* species: *L. crispatus*, *L. gasseri*, *L. iners* and *L. jensenii*.²

As the name implies, the primary function of *Lactobacillus* species is to produce lactic acid to support vaginal acidity. *L. crispatus*, *L. gasseri*, and *L. jensenii* do a great job at this. *L. iners*, on the other hand, is more unpredictable. This is because *L. iners* produces L-actic acid, rather than D-lactic acid, which is less successful at promoting vaginal acidity.⁴ VMBs dominated by *L. iners* have more fluctuations in community composition and are more susceptible to infection.

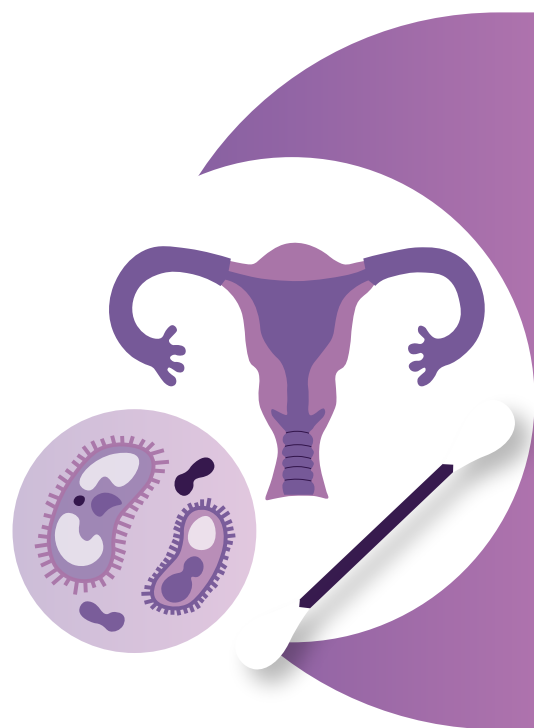
Lactobacillus species also produce bacteriocins (antimicrobial compounds) and take up real estate along the vaginal walls so that unwanted microbes cannot thrive.^{1,2} These mechanisms support thriving mucosal health to stave off vaginal dryness and discomfort. If *Lactobacillus* abundance drops, unwanted microbes have an opportunity to take over. This can lead to a thinning of the vaginal mucosa, vaginal dryness, abnormal discharge, itching, pain, increased risk of infection and other adverse symptoms.⁴

Estrogen and the Vaginal Microbiome

Estrogen plays a massive role in VMB composition because it increases the availability of glycogen—the primary food source for *Lactobacillus* species. Menstruation, menopause and breastfeeding are all marked by a significant drop estrogen levels, which can lead to vaginal dysbiosis, itching, dryness and discomfort. Pregnancy and puberty are associated with an increase in estrogen which can benefit *Lactobacillus* abundance and overall vaginal health.

Similarly, estrogen-based contraceptives are beneficial to the VMB, while progestin-only local release contraceptives are associated with less-stable microbial composition and lower abundance of *Lactobacillus spp.*^{10,11}

Supporting healthy estrogen levels can also be accomplished by maintaining gut health. For example, the estrobolome is a group of gut bacteria that recycle estrogen which would otherwise be excreted in the stool.⁹ Estrobolome functioning must be balanced as too much or too little estrobolome activity may support an imbalance of serum estrogen levels. The best way to measure estrobolome health is through a functional analysis stool test. Furthermore, research shows that certain gut microbes activate the ESR1 gene to promote glycogen availability in the vagina.



General Healthy Guidelines for Vaginal Health

Lifestyle Recommendations and Tips

- Avoid extreme exercise.¹⁰
- Condoms and sexual hygiene may reduce the occurrence of vaginal dysbiosis.¹²⁻¹⁴
- Wear loose fitting, breathable undergarments.
- Practice stress-reducing techniques include cold water plunges and/or applying cold water to the face, belly breathing, slow movements, yoga, spending time with people who make you feel relaxed and comfortable, and spending time in nature.¹⁵⁻¹⁷
- Avoid intravaginal washing, such as douching.^{18,19}
- Avoid excessive, recurrent use of antibiotics, if possible. Alternatively, consuming probiotics while on an antimicrobial therapy can support vaginal acidity and redox potential of the vaginal fluid.²⁰⁻²²
- Traditional tampons and pads are shown to disrupt the vaginal microbiome.²⁴ While alternatives have not been clinically studied, clinicians often recommend the use of reusable menstrual cup made of medical grade silicon.
- Avoid sitting in chlorinated water for extended periods of time.²⁵
- If you regularly smoke tobacco, talk to a support group or therapist about quitting.
- Support estrogen recycling within the gut by consuming spore-based probiotics and precision prebiotics.²⁶ These supplements support gut microbial diversity and help maintain estrobolome functioning.

Nutrition Guidelines and Tips

- Consume raw and lightly cooked vegetables including kale, spinach, cauliflower, carrots, beets, sea vegetables and seafood.^{2,27-29}
- Fermented foods such as yogurt, kim chi, kombucha, and tempeh may be beneficial for the vaginal microbiome.^{2,27-29}
- Specific nutrients are also associated with a healthy vaginal microbiome, such as vitamins A, C, D, E, beta carotene, calcium, zinc, selenium.²³ These nutrients can be found in yellow and red fruits and vegetables, fortified milk and eggs, nuts, and seeds.
- Diets rich in sugar and fat is also linked to a higher incidence of bacterial vaginosis, while vegetarian diets are linked to increased diversity in the VMB.,¹⁰
- Polyphenols and flavonoids are shown to support urogenital health and may even reduce the risk of UTI. ²⁹⁻³¹ These nutrients are found in brightly colored fruits and vegetables, particularly in cranberries, pomegranate and other red colored fruits.

Probiotics and Supplements

Research suggests that the gut acts as a microbial reservoir for vaginal microbes.³¹ Probiotics containing lactic acid producing bacteria can maintain the vaginal pH and reduce the growth of pathogenic bacteria.³² Lactic-acid producing probiotics include *B. coagulans*, *Sacchromyces boulardii*, *L. reuteri*, *L. rhamnosus*, *L. salivarius* and *L. acidophilus*.³³⁻³⁷

B. subtilis can also promote a favorable vaginal environment by lowering inflammatory cytokine production.^{38,39} Inflammation reduces availability of vaginal glycogen, manganese and magnesium which can lower the abundance of beneficial *Lactobacilli* species.

Microbe-specific supplement recommendations are listed below:

Lactobacillus species

If the abundance of *Lactobacillus* species is low, this could indicate vaginal dysbiosis or increased risk of bacterial vaginosis, vulvovaginal candidiasis, STIs, pelvic inflammatory disease and spontaneous pre-term birth. Low *Lactobacillus* is indicative of CST IV. To support healthy *Lactobacillus* levels, follow general healthy guidelines for vaginal health as listed above.

Opportunistic Bacteria

Prevotella copri
Ureaplasma spp
Mobiluncis spp
Megasphaera spp
Gardnerella spp
Atopobium spp

Boric acid suppositories can help break up the biofilms created by these species. Probiotics (*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *L. salivarius*, *Lactobacillus fermentum*, *B. coagulans*) may help increase vaginal acidity and support healthy levels of these microbes. Sea buckthorn may help reduce vaginal atrophy and vaginal dryness.

Streptococcus agalactiae
(Group B streptococcus)

Streptococcus salivarius and *Lactobacillus salivarius* are shown to eliminate Group B streptococcus from the vaginal microbiome.

Pathobionts

Escheria spp

Probiotics such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *L. salivarius*, *Lactobacillus fermentum*, *B. coagulans*, and *Sacchromyces boulardii* may help increase vaginal acidity and support healthy levels of these microbes. Bacillus spore-forming probiotics may help lower abundance of *E. coli* in the gut, thereby reducing the microbial reservoir of this pathobiont. Cranberry extract may help reduce adhesion of *E. coli* in urogenital tracts.

Enterococcus spp

Probiotics such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *L. salivarius*, *Lactobacillus fermentum*, *B. coagulans*, and *Sacchromyces boulardii* may help increase vaginal acidity and support healthy levels of these microbes.

Consuming *Bacillus* spore forming strains improves the composition of the gut microbiome, which may help lower the microbial reservoir of *Enterococcus* in the gut

Staphylococcus spp

Probiotics such as *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *L. salivarius*, *Lactobacillus fermentum*, *B. coagulans*, and *Sacchromyces boulardii* may help increase vaginal acidity and support healthy levels of these microbes.

Consuming *Bacillus* spore forming strains improves the composition of the gut microbiome, which may help lower the microbial reservoir of *Staphylococcus* in the gut

Opportunistic Fungi

Candida spp

Caprylic acid oral supplements are shown to support healthy levels of *Candida*.

Boric acid suppositories can help break up biofilms that *Candida* is attached to *Lactobacillus* and *Sacchromyces boulardii* probiotics are shown to support healthy levels of *Candida*. Lactoferrin can reduce iron availability, a necessary nutrient for *Candida*. Research shows Lactoferrin supports healthy levels of this microbe
Additional Nutrition Recommendations for *Candida* overgrowth: Avoid high sugar, wheat and refined carbohydrates Consume alcohol and caffeine sparingly Consume fermented dairy products, if tolerated.

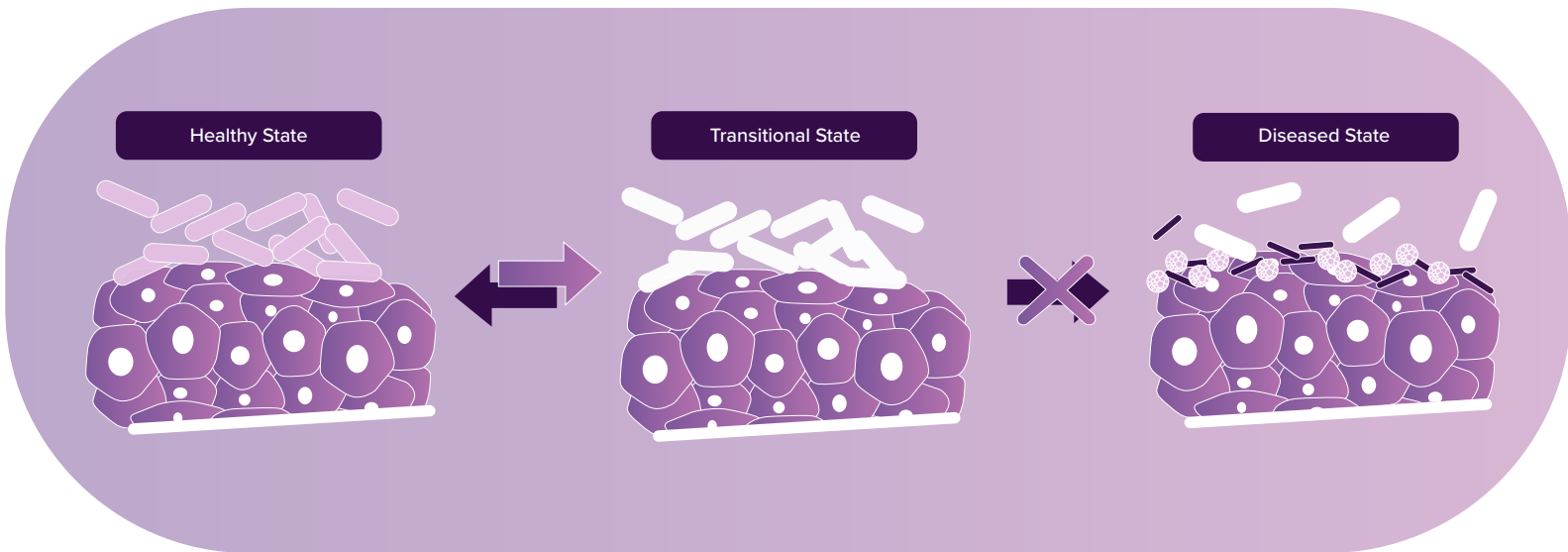


Community State Types

The composition of women's VMB falls under 1 of 5 different community state types. This can change over time depending on a number of biological and lifestyle factors. The type of community state type can be a determinant of both positive and adverse outcomes.

Type I

L. crispatus dominate. This is considered one of the healthiest CSTs, marked by low pH (< pH 4) and maintains stability throughout a women's menstrual cycle. Type I can occasionally transition towards a *L. iners*-dominate microbiome due to sexual activity, menses and pregnancy.^{5,6}



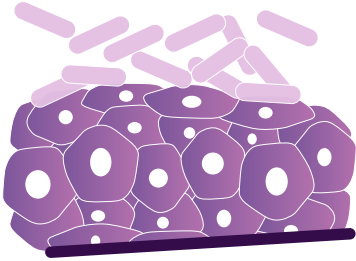
Type II

L. gasseri dominate. While type II can be more dynamic than type I, it is not commonly associated with diseased states. Type II is associated with a vaginal pH of 4.4. Women with type II may experience a transition to *L. crispatus*-dominate microbiome (type I) during pregnancy, though this is an uncommon occurrence. Type II is not a common CST found in women.

Type III

L. iners dominate. While most *Lactobacillus* species are beneficial, *L. iners* is an exception. This is because *L. iners* produces *L-lactic* acid which is less effective at inhibiting unwanted microbial species, as compared to D-lactic acid. Women with type III can experience a transition to a CST dominated by BV-associated bacteria. *L. iners* is particularly sensitive to environmental changes and may be due to a loss of glycogen availability. Type III is associated with a pH greater than 4.5 and higher baseline concentrations of pro-inflammatory molecules such as TNF-a, interleukin-1a and interleukin 18. These factors can damage vaginal mucosa.²

Healthy/ Transitional State

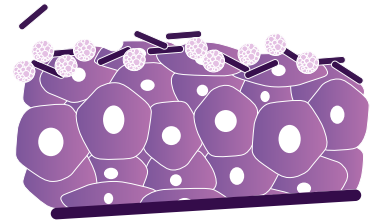


Facilitate Vaginal Dysbiosis

Restricted metabolic repertoire of *L. iners* and their dependence on the nutrients from host causing it to highly sensitive to environmental changes

Produced *L.lactic* acid which is insufficient to inhibit progression of pathogens during vaginal infection.

Diseased State



Type IV

Non-lactobacillus dominate and high microbial diversity. Group IV is most closely associated with high Nugent scores (>7), which indicates higher risk of BV. Type IV is the least stable and shows the most fluctuations in composition throughout a women's menstrual cycle. CST IV is the most highly associated with BV, STI's, preterm birth, pelvic inflammatory disease, and miscarriage. Due to the high prevalence of non-lactic-acid producing species, type IV tends to have low organic acid production, higher pH and vaginal mucosal disruption.

Subset A

Characterized by modest amounts of *L. iners* and high abundance of *G. vaginalis*, *A. vaginae*, *Prevotella* spp.

Subset B

Non-lactobacillus dominate and high microbial diversity. Characterized by high abundance of *A. vaginae*, *Leptotrichia* spp, *Mobiluncis* spp. CST type IV B contains the highest amounts of BV associated bacteria.

Type V

L. jensenii dominate. Scientists are still researching the specifics of *L. jensenii*-dominate vaginal microbiomes. We know that type V is associated with a pH of 4.2 and healthy vaginal mucosa. Type V is an uncommon CST found in women

1. García-Velasco, et al. What fertility specialists should know about the vaginal microbiome: a review. *Reproductive BioMedicine Online*. 2017;35(1): 103-112
2. Ravel J, et al. Vaginal microbiome of reproductive-age women. *Proc Natl Acad Sci U S A*. 2011 Mar 15;108 Suppl 1(Suppl 1):4680-7.
3. Boskey, et al. Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Human Reproduction*. 2001; 16(9): 1809–1813.
4. Feng, et al. Oxidative stress tolerance and antioxidant capacity of lactic acid bacteria as probiotic: a systematic review. *Gut Microbes*. 2020; 12(1):180194
5. Barrientos-Durán A, et al. Reviewing the Composition of Vaginal Microbiota: Inclusion of Nutrition and Probiotic Factors in the Maintenance of Eubiosis. *Nutrients*. 2020;12(2):419.
6. Lee S, Oh KY, Hong H, et al. Community State Types of Vaginal Microbiota and Four Types of Abnormal Vaginal Microbiota in Pregnant Korean Women. *Front Public Health*. 2020;8:507024.
7. Chee, et al. Vaginal microbiota and the potential of *Lactobacillus* derivatives in maintaining vaginal health. *BMC*. 2020;19:203.
8. Eschenbach, et al. Influence of the normal menstrual cycle on vaginal tissue, discharge, and microflora. *Clinical Infectious Diseases*. 2000; 30(6): 901–907
9. Kwa, et al. The Intestinal Microbiome and Estrogen Receptor-Positive Female Breast Cancer. *Journal of the National Cancer Institute*. 2016; 108(8)
10. Song, et al. Daily Vaginal Microbiota Fluctuations Associated with Natural Hormonal Cycle, Contraceptives, Diet, and Exercise. *ASM Journals*. 2020; 5(4). DOI: 10.1128/mSphere.00593-20
11. Setty, et al. Vaginal estrogen use and effects on quality of life and urogenital morbidity in postmenopausal women after publication of the Women's Health Initiative in New York City. *Menopause*. 2016; 23(1): 7–10
12. Plummer, et al. Sexual practices have a significant impact on the vaginal microbiota of women who have sex with women. *Scientific Reports*. 2019; 9(1)
13. Marfatia, et al. Condoms: Past, present, and future. *Indian journal of sexually transmitted diseases and AIDS*. 2015; 36(2): 133-9
14. Ma L, Lv Z, Su J, Wang J, Yan D, Wei J, et al. Consistent condom use increases the colonization of *Lactobacillus crispatus* in the vagina. *PLoS One*. 2013;8:e70716
15. Nansel, et al. The Association of Psychosocial Stress and bacterial vaginosis in a longitudinal cohort. *American Journal of Obstetrics and Gynecology*. 2006;194(2): 381–386.
16. Pandey, et al. Impact of stress on female reproductive health disorders: Possible beneficial effects of shatavari (*Asparagus racemosus*). *Biomedicine & Pharmacotherapy*. 2018;103:46–49.
17. Brotman, et al. Association between Cigarette Smoking and the vaginal microbiota: A pilot study. *BMC Infectious Diseases*. 2014; 14(1)
18. Lokken, et al. Association between vaginal washing and detection of *Lactobacillus* by culture and quantitative PCR in HIV-seronegative Kenyan women: A cross-sectional analysis. *Sex. Transm. Infect.* 2019; 95(6), 455–461
19. Sabo, et al. Association between vaginal washing and vaginal bacterial concentrations. *PLoS ONE*. 2019; 14(1).
20. 33 Akgül, et al. The role of probiotics in women with recurrent urinary tract infections. *Turkish journal of urology*. 2018; 44(5): 377-383.
21. Ma, D., Chen, Y., and Chen, T. Vaginal microbiota transplantation for the treatment of bacterial vaginosis: a conceptual analysis. *FEMS Microbiol. Lett.* 2019; 366:fnz025.
22. Sgibnev, A., and Kremleva, E. Probiotics in addition to metronidazole for treatment *Trichomonas vaginalis* in the presence of BV: a randomized, placebo-controlled, double-blind study. *Eur. J. Clin. Microbiol. Infect. Dis.* 2020; 39: 345–351.

23. Tuddenham, et al. Associations between dietary micronutrient intake and molecular-Bacterial Vaginosis. *Reproductive Health*. 2019; 16:151.
24. Noyes N, et al. Associations between sexual habits, menstrual hygiene practices, demographics and the vaginal microbiome as revealed by Bayesian network analysis. *PLoS One*. 2018;13(1):e0191625.
25. Ram H, Dastager SG. Re-purposing is needed for beneficial bugs, not for the drugs. *Int Microbiol*. 2019 Mar;22(1):1-6. doi: 10.1007/s10123-018-00049-x. Epub 2018 Dec 11. PMID: 30810942.
26. Salliss ME, et al. The role of gut and genital microbiota and the estrobolome in endometriosis, infertility and chronic pelvic pain. *Hum Reprod Update*. 2021 Dec 21;28(1):92-131
27. 18Tohill, et al. Nutritional biomarkers associated with gynecological conditions among US women with or at risk of HIV infection. *The American Journal of Clinical Nutrition*. 2007 (85):1327–1334
28. 19Thoma, et al. Bacterial vaginosis is associated with variation in dietary indices. *The Journal of Nutrition*. 2011; 141(9)
29. 20Kontiohari, et al. Dietary factors protecting women from urinary tract infection. *The American Journal of Clinical Nutrition*. 2003; 77(3): 600–604
30. 21Howell, et al. Dosage effect on uropathogenic *Escherichia coli* anti-adhesion activity in urine following consumption of cranberry powder standardized for proanthocyanidin content: a multicentric randomized double blind study. *BMC Infect Dis*. 2010; 10(94)
31. 22Marrazzo, et al. Extravaginal Reservoirs of Vaginal Bacteria as Risk Factors for Incident Bacterial Vaginosis. *The Journal of Infectious Disease*. 2012; 205(10): 1580–1588
32. Mizgier, et al. The role of diet and probiotics in prevention and treatment of bacterial vaginosis and vulvovaginal candidiasis in adolescent girls and non-pregnant women. *Ginekologia Polska*. 2020; 91(7): 412–416
33. 26 Russo R, et al. Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. *Arch Gynecol Obstet*. 2018;298(1):139-145.
34. 27 Russo R, et al. Evidence-based mixture containing *Lactobacillus* strains and lactoferrin to prevent recurrent bacterial vaginosis: a double blind, placebo controlled, randomised clinical trial. *Benef Microbes*. 2019;10(1):19-26.
35. 28 Russo R, et al. Randomised clinical trial in women with Recurrent Vulvovaginal Candidiasis: Efficacy of probiotics and lactoferrin as maintenance treatment. *Mycoses*. 2019;62(4):328-335
36. 29 De Alberti D, et al. Lactobacilli vaginal colonisation after oral consumption of Respecta® complex: a randomised controlled pilot study. *Arch Gynecol Obstet*. 2015;292(4):861-867
37. 30 Patras KA, et al. *Streptococcus salivarius* K12 Limits Group B *Streptococcus* Vaginal Colonization. *Infect Immun*. 2015;83(9):3438-3444.
38. Coppi, et al. Results of treatment with *Bacillus subtilis* spores (Enterogermina) after antibiotic therapy in 95 patients with infection calculosis. *Chemioterapia*. 1985;4(6):467-70
39. Dound YA, Jadhav SS, Devale M, Bayne T, Krishnan K. The effect of Probiotic *Bacillus subtilis* HU58 on Immune function in Healthy Human. *The Indian Practitioner*. 2019;70(9):15-20.