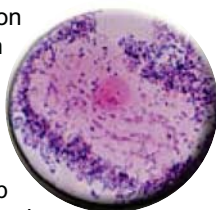


MEDICAL DIAGNOSTIC LABORATORIES, L.L.C.

Infectious Vaginitis

Vaginitis is one of the most common reasons women seek medical attention and accounts for 10 million office visits annually (1). Although common in adult women, it is uncommon in prepubertal girls. The normal vaginal ecosystem is composed of a dynamic relationship between *Lactobacillus* spp. other normal flora and their metabolic by-products, glycogen, estrogen, and vaginal pH (2). Vaginitis occurs as a result of a shift in the vaginal flora. This shift can result either through the introduction of a pathogen or changes in the vaginal ecosystem that allow a pathogen to proliferate or push asymptomatic colonization into symptomatic infection. Despite the variety of causes of vaginitis, in approximately 90% of cases it is thought to occur secondary to a triad of infectious agents including Bacterial Vaginosis (BV), Candidiasis, and Trichomoniasis (2).



BACTERIAL VAGINOSIS

Bacterial vaginosis (BV) is a leading cause of abnormal vaginal discharge and odor and is the cause of costly obstetric and gynecologic infectious complications worldwide. *Lactobacillus* species are the predominant vaginal bacteria during women's reproductive years (3, 4). A large number of commensal bacterial species also inhabit the same niche in healthy women, but at three log-fold lower numbers than those of the *Lactobacillus*. In addition, vaginal microorganisms that are either associated with BV, or are likely etiological agents of BV, can often be detected at low numbers in the absence of BV.

Epidemiology

BV has been studied largely among self-selected women attending a variety of clinics. Since more than 50% of women with BV are asymptomatic, large numbers of women with the condition are not included in studies of this type. The prevalence of BV in participants is similar to those found among non-pregnant women with similar demographic characteristics. The prevalence of BV in the U.S. is highest among African American women and lowest among Asian American women; highest among women with multiple sexual partners and lowest among women with no history of heterosexual contact (2, 5). BV-associated microorganisms, including *Mycoplasma hominis*, *G. vaginalis*, *Mobiluncus* spp., and *Bacteroides fragilis* are recovered from 17% to 52% of urethral cultures from male partners of women with BV. Furthermore, the same *G. vaginalis* biotypes are isolated from heterosexual couples (6, 7).

Pathogenesis

BV does not fulfill Koch's postulates, i.e., no single organism is the etiological agent of this disease. While specific vaginal microorganisms are associated with BV, the current perception is that it results from a complex change in the microbial

ecosystem of the vagina. BV is characterized by a substantive decrease or total elimination of the *Lactobacillus* species and a concomitant multi log-fold increase in the number of facultative and anaerobic Gram-positive and Gram-variable bacteria and other microbes including *Gardnerella vaginalis*, *Bacteroides* spp., *Mobiluncus* spp., *Ureaplasma urealyticum*, and *Mycoplasma hominis*. (4, 8-10).

Recent studies of the vaginal flora using molecular techniques have identified several novel bacterial species that are more prevalent in women with BV compared to women without BV. The first of these species to be identified was *Atopobium vaginae*, which was initially described after isolation from the vagina of a healthy woman (11). Subsequent studies using Polymerase Chain Reaction (PCR)-based detection showed that *A. vaginae* was more prevalent in women with BV than in healthy women, and that the sensitivity and specificity of *A. vaginae* detection in diagnosing BV was comparable to that of *G. vaginalis* detection (12-15). More recently, other fastidious vaginal microorganisms (FVM) including *Megasphaera* and *Eggerthella* species as well as three as of yet unclassified microorganisms termed Bacterial Vaginosis Associated Bacteria (BVAB): BVAB1, BVAB2, and BVAB3, have also been shown by molecular techniques to be prevalent in women with BV (16-17).

The mechanism of how the vaginal flora changes so distinctly in BV is unknown. Lactic acid is produced by vaginal epithelial cells and vaginal microorganisms including *Lactobacillus* spp. Consequently, the vaginal pH in healthy women ranges between pH 3.8 to 4.2. This acidic environment is an important factor in the maintenance of the balance between the commensal and pathogenic microorganisms. Attachment and growth of lactobacilli are favored in the acidic vaginal environment while the attachment of BV-associated microorganisms is reduced (18, 19). Conversely, higher pH tends to displace lactobacilli from vaginal epithelial cell receptor sites and to maximize adherence of *G. vaginalis*. Risk factors for BV include douching, menstruation, and antibiotic use, all of which either directly or indirectly raise the vaginal pH above the optimum for *Lactobacillus*-dominated microflora. The production of hydrogen peroxide by specific species of lactobacilli also appears to play an important role in maintaining the healthy vaginal microflora (19, 20). In vitro studies have demonstrated that hydrogen peroxide-producing lactobacilli are toxic to *G. vaginalis* and *Bacteroides* spp. (17). In addition, the loss of the hydrogen peroxide-producing *Lactobacillus* spp. from the vaginal flora is associated with BV (21).

Cooperative interactions between BV-associated bacteria have been shown. In vitro studies by Pybus and Onderdonk (22) demonstrated a relationship between two of the predominant organisms in BV, *G. vaginalis* and *Bacteroides* spp. (22). Amino acids produced by *G. vaginalis* are utilized by *Bacteroides* to produce ammonia and short chain fatty

acids, such as succinate and isovalerate. The growth of *G. vaginalis* is further enhanced by the presence of ammonia, which is produced during the growth of *Bacteroides*. More recently, it was demonstrated that a biofilm consisting mainly of *A. vaginae* and *G. vaginalis* was present on the vaginal mucosa after metronidazole treatment (23).

Gynecological Complications

BV is related to considerable, and possibly preventable, infectious morbidity in non-pregnant women. The sequelae of BV now include endometritis, pelvic inflammatory disease, post surgical abortion infections, post-hysterectomy infection, increased risk of HIV acquisition, and possibly cervical intraepithelial neoplasia. Constituents of BV are *Bacteroides* spp., *Mobiluncus* spp., *G. vaginalis* and Mycoplasmas, which are all associated with laparoscopically confirmed nonchlamydial, nongonococcal pelvic inflammatory disease (PID) endometritis, salpingitis, and peritonitis (24). Women with PID are 7.5 times more likely to have BV compared with women who do not have PID. Both circumstantial and direct evidence link BV and endometritis. Research by Wolner-Hanssen and co-workers (42) identified BV 3.8 times more often among women using oral contraceptives who were examined for menorrhagia compared with women who did not report this complaint. Two studies by Larsson and co-workers demonstrate resolution of menorrhagia following successful treatment of BV and *Mobiluncus* spp. (25, 26). BV has been shown to significantly increase the risk of postsurgical infection up to four times among women undergoing pregnancy termination and three- to six-times among women having abdominal hysterectomies.

Diagnosis

The diagnosis of BV is complicated by the polymicrobial nature of the condition. In a clinical setting, the most common method of BV diagnosis is based on the observation of specific signs of disease known as the "Amsel criteria":

- Homogeneous, thin adherent gray-white discharge
- Vaginal fluid pH > 4.5
- Release of an amine odor with alkalization of the vaginal fluid
- Presence of vaginal epithelial cells with borders obscured with adherent, small bacteria called "clue" cells

Of the four (4) Amsel criteria, only the presence clue cells is specific for BV. Discharge, odor, and elevated pH are associated with, but are not individually specific for, BV. For example, the presence of semen and *Trichomonas vaginalis* in the vagina also increase vaginal pH.

The Nugent score was proposed as a diagnostic test for BV that required less subjective interpretation than the Amsel criteria (27). Used mostly in a laboratory setting, the Nugent score is a semi-quantitative evaluation of vaginal Gram-positive and Gram-variable bacterial morphotypes by Gram stain that accounts for the numbers of *Lactobacillus*, *Gardnerella vaginalis*, *Prevotella*, and *Mobiluncus* species. In this way, vaginal flora is categorized as normal (*Lactobacillus* predominant), intermediate (mixed flora), and BV (anaerobe predominant) (28). Nugent scores have a sensitivity of 62% to 100%, and a positive predictive value of 76% to 100% compared with Amsel criteria (29). Despite certain advantages over the Amsel criteria, the accurate and reproducible Gram-stain scoring of the bacteria in vaginal smears requires experienced laboratory personnel (30). In addition, variations

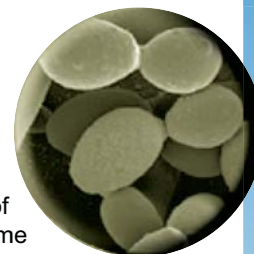
in the vaginal flora including the decline in and loss of *Lactobacillus* species that occur naturally with changing hormonal status in populations such as post-menopausal women, may reduce the usefulness of the Nugent score (31).

Other laboratory methods that are available for the diagnosis of BV include culture, Papanicolaou smears, and PCR-based detection of the microorganisms associated with BV (29). It should be noted, however, that culture of a single microorganism such as *G. vaginalis* does not provide an accurate diagnosis of the clinical condition (8). PCR analysis of BV has a sensitivity of up to 98.5% and a specificity of 95.6% to 100% compared to clinical criteria (32). MDL has introduced an ultrasensitive, PCR-based assay to identify pathogens that are associated with BV.

CANDIDIASIS

Epidemiology

Candidiasis accounts for 20% to 25% of cases of Infectious Vaginitis. It has been estimated that 75% of women will experience at least one (1) episode of vulvovaginal candidiasis in their lifetime (33). A study of female college graduates reported at least one (1) lifetime physician-diagnosed and treated vaginal yeast infection and 20% of women reported infection within the past year (33). Although *Candida albicans* can be recovered from 80% to 90% of women with vulvovaginal candidiasis, non-*albicans* infections are typically associated with recurrence (33). It is believed that widespread use of topical antifungal therapy in short courses may contribute to this selection of non-*albicans* yeast infections due to the fact that they tend to be less susceptible to these agents.



Pathogenesis

It has been proposed that mechanisms of adherence contribute to the virulence of *Candida* species, although the mechanism by which *Candida* produces disease is not well understood. In fact, there tends to be little difference between species isolated from symptomatic patients and asymptomatic carriers (33). However, filamentous forms of *Candida*, possessing hyphae and pseudohyphae, have been observed to penetrate vaginal epithelial cells and are thought to be an important pathologic feature.

Candida species have extraordinary phenotypic plasticity which allows them to adapt to the changing environment within its host. This ability enables *Candida* species to evade the host immune system, increases adhesion to human epithelial cells, and affects drug susceptibility (34). *Candida* species secrete extracellular hydrolytic enzymes, such as aspartyl proteinases, to disrupt host cell membranes in an attempt to facilitate adhesion and tissue invasion (35).

Risk factors for candidiasis include uncontrolled diabetes mellitus, recent antibiotic therapy, immunosuppression, pregnancy, and hormone replacement therapy. Symptoms typically include perivaginal pruritus, vulvovaginal swelling, and dysuria. Tiny papules, called satellite lesions, may surround areas of erythema. Vaginal discharge, when present, is typically thick and white, although it may present as thin and loose resembling discharge associated with other disorders.

Diagnosis

Despite the commonly held belief that patients can intuitively self-diagnose yeast infections, Ferris et al., observed that vulvovaginal candidiasis was confirmed in only 33.7% of women who self diagnosed yeast infection (36). Traditional diagnosis of Candida infection is slow and complicated and relies heavily on microscopic examination, evaluation of KOH preparations, and vaginal culture. The ability to diagnose and identify candidiasis may be enhanced by the use of molecular techniques, such as Polymerase Chain Reaction (PCR).

TRICHOMONIASIS

Epidemiology

Trichomoniasis is caused by the protozoan *Trichomonas vaginalis*. Incidences of Trichomoniasis vary from 11% to 26% infection rates in female sexually transmitted disease (STD) clinics to as high as 50% among newly incarcerated women (36). Approximately 10% to 50% of infected women remain asymptomatic (36). Although traditionally thought of as a sexually transmitted disease, there are occasional incidences of nonvenereal transmission. It has been documented that *T. vaginalis* can survive in moist environments and on fomites for several hours (36).



Symptoms of infection, when present, will often be exacerbated during the menstrual cycle and may include vulvovaginal soreness or irritation, pain upon urination, painful intercourse, inflammation of the external genitalia, abdominal discomfort and a yellow/green foamy discharge which may have a fishlike odor. In women, *T. vaginalis* infection can cause adverse outcomes of pregnancy, cervical neoplasia, and atypical pelvic inflammatory disease. Complications in men include non-gonococcal urethritis, prostatitis, epididymitis, urethral disease, and infertility. *T. vaginalis* has a high association with other sexually transmitted diseases and may increase the risk of HIV transmission in both men and women. Therefore, patients found to be infected with *T. vaginalis* should also be screened for other STDs including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, and HIV.

Pathogenesis

Trichomonas vaginalis colonizes both the male and female urogenital tracts. This protozoan possesses four free anterior flagella and a fifth which is embedded in the undulating membrane. They are responsible for its characteristic twitching motility. The pathogenesis of *T. vaginalis* is due, in part, to multiple adhesion factors which provide an increased ability to cytoadhere to vaginal epithelial cells resulting in micro ulcerations.

Diagnosis

Clinically, the most common approach to diagnosis is by demonstrating the presence of the trichomonad in genital specimens by microscopic visualization in a wet mount preparation. However, this technique will only detect *T. vaginalis* infection in 60% of infected women (36). Due to the fact that *T. vaginalis* infection is associated with a shift in vaginal flora hallmarked by a reduction in Lactobacillus, an increase in anaerobes and *Gardnerella vaginalis*, a pH above 4.5, clue cells upon microscopic examination and a positive whiff test; many women with *T. vaginalis* infection will meet the diagnostic criteria for Bacterial Vaginosis. The use of molecular diagnostic methods, such as Polymerase Chain Reaction (PCR) testing, offers clinicians a highly sensitive and specific means of identification of *Trichomonas vaginalis* with a rapid turnaround time.

MDL has developed highly sensitive and specific Real-Time PCR based assays for the detection of the aforementioned pathogens utilizing the **OneSwab**® platform. Benefits of this system include:

- Real-Time PCR
- Simple & convenient sample collection
- No refrigeration required before or after collection
- Specimen viability up to five (5) days
- Test additions available up to 30 days
- 24-48 hour turnaround time
- High diagnostic specificity and sensitivity
- One vial, multiple pathogens

Table 1. Vaginal Infections: Diagnostic Clues (2, 35, 40)

	Clinical Signs	Discharge Characteristics	Vaginal pH	Microbiology	Sexually Transmitted?
Bacterial Vaginosis	Usually normal appearance of tissue. Discolored vaginal discharge which adheres to the vaginal wall. NOT accompanied by leukorrhea, vulvar burning, or pruritis.	Color: off-white Consistency: creamy Odor: Fishy or musty odor which may increase after sexual intercourse	>4.5	Polymicrobial; mostly normal flora, but can include comma-shaped, gram-variable anaerobic <i>Mobiluncus</i> rods, <i>Gardnerella vaginalis</i> , or clue cells (> 20 % of the epithelial cells), but few WBCs, few lactobacilli. Pap smear may indicate coccobacillary shift of flora.	Probably not
Candida species	Vulvar pruritus, indicating spread of fungus distally onto the vulva. Hyperemic vagina, vulvar and vaginal erythema, or excoriated vulva.	Color: white or off-white if mixed infection present Consistency: "curdled" Odor: not malodorous unless a mixed infection is present	Normal range of 3.8-4.2	Hyphae or budding yeast visible in 50% to 70% of cases. Fungal elements on Pap smear could indicate colonization, not infection.	Patients may infect the glans penis of their partners
Trichomonas vaginalis	Irritation and soreness of the vulva, perineum, and thighs, with dyspareunia and dysuria. "Strawberry cervix" with punctate cervical microhemorrhages visible in 25% of cases. Asymptomatic up to 50% of the time.	Color: greenish-yellow Consistency: Frothy and purulent Odor: foul-smelling	> 4.5 (70% of cases)	Flagellated protozoa visible on wet mount (≈ 60%); Pap smear sensitive for trichomonads (70%).	Yes

References:

1. **Culhane JF, Rauh V, McCollum KF, et al.** 2002. Exposure to chronic stress and ethnic differences in rates of bacterial vaginosis among pregnant women. *Am J Obstet Gynecol.* **187**:1272-6.
2. **Egan ME, Lipsky MS.** Diagnosis of Vaginitis. 2000. *Am Fam Physician.* **62(5)**: 1095-104.
3. **Giorgi A, Torriani S, Dellaglio F, et al.** 1987. Identification of vaginal lactobacilli from asymptomatic women. *Microbiologica.* **10**:377-84.
4. **Spiegel CA.** 1991. Bacterial vaginosis. *Clin Microbiol Rev.* **4**:485-502.
5. **Goldenberg RL, Iams JD, Mercer PJ, et al.** 1998. The preterm prediction study: the value of new vs. standard risk factors in predicting early and all spontaneous preterm births. NICHD MFMU Network. *Am J Public Health.* **88**:233-8.
6. **Piot P, Van Dyck E, Peeters M, et al.** 1984. Biotypes of *G. vaginalis*. *J Clin Microbiol.* **20**:677-9.
7. **Zarifard MR, Saifuddin M, Sha BE, et al.** 2002. Detection of bacterial vaginosis-related organisms by real-time PCR for Lactobacilli, Gardnerella vaginalis and Mycoplasma hominis. *FEMS Immunol Med Microbiol.* **34**: 277-81.
8. **Eschenbach DA.** 1989. Bacterial vaginosis: emphasis on upper genital tract complications. *Obstet Gynecol Clin North Am.* **16**:593-610.
9. **Hillier SL, Holmes KK.** 1990. Bacterial Vaginosis, p. 547- 559. In K. K. Holmes, P.-A. Mardh, and P. F. Sparling (ed.), Sexually Transmitted Diseases, vol. 2nd. McGraw-Hill Inform. Services Co., NY.
10. **Larsen B, Monif GR.** 2001. Understanding the bacterial flora of the female genital tract. *Clin Infect Dis.* **32**:e69-77.
11. **Rodriguez JM, Collins MD, Sjoden B, et al.** 1999. Characterization of a novel *Atopobium* isolate from the human vagina: description of *Atopobium vaginae* sp. nov. *Int J Syst Bacteriol.* **49**:1573-6.
12. **Hebb JK, Cohen CR, Astete SG, et al.** 2004. Detection of novel organisms associated with salpingitis, by use of 16S rDNA polymerase chain reaction. *J Infect Dis.* **190(12)**:2109-20.
13. **Verstraelen H, Verhelst R, Claeys G, et al.** 2004. Culture-independent analysis of vaginal microflora: the unrecognized association of *Atopobium vaginae* with bacterial vaginosis. *Am J Obstet Gynecol.* **191(4)**:1130-2.
14. **Ferris MJ, Maszta A, Aldridge KE, et al.** 2004. Association of *Atopobium vaginae*, a recently described metronidazole resistant anaerobe, with bacterial vaginosis. *BMC Infect Dis.* **4**:5.
15. **Bradshaw CS, Tabrizi SN, Fairley CK, et al.** 2006. The association of *Atopobium vaginae* and *Gardnerella vaginalis* with bacterial vaginosis and recurrence after oral metronidazole therapy. *J Infect Dis.* **194**:828-36.
16. **Fredricks DN, Fiedler TL, Thomas KK, et al.** 2007. Targeted Polymerase Chain Reaction for the Detection of Vaginal Bacteria Associated with Bacterial Vaginosis. *J Clin Microbiol.* **45(10)**:3270-6.
17. **Thies FL, Konig W, Konig B.** 2007. Rapid characterization of the normal and disturbed vaginal microbiota by application of 16S rRNA gene terminal RFLP fingerprinting. *J Med Microbiol.* **45(10)**:3270-6.
18. **Hillier SL, Holmes KK.** 1990. Bacterial Vaginosis, p. 547- 559. In K. K. Holmes, P.-A. Mardh, and P. F. Sparling (ed.), Sexually Transmitted Diseases, vol. 2nd. McGraw-Hill Inform. Serv. Co., NY.
19. **Redondo-Lopez V, Cook RL, Sobel JD.** 1990. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis.* **12**:856-72.
20. **Culhane JF, Rauh V, McCollum KF, et al.** 2002. Exposure to chronic stress and ethnic differences in rates of bacterial vaginosis among pregnant women. *Am J Obstet Gynecol.* **187**:1272-6.
21. **Klebanoff SJ, Hillier SL, Eschenbach DA, et al.** 1991. Control of the microbial flora of the vagina by H₂O₂-generating lactobacilli. *J Infect Dis.* **164(1)**:94-100.
22. **Pybus V, Onderdonk AB.** 1997. Evidence for a commensal, symbiotic relationship between Gardnerella vaginalis and Prevotella bivia involving ammonia: potential significance for bacterial vaginosis. *J Infect Dis.* **175**:406-13.
23. **Swidsinski A, Mendling W, Loening-Baucke V, et al.** 2008. An adherent Gardnerella vaginalis biofilm persists on the vaginal epithelium after standard therapy with oral metronidazole. *Am J Obstet Gynecol.* **198(1)** 97.e1-97.e6.
24. **Sweet RL.** 1995. Role of bacterial vaginosis in pelvic inflammatory disease. *Clin Infect Dis.* **20 Suppl 2**:S271-5.
25. **Goldenberg R L, Iams JD, Mercer BM, et al.** 1998. The preterm prediction study: the value of new vs. standard risk factors in predicting early and all spontaneous preterm births. NICHD MFMU Network. *Am J Public Health.* **88**:233-8.
26. **Hillier SL.** 1993. Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol.* **169**:455-9.
27. **Nugent RP, Krohn MA, Hillier SL.** 1991. Reliability of diagnosing bacterial vaginosis is improved by a standardized method of gram stain interpretation. *J Clin Microbiol.* **29(2)**:297-301.
28. **Klebanoff SJ, Hillier SL, Eschenbach DA, et al.** 1991. Control of the microbial flora of the vagina by H₂O₂-generating lactobacilli. *J Infect Dis.* **164**:94-100.
29. **Hillier SL.** 1993. Diagnostic microbiology of bacterial vaginosis. *Am J Obstet Gynecol.* **169**:455-9.
30. **Forsum U, Jakobsson T, Larsson PG, et al.** 2002. An international study of the interobserver variation between interpretations of vaginal smear criteria of bacterial vaginosis. *Apmis.* **110(11)**:811-8.
31. **Cauci S, Driussi S, De Santo D. et al.** 2002. Prevalence of bacterial vaginosis and vaginal flora changes in peri- and postmenopausal women. *J Clin Microbiol.* **40(6)**:2147-52.
32. **Korn AP, Bolan G, Padian N, et al.** 1995. Plasma cell endometritis in women with symptomatic bacterial vaginosis. *Obstet Gynecol.* **85**:387-90.
33. **Mandell GL, Bennett JE, Dolin R.** Mandell, Douglas, and Bennett's Principles and Practices of Infectious Diseases, 6th edition, Vol 2. Philadelphia: Churchill Livingstone; 2005:2269-1357-71.
34. **Calderone RA.** 2002. Candida and Candidiasis. ASM Press, Washington, DC. P. 123-42.
35. **Naglik JR, Challacombe SJ, Hube B.** 2003. Candida albicans secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev.* **67(3)**: 400-28.
36. **Giorgi A, Torriani S, Dellaglio F, et al.** 1987. Identification of vaginal lactobacilli from asymptomatic women. *Microbiologica.* **10**:377-84.
37. **Eschenbach DA.** 1989. Bacterial vaginosis: emphasis on upper genital tract complications. *Obstet Gynecol Clin North Am.* **16**:593-610.
38. **Platz-Christensen JJ, Sundstrom E, Larsson PG.** 1994. Bacterial vaginosis and cervical intraepithelial neoplasia. *Acta Obstet Gynecol Scand.* **73**:586-8.
39. **Thomason JL, Gelbart SM, Anderson RJ, et al.** 1990. Statistical evaluation of diag. criteria for bacterial vaginosis. *Am J Obstet Gynec.* **162**:155-60.
40. **Wolner-Hanssen P, Westrom L.** 1983. Second-look laparoscopy after acute salpingitis. *Obstet Gynecol.* **61**:702-4.
41. **Plourd DM.** 1999. Practical Guide to Diagnosing and Treating Vaginitis. Accessed online October 10, 2008 at: <http://www.medscape.com/viewarticle/408848>.

