VOLUME - XVI
ISSUE - XCIII
MAY/JUN 2019

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Vaginitis, also known as vulvovaginitis, is inflammation of the vagina and vulva. Symptoms may include itching, burning, pain, discharge, and a bad smell. Certain types of vaginitis may result in complications during pregnancy.

The three main causes are infections, specifically bacterial vaginosis, vaginal yeast infection, and trichomoniasis. Other causes include allergies to substances such as spermicides or soaps or as a result of low estrogen levels during breast-feeding or after menopause. More than one cause may exist at a time. The common causes vary by age.

Diagnosis generally include examination, measuring the pH, and culturing the discharge. Other causes of symptoms such as inflammation of the cervix, pelvic inflammatory disease, cancer, foreign bodies, and skin conditions should be ruled out.

Treatment depends on the underlying cause. Infections should be treated. Sitz baths may help with symptoms. Soaps and feminine hygiene products such as sprays should not be used. About a third of women have vaginitis at some point in time. Women of reproductive age are most often affected. The **"DISEASE DIAGNOSIS"** segment delves deep into the various CLINOCODIAGNOSTIC aspects of Vaginitis.

"INTERPRETATION" portion highlights a very common disorder termed as BACTERIAL VAGINOSIS. Though not dangerous in itself, it predisposes to other sinister STDs.

What we are discussing in **"INTERPRETATION"** is all about bacteria and the Primary investigation when t dealing with bacteria is GRAM's STAIN. Hence **"TROUBLESHOOTING"** part of this issue discusses GRAM's STAIN. What can possibly go wrong, how to identify and thereafter rectify the issues involved.

The fourth fixed component., viz., **"BOUQUET"** is very much there. Flip over please.

DISEASE DIAGNOSIS

VAGINITIS

PRACTICE ESSENTIALS

Vaginitis (inflammation of the vagina) is the most common gynecologic condition encountered in the office. It is a diagnosis based on the presence of symptoms of abnormal discharge, vulvovaginal discomfort, or both. Cervicitis may also cause a discharge and sometimes occurs with vaginitis. Discharge flows from the vagina daily as the body's way of maintaining a normal healthy environment. Normal discharge is usually clear or milky with no malodor. A change in the amount, color, or smell; irritation; or itching or burning could be due to an imbalance of healthy bacteria in the vagina, leading to vaginitis. The most common causes of vaginitis in symptomatic women are bacterial vaginosis (40-45%), vaginal candidiasis (20-25%), and trichomoniasis (15-20%); yet 7-72% of women with vaginitis may remain undiagnosed. The workup for patients with vaginitis depends on the risk factors for infection and the age of the patient. Accurate diagnosis may be elusive, and care must be taken to distinguish vaginitis from other infectious and noninfectious causes of symptoms. All women presenting with abnormal vaginal discharge should have a careful pelvic examination. Condition-specific tests (ie, colposcopy and cervical biopsies) are indicated for suspected cervical cancer. Studies that may be performed in cases of suspected vaginitis include saline wet mount, the so-called whiff test, pH testing, culture, nucleic acid amplification testing, and a number of other second-line tests (see Presentation, DDx, and Workup). Treatment of vaginitis varies by cause and is directed at the relevant pathogen. Inpatient care usually is not indicated, unless serious pelvic infections arise or evidence of systemic infection in an immunocompromised host is present (see Treatment).

PATHOPHYSIOLOGY

A complex and intricate balance of microorganisms maintains the normal vaginal flora. Important organisms include lactobacilli, corynebacteria, and yeast. Aerobic and anaerobic bacteria can be cultured from the vagina of prepubertal girls, pubertal adolescents, and adult women. A number of factors can change the composition of the vaginal flora, including the following:

- Age
- Sexual activity (or abuse)
- Hormonal status
- Hygiene
- Immunologic status
- Underlying skin diseases

The normal postmenarchal and premenopausal vaginal pH is 3.8-4.2. At this pH, growth of pathogenic organisms usually is inhibited. Disturbance of the normal vaginal pH can alter the vaginal flora, leading to overgrowth of pathogens. Factors that alter the vaginal environment include feminine hygiene products, contraceptives, vaginal medications, antibiotics, sexually transmitted diseases (STDs), sexual intercourse, and stress. The overgrowth of normally present bacteria, infecting bacteria, or viruses can cause symptoms of vaginitis. Chemical irritation also can be a significant factor. Atrophic vaginitis is associated with hypoestrogenism, and symptoms include dyspareunia, dryness, pruritus, and abnormal bleeding. A state of decreased estrogen can result in an altered risk of infection. Based on data from 11 countries, Kenyon and Colebunders found evidence that the risk of bacterial vaginosis is increased in women whose male sexual partner is concurrently having sexual relations with other partners. The age of the patient affects the anatomy and physiology of the vagina. Prepubertal children have a more alkaline vaginal pH than do pubertal and postpubertal adolescents and women. The vaginal mucosa is squamous epithelium, vaginal mucous glands are absent, the normal vaginal flora is similar to that of postmenopausal women (eg, gram-positive cocci and anaerobic gram-negatives are more common), and the labia are thin with a thin hymen. Pubertal and postpubertal adolescents and women have a more acidic vaginal pH, a stratified squamous vaginal mucosa, vaginal mucous glands, a normal vaginal flora dominated by lactobacilli, thick labia, and hypertrophied hymens and vaginal walls. Loss of vaginal lactobacilli appears to be the primary factor in the changes leading to bacterial vaginosis. Recurrences of vaginitis are associated with a failure to establish a healthy vaginal microflora dominated by lactobacilli.

ETIOLOGY

Approximately 90% of all cases of vaginitis are thought to be attributable to 3 causes: bacterial vaginosis, vaginal candidiasis (or vulvovaginal candidiasis [VVC]), and *Trichomonas vaginalis* infection (trichomoniasis). Bacterial vaginosis is the most common cause of vaginitis, accounting for 50% of cases. As previously mentioned, bacterial vaginosis is caused by an overgrowth of organisms such as *Gardnerella vaginalis* (a gram-variable coccobacillus), *Mobiluncus* species, *Mycoplasma hominis*, and *Peptostreptococcus* species. Risk factors include pregnancy, intrauterine device (IUD) use, and frequent douching. Candida species (including *C albicans, C tropicalis*, and *C glabrata*) are airborne fungi that are natural inhabitants of the vagina in as many as 50% of women. Vaginal candidiasis is the second most common cause of vaginitis. In 85-90% of cases, it is caused by *C albicans*, and in 5-10%, it is caused by *C glabrata* or *C parapsilosis*. Risk factors include oral contraceptive use, IUD use, young age at first intercourse, increased frequency of intercourse, receptive cunnilingus, diabetes, HIV or other immunocompromised states, long-term antibiotic use, and pregnancy. *T vaginalis* infection, the third most common cause of vaginitis, is caused by trichomonads. *T vaginalis* is an oval-shaped or fusiform-shaped flagellated protozoan that is 15 µm long (the size of a leukocyte). These organisms primarily infect vaginal epithelium; less commonly, they infect the endocervix, urethra, and Bartholin and Skene glands. Trichomonads are transmitted sexually and can be identified in as many as 80% of male partners of infected women. Risk factors include tobacco use, unprotected intercourse with multiple sexual partners, and the use of an IUD. A study by Mercer et al suggested that symbionts, commensals, and concomitant infections impact the adaptive immune response to *T vaginalis*, finding that the presence of *M hominis* in vitro led to greater diversity in the inflammatory cytokine secretion response to *T vaginalis*. Noninfectious vaginitis is usually due to allergic reaction or irritation. Another common cause is atrophic vaginitis due to estrogen deficiency. Common preventable causes of candidal vaginitis or bacterial vaginosis include damp or tight-fitting clothing, scented detergents and soaps, feminine sprays, and poor hygiene. For related information, see the Women's Sexual Health Resource Center.

EPIDEMIOLOGY

Vaginitis is common in adult women and uncommon in prepubertal girls. Bacterial vaginosis accounts for 40-50% of vaginitis cases; vaginal candidiasis, 20-25%; and trichomoniasis, 15-20%. In US women of childbearing age, bacterial vaginosis is the most common vaginal infection. An estimated 7.4 million new cases of bacterial vaginosis occur

each year. National data show that the prevalence is 29%. However, the rate varies in different subpopulations: it is 5-25% in college students and 12-61% in patients with STDs. In the United States, as many as 16% of pregnant women have bacterial vaginosis. A 50-60% prevalence is found in female prison inmates and commercial sex workers. Eighty-five percent of those with bacterial vaginosis are asymptomatic. More than a billion dollars is estimated to be spent annually on both self-treatment and visits to a medical provider. An estimated 3 million cases of trichomoniasis occur each year in the United States. The worldwide prevalence of trichomoniasis is 174 million; these cases account for 10-25% of all vaginal infections.

Age- and race-related demographics

All age groups are affected. The highest incidence is noted among young, sexually active women. Vaginitis affects all races. The highest incidence of bacterial vaginosis is in blacks (23%), and the lowest is in Asians (6%). Prevalence increases with age among non-Hispanic black women. The incidence is 9% in whites and 16% in Hispanics.

PROGNOSIS

Overall, the prognosis is very good: most of those infected are cured. However, recurrent vaginal infections can lead to chronic irritation, excoriation, and scarring. These, in turn, can lead to sexual dysfunction. Psychosocial and emotional stresses are not uncommon. Although treatment of bacterial vaginosis has not been documented to prevent HIV, bacterial vaginosis and sexually transmitted infections, including trichomoniasis, are considered to be risk factors for HIV. Chronic vaginal infection can facilitate the transmission of various STDs, including HIV. Complications of bacterial vaginosis include endometritis and pelvic inflammatory disease (PID). Untreated bacterial vaginosis may result in complications (eg, vaginal wound infections) after gynecologic surgical procedures. In pregnancy, *Trichomonas* infection and bacterial vaginosis are associated with an increased risk of adverse pregnancy outcomes, including preterm labor, premature rupture of membranes, preterm delivery, low birth weight, and postpartum endometritis.

PATIENT EDUCATION

Safe sex and STD counseling may help decrease the rates of reinfection. Discuss further preventive efforts, including proper hygiene and toilet techniques, when it is appropriate to do so. Remind patients that douching can spread a vaginal or cervical infection into the uterus, increasing the likelihood of PID; douching can also be associated with endometritis. Educate patients regarding use of topical creams for treatment of vaginitis (eg, candidal vaginitis, bacterial vaginosis) as necessary.

HISTORY

A carefully documented history is vital for establishing the diagnosis. Adults and children must be questioned regarding specific aspects of the symptoms of vaginitis. Essential information to obtain during the history includes the onset of symptoms, previous occurrences, associated abdominal pain, trauma, and urinary or bowel symptoms. Vaginal bleeding in prepubertal females is always abnormal and warrants a full investigation. In adults, as noted (see Etiology), the most common conditions resulting in symptoms of vaginitis include vaginal candidiasis, trichomoniasis, and bacterial vaginosis; accordingly, particular attention should be paid to symptoms suggesting these possible causes. Patients with vaginitis almost always present with a chief complaint of abnormal vaginal discharge. Ascertain the following attributes of the discharge:

- Quantity
- Duration
- Color
- Consistency
- Odor

Obtain a history of the following:

- Previous similar episodes
- Sexually transmitted infection
- Sexual activities
- Birth control method
- Last menstrual period
- Douching practice
- Use of personal hygiene products
- Antibiotic use
- General medical history
- Systemic symptoms (eg, lower abdominal pain, fever, chills, nausea, and vomiting)

Bacterial vaginosis

Bacterial vaginosis is asymptomatic in up to 50% of women. If a discharge is present, it is typically thin, homogeneous, malodorous, and grayish white or yellowish white in color. Vaginal pain or vulvar irritation is uncommon. Pruritus may occur. Bacterial vaginosis is common in pregnant women and is associated with preterm birth. In pregnant women with symptomatic bacterial vaginosis who have a history of preterm birth, administration of treatment early in pregnancy has been shown to decrease the incidence of preterm birth.

Vaginal candidiasis

Candidiasis is a fungal infection common in women of childbearing age. Pruritus is the most common symptom. This is accompanied by a thick, odorless, white vaginal discharge (with an appearance similar to that of cottage cheese), which can be minimal. Usually, associated vulvar candidiasis is present, commonly with vulvar burning, dyspareunia, and vulvar dysuria (a burning sensation arising when urine comes into contact with vulvar skin). Patients often have a history of recurrent yeast infection or recent antibiotic treatment. Symptoms of candidiasis often begin just before menses. Precipitating factors include immunosuppression, diabetes mellitus, pregnancy, and hormone replacement therapy. Candidiasis is usually not contracted from a sexual partner. About 75% of all women have at least 1 episode of candidiasis in their lifetime. Recurrent episodes may indicate underlying immunodeficiency or diabetes.

Trichomoniasis

T vaginalis infection is the most common nonviral STD in the world. Many patients (20-50%) are asymptomatic. If discharge is present, it is usually copious and frothy and can be white, gray, yellow, or green (the yellow and green colors are due to the presence of white blood cells [WBCs]). Local pain and irritation are common. Dysuria (20%), pruritus (25%), and postcoital bleeding due to cervicitis are other possible symptoms. Symptoms often peak just after menses. Trichomoniasis is associated with risk factors for other STDs; accordingly, a history of multiple sexual partners should be elicited. Infection during pregnancy has been associated with preterm deliveries and low-birth-weight infants. Trichomoniasis is rare in prepubertal children. Sexual abuse should be suspected if symptoms are present. Symptoms include a copious frothy discharge, local pain, irritation, and, occasionally, pruritus.

Other conditions

In women with chronic vaginitis, atrophic vaginitis and hypoestrogenism must be considered. Elicit an accurate menstrual history, along with statuses such perimenopause, postmenopause, postpartum, and lactation. Ask about medications such as depot leuprolide (Lupron) and

antiestrogen medications used for breast cancer. Vulvovaginitis has multiple nonvenereal causes in prepubertal children; however, if a vaginal discharge suggests an STD, question all children (or their caretakers) regarding possible sexual abuse. Symptoms of vulvovaginitis in prepubertal girls generally include localized pain, dysuria, pruritus, erythema, and discharge. Bacteria that can cause vulvovaginitis include streptococcal species (including group A streptococci), *Escherichia coli*, and *Shigella sonnei*. Symptoms (eg, pharyngitis and diarrhea) may result from infections in areas of the body other than the vagina. A *Shigella* infection may result in a bloody vaginal discharge without symptoms of diarrhea. A patient with group A streptococcal infection may present with itching or painful defecation. Purulent discharge may develop insidiously. Viral infections may cause symptoms of vulvovaginitis. Elicit a history of recent viral infections, including varicella. Herpes simplex viruses (HSVs), particularly HSV-1 transmitted via autoinoculation from the oral mucosa, might be present; elicit a history of recurrent oral herpes or digital herpes in the caretaker of a child in diapers. If candidal vulvovaginitis is considered (it is rare in healthy prepubertal girls), the history should include recent antibiotic use, possible diabetes mellitus, immunosuppression, and underlying skin disease. Ask about a family history of mucocutaneous candidiasis. Consider helminthic infections (eg, *Enterobius vermicularis* infections) resulting in pruritus of the genital area. Ask about contact with pinworm-infected children, itching (particularly at night), and vaginal pain. Ask questions to exclude the possibility of a foreign body in the vagina, chemical irritation (eg, recent bubble baths, washing hair with shampoo while bathing, douching, use of feminine hygiene sprays, colored or scented toilet papers, panty liners), latex, semen, mechanical irritation, and poor hygiene. Foreign bodies in the vagina result in a persistent, foul-smelling, serosanguineous discharge. Contact dermatitis from unusual exposures may occur; ask about this possibility and about bathing patterns. Obtain a history of recent trauma to the vaginal area and a history of urination and defecation patterns and problems to exclude possible anatomic abnormalities (eg, rectovaginal fistula). Lichen sclerosis et atrophicus may be seen in prepubertal children and in postmenopausal women. Symptoms of chronic fissures, pain, or pruritus are often present. Rectal fissures may lead to chronic constipation in children.

VAGINITIS CLINICAL PRESENTATION

Physical Examination

The physical examination of pubertal and adult women should include a complete pelvic examination. The Tanner stage of development should be noted. The examination for prepubertal girls should be performed as described in Pediatrics, Child Sexual Abuse.

Bacterial vaginosis

Physical findings in bacterial vaginosis include a homogeneous, frothy vaginal discharge that is grayish-white to yellowish-white in color. The discharge appears adherent to the vaginal mucosa. Typically, no underlying erythema exists. As many as 50% of women with bacterial vaginosis are asymptomatic. Bacterial vaginosis can be diagnosed if 3 of the following 4 Amsel criteria are present (see Workup):

- Homogeneous, white, adherent discharge
- Vaginal pH higher than 4.5
- Amine (fishy) smell from vaginal discharge when potassium hydroxide (KOH) is added (whiff test)
- Clue cells on wet mount

Vaginal candidiasis

Vaginal candidiasis may present with a well-demarcated erythema of the vulva with satellite lesions (discrete pustulopapular lesions) surrounding the redness. The vulva, vagina, and surrounding areas may be edematous and erythematous, possibly accompanied by excoriations and fissures. A thick, adherent, cottage cheese–like vaginal discharge may be seen. The cervix usually appears normal.

Trichomoniasis

In trichomoniasis, the vulva may appear erythematous and edematous, with excoriation. Look for a copious, frothy, homogeneous vaginal discharge that can be white, gray, yellow, or green. Small punctate cervical and vaginal hemorrhages with ulcerations may be observed. So-called strawberry cervix, or colpitis macularis, is highly specific for *Trichomonas* infection, and 2-5% of patients will have this finding on examination. Because diagnosis of *Trichomonas* infection on the basis of clinical signs and symptoms is unreliable, laboratory confirmation is mandatory.

Other conditions

Physical findings associated with cervicitis from STDs include excessive vaginal discharge, erythema, and edema of the cervix. Fever, cervical motion, or abdominal or adnexal tenderness may indicate upper genital tract infection (eg, cervicitis or PID). Cervical ectopy or eversion may cause discharge with no apparent infectious cause. Physical findings associated with atrophy, dysplasia, and vulvar vestibulitis syndrome include localized atrophy or infection in skin and mucous membranes. In about 50% of all cases of mucopurulent discharge in women, the etiology is unknown. Vaginal foreign bodies in adults include forgotten tampons; in children, pieces of toilet tissue typically are found. Findings of foul odor and irritation with a purulent discharge are common. A patient with pinworms may present with few physical findings. Occasionally, there may be erythema and excoriations around the perianal area. In severe cases, eggs or dead female nematodes may be seen on examination of the anal area. Perianal streptococcal dermatitis usually results in a beefy-red perineal area that is edematous and tender. Fissures, drainage, and hemorrhagic spotting may be present.

Complications

Bacterial vaginosis has been associated with pelvic inflammatory disease (PID), endometritis, and vaginal cuff cellulitis when invasive procedures have been performed. Such procedures include endometrial biopsies, cesarean section, uterine curettage, and intrauterine device (IUD) placement. During pregnancy, bacterial vaginosis and trichomoniasis are associated with an increased risk of premature rupture of membranes, preterm labor, low birth weight, and preterm delivery. Systemic disease resulting from the spread of gonorrhea may occur.

VAGINITIS DIFFERENTIAL DIAGNOSES

Diagnostic Considerations

In addition to the conditions listed in the differential diagnosis, other problems to be considered include the following:

- Atrophic vaginitis
- Cervical polyp
- Contact dermatitis
- *Entamoeba histolytica* infection
- Excessive desquamation of normal vaginal epithelium
- Large cervical ectropion
- Lichen sclerosis
- Lichen simplex chronicus
- Vaginal adenosis
- Vaginal cancer

- Vaginal intraepithelial neoplasia
- Vaginal ulcers
- Vaginitis emphysematosa (multiple gas-filled cysts on the vaginal and cervical mucosa)

Differential Diagnoses

- Cervicitis
- Child Sexual Abuse in Emergency Medicine
- Cystitis, Nonbacterial
- Cytomegalovirus (CMV)
- Foreign Bodies, Rectum
- Herpes Simplex
- Pinworms in Emergency Medicine
- Postpartum Infections
- Rehabilitation for Paget Disease
- Salmonella Infection in Emergency Medicine
- Sexual Assault
- Ureaplasma Infection
- Urinary Tract Infection, Female
- Varicella-Zoster Virus (VZ)

VAGINITIS WORKUP

Approach Considerations

The workup for patients with vaginitis depends on the risk factors for infection and the age of the patient. All women presenting with abnormal vaginal discharge should have a careful pelvic examination. Condition-specific tests (ie, colposcopy and cervical biopsies) are indicated for suspected cervical cancer. Studies that may be performed in cases of suspected vaginitis include saline wet mount, the so-called whiff test, pH testing, culture, nucleic acid amplification testing, and a number of other second-line tests.

Saline Wet Mount

In a saline wet mount test, a drop of vaginal discharge is placed on a slide with 1-2 drops of 0.9% isotonic sodium chloride solution and examined under high power (×400). This test is 60% sensitive and 98% specific for bacterial vaginosis. Clue cells are vaginal epithelial cells covered with many vaginal rods and cocci bacteria, creating a stippled or granular appearance. A decreased number of lactobacilli are observed, and white blood cells (WBCs) are absent. In patients with vaginal candidiasis, the test reveals hyphae and budding yeast forms. In symptomatic women with trichomoniasis, saline wet mount is 80-90% sensitive for *T vaginalis* infection. Large numbers of WBCs (>10 per high power field [hpf]) and epithelial cells are observed.

Whiff Test

In the whiff test, vaginal discharge is placed on a slide with 10% potassium hydroxide (KOH) solution. A positive test result is the release of an amine (fishy) odor after the addition of KOH to the discharge. The odor is due to the release of amines such as putrescine, cadaverine, histamine, and trimethylamine. Bacterial vaginosis is associated with an intense amine odor on this test; however, the whiff test is not highly sensitive or specific for diagnosing this condition. A negative whiff test result is 65%-85% sensitive for candidal infection; as many as 30% of symptomatic candidiasis cases show false-negative results. The whiff test may be positive with *Trichomonas* vaginitis. Vaginal pH can be determined with litmus paper. A pH greater than 4.5 is often found in patients with *Trichomonas* infection or bacterial vaginosis (84-97% sensitivity, 57-78% specificity). Recent intercourse, douching, cervical mucus, and blood can lead to false-positive results.

- Bacterial vaginosis - pH is 5.0-6.0
- Vaginal candidiasis - pH is less than 4.5
- *T vaginalis* infection - pH is 5.0-7.0

Cultures

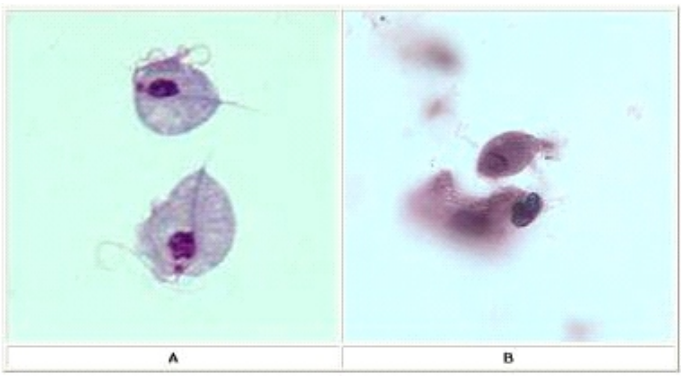
Cultures are of little use in diagnosing bacterial vaginosis and therefore are not generally indicated or recommended in this setting. Cultures with Nickerson or Sabouraud mediums should be performed in refractory or recurrent cases of vaginal candidiasis. Culture using Diamond medium or Trichosel broth is recommended for detection of trichomonads and should be used when infection is suspected but cannot be confirmed by other means. InPouch TV is 90-95% specific and 100% sensitive for culturing *T vaginalis*. Gonorrhea usually causes a cervicitis, not a vaginitis, and may be asymptomatic. Symptomatic *Neisseria gonorrhoeae* infection usually results in a purulent discharge. Obtain cultures of the vagina (in prepubertal patients), cervix (in pubertal and adult patients), oral pharynx, and rectum if gonococcal vulvovaginitis is suspected. Obtain cultures by using a cotton-tipped swab and Thayer-Martin media on chocolate agar, incubated in a carbon dioxide–rich environment. Test for chlamydial vulvovaginitis via culture in prepubertal girls and in patients who show signs of abuse or sexual assault. Obtain rectal *Chlamydia* swabs.

Nucleic Acid Amplification

The use of nucleic acid amplification tests (NAATs) has been implemented in many office and emergency settings. Tests such as polymerase chain reaction (PCR) can be performed by using swabs of the cervix or vagina or by collecting a urine sample. NAATs may be performed as a screen in pubertal and adult women. They may also be used for initial screening in prepubertal children, but in view of medicolegal concerns, confirmation testing should be ensured. DNA amplification assays of genital tract specimens are both sensitive and specific. First-void urine specimens for NAATs have also been shown to be sensitive and specific in females. They are less invasive than swabs, and with confirmation (eg, repeat testing with a different NAAT), urine NAATs may be used for the evaluation of chlamydial infection and gonorrhea in cases of suspected sexual abuse. Although NAATs are generally performed to test for these common sexually transmitted diseases, their utilization for the diagnosis of bacterial vaginosis has also been studied, and they have been shown to be potentially more sensitive and specific than Gram staining and clinical diagnosis. The Affirm DNA hybridization method is 80% sensitive for *Trichomonas* and 94% sensitive for bacterial vaginosis. Oligonucleotide probes detect high (> 10/mL) concentrations of *Gardnerella vaginalis* and can also can detect *Candida*. Antigen-detecting immunoassays, the Trichomonas Rapid Test (an enzyme-linked immunosorbent assay [ELISA] strip test with 80% sensitivity), DNA probes, and PCR are useful for detecting trichomonads.

Staining (Giemsa, Papanicolaou, Schiff)

Gram stain is 89-97% sensitive and 79-85% specific for detecting bacterial vaginosis. On Gram stain, clue cells are identified as epithelial cells covered by small gram-negative rods. Gram stain or culture on Nickerson media and Sabouraud agar may enhance diagnosis of vaginal candidiasis. The Papanicolaou test (Pap smear) may have frequent false-positive results for yeast. The Papanicolaou test is not accurate in the diagnosis of Trichomonas infections: Pap smears may reveal trichomonads but have high false-positive and false-negative rates. *T vaginalis* may be identified with Giemsa staining of in vitro culture specimens (see the image on next page).



(A) Two trophozoites of *Trichomonas vaginalis* obtained from in vitro culture, stained with Giemsa. (B) Trophozoite of *T vaginalis* in vaginal smear, stained with Giemsa.

Other Tests

The latex agglutination test employs polyclonal antibodies reactive against multiple species of *Candida*. Gas-liquid chromatography can be used to detect the succinate-to-lactate ratio in vaginal fluid to assist in the diagnosis of bacterial vaginosis. Succinate and lactate are metabolites produced by anaerobic gram-negative rods and lactobacilli, respectively. A cross-sectional study that involved 1,740 patients with vaginitis symptoms by Gaydos et al reported that a vaginal swab molecular-based test collected by patients by themselves or by clinicians can accurately diagnose most common bacterial, fungal, and protozoan causes of vaginitis. The vaginal swab test sensitivity for bacterial vaginosis was 90.5% (95% confidence interval [CI] 88.3-92.2%) and specificity was 85.8% (95% CI 83.0-88.3%). For Candida, the test sensitivity was 90.9% (95% CI 88.1-93.1%) and specificity was 94.1% (95% CI 92.6-95.4%) and test sensitivity was 93.1% (95% CI 87.4-96.3%) and specificity was 99.3% (95% CI 98.7-99.6%) for trichomoniasis.

Histologic Findings

T vaginalis infection can be confused with koilocytotic atypia, caused by the human papillomavirus, and may mimic findings of mild dysplasia. Bacterial vaginosis may produce inflammation and atypical squamous cells of undetermined significance (ASCUS) on Papanicolaou tests. In addition, bacterial vaginosis may be linked with cervical intraepithelial neoplasia (CIN).

VAGINITIS TREATMENT & MANAGEMENT

Approach Considerations

Treatment of vaginitis may include sitz baths and instruction regarding proper toilet and hygiene techniques. Many women assume vaginal symptoms are the result of a sexually transmitted disease (STD), which is often not the case. A patient's idea of vaginal normality may be inaccurate and result in increased or unnecessary treatment seeking. Also educate patients regarding the following:

- Avoiding irritants in the vaginal area, such as perfumes, soaps, and panty liners, among others.
- After swimming or exercise, which keeps the vaginal area moist, air-drying the area or changing the underwear.
- Always cleaning the area from front to back.

Management considerations

Intravaginal imidazoles (see Pharmacologic Therapy) can be purchased over the counter and have proven efficacy for vaginal candidiasis. Patients may purchase and utilize these medications without a doctor's advice or prescription and the choice of treatment can be based on personal preference since they appear to be equally effective. Vaginal anti-itch creams provide only symptomatic relief. Homeopathic treatments for vaginitis (boric acid, tea tree oil, live acidophilus, garlic) have not been well studied but may have some efficacy. If the patient shows no improvement, despite symptomatic or over-the-counter treatment, refer her for further workup of possible STDs and other infectious causes of vulvovaginitis. When a patient is seen for suspected vaginitis in the emergency department (ED), there is usually no need for active treatment. However, prepubertal girls with vulvovaginitis caused by a foreign body in the vagina may require sedation for removal of the foreign body. Treatment of vaginitis varies by cause and is directed at the relevant pathogen. Inpatient care usually is not indicated, unless serious pelvic infections arise or evidence of systemic infection in an immunocompromised host is present. Parenteral treatment of infectious causes of vaginitis is rarely indicated. Complicated cases of certain infections (eg, gonorrhea, chlamydial infection) may require parenteral treatment. A German study has suggested that a 6-day vaginal application of dequalinium chloride (10 mg) is a safe and effective treatment option for mixed vaginal infections or those with an uncertain diagnosis. The investigators noted this antimicrobial antiseptic agent has broad bactericidal and fungicidal activity with a low risk for antimicrobial resistance and posttreatment vaginal infections. Atrophic vaginitis can be treated with lubricants, estrogen vaginal cream, tablets, and rings, among others. A study by Shen et al indicated that low-dose estrogen therapy in atrophic vaginitis causes a rise in the proportion of *Lactobacillus* species in the vaginal microbiome while reducing the proportion of *Gardnerella*. In addition, serum estradiol levels rose four-fold in the study, vaginal pH was reduced, and the vaginal maturation index almost doubled. Vaginal suppositories containing human *Lactobacillus* strains are under investigation, as are changes in formulation strategies to improve pharmacologic delivery and treatment modalities.

Other issues to consider

Patients who are immunocompromised, such as those with HIV infection, should be treated with the same regimens as other patients. Before initiating treatment with any drugs that should not be used during pregnancy, determine the possibility of pregnancy, test for pregnancy as appropriate, and maintain proper documentation. However, pregnancy should not delay treatment. In cases of suspected sexual assault or child sexual abuse, proper documentation may assist with possible subsequent legal action.

PHARMACOLOGIC THERAPY

Bacterial vaginosis

Recommended regimens for bacterial vaginosis include the following:

- Metronidazole 500 mg orally twice a day for 7 days
- Metronidazole gel 0.75%, 1 full applicator (5 g) intravaginally, once a day for 5 days
- Clindamycin cream 2%, 1 full applicator (5 g) intravaginally at bedtime for 7 days

Alternative regimens include the following:

- Clindamycin 300 mg orally twice a day for 7 days
- Clindamycin ovules 100 mg intravaginally once at bed time for 3 days

Patients should be advised to avoid alcohol consumption during and 24 hours after treatment with metronidazole. Clindamycin cream is oil-based and might weaken latex condoms and diaphragms for 5 days after use. Clindamycin should not be used in the second half of pregnancy. Routine follow-up visits are unnecessary. Routine treatment of sex partners is not recommended. The recurrence rate is 20-40% after 1 month. Twice weekly metronidazole gel for 6 months may reduce

recurrences. Regimens for pregnant women with bacterial vaginosis include the following:

- Metronidazole 500 mg orally twice a day for 7 days
- Metronidazole 250 mg orally 3 times a day for 7 day
- Clindamycin 300 mg orally twice a day for 7 day

Pregnant women should have a follow-up visit 1 month after completion of treatment. Treatment regimens in patients with HIV are the same as in patients without HIV, but bacterial vaginosis appears to be more persistent in women who are HIV positive. Therapy is not recommended for male partners, but female partners of women with BV should be examined and treated.

Vaginal candidiasis

For the purposes of treatment, vaginal candidiasis, also referred to as vulvovaginosis candidiasis (VVC), may be broadly classified as either complicated or uncomplicated, as follows:

- Uncomplicated - Sporadic or infrequent VVC; mild-to-moderate VVC likely to be caused by *C albicans* and occurring in nonimmunocompromised women
- Complicated - Recurrent VVC; severe VVC; VVC caused by a species other than *C albicans* or occurring in immunocompromised women

Recommended regimens for intravaginal agents are as follows:

- Butoconazole 2% cream 5 g intravaginally for 3 days
- Butoconazole 2% cream 5 g (butoconazole 1 sustained release), single intravaginal application
- Clotrimazole 1% cream 5 g intravaginally for 7-14 days
- Clotrimazole 100 mg vaginal tablet for 7 days
- Clotrimazole 100 mg vaginal tablet, 2 tablets for 3 days
- Miconazole 2% cream 5 g intravaginally for 7 days
- Miconazole 100 mg vaginal suppository, 1 suppository for 7 days
- Miconazole 200 mg vaginal suppository, 1 suppository for 3 days
- Miconazole 1200 mg vaginal suppository, 1 suppository for 1 day
- Nystatin 100,000 unit vaginal tablet, 1 tablet for 14 days
- Terconazole 80 mg vaginal suppository, 1 suppository for 3 days

The recommended regimen for the oral agent fluconazole is a 150 mg oral tablet in a single dose. It should be kept in mind that the oil-based cream and suppositories might weaken latex condoms. Patients are instructed to return only if symptoms persist or recur within 2 months of the onset of initial symptoms. Routine treatment of sex partners is not indicated.

Recommendations for complicated VVC are as follows:

- Recurrent VVC (≥4 episodes of symptomatic VVC in 1 y) - 7-10 days of topical therapy or a 100 mg or 150 mg oral dose of fluconazole every third day for a total of 3 doses (days 1, 4, and 7); for maintenance, oral fluconazole 100 mg / 150 mg weekly for 6 months
- Severe VVC - 7-14 days of topical azole therapy or 150 mg of oral fluconazole repeated in 72 hours; adjunctive use of nystatin cream or low-potency steroid cream may be beneficial
- Non-*albicans* VVC - 7-14 days of nonfluconazole therapy; 600 mg of boric acid in a gelatin capsule vaginally twice daily for 14 days
- VVC in compromised hosts - 7-14 days of topical therapy
- VVC in pregnant patients - 7 days of topical agents; fluconazole is contraindicated

Trichomoniasis

Recommended regimens for *T vaginalis* infection include the following:

- Metronidazole 2 g orally in a single dose (or 500 mg orally twice a day for 7 days)
- Tinidazole 2 g orally in a single dose

Metronidazole is the treatment of choice both for patients who are immunocompetent and for those who are immunocompromised. Because trichomonads often infect the urethra and the Skene and Bartholin glands, metronidazole gel is considerably less efficacious than an oral preparation; therefore, use of the gel is not recommended. Sex partners of patients with *T vaginalis* infection should be treated, and intercourse should be avoided until both partners have been treated and are asymptomatic. Pregnant women with trichomoniasis may be treated with 2 g of metronidazole in a single dose. Lactating women should withhold breastfeeding during treatment and for 12-24 hours after the last dose of metronidazole. For women taking tinidazole, breastfeeding should be interrupted during treatment and for 3 days after the last dose.

Topical treatment with nonoxynol-9 and povidone-iodine douches has been shown to be effective in treating *T vaginalis* infection in women unable to use metronidazole. Further studies are needed to confirm this preliminary finding. A vaccine containing killed "aberrant lactobacilli" is available in Europe. This vaccine has not been evaluated in well-controlled, double-blind prospective trials

DIET AND ACTIVITY

Acidophilus supplements in the diet may help prevent vaginitis, especially if patients are taking antibiotics. In addition, an increase in the intake of garlic seems to help vaginitis symptoms and prevention. Patients should be instructed to abstain from sexual activity and from douching until a diagnosis has been made. Patients also should abstain from unprotected sexual activity (sexual activity without proper male condom use) until the infection has been treated. Reducing simple carbohydrates, refined foods, and alcohol helps to reduce frequent/persistent yeast infection.

PREVENTION

Although safe sexual practices have not extensively evaluated as means of preventing vaginitis, they may play a role in reducing the incidence of bacterial vaginosis and *T vaginalis* infections. Good hygiene, avoiding tight undergarments, wearing 100% cotton underwear, and keeping the area dry also may play a role in preventing candidal infections. No studies show any benefit to douching as a treatment or prevention for vaginitis; douching may actually exacerbate symptoms. Tampon use does not seem to be associated with vaginitis.

LONG-TERM MONITORING

In asymptomatic women, follow-up care is not indicated. However, in women who are pregnant or have recurrent infections, a follow-up evaluation should be performed 1 month after completion of treatment. Techniques of proper genital hygiene should be recommended. Refer for sexual abuse evaluation all children in whom vaginitis was caused by an STD (see Pediatrics, Child Sexual Abuse). Treat sexual partners of patients with identified STDs. Consider treatment of partners in cases of trichomoniasis. In addition, consider treatment of partners in cases of bacterial vaginosis if chronic or recurrent infections develop. In cases of recurrent or resistant vaginal candidiasis, yeast culture, glucose intolerance test, and HIV testing should be offered. In women with recurrent candidal vaginitis that requires longer fluconazole therapy, fluconazole resistance may be related to overexpression of Plr1 protein (Pir1p).

Medication Summary

The goals of pharmacotherapy in vaginitis are to reduce morbidity, prevent complications, and eradicate the infection. Drugs used for infectious causes of vaginitis may be applied topically or may require oral or parenteral administration.

INTERPRETATION

BACTERIAL VAGINOSIS

Important Vaginal Pathogens include: *Trichomonas vaginalis. Chalamydia* infections, yeast infections and bacterial vaginosis. While *Neisseria gonorrhoea* can be isolated from the vagina, a cervical swab is a superior specimen for detecting gonnococcus (G.C.) Bacterial vaginosis is characterized by a thin malodorous vaginal discharge due to an alteration of bacterial flora caused by a shift in the acidity (pH) of the vagina. It's referred to as a 'vagin**osis**' rather than an 'vagin**itis**' because '**itis**' implies an inflamation which would be accompanied by a proliferation of white blood cells (wbc's). Here, in a bacteria vaginosis, the bacteria flourish without a significant increase in wbc's.

A SEVERAL MARKERS PROVIDE CLUES IN THE DIAGNOSIS OF BACTERIAL VAGINOSIS.

- **Potassium hydroxide** - a drop added to sample of the discharge produces a fishy odour due to amines released.
- **pH measurement** - loss of normal acidity (generally considered >4.5) may suggest a vaginosis diagnosis, however it is not very definitive therefore not usually done in the lab.
- Observation and **bacterial enumeration** of genera present in a vaginal gram stain. Vaginosis is characterized by a decrease by the lactic acid producing lactobacilli (*hence the name*) and an increase in the specific organisms, *Gardnerella vaginalis, Mobiluncus* species, and anaerobic organisms such as *Bacterioides* & *Prevotella* species. The relative proportion and or presence of these organisms can suggest bacterial vaginosis.
- **Clue Cells** - the presence of epithelial cells stippled with gram variable bacilli is suggestive of bacterial vaginosis. In Scanning the gram stain, the presence of these epithelial cells coated with gram variable bacilli appear purplish in comparison to the regular pinkish-red epithelial cells present. Why these bacteria tend to adhere is not fully understood.

SPECIFIC DESCRIPTION OF BACTERIAL FORMS PRESENT

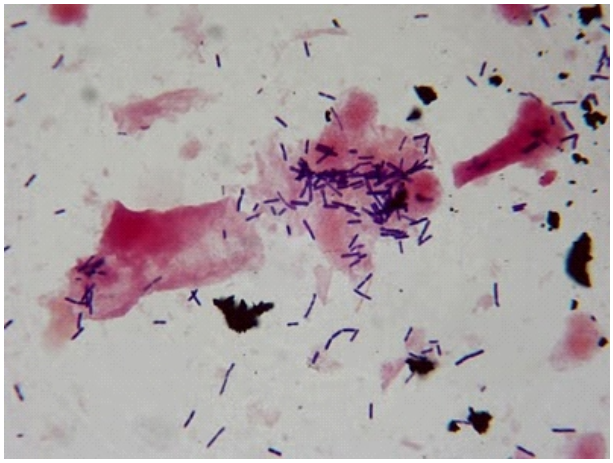
- Probably the most noticeable bacterial form present in bacterial vaginosis is referred to as 'gram variable' bacilli. These are the ***Gardnerella vaginalis*** bacteria whose individual cells can stain both purple and red with the gram stain. That is, some entire cells may be either gram positive (purple-blue) or gram negative (pinkish-red) or both. They are relatively small or short cells. The irregular gram staining properties historically have contributed to placing the organisms in different genera. Previously called both *Corynebacterium vaginale* (a gram positive genera), and *Haemophius vaginalis*, (a gram negative genera), before being given it's own genera of *Gardnerella*. It is a somewhat fastidious organism.
- ***Mobiluncus* species** - various species of the genus may be present in bacterial vaginosis and are observed, if present, as gram negative curved bacilli.
- **Anaerobic bacteria** such as ***Bacteroides*** and ***Prevotella*** also are disproportionate and can be seen as smaller straight gram negative bacilli

The bacteria mentioned above may be present in the normal vagina in small amounts. When whatever causes the conditions to change, these bacteria can overgrow the normal flora and therby cause vaginosis.

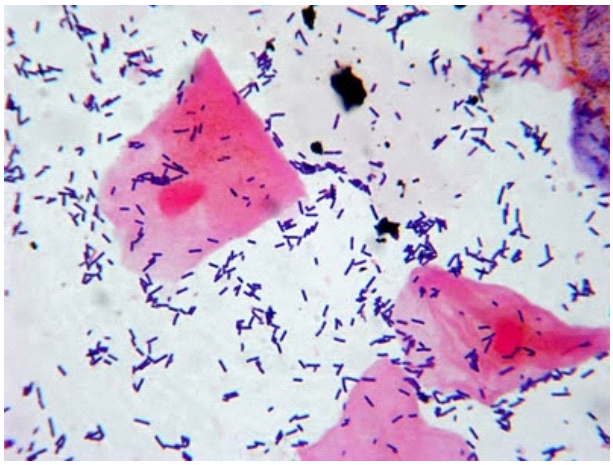
- ***Lactobacillus* species** are regular gram positive bacilli (purple-blue rods) which can vary in shape depending on the species and environment. The lactic acid they produce within the healthy vagina keep it slightly acidic which restricts the growth of other species of bacteria.

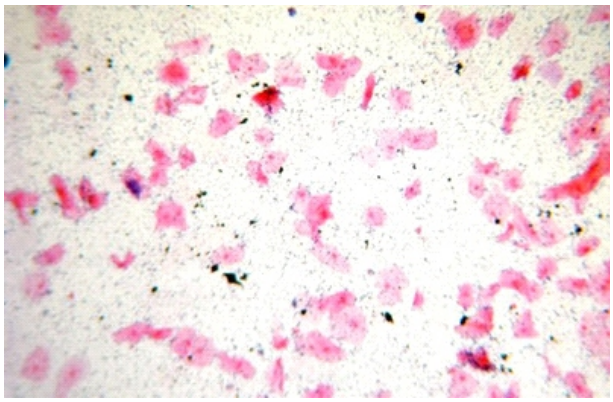
A guide for enumerating the proportion of the various bacteria forms present in a vaginal smear has been created and is known as the Nugent Score. By scoring the various bacterial forms and noting the presence of Clue Cells, one can standardize the criteria for determining if the patient does indeed have bacterial vaginosis. While bacterial vaginosis is a genital, or perhaps a sexual disease, it is not considered a sexually transmitted disease. Sexual contact is not responsible for it's presence nor is it spread through sexual contact. It arises from changes within host allowing for the disproportionate proliferation of native bacterial species.

Now for some photos of what is described above;

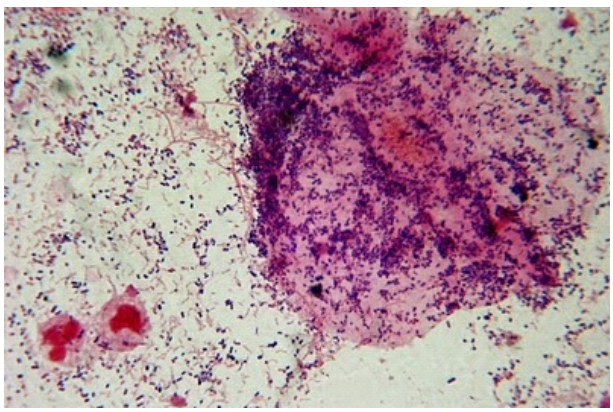


Normal vaginal fora
Gram stain showing a couple of epithelial cells and numerous gram positive lactobacilli (purple rods). Note too that there are few if any white blood cells. Above & Below
(100X, Gram Stain,)

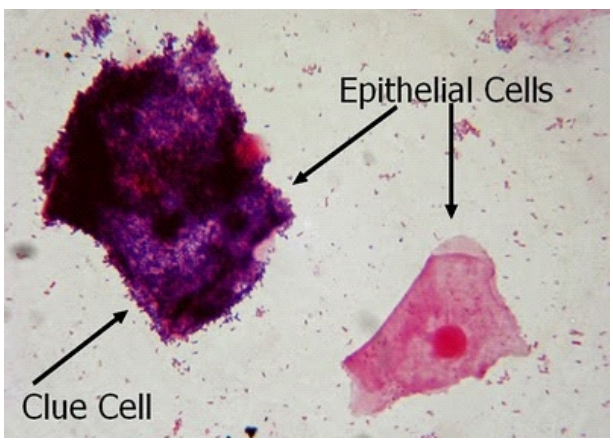




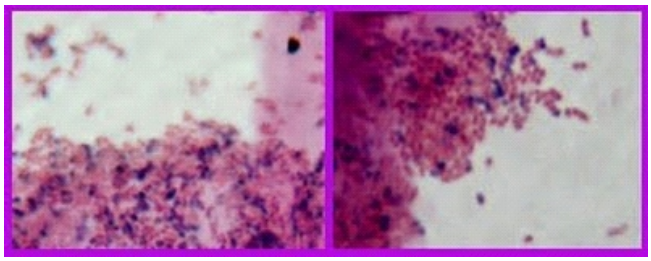
Normal Vaginal Flora
All epithelial cells appear similar with no 'Clue Cells' which would appear bluish due to all the *Gardnerella vaginalis* organisms adhering to the cells.
(100X, Gram Stain,)



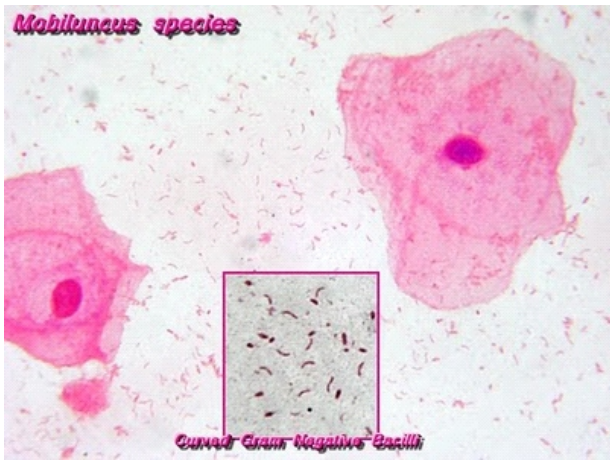
Gram of bacterial vaginosis
Gram stain (1000X enlarged) showing epithelial cell coated with gram variable bacilli (Clue Cell). Note gram variable bacilli (*Gardnerella vaginalis*), gram negative curved bacilli (*Mobiluncus* sp.) & regular gram negative bacilli. Straight gram positive lactobacilli as seen in the previous photo are absent.
(1000X, Gram Stain,)



'Clue Cell' in vaginal swab gram stains. Here two epithelial cells are seen however the one on the left is 'coated' with gram variable bacilli (*Gardnerella vaginalis*) making the cell appear purplish. On lower power scanning, these are usually seen with some frequency in a patient experiencing bacterial vaginosis.
(1000X, Gram Stain,)

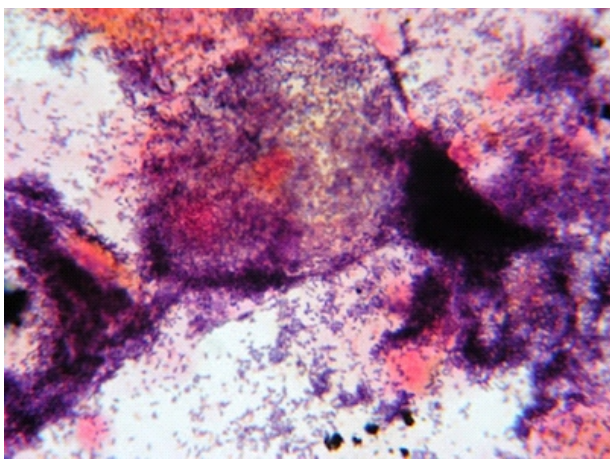


A couple enlarged (1000X) gram stains of gram variable bacilli. Small, short cells are gram variable -gram negative (pink), gram positive (blue) or both at once.

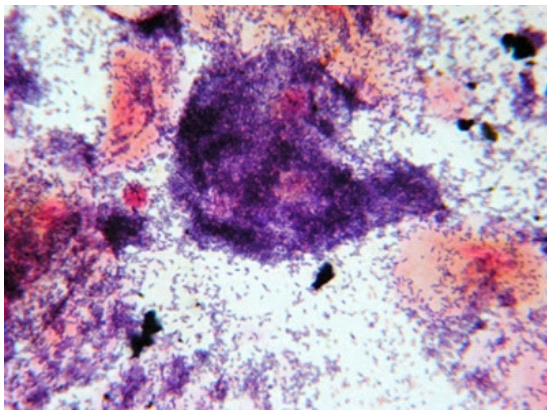


Gram Stain (1000X) of *Mobiluncus* species (curtisii)
Mobiluncus species contribute to Bacterial vaginosis. They appear as gram negative (pink) curved bacilli - (look like pink parentheses) Present are also two large pink epithelial cells

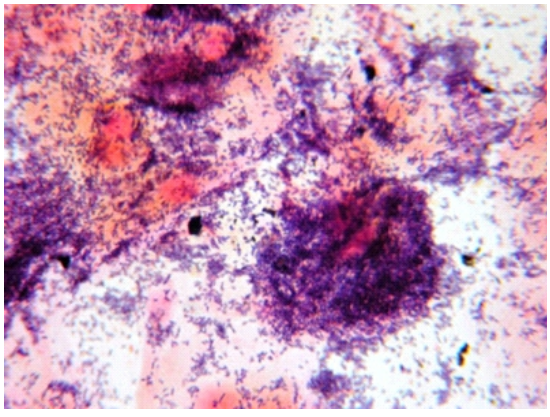
The next series of photos illustrate the condition of bacterial vaginosis where normal vaginal flora is greatly diminished and replaced primarily by *Gardnerella vaginalis* but possibly also *Mobiluncus curtisii* and other organisms such as the anaerobic gram negative *Bacteroides* or *Prevotella* species.



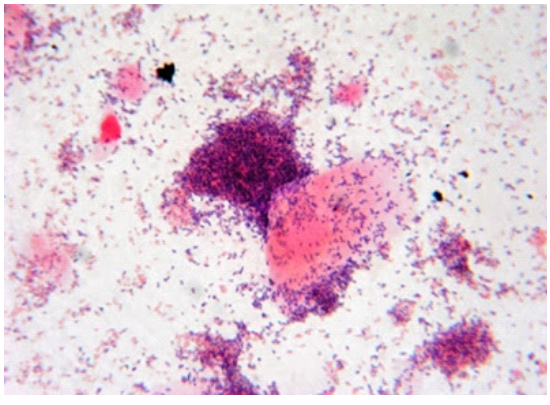
Bacterial vaginosis - usually appears as rather 'granular' (my personal description) as the entire field is usually stippled with the tiny gram-variable *Gardnerella vaginalis*. Compare this appearance to the normal vaginal flora which follows shortly below.
(1000X, Gram Stain,)



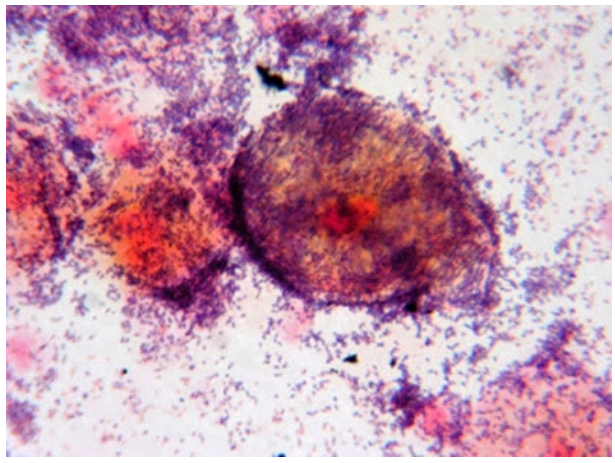
Bacterial vaginosis - as above, a very 'granular' appearance throughout the field with epithelial cells and epithelial cells stippled with *Gardnerella vaginals* (Clue Cell).
(1000X, Gram Stain,)



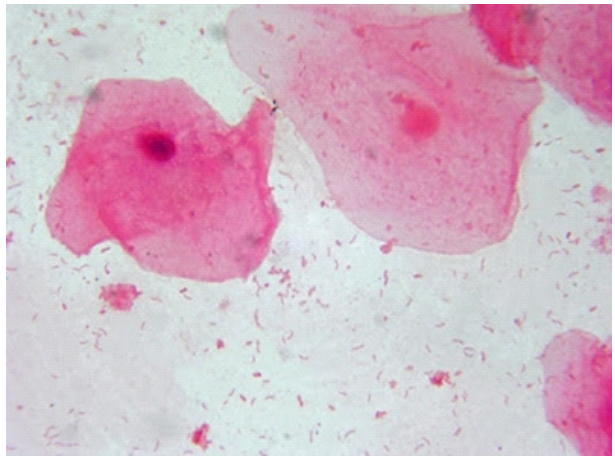
Bacterial vaginosis - distinctive granular appearance. Entire microscope field is often filled with these small gram-variable *Gardnerella vaginalis* usually appearing primarily purple-blue from the crystal violet in the gram stain. The Nugent score which assists in making a definitive decision on whether the flora present indicate bacterial vaginosis is really not necessary when the specimen looks as above.
(1000X, Gram Stain,)



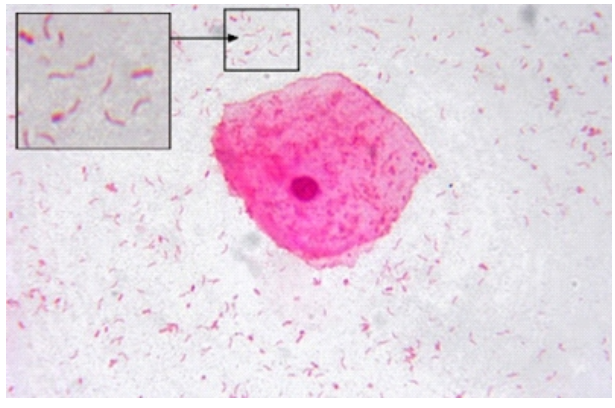
Bacterial vaginosis - one epithelial cell stippled (covered) with the small gram-variable *Gardnerella vaginalis* (clue cell) and one epithelial cell showing less coverage, equal to numbers you see throughout the field.
(1000X, Gram Stain,)



Bacterial vaginosis - primarily with the gram-variable *Gardnerella vaginalis*.
(1000X, Gram Stain,)



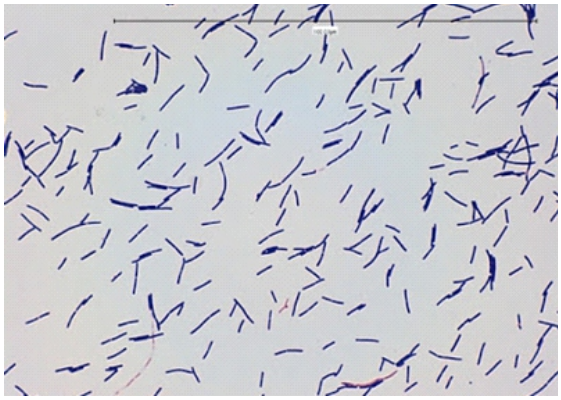
Mobiluncus species - appear as small curved gram negative bacilli. They may be present in variable quantities in bacterial vaginosis.
(100X, Gram Stain,)



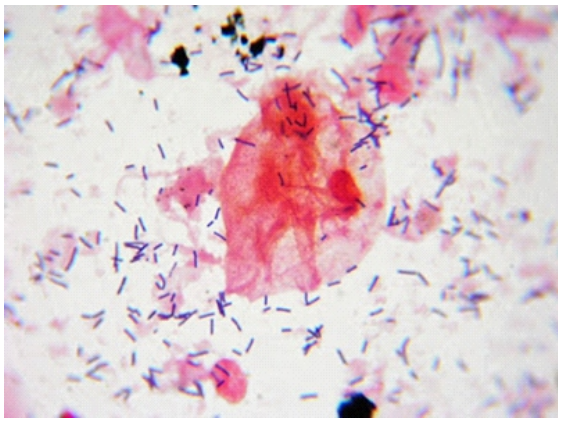
Mobiluncus species - another view showing the rather small, curved gram-negative bacilli which may accompany *Gardnerella vaginalis* in bacterial vaginosis.
(1000X, Gram Stain,)

Gram stains of normal vaginal fora and its consituents follow;

Lactobacilli - *Lactobacillus* species are the predominant flora found in the vagina and appear as gram postivie (purple-blue) rods. Lactic acid produced by these bacteria lowers the pH of the vagina and normal creates an environment which is not favorable to the growth of many other bacteria including *Gardnerella vaginalis*. When the natural occurring commensal flora changes, invading organisms can capitalize and displace and invade.
(100X, Gram Stain,)

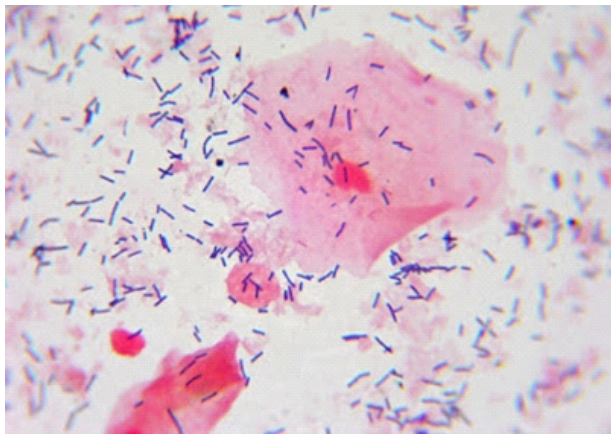


Lactobacillus species - another view as above. These should constituent the primary flora of the human vagina.
(1000X, Gram Stain,)

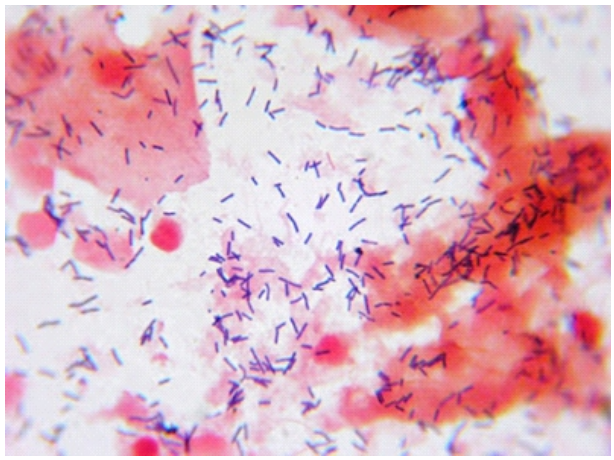


Appearance of normal vaginal flora - Here we see an single epithelial cell in the center of the photo and numerous larger gram positive bacilli (rods). The appearance of this photo is distinctly different from the previous bacterial vaginosis examples.

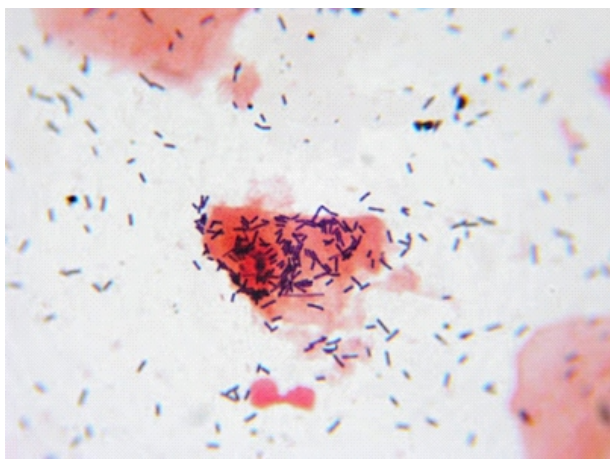
The overall appearance is not so 'granular' as the tiny *Garnerella vaginalis* is not present, or at least present in any appreciable quantities. While white blood cells may be seen, they are not present in large quantities (pus) which differentiates 'vaginitis' from 'vaginosis'.
(1000X, Gram Stain,)



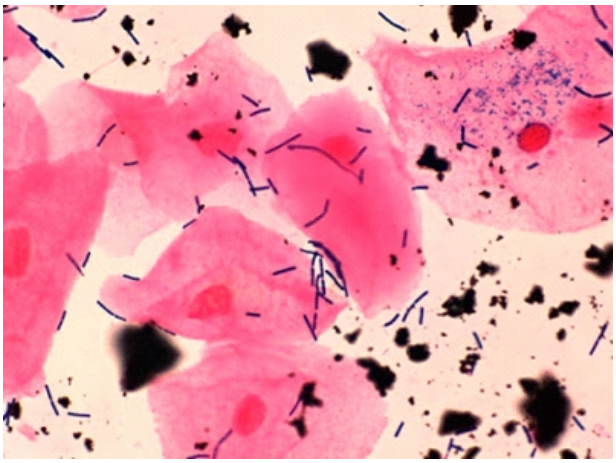
Appearance of normal vaginal flora
(1000X, Gram Stain,)



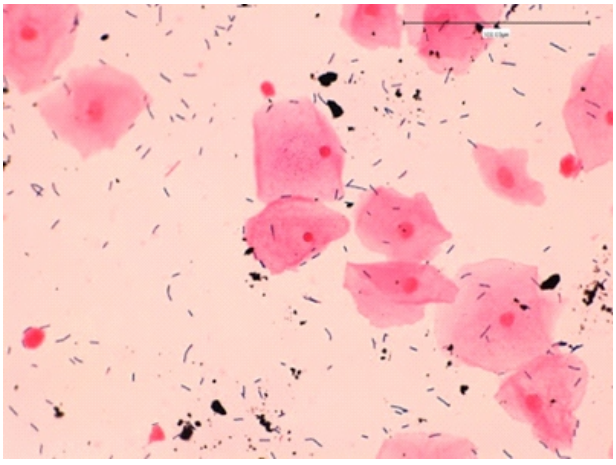
Appearance of normal vaginal flora - lactobacilli, epithelial cells and occasional white blood cells.
(1000X, Gram Stain,)



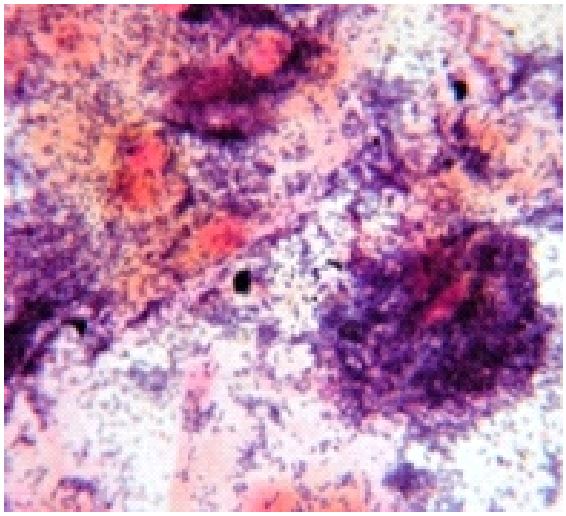
Appearance of normal vaginal flora - lactobacilli accumulated around an epithelial cell, not to be confused with 'clue cells'.
(1000X, Gram Stain,)



Appearance of normal vaginal flora -numerous epithelial cells with lactobacilli distributed throughout the microscopic field. No WBC's seen in this photo.
(1000X, Gram Stain,)

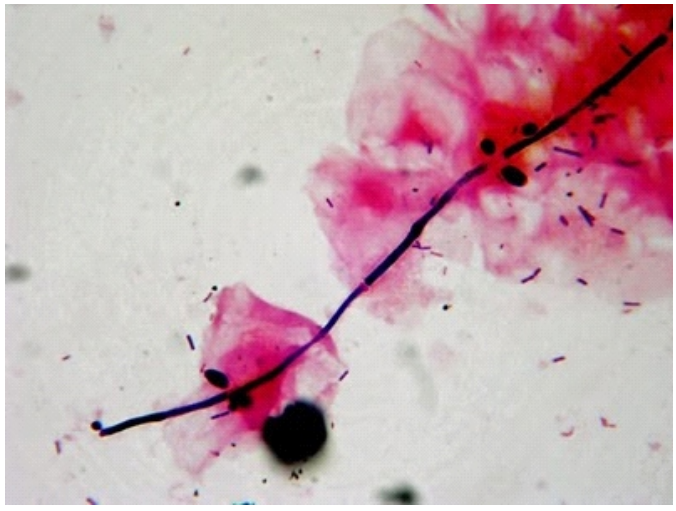


Appearance of normal vaginal flora - numerous epithelial cells, occasional wbc's and the comparably (to *G.vaginalis*) larger gram positive lactobacilli throughout the field.
(400X, Gram Stain,)



Bacterial vaginosis
For direct comparison
(Both 1000X, Gram Stain)

Typical normal vaginal gram stain
For direct comparison
(Both 1000X, Gram Stain)



Just for comparison: Vaginal Gram Stain of Yeast Infection (1000X)

Note: epithelial cells with yeast cells (purple) and yeast pseudohyphae (long purple thread-like structure).

TROUBLESHOOTING

THE GRAM STAIN

Gram staining is an empirical method of differentiating bacterial species into two large groups (Gram-positive and Gram-negative) based on the chemical and physical properties of their cell walls. The method is named after its inventor, the Danish scientist Hans Christian Gram (1853-1938), who developed the technique in 1884 (Gram 1884). The importance of this determination to correct identification of bacteria cannot be overstated as all phenotypic methods begin with this assay.

THE BASIC METHOD

1. First, a loopful of a pure culture is smeared on a slide and allowed to air dry. The culture can come from a thick suspension of a liquid culture or a pure colony from a plate suspended in water on the microscope slide. Important considerations:
   - Take a small inoculum—don't make a thick smear that cannot be completely decolorized. This could make gram-negative organisms appear to be gram-positive or gram-variable.
   - Take a fresh culture—old cultures stain erratically.
2. Fix the cells to the slide by heat or by exposure to methanol. Heat fix the slide by passing it (cell side up) through a flame to warm the glass. Do not let the glass become hot to the touch.
3. Crystal violet (a basic dye) is then added by covering the heat-fixed cells with a prepared solution. Allow to stain for approximately 1 minute.
4. Briefly rinse the slide with water. The heat-fixed cells should look purple at this stage.
5. Add iodine (Gram's iodine) solution (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye, making it more difficult to decolorize and reducing some of the variability of the test.
6. Briefly rinse with water.
7. Decolorize the sample by applying 95% ethanol or a mixture of acetone and alcohol. This can be done in a steady stream, or a series of washes. The important aspect is to ensure that all the color has come out that will do so easily. This step washes away unbound crystal violet, leaving Gram-positive organisms stained purple with Gram-negative organisms colorless. The decolorization of the cells is the most "operator-dependent" step of the process and the one that is most likely to be performed incorrectly.
8. Rinse with water to stop decolorization.
9. Rinse the slide with a counterstain (safranin or carbol fuchsin) which stains all cells red. The counterstain stains both gram-negative and gram-positive cells. However, the purple gram-positive color is not altered by the presence of the counter-stain, it's effect is only seen in the previously colorless gram-negative cells which now appear pink/red.
10. Blot gently and allow the slide to dry. Do not smear.

What's Going On?

Bacteria have a cell wall made up of peptidoglycan. This cell wall provides rigidity to the cell, and protection from osmotic lysis in dilute solutions. Gram-positive bacteria have a thick mesh-like cell wall, gram-negative bacteria have a thin cell wall and an outer phospholipid bilayer membrane. The crystal violet stain is small enough to penetrate through the matrix of the cell wall of both types of cells, but the iodine-dye complex exits only with difficulty (Davies et al. 1983). The decolorizing mixture dehydrates cell wall, and serves as a solvent to rinse out the dye-iodine complex. In Gram-negative bacteria it also dissolves the outer membrane of the gram-negative cell wall aiding in the release of the dye. It is the thickness of the cell wall that characterizes the response of the cells to the staining procedure. In addition to the clearly gram-positive and gram-negative, there are many species that are "gram-variable" with intermediate cell wall structure (Beveridge and Graham 1991). As noted above, the decolorization step is critical to the success of the procedure. Gram's method involves staining the sample cells dark blue, decolorizing those cells with a thin cell wall by rinsing the sample, then counterstaining with a red dye. The cells with a thick cell wall appear blue (gram positive) as crystal violet is retained within the cells, and so the red dye cannot be seen. Those cells with a thin cell wall, and therefore decolorized, appear red (gram negative). It is a prudent practice to always include a positive and negative control on the staining procedure to confirm the accuracy of the results (Murray et al 1994) and to perform proficiency testing on the ability of the technicians to correctly interpret the stains (Andserson, et al. 2005).

Excessive Decolorization

It is clear that the decolorization step is the one most likely to cause problems in the gram stain. The particular concerns in this step are listed below (reviewed in McClelland 2001)

1. **Excessive heat during fixation:** Heat fixing the cells, when done to excess, alters the cell morphology and makes the cells more easily decolorized.
2. **Low concentration of crystal violet:** Concentrations of crystal violet up to 2% can be used successfully, however low concentrations result in stained cells that are easily decolorized. The standard 0.3% solution is good, if decolorization does not generally exceed 10 seconds.
3. **Excessive washing between steps:** The crystal violet stain is susceptible to wash-out with water (but not the crystal violet-iodine complex). Do not use more than a 5 second water rinse at any stage of the procedure.
4. **Insufficient iodine exposure:** The amount of the mordant available is important to the formation of the crystal violet – iodine complex. The lower the concentration, the easier to decolorize (0.33% – 1% commonly used). Also, QC of the reagent is important as exposure to air and elevated temperatures hasten the loss of Gram's iodine from solution. A closed bottle (0.33% starting concentration) at room temperature will lose >50% of available iodine in 30 days, an open bottle >90%. Loss of 60% iodine results in erratic results.
5. **Prolonged decolorization:** 95% ethanol decolorizes more slowly, and may be recommended for inexperienced technicians while experienced workers can use the acetone-alcohol mix. Skill is needed to gauge when decolorization is complete.
6. **Excessive counterstaining:** As the counterstain is also a basic dye, it is possible to replace the crystal violet—iodine complex in gram- positive cells with an over-exposure to the counterstain. The counterstain should not be left on the slide for more than 30 seconds.

ALTERNATIVES TO THE GRAM STAIN

Gram's staining method is plainly not without its problems. It is messy, complicated, and prone to operator error. The method also requires a large number of cells (although a membrane-filtration technique has been reported; Romero, et al 1988). However, it is also central to phenotypic microbial identification techniques. This method, and it's liabilities, are of immediate interest to those involved in environmental

monitoring programs as one of the most common isolates in an EM program, Bacillus spp., will frequently stain gram variable or gram negative despite being a gram-positive rod (this is especially true with older cultures). The problems with Gram's method have lead to a search for other tests that correlate with the cell wall structure of the gram-positive and the gram-negative cells. Several improvements/ alternatives to the classical gram stain have appeared in the literature.

KOH String Test

The KOH String Test is done using a drop of 3% potassium hydroxide on a glass slide. A visible loopful of cells from a single, well-isolated colony is mixed into the drop. If the mixture becomes viscous within 60 seconds of mixing (KOH-positive) then the colony is considered gram-negative. The reaction depends on the lysis of the gram-negative cell in the dilute alkali solution releasing cellular DNA to turn the suspension viscous. This method has been shown effective for food microorganisms, and for Bacillus spp , although it may be problematic for some anaerobes. This test has the advantage of simplicity, and it can be performed on older cultures. False negative results can occur in the test by using too little inoculum or too much KOH (DNA-induced viscosity not noticeable). False positive results can occur from too heavy an inoculum (the solution will appear to gel, but not string), or inoculation with mucoid colonies. This can serve as a valuable adjunct to the tradition gram stain method.

Aminopeptidase Test

L-alanine aminopeptidase is an enzyme localized in the bacterial cell wall which cleaves the amino acid L-alanine from various peptides. Significant activity is found almost only in Gram-negative microorganisms, all Gram-positive or Gram-variable microorganisms so far studied display no or very weak activity . To perform the test, the reagent is used to make a suspension (with the bacteria). Aminopeptidase activity of the bacteria causes the release of 4-nitroaniline from the reagent, turning the suspension yellow. The test is especially useful for non-fermenters and gram-variable organisms, and is a one step test with several suppliers of kits. Results of the test are available in 5 minutes.

Fluorescent Stains

A popular combination of fluorescent stains for use in gram staining (particularly for flow-cytometry) involves the use of the fluorescent nucleic acid binding dyes hexidium iodide (HI) and SYTO 13. HI penetrates gram-positive but not gram-negative organisms, but SYTO 13 penetrates both. When the dyes were used together in a single step, gram-negative organisms are green fluorescent by SYTO 13 while gram-positive organisms are red-orange fluorescent by HI which overpowers the green of SYTO 13 (Mason et al 1998). There are commercial kits available for this procedure, which requires a fluorescent microscope or a flow cytometer. Sizemore et al (1990) developed a different approach to fluorescent labeling of cells. Fluorescence-labeled wheat germ agglutinin binds specifically to N-acetylglucosamine in the outer peptidoglycan layer of gram-positive bacteria. The peptidoglycan layer of gram-negative bacteria is covered by a membrane and is not labeled by the lectin. A variant of this method has also been used to "gram stain" microorganisms in milk for direct measurement by flow cytometry.

LAL-based Assay

Charles River Laboratories has just released a product to be used with their PTS instrument – the PTS Gram ID (Farmer 2005). This methodology makes use of the same reaction used for the chromogenic LAL test. Gram-negative organisms, with bacterial endotoxin, initiate the LAL coagulase cascade which results in activation of the proclotting enzyme, a protease. In the LAL test, this enzyme cleaves a peptide from the horseshoe crab coagulen, resulting in a clot. It can also cleave a peptide from a synthetic substrate, yielding a chromophore (p-nitroaniline) which is yellow and can be measured photometrically at 385 nm . Gram-positive organisms, lacking endotoxin, do not trigger the color change in this method, while gram-negative organisms do trigger it. Results are available within 10 minutes.

Summary

The differentiation of bacteria into either the gram-positive or the gram-negative group is fundamental to most bacterial identification systems. This task is usually accomplished through the use of Gram's Staining Method. Unfortunately, the gram stain methodology is complex and prone to error. This operator-dependence can be addressed by attention to detail, and by the use of controls on the test. Additional steps might include confirmatory tests, of which several examples were given. As with all microbiology assays, full technician training and competent review of the data are critical quality control steps for good laboratory results.

BOUQUET

Brain Teasers

1. **HRP 2 is used to detect which malarial parasite?**
   - A. P. vivax
   - B. P. falciparum
   - C. P. malariae
   - D. P. ovale.

2. **Pf HRP 2 does not detect_____ stage of Falciparum malaria.**
   - A. Gametocytes
   - B. Trophozoites
   - C. Immature schizonts
   - D. Mature schizonts.

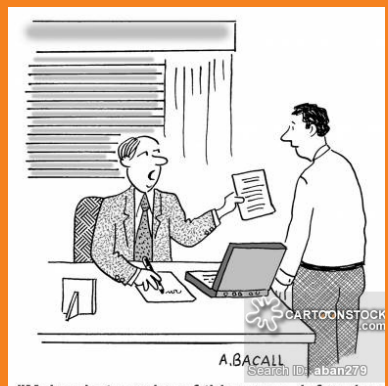
3. **In relation to Pf HRP 2, what does H stand for?**
   - A. Histamine
   - B. Histidine
   - C. Horse
   - D. Human.

4. **Post-treatment Pf HRP 2 levels can remain elevated up to_____.**
   - A. 3 days
   - B. 7 days
   - C. 15 days
   - D. 30 days.

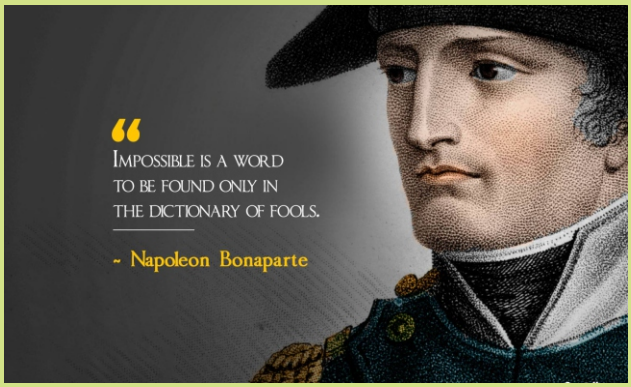
ANSWER: 1. B, 2. A, 3. B, 4. C

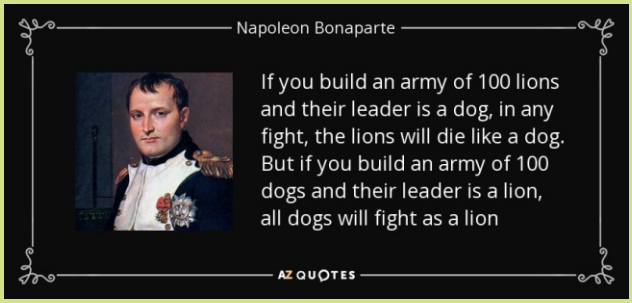
BOUQUET

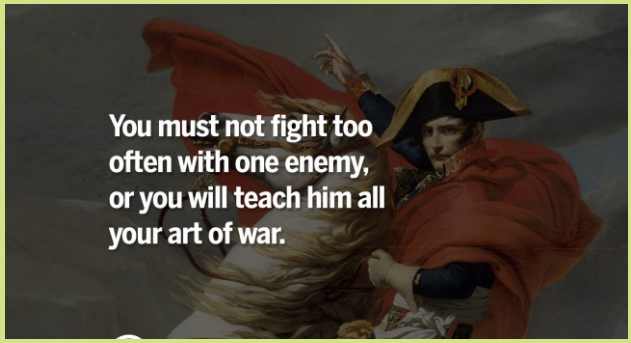
In Lighter Vein



Wisdom Whispers







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