VOLUME - XIX
ISSUE - CXI
MAY/JUN 2022

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

CONTENTS

Editorial

Syphilis is a sexually transmitted disease caused by the bacterium *Treponema pallidum* subspecies *pallidum*. The signs and symptoms of syphilis vary depending in which of the four stages it presents (primary, secondary, latent, and tertiary). The primary stage classically presents with a single chancre (a firm, painless, non-itchy skin ulceration usually between 1 cm and 2 cm in diameter) though there may be multiple sores. In secondary syphilis, a diffuse rash occurs, which frequently involves the palms of the hands and soles of the feet. There may also be sores in the mouth or vagina. In latent syphilis, which can last for years, there are few or no symptoms. In tertiary syphilis, there are gummas (soft, non-cancerous growths), neurological problems, or heart symptoms. Syphilis has been known as "the great imitator" as it may cause symptoms similar to many other diseases.

Syphilis is most commonly spread through sexual activity. It may also be transmitted from mother to baby during pregnancy or at birth, resulting in congenital syphilis. Other diseases caused by *Treponema* bacteria include yaws (*T. pallidum* subspecies *pertenue*), pinta (*T. carateum*), and nonvenereal endemic syphilis (*T. pallidum* subspecies *endemicum*). These three diseases are not typically sexually transmitted. Diagnosis is usually made by using blood tests; the bacteria can also be detected using dark field microscopy. It is recommended that all pregnant women be tested.

The risk of sexual transmission of syphilis can be reduced by using a latex or polyurethane condom. Syphilis can be effectively treated with antibiotics. The preferred antibiotic for most cases is benzathine benzylpenicillin injected into a muscle. In those who have a severe penicillin allergy, doxycycline or tetracycline may be used. In those with neurosyphilis, intravenous benzylpenicillin or ceftriaxone is recommended. During treatment people may develop fever, headache, and muscle pains, a reaction known as Jarisch–Herxheimer. SYPHILIS is the disease discussed under "**DISEASE DIAGNOSIS**".

There is an array/ battery of tests available for diagnosing Syphilis, sometimes confusing for some individuals. "**INTERPRETATION**" highlights all diagnostic aspects related to this sTD.

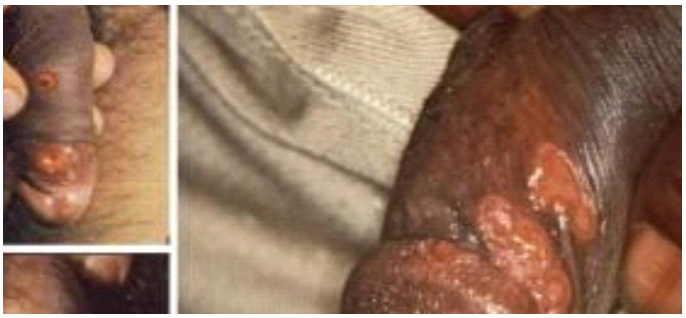
As ICTs or rapid flow through or lateral flow kits are often used currently. "**TROUBLESHOOTING**" outlines all issues concerned with ICTs. Needless to mention that "**BOUQUET**" is an integral part of every issue – including this one.

DISEASE DIAGNOSIS

SYPHILIS

Background

Syphilis is an infectious venereal disease caused by the spirochete *Treponema pallidum*. Syphilis is transmissible by sexual contact with infectious lesions, from mother to fetus in utero, via blood product transfusion, and occasionally through breaks in the skin that come into contact with infectious lesions. If untreated, it progresses through 4 stages: primary, secondary, latent, and tertiary. The image below depicts the characteristic chancre observed in primary syphilis.



Syphilis. These photographs depict the characteristic chancre observed in primary syphilis.

Syphilis has a myriad of presentations and can mimic many other infections and immune-mediated processes in advanced stages. Hence, it has earned the nickname "the great impostor." The complex and variable manifestations of the disease prompted Sir William Osler to remark, "The physician who knows syphilis knows medicine." Many famous persons throughout history are thought to have suffered from syphilis, including Bram Stoker, Henry VIII, and Vincent Van Gogh. Since the discovery of penicillin in the mid-20th century, the spread of this once very common disease has been largely controlled, but efforts to eradicate the disease entirely have been unsuccessful.

Pathophysiology

Three genera of spirochetes cause human infection:

- *Treponema*, which causes syphilis, yaws, and pinta
- *Borrelia*, which causes Lyme disease and relapsing fever
- *Leptospira*, which causes leptospirosis

The particular spirochete responsible for syphilis is *Treponema pallidum*. *T pallidum* is a fragile spiral bacterium 6-15 micrometers long by 0.25 micrometers in diameter. Its small size makes it invisible on light microscopy; therefore, it must be identified by its distinctive undulating movements on darkfield microscopy. It can survive only briefly outside of the body; thus, transmission almost always requires direct contact with the infectious lesion. Syphilis is usually classified into 4 stages: primary, secondary, latent, and tertiary. It can be either acquired or congenital. That is, it can be transmitted either by intimate contact with infectious lesions (most common) or via blood transfusion (if blood has been collected during early syphilis), and it can also be transmitted transplacentally from an infected mother to her fetus.

Acquired syphilis

In acquired syphilis, *T pallidum* rapidly penetrates intact mucous membranes or microscopic dermal abrasions and, within a few hours, enters the lymphatics and blood to produce systemic infection. Incubation time from exposure to development of primary lesions, which occur at the primary site of inoculation, averages 3 weeks but can range from 10-90 days. Studies in rabbits show that spirochetes can be found in the lymphatic system as early as 30 minutes after primary inoculation, suggesting that syphilis is a systemic disease from the outset. The central nervous system (CNS) is invaded early in the infection; during the secondary stage, examinations demonstrate that more than 30% of patients have abnormal findings in the cerebrospinal fluid (CSF). During the first 5-10 years after the onset of untreated primary infection, the disease principally involves the meninges and blood vessels, resulting in meningovascular neurosyphilis. Later, the parenchyma of the brain and spinal cord are damaged, resulting in parenchymatous neurosyphilis. Go to Neurosyphilis for complete information on this topic. Regardless of the stage of disease and location of lesions, histopathologic hallmarks of syphilis include endarteritis (which in some instances may be obliterative in nature) and a plasma cell–rich infiltrate. Endarteritis is caused by the binding of spirochetes to endothelial cells, mediated by host fibronectin molecules bound to the surface of the spirochetes. The resultant endarteritis can heal with scarring in some instances. The syphilitic infiltrate reflects a delayed-type hypersensitivity response to *T pallidum*, and in certain individuals with tertiary syphilis, this response by sensitized T lymphocytes and macrophages results in gummatous ulcerations and necrosis. Antigens of *T pallidum* induce host production of treponemal antibodies and nonspecific reagin antibodies. Immunity to syphilis is incomplete. For example, host humoral and cellular immune responses may prevent the formation of a primary lesion on subsequent infections with *T pallidum*, but they are insufficient to clear the organism. This may be because the outer sheath of the spirochete is lacking immunogenic molecules, or it may be because of down-regulation of helper T cells of the TH1 class. Primary syphilis is characterized by the development of a painless chancre at the site of transmission after an incubation period of 3-6 weeks. The lesion has a punched-out base and rolled edges and is highly infectious. Histologically, the chancre is characterized by mononuclear leukocytic infiltration, macrophages, and lymphocytes. The inflammatory reaction causes an obliterative endarteritis. In this stage, the spirochete can be isolated from the surface of the ulceration or the overlying exudate of the chancre. Whether treated or not, healing occurs within 3-12 weeks, with considerable residual fibrosis. Secondary syphilis develops about 4-10 weeks after the appearance of the primary lesion. During this stage, the spirochetes multiply and spread throughout the body. Secondary syphilis lesions are quite variable in their manifestations. Systemic manifestations include malaise, fever, myalgias, arthralgias, lymphadenopathy, and rash. Widespread mucocutaneous lesions are observed over the entire body and may involve the palms, soles, and oral mucosae. Most often, the lesions are macular, discrete, reddish brown, and 5 mm or smaller in diameter; however, they can be pustular, annular, or scaling. Vesicular rash is typically absent. All such lesions contain treponemes. Of these, wet mucous patches are the most contagious. Histologically, the inflammatory reaction is similar to but less intense than that of the primary chancre. Other skin findings of secondary syphilis are condylomata lata and patchy alopecia. Condylomata lata are painless, highly infectious gray-white lesions that develop in warm, moist sites. The alopecia is characterized by patchy hair loss of the scalp and facial hair, including the eyebrows. Patients with this finding have been referred to as having a "moth-eaten" appearance. During secondary infection, the immune reaction is at its peak and antibody titers are high. Latent syphilis is a stage at which the features of secondary syphilis have resolved, though patients remain seroreactive. Some patients experience recurrence of the infectious skin lesions of secondary

syphilis during this period. About one third of untreated latent syphilis patients go on to develop tertiary syphilis, whereas the rest remain asymptomatic. Currently, tertiary syphilis disease is rare. When it does occur, it mainly affects the cardiovascular system (80-85%) and the CNS (5-10%), developing over months to years and involving slow inflammatory damage to tissues. The 3 general categories of tertiary syphilis are gummatous syphilis (also called late benign), cardiovascular syphilis, and neurosyphilis. Gummatous syphilis is characterized by granulomatous lesions, called gummas, which are characterized by a center of necrotic tissue with a rubbery texture. Gummas principally form in the liver, bones, and testes but may affect any organ. Histological examination shows palisaded macrophages and fibroblasts, as well as plasma cells surrounding the margins. Gummas may break down and form ulcers, eventually becoming fibrotic. Treponemes are rarely visualized or recovered from these lesions. Cardiovascular syphilis occurs at least 10 years after primary infection. The most common manifestation is aneurysm formation in the ascending aorta, caused by chronic inflammatory destruction of the vasa vasorum, the penetrating vessels that nourish the walls of large arteries. Aortic valve insufficiency may result. Neurosyphilis has several forms. If the spirochete invades the CNS, syphilitic meningitis results. Syphilitic meningitis is an early manifestation, usually occurring within 6 months of the primary infection. CSF shows high protein, low glucose, high lymphocyte count, and positive syphilis serology. Meningovascular syphilis occurs as a result of damage to the blood vessels of the meninges, brain, and spinal cord, leading to infarctions causing a wide spectrum of neurologic impairments. Parenchymal neurosyphilis includes tabes dorsalis and general paresis. Tabes dorsalis develops as the posterior columns and dorsal roots of the spinal cord are damaged. Posterior column impairment results in impaired vibration and proprioceptive sensation, leading to a wide-based gait. Disruption of the dorsal roots leads to loss of pain and temperature sensation and areflexia. Damage to the cortical regions of the brain leads to general paresis, formerly called “general paresis of the insane,” which mimics other forms of dementia. Impairment of memory and speech, personality changes, irritability, and psychotic symptoms develop and may advance to progressive dementia. The Argyll-Robertson pupil, a pupil that does not react to light but does constrict during accommodation, may be seen in tabes dorsalis and general paresis. The precise location of the lesion causing this phenomenon is unknown.

Congenital syphilis

Congenital syphilis, discussed briefly here, is a veritable potpourri of antiquated medical terminology. The treponemes readily cross the placental barrier and infect the fetus, causing a high rate of spontaneous abortion and stillbirth. Within the first 2 years of life, symptoms are similar to severe adult secondary syphilis with widespread condylomata lata and rash. “Snuffles” describes the mucopurulent rhinitis caused by involvement of the nasal mucosae. Later manifestations of congenital syphilis include bone and teeth deformities, such as “saddle nose” (due to destruction of the nasal septum), “saber shins” (due to inflammation and bowing of the tibia), “Clutton's joints” (due to inflammation of the knee joints), “Hutchinson's teeth” (in which the upper incisors are widely spaced and notched), and “mulberry molars” (in which the molars have too many cusps). Tabes dorsalis and general paresis may develop as in adults, with 8th cranial nerve deafness and optic nerve atrophy as well as a variety of other ophthalmologic involvement leading to blindness being additional features.

Etiology

The cause of syphilis is infection with the spirochete *T pallidum. T pallidum* is solely a human pathogen and does not naturally occur in other species. *T pallidum* has, however, been cloned in *Escherichia coli* and has been used experimentally in rabbits. Transmission of *T pallidum* occurs via penetration of the spirochetes through mucosal membranes and abrasions on epithelial surfaces. It is primarily spread through sexual contact but can be spread by exposure to blood products and transferred in utero. *T pallidum* is a labile organism that cannot survive drying or exposure to disinfectants; thus, fomite transmission (eg, from toilet seats) is virtually impossible. Unprotected sex is the major risk factor for the acquisition of syphilis, especially among men who have sex with men (MSM), who accounted for 83.7% of all syphilis cases in the United States.

Epidemiology

International statistics

Internationally, the prevalence of syphilis varies by region. Syphilis remains prevalent in many developing countries and in some areas of North America, Asia, and Europe, especially Eastern Europe. The highest rates are in South and Southeast Asia, followed closely by sub-Saharan Africa. The third highest rates are in the regions of Latin America and the Caribbean. In some regions of Siberia, as of 1999, prevalence was 1300 cases per 100,000 population.

Age distribution of syphilis

Syphilis is most common during the years of peak sexual activity. Most new cases occur in men and women aged 20-29 years. In 2013, the rate of primary and secondary syphilis was highest in people aged 25-29 years (27 per 100,000). The incidence of congenital syphilis has increased to 11.6 cases per 100,000 live births in 2014, the highest congenital syphilis rate reported since 2001. The number of congenital syphilis cases declined in the United States during 2008-2012, from 446 to 334 cases (10.5 to 8.4 cases per 100,000 live births) but is increasing; from 2012-2014, the number of reported congenital syphilis cases in the United States increased from 334 to 458.

Sex distribution of syphilis

Men are affected more frequently with primary or secondary syphilis than women. This difference has varied over time. Male-to-female ratios of primary and secondary syphilis increased from 1.6:1 in 1965 to nearly 3:1 in 1985. After, the ratio decreased, reaching a nadir in 1994-95. The past decade has seen a sharp rise in syphilis cases among men, driven mostly by the MSM community. Males with primary and secondary syphilis outnumber females 10 to 1. Among women, the reported primary and secondary syphilis rate increased from 0.9 to 1.5 per 100,000 population per year during 2005- 2008 and decreased to 0.9 in 2013.

Prevalence of syphilis by race or ethnicity

In the United States, syphilis is more prevalent among persons of minority race and ethnicity. Non-Hispanic blacks are at higher risk for syphilis than all other racial groups. In 2013, the primary and secondary syphilis rate among black men was 5.2 times that among white men (27.9 vs 5.4 cases per 100,000 population); the rate among black women was 13.3 times that among white women (4 vs 0.3). The rate among Hispanic men was 2.1 times that among white men (11.6 vs 5.4), and the rate among Hispanic women was 2.7 times that among white women (0.8 vs 0.3). These disparities were similar to disparities observed in 2005 and represent an increase in syphilis rates in all racial groups.

HIV and syphilis co-infection

Syphilis acquisition increases the risk of HIV acquisition by 2- to 5-fold and makes transmission of HIV more efficient via various methods. First, primary syphilis infection causes a genital ulcer, which disrupts the mucous membrane, making it more vulnerable to penetration by the HIV

virus. Second, genital ulcers bleed easily during sex, increasing the risk of viral transmission. Third, genital ulcers attract CD4 cells to the ulcer surface, increasing targets for the HIV virus to infect. Fourth, the risk behaviors associated with acquiring syphilis also increase the likelihood of acquiring HIV. The rate of HIV and syphilis co-infection is high. More than 50% of MSM with syphilis are also infected with HIV, and this number increases with each recurrence.

Prognosis

The morbidity of syphilis ranges from the relatively minor symptoms of the primary stages of infection to the more significant constitutional systemic symptoms of secondary syphilis and the significant neurological and cardiovascular consequences of tertiary disease. Since latent syphilis can persist for years or decades, the manifestations of tertiary syphilis often occur much later in life, causing significant morbidity and even death if not identified and treated. These figures have continued to increase since the emergence of the AIDS epidemic, since genital ulcer diseases (including syphilis) are cofactors for the sexual transmission of HIV. Additionally, untreated patients who are HIV seropositive have an increased risk for rapid progression to neurosyphilis. In addition, patients with HIV are at greater risk for development or relapse of early symptomatic neurosyphilis for up to 2 years after treatment with intramuscular or intravenous penicillin. The morbidity and mortality of untreated syphilis must be estimated from the limited data available regarding its natural course. These data are largely from one retrospective study of autopsies and two prospective studies, most notably the famous Tuskegee Study of Untreated Syphilis in the Negro Male, which fell under serious ethical scrutiny in later years for exploiting a vulnerable patient population and not offering treatment for the disease when it became available after the study was underway. These data indicate that approximately one third of patients left untreated will develop late complications, with 10% of the total developing cardiovascular syphilis; 6%, neurosyphilis; and 16%, gummatous syphilis. Mortality rates in general are greater among those affected, and late complications appear to occur more commonly in men than in women. For patients diagnosed with either primary or secondary syphilis (without auditory/neurologic/ocular involvement), the prognosis is good following appropriate treatment. *T pallidum* remains highly responsive to the penicillins, and cure is likely. Among patients diagnosed with tertiary syphilis, the prognosis is less sanguine. Twenty percent of untreated patients with tertiary syphilis die of the disease, making syphilis one of the few sexually transmitted diseases (SDTs) capable of killing its host. However, with adequate treatment, 90% of patients with neurosyphilis have a clinical response. Overall prognosis for tertiary syphilis depends on the extent of scarring and tissue damage, as treatment arrests further damage and inflammation but cannot reverse previous tissue damage. For example, the prognosis or advanced symptomatic disease in cardiovascular syphilis is poor. In contrast, syphilitic gummas typically resolve promptly with high-dose penicillin. Congenital syphilis is the most serious outcome of syphilis in women. It has been shown that a higher proportion of infants are affected if the mother has untreated secondary syphilis, compared to untreated early latent syphilis. Since T pallidum does not invade the placental tissue or the fetus until the fifth month of gestation, syphilis causes late abortion, stillbirth, or death soon after delivery in more than 40% of untreated maternal infections. Neonatal mortality usually results from pulmonary hemorrhage, bacterial superinfection, or fulminant hepatitis. For patients who are pregnant and have early syphilis, it is likely that the mother will deliver a child not infected by syphilis (assuming the mother was treated appropriately).

Clinical Presentation

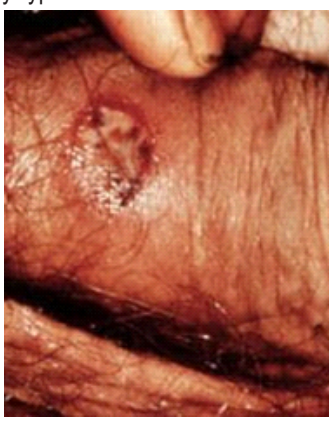
History

Physicians must keep a high index of suspicion for the diagnosis of syphilis, as the manifestations of syphilis (particularly advanced syphilis) are nonspecific and may masquerade as many other diseases. Rigorous attention to the time course of symptoms is required for proper staging. Obtain a thorough sexual and social history, including the number of sexual partners, condom use, history of STDs in the patient and their partners, intravenous (IV) drug use, and exposure to blood products. In children and infants, seek a maternal history, history of exposure to individuals with syphilis or blood products, and a history of sexual abuse.

Primary syphilis

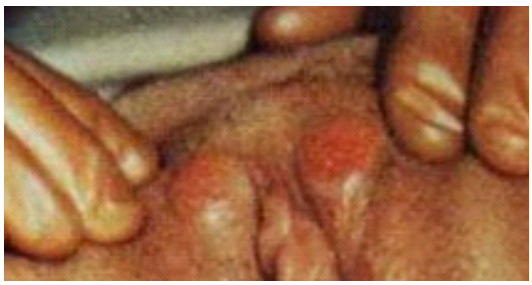
Primary syphilis occurs 10-90 days after contact with an infected individual. It manifests mainly on the glans penis in males and on the vulva or cervix in females. Ten percent of syphilitic lesions are found on the anus, fingers, oropharynx, tongue, nipples, or other extragenital sites. Regional nontender lymphadenopathy follows invasion. Lesions (chancres) usually begin as solitary, raised, firm, red papules that can be several centimeters in diameter. The chancre erodes to create an ulcerative crater within the papule, with slightly elevated edges around the central ulcer (see the images below). It usually heals within 4-8 weeks, with or without therapy.

Syphilis. These photographs depict the characteristic chancre observed in primary syphilis



Syphilitic chancre

Although genital chancres are frequently solitary, they may be multiple in some patients. Sometimes they appear as “kissing” lesions on opposing skin surfaces—for example, the labia (see the image below).

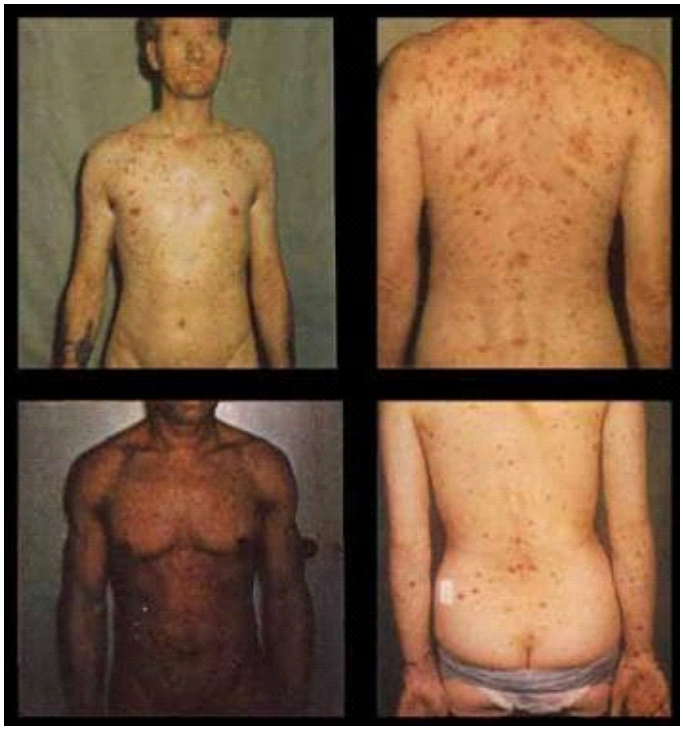


Syphilis. This photograph depicts primary syphilis "kissing" lesions.

Secondary syphilis

Secondary syphilis manifests in various ways. It usually presents with a cutaneous eruption within 2-10 weeks after the primary chancre and is most florid 3-4 months after infection. The eruption may be subtle; 25% of patients may be unaware of skin changes. A localized or diffuse

mucocutaneous rash (generally nonpruritic and bilaterally symmetrical) with generalized nontender lymphadenopathy is typical (see the image below). Patchy alopecia and condylomata lata may also be observed.



Syphilis. These photographs show the disseminated rash observed in secondary syphilis.

Mild constitutional symptoms of malaise, headache, anorexia, nausea, aching pains in the bones, and fatigue often are present, as well as fever and neck stiffness. A small number of patients develop acute syphilitic meningitis and present with headache, neck stiffness, facial numbness or weakness, and deafness. Other less-common manifestations include GI involvement, hepatitis, nephropathy, proctitis, arthritis, and optic neuritis.

Latent syphilis

Latency may last from a few years to as many as 25 years before the destructive lesions of tertiary syphilis manifest. Affected patients may recall symptoms of primary and secondary syphilis. They are asymptomatic during the latent phase, and the disease is detected only by serologic tests. Latent syphilis is divided into early latent and late latent. The distinction is important because treatment for each is different. The early latent period is the first year after the resolution of primary or secondary syphilis. Asymptomatic patients who have a newly active serologic test after having a serologically negative test result within 1 year are also considered to be in the early latent period. Late latency syphilis is not infectious; however, women in this stage can spread the disease in utero.

Tertiary syphilis

Tertiary (late) syphilis is slowly progressive and may affect any organ. The disease is generally not thought to be infectious at this stage. Manifestations may include the following:

- Impaired balance, paresthesias, incontinence, and impotence
- Focal neurologic findings, including sensorineural hearing and vision loss
- Dementia
- Chest pain, back pain, stridor, or other symptoms related to aortic aneurysms

The lesions of gummatous tertiary syphilis usually develop within 3-10 years of infection. The patient complaints are usually secondary to bone pain, which is described as a deep boring pain characteristically worse at night. Trauma may predispose a specific site to gumma involvement. CNS involvement may occur, with presenting symptoms representative of the area affected (ie, brain involvement [headache, dizziness, mood disturbance, neck stiffness, blurred vision] and spinal cord involvement [bulbar symptoms, weakness and wasting of shoulder girdle and arm muscles, incontinence, impotence]). Some patients may present up to 20 years after infection with behavioral changes and other signs of dementia, which is indicative of paresis.

Congenital syphilis

Early congenital syphilis occurs within the first 2 years of life. Late congenital syphilis emerges in children older than 2 years. A small percentage of infants infected in utero may have a latent form of infection that becomes apparent during childhood and, in some cases, during adult life. The earliest symptom that occurs prior to age 2 years is rhinitis (snuffles), soon followed by cutaneous lesions. After age 2 years, parents may note problems with the child's hearing and language development and with vision. Facial and dental abnormalities may be noted.

Physical Examination

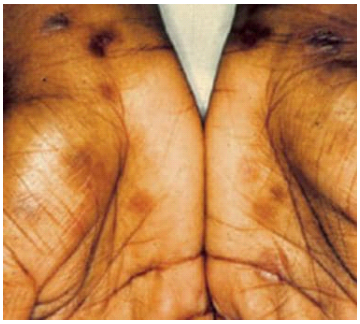
Conduct the physical examination with the manifestations of primary, secondary, and tertiary syphilis in mind. The lesions and exanthem of primary and secondary syphilis are infectious; thus, gloves and other relevant personal protective equipment must be worn.

Primary syphilis

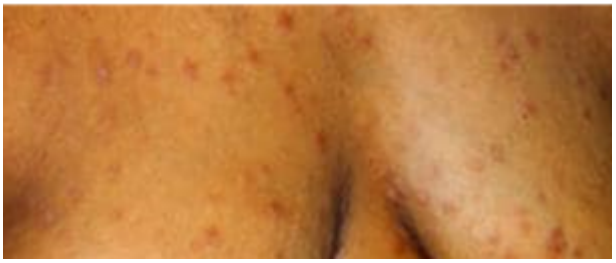
The patient is typically afebrile, with a chancre at the site of inoculation, often accompanied by inguinal adenopathy. The chancre of primary syphilis usually begins as a single, painless papule that rapidly becomes eroded and indurated, with a surrounding red areola. The edge and base of the ulcer have a cartilaginous (buttonlike) consistency on palpation. Although classic chancres are not painful, they can become so if suprainfected with bacteria or if located in the anal canal. Atypical primary lesions are common and may manifest as a papular lesion without subsequent ulceration or induration. The primary lesion usually is associated with regional lymphadenopathy that may be unilateral or bilateral. Inguinal adenitis is usually discrete, firm, mobile, and painless, without overlying skin changes. Chancres usually are located on the penis in heterosexual men, but in homosexual men, they may be found in the anal canal, mouth, or external genitalia. Common primary sites in women include the cervix and labia. Extragenital chancres occur most commonly above the neck, typically affecting the lips or oral cavity. The lesion is highly infectious; when abraded, it exudes a clear serum containing numerous *T pallidum* organisms.

Secondary syphilis

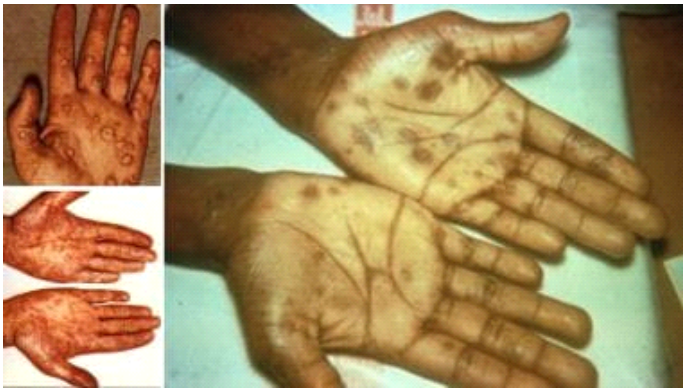
Secondary syphilis may present in many different ways but usually includes a localized or diffuse mucocutaneous rash and generalized nontender lymphadenopathy. The exanthem may be macular, papular, pustular, or mixed (see the images below).



Secondary syphilis - Exanthem

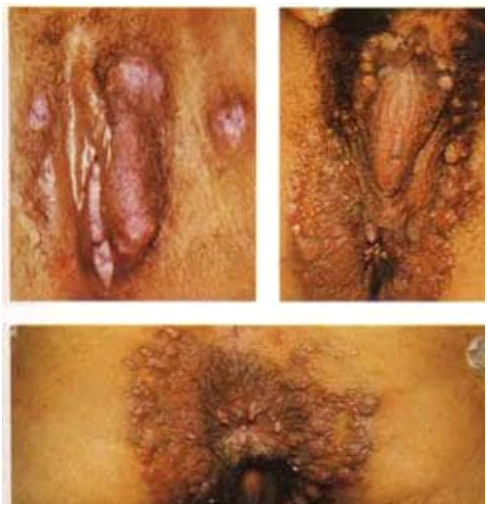


Secondary syphilis - Exanthem
Initial lesions are bilaterally symmetric, pale red to pink (in light-skinned persons) or pigmented (in dark-skinned persons), discrete, round, nonpruritic macules that measure 5-10 mm in diameter and are distributed on the trunk and proximal extremities. After several days or weeks, red papular lesions 3-10 mm in diameter appear. These lesions often become necrotic and are distributed widely with frequent involvement of the palms and soles (see the image below).



Syphilis. Palmar lesions observed in secondary syphilis

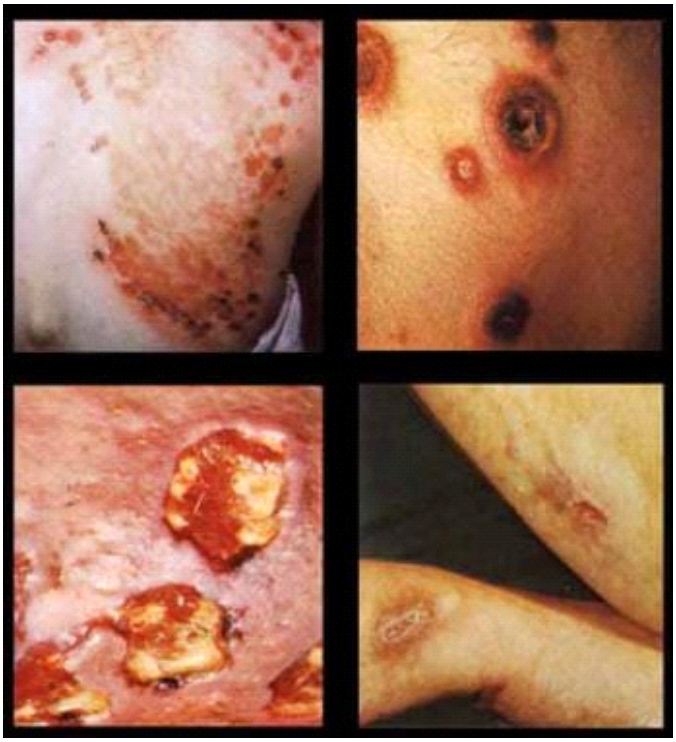
Tiny papular follicular syphilids involving hair follicles may result in patchy alopecia. In addition to the classic moth-eaten alopecia, a diffuse alopecia also has been reported.
Reddish-brown papular lesions on the penis or anogenital area can coalesce into large elevated plaques up to 2-3 cm in diameter, known as condylomata lata (see the image below). Lesions usually progress from red, painful, and vesicular to "gun metal grey" as the rash resolves. Condylomata lata are highly infectious. They are sometimes confused with condylomata acuminata or venereal warts.



These photographs illustrate examples of condylomata lata. The lesions resemble genital warts (condylomata acuminata). Fluids exuding from these lesions are highly infectious. From 10-15% of patients with secondary syphilis develop superficial mucosal erosions, usually painless, on the palate, pharynx, larynx, glans penis, vulva, or in the anal canal and rectum. These mucous patches are circular silver-gray erosions with a red areola. The erosions harbor treponemes and can transmit disease. Ocular abnormalities, such as iritis, are a rare clinical finding, although anterior uveitis has been reported in 5-10% of patients with secondary syphilis. Less common findings include periostitis, arthralgias, meningitis, nephritis, hepatitis, proctitis, and ulcerative colitis. Go to Interstitial Keratitis for complete information on this topic. Thirty percent of patients experience recurring symptoms after the primary or secondary stage of syphilis. Lesions are less numerous but are still infectious.

Tertiary syphilis

Symptomatic tertiary syphilis is the result of a chronic, progressive inflammatory process that eventually produces clinical symptoms years to decades after the initial infection. The liver and skeleton are possible sites of infection; such infections are characterized by fever, jaundice, anemia, and/or nighttime skeletal pain. Gummatous syphilis is characterized by coalescent granulomatous lesions, called gummas, that usually affect skin, bone, and mucous membranes but may involve any organ system, often causing local destruction of the affected organ system (see the image below). Cutaneous gummas are indurated, nodular, papulosquamous or ulcerative lesions that form characteristic circles or arcs with peripheral hyperpigmentation. They may mimic other granulomatous ulcerative lesions and may be histologically indistinguishable from them.



Syphilis. These photographs show close-up images of gummas observed in tertiary syphilis.

Although gummas may be identified on the skin, in the mouth, and in the upper respiratory tract, they appear most commonly on the leg just below the knee. Gummas may be multiple or diffuse but usually are solitary

lesions that range from less than 1 cm to several centimeters in diameter. They are generally asymmetric and grouped together. Cardiovascular syphilis usually involves the aorta, though other large arteries may be affected as well. Invading treponemes cause scarring of the tunica media. Over many years, the inflammatory scarring weakens the aortic wall, leading to aneurysm formation, which causes incompetence of the aortic valve and narrowing of the coronary ostia. The most common clinical finding on cardiovascular examination is a diastolic murmur with a tambour quality, secondary to aortic dilation with valvular insufficiency.

Neurosyphilis may be either asymptomatic or symptomatic.

In asymptomatic neurosyphilis, no signs or symptoms are present, but CSF abnormalities are demonstrable, including possible pleocytosis, elevated protein, decreased glucose, or a reactive CSF Venereal Disease Research Laboratory (VDRL) test.

Symptomatic neurosyphilis may manifest in the following three forms:

- Syphilitic meningitis, cranial neuritis, or meningovascular disease
- Meningovascular neurosyphilis
- Parenchymatous neurosyphilis

Syphilitic meningitis, cranial neuritis, and meningovascular disease usually develop within 6 months to several years of initial infection. Patients with syphilitic meningitis present with typical symptoms of meningitis, including headache, nausea and vomiting, and photophobia, but are typically afebrile. Patients may exhibit cranial nerve abnormalities. Meningovascular syphilis typically manifests 5-10 years after infection and is the result of endarteritis, inflammation of small blood vessels of the meninges, brain, and spinal cord. Patients may present with CNS vascular insufficiency or outright stroke. The most common presentation of meningovascular syphilis (diffuse inflammation of the pia and arachnoid along with widespread arterial involvement) is an indolent stroke syndrome involving the middle cerebral artery. Cranial nerve palsies and pupillary abnormalities occur with basilar meningitis. Parenchymatous neurosyphilis results from direct parenchymal CNS invasion by *T pallidum* and is usually a late development (15-20 years after primary infection). It includes general paresis and tabes dorsalis. Paretic syphilis is the result of widespread parenchymal invasion that causes individual cell death and brain atrophy. Tabes dorsalis is the result of damage to the sensory nerves in dorsal roots, producing ataxia and loss of pain sensation, proprioception, and deep tendon reflexes in joints. Patients with parenchymatous neurosyphilis present with ataxia; incontinence; paresthesias; and loss of position, vibratory, pain, and temperature sensations. Paresis and dementia, with changes in personality and intellect, may develop. Tabes dorsalis presents with signs of demyelination of the posterior columns, dorsal roots, and dorsal root ganglia (eg, ataxic wide-based gait and foot slap, areflexia and loss of position, deep pain and temperature sensations). Deep ulcers of the feet can result from loss of pain sensation. Argyll Robertson pupil, which occurs almost exclusively in neurosyphilis, is a small irregular pupil that reacts normally to accommodation but not to light. Rare findings include iritis, with possible adhesion of the iris to the anterior lens, producing a fixed pupil (not to be confused with Argyll Robertson pupil). Go to Neurosyphilis for complete information on this topic.

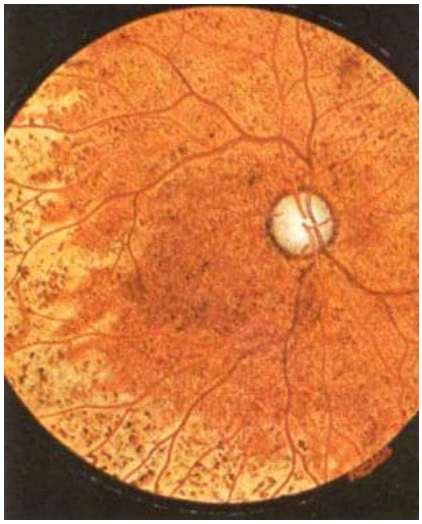
Congenital syphilis

The manifestations of untreated congenital syphilis can be divided into those that are expressed prior to age 2 years (early) or after age 2 years (late). Clinical evidence of early congenital syphilis is similar to that of secondary syphilis in adults. Early signs and symptoms include development of a diffuse rash, characterized by extensive sloughing of the epithelium, particularly on the palms, soles, and skin around the mouth and anus. The rash has a higher probability of being atypical and can be vesicular or bullous instead of the characteristic reddish brown macular rash. A compilation of early clinical presentations of congenital syphilis in 9 studies involving a total of 212 infants included abnormal bone radiographs (61%); hepatomegaly (51%); splenomegaly (49%); petechiae (41%); other skin rashes (35%); anemia (34%); lymphadenopathy (32%); jaundice (30%); pseudoparalysis, often due to pain secondary to osteochondritis (28%); and snuffles (23%).

A classic mucocutaneous sign is depressed linear scars radiating from the orifice of the mouth (perioral fissures), termed rhagades (Parrot lines).

Additional symptoms of early congenital syphilis include the following:

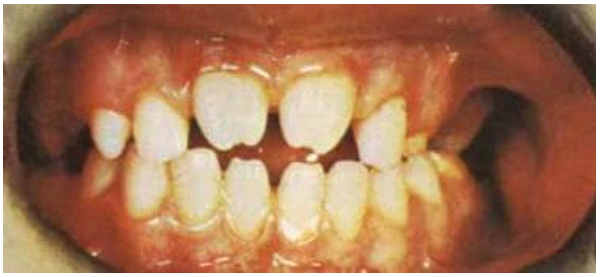
- Hemorrhagic rhinitis
- Periostitis
- Mucous patches
- Hydrops
- Glomerulonephritis
- Thrombocytopenia
- Neurologic involvement
- Ocular involvement (see the image below.)



Syphilis. This photograph illustrates chorioretinitis of congenital syphilis. View Media Gallery

The clinical manifestations of untreated congenital neurosyphilis present in 25% of patients older than age 6 years and correspond to those of adult neurosyphilis. Cardiovascular abnormalities are rare. Findings include the following :

- Bony abnormalities, including prominent frontal bones, depression of nasal bridge, abnormal maxilla development, anterior tibial bowing, frontal bossing of Parrot, and Higoumenakia sign (unilateral irregular enlargement of the sternoclavicular portion of the clavicle secondary to periostitis)
- Clutton joints (arthritis of both knees)
- Interstitial keratitis
- Hutchinson incisors (centrally notched and widely spaced, peg-shaped, upper central incisors; see the image below)
- Mulberry molars (sixth-year molars with multiple poorly developed cusps)
- Cranial nerve VIII involvement - Deafness
- Paroxysmal cold hemoglobinuria
- Gummatous involvement - Gummatous periostitis occurs in patients aged 5-20 years and tends to cause destructive lesions of the palate and nasal septum (saddle nose).
- Corneal opacities



Syphilis. This photograph shows an example of Hutchinson teeth in congenital syphilis. Note notching.

Complications

Complications of syphilis may include the following:

- Cardiovascular disease - Aortic aneurysm
- CNS disease - Dementia, stroke
- Membranous glomerulonephritis
- Paroxysmal cold hemoglobinemia
- Irreversible end-organ damage
- Disfigurement by gummas

Differential Diagnoses

Diagnostic Considerations

Syphilis, a reportable disease, is tracked by the Centers for Disease Control and Prevention (CDC). Syphilis has an extensive differential diagnosis. In particular, the extremely variable manifestations of tertiary syphilis produce an extremely broad differential diagnosis, and care must be taken to consider syphilis in cardiac, dermatologic, and neurologic disorders as is relevant. Patients diagnosed with syphilis should also be tested for other sexually transmitted diseases (STDs), including chlamydia, gonorrhea, trichomoniasis, bacterial vaginosis, and HIV infection.

When making a primary diagnosis of a generalized rash or an STD, always include syphilis in the differential diagnoses because of its varying manifestations.

Other problems to consider

Other problems to consider include the following:

- Brain tumors
- Carcinoma
- Congestive heart failure
- Fungal infection (superficial and deep)
- Lymphoma
- Mycotic infection
- Other CNS infections
- Sarcoid
- Seizures
- Stroke
- Trauma
- Traumatic superinfected lesions
- Venereal chlamydial infections

Differential Diagnoses

- Candidiasis
- Chancroid
- Condyloma Acuminatum
- Urinary Tract Infection (UTI) and Cystitis (Bladder Infection) in Females
- Dermatologic Manifestations of Herpes Simplex
- Drug Eruptions
- Genital Warts
- Granuloma Inguinale (Donovanosis)
- Herpes Zoster
- HIV Infection and AIDS
- Lymphogranuloma Venereum (LGV)
- Urethritis
- Urinary Tract Infection (UTI) in Males
- Urinary Tract Infections in Pregnancy
- Varicella-Zoster Virus (VZV)
- Yaws

Workup

Approach Considerations

T pallidum cannot be cultivated in vitro and is too small to be seen under the light microscope. Serologic testing is considered the standard method of detection for all stages of syphilis. (Note, however, that serologic tests cannot be used to differentiate the different species of the treponeme family—for example, yaws.) In suspected acquired syphilis, the traditional approach has been to first perform nontreponemal serology screening using the Venereal Disease Research Laboratory (VDRL), rapid plasma reagin (RPR), or the recently developed ICE Syphilis recombinant antigen test. Sensitivity of the VDRL and RPR tests are estimated to be 78-86% for detecting primary syphilis, 100% for detecting secondary syphilis, and 95-98% for detecting tertiary syphilis. Specificity ranges from 85-99% and may be reduced in individuals who have coexisting conditions (ie, collagen vascular disease, pregnancy, intravenous drug use, advanced malignancy, tuberculosis, malaria, viral and rickettsial diseases). VDRL test results turn positive 1-2 weeks after chancre formation. Nontreponemal tests usually become nonreactive with time after treatment. Serology values in patients with HIV infection may take longer to fall than in patients without HIV infection. In some patients, nontreponemal antibodies can persist, sometimes for life, a conditioned referred to as "serofast." Because of the possibility of false-positive results, confirmation for any positive or equivocal nontreponemal test result should follow with a treponemal test, such as the fluorescent treponemal antibody-absorption (FTA-ABS), microhemagglutination assay *T pallidum* (MHA-TP), *T pallidum* hemagglutination (TPHA), and *T pallidum* particle agglutination (TPPA) tests. Treponemal enzyme immunoassay (EIA) for immunoglobulin G (IgG) and immunoglobulin M (IgM) may be performed. FTA-ABS is commonly used as a confirmatory test following positive VDRL or RPR test findings. FTA-ABS has a sensitivity of 84% for detecting primary syphilis infection and almost 100% sensitivity for detecting syphilis infection in other stages. Its specificity is 96%. Some labs have adopted reverse sequence screening in order to reduce time, labor, and costs. Reverse screening test sera first by automatable treponemal enzyme and chemiluminescence immunoassays (EIA/CIA), followed by testing of reactive sera with a nontreponemal test. Results of the first direct comparison of traditional and reverse screening suggest reverse screening may not be as inferior to traditional testing as previously thought. Six out of 1000 patients tested were falsely reactive by reverse screening, compared to none by traditional testing. However, reverse screening identified 2 patients with possible latent syphilis that were not detected by RPR. The CDC recommends traditional testing, but if reverse screening is used all sera that produce reactive EIA/CIA results should be reflexively tested with a quantitative nontreponemal test. Sera with discordant results should be reflexively tested with a confirmatory TPPA test. If the result is positive, the patient should be offered treatment if no treatment history can be elucidated. Darkfield microscopy is a possible mode of evaluating moist cutaneous lesions, such as the

chancre of primary syphilis or the condyloma lata of secondary syphilis. If darkfield microscopy is not available, direct immunofluorescence staining of fixed smears (direct fluorescent antibody *T pallidum* [DFA-TP]) is an option. Both procedures detect the causative organism at a rate of approximately 85-92%. Slit-lamp examination and ophthalmic assessment can be used to differentiate between acquired and congenital syphilis (presence of interstitial keratitis) in patients with latent infection of uncertain duration. Diagnosis of neurosyphilis can be challenging. The VDRL test for CSF (VDRL-CSF) is highly specific but has low sensitivity. Therefore, the diagnosis of neurosyphilis usually depends on a combination of CSF cell count, CSF protein, and clinical manifestations with or without a reactive VDRL-CSF. Some specialists recommend performing an FTA-ABS test on CSF. The CSF FTA-ABS is less specific for neurosyphilis than the VDRL-CSF, but it is highly sensitive. A negative CSF FTA-ABS test result effectively rules out neurosyphilis. Patients with confirmed syphilis infections should be tested for other STDs, including HIV infection. If the HIV test is negative, the patient should be retested for HIV in 3 months except in areas with low HIV prevalence.

Diagnosis of syphilis in pregnant women

Advised screening all pregnant women for syphilis infection at the first prenatal visit. High-risk women (eg, uninsured women, women living in poverty, sex workers, illicit drug users, those with other STDs, those living in communities with high syphilis morbidity) should also be tested in the third trimester and at delivery. If the test results are positive for syphilis, the treatment of choice is parenteral benzathine penicillin G. Dosage and the length of treatment depend on the stage and clinical manifestations of the disease. Recommended STD treatment guidelines, pregnant women who are seropositive should be considered infected unless there is evidence of adequate treatment in the medical records and sequential serologic antibody titers have decreased by 4-fold. Serologic titers should be checked monthly if the patient is at risk for reinfection or lives in an area with high syphilis prevalence. Additionally, any woman who delivers a stillborn infant after 20 weeks' gestation should also be tested for syphilis. No infant should leave the hospital without the maternal serologic status having been determined at least once during pregnancy.

Diagnosis of congenital syphilis

Consider congenital syphilis and sexual abuse in all children who present with syphilis. Most infants with congenital syphilis are born to mothers with syphilis who were either not treated in pregnancy or treated too late during pregnancy. Treponemal tests (ie, TPPA, FTA-ABS, EIA, CIA) using neonatal serum are not recommended owing to passive transfer of IgG antibodies, which react with the reagents in these tests. All infants born to mothers with syphilis should be evaluated with a quantitative nontreponemal serologic test (RPR or VDRL) performed using the neonate's serum. The treatment algorithm below is provided in the 2015 CDC congenital syphilis guidelines for determining the need for further diagnostic workup and/or treatment.

Proven or highly probable congenital syphilis

Proven or highly probably congenital syphilis is defined as any one of the following:

- Abnormal physical examination findings that are consistent with congenital syphilis
- A serum quantitative nontreponemal serologic titer that is fourfold higher than the mother's titer
- A positive darkfield test result or positive PCR result using lesions or body fluid(s)

The recommended evaluation in proven or highly probable congenital syphilis includes the following:

- CSF analysis for VDRL, cell count, and protein
- Complete blood cell (CBC) count with differential and platelet count
- Other tests as clinically indicated (eg, radiography of long bones, chest radiography, liver function tests, neuroimaging, ophthalmologic examination, and auditory brain stem response)

Possible congenital syphilis

Congenital syphilis should be considered possible in any neonate who has normal physical examination findings and a serum quantitative nontreponemal serologic titer equal to or less than fourfold the maternal titer in addition to one of the following:

- Mother was not treated, inadequately treated, or has no documentation of having received treatment
- Mother was treated with erythromycin or a regimen other than those recommended in these guidelines (ie, a non–penicillin G regimen)
- Mother received recommended treatment less than 4 weeks before delivery

The recommended evaluation in possible congenital syphilis is as follows:

- CSF analysis for VDRL, cell count, and protein
- CBC count, differential, and platelet count
- Long-bone radiography

Less-likely congenital syphilis

Congenital syphilis is considered less likely in any neonate who has normal physical examination findings and a serum quantitative nontreponemal serologic titer equal to or less than fourfold the maternal titer and both of the following:

- Mother was treated during pregnancy, treatment was appropriate for the stage of infection, and treatment was administered more than 4 weeks before delivery
- Mother has no evidence of reinfection or relapse

No evaluation is recommended in these cases.

Unlikely congenital syphilis

Congenital syphilis is considered unlikely in any neonate who has normal physical examination findings and a serum quantitative nontreponemal serologic titer equal to or less than fourfold the maternal titer and both of the following:

- Mother's treatment was adequate before pregnancy
- Mother's nontreponemal serologic titer remained low and stable (ie, serofast) before and during pregnancy and at delivery (VDRL <1:2; RPR <1:4)

No evaluation is recommended in these cases.

For more information, see the 2015 CDC guidelines for congenital syphilis treatment.

Imaging Studies

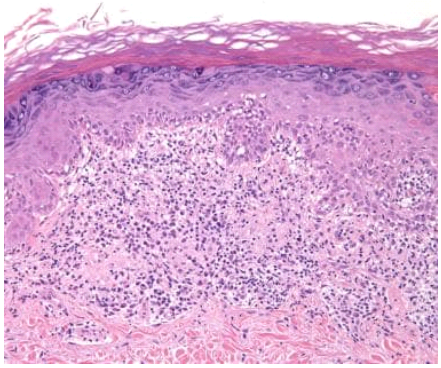
Imaging studies should be performed depending on the organ system involved. For example, granulomatous disease of the liver can be seen on computed tomography (CT) of the abdomen. Obtain chest radiography in patients with tertiary syphilis to screen for aortic dilatation. Linear calcification of the ascending aorta on chest films suggests asymptomatic syphilitic aortitis. Radiologic abnormal findings commonly seen with advanced gummas of bone include periostitis, destructive osteitis, or sclerosing osteitis. Angiography may be useful to distinguish between abdominal aneurysms of syphilitic versus arteriosclerotic origin. About 10% of syphilitic aneurysms occur superior to the renal arteries, while arteriosclerotic abdominal aneurysms usually are found inferior to the renal arteries.

Lumbar Puncture

Invasion of the central nervous system (CNS) by treponemes occurs in 30-40% of patients with primary or secondary syphilis; however, no studies show this to be a predictor of poor neurologic outcome. According to the 2015 CDC STD treatment guidelines, CSF laboratory abnormalities are common in persons with early syphilis, even when clinical neurological findings are absent. If clinical evidence of neurological involvement is found, a CSF examination should be performed. Current guidelines state that physicians should evaluate CSF in individuals with late latent syphilis if treatment fails or if neurologic or ocular symptoms are present. It is also indicated if there are other changes indicative of tertiary syphilis (eg, gumma, aortitis). LP should be performed in patients suspected of having neurosyphilis with no contraindication. There is no single test available for the definitive diagnosis of neurosyphilis; rather, the clinical symptoms, serology, and CSF values (CSF cell count or protein and a reactive CSF-VDRL) must be used in combination to determine the diagnosis. CSF examination is the only means by which the occurrence of asymptomatic neurosyphilis in latent syphilis can be excluded; however, it is not recommended unless the patient is asymptomatic or fails to respond serologically to treatment. Examination of the CSF should include the VDRL test, cell count, and protein level. Abnormalities of any of these measurements combined with a suggestive history and examination strongly indicate the presence of neurosyphilis. A positive VDRL test result is highly sensitive for active syphilis. Among persons with HIV infection and active syphilis, the CSF leukocyte count is usually elevated (>5 WBCs/µL), so more than 20 cells/µL should be considered positive.

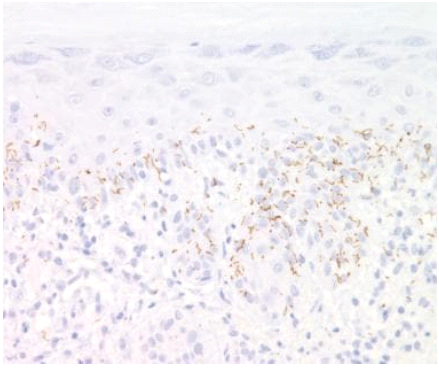
Histologic Findings

The primary lesion of syphilis is a chancre. Histologically, skin and mucosal lesions show a perivascular and perijunctional infiltrate of lymphocytes, plasma cells, and macrophages. At times, capillary endothelial proliferation and subsequent obliteration of small blood vessels may be appreciable. Focal erosion or ulceration is common. The inflammatory reaction of secondary syphilis is histologically similar to that of the primary chancre but is less intense. Skin lesions are typified by a "lichenoid-psoriasiform" configuration with a perijunctional infiltrate of lymphocytes, histiocytes, and plasmacytes (see the image below). Often the histiocytic component of the infiltrate is prominent, and thus the biopsy may assume a "lichenoid-granulomatous" configuration.



Lues hematoxylin and eosin stain. Histopathological examination shows a lichenoid infiltrate that is stereotypical of the secondary stage of syphilis. Note that vacuolar alteration of the superjacent epithelium can be seen much like a noninfectious form of lichenoid dermatitis. The subjunctional infiltrate is rich in histiocytes and plasmacytes. At times, an overtly granulomatous lichenoid infiltrate can be seen.

Small numbers of neutrophils may be included in the perijunctional infiltrate, and neutrophils may also be present in an expanded overlying stratum corneum. Organisms are readily demonstrable using *T pallidum* immunoperoxidase staining during the secondary stage (see the image below).



Lues TP stain. Immunoperoxidase staining for *T pallidum* highlights many slender coiled organisms residing in the perijunctional zone. Occasionally, organisms can also be found in the upper dermis or around adnexal structures. In tertiary syphilis, histological examination shows gummas consisting of granulomatous inflammation with central necrosis flanked by plump or palisaded macrophages and fibrocytes surrounded by large numbers of mononuclear leukocytes, including many plasma cells. Treponemes are rare in these lesions and typically cannot be cultured or visualized. Aortitis reveals inflammatory scarring of the tunica media, secondary to obliterative endarteritis of the vasa vasorum. Uneven loss of the medial elastic fibers and muscle cells may be evident.

Treatment & Management

Approach Considerations

Key principles for the treatment of syphilis include the following:

- Penicillin is the drug of choice to treat syphilis.
- Doxycycline is the best alternative for treating early and late latent syphilis. Syphilis associated with HIV infection does not require any enhanced antimicrobial therapy.
- In the treatment of late syphilis by weekly injections, missing a dose of penicillin for a period of 10-14 days does not require restarting the entire course of injections.
- The exception to this is in the case of pregnant women in whom there is no latitude for missing a dose of penicillin.
- There is evidence that an interval of 7-9 days between doses may produce better results.
- CSF testing to detect neurosyphilis is strongly recommended in patients with tertiary syphilis or with neurological signs or symptoms consistent with neurosyphilis and in patients without symptoms whose serologic titers do not decline appropriately after being treated with recommended therapy.
- Reinfection rates among MSM are high, so frequent serological testing in this group is recommended.
- CDC recommends the use of the RPR-based screening algorithm. When there is a low epidemiologic risk or clinical probability of syphilis, the positive predictive value of an isolated unconfirmed reactive treponemal chemoluminescence test or enzyme immunoassay is low.

INTERPRETATION

SYPHILIS TESTS

- Venereal Disease Research Laboratory
- VDRL
- Rapid Plasma Reagin
- RPR Test
- RPR Titer
- Fluorescent Treponemal Antibody Absorption Test
- FTA-ABS
- Treponema pallidum Particle Agglutination Assay
- TPPA
- Microhemagglutination Assay
- MHA-TP
- Darkfield Microscopy
- Automated Immunoassays for Syphilis Antibodies
- Treponema pallidum by PCR

Syphilis Detection Tests

Test Quick Guide

Syphilis is a sexually transmitted disease (STD) caused by the bacteria Treponema pallidum. A syphilis infection is spread through contact with a syphilic sore, also called a chancre, usually during vaginal, anal, or oral sex. Syphilis can also be spread from a parent to a fetus in pregnancy or to an infant during childbirth. If not treated appropriately, syphilis may cause severe damage to internal organs. Testing for syphilis is used in patients who have symptoms suggestive of this infection. Syphilis testing is also recommended to screen for syphilis in certain groups of people at an increased risk of infection or of transmitting the infection to others. Syphilis testing can be conducted using a sample of blood, a swab of fluid taken from a sore, or a sample of spinal fluid.

About the Test

Purpose of the test

The purpose of syphilis testing is to identify a syphilis infection. Syphilis tests may be prescribed for screening, diagnosis, or monitoring:

- Screening for syphilis is testing for the infection in people without signs or symptoms of syphilis. Groups who benefit from screening include those at high risk of contracting syphilis as well as groups more likely to transmit this infection to others.
- Diagnostic testing is recommended for people who have signs or symptoms that could be caused by syphilis. Syphilis can cause a wide range of symptoms, so doctors may recommend testing for syphilis even when symptoms aren't severe or specific to this condition. Generally, diagnosing syphilis requires two tests: an initial screening test and a second confirmatory test.
- Monitoring after treatment for syphilis is important to make sure that patients are responding to the prescribed treatment. Tests used to monitor patients include a physical exam to assess for changes in observable symptoms as well as laboratory blood tests to confirm a response to treatment.

What does the test measure?

Most forms of syphilis testing look for syphilis antibodies. Syphilis antibodies are substances in the blood that are made by the body's immune system in people who come into contact with the bacteria that causes syphilis. Several types of antibody blood tests may be used to detect a syphilis infection.

Nontreponemal antibody tests detect antibodies that are not specific to the Treponema pallidum bacteria that causes syphilis. Although these antibodies are usually produced when a person has syphilis, they can also be produced in response to other conditions. People usually test negative for these antibodies after successful treatment for syphilis infection. Nontreponemal antibody tests are usually used as an initial screening test and a positive result must be confirmed with another type of test. There are several types of nontreponemal antibody tests that may be used to detect and monitor syphilis:

- Rapid plasma reagin (RPR) test: This test looks for the reagin antibody, which is often produced by the body in response to a syphilis infection.
- Venereal Disease Research Laboratory (VDRL): A VDRL test measures antibodies that are often produced within one to two weeks after an infected person develops an initial sore. VDRL testing can be performed on blood or spinal fluid.

Treponemal antibody tests detect antibodies that are produced by the body only after infection with the bacteria that causes syphilis. Treponemal antibodies are detectable in the body earlier than nontreponemal antibodies and typically remain indefinitely. A person can still test positive for treponemal antibodies after completing syphilis treatment. This means that treponemal antibody tests cannot distinguish between a current and a past syphilis infection. Treponemal tests are usually performed to confirm an infection after a patient has a positive result on a nontreponemal screening test. Some of the most common treponemal tests include:

- Fluorescent treponemal antibody absorption (FTA-ABS) test
- Microhemagglutination assay for antibodies to Treponema pallidum (MHA-TP)
- Treponema pallidum hemagglutination assay (TPHA)
- Treponema pallidum enzyme immunoassay (TP-EIA)
- Chemoluminescence immunoassays (CLIA)

Most of the time, nontreponemal antibody tests are used to screen patients for syphilis while treponemal tests are used to confirm a patient's diagnosis. In some cases, a patient's doctor may start by screening a patient with a treponemal antibody test and then confirm a positive result using a nontreponemal antibody test. This testing strategy is called reverse sequence testing.

Less common methods to detect syphilis look for the bacterium itself or test for its genetic material. These test include:

- **Darkfield microscopy:** This method of detecting syphilis uses a sample of fluid from a skin sore or a lymph node. The sample is analyzed using a specially designed microscope under which the Treponema pallidum bacteria look bright against a dark background.
- **Polymerase chain reaction (PCR) testing:** PCR testing detects the genetic material, called DNA, of the Treponema pallidum bacteria.

When should I get a syphilis test?

Adults without symptoms should be screened for syphilis only if they are at an increased risk of infection or of transmitting the infection to others. Factors that increase a person's risk of contracting syphilis and indicate the need for annual screening include:

- Being male and under age 29
- Having a history of incarceration
- Involvement in commercial sex work
- Living in an area with high syphilis infection rates
- Being Black or African American

Other groups also benefit from regular screening at certain intervals.

These groups include:

- **Pregnant people** at first prenatal visit, at 28 weeks, and again at delivery if high risk.
- **Men and anyone with a penis** who is sexually active and has sex with another person with a penis should be tested annually. More regular testing may be recommended if they have additional risk factors.
- **Transgender and gender diverse people** should be screened annually depending on sexual behaviors and risk of exposure.
- **People diagnosed with HIV** may be screened at their first HIV evaluation, then one or more times each year depending on their behaviors and local infection rates.

Anyone with symptoms of syphilis should receive diagnostic testing. Without treatment, a syphilis infection progresses through three stages: primary syphilis, secondary syphilis, and tertiary syphilis. These three stages are separated by periods in which no symptoms are present. In any stage of syphilis, the infection can spread to the brain and nervous system, called neurosyphilis. Syphilis can also spread to the eye, called ocular syphilis, or to the ears, called otosyphilis.

The signs and symptoms of syphilis vary based on the stage of an infection:

SYPHILIS SYMPTOMS	
DISEASE STAGE	**SYMPTOMS**
Primary Syphilis	■ One or more painless sores, or chancres, that appear near the site of infection, usually the penis, vulva, or vagina
Secondary Syphilis	■ Swollen lymph nodes ■ A widespread skin rash on the palms of the hands or the soles of the feet ■ Wart-like growths near moist areas of the skin, such as the mouth, armpits, genitals, and anus ■ Fever ■ Fatigue ■ Loss of appetite ■ Weight loss ■ Less commonly, infection of the liver, eyes, or nervous system
Tertiary Syphilis	■ Inflammatory masses, called gumma, of the skin, bones, or internal organs ■ Deep bone pain ■ Heart damage
Neurosyphilis	■ Headaches ■ Lack of coordination ■ Numbness ■ Paralysis of body parts ■ Dementia, a mental disorder that causes memory loss
Ocular Syphilis	■ Vision changes ■ Blindness
Otosyphilis	■ Ringing in the ears ■ Hearing loss ■ Vertigo, ■ A rapid jerking movement of the eyes called nystagmus.

Syphilis Test Results

Interpreting test results

The way in which the results of syphilis tests are reported depend on the type of test conducted.

Results of **nontreponemal testing** may be reported as positive, also called reactive, or negative, also called non-reactive. If positive, the results may also indicate the amount of antibody present in the sample used for testing.

A positive nontreponemal test result means that a patient may have syphilis. A follow-up treponemal test is required to confirm a positive diagnosis. Negative test results indicate that a patient may not have syphilis, although additional testing may be needed if a patient is experiencing symptoms.

For **treponemal testing**, results are typically reported as reactive or nonreactive. A reactive test result indicates that a patient has had syphilis at some point in the past. Because a patient who has been treated for syphilis can continue having reactive results indefinitely, doctors take into account a person's health history when interpreting positive test results. A nonreactive treponemal test result indicates that antibodies to syphilis weren't detected and a patient is unlikely to have an infection.

It's important to discuss test results with a doctor or other health care professional who can help patients understand their test results.

Are test results accurate?

Both nontreponemal and treponemal antibody tests are important methods for detecting syphilis infections and preventing complications of this disease. Inaccurate results can occur, which is why doctors use strategies such as performing multiple tests to confirm positive results and taking a careful medical and sexual history to help interpret test results correctly.

Several conditions can cause a false positive result on a **nontreponemal test**, which means that the test result is positive despite the patient not having a syphilis infection. These conditions include IV drug use, HIV/AIDS, hepatitis B, Lyme disease, certain types of pneumonia, malaria, and autoimmune diseases.

False negative nontreponemal test results occur when a person tests negative, but they actually do have a syphilis infection. The most common reason for a false-negative result is that the antibodies detected in this type of syphilis test may not develop until 3 to 6 weeks after infection. Nontreponemal testing is also less accurate in patients in the tertiary stage of syphilis.

False positive test results can also occur in **treponemal testing** in patients who have been successfully treated for syphilis in the past. False negative test results may occur in treponemal tests during the first several weeks after infection.

Do I need follow-up tests?

In patients who are treated for syphilis, doctors measure treatment effectiveness by conducting nontreponemal tests and monitoring symptoms. The frequency of testing during treatment depends on the stage of a patient's infection and whether or not the patient is also diagnosed with HIV.

If the results of nontreponemal tests are negative, testing may be repeated after six weeks if doctors believe that a syphilis infection is likely.

Patients with syphilis should also be tested for other STDs, both at diagnosis and six months later.

TROUBLESHOOTING

UNDERSTAND ICT TESTS

Introduction

Immunochromatography assay (ICA), namely lateral flow test, is a simple device intended to detect the presence or absence of the target analyte. The concept of immune-chromatography is a combination of chromatography (separation of components of a sample based on differences in their movement through a sorbent) and immunochemical reactions. The most widespread immunochromatographic system is the test strip (Figure 1).

Developing an ICA

Strips used for ICA contain four main components:

1. Sample Application Pad

It is made of cellulose and/or glass fiber and sample is applied on this pad to start the assay. Its function is to transport the sample to other components. Sample pad should be capable of transportation of the sample in a smooth, continuous and homogenous manner. This pretreatment may include separation of sample components, removal of interferences, adjustment of the pH, etc. analyte sample should be added to the sample application pad to start the test.

2. Conjugate Pad

It is the place where labeled biorecognition molecules (labeled antibodies, usually nano colloid gold particle) are dispensed. Material of conjugate pad should immediately release labeled conjugate upon contact with moving liquid sample. Labeled conjugate should stay stable over entire life span of the lateral flow strip. Any variations in dispensing, drying or release of conjugate can change the results of assay significantly. Poor preparation of labeled conjugate can adversely affect sensitivity of the assay. Glass fiber, cellulose, polyesters and some other materials are used to make conjugate pad.

3. Substrate (Nitrocellulose) Membrane

It is highly critical in determining sensitivity of ICA. Test and control lines are drawn over this piece of membrane. So an ideal membrane should provide support and good binding to capture probes (antibodies, etc.). Nonspecific adsorption over test and control lines may affect results of assay significantly, thus a good membrane will be characterized by lesser non-specific adsorption in the regions of test and control lines. Proper dispensing of bioreagents, drying and blocking play a role in improving sensitivity of the assay.

4. Adsorbent Pad

It works as sink at the end of the strip. It also helps in maintaining flow rate of the liquid over the membrane and stops back flow of the sample. Adsorbent capacity to hold liquid can play an important role in results of assay.

All these components are fixed or mounted over a backing card. Materials for backing card are highly flexible because they have nothing to do with ICA except providing a platform for proper assembling of all the components. Thus, backing card serves as a support and it makes easy to handle the strip.

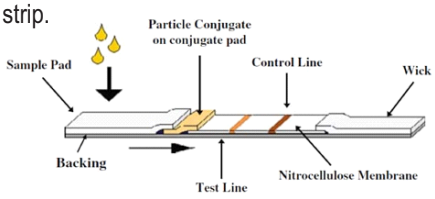


Figure 1. Typical layout of a lateral flow test strip.

Major steps in ICA are:

(i) Preparation of labeled antibody and capture antibody against target analyte;

(ii) Immobilizing the labeled antibody onto conjugate pad, and the capture antibody onto the strip membrane to form the Test/Control line.

(iii) Assembling of all components onto a backing card after dispensing of reagents at their proper pads.

(iv) Add samples and buffer onto sample pad.

(v) Wait the sample flow through the test and control line for 5-10min.

(vi) Read the result when the color reveal.

ICA Format

Lateral flow assay basically combines a number of variants such as formats, biorecognition molecules, labels, detection systems and applications. There are three types of ICAs based on detection format, which are:

1. Sandwich Assay

In this assay format, label (Enzymes or nanoparticles or fluorescence dyes) coated antibody is immobilized at conjugate pad. This is a temporary adsorption which can be flushed away by flow of any buffer solution. A capture antibody against target analyte is immobilized over test line. A secondary antibody against labeled antibody is immobilized at control zone (Figure 2).

To start a test, sample containing the analyte is applied to the sample application pad and it subsequently migrates to the other parts of strip. At conjugate pad, target analyte is captured by the immobilized labeled antibody and results in the formation of analyte-labeled antibody complex. This complex now reaches to nitrocellulose membrane and moves under capillary action. At test line, analyte-labeled antibody complex is captured by another antibody which is primary to the analyte. Analyte becomes sandwiched between labeled and primary antibodies forming labeled antibody-analyte-primary antibody complex. Excess labeled antibody will be captured at the control zone by secondary antibody. Buffer or excess solution goes to absorption pad. Intensity of color at test line corresponds to the amount of target analyte and is measured with an optical strip reader or visually inspected. Appearance of color at control line ensures that a strip is functioning properly.

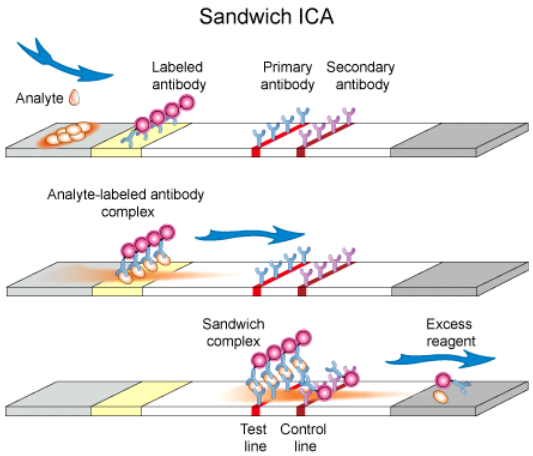


Figure 2. Schematic diagram of Sandwich ICA.

2. Competitive Assay

Competitive format has two layouts. In the first layout, solution containing target analyte is applied onto the sample application pad and prefixed labeled antibody gets hydrated and starts flowing with moving

liquid. Test line contains pre-immobilized antigen (same analyte to be detected) which binds specifically to label conjugate. Control line contains pre-immobilized secondary antibody which has the ability to bind with labeled antibody. When liquid sample reaches at the test line, pre-immobilized antigen will bind to the labeled conjugate in case target analyte in sample solution is absent or present in such a low quantity that some sites of labeled antibody conjugate were vacant. Antigen in the sample solution and the one which is immobilized at test line of strip compete to bind with labeled conjugate (Figure 3.). In another layout, labeled analyte conjugate is dispensed at conjugate pad while a primary antibody to analyte is dispensed at test line. After application of analyte solution, a competition takes place between analyte and labeled analyte to bind with primary antibody at test line.

Such format suits best for low molecular weight compounds which cannot bind two antibodies simultaneously. Absence of color at test line is an indication for the presence of analyte while appearance of color both at test and control lines indicates a negative result.

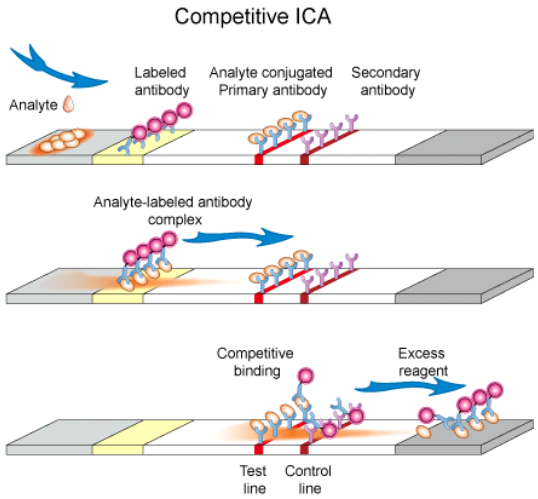


Figure 3. Schematic diagram of competitive ICA.

3. Multiplex Detection Assay

Multiplex detection format is used for detection of more than one target species and assay is performed over the strip containing test lines equal to number of target species to be analyzed. It is highly desirable to analyze multiple analytes simultaneously under the same set of conditions. Multiplex detection format is very useful in clinical diagnosis where multiple analytes which are inter-dependent in deciding about the stage of a disease are to be detected. Lateral flow strips for this purpose can be built in various ways, for example, by increasing length and test lines on conventional strip, making other structures like parallel threads, stars or T-shapes. Shape of strip for ICA will be dictated by number of target analytes.

Lateral flow immunoassays represent a well-established and very appropriate technology when applied to a wide variety of point-of-care (POC) or field use applications.

The advantages of the lateral flow immunoassay system (LFIA) are well known:

- Relative ease of manufacture - equipment and processes already developed and available. Easily scalable to high-volume production
- Stable - shelf-lives of 12-24 months often without refrigeration
- Ease of use: minimal operator-dependent steps and interpretation
- Can handle small volumes of multiple sample types
- Can be integrated with onboard electronics, reader systems, and information systems
- Relatively low cost and short timeline for development and approval
- Market presence and acceptance - minimal education required for users and regulators

Traditionally designed lateral flow immunoassays, however, have also suffered from performance limitations, most notably sensitivity and reproducibility. Some of these issues are listed below:

- Miniaturization of sample volume requirements below microliter level
- Multiplexing: simultaneous analysis of multiple markers difficult
- Integration with onboard electronics and built-in QC functions challenging
- Sensitivity issues in some systems
- Test-to-test reproducibility challenging - limits applications in quantitative systems

Benefit from its rapid test procedure and naked eyes visible characteristics, lateral flow immunoassays have achieved broad penetration in a variety of markets. Here we summarize some of the applications in figure 4.

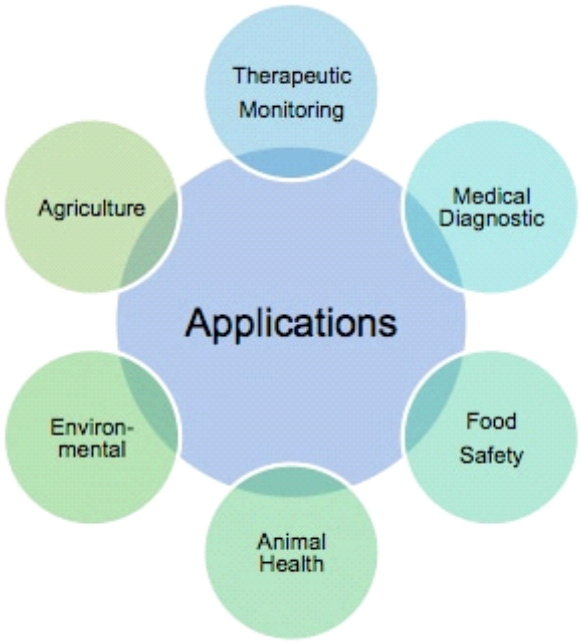
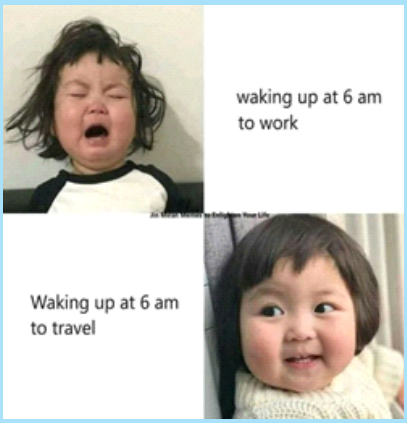


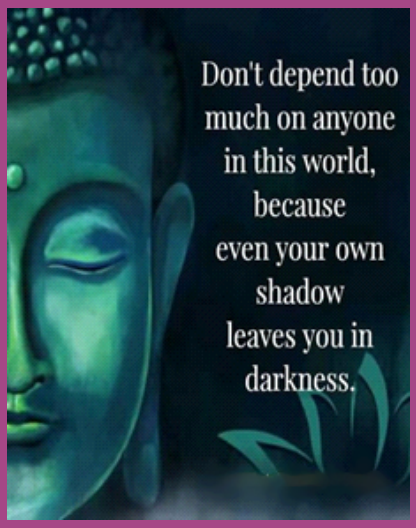
Figure 4. Applications of Immunochromatography assay.

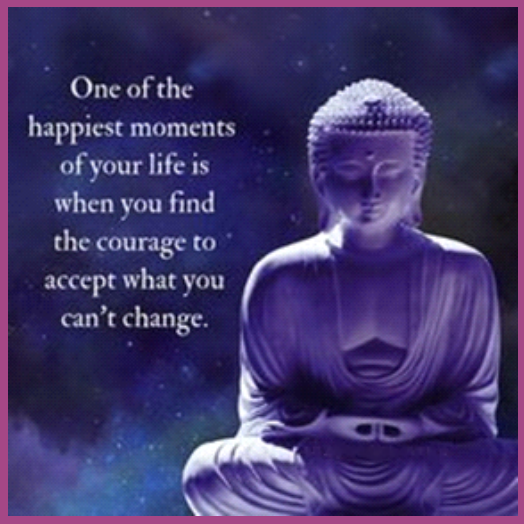
BOUQUET

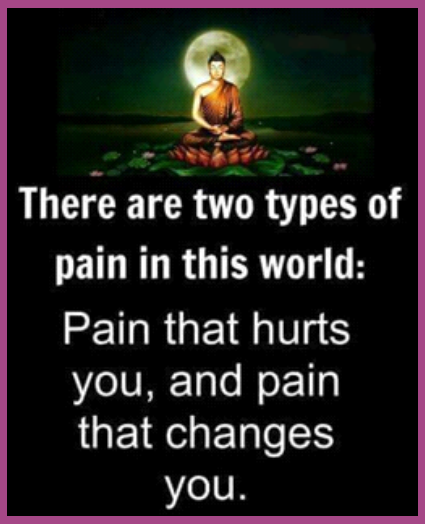
In Lighter Vein



Wisdom Whispers







Brain Teasers

1. **The size of HIV virion is approximately nanometers in diameter.**
   A. 100 C. 300
   B. 200 D. 400.

2. **The HIV virus has strands.**
   A. 2 RNA strands C. 1 RNA and 1 DNA strand
   B. 2 DNA strands D. None of the above.

3. **Which of the following are HIV viral enzymes**
   A. Reverse transcriptase C. Protease
   B. Integrase D. All of the above.

4. **Which of the following is not an HIV core protein?**
   A. p7 C. gp120
   B. p9 D. p24.

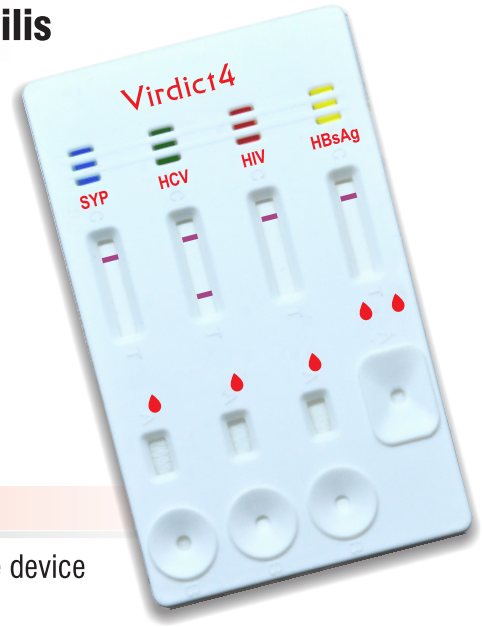
ANSWER: 1: A, 2: A, 3: D, 4: C

Virdict4

Integrated Test for:
• HIV • HCV • HBsAg • Syphilis

Sensitivity & Specificity*

Analyte	Sensitivity	Specificity
HIV 1 & 2	100%	100%
HCV	100%	100%
HBsAg	100%	100%
Syphilis	>95%	100%



Virdict4 *Synergistic Benefits:*

Influences Detection of individual & coinfections

i) Detects and differentiates HIV, HBsAg, HCV and syphilis in single device
ii) Facilitates detection of coinfections
iii) Facilitates timely treatment and prevents onward transmission benefits the patient & the society

Single test suitable for different profile prescriptions benefits the laboratory & Clinicians

i) Presurgical screening
ii) STI screening
iii) ANC (Antenatal care) screening
iv) TTI (transfusion transmitted infections) screening in resource limited settings

Minimizes procedural & labelling errors

i) Single labelling for 4 individual tests
ii) Uniform sample and buffer volumes for the Antibody detection tests (HIV, HCV & Syp.) enhances procedural compliance

Convenience from performance to procurement

i) Sensitivity and specificity within recommended guidelines
ii) Minimizes analytical variables
iii) One brand for 4 different assays
iv) Single vendor for procurement
v) Single dropper, pouch and desiccator handling reduces biohazard and waste burden

*Data on File Tulip Diagnostics (P) Ltd.

Influencing Trends of "Integrated" Testing

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
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