

For the use of Registered Medical Practitioners and Laboratories only



TECHNICAL SERIES

Syphilis Diagnosis



TULIP DIAGNOSTICS (P) LTD.

Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex,
Post Office, Goa - 403 202. INDIA. Telephone Nos.: (0832) 2458545-51 Fax:(0832)2458544
E-mail: tulip@goatelecom.com Website:<http://www.tulipgroup.com>



...Setting trends



Other Technical Series published by TULIP:

1. Lupus Anticoagulants
– Basic concepts and Laboratory Diagnosis
2. Monitoring oral anticoagulant therapy
– Concepts & Practice
3. Mycobacterium Tuberculosis
– AFB staining, Culture and sensitivity
4. Quality Assurance for routine Haemostasis
Laboratory
5. Anti Human Globulin Reagent
- Basic Concepts and Practice

Foreword

TULIP Diagnostics (P) Ltd. is a part of the innovative **TULIP** Group of companies based at Goa, India.

The group's commitment in building products of international standards, through indigenous R&D has accorded the company virtual leadership in most product segments in the Indian marketplace. Its state-of-art manufacturing facility conforms to the strictest FDA (India) and GMP regulations. In its efforts to build world-class Quality products, the group has recently received the ISO 9001(2000) certification from TUV.

It is this commitment to Quality, which has given the group international acclaim. The products are now exported to over 45 countries globally with an ever-increasing user base.

With decades of experience in *in-vitro* diagnostics (IVD), **TULIP** has created a strong knowledge base. **TULIP** believes that in the knowledge-based society of the 21st century, regular upgradation of knowledge is essential not only for better diagnosis and patient care, but also to improve the overall quality of life.

Publishing of **Technical Series** is one such initiative to make available to the Laboratory professionals and clinicians updated knowledge that is vital for them to set trends in their day-to-day practice.

Contents

Introduction.....	1
Mode of Transmission	1
Clinical Manifestations.....	2
Clinical Manifestations in special populations.....	5
Immunology of infection.....	7
Diagnosis	9
Serological Tests.....	9
The Prozone Phenomenon – Diagnostic implications in syphilis.....	18
Treatment/ Medication.....	19
Glossary of terms.....	20
Bibliography.....	21

INTRODUCTION

Syphilis, which is also called *lues* (from a Latin word meaning plague), has been a major public health problem since the sixteenth century. The disease was treated with mercury or other ineffective remedies until World War I, when effective treatments based on arsenic or bismuth were introduced. Antibiotics succeeded these after World War II. It is estimated that about 50 million persons worldwide need treatment for syphilis with 12 million new cases added every year.

MODE OF TRANSMISSION

Syphilis is caused by *Treponema pallidum*, a spirochete. It is a thin spiral or coil-shaped bacterium that enters the body through the mucous membranes or breaks in the skin. In around 90% of cases, the spirochete is transmitted by sexual contact.

Causative organisms and characteristics of different pathogenic treponemal infections

	Syphilis	Bejel - endemic syphilis	Yaws	Pinta
Causative organism	<i>T. pallidum</i> subsp. pallidum	<i>T. pallidum</i> subsp. Endemicum	<i>T. pallidum</i> subsp. pertenue	<i>T. carateum</i>
Geographic Distribution	Worldwide	Mid. East, Africa	Africa, Pacific	Central & South America
Preferred climate	All	Subtropical	Tropical	Warm
Typical age of infection in yrs	15-40	1-10	1-15	10-30
Main mode of transmission	Sexual, congenital	skin contact	skin contact	skin contact
Late complications				
- skin / mucosa	+	+	+	+
- bone / cartilage	+	+	+	-
- CNS	+	-	-	-
- heart / circulation	+	-	-	-

Other means of transmission include passage through the placenta (vertical transmission), kissing, transfusion of fresh blood, and direct inoculation such as by needlestick. Transmission by blood transfusion is rare, not only because blood products are screened for the disease, but also because the spirochetes do not survive more than 3 days in stored blood. However, there have been some recent reports, that the spirochete can survive upto 5 days, at 4°C, in stored blood. So it is mandatory to screen all blood samples before transfusion. To avoid vertical transmission, all pregnant women should also be routinely screened.

CLINICAL MANIFESTATIONS

Primary syphilis

Primary syphilis is the stage of the organism's entry into the body. The first signs of infection are not always noticed. After an incubation period ranging between 10 - 90 days, the patient develops a chancre, which is a small blister-like sore about 0.5 inches (13 mm) in size. Most chancres are on the genitals, but may also develop in or on the mouth or on the breasts. Rectal chancres are common in male homosexuals. Chancres in women are sometimes overlooked if they develop in the vagina or on the cervix. The chancres are not painful and disappear in 3-6 weeks even without treatment. They resemble the ulcers of lymphogranuloma venereum, herpes simplex virus, or skin tumors.

About 70% of patients with primary syphilis also develop swollen lymph nodes near the chancre. The nodes may have a firm or rubbery feel when touched but are not usually painful.

Secondary syphilis

Syphilis enters its secondary stage between 6 weeks - 6 months after contracting the infection. Chancres may still be present but are usually self-healing. Secondary syphilis is a systemic infection marked by the eruption of skin rashes and ulcers in the mucous membranes.

The skin rash may mimic a number of other skin disorders such as drug reactions, rubella, ringworm, mononucleosis, and pityriasis rosea. Characteristics that point to syphilis include:

- A coppery color skin rash
- Absence of pain or itching
- Occurrence of rash on the palms of hands and soles of feet.

The skin eruption may resolve in a few weeks or may last as long as a year. The patient may also develop condylomata lata, which are weepy pinkish or grey areas of flattened skin in the moist areas of the body. *The skin rashes, oral and genital ulcers,*

and condylomata lata are all highly infectious.

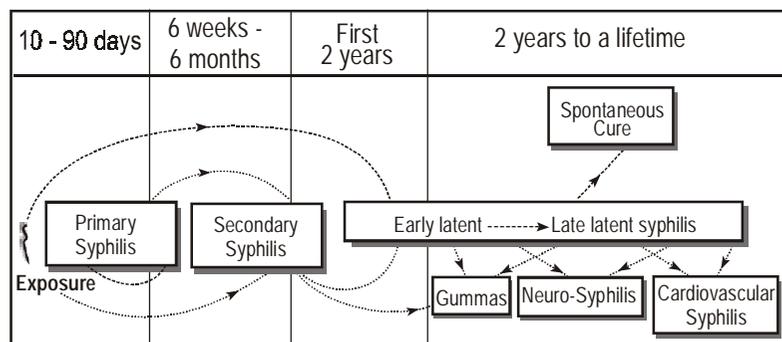
About 50% of patients with secondary syphilis develop swollen lymph nodes in the armpits, groin, and neck areas; about 10% develop inflammations of the eyes, kidney, liver, spleen, bones, joints, or the meninges (membranes covering the brain and spinal cord). Patients may also have flu like general illness with a low fever, chills, loss of appetite, headaches, runny nose, sore throat, and aching joints.

Latent syphilis

Latent syphilis is a phase of the disease characterized by relative absence of clinical symptoms. The term latent does not mean that the disease is not progressing or that the patient cannot infect others. For example, *pregnant women can transmit syphilis to their unborn children during the latency period of the infection.*

The latent phase is sometimes divided into *early latency* (less than two years after infection) and *late latency*. During early latency, patients are at risk for spontaneous relapses marked by recurrence of the ulcers and skin rashes of secondary syphilis. In late latency, these recurrences are less likely. Late latency may either resolve spontaneously or continue for the rest of the patient's life.

Course of untreated syphilis



Tertiary syphilis

Untreated syphilis progresses to a third or tertiary stage in about 35-40% of patients. *Patients with tertiary syphilis cannot infect others with the disease.* It is thought that the symptoms of this stage are a delayed hypersensitivity reaction to the spirochetes. Some patients develop so-called benign late syphilis, which begins between 3 - 10 years after infection and is characterized by the development of gummas. Gummas are rubbery

tumor-like growths that are most likely to involve the skin or long bones but may also develop in the eyes, mucous membranes, throat, liver, or stomach lining. Gummas are increasingly uncommon since the introduction of antibiotics for treating syphilis. Benign late syphilis is usually rapid in onset and responds well to treatment.

Cardiovascular syphilis

Cardiovascular syphilis occurs in 10-15% of patients who have progressed to tertiary syphilis. It develops between 10 and 25 years after infection and often occurs together with neurosyphilis. Cardiovascular syphilis usually begins as an inflammation of the arteries leading from the heart and causes heart attacks, scarring of the aortic valves, congestive heart failure, or the formation of an aortic aneurysm.

Neurosyphilis

About 8% of patients with untreated syphilis will develop symptoms in the central nervous system that include both physical and psychiatric symptoms. Neurosyphilis can appear at any time, from 5-35 years after the onset of primary syphilis. Neurosyphilis is classified into four categories:

- 1. Asymptomatic.** In this form of neurosyphilis, the patient's spinal fluid gives abnormal test results but there are no symptoms affecting the central nervous system.
- 2. Meningovascular.** This type of neurosyphilis is marked by changes in the blood vessels of the brain or inflammation of the meninges (the tissue layers covering the brain and spinal cord). The patient develops headaches, irritability, and visual problems. If the spinal cord is involved, the patient may experience weakness of the shoulders and upper arm muscles.
- 3. Tabes dorsalis.** Tabes dorsalis is a progressive degeneration of the spinal cord and nerve roots. Patients lose the sense of perception of their body position and orientation in space (proprioception), resulting in difficulties in walking and loss of muscle reflexes. They may also have shooting pains in the legs and periodic episodes of pain in the abdomen, throat, bladder, or rectum. Tabes dorsalis is sometimes called locomotor ataxia.
- 4. General paresis.** General paresis refers to the effects of neurosyphilis on the cortex of the brain. The patient has a slow but progressive loss of memory, ability to concentrate, and interest in self-care. Personality changes may include irresponsible behavior, depression, delusions of grandeur, or complete psychosis. General paresis is sometimes called dementia paralytica, and is most common in patients over 40 years of age.

CLINICAL MANIFESTATION IN SPECIAL POPULATIONS

Congenital syphilis

Almost all infants born to mothers with untreated primary or secondary syphilis will be infected, whereas the infection rate drops to 40% if the mother is in the early latent stage and 6-14% if she has late latent syphilis.

The prognosis for early congenital syphilis is poor: about 54% of infected fetuses die before or shortly after birth. Those who survive may look normal at birth but show signs of infection between three and eight weeks later.

Diagnostic classification of congenital syphilis

Laboratory criteria for diagnosis

Demonstration of *Treponema pallidum* by darkfield microscopy, fluorescent antibody, or other specific stains in specimens from lesions, placenta, umbilical cord, or autopsy material.

Case classification

Probable: A condition affecting an infant whose mother had untreated or inadequately treated* syphilis at delivery, regardless of signs in the infant, or an infant or child who had a reactive treponemal test for syphilis and any one of the following:

- Any evidence of congenital syphilis on physical examination
- Any evidence of congenital syphilis on radiographs of long bones
- A reactive CSF VDRL Test
- An elevated CSF cell count or protein (without other cause)
- A reactive fluorescent treponemal antibody absorbed 19S class-IgM antibody test or IgM enzyme-linked immunosorbent assay

Confirmed: A case that is laboratory confirmed

Also includes mother with serologic evidence of relapse or reinfection after treatment or with a documented history of treatment of insufficient serologic follow-up after appropriate treatment.

From " Case Definitions for Infectious Conditions Under Public Health Surveillance."

Morbidity and Mortality Weekly Report, Vol.46, No.RR-10. Pp.37-38

Centers for Disease Control and Prevention. U.S. Public Health Service

U.S. Department of Health and Human Services, 1997.

Infants with **early congenital syphilis** have systemic symptoms that resemble those of adults with secondary syphilis. There is a 40-60% chance that the child's central nervous system will be infected. These infants may have symptoms ranging from jaundice, enlargement of the spleen and liver, anemia, skin rashes, condylomata lata, inflammation of the lungs, "snuffles" (a persistent runny nose), and swollen lymph nodes.

Late congenital syphilis

Children who develop symptoms after the age of two years are said to have late congenital syphilis. The characteristic symptoms include facial deformities (saddle nose), Hutchinson's teeth (abnormal upper incisors), saber shins, dislocated joints, deafness, mental retardation, paralysis, and seizure disorders.

As far as possible, all pregnant women should be tested for syphilis at their *first prenatal visit*. It should be *repeated in the third trimester and at delivery* if the patient is at high risk or in communities or populations with high prevalence of syphilis.

Sero-titers may increase slightly when serofast women who were previously adequately treated for syphilis become pregnant. It is generally less than a fourfold increase. Serologic response to treatment is the same as for non-pregnant women. As far as possible, no infant should be discharged from the hospital without the serological status of the mother being known.

HIV patients

Syphilis has been closely associated with HIV infection since the late 1980s. Syphilis sometimes mimics the symptoms of AIDS. Conversely, AIDS appears to increase the severity of syphilis in patients suffering from both diseases. AIDS also speeds up the development or appearance of neurosyphilis. Although standard serologic tests for syphilis are considered a relatively reliable method of diagnosis in patients with HIV infection, false-negative treponemal and nontreponemal tests occur somewhat more frequently in patients who have HIV infection. If suspicious lesions are present in a patient with HIV infection, but serologic tests are negative, biopsy of the lesion should be performed for microscopic examination to identify spirochetes.

Ocular syphilis

Syphilis is the commonest bacterial intraocular infection in HIV-infected patients. Vision loss in patients with syphilis occurs most frequently as a result of either uveitis or optic nerve disease, which may manifest itself as papillitis, perineuritis or retrobulbar optic neuropathy, although uveitis appears to be the most common complication.

IMMUNOLOGY OF SYPHILIS INFECTION

Immune response to the first infection

The activation of the cellular & humoral immune system takes place during the incubation period. Approximately 4 days after invasion by the organism, *T.pallidum*-specific antibodies of IgM class are synthesized in the host. At 10-21 days post infection these antibodies reach titers which can be detected using appropriate IgM antibody assays. IgG antibodies are detectable a few days later. It has not been entirely elucidated why symptoms of secondary syphilis occur about 3-6 weeks after the primary complex, at a time when the patient appears to have immunological control over the local infection. During the stage of the infection hematogenous spread of the treponemes throughout the body, eruption of skin rashes and the development of systemic immunity occur. Although the body is protected against reinfection during this stage, it is unable to eliminate the causative organism. The manifestations of the secondary stage, such as recurrent skin eruptions lasting from weeks to months, seem to be related to the development of systemic immunity.

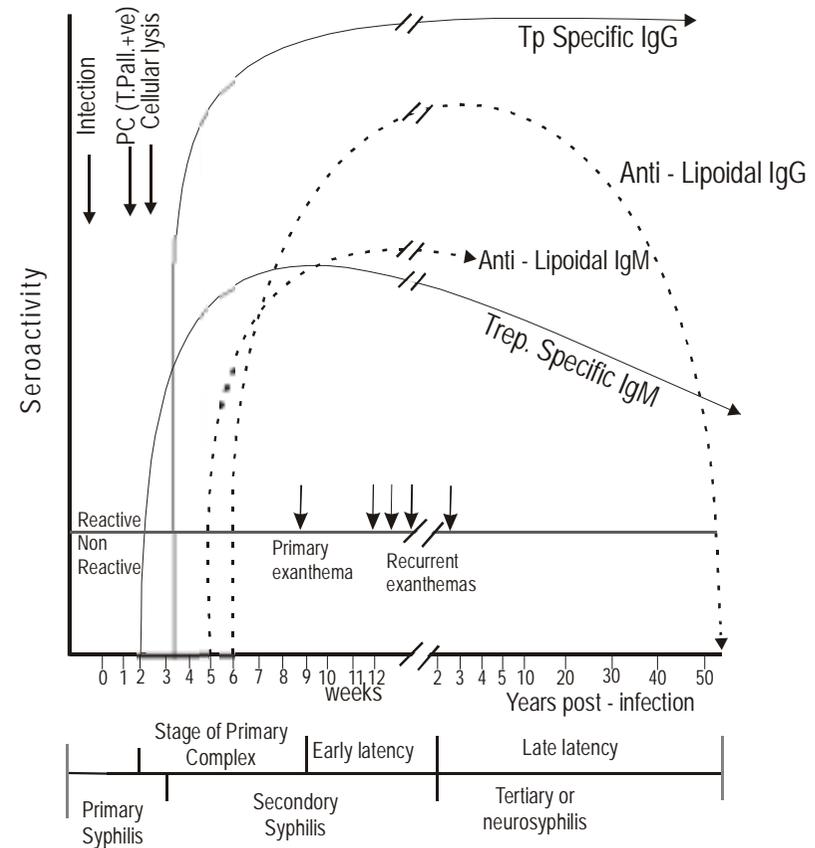
If the host has immunological control over the *T.pallidum* infection to the point where no further clinically apparent eruptions occur, the period of late latency is said to be present. During this phase a delayed type of hypersensitivity reaction against the causative organism is detectable. This reaction, together with the humoral immune response, is considered to be crucial for the suppression of infection. The elimination of treponema from the host is not linked to this and the mechanism of *T.pallidum* persistence in the infected patient has not been elucidated. It is possible that the causative organism survives within so-called "immunological niches", such as CNS.

After another latency period, which may last more than 20 yrs, clinical manifestations of tertiary syphilis may occur. The immunological mechanisms leading to this are unknown.

Immune response after specific therapy

Curative treatment of the infection results in the disappearance of *T.pallidum*-specific IgM antibodies from patient's serum usually within 3-12 months. The reduction of IgG antibodies depends on the time interval between infection & first antibiotic treatment. If this interval is short the infection may resolve without "immunological scars" i.e., all serological tests may again have negative results. If the time interval amounts to several months or even years many clones of memory cells will have developed, containing the information for the production of *T.pallidum*-specific IgG antibodies. Under these circumstances, it is possible that the antibody concentration will not decrease below the lower limit of detection in some assays. In such cases there is a residual, so-called, immunological IgG scar, i.e., persistence of IgG, which may remain detectable for life.

Sero-response during various stages of syphilis infection



Immune response after the second or multiple reinfections

In the case of second or multiple reinfections different pathophysiological mechanisms control the synthesis of antibodies. Second or multiple contacts with the antigens boost production of pre-formed IgG antibodies. Immediately after infection these antibodies show a steep rise in titer. IgG synthesis simultaneously leads to the *in vivo* inhibition or suppression of the synthesis of specific IgM antibodies. These IgM antibodies are either not detectable in serum of patients, or only after a time delay of 2-4 weeks.

DIAGNOSIS

Patient history and physical diagnosis

The diagnosis of syphilis is often delayed because of the variety of early symptoms, the varying length of the incubation period, and the possibility of not noticing the initial chancre. Patients do not always connect their symptoms with recent sexual contact. Women may be diagnosed in the course of a gynecological checkup. Definite diagnosis, however, depends on the results of laboratory blood tests.

Direct visualization of the spirochetes

T. pallidum is too thin to be observed by light microscopy. Therefore, *darkfield microscopy* or electron microscopy is required to visualize the organism. No effective method exists for culturing *T. pallidum* in the routine clinical laboratory setting. It has been cultured *in vivo* most commonly by inoculating rabbits (RIT: rabbit infectivity testing). This experimental model for detection through propagation in rabbits is impractical, expensive and requires too much time to be practically useful.

The presence of characteristic organisms in a specimen obtained from a typical lesion of very early syphilis is diagnostic, even in the absence of positive serologic tests. Dark-field microscopy of material from a genital lesion demonstrates corkscrew-like organisms moving with a spiraling motion. Oral and anal lesions cannot be used as sources for specimens because of the presence of nonpathogenic treponemes.

SEROLOGICAL TESTS

Serological tests for syphilis may be classified into two distinct groups - *Non-treponemal & Treponemal* tests. Serology cannot distinguish between the different treponematoses (syphilis, yaws, pinta, and bejel). In practice, serological tests for syphilis are used for:

- Screening asymptomatic individuals with no history suggestive of syphilis, such as pregnant women.
- Screening genitourinary medicine clinic attenders at recent risk of acquiring a sexually transmitted infection.
- Screening blood and organ/tissue donors.
- Detecting or excluding current or past syphilis in patients with HIV infection.
- Testing patients whose history or clinical signs are consistent with syphilis – for example, genital ulceration or chronic neurological illness.
- Confirmatory testing of specimens reactive in screening tests for syphilis.
- Assessment of the stage of infection and monitoring the therapeutic response.

Performance characteristics of non treponemal tests

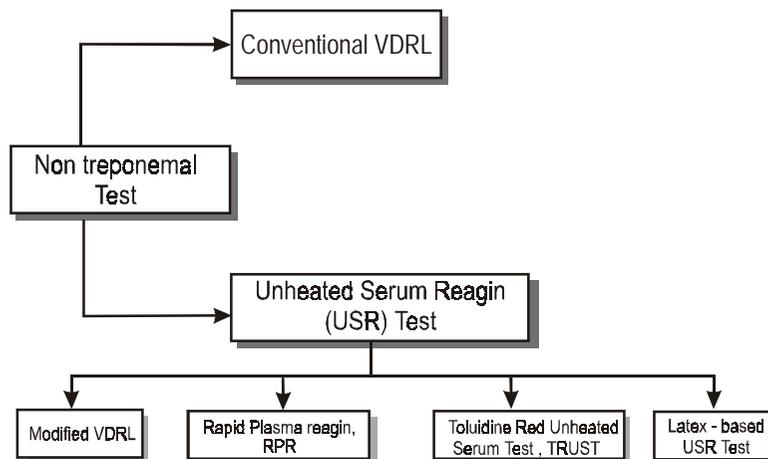
Parameter	Conventional VDRL	Modified VDRL	RPR	TRUST	Latex
Sample used	Serum /Plasma/ CSF	Serum /Plasma/ CSF	Serum / Plasma	Serum / Plasma	Serum / Plasma
Sample Inactivation	Required @ 56° C	Not Required	Not Required	Not Required	Not Required
Test Done On	Glass Concavity Slide	Glass Concavity Slide	Disposable Plastic Cards	Disposable Plastic Cards	Disposable Plastic Cards
Reagents	•Cardiolipin Antigen •Buffer	Mod. VDRL Reagent	Carbon Antigen	Toluidine Red Antigen	Latex Reagent
Stability	Reconstituted Stability: 24 Hrs.	•Ready to use •Stability > 1 yr.	•Ready to use •Stability > 1 yr.	•Ready to use •Stability > 1 yr.	•Ready to use •Stability > 1 yr.
Test duration	4 Minutes	4 Minutes	8 Minutes	8 Minutes	6 Minutes
Additional material provided with the kit	None	•Pos. Control •Mod.VDRL reagent •Sample dropper	•Pos. Control •Neg. Control •Dispensing pipettes •Mixing sticks, •Reaction Cards	•Pos. Control •Neg. Control •Dispensing pipettes •Mixing sticks, •Reaction Cards	•Pos. Control •Neg. Control •Dispensing pipettes •Mixing sticks, •Reaction Cards
Relative sensitivity in different stages of Syphilis	Primary- 70% Secondary- 99% Late - 1%	Primary- 70% Secondary- 99% Late - 1%	Primary - 80% Secondary-99% Late - 0%	Primary - 80% Secondary-99% Late - 0%	Primary - 80% Secondary-99% Late - 0%
Final reaction	Opaque floccules against clear background	White/Opaque floccules against clear background	Black aggregates against white background	Red/Pink aggregates against white background	White aggregates against black background
Reading	Macroscopic + Microscopic	Macroscopic + Microscopic	Macroscopic only	Macroscopic only	Macroscopic only
Reaction grading	•Non-reactive •Weakly reactive •Reactive	•Non-reactive •Weakly reactive •Reactive	•Non-reactive •Weakly reactive •Reactive	•Non-reactive •Weakly reactive •Reactive	•Non-reactive •Weakly reactive •Reactive

Nontreponemal tests

Nontreponemal antigen tests are used for screening as well as monitoring the patient's response to treatment. They measure the presence of reaginic antibodies, which are a group of anti-lipoidal antibodies. They detect both IgM and IgG antibodies (reagin) directed against the cardiolipin-lecithin- cholesterol antigen. The reaction may be microscopic (Venereal Disease Research Laboratory test e.g. VDRL) or macroscopic (Rapid Plasma Reagin e.g. RPR). They are not specific for *T. pallidum* and their sensitivity varies with stage of infection. A positive nontreponemal test must be confirmed with a treponemal test for a diagnosis of syphilis. After adequate treatment, 97% of patients with primary syphilis should have a negative RPR/VDRL within one year. The greater the duration of untreated disease the greater the time needed to achieve seronegativity.

In the **VDRL test**, a sample of the patient's serum is mixed with the antigen (cardiolipin-lecithin- cholesterol antigen). If the mixture forms clumps (flocculation), it is reactive or positive. The serum sample can be diluted several times to determine the concentration of reagin in the patient's blood. One of the major disadvantages of conventional VDRL test is that the serum needs to be decplemented by heating prior to testing to reduce interference from complements.

Different format for Non - treponemal Tests



Unheated Serum Reagin (USR) tests solve this problem. Here the reagent is ready-to-use and no serum heating is required. *Chelating agents* are added to neutralize the interference due to complements. Also, a buffer is added to stabilize the emulsion. Several types of USR tests are available. These types of tests show a high incidence (8-10%) of false negatives due to the prozone phenomenon. For this reason its preferable to run the tests at two dilutions.

The **Rapid Plasma Reagin (RPR)** test works on the same principle as the VDRL. Here charcoal particles are used to visualize the results.

TRUST utilizes toluidine Red dye instead of charcoal.

Another format is using cardiolipin-coated polystyrene **LATEX** particles for visualization. Of late the LATEX-based tests are becoming popular owing to their good readability. Nontreponemal tests can yield both false-negative and false-positive results. False-positive results can be caused by other infectious diseases, including mononucleosis, malaria, leprosy, rheumatoid arthritis, and lupus. False-negatives can occur when patients are tested too soon after exposure to *Treponema pallidum*; it takes about 14-21 days after infection for reaginic antibodies to appear in the blood.

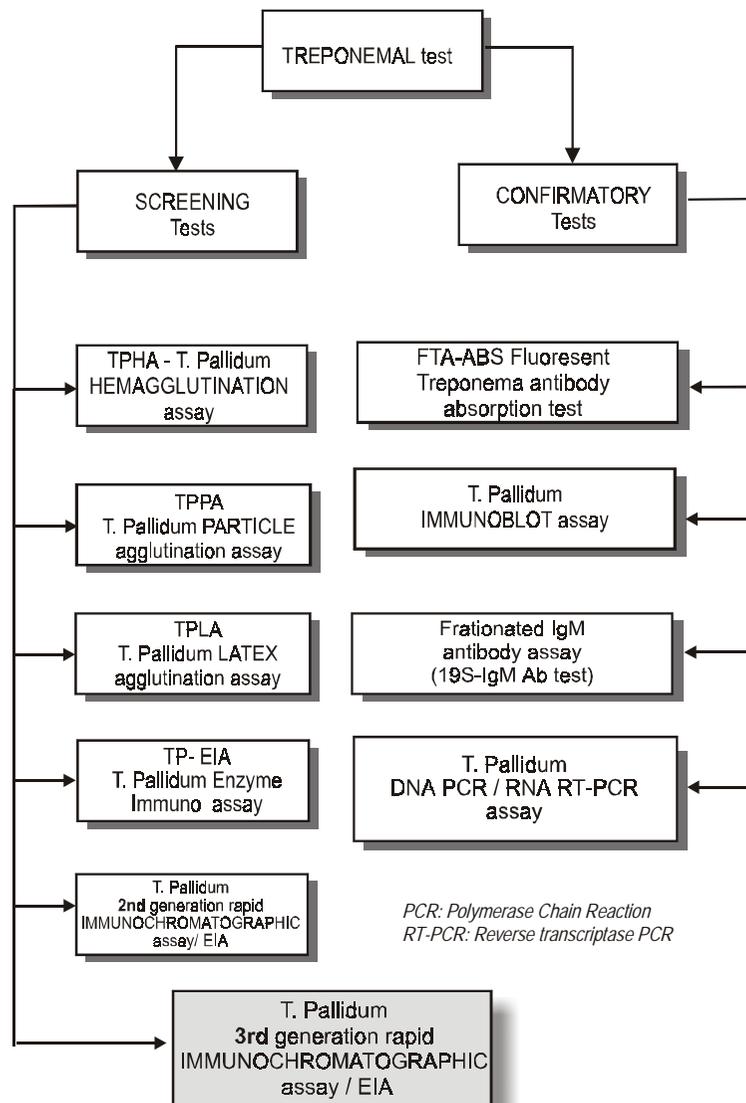
Treponemal tests

The period between latent syphilis and the development of late syphilis, which can either be benign or involve the cardiovascular / central nervous system, is unpredictable. The fact that it is unpredictable is important because it is not known which patients will develop the late manifestations. *Treatment, at this stage, prevents the late manifestations.*

Non-treponemal tests like VDRL or RPR lacks sensitivity for late disease. Thus it is preferable to screen patients with treponemal tests. Recently in the US, treponemal tests have been introduced for **screening in blood banks** and their use has been also been advocated for **screening psychiatric patients** because of their greater ability to detect late stage infection. Treponemal tests measure the presence of antibodies that are specific for *T. pallidum*. The various types of treponemal tests are:

- **TPHA (Treponema pallidum hemoagglutination assay)**: In the TPHA tests, mammalian/avian red blood cells are coated with *T. pallidum* antigen. The RBCs agglutinate if the patient's serum contains antibodies for syphilis. There are reports of inconsistent results due to improper antigen uptake on the cell wall of the RBCs. Also, the use of erythrocytes of animal origin leads to non-specific reactions with some human sera. This may be due to the presence of heterophilic antibodies found in some human sera. Also, TPHA has a variable sensitivity in the early stage of infection.

Different format for TREPONEMAL tests



● **Fluorescent treponemal antibody absorption (FTA-ABS) tests:** In the FTA-ABS, the patient's serum is mixed with a preparation that prevents interference from antibodies to other treponemal infections. The test serum is added to a slide containing *T. pallidum*. In a positive reaction, syphilitic antibodies in the sample coat the spirochetes on the slide. The slide is then stained with fluorescein, which causes the coated spirochetes to fluoresce when the slide is viewed under ultraviolet (UV) light. The FTA-ABS is still generally regarded as the 'gold standard', but it has a number of limitations. It is a subjective test and difficult to standardise. Both false positive & false negative results has been reported in FTA-ABS. Moreover, this is not a practical test to be carried out in blood banks & pathological laboratories.

● **Treponema pallidum (Tp) Immunoblot assay:** The Tp proteins are separated by SDS-PAGE (Sodium dodecyl sulphate – PolyAcrylamide Gel Electrophoresis) and IgG and IgM antibodies are detected by from HRP (horse radish peroxidase) – labeled antisera. Although immunoblotting has been suggested as a possible confirmatory test, further evaluation is needed in order to define its precise role.

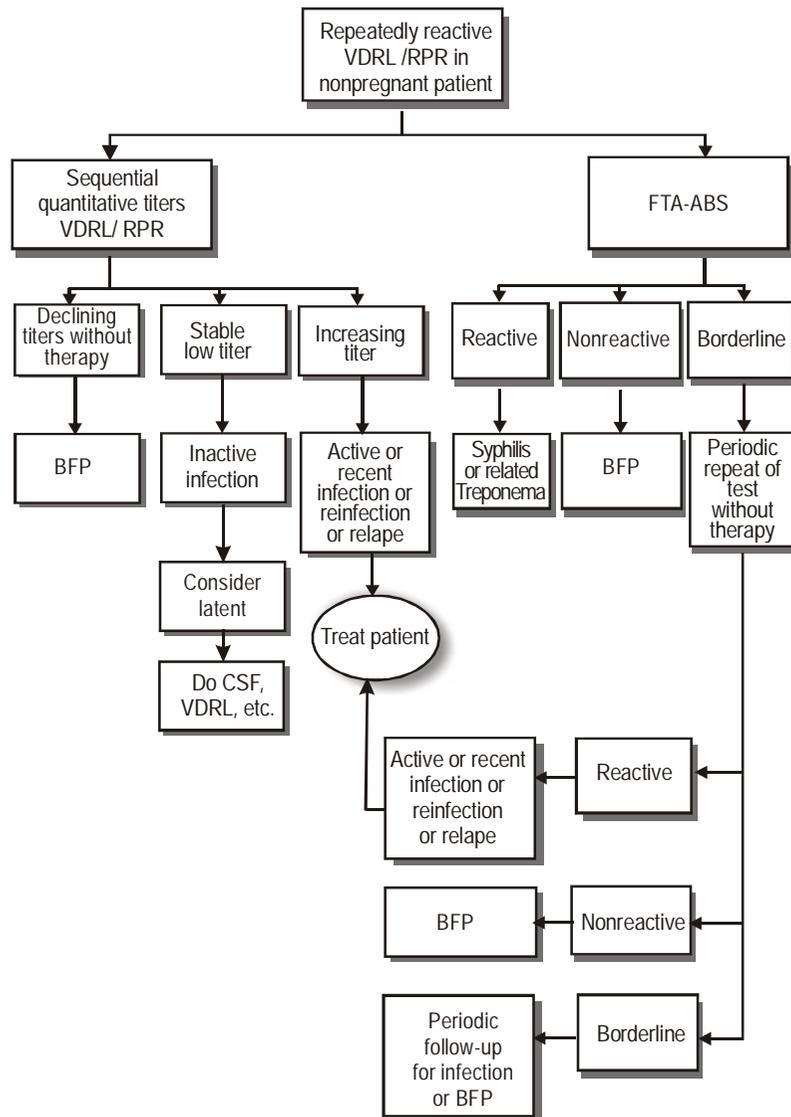
● **Fractionated IgM Antibody assay (19S IgM Ab test):** Here IgM & IgG antibodies in patient's serum is separated by column chromatography or ultracentrifugation. Then they are detected by means of indirect immunofluorescence This test is sensitive, but very laborious. It cannot be automated and only a limited number of samples can be processed at one time. It requires highly skilled technologists in order to obtain reproducible "readings".

● **Treponema pallidum Enzyme immunoassay (Tp-EIA):** In this treponemal assay, *T. pallidum*-specific antibodies are detected. Several methodologies are available from different manufacturers.

For example, in the **Differential Conjugate System** in an IgM assay, microtitre wells coated with *T. pallidum* antigens are exposed to test specimens, which may contain specific antibodies. After an incubation period, unbound components in the test sample are washed away. Now an anti-human IgM (μ chain specific) conjugate is added. Following a second wash cycle, specifically bound enzyme conjugate is detected by reaction with a chromogenic substrate. The assay is measured spectrophotometrically to indicate the presence or absence of IgM treponemal antibodies. The drawback of this system is the interference caused by co-specific IgG (in specimen) that compete with specific IgM antibodies. They block or "mask" the reaction sites (epitopes) and cause false negative reactions.

The IgM assays with some manufacturers use the **sample pre-treatment (absorption) method**. Here the test specimen is incubated with anti- human IgG for neutralising IgG class Anti-Tp antibodies. IgG is bound & removed from the mixture by centrifugation. The supernatant contains unbound/un-neutralised IgM, which is assayed

Flow chart for positive serologic test for syphilis



BFP = Biological False Positive

with specific Tp-antigens bound to the solid phase, specific Anti-human IgM enzyme-conjugate & chromogenic substrate. The disadvantage in this system is that there may be non-uniform /inadequate antibody absorption during the pre-treatment phase. This may lead to false negative results.

The interference of co-specific IgG can be overcome in the **µ-capture IgM assay**. Antibodies against human IgM (µ chain specific) are coated on the inner surfaces of microtitre wells. Patient serum is dispensed into the wells and, during incubation; a proportion of the total serum IgM is "captured" on the coated plastic. Unbound serum components are washed away. Surface-bound IgM antibodies to *T. pallidum* are subsequently traced by incubation with the Tp antigen-conjugate reagent. Unbound conjugate is washed away and surface-bound conjugate is detected by reaction with a chromogenic substrate.

But, again, as a screening test, only IgM assay may not provide adequate information to take effective clinical decisions e.g., in a blood bank, a pre-natal clinic or in an HIV patient. At the same time, it is not practical to screen all negatives (over 90% in general population are negative) once again for presence of IgG antibodies. Thus, ideally, a screening test should detect both IgG & IgM antibodies. This would make re-testing of IgM negatives redundant.

The latest double antigen sandwich **3rd Generation EIA** uses specific treponemal antigens at both solid phase and conjugate (also refer 3rd generation Immunochromatographic assay). Both IgG & IgM antibodies can be detected by this method. However, Tp-EIA requires skilled personnel & automation to consistently deliver reproducible results.

● **T. pallidum 2nd Generation Rapid Immunochromatographic assay:** Immunochromatographic assays are rapid, simple and easy-to-perform tests where investment in skilled manpower & automation is greatly reduced. The 2nd Generation Immunochromatographic assays use a Tp-recombinant antigen, at one site i.e., the solid phase, bound to the membrane. The conjugate pad contains immunoglobulin-binding protein like **Protien-A** (derived from *Staphylococcus aureus*) or **anti-human immunoglobulins** to Tp (raised in goat/rabbit) conjugated with a **dye** (instead of colloidal gold). Protein A binds to the F_c fragment of most mammalian IgGs. Each Protein A molecule can bind 2- 4 IgG molecules. Also, anti-human IgG, as used in some 2nd generation tests, binds to human IgG only. Thus, the 2nd Generation Immunochromatographic assays detect only total IgG and not IgM in the sample. False negatives can occur due to prozone effect because the total IgG titre in sera is much higher than Tp-specific IgG alone. This is the reason, these tests are recommended to be performed with diluted sample & not with neat sera.

- **T. pallidum 3rd Generation Rapid Immunochromatographic assay:** For maximum sensitivity at all stages of syphilis, it is important that tests should detect both types of anti-treponemal antibodies i.e., IgG & IgM. The problems faced in 2nd Generation assays, has been overcome after the introduction of The 3rd Generation

Causes of False - Positive Serologic Tests for Syphilis

Non treponemal Tests

<i>Acute condition(<6 months)</i>	<i>Chronic condition(>6 months)</i>
1. Pneumonia	1. Liver disease
2. Viral	2. Malignancy
3. Pneumococcal	3. Intravenous drug use
4. Mycoplasma	4. Aging
5. Hepatitis	5. Connective tissue disorders
6. Tuberculosis	6. Multiple Blood transfusions
7. Mononucleosis	
8. Chancroid	
9. Chickenpox	
10. HIV infection	
11. Measles	
12. Malaria	
13. Immunizations	
14. Pregnancy	
15. Laboratory error	

Treponemal Tests

<i>Acute condition(<6 months)</i>	<i>Chronic condition(>6 months)</i>
1. Mononucleosis	1. Systemic lupus erythematosus
2. Lyme disease	
3. Leprosy	
4. Malaria	

Immunochromatographic assays. They detect both IgG & IgM types of antibodies and can be used for both screening & confirmation. It is a double antigen sandwich immunoassay, where a cocktail of **two** specific Tp recombinant antigens (double antigen sandwich) are used at two sites – one in the conjugate pad (conjugated to **colloidal gold**) and the other immobilised on the membrane at the test line. These antigens are recombinant (not purified from Tp as in some 2nd generation assays) and isolated in such a way that the most immunodominant epitopes are expressed. The recombinant Tp antigen-colloidal gold conjugate forms a complex with Tp specific antibodies in the sample that flows on the membrane. This complex moves further on the membrane to the test region where it is immobilized by the specific recombinant Tp antigens leading to the formation of a coloured band. As in the 2nd Generation assay, these tests are simple & rapid – takes 15 mins. Due to the use of specific recombinant Tp-antigens at two sites (sandwich assay), both IgG & IgM antibodies are detected. The problem of prozone effect is also overcome. The sample need not be diluted and can be tested neat. Thus, The 3rd Generation Immunochromatographic assays are currently the best available for rapid diagnosis of syphilis.

THE PROZONE PHENOMENON – Diagnostic implications in syphilis

The **Prozone phenomenon** exhibited by some sera, which give effective agglutination reactions when diluted several fold, but do not visibly react with the antigen when undiluted. The phenomenon is not simply due to antibody excess, but often involves a

Algorithm for Syphilis Diagnosis in different patient groups

	Initially use	If positive, use
Pre - natal / Ante- natal	Treponemal test	Non-treponemal test for titre
Blood transfusion	Non-treponemal test	Treponemal test to rule out BFP
Random Screening	Treponemal test	
Rx monitoringNon-treponemal test for titre.....	
Organ transplantation	Treponemal test	Non-treponemal test for titre
Patients @ VD clinics	Treponemal test	Non-treponemal test for titre
Psychiatric patients (Neurosyphilis)	Treponemal test	Non-treponemal test for titre
HIV Patients	Treponemal test	Non-treponemal test for titre

special class of antibodies (blocking or incomplete), which react with the corresponding antigen in an anomalous manner. The bound antibody not only fails to elicit agglutination, but also actively inhibits it. The prozone phenomenon can occur in pregnancy, and request for dilution of sera should be made.

Prozone phenomenon is often encountered in non-treponemal tests. The non-treponemal tests like VDRL or RPR are economical & simple for early syphilis. Other than Biological False Positives (BFP), the main limitations of non-treponemal tests are:

- Poor sensitivity in late stage infection, and
- False negatives due to *Prozone phenomenon* in early infection

Due to *Prozone phenomenon* the reaction may show "roughness" instead of clear-cut positive or negative. So it is recommended to dilute the serum and run in duplicate along with the neat sample. The *Prozone phenomenon* is a greatly underestimated limitation of this type of tests. It has been described that prozone were more likely in HIV positive patients due to high antibody levels resulting from B-cell dysregulation. There are reports of prozone in women during pre-natal screening and congenitally infected infants resulting from this misdiagnosis. The prozone reaction can be as high as 8% in secondary & 10% in early latent syphilis.

TREATMENT / MEDICATION

Syphilis is treated with antibiotics given either intramuscularly (benzathine penicillin G or ceftriaxone) or orally (doxycycline, minocycline, tetracycline, or azithromycin). Neurosyphilis is treated with a combination of aqueous crystalline penicillin G, benzathine penicillin G, or doxycycline. It is important to keep the levels of penicillin in the patient's tissues at sufficiently high levels over a period of days or weeks because the spirochetes have a relatively long reproduction time. Penicillin is more effective in treating the early stages of syphilis than the later stages. Separate medications for the skin rashes or ulcers of secondary syphilis are not usually prescribed. The patient is advised to keep them clean and dry, and to avoid exposing others to fluid or discharges from condylomata lata.

Pregnant women should be treated as early in pregnancy as possible. Infected fetuses can be cured if the mother is treated during the second and third trimesters of pregnancy. Infants with proven or suspected congenital syphilis are treated with either aqueous crystalline penicillin G or aqueous procaine penicillin G. Children who acquire syphilis after birth are treated with benzathine penicillin G.

Jarisch-Herxheimer reaction

The Jarisch-Herxheimer reaction, first described in 1895, is a reaction to penicillin treatment that may occur during the late primary, secondary, or early latent stages. The patient develops chills, fever, headache, and muscle pains within two to six hours after the penicillin is injected. The chancre or rash gets temporarily worse. The Jarisch-Herxheimer reaction, which lasts about a day, is thought to be an allergic reaction to toxins released when the penicillin kills massive numbers of spirochetes.

GLOSSARY OF TERMS
Aortic aneurysm

Aortic aneurysm is an abnormal bulging or swelling of a portion of the aorta. It occurs when a weakness develops in the part of the wall of the aorta.

Chancres

The initial skin ulcer of primary syphilis, consisting of an open sore with a firm or hard base.

Condylomata lata

Highly infectious patches of weepy pink or gray skin that appear in the moist areas of the body during secondary syphilis.

Dark field

A technique of microscope examination in which light is directed at an oblique angle through the slide so that organisms look bright against a dark background.

General paresis

A form of neurosyphilis in which the patient's personality, as well as his or her control of movement, is affected. The patient may develop convulsions or partial paralysis.

Gumma

A symptom that is sometimes seen in tertiary syphilis, characterized by a rubbery swelling or tumor that heals slowly and leaves a scar.

Lues maligna

A skin disorder of secondary syphilis in which areas of ulcerated and dying tissue are formed. It occurs most frequently in HIV-positive patients.

Lymphogranuloma venereum

Lymphogranuloma venereum (LGV) is a sexually transmitted disease that primarily infects the lymphatics. LGV synonyms include lymphopathia venerea, tropical bubo, climatic bubo, strumous bubo, poradenitis inguinales, Durand-Nicolas-Favre disease, and lymphogranuloma inguinale. LGV is caused by *Chlamydia trachomatis*.

Mononucleosis

Infectious mononucleosis (IM) is a clinical syndrome. IM represents the immunopathologic expression that occurs under a specific set of circumstances and in response to infection with the Epstein-Barr virus (EBV).

Pityriasis Rosea

Pityriasis rosea (PR) is a common benign papulosquamous disease that was originally described in 1860 by Camille Melchior Gibert. Pityriasis denotes fine scales and rosea translates as rose colored or pink. PR can have a number of clinical variations. Its diagnosis is important, as it may resemble secondary syphilis. PR has often been considered to be a viral disease. Till date, no single virus has been proven to cause the disease.

Serofast

It is expected that the non-treponemal test will eventually become non-reactive after treatment. However, in some patients, non-treponemal antibodies can persist at a low titre for a long period, sometimes for the remainder of their lives. This response is referred to as serofast.

Spirochete

A type of bacterium with a long, slender, coiled shape. Syphilis is caused by a spirochete.

Tabes dorsalis

A progressive deterioration of the spinal cord and spinal nerves associated with tertiary syphilis.

Uveitis

Uveitis is defined as inflammation of one or all parts of the uveal tract of the eye. Components of the uveal tract include the iris, the ciliary body, and the choroid. The exact pathophysiology of uveitis is unknown. In general, uveitis is caused by an immune reaction.

BIBLIOGRAPHY

1. Jr. Pelczar MJ et al. Microbiology. Tata-McGraw Hill. 2001.
2. Gale Encyclopedia of Alternative Medicine. Gale Group 2001.
3. Philippe G. Kestelyn *et al.* HIV/AIDS and blindness. Bulletin of the World Health Organization, 2001, 79 (3).
4. Serological diagnosis of syphilis SI Egglestone and AJL Turner for the PHLS Syphilis Serology Working Group. Communicable Disease and Public health. Vol 3 no. 3. September 2000.
5. Birnbaum Nina R. *et al.* University of California, San Francisco School of Medicine, San Francisco, California. Resolving the Common Clinical Dilemmas of Syphilis. American Academy of Family Physicians. April 15, 1999.
6. Lothar Thomas. Clinical Laboratory Diagnostics - Use and assessment of Clinical Laboratory Results. TH-Books. 1998.
7. Hermanson Greg T. Bioconjugate Techniques. Academic Press. 1996.
8. Mackie & McCartney. Practical Medical Microbiology. Churchill-Livingstone. 1996.
9. Walker John M. The Protein Protocols Handbook. Humana Press. 1996.
10. Young H. Screening with Captia Syphilis-G. Comparison with other Treponemal and non-treponemal tests. AABB meeting, San Diego, California, USA. 1994.
11. Ramskill S. Evaluation & review of Captia Syphilis-G assay when used for screening in blood bank. AABB meeting, San Diego, California, USA. 1994.
12. Burdon RH & Knippenberg van PH. Laboratory Techniques in Biochemistry and Molecular Biology. Elsevier. Volume 15. 1993.
13. Wallach J. Interpretation of Diagnostic Tests. A synopsis of Laboratory medicine. Fifth edition. 1992.
14. SYPHILIS. Section 2. STD/HIV Prevention Training Center of New England. A Project of the Division of STD Prevention, Massachusetts Department of Public Health, USA.
15. Data on File. Tulip Diagnostics (P) Ltd., INDIA.