SYPHILIS

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DiaSorin
The Diagnostic Specialist

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1. Microorganism and disease

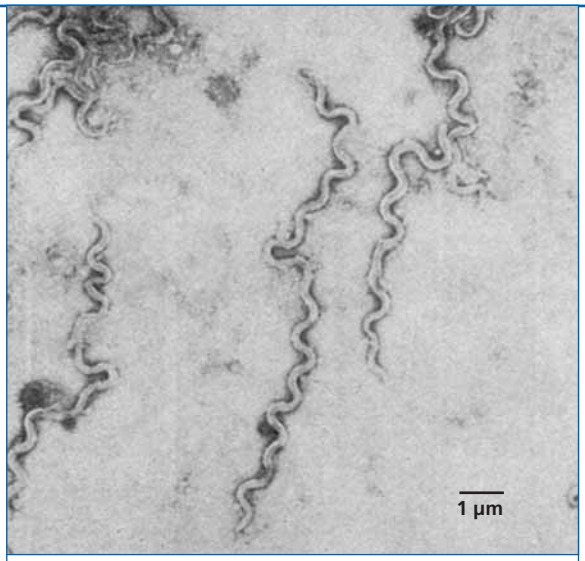
Syphilis is a systemic infectious disease caused by the *Treponema pallidum* subspecies *pallidum*. It is generally acquired by direct sexual contact and features treponemes-containing lesions. The infection can also affect a foetus if the pathogen crosses the placental barrier. The only known hosts are human beings.

1.1 The microorganism

Treponema pallidum belongs to the genus *Treponema*, *Spirochaetales* order and Spirochaetaceae family, together with *Borrelia* and *Leptospira*.

This genus includes three other human pathogenic subspecies and at least six saprophytic bacteria present in normal digestive and genital tract flora and the oral cavity. The pathogenic subspecies are *pallidum*, which causes venereal syphilis, *endemicum*, responsible for non-venereal endemic syphilis also known as bejel and *pertenue*, which produces yaws. Due to insufficient genetic information, another pathogenic treponeme, *Treponema carateum*, which causes pinta, has not yet been formally classified as a subspecies (Cf. Table 1).[1] They all present higher than 95% genetic homology and are morphologically indistinguishable from each other.[1–4]

T. pallidum has a thin, regular helical shape with no terminal hook. Its length ranges from 6 to 20 µm and its diameter from 0.10 to 0.20 µm. Its small size makes it invisible to light microscopy, but it can be identified with phase-contrast microscopy or staining methods, which increase bacterial thickness[1,3] (Cf. Picture 1).



Picture 1: *T. pallidum* Nichols cell negative staining (Electron microscopy, X 2,500 Bar, 1 µm).
From Dettori G, Amalfitano G, Polonelli L et al. Electron microscopy studies of human intestinal spirochetes. *Eur J Epidemiol* 1987;3:187-95. **Authorised reproduction**

Biochemically, *T. pallidum* consists of 70% proteins, 20% mainly high cardiolipid-content phospholipids and 7% carbohydrates. Metabolically, it is a low-activity microorganism, which uses enzymatic host mechanisms.

The bacterium can only produce ATP by glycolysis. Given this low energy production, its tissue generation time is extremely long, from 30 up to 33 hours. *T. pallidum* cannot be cultivated in the conventional sense but can be reproduced in specific tissues, such as mouse testes by using the Nichols strain. It is thermolabile; some of its enzymes cannot withstand temperatures other than those of

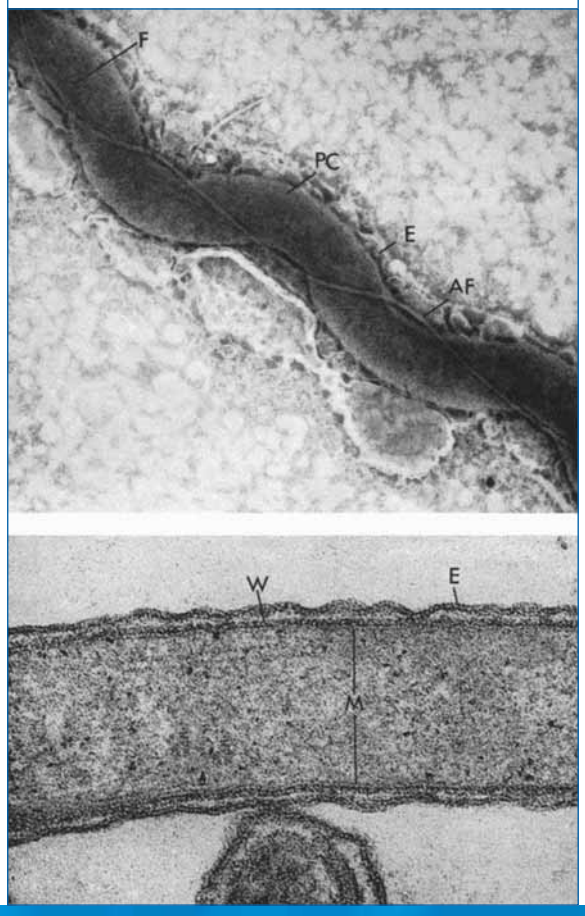
Table 1: Pathology produced by pathogenic treponemes

	subsp. *pallidum*	subsp. *pertenue*	subsp. *endemicum*	subsp. *carateum*
Disease	Syphilis	Yaws	Bejel	Pinta
Infection	Systemic	Cutaneous	Cutaneous	Cutaneous
Transmission	Sexual Congenital	Not sexual	Not sexual	Not sexual
Distribution	Worldwide	Tropical	Deserts	Deserts
Age	Sexual activity	All	All	All
Lesion	Syphiloma	Papilloma on exposed skin	Dermal, perioral	Dermal on dorsum of the foot

the human body and it survives for a short time outside its host. A lack of superoxide dismutase, catalase, and peroxidase makes it vulnerable to oxygen activity, although the TpO823 and TpO509 enzymes are believed to protect against oxidation-induced stress.[4,6,8]
The structure of *T. pallidum* (Cf. Picture 2) is cytoplasm surrounded by an elastic cellular membrane containing a thin peptidoglycan layer. The flagella move within the peri-plasma space, spinning contrary to the helix and causing bacterium extensibility and flexibility. The extremely fluid external membrane contains phospholipids and a very small amount of proteins, among which TpN47 presents as the most abundant and immunogenic.[1] Three rotary motion fibrils are inserted onto the sharpened ends.

Picture 2: *T. pallidum Nichols* cell negative staining (Electron microscopy, X 50,000) and thin sections (Electron microscopy, X 160,000). E: external membrane, W: cell wall, M: cytoplasmic membrane, PC: protoplasmic cylinder, AF: axial filaments, F: fibrils

From Jackson S, Black SH. Ultrastructure of *T. pallidum Nichols* following lysis by physical and chemical methods. I. Envelope, wall, membrane and fibrils. *Arch Mikrobiol* 1971;76:308-24. **Authorised reproduction**



The genome is a small circular chromosome whose DNA contains 1,138,006 bp with 1,041 open reading frames (ORFs), only 55% of which have been assigned a biological role, while 17% codify hypothetical proteins, and 28% represent new genes.[4,5] Another 5% codify for 18 specific amino acid, carbohydrate and cation carriers. Mobility-related proteins are encoded in 36 highly conserved ORF.[4,6] The virulence factors appear to be related to the different gene expression patterns of tpr (T*reponema pallidum repeat*), responsible for certain external membrane proteins. Cytotoxicity versus neuroblasts and other cells is due to an unknown mechanism and is not attributed to the production of exotoxins or lipopolysaccharides. Unlike most pathogenic bacteria, very few mobile elements are evident in the genome, which explains its great genetic stability,[6] and certain strains have been observed to have a mutation that enables resistance to macrolids and other related drugs.[7] It has been recently reported that a 15 kDa lipoprotein (tpp15) can discriminate *T. pallidum* subspecies *pallidum* from subspecies *endemicum* and *pertenue*.[4] Most proteins and lipopolysaccharides described were obtained from the *T. pallidum* Nichols; when naming its lipoproteins or genes, the prefix *TpN* or *tpn* is used, followed by the corresponding molecular mass. Polypeptide TpN47 is therefore expressed by gene *tpn47* (Cf. Table 2). For each of the 12 genes of the *tpr* family there is an alternative name (Cf. Table 3). The external membrane-exclusive proteins are known as Tromp, Tpm or Tro (Treponemal rare outer membrane proteins) and are named after their molecular weight. They consist of 20 proteins.[5,6]
Comparison of the *T. pallidum* genome with that of another spirochete, *Borrelia burgdorferi,* shows that 46% of *T. pallidum* ORFs have orthologs in *B. burgdorferi*. A total of 115 ORFs shared by *T. pallidum* and *B. burgdorferi* encode proteins of unknown biological function.[5,8,9]

1.2. Clinical aspects

Untreated syphilis is a contagious systemic disease featuring sequential clinical stages added to years of latency and is classed as sexually transmitted disease (STD). It can affect several organs

simultaneously and produce clinical conditions similar to some other diseases. Its primary mode of infection is by direct contact with a productive lesion or transplacental transmission.

1.2.1. Pathogenesis

Treponema penetrates microscopic skin lesions but can also cross intact barriers. Incubation

Table 2: Nomenclature and some properties of treponemal polypeptides most often used in serological diagnosis

Identification	Other names and functions	Reactivity in syphilis
Flagellar Polypeptides		
TpN 15		67%
TpN 17		89%
TpN 24,27,28		
TpN 29		
TpN 30	Flagellin B3	*
TpN 33	Flagellin B2	*
TpN 34	Flagellin B1	
TpN 35		
TpN 37a	Flagellin A	35%
TpN 39	Basic membrane protein	
TpN 44		
TpN 47	Surface antigen	92%
TpN 60	Common antigen	
External membrane proteins		
Tromp 1	31 kDa (TroA)	
Tromp 2	28 kDa	
Tromp 3	65 kDa	
Polypeptides shared with flagella		
Tpm A	45 kDa	PCR target
Antioxidant proteins		
TpO 823	Superoxide to peroxide	
TpO 509	Hydroperoxide reductase	

* Normal sera can contain low titre antibodies

Table 3: *Tpr* membrane protein genes

Family	*Tpr A*	*Tpr B*	*Tpr C*	*Tpr D*	*Tpr E*	*Tpr F*	*Tpr G*	*Tpr H*	*Tpr I*	*Tpr J*	*Tpr K*	*Tpr L*
Subfamily*	III	III	I	I	II	I	II	III	I	II	III	III

* Based on DNA homology

time may depend on the inoculum and the host immune status. Dark-field microscopy studies on the infective dose showed that 2, 70, and 2 x 10^5 of treponemal inoculum produced a lesion respectively in 47, 70 and 100% of subcutaneously infected mice.[2–4]
T. pallidum acts on the cells at its point of entry, causes the primary lesion and rapidly spreads throughout the lymphatic and haematological systems. *T. pallidum* damages intercellular junctions in the vascular endothelium, penetrates perivascular space and destroys blood vessels, leading to obliterating endarteritis and periarteritis, which interrupt area blood flow and cause an ulcer. Initial symptoms are local but the infection starts spreading during the first hour after transmission. Both the onset of circulating immune complexes and direct treponemes action are believed to elicit transitional immune suppression, culminating in renewed response to free treponemes, based on the lympho-plasmacytic and macrophage cells producing the generalised roseola lesions typical of the disease.[2,3,6] Clinical symptoms are very infrequent two years later. A typical microscopic lesion consists of a granuloma in the disease later stage (Cf. Table 4).

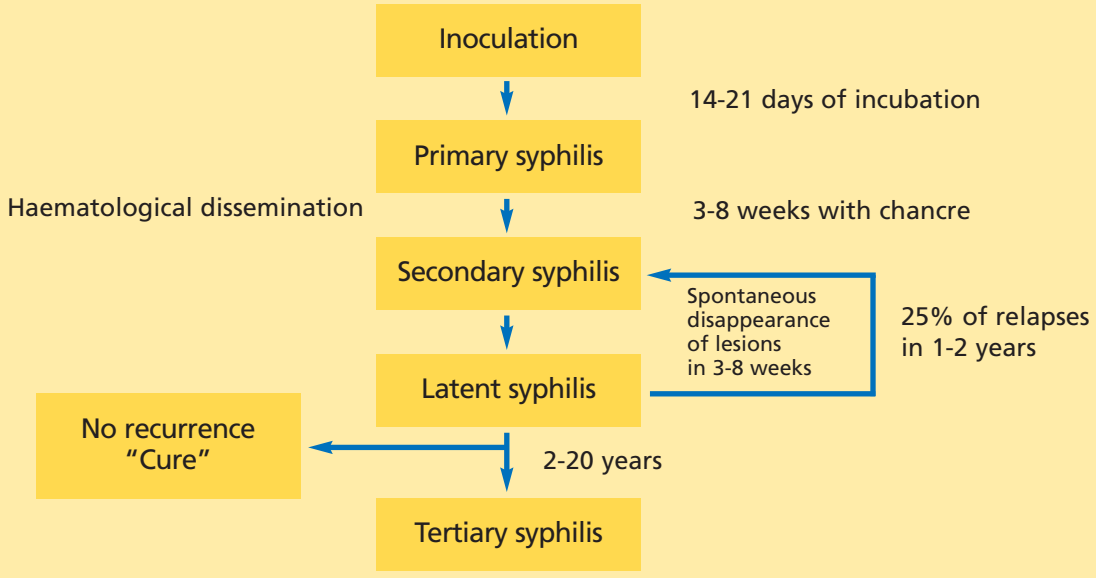
1.2.2. Clinical stages of untreated syphilis in adults

Studies by Boeck and Gjestland between 1891 and 1949, later revised by Clark in 1955[11-13] and the 1932 Tuskegee Study[14,15] concluded that one-third of untreated syphilis patients develop symptoms of benign, neurological or cardiovascular tertiary syphilis, the mortality rate of untreated syphilis being 17% in men and 8% in women. Another one-third of patients recovered with RPR (Rapid Plasma Reagin test) negativisation and the remaining cases developed no symptoms of syphilis, although RPR was still reactive (Cf. Table 5). It was also shown that the Afro-American population is more prone to developing cardiovascular complications while the white population is more likely to develop neurosyphilis. These studies also showed that women infected during the early months of pregnancy develop more severe foetal and neonatal pathologies than those in whom infection first occurs during the second pregnancy half.

1.2.2.1. Primary stage. Primary syphilis

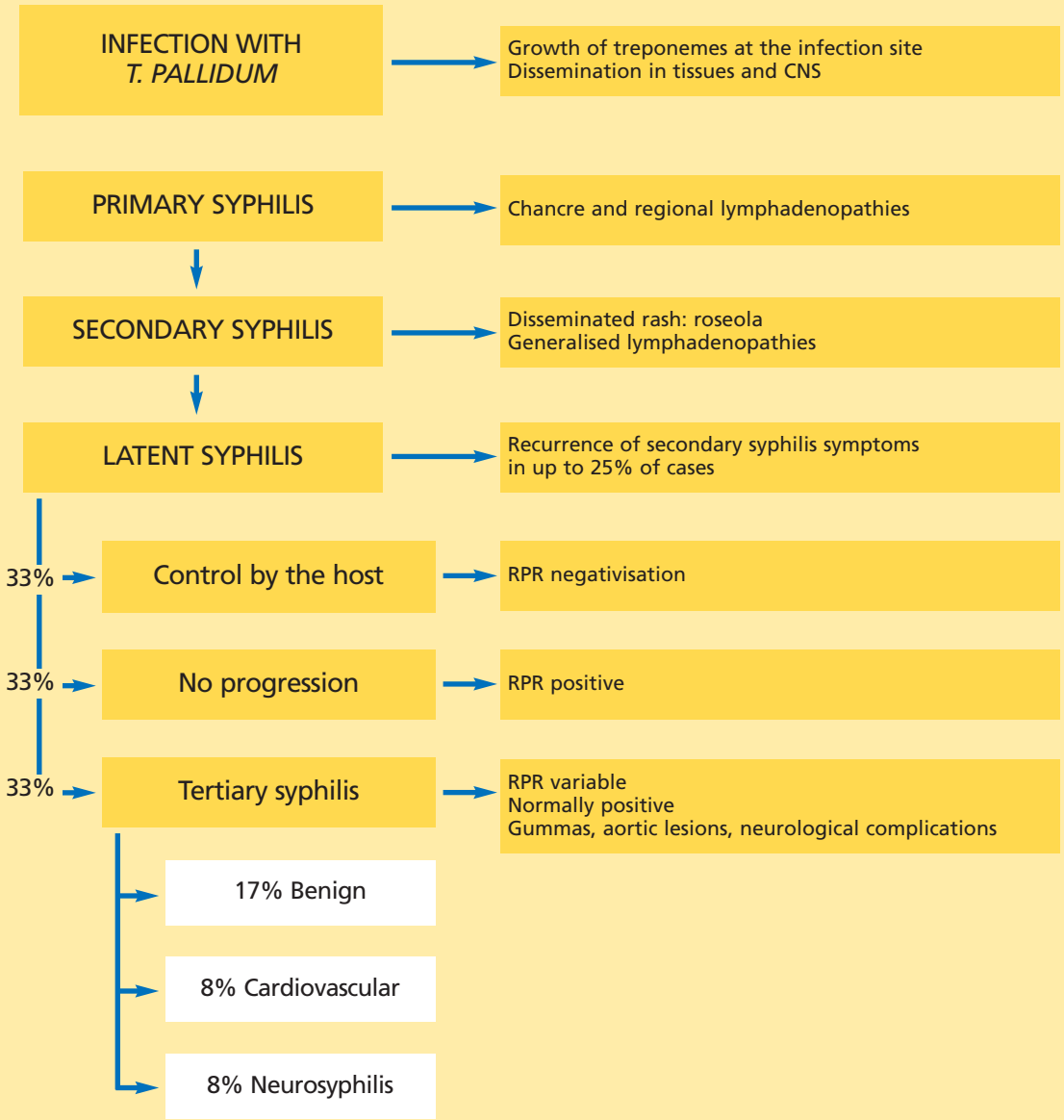
A non-purulent ulcer appears at the inoculation site, after an incubation period of 12 to 90 days

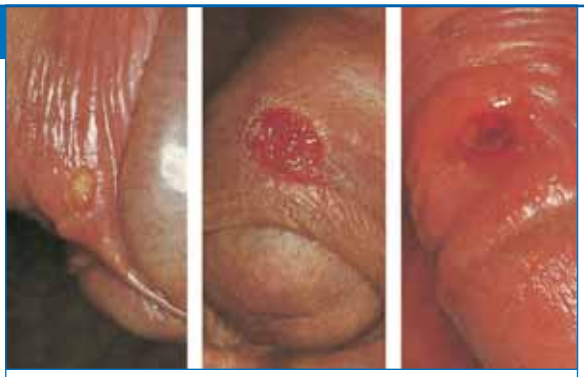
Table 4: Pathogenesis



with an average of 3 weeks. This syphilitic chancre (Cf. Picture 3) disappears spontaneously a few weeks later. The features typically associated with this lesion are hardness, mild pain, and regional adenopathy. Extragenital chancres are softer but more painful. The surface is covered with a fibrin, necrotic material and polymorphonuclear leucocyte exudate with an inflammatory infiltrate in the peri-lesion area. The lesions are replete with treponemes.[1,2] *T. pallidum* congregates in the lymphatic regions within a few hours of this initial stage and spreads rapidly through the bloodstream to all body organs, including the central nervous system (CNS) with systemic infection. The Centers for Disease Control and Prevention (CDC) divides this initial stage into two groups: early primary syphilis and late primary syphilis, according to whether treponeme activity is still detected in the lesions of a seropositive patient or seropositive conditions associated with a healed primary lesion. During this 3 to 4 weeks period all serological tests will yield positive results.[2,6,16]

Table 5: Evolution of untreated syphilis





Picture 3: Syphilitic chancre

From Braun-Falco O, Plewig G, Wolff H, Burgdorf W, Landthaler M (eds) Dermatologie und Venerologie, Vol 5. Springer, Berlin Heidelberg New York, 2005. **Authorised reproduction**

1.2.2.2. Secondary stage. Secondary syphilis

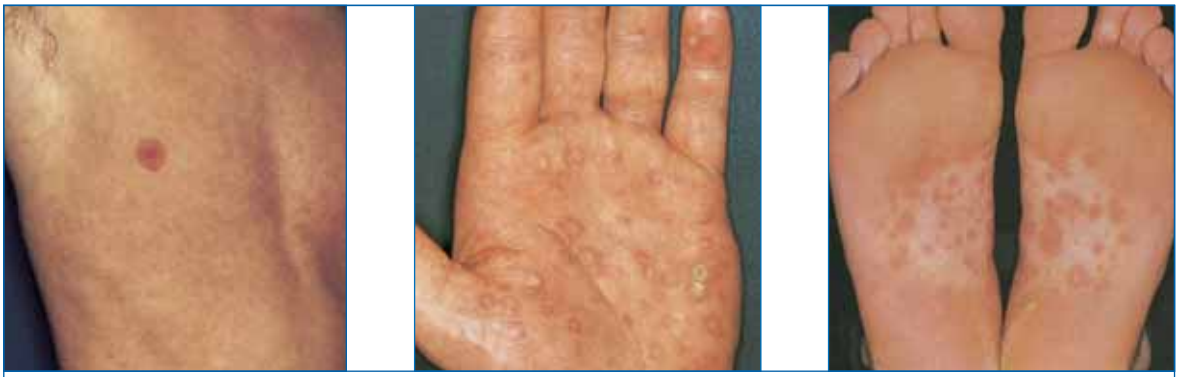
No clear distinction can always be made between primary and secondary stages, as the disease becomes generalised in untreated patients and the secondary stage starts with the onset of variable intensity systemic symptoms, such as mild fever, a sore throat, oral or genital mucous patches, hepatitis, gastrointestinal disorders, painful cervical adenopathy, bone aching, nephrosis etc. Over 30% of patients present with cerebrospinal fluid (CSF) abnormalities that either evolve or persist, subject to aseptic meningitis. Facial and auditory nerve impairment and optical neuritis are also not infrequent. Typical lesions of this stage include dermal syphilitic roseola (Cf. Picture 4) and flat genital and anal warts. Roseola presents as a variable size macular, macular-papular, follicular or pustular copper-colour rash affecting palm and plant areas. Lesions contain a large number of treponemes per gram tissue and are highly contagious if opened. 25% of patients show similar decreasing intensity outbreaks during early latency to total remission in late latency without treatment and after initial spontaneous symptomatic remission, during the first and second disease year.

90% recurrence occurs the first year, 94% the second two and the remainder in the following four years, so it can be safely stated that the boundary between early and late latency syphilis is at over one year of evolution and early latency patients are still infectious and can relapse in 25% of cases due to treponemes-replete lesions.

1.2.2.3. Tertiary stage. Tertiary or late syphilis (benign, cardiovascular and neurosyphilis)

The third stage of the disease only appears in one third of untreated infected patients – benign 17%; neurosyphilis 8% and cardiovascular syphilis 8% respectively. The remaining patients never reach this stage but retain latent infection or so-called biological healing. Studies on spontaneous disease evolution indicated that 33% of patients healed with no treatment and negative reaction tests while another 33% developed no progression symptoms even though tests continued to yield positive results (Cf. Table 5).

The most frequent type is so-called benign late syphilis featuring the formation of gummas consisting of a very low treponemes charge destructive granulomatous lesions. The most commonly affected areas are the skin, bones and liver. The term benign implies that these lesions rarely cause physical disability or death. They can however cause severe complications



Picture 4: Syphilitic roseola

By Braun-Falco O, Plewig G, Wolff H, Burgdorf W, Landthaler M (eds) Dermatologie und Venerologie, Vol 5. Springer, Berlin Heidelberg New York, 2005. **Authorised reproduction**

when present in important organs, such as the heart and brain. A gumma can appear 2 to 45 years after secondary lesions healing, the average being 15 years.

Specific disease signs and symptoms leading to cardiovascular syphilis may appear in this period. This clinical form is rare and presents 10 to 30 years after initial infection. The primary lesion consists of aortitis typically located in the ascending aorta. The best-documented complications are aortic failure, angina and aneurism.

Secondary syphilis patients frequently show treponemal invasion of the CSF but few develop permanent CSF abnormalities or neurosyphilis symptoms. Neurosyphilis has been divided into categories, each representing a different stage of progression where one often overlaps another. Asymptomatic neurosyphilis is present in one-third of cases and is defined by the presence of CSF changes, which include cell and protein increases or VDRL (Venereal Disease Research Laboratory) reactivity. It is usually diagnosed in the 12 to 18 months following primary infection. Meningovascular syphilis represents 10% of diagnoses and appears 4 to 7 years after infection, usually presenting with a diffuse encephalitis syndrome. Parenchymatous syphilis is currently a very rare form that presents between 5 and 25 years after infection, as generalised paresis or tabes dorsalis. The primary disorders in the paresic form affect cognitive and memory functions, with the progressive appearance of irritability and personality disturbances. In tabes dorsalis, the posterior medullary arteries present trophic lesions and distal neuropathies and pupil-motor structures are destroyed, accompanied by optic nerve atrophy.[2–4,6,16]

1.2.3. Congenital syphilis

In pregnant women with syphilis, *T. pallidum* can cross the placental barrier and interfere with foetal development. Transplacental infection can lead to miscarriage, low newborn weight, premature birth or perinatal mortality. Crossing the placenta becomes easier after the third or fourth month of pregnancy. Vertical transmission is estimated at 70 to 100% of pregnant women with primary syphilis, 40% for those in the early latent stage and 10% for those in the later latent stage.[17,18] *T. pallidum* reaches the foetus via the bloodstream and the primary stage is bypassed. Treponemes and infection effects can nonetheless be detected in all tissues.[25] The neonatal disease is usually symptomatic if the mother is infected during pregnancy and asymptomatic if she became pregnant during a latent phase.[19] Foetal infection prior to the fourth month of pregnancy is rare, as passage of the treponemes through the placenta requires an as yet undeveloped active mechanism. Neonatal syphilis infection may also occur during delivery by contact with a productive lesion. Congenital syphilis can be early or late. The early form that can present before the second year of life may be fulminating. Clinical manifestations can be generalised and already present at 3 months after birth as mucocutaneous lesions, such as serohaemorrhagic rhinitis with a high treponemes charge and a desquamating maculopapular rash. There may be Parrot's pseudo-paralysis osteochondritis and perichondritis, hepatic involvement, anaemia, severe pneumonia or pulmonary haemorrhage, glomerulonephritis etc. The development of interstitial keratitis is quite frequent in latent untreated syphilis, appearing 6 to 12 months after birth. Symptomatic or asymptomatic neurosyphilis is also common.[1,2,4,16] Late-stage syphilis can cause deformation of the bones and teeth, deafness caused by lesions of the VIII nerve and other tertiary disease symptoms. Table 6 illustrates all untreated and congenital syphilis stages and evolution.

1.3. Epidemiology

The infection spreads by intimate contact with lesions containing high concentrations of primary, secondary and early latent stage treponemes. The most common mode of contagion is sexual, the second most common one being transplacental mother-to-foetus transmission. The sexual transmission rate in productive phases is estimated at 60%. Most children with syphilis are infected *in utero* but newborns can also become infected at birth by contact with one of the mother's open lesions. Late latent and latent syphilis are

rarely contagious.
The disease is a source of public concern. The World Health Organization (WHO) estimates 12 million new cases occur that every year, 90% of which in developing countries (Cf. Figure 1). The re-emergence of syphilis in Eastern countries has contributed to the spread of HIV. The groups at highest risk in the Western countries seem to be homosexuals and intravenous drug users, whose infection rates increase rapidly.[21,22]

1.4. Immune response

SDS-PAGE (Sodium Dodecyl Sulphate-PolyAcrylamide Gel Electrophoresis) studies have shown that *T. pallidum* produces around 60 antigens, among which p47, p37, p35, p33, p30, p17 and p15 are the most useful proteins for serological diagnosis. The *T. pallidum* external membrane is scantly antigenic, which explains its long-lasting persistence in the body.[20] Normal human serum may occasionally contain small amounts of reactive antibodies that counteract *T. pallidum* antigens TpN47, TpN33 and TpN30.
Immune response is very complex.[6] Humans and animals alike present progressive and high membrane Tpr protein reactivity, with a maximum level 45 to 60 days after infection. IgM and IgG *T. pallidum* antibodies are found in primary and secondary active syphilis but IgM antibodies decrease in later stages and after treatment. Reactivity appears to correlate with symptom intensity. This response can eliminate most treponemes in

Table 6: Clinical stages of untreated syphilis in adults

	Primary	Secondary			Tertiary
	Early primary and Late primary*	Secondary	Early latent	Late latent	
Time	3 weeks to 3 months. Average 21 days	6 weeks to 6 months	< 1 year	> 1 year	10-20 years
Serology	Variable/Negative	Reaginic (+) Treponemal (+)	Reaginic (+) Treponemal (+)	Reaginic (+) or (-) Treponemal (+)	Reaginic Variable Treponemal (+)
Clinical	Chancre: single or multiple with spontaneous healing Regional lymphadenopathies CSF abnormalities in 40% of cases	Syphilitic roseola Constitutional syndrome Papulae Muscular lesions Oropharyngeal inflammation Warts Adenopathies Alopecia (7%)	Asymptomatic or recurrences in 25%	Asymptomatic Spontaneous healing?	Benign late syphilis Gummas Neurosyphilis** Cardiovascular syphilis

Congenital syphilis		
Time	Early < 2 years	Late > 2 years
Clinical	Disseminated acute infection; Mucocutaneous lesions; Osteochondritis; Anaemia; Hepatosplenomegaly; Neurosyphilis	Interstitial keratitis; Lymphadenopathies; Hepatosplenomegaly; Skeletal disorders; Hutchinson's teeth; Neurosyphilis

*Nomenclature accepted by the CDC. **Sometimes it appears extremely soon, two years after infection.

early lesions but is unable to completely eradicate the infection.
Opsonized treponemes bacteriolysis and phagocytosis are the organism's first cleaning mechanisms.
Kinetic studies have shown that antibodies appear against various Tpr proteins[23,24] throughout the infected areas, suggesting that *T. pallidum* could express different antigenic patterns during infection, depending on the infecting strain. Different *tpr* gene product location and some sequence variations could contribute to explaining the lack of immune response and the persistence of treponemes in infected patients, as well as the lack of antibody re-infection protection, added to current difficulties in vaccine development.[10] TprK is well known to be a highly efficient opsonizing antibody receptor in the healing process, where its antibodies protect against re-infection of homologous but not heterologous strains.[16,17,23,24] TprA antibodies appear to confer re-infection resistance. Serum reactivity gradually decreases in untreated patients.
Treponemal lipopeptides feature high anti-inflammatory potential, so cellular response is fast, mainly CD4 lymphocytes in primary chancres and CD8 lymphocytes in secondary syphilitic lesions.

2. Microbiological diagnosis

The diagnosis of syphilis depends on clinical findings, detecting treponemes in open tissue lesions and the reactivity of specific serological tests for identifying components or specific *T. pallidum* antibodies and non-specific tests known as reaginic (Cf. Table 7). Exudates, tissues and blood can be made resort to for diagnosing congenital syphilis, with blood samples from the mother, foetus, umbilical cord or the newborn baby.

2.1. Direct diagnosis

Treponemes detection using standard staining procedures in lesion transudates is difficult but the bacterium can be identified with dark-field microscopy and fluorescent staining when the sample is properly collected (Cf. Table 8).

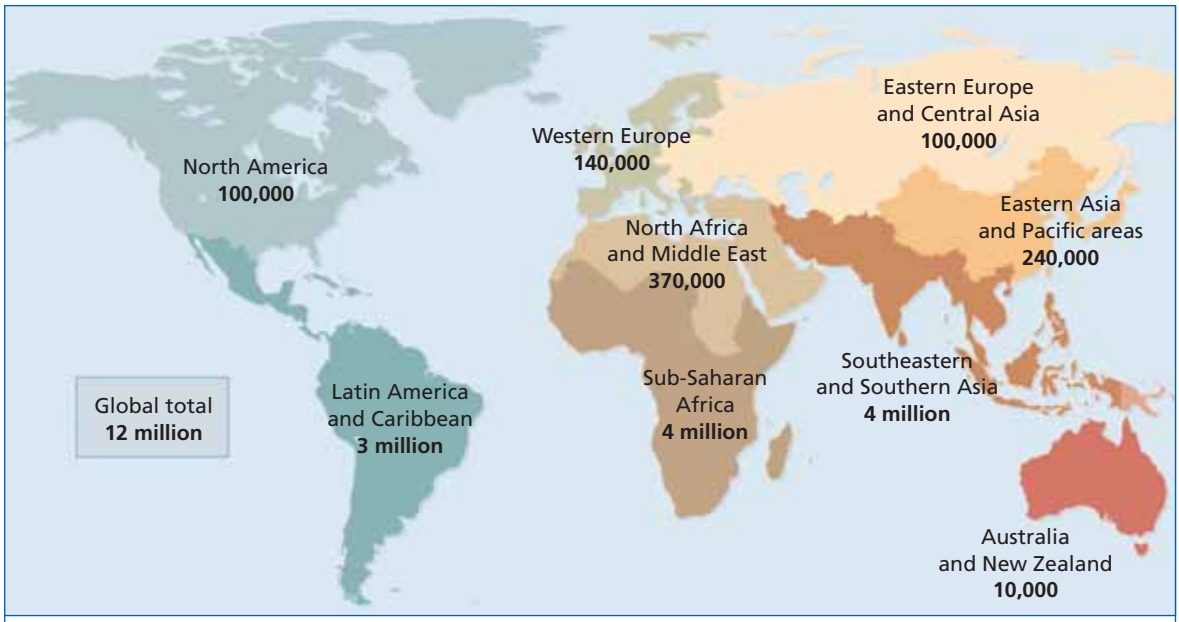


Fig. 1: Estimated annual number of new cases of syphilis among adults
Data released by the World Health Organization, 1999

2.1.1 Dark-field microscopy: living treponemes visualization

Identifying *T. pallidum* by direct examination of lesion exudate is an outstanding test for diagnostic confirmation. The advantages of this procedure are high speed and low cost. Short intervals between sampling and visualization are essential for good technical results, which is why operators should have the entire necessary equipment ready before specimens are taken. The literature describing how to obtain different samples in different lesion locations is excellent.[25] Direct treponeme observation can give positive results even before serum reactivity tests. It is certainly the most efficient diagnostic procedure in the event of open lesions available for sampling; including chancres, warts, roseola and tertiary syphilitic lesions and can also be performed on suspect ganglion aspirates.
Visualization is only considered positive when living treponemes are detected and their morphology and movements are analysed, in which case the test is highly predictive, provided other treponemal infections can be excluded. A negative result for the direct test does not exclude the disease, as only few treponemes may be present, depending on disease evolution stage and treatment management.[16,25] This technique does not distinguish between different pathogenic treponemes, though they can be morphologically differentiated from *T. refringens* and *T. denticola* saprophytes. The sensitivity rate of this test is 75 to 80%.[6,25]
Samples from suspect lesions at the mouth and close to the rectum should not be tested with this method, due to the high risk of confusing treponemes with other saprophytic spirochetes. The efficiency of this test is very low on CSF samples.[4,25]

2.1.2. Direct fluorescence (DFA-TP)

This fluorescent-antibody test has the same features as dark-field microscopy but living treponemes are not required. It uses specific conjugates, which differentiate pathogenic treponemes and saprophytes. Test specificity is improved by using monoclonal antibodies for the 37 kDa flagella protein. This technique can also be adapted to stain tissue sections (DFAT-TP) and used in placental histopathology.

Table 7: Standard test for diagnosis of syphilis

- Direct tests:
  - Microscopic examination
  - PCR
- Indirect tests (Serology):
  - NON TREPONEMAL
    - VDRL
    - RPR
  - TREPONEMAL
    - ELISA
    - Chemiluminescence
- Confirmatory tests:
  - Western blot
  - LIA
  - PCR

2.1.3. Rabbit inoculation (RIT)

Isolating treponemes with the rabbit infectivity test (RIT) is a very sensitive and specific but complex and expensive procedure that requires infrastructures unavailable in conventional laboratories. It is the most sensitive method for detecting the infectious treponemes PCR results are compared with. Its sensitivity level is 100% when the infective dose contains more than 10 to 20 treponemes.[25,40]

2.1.4. Molecular tests (PCR)

Polymerase Chain Reaction (PCR) technology is not as sensitive as the RIT and is still considered experimental. It uses gene primers that encode for 47 kDa surface antigen immune-dominant proteins and 39 kDa basic membrane protein *tpnA* genes. Sensitivity for CSF and neonatal serum is 60 and 67% respectively. Only for amniotic fluid it reaches a sufficient sensitivity and could be considered a diagnostic test.[26] Other methods describe amplification of the *tmpA* 45 kDa membrane protein gene but results have not been compared with RIT.[28,29] Molecular tests can be

Table 8: Characteristics of direct diagnosis

- If positive:
  - Immediate diagnosis
  - Very specific
  - Very early: even prior to seroconversion
- If negative:
  - Serology is required
- False negatives:
  - When only a few treponemes are present
  - Due to previous topical or systemic treatments

very useful in congenital syphilis where antibody transport can affect the diagnosis, in neurosyphilis[27] for which VDRL features 50% sensitivity and in early primary syphilis without seroconversion.[28]

2.1.5. Placental histopathology

Study of the placenta significantly increases the number of diagnoses performed on full-term infants and to a lesser extent on premature and stillbirths.[30]

2.2. Indirect diagnosis: serological tests

Serological diagnosis is the laboratory tool used the most to diagnose this infection. Reaginic tests detect non-specific treponemal antibodies (Cf. Table 9), while treponemal tests measure specific antibodies. They should preferably be conducted in serum. Each is complementary to the other and their prediction value is optimised when performed simultaneously. They are also necessary to support treponeme visualization diagnosis.

2.2.1. Reaginic tests

Several different techniques have been used to diagnose syphilis ever since 1906, when Wassermann et al. adapted the complement fixation test. The historical development of these tests reflects the extreme importance of this disease in the past.[16]

All these tests use alcohol antigen solutions containing standard mixtures of purified and stabilised cardiolipins, cholesterol and lecithins. Together, they measure the serum level of IgG and IgM immunoglobulins against substances in inflammation and/or treponemes damaged tissues.[1,4]

They can be used qualitatively or quantitatively. Quantitative tests are the most informative as they set a reference for reactivity measured versus biological changes during infection, natural evolution or after treatment. All reaginic test procedures have approximately the same sensitivity and specificity but reactivity expressed as serum titre can vary according to antigen quality. The same reagent batch should therefore be used for all titration samples when comparing titres. The following techniques are the most widely used for diagnosis.

2.2.1.1. VDRL (Venereal Disease Research Laboratory)

This test can be applied on serum or plasma and the sample may not require heating according to the brand used, as well as on un-heated CSF. The reaction obtained with a positive sample is flocculation with a non-particulate antigen, so microscopy should be used for reading. Test results are largely subject to proper procedure completion and all parameters involved should be controlled by following all related reagent preparation and use instructions. The test measures both IgG and IgM antibodies simultaneously.

2.2.1.2. RPR (Rapid Plasma Reagin)

Plasma and serum may be used. This method is a variation of VDRL, in which carbon particles are added to VDRL to facilitate macroscopic flocculation reading. The commonest method uses an 18-mm round plate as a test base. This method should not be employed with CSF.

The patient's sample is mixed in both tests with the antigen in a preset diameter circular vessel. All antibodies present match up and cause a reaction read microscopically at 100 magnification in the case of VDRL, or

macroscopically if the reagent contains carbon, such as RPR. Even though it can be stabilised and kept for several days, the VDRL antigen should be prepared again each time by adding 1% benzoic acid. It must be remembered that VDRL is the only validated test to be used together with CSF, as well as the only tool useful for diagnosing neurosyphilis. RPR can be performed on micro-titration plates, provided reaction and reading times are changed. They do not detect specific treponemal antibodies, so positivity is not synonymous with syphilitic infection. These two tests are the only ones to be used for monitoring treatment efficacy.

2.2.1.3. VDRL and RPR sensitivity and specificity

False negative results: VDRL and RPR can cause prozone reactions with some high-reactivity samples. This effect sometimes occurs in patients with secondary syphilis, which is why titration is recommended whenever reactivity could be interpreted as uncertain or unusual or in the case of positive treponemal testing. False negative results may also occur when a procedure is completed improperly, such as the antigen being distributed on a sample not previously placed on the entire test area surface. Reagent temperature is also important for sensitivity, especially when testing samples from patients at a very early disease stage.

False positive results: they can be transitory or permanent, depending on whether they last less than or more than 6 months. Generally, titres do not exceed 1/8; but can be much higher in some cases.
Table 10 illustrates the most common causes of these results and Table 12 gives a summary of sensitivity and specificity.

2.2.2. Treponemal tests

These tests also sometimes produce false positive results, especially with EBV mononucleosis, leprosy, collagen diseases and intravenous drug addiction. They also show cross-reactive results with *Borrelia* antigens and other pathogenic treponemes, which is why preliminary adsorption of treponemal membrane containing serum is recommended to minimise or eliminate cross-reactivity.
These tests are not designed for treatment monitoring, since 85 to 90% of results on treated and cured patients are usually positive (Cf. Table 11).
The following tests are the most widely used in laboratories.

Table 9: Characteristics of reaginic tests

- Advantages
  - Easy to perform and low cost
  - Useful for monitoring treatment. Titres rise and fall according to disease status
- Drawbacks
  - Prozone reaction
  - Cross-reactivity
  - Low sensitivity in initial stages
  - False positive results in some clinical situations

2.2.2.1. TPHA (Treponema Pallidum Haemaglutination Assay or Microhaemagglutination)

This test procedure is validated for serum only. Sheep erythrocytes are coated with native Nichols strain treponemal antigens extracted by sonication by previous Reiter strain membrane sample absorption. The sample is tested in a 1/80 solution, so TPHA is one of the simplest methods to follow. Result usefulness has not been demonstrated yet and the test is not validated for use with CSF. It produces fewer false positive results than FTA-ABS and some trials indicate suitable use for screening. TPHA presents the greatest sensitivity of all tests to differences between stages[32] and should therefore be always used together with RPR or VDRL. It also is a good indicator for latent or cured infections after the primary stage. Test results can present very different patterns if patients are treated early. Replacing erythrocytes with TPPA gelatin particles has improved test stability. Testing with HATTS geese red blood cells is scantly reproducible. The most important improvement

Table 10: Some common clinical situations producing false-positive results in syphilis tests		
VDRL and RPR		Treponemal
Transitory	Permanent	Permanent
Hepatitis	Connective diseases	Endemic treponematosis
Mononucleosis caused by EBV	Immune system diseases	Immune system diseases
Viral pneumonia	Drug addiction	Lyme disease
Malaria	Leprosy and tuberculosis	Leprosy
Vaccinations	Malignant diseases	Malignant disease
Pregnancy	HIV infection	HIV infection
Cardiovascular disease	Cardiovascular disease	Cardiovascular disease
Technical error	Multiple transfusions	Multiple transfusions

in syphilis diagnosis over the past two years has been consolidating ELISA treponemal tests and chemiluminescence. These detect IgG and IgM antibodies either separately or simultaneously. The use of TpN15, 17, and 47 recombinant proteins has greatly improved performance (Cf. Fig. 2).

2.2.2.2. ELISA for IgG detection

This test was designed mostly for use with serum. Research has proven its high sensitivity and specificity, comparable both with TPHA and FTA-ABS, so ELISA IgG can be used instead of TPHA and FTA-ABS treponemal tests. Several trials have shown its outstanding rate of >92% sensitivity and >94% specificity, depending on the antigen used, both on treated and untreated patients. ELISA presents outstanding serum negative and serum positive selection capability, considering that serum negative patients rarely show indices >0.3, compared to >1.1 obtained with positive samples.[30–32] These tests enable automation and objective reading and the method features improved specificity.

2.2.2.3. ELISA for IgM detection

This is the diagnostic test of choice for early congenital and neonatal syphilis featuring 93 to 99% sensitivity[30,31] and possible use with serum. Its capture method is the most sensitive test for detecting this type of immunoglobulin and is even better than FTA-ABS IgM. The test is a significant indicator in primary infection and symptomatic untreated stages. Even treatment monitoring has been successfully performed with this method. Its decrease in concentration becomes clear in 12 weeks and can be detected in persistent low titre infection. The disease cannot be classed as uncured or active only based on positive results. The main issue of this test is false negative results generation for infected pre-reactive children, which is why a negative result in a newborn child previously exposed to the disease cannot exclude possible congenital syphilis.

2.2.2.4. Chemiluminescence

This is the most recently developed technique and can be performed using either serum or

Table 11: Treponemal Tests: limitations

- More expensive
- Cross-reactivity with other treponemes
- False positive results
- Unsuitable for monitoring treatment: 85% of cured patients test positive

plasma. It detects total antibodies. The tests use TpN17 recombinant protein or a mixture of TpN15, TpN17 and TpN47 as antigens. Research has indicated 99.9% specificity and 99.2% sensitivity. With respect to Western blot, sensitivity is somewhat higher than ELISA and TPHA (95.4 and 94.7% respectively), and the test is more sensitive and specific than RPR at any infection stage. Completion of this automated test is fast and simple.[32]

2.2.2.5. Immunochromatography

This is performed with nitrocellulose membranes where 47, 17, or 15 kDa recombinant peptides and labelled IgG anti-globulins are located.

By capillary force particles conjugated with analytes migrate towards the peptide area and test results can be read in few minutes. This test is quick and easy as it is designed for completion outside the laboratory and enables fast clinical decisions. Some commercial tests offer sensitivity results similar to or greater than RPR, achieving some 95% at certain disease stages.[30,33,34]

2.2.2.6. Western blot

This is a confirmatory test, in which either anti-IgG or anti-IgM can be used for detection. Western blot is extremely useful for disease confirmation. There is widespread consensus on the proteins to be evaluated with this technique, and TpN14, TpN15, TpN17, TpN37, TpN44, TpmA and TpN48 reactivity is considered the most specific.[35,36] There are different patterns according to the disease stage, since the early latent condition produces the greatest number of reactive bands and p14 and p15 antibodies are always positive after the primary stage. Cross-reactivity is exhibited with *Borrelia*, autoimmune diseases, some HIV infections and tests on elderly individuals and pregnant women. When reactivity is evident with anti-IgM, the test is as sensitive and specific as FTA-ABS, representing the main tool for congenital syphilis diagnosis. In these circumstances, it is more sensitive and specific than FTA-ABS IgM. Its sensitivity and specificity reach 99–100% when strict reading criteria are employed. Other similar tests include line immunoassay (LIA), which uses synthetic or recombinant proteins TpN47, TpN17, and TpN15. Sensitivity is 99.6%, and specificity ranges between 99.3% and 99.5%. As a confirmatory test, it has the advantage of yielding very few false-positive reactions.[37]

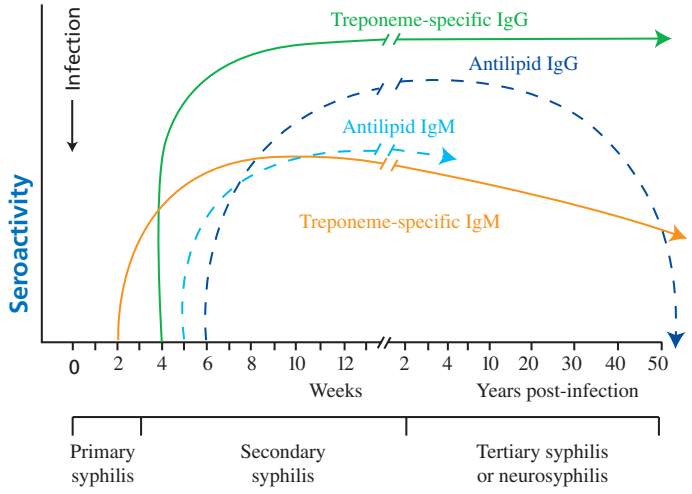


Fig. 2: Antibody patterns during treponemal infection

2.2.2.7. Other tests

Other less frequently used tests include:

FTA-ABS 200 (Fluorescent Treponemal Antibody Absorption Test). The treponemal antigen is the Nichols strain and the absorbent is Reiter strain. It can be performed both with serum and CSF.

FTA-ABS 200 DS (Fluorescent Treponemal Antibody Absorption Test with Double Staining). The treponemal antigen is the Nichols strain and the absorbent the Reiter. It is used with serum and CSF. It uses a tetra-methyl-rhodamine isothiocyanate labelled IgG as an antiserum and a fluorescein isothiocyanate conjugated anti-treponemal serum as a contrast medium. The oldest in this category is FTA-ABS IgG with its different variants. This complex test is subject to multiple errors if all reagents are not previously standardised against one another. Reading is also less objective and yields approximately 1% false positive results. The use of FTA-ABS IgM in serum to diagnose acute or congenital syphilis has been recently seriously questioned due to its low sensitivity

and specificity. Its use with modified FTA-ABS 19S IgM should be preceded by serum fractioning, though this method does not improve sensitivity and the false-negative rate is 30 to 35%. Hence, only a positive result achieved with this test would confirm the diagnosis. The sensitivity of all IgM tests is very low in asymptomatic forms of congenital syphilis; so only positive results confirm diagnosis. The complexity required to carry out this test properly combined with its low sensitivity means it is useless. The same occurs with FTA-ABS 19S IgM for serum and FTA-ABS for 1/5 diluted CSF.

3. Use of diagnostic tests. Clinical interpretation of results

Four tests are available to diagnose any disease form: two direct, treponemes visualization test and PCR, where positive results confirm the diagnosis, and two indirect, treponemal and reaginic tests. Each has a different biological meaning. Western blot and LIA should also be considered as confirmatory tests. The importance, accuracy and immediacy of direct diagnostic tests are often forgotten in an ambulatory setting and diagnosis is only based on indirect tests. We must remember that finding *T. pallidum* or detecting one of its components, namely DNA, ensures correct diagnosis. In the event of impossible diagnosis, serology becomes a valuable tool, as it is unique for assessing treatment efficacy. There however is a widespread opinion that the use of serological tests is predetermined, in other words, that reaginic tests should be used to analyse a greater number of samples and treponemal tests should be used to confirm the positive results from other tests.

This can be explained by technical difficulties in performing routine immunofluorescence (FTA) tests in patients with suspected infections. Their use can no longer be justified merely as confirmatory tests today, with the development of new automated treponemal tests, simpler technical methods and improved sensitivity and specificity. Though this is still common practice, treponemal tests, ELISA and chemiluminescence particularly, should be included in first-line serological diagnosis[38,39] rather than being used only for confirmation.

With this introduction, two diagnostic algorithms for adults to be performed on serum are proposed below, bearing direct test results in mind (Cf. Figures 3 and 4).

Table 12: Sensitivity and specificity of some serological tests for syphilis diagnosis*

Technique	Primary	Secondary	Latent	Late	Specificity Not syphilis
Dark-field microscopy	76%	76%	/	/	/
VDRL	78% (74-87)	100%	96% (88-100)	71%	98% (96-99)
RPR	86% (77-99)	100%	98% (95-100)	73%	98% (36-99)
Chromatography	92%	96%	96%	95%	95%
FTA-ABS	84% (70-100)	100%	100%	96%	97% (94-100)
FTA-ABS (Congenital S.**)	77%	/	/	/	/
TPHA	76% (69-90)	100%	97% (97-100)	94%	99% (98-100)
ELISA IgG	98%	100%	64%	96%	98% (70-99)
ELISA IgM	93%	85%	?	?	99%
ELISA IgM (Congenital S.**)	90%	/	/	/	/
Western blot	99%	100%	100%	99%	100%
W. blot IgM (Congenital S.**)	83%	/	/	/	/
Chemiluminescence	99%	100%	100%	99%	99%
PCR	95%	95%	95%	95%	> 99%

*Modified from references 1 and 2. The figures in parentheses indicate value ranges in different series.

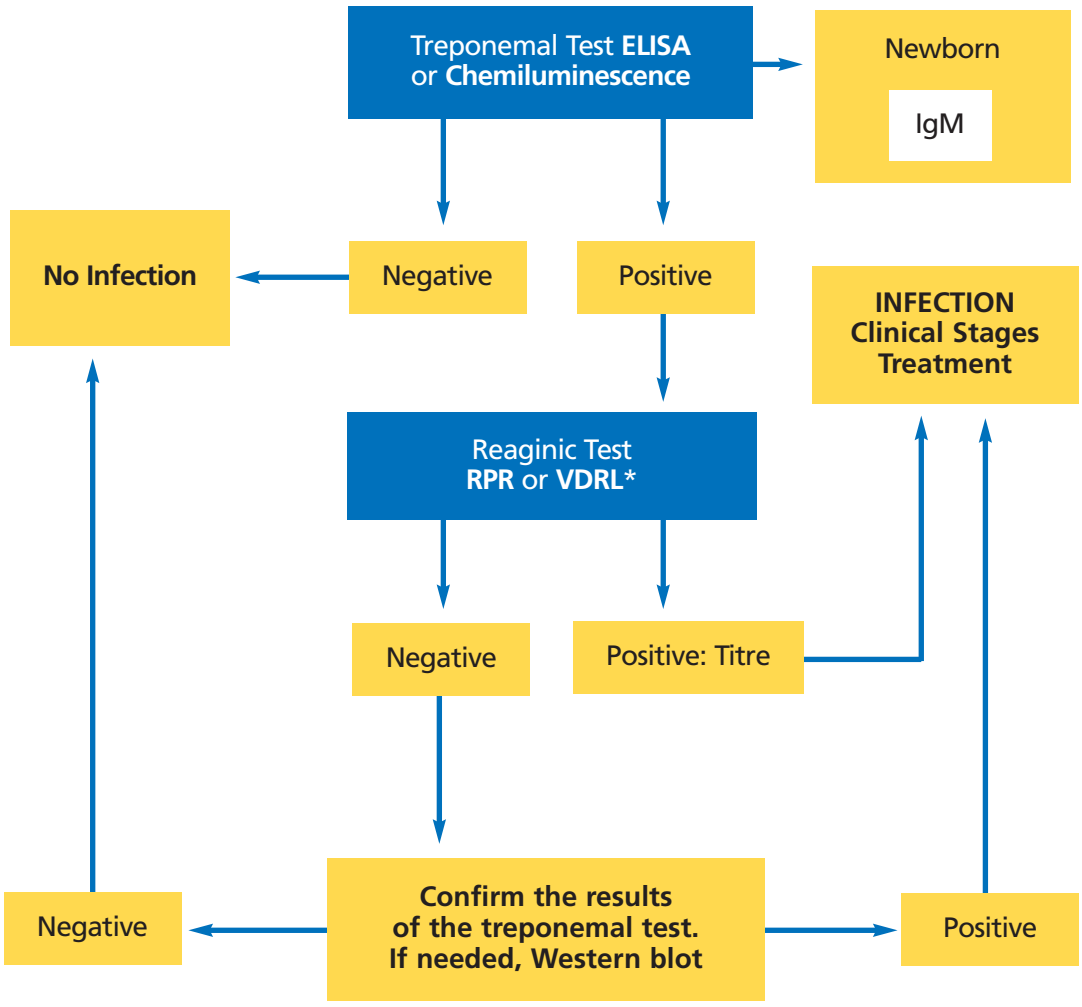
** Symptomatic congenital

3.1. Screening of donors, pregnant women and HIV-positive individuals

The benefits obtained by performing systematic screening tests for syphilis in blood donors, donated organs or unfrozen tissues, as well as in pregnant women and HIV-positive individuals are evident in diagnosis.

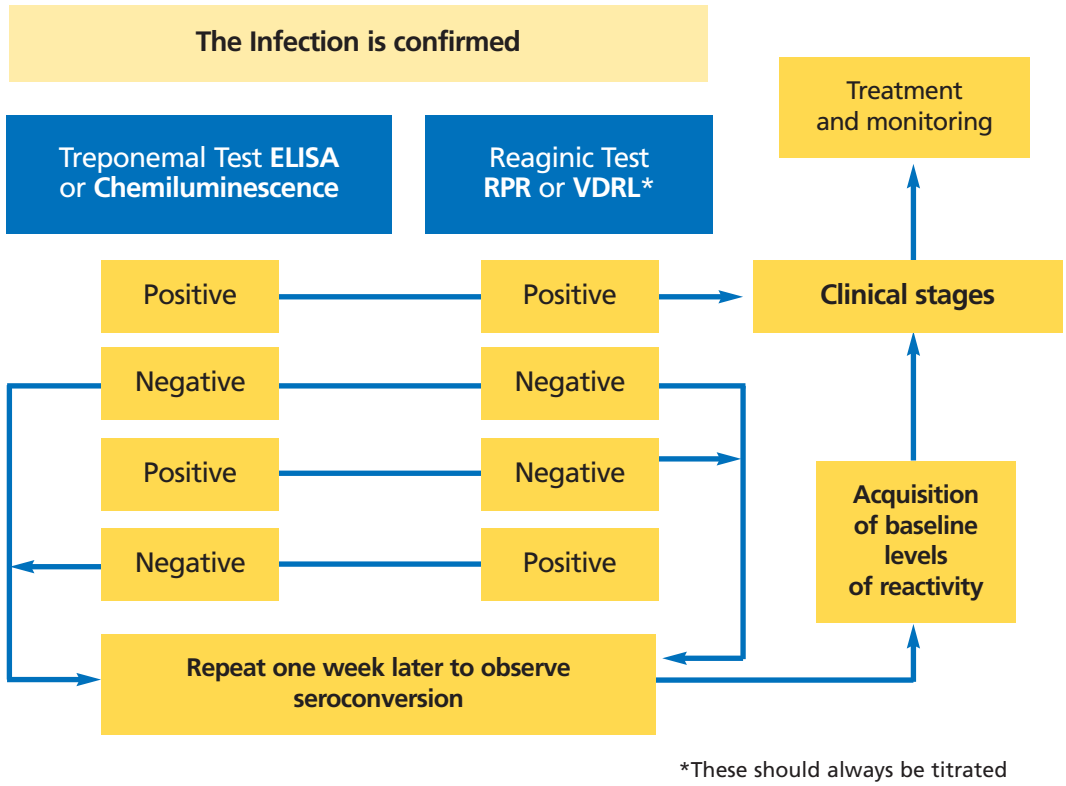
Pregnant women should not be excluded from serological marker surveys to prevent congenital or neonatal syphilis. The foetal infection transmission rate in pregnant women relates to the gestation period or in-pregnancy illness stage onset. Some groups of HIV-positive subjects, prostitutes and male homosexuals particularly, present greater prevalence of syphilis, in which cases screening is highly useful for diagnosis. Treponemes transmission from unfrozen organs or infected donor tissues is very unlikely and blood product refrigeration destroys treponemes after blood donation. Some pregnant women properly treated for syphilis in the past present moderately increased reaginic test residual titres. They are mostly cases of a non-specific increase of less than 2 titres, which does not

Fig. 3: Diagnosis of infection by *T. pallidum*: negative direct observation



* These should always be quantified

Fig. 4: Diagnosis of *T. pallidum* infection: positive direct observation



necessarily indicate re-infection or recurrence and correct clinical re-assessment is of primary importance. All tests can produce false positive results in healthy expectant mothers and doubtful reactivity in HIV-positive individuals. The challenge thus is to avoid false negative results. ELISA and chemiluminescence sensitivity and specificity are higher than for RPR, VDRL and FTA. The most frequently used strategies are RPR, VDRL or treponemal tests. The first option is a simple and inexpensive way of studying populations. The disadvantages are poor sensitivity in late-stage diseases and prozone reactions in early-stage infections. The second option is preferred because of its greater sensitivity; though it can produce false-positive results in cured patients, one advantage being that tests can be automated. We recommend identifying a grey zone between the 0.9 and 1.1 indices, though this should be adjusted according to the population analysed.

3.2. Disease diagnosis

3.2.1. Primary syphilis in adults

It must be stated that untreated patients can also present negative serological results even several weeks after the onset of chancre. Ensuring that direct diagnostic tests are carried out during this period is therefore vital. Any pattern of serological results is possible in a primary infection, depending on time elapsed. During this stage, IgG and IgM treponemal tests are positive in 99% of patients. TPHA is less sensitive than VDRL or RPR during this period and all those tests are less sensitive than ELISA and IgG/IgM chemiluminescence. If the disease is suspected to be at this stage, TPHA should thus not be the only test used, as a false-negative diagnosis for syphilis might result. Therapy during this period and the following stages can significantly alter serological results (Cf. Table 13).

3.2.2. Secondary syphilis

Any reaginic or treponemal test will be positive during this stage, ELISA IgM can even be positive in 80 to 90% of patients. It must be remembered that direct diagnosis is also possible by examining secondary lesions, replete with treponemes at this stage.

3.2.3. Early latent or under one year evolution syphilis

Reaginic and treponemal tests usually yield positive results. Reaginic tests reveal elevated titres if a patient has not been treated and a slow but progressive spontaneous titre reduction is observed throughout the year. Treatment accelerates this decrease. Some patients continue to be reactive to ELISA IgM at very low levels.

3.2.4. Late latent or more than one year evolution syphilis

Treponemal tests are generally reactive during this period, while RPR or VDRL results are highly variable though usually negative depending on infection age. When a diagnosis is made at this stage, the patient should be tested for neurosyphilis even in the absence of clinical symptoms. In cases of clinical history with no evident previous infection, a positive ELISA or TPHA serological pattern and a negative RPR or VDRL should be made resort to using a test such as the Western blot to monitor treponemal reactivity. Some serum positives for ELISA IgM at very low titres are sometimes found, though infrequently. This reactivity has no diagnostic value unless an elevation of any other type of antibody is exhibited, in which case a relapse could be imminent.

3.2.5. Tertiary syphilis

Serological diagnosis is more difficult and almost always hypothetical, so the patient's history and therapy must be well known. As a general rule, clinical data tend to be confused. In these clinical forms, treponemal tests are positive in 97% of cases, and reaginic tests negative in at least 25 to 30% of patients. Once again, the Western blot confirms antibody specificity and an examination for clinical symptoms of tertiary syphilis is recommended if under suspicion, such as gummas, cardiovascular syphilis, neurosyphilis, for instance. A CSF survey is absolutely necessary.

3.2.6. Neurosyphilis

Treponemes visualization in CSF is highly unlikely, so direct diagnosis of neurosyphilis is difficult. A definite diagnosis can be made if the patient has a positive serum treponemal test and a reactive VDRL in CSF, added to >5–10 mg/mL cellular and >40–100 mg/dL protein increases. Cells should decrease to normal levels under treatment, followed by protein normalization. The serial VDRL titre study does not always present significant VDRL decrease.
It must be remembered that 40% of the CSF elicits transitory alterations with no fatal evolution to neurosyphilis in the acute phase of the disease. Similarly, a patient can have neurosyphilis with no CSF alteration.[3,4,40,41] This clinical form can sometimes present rather soon, two or three years after infection.

3.2.7. Re-infection

Re-infection is possible in high-risk lifestyle patients. This condition presents as a difficult serological diagnosis with no knowledge of patient history, measurements and evolution of previous infection tests and treatment administered. Serum studies yield positive reaginic test result, more likely at higher titres than prior to re-infection, added to positive ELISA IgG testing or TPHA in the event of re-infection. ELISA IgM can present positive results in 58 to 65% of re-infected patients, in these circumstances. IgM peak reactivity is always less than in the previous infection. There may however be increased reactivity in some cases, when IgM is not completely negative after treatment and there are two samples. Studies show that increased reactivity to this marker is higher in patients who were not on a suitable treatment regime for their previous infection.

3.2.8. Congenital syphilis

Microscopy or PCR treponemes detection on newborn tissues, placenta, or umbilical cord is the definitive diagnostic tool. Various factors should be considered when performing serological tests for congenital syphilis: firstly, the mother should be seropositive and secondly, gestation promotes the occurrence of false positive results. The best sample on which to identify foetal risk is the maternal serum[1,2] in women with a history of past or suspected syphilis. The results obtained with this method are easier to interpret than those from umbilical cord blood tests. Some pregnant women with previous properly treated syphilis present evidence of moderately increased residual reaginic test titres. Non-specific increases of less than 2 titres occur in most cases, but this does not necessarily indicate re-infection or relapse, so accurate clinical re-assessment is absolutely necessary.[25] The evidence of RPR or VDRL newborn titres 3-4 times higher than the mother's is generally accepted as a diagnosis of infection; however, blood analysis of *in utero* infected newborns show that only 20% present reaginic test titres higher than the mother's, so congenital infection cannot be ruled out, even if reaginic titres are lower.[39,42] Reaginic and treponemal IgG antibodies transferred to the newborn infant disappear in 12 to 18 months, with an average of 2 to 3 weeks. Congenital infection is therefore suggested and the infant should be treated immediately if the antibody titre increases or

Table 13: *T. pallidum* infection in adults: Interpretation of most common results

		Stages with lesions and positive direct diagnosis				Stages with lesions or negative direct diagnosis	
Test priority		❶	❷	❸	❹	❺	❻
1°	Direct diagnosis	+	+	+	+	-	-
2°	Treponemal IgG/IgM	+	-	+	-	+	+
3°	Reaginic, titrated	+	+	-	-	+	-
4°	Specific IgM	Unnecessary. Elevated titres when positive				May be useful. Low titres if positive	
5°	Western blot or LIA IgG/IgM	Unnecessary				Unnecessary	Useful
6°	PCR	Unnecessary				If positive: diagnosis is certain	
Treatment and control with VDRL or RPR							

❶❷❸❹ With positive direct diagnosis, diagnosis of infection is CERTAIN. Serological tests may produce variable results, but all will turn out positive within a short time. Detection of IgM is usually positive. It is important to know RPR titre, even though a particular diagnosis is obtained.

❺❻ Whenever direct diagnosis is negative, diagnosis is hypothetical. Direct diagnosis can be negative when few treponemes exist or when treatment is under way, and diagnosis is probable even if serological tests are positive. If the PCR is positive, diagnosis is certain. The results of IgM tests may be variable: concentration depends on the disease evolution, and positive reaction in low, oscillating concentrations in later stages may indicate possible infection activity.

❺ This test pattern corresponds to primary and secondary syphilis and untreated infections for less than one or two years. In primary stages, the RPR or VDRL titres are usually greater than 1/64–1/28. They gradually decrease spontaneously or in response to treatment until they reach very low levels. During this period, CSF study is not normally needed.

❻ Typical of cured syphilis and also of later stages. It is difficult to interpret. If reactivity is not clear, or the patient's history is contradictory, confirmation should be obtained with another sample or through Western blot analysis before taking this result as diagnosis. In adults it is almost always necessary to study CSF to rule out neurosyphilis.

remains the same during the first 6 months of life. The presence of IgM in newborn blood by ELISA or Western blot would also confirm the diagnosis. The diagnosis of congenital syphilis should always be combined with an CSF test to exclude possible neurosyphilis.

Cellular and biochemical alterations, added to positive VDRL, are evident in this case. The diagnosis of neurosyphilis in these children is difficult, and the use of PCR and Western blot IgM should not be discounted[40] (Cf. Table 14) even though CNS infection can be identified by physical examination, radiology, reaginic tests, IgM evidence and CSF abnormalities. According to the CDC, confirmation of a case of congenital syphilis requires treponemal visualisation or the PCR results must be positive in cord sample, placenta, nasal secretion or skin lesions otherwise diagnosis is classed as probable.[1] The following cases should be considered as probable congenital syphilis given vertical transmission gravity and the mildness and short duration of adverse events following treatment.

- A newborn with signs of congenital syphilis and negative ELISA IgM, with an infected mother who is untreated or has not received adequate treatment.
- A newborn without symptoms and with negative ELISA IgM, whose mother was not treated or did not receive adequate treatment during the early infection. The newborn might be

Table 14: *T. pallidum* infection in pregnant women: interpretation of most common results in newborns

		With lesions and positive direct diagnosis				With a negative direct diagnosis			
Test priority		❶	❷	❸	❹	❺	❻	❼	❽
1°	Direct diagnosis	+	+	+	+	-	-	-	-
2°	Treponemal IgM	+	-	+	-	+	+	-	-
3°	Reaginic, titrated	+	+	-	-	+	-	+	-
4°	Treponemal IgG	Positive (maternal and newborn)				Positive (maternal and newborn)			
5°	Western blot / LIA IgM	If positive, IgM reactivity and infection are confirmed							
6°	PCR	Unnecessary				If positive: diagnosis is certain			

Direct diagnosis: visualization of the treponemes in lesions or positive PCR in cord, placenta, nasal secretion or dermal lesion.

❶❷❸❹ With positive direct diagnosis, diagnosis of infection is CERTAIN. Treponemal IgG tests are invariably positive due to the presence of antibodies of maternal or infant origin. Their quantification is useless. In these symptomatic patients, RPR and VDRL are almost always extremely reactive and their titration is important to monitor treatment. The remainder of the tests only has complementary value for diagnosis.

❺❻❼❽ Serological diagnosis is the only method possible in infected asymptomatic newborns. Treponemal IgG tests are invariably positive, due to the presence of antibodies of maternal or infant origin. Their quantification is useless.
Specific IgM should be positive and its presence confirms diagnosis. Western blot analysis is the serological test giving most information on IgM reactivity. To exclude neurosyphilis, a CSF study is needed.

❺❼ The criteria for congenital infection are fulfilled if RPR or VDRL elicit concentrations significantly higher than the mother's. A CSF study is needed to exclude neurosyphilis.

❼❽ These are the most common and difficult profiles to interpret. To evaluate their meaning, RPR or VDRL titres must be compared with the mother's serology. A CSF study is needed to exclude neurosyphilis.

in the infection incubation period where the IgM test could be a false negative simply because antibodies have not developed yet.

- A newborn without symptoms with negative ELISA IgM, whose mother received no or inadequate treatment in the latent period of the disease. This represents a risk of syphilis for the newborn.
- A newborn with or without symptoms, with positive ELISA IgM, whose mother is seropositive and untreated, will probably suffer from congenital syphilis.
- The evidence of a cellular and protein increase in CSF added to reactive VDRL and/or positive IgM would facilitate diagnosis confirmation in all these cases (Cf. Table 15).

3.2.9. Diagnosis peculiarities in HIV-positive individuals

Treponemal infection in HIV-positive individuals is greatly prevalent, probably because both pathogens share certain acquisition factors that are very common in the population under review. Most HIV patients respond well to treponemal infection. There are exceptions, however, such as the presence of particularly high or low titres, reaginic test false positive results, gradually disappearing reactivity in treponemal tests, the persistence of reaginic test titres despite adequate treatment and no serological response in proven syphilis cases. All these circumstances complicate diagnosis in some patients, and cause the risk of results being improperly taken to be false positive results. Clinical data and caution are even more fundamental, due to the fast progression of syphilis in these patients.

4. Treatment monitoring

Only RPR and VDRL reaginic tests are fundamental in evaluating treatment efficacy. VDRL titres should decrease significantly at least four times 3 to 4 months from treatment onset, and eight times after 8 to 12 months if therapy is adequate and effective. RPR decreases less and both tests are less accurate in HIV-positive individuals.[39] Titres generally decrease slightly in 25 to 40% of patients if treatment begins in the latent or late stages, or in cases of repeated infections with moderate or low reaginic titres. Many properly treated patients commonly present reactivity persistence for some time at titres of 1/2 or neat serum, but this is not failed treatment or re-infection. The same antigen type should always be used to perform this evaluation considering the different RPR and VDRL sensitivity levels. ELISA IgM can also be used to assess therapy efficacy if it is administered during the primary stage or very early in the secondary stage. Concentration of this antibody decreases significantly in 75% of patients.

Table 15: Defining a congenital syphilis case (CDC, 1993)

- Confirmed case
  - Visualization of *T. pallidum* in lesions of placenta, cord, nasal secretion, etc.
- Hypothetical diagnosis
  - A child whose mother has received no or inadequate treatment
  - A child with positive serological tests and:
    - Physical evidence
    - CSF with positive VDRL
    - CSF with cellular or biochemical alterations
    - Osteitis of long bones
  - A child with a:
    - VDRL or RPR more elevated than in the mother
    - Positive IgM

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