SPIROCHETES

Spirochaetes (also spelled **spirochetes**) belong to a phylum of distinctive diderm (double-membrane) bacteria, most of which have long, helically coiled (corkscrew-shaped) cells. Spirochaetes are chemoheterotrophic in nature, with lengths between 5 and 250 µm and diameters around 0.1–0.6 µm. Spirochaetes are distinguished from other bacterial phyla by the location of their flagella, sometimes called axial filaments, which run lengthwise between the bacterial inner membrane and outer membrane in periplasmic space (Fig. 101). These cause a twisting motion which allows the spirochaete to move about.

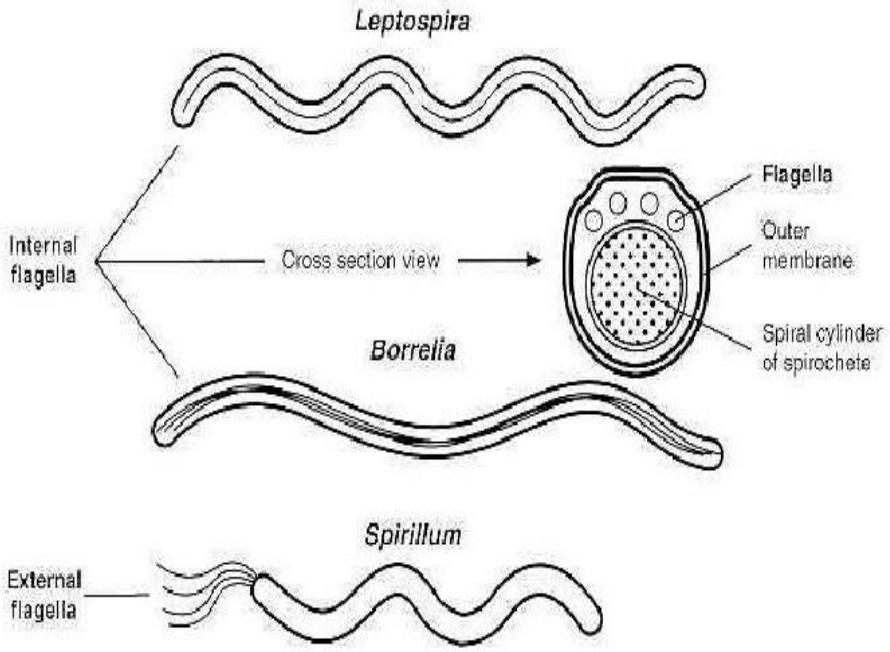

Fig. 1 Spirochetes - structure.

When reproducing, a spirochaete will undergo asexual transverse binary fission. In addition, the spirochetes are microaerophilic or anaerobic and are extremely sensitive to oxygen toxicity. The complete genome sequence has revealed there are no genes for catalase or superoxide dismutase.

The order of Spirochaetales is divided into two families:

1. Spirochaetaceae
2. Leptospiraceae

Two of the four genera of Spirochaetaceae, Treponema and Borrelia, include species that are pathogenic to man. Among Leptospiraceae, only one genus, Leptospira, has pathogenic species.

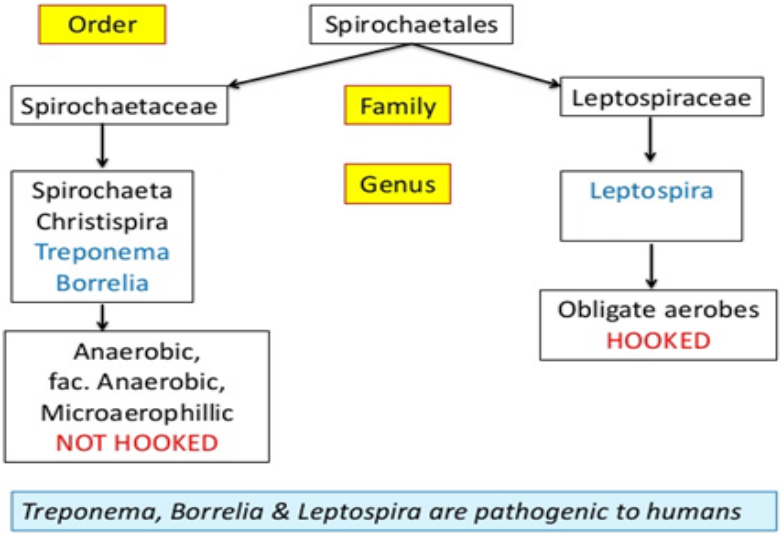

Fig. 2 The order *Spirochaetales*.

Disease-causing members are *Leptospira* species, *Borrelia burgdorferi*, *B. garinii*, *B. afzelii*, *Borrelia recurrentis*, *Treponema pallidum* subspecies, *Brachyspira pilosicoli* and *Brachyspira aalborgi* (Table 6).

Disease-causing members	Disease
***Leptospira* species**	**leptospirosis**
Borrelia burgdorferi	**Lyme disease**
B. garinii	
B. afzelii	
Borrelia recurrentis	**relapsing fever**
***Treponema* species**	**treponematoses**
Brachyspira pilosicoli	**intestinal spirochaetosis**
Brachyspira aalborgi	

Table 1 Disease - causing members of Spirochetes.

TREPONEMA PALLIDUM

Treponema pallidum is a spirochaete bacterium with subspecies that cause treponemal diseases such as syphilis, bejel, pinta, and yaws. Classification of the pathogenic treponemes is based primarily upon the clinical manifestations of the respective diseases they cause (Table 7).

Subspecies	Disease
T. pallidum* subsp *pallidum	**Venereal syphilis**
T. pallidum* subsp *pertenue	**Yaws**
T. pallidum* subsp *endemicum	**Endemic syphilis**
T. carateum	**Pinta**

Table 2 Classification of the pathogenic treponemes.

SYPHILIS

The clinical course of syphilis evolves through three phases. The initial or **primary phase (primary syphilis)** is characterized by one or more skin lesions **(chancres)** at the site where the spirochete penetrated. Although spirochetes are disseminated in the blood soon after infection, the chancre represents the primary site of initial replication. Histologic examination of the lesion reveals endarteritis and periarteritis (characteristic of syphilitic lesions at all stages) and infiltration of the ulcer with polymorphonuclear leukocytes and macrophages. Phagocytic cells ingest spirochetes, but the organisms often survive. Clinical manifestations of the primary stage include regional lymphadenopathy. In the **secondary phase (secondary syphilis),** the clinical signs of disseminated disease appear, with prominent skin lesions dispersed over the entire body surface. Spontaneous remission may occur after the primary or secondary stages, or the disease may progress to the **late phase (tertiary syphilis)** of disease, in which virtually all tissues may be involved. Tertiary syphilis may include gummatous syphillis where gummatous lesions may form on any organ or tissue, cardiovascular syphilis, which usually manifests as aortic disease and neurosyphillis. Neurosyphilis can manifest as acute syphilitic meningitis, meningovascular syphilis or as paresis or tabes dorsalis. It usually arises in tertiary syphilis, but can occur as early as 3 months post infection. Over 40% of patients with secondary syphilis experience some central nervous system involvement Each stage represents localized multiplication of the spirochete and tissue destruction. Although replication is slow, numerous organisms are present in the initial chancre, as well as in the secondary lesions, making the patient highly infectious at these stages.

Congenital Syphilis

In utero infections can lead to serious fetal disease, resulting in latent infections, multiorgan malformations, or death of the fetus. Most infected infants are born without clinical evidence of the disease, but rhinitis then develops and is followed by a widespread desquamating maculopapular rash. Teeth and bone malformation, blindness, deafness, and cardiovascular syphilis are common in untreated infants who survive the initial phase of disease.

SYPHILIS – LABORATORY TESTING

Syphilis has several clinical manifestations, making laboratory testing a very important aspect of diagnosis. The etiological agent, *Treponema pallidum*, cannot be cultured, and there is no single optimal alternative test. Serological testing is the most frequently used approach in the laboratory diagnosis of syphilis. Syphilis has diverse clinical manifestations and shares many clinical features with other treponemal and nontreponemal diseases. Therefore, it is mandatory that the clinical diagnosis is always supported by appropriate laboratory tests and that the test results are interpreted with reference to the patient's history and physical examination findings.

Although [1]*T.pallidum* cannot be grown in culture, there are many tests for the direct and indirect diagnosis of syphilis. Direct diagnostic methods include the detection of *T. pallidum* by microscopic examination of fluid or smears from lesions, histological examination of tissues or nucleic acid amplification methods such as polymerase chain reaction (PCR). Indirect diagnosis is based on serological tests for the detection of antibodies.

TREPONEMA PALLIDUM - CULTIVATION

T. pallidum is an obligate human parasite, which does not survive outside its mammalian host and cannot be cultivated continuously under in vitro conditions. Optimal conditions for time-limited cultivation in tissue culture consisted of temperature between 33°C-35°C, atmospheric oxygen concentration in the 1,5% - 5% range, 20% fetal bovine serum in the culture medium and the testes extract. Stable propagation of *T. pallidum* strains can only be achieved in mammalian hosts, usually rabbits.

TREPONEMA PALLIDUM - MICROSCOPY

Because *T. pallidum* is too thin to be seen by light microscopy, **darkfield microscopy** or **special fluorescent stains** must be used. The diagnosis of primary, secondary, or congenital syphilis can be made rapidly by darkfield examination of the exudate from skin lesions (Fig. 103).

Fig. 3 *T. pallidum*- darkfield microscopy.

[1] T. - Treponema

Darkfield microscopy method may be used in the early stages of syphilis when a suspected syphilis sore (chancre) is present. It involves obtaining a scraping of the sore, placing it on a slide, and examining it with a special dark-field microscope. Material collected from oral and rectal lesions should not be examined because nonpathogenic spirochetes can contaminate the specimen. Because of the limitations of darkfield microscopy, a more useful test for detecting *T. pallidum* is the **direct fluorescent antibody test (**uses a labeled antibody to directly react with the antigen).
Fluorescein-labeled antitreponemal antibodies are used to stain the bacteria. A monoclonal antibody reagent is available that is specific for pathogenic treponemes, so oral and rectal specimens can be examined.

TREPONEMA PALLIDUM - SEROLOGICAL TESTS (NONTREPONEMAL AND TREPONEMAL TESTS)

Serological tests fall into two categories: nontreponemal tests for screening, and treponemal tests for confirmation.

Nontreponemal tests

Nontreponemal tests measure immunoglobulin G (IgG) and IgM antibodies (also called **reagins**) developed against lipids released from damaged cells during the early stage of disease and that appear on the cell surface of treponemes. The antigen used for the nontreponemal tests is **cardiolipin,** which is derived from beef heart.

	Test
VDRL	Venereal Disease Research Laboratory
RPR	Rapid Plasma Reagin
USR	Unheated Serum Reagin (modification of the VDRL test)

Table 3 Nontreponemal tests.

The two tests used most commonly are the **Venereal Disease Research Laboratory (VDRL) test** and the **rapid plasma reagin (RPR) test.** Both tests measure the flocculation of cardiolipin antigen by the patient's serum, both tests can be performed rapidly. Only the VDRL test should be used to test CSF from patients with suspected neurosyphilis. Other nontreponemal tests in use include the unheated serum reagin (USR) test and the toluidine red unheated serum test (TRUST). All nontreponemal tests have essentially the same sensitivity (70% to 85% for primary disease, 100% for secondary disease, 70% to 75% for late syphilis) and specificity (98% to 99%).

VDRL test

Venereal Disease Research Laboratory test utilizes an antigen which consists of cardiolipin, cholesterol and lecithin. The antigen particles appear as short rod forms at magnification of about 100x. Aggregation of these particles into large or small clumps is interpreted as positivity (reactive) (Fig. 104).

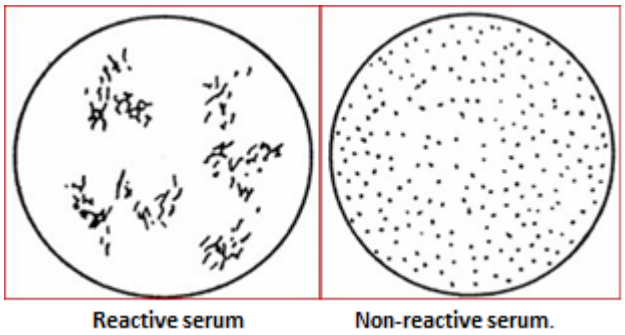

Fig. 4 Venereal Disease Research Laboratory test.

RPR test

Rapid plasma reagin (RPR) test is the most commonly used non-treponemal test for the diagnosis of syphilis. The test takes the form of a flocculation assay in which a cardiolipin antigen and the patient's anti-cardiolipin antibodies form an antigen–antibody lattice, which can be visualised when carbon particles are trapped within it.

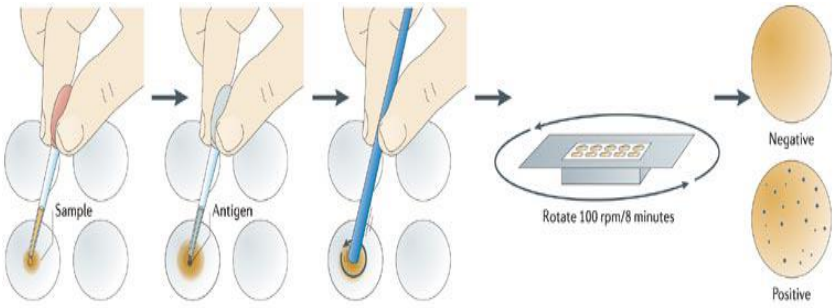

Fig. 5 Rapid plasma reagin test.

Treponemal tests

Treponemal tests use *T. pallidum* as the antigen and detect specific anti - *T. pallidum* antibodies. The treponemal test results can be positive before the nontreponemal test results become positive in early syphilis, and they can remain positive when the nonspecific test results revert to negative in some patients who have late syphilis.

Test	
FTA-ABS	Fluorescent Treponemal Antibody-Absorption
MHA-TP	Microhemagglutination *Treponema pallidum*
TP-PA	*T. pallidum* particle agglutination

Table 4 Treponemal tests.

Historically, the most commonly used treponemal test was the **fluorescent treponemal antibody-absorption (FTA-ABS) test.**

FTA-ABS test

FTA-ABS (**Fluorescent Treponemal Antibody-Absorption)** test is an indirect fluorescent antibody test. The patient's serum, which has been diluted 1:5 in sorbent (an extract from cultures of *Treponema phagedenis*, Reiter treponeme), is layered on a microscope slide to which *T. pallidum* subspecies *pallidum* has been fixed. If the patient's serum contains antibody, the antibody will coat the treponeme. Next, fluorescein isothiocyanate (FITC)-labeled antihuman immunoglobulin is added; this combines with the patient's IgG and IgM antibodies that are adhering to *T. pallidum*, and results in a visible test reaction when examined by fluorescence microscopy.

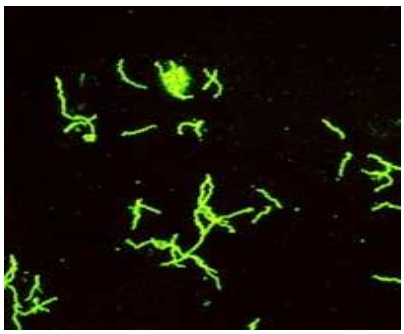

Fig. 6 Fluorescent Treponemal Antibody-Absorption Test - visible test reaction.

TP-PA test

***Treponema pallidum* Particle Agglutination (TP-PA) test** or one of a number of specific **enzyme immunoassays (EIAs).** The TP-PA test is a microtiter agglutination test, based on the agglutination of colored gelatin particle carriers sensitized with *T. pallidum* antigen. Patient sera are incubated with sensitized particles in microtiter wells and unsensitized gelatin particles in control wells. Patient sera containing specific antibodies will react only with the

antigen sensitized colored gelatin particles to form a smooth mat of agglutinated particles in the microtitration tray (+ or ++). A compact button formed by the settling of the non-agglutinated particles in the microtiter wells containing sensitized particles indicates lack of specific antibody in patient sera (-). A variety of specific EIAs have been developed and appear to have sensitivities (80% to 95% for primary disease, 100% for secondary and late syphilis) and specificities (96% to 99%) similar to the FTA-ABS and TP-PA tests.

Fig. 7 *Treponema pallidum* Particle Agglutination Test.

MHA-TP test

Microhemagglutination *Treponema pallidum* test is based on the principle of agglutination and pattern recognition. The test uses fixed chicken erythrocytes sensitized with components of the pathogenic *T. pallidum* (Nichols Strain). Hemagglutination occurs in the presence of *Treponema pallidum* antibodies in specimens.

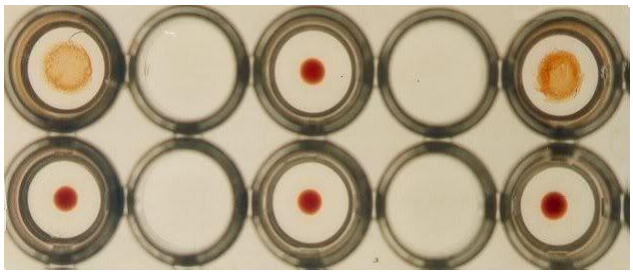

Fig. 8 Microhemagglutination *Treponema pallidum* test.

The upper, left-hand well contains a positive control test. The red cells have had treponemal antigens attached and antibodies in the serum have caused these cells to agglutinate and form a mat across the bottom of the well. These antibodies can be presumed to be specific for treponemes, since identical red cells that have not had the treponemal antigens attached do not cause haemagglutination, as seen in the bottom, left-hand well. A negative serum test is shown in the centre well, where no agglutination is observed. On the upper, right-hand of the

well is a patient's sample. The agglutination formed by the patient's serum support positive syphillis infection.

Interpretation of the results of serological tests

Because positive reactions with the nontreponemal tests develop late during the first phase of disease, the serologic findings are negative in many patients who initially have chancres. However, serologic results are positive within 3 months in all patients and remain positive in untreated patients with secondary syphilis. The antibody titers decrease slowly in patients with untreated syphilis, and serologic results are negative in approximately 25% to 30% of patients with late syphilis. Thus the limitation of the nontreponemal tests is reduced sensitivity in early primary disease and late syphilis. Although the results of treponemal tests generally remain positive for the life of the person who has syphilis, a negative test is unreliable in patients with AIDS.
Successful treatment of primary or secondary syphilis and, to a lesser extent, late syphilis, leads to reduced titers measured in the VDRL and RPR tests. Thus these tests can be used to monitor the effectiveness of therapy, although seroreversion is slowed in patients in an advanced stage of disease, those with high initial titers, and those who have previously had syphilis. The treponemal tests are influenced less by therapy than are the VDRL and RPR tests, with seroreversion observed in less than 25% of patients successfully treated during the primary stage of the disease.

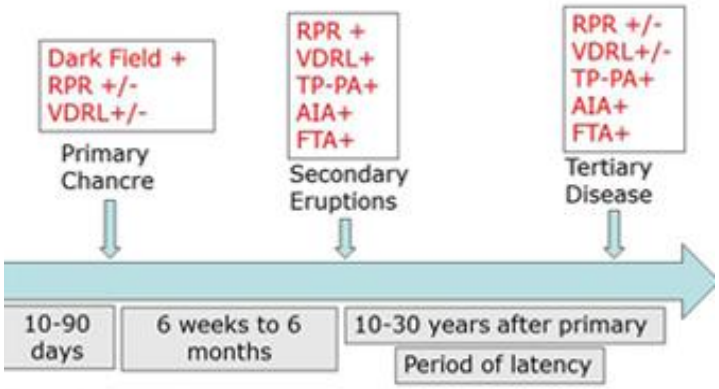

Fig. 9 Syphilis - stages and possible results.

Diagnosis of neurosyphilis and congenital syphilis

Diagnosis of neurosyphilis and congenital syphilis can be problematic. The diagnosis of neurosyphilis is based on clinical symptoms and laboratory findings.
A VDRL test on cerebrospinal fluid (CSF) is highly specific but not sensitive. Thus a positive VDRL confirms the diagnosis, but a negative test does not rule out neurosyphilis. In contrast,

the FTA-ABS CSF test has high sensitivity but low specificity because of passive transfer of antitreponemal antibodies from blood to CSF. In this case, a positive FTA-ABS CSF test is consistent with neurosyphilis but is not diagnostic, and a negative test would essentially rule out the diagnosis.

Positive serologic test results in infants of infected mothers can represent a passive transfer of antibodies or a specific immunologic response to a congenital infection. These two possibilities are distinguished by measuring the antibody titers in the sera of the infant during a 6-month period. The antibody titers in noninfected infants decrease to undetectable levels within 3 months of birth but remain elevated in infants who have congenital syphilis.

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