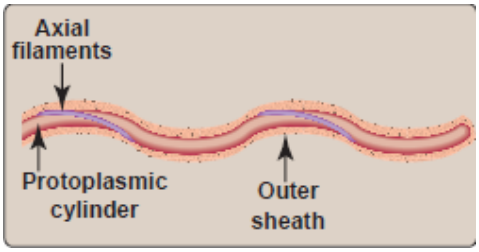
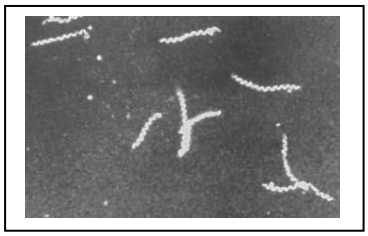
Spirochetes

long, slender, motile, flexible, undulating, gram-negative bacilli that have a characteristic corkscrew or helical shape.
Depending on the species, they can be microaerophilic, aerobic, or anaerobic.
Some species can be grown in laboratory culture (either cell free culture or tissue culture), whereas others cannot.
Some species are free living, and some are part of the normal flora of humans and animals. Spirochetes that are important human pathogens are confined to three genera:
Treponema (Treponema pallidum causes syphilis), Borrelia (causes Lyme disease and relapsing fever), and Leptospira (causes leptospirosis).

Spirochetes have a unique structure that is responsible for motility?
the spirochete cell has a central protoplasmic cylinder bounded by a plasma membrane and a typical gram-negative cell wall. Unlike in other bacilli, this cylinder is enveloped by an outer membrane composed of glycolipids and lipoproteins. Between the peptidoglycan and the outer sheath are located multiple periplasmic flagella that do not protrude from the cell but are oriented axially. Bundles of these endoflagella (axial filaments) span the entire length of the cell and are anchored at both ends. Although the mechanics are not totally clear, it is likely that these axial periplasmic flagella rotate like the external flagella of other motile bacteria, propelling the cell in a corkscrew-like manner.

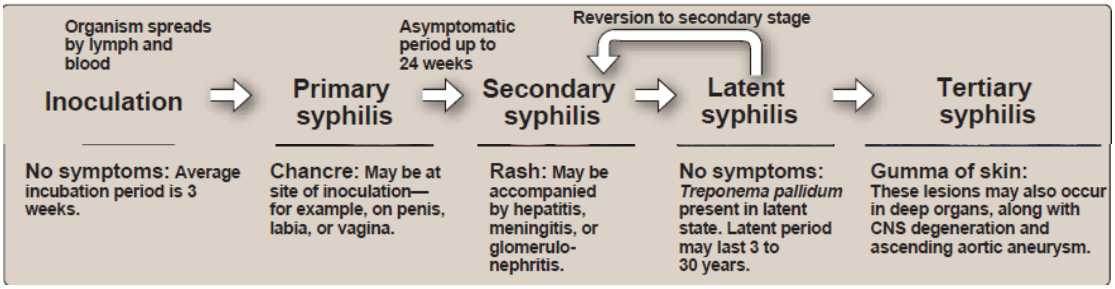




TREPONEMA PALLIDUM
Syphilis is primarily a sexually transmitted infection caused by the spirochete *T. pallidum*. Starting with a small lesion (chancre), several progressive stages of the disease can span a period of 30 years or more, often ending in syphilitic dementia or cardiovascular damage.

Transmission of T. pallidum is almost always by **sexual contact or transplacentally**. The organism enters the body through a break in the skin or by penetrating mucous membranes such as those of the genitalia.

congenital syphilis**:** T. pallidum can be transmitted through the placenta to a fetus after the first 10 to 15 weeks of pregnancy. Infection can cause fetal or infant death or spontaneous abortion.



LABORATORY FEATURES

Specimens: Serous fluid from chancres and secondary skin lesions (from moist areas) to detect motile treponemes.
Note: Within a few hours of antibiotic treatment, spirochaetes will not be found in lesions. Sometimes the organisms can be detected in lymph gland fluid. It is important therefore to ask patients whether they have taken antibiotics to treat their infection. When a specimen is collected at the healing stage of the chancre, the organisms may be too few to detect by dark-field microscopy. Blood should always be collected for antibody testing when syphilis is suspected but no treponemes are detected microscopically.

A blood sample (3–5 ml) is required for serological testing.

Morphology

T. pallidum cannot be seen in Gram stained smears. The organisms are best seen by dark-field microscopy in serous fluid collected from a primary chancre or secondary skin lesion (about 50–80% sensitive). The organisms have a bending and slowly rotating motility and may be seen lengthening and shortening.

Culture

Pathogenic treponemes have not yet been reproducibly cultured in the routine laboratory. The organisms, however, **are able to survive in some fluids, including donated blood.** The transmission of *T. pallidum* by blood transfusion can be avoided by storing all donor blood at 2–6 ºC for

3–5 days and collecting blood from low risk donors or preferably from donors previously serologically screened for syphilis.

Serological diagnosis of syphilis

A person infected with *T. pallidum* produces two types of antibody:

_ Non-specific antibody that reacts with cardiolipin antigen in non-specific syphilis tests.

Cardiolipin

This is a phospholipid substance extracted from beef heart tissue.

It is thought that a similar substance to cardiolipin is released from tissue damaged by treponemal infection and possibly also from the treponemes, and this substance (often referred to as reagin) stimulates the production of anti-cardiolipin antibodies which can be detected in the serum of patients.

_ Specific treponemal antibody that reacts with treponemal antigen in specific syphilis tests.

NON-SPECIFIC CARDIOLIPIN (REAGIN) TESTS: include:

– VDRL (Venereal Diseases Research Laboratory) test which is read microscopically.

– RPR (Rapid plasma reagin) test which is read macroscopically.

VDRL

In the VDRL test, heat-inactivated serum (to destroy complement) is reacted with freshly prepared cardiolipin- cholesterol-lecithin antigen and the resulting flocculation (suspended antigen-antibody complex)

RPR

In the RPR test, the cardiolipin-cholesterol-lecithin antigen has choline chloride added to it which removes the need for heat-inactivation of samples and enables plasma as well as serum to be used in the test. It also enhances the reactivity of the antigen.

In the RPR test, the patient's serum or plasma is spread within a marked circular area on a plastic coated card, antigen is added, and the mixture rotated at 100 rpm for 8 minutes using a mechanical rotator. Reactive tests are quantitated to obtain the antibody titre.

SPECIFIC TREPONEMAL TESTS

A specific treponemal test is performed when a person gives a reactive non-specific cardiolipin test or in late stage syphilis when a cardiolipin test may be non-reactive.

Specific treponemal tests include:

– TPHA (*T. pallidum* haemagglutination assay)

– TPPA (*T. pallidum* particle agglutination assay)
– FTA-ABS (Fluorescent treponemal antibody absorption) test

TPHA
In the TPHA, patient's diluted serum samples are mixed in the wells of a microtitration plate with sheep or avian red cells coated (sensitized) with *T. pallidum* antigen (Nichol's strain). If antibody is present, the sensitized cells are agglutinated

TPPA
The TPPA test is similar to the TPHA test except that gelatin particles instead of red cells are sensitized with *T. pallidum* antigen, making the reagent more stable.

FTA-ABS
The fluorescent antibody test is the first serological test to become positive following infection, i.e. 3–4 weeks after infection. It is an expensive test mainly performed in reference public health laboratories.

Main differences between non-specific and specific syphilis antibody tests

Non-specific cardiolipin tests
● Screening tests.
● Indicate possible active disease.
● Antibody titre falls with effective treatment.
● Biological false positives occur (in the presence of other conditions including viral infections, autoimmune diseases, leprosy, tuberculosis, and malaria)

Specific treponemal tests
● Positive test indicates present or past infection.
● Higher specificity than cardiolipin tests.
● Used to check positive cardiolipin test reaction.
● Not suitable for assessing response to treatment.
● Tests often remain positive for many years due to persistence of IgG.