



RPR test for Venereal Disease (Syphilis)

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Syphilis

- **Syphilis** is a chronic systemic infectious , venereal disease, sexually transmitted infection caused by the bacterium *Treponema pallidum* .
- *Treponema pallidum* subspecies *pallidum* is a spiral-shaped (spirochete), gram negative & highly mobile bacterium.
- that has never been cultured successfully on artificial culture media and it does not take up Gram's stain.

Signs and symptoms

- The signs and symptoms of syphilis vary depending in which of the four stages it presents .
- Primary syphilis
- Secondary syphilis
- Latent syphilis
- Tertiary syphilis

Transmission

- Syphilis is transmitted primarily by :
- Sexual contact
- Transmitted from mother to her fetus during pregnancy or at birth, resulting in congenital syphilis .

- The spirochete bacteria is able to pass through intact mucous membranes or compromised skin.

Diagnosis

- Syphilis is difficult to diagnose clinically during early infection. Confirmation is either via :
 1. **Blood tests**... are more commonly used, as they are easier to perform...
 2. **Dark field microscopy** ..(direct visual inspection using).
- Diagnostic tests are unable to distinguish between the stages of the disease.

Syphilis

- **Early: chancre: 90% seropositive within 3 weeks**
- **Secondary: 6-8 weeks 100% seropositive**
- **Latent: seropositive, symptoms absent**
- **Tertiary: Years later, new lesions any body part**





Syphilis – *Treponema pallidum*



Syphilis – *Treponema pallidum* on darkfield

Blood tests

- Blood tests are divided into 2 different types of serologic tests classified based on the type of antigen, antibodies are directed against:
 1. **Non treponemal antibodies**, detect antibodies directed against lipoidal antigens, damaged host cells, and possibly from treponemal.
 2. **Treponemal antibodies**, detect antibody to *T. pallidum* proteins.
 3. **Both tests are used to confirm the infection and determine whether the disease is active.**



Non treponemal tests

Standard Nontreponemal Tests

- All of the current non-treponemal procedures for syphilis are **flocculation tests** using **cardiolipin, lecithin and cholesterol** as an antigen.
- All non-treponemal tests are performed in a similar manner: after human serum or plasma is mixed with the antigen and rotated for a few minutes, the flocculation (suspended antigen-antibody complex) can be observed.
- The reaction can be read by naked eye in macroscopic tests or by using microscope in microscopic tests.

Types of nontreponemal tests

- Two types of nontreponemal tests are available :
- Rapid Plasma Reagin (RPR),
- Venereal Disease Research Laboratory (VDRL) test,
- These tests usually react with immunoglobulin M(IgM) and immunoglobulin G (IgG) antibodies.
- The results of these tests are semi quantitative, reflecting the activity of the infection, and are reported as a titer of the antibody reflecting the number of dilutions where activity is still detected.
- Seropositive occurs around 3 weeks, but can take up to 6 weeks.
- Consequently, patients can present with primary syphilis and have initially negative serologic tests.
- Titers will normally decline over time, often to undetectable titers after successful treatment.

❑ **Nontreponemal tests are used initially, detect antibody to cardiolipin called reagin and include:**

➤ **VDRL: flocculation, prone to false positives**

✦ Antigen composed of cardiolipin, cholesterol, lecithin

✓ **RPR: modified VDRL with charcoal particles (Carbone), more sensitive**

❑ **Treponemal tests: detect antibody to spirochete itself**

Rapid Plasma Reagin test (RPR)

- The **Rapid Plasma Reagin test (RPR)** is a type of rapid diagnostic that looks for non-specific antibodies in the blood of the patient that may indicate a syphilis infection.
- It is one of several nontreponemal tests for syphilis.
- The term **reagin** means that this test does not look for antibodies against the bacterium itself, *Treponema pallidum*, but rather for antibodies **against substances released by cells when they are damaged by *T. pallidum***(cardiolipin and lecithin).

False-positive results

False-positive nontreponemal tests have been described in systemic infections such as:

- Tuberculosis.
- Rickettsial diseases.
- Endocarditic.
- and also during pregnancy.

There are a number of limitations associated with nontreponemal

- Firstly, they lack sensitivity in late stage infection.
- 30% of patients with late latent or late active syphilis will show a non-reactive result.
- Secondly, 1-2% of patients with secondary syphilis exhibit a prozone reaction.
- Prozone occurs when an excess of antibody in undiluted serum inhibits flocculation with the antigen, giving rise to weakly reactive, atypical or occasionally false negative results.
- Finally, antibodies detected by nontreponemal tests are not only produced as a consequence of treponemal infection, but also in response to other conditions where tissue damage occurs

- Because of the possibility of false positives with nontreponemal tests, **confirmation is required with a treponemal test, such as**
- Treponemal pallidum particle agglutination (TPHA) or
- Fluorescent treponemal antibody absorption test (FTA-Abs).
- Treponemal antibody tests usually become positive **two to five weeks** after the initial infection

Table 1 Interpretation of serologic tests in syphilis

Treponemal test	Nontreponemal test	Possible interpretations
Nonreactive	Nonreactive ^a	1. Absence of syphilis 2. Very early syphilis before seroconversion
Reactive^b	Nonreactive	1. Prior treated syphilis 2. Untreated syphilis 3. False-positive treponemal test ^c
Reactive	Reactive with or without a measurable titer	1. Active syphilis 2. Recently treated syphilis with nontreponemal titers that have not yet become nonreactive 3. Treated syphilis with persistent titers ^d
Nonreactive	Reactive ^a	1. False-positive nontreponemal test

^aUsually not performed if the initial treponemal test is negative.

^bBy 2 different methods if the nontreponemal test is nonreactive.

^cCommonly seen among African immigrants with previous exposure to endemic treponematoses.

^dSuccessful treatment is usually considered with a fourfold decline in titers (e.g., from 1:32 to 1:8).

Diagnostic tests for syphilis

Direct detection methods

Although these methods are not widely available, there are several tests that can be used to directly detect the organism. These include:

□ ELISA – screening or diagnosis IgG, IgM

□ Dark-field microscopy,

□ PCR,

□ and direct fluorescent antibody testing for *T pallidum*.

□ In some cases, these tests may allow the diagnosis of syphilis prior to a serologic response.

Syphilis Testing

VDRL

- ❑ Must heat serum to 56C for 30 minutes prior to testing to inactivate complement which can cause a false positive.
- ❑ Antigen must be prepared daily.
- ❑ Test read microscopically
- ❑ VDRL can be performed on CSF.

RPR

- ❑ Modified commercially prepared antigen attached to charcoal.
- ❑ Serum does not have to be heated.
- ❑ Plasma can be used.
- ❑ Read macroscopically
- ❑ RPR CANNOT be performed on **Cerebrospinal fluid CSF or cord blood.**

Thank You

