Rapid Plasma Reagin (RPR) Test for the diagnosis of Syphilis

Introduction of Rapid Plasma Reagin (RPR) Test

- Rapid plasma reagin (RPR) is macroscopic, non treponemal, flocculation card test used to screen for syphilis caused by *Treponema pallidum*.
- RPR is simple test can be done within few minutes.
- This test is less sensitive than treponemal test in early syphilis infection.
- Two types of antibodies are produced in syphilis i.e. autoantibody (cardiolipin) response and treponemal antibody response.
- Autoantibodies are produced in 2-3 weeks of treponemal infection due to tissue damage. These auto antibodies are often referred to as cardiolipin antibodies because they can be detected in serological test using cardiolipin antigen.
- This test doesn’t look for antibodies against actual bacterium but rather for antibodies against substances released by cells they are damaged by *Treponema pallidum*.
- The anti-lipodial antibodies are antibodies that are not produce only in syphilis infection but also in other non treponemal disease of an acute and chronic nature in which tissues are damaged.
- RPR measures IgM and IgG antibodies to lipodial materials released from damaged host cells as well as lipoprotein like material and possibly cardiolipin released from treponems.
- Antigen used in RPR test contain cardiolipin lecithin, cholesterol, 10% choline chloride, EDTA, charcoal in buffer.
- This test cannot be performed on CSF. Serum or plasma can be used for testing, serum not heated.
- This test tends to give negative results during late syphilis.

Reagents used for Rapid Plasma Reagin (RPR) Test

**RPR antigen suspension**

RPR antigen suspension is a stabilized combination of 0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 10% choline chloride, 0.0125M EDTA, 0.01875% charcoal, 0.01M Na2HP04, 0.01M KH2P04, 0.1% thimerosal in distilled water.
Control serum samples

Control serum samples are lyophilized reactive (R), minimally reactive (Rm), and nonreactive (N) control serum specimens on a card, or liquid or lyophilized serum samples of graded reactivity. If quantitative tests are to be performed, a control serum that can be titered to at least a 1:4 dilution should be used.

0.9% Saline

Add 0.9 gm of dry sodium chloride (ACS) to 100 ml of distilled water.

Diluent

Prepare a 2% solution of human serum in 0.9% saline, by diluting a human serum nonreactive for syphilis 1:50 in 0.9% saline.

Principle of Rapid Plasma Reagin (RPR) Test

RPR is 18mm circle card test is a macroscopic flocculation test for syphilis. The antigen is prepared from modified VDRL (Venereal Disease Research Laboratory), antigen suspension containing choline chloride and EDTA (ethylenediamine tetraacetic acid) to enhance stability of suspension, finely divided charcoal particles as visualizing agents. In this test antigen is mixed with unheated serum on plastic –coated card. This test measures IgM & IgG antibodies to lipodial material released from damaged host cells as well as possibly cardiolipin released from treponems. If antibodies are present, they combines with lipid particles of the antigen, causing them to agglutinate .The charcoal particles co-agglutinate with antibodies and shows black clumps on white cards. If antibodies are not present, the test mixture is uniformly gray.

Procedure of Rapid Plasma Reagin (RPR) Test

Qualitative Test

- Place 50µl of serum or plasma on 18mm circle of RPR test using a disposable dispenstirs or a safety pipetting device.
- Spread serum or plasma to fill the entire circle. Don’t spread the specimen beyond the confines of the circle.
- Gently shake the antigen dispensing bottles to re-suspend the particle.
- Dispense several drop[s of antigen (17µl of ag) suspension to each circle containing serum or plasma.
- Mix the suspension well in one direction.
- Rotate card for 4-8 mins and observed for flocculation.
Quantitative Test

- Dilute the endpoint titre all serum specimen with rough non-reactive results in qualitative test. Test each specimen undiluted (1:1) and in 1:2, 1:4, 1:8, 1:16 dilution.
- Place 50µl of 0.9% saline in circles. Don’t spread saline.
- Using safety pipette device, place 50µl of serum in circle labeled 1 and 50µl of serum in circle 2. Mix the saline and serum in circles.
- Transfer 50µl from circle 2 (1:2) to circle3, & mix
- Transfer 50µl from circle 3 (1:4) to circle 4 & Mix
- Same way transfer 50µl from circle (1:8) to circle (1:16), mix and discard the last 50µl.
- Spread the serum dilution using clean dispensers to fill entire circle.
- Gently shake the dispensing bottles to re-suspend the antigen particles.
- Add (17µl of ag) antigen suspension in each circle.
- Place the card in rotator for 8 min at 100v 2rpm under humidifying cover.
- Remove card from rotator and tilt the card by hand (three or four to and fro motions) to aid in differentiating non-reactive from minimally reactive results.

Result Interpretation of Rapid Plasma Reagin (RPR) Test
Positive Result (Reactive): Clumping (Characteristic clumping ranging from marked and intense (reactive) to Reactive (R) slight but definite (minimally to moderately) reactive).

Negative Result (Non-Reactive): No Clumping or slight roughness.

Note: A negative RPR Test result does not mean the patient is free of syphilis, particularly if the exposure was recent. It can take up to several weeks for antibodies to reach detection levels in the blood. As a result, repeat RPR testing may be required at a later date.

- RPR test is used as screening test, positive about 70% of primary and 98% of secondary syphilis.
- RPR card test is an aid in diagnosis of syphilis. The RPR card test predictive value in a serological diagnosis of syphilis increased when combined with a reactive treponemal test.
- A reactive RPR card test suggest past or present infections with pathogenic treponems, however, it may also be a false –positive reactions. False –positive result can results from laboratory error as well as serum antibody unrelated to syphilis infection.
- RPR test can be used quantitatively by dilution of patient’s serum in normal saline. The reagin titre reflect the activity of disease. Four fold or greater rise in reagin titres usually occurs during evolution of primary syphilis. Sensitivity 60-70% in primary stage. Sensitivity is 100% and titre of 1:32 or higher is seen in secondary syphilis.
- Reagin test usually turn non-reactive 6-18 months after effective therapy of syphilis depending on the stages of disease at which treatment is given.
- RPR test may be positive in low titre when treatment is started late. In late syphilis (cardiovascular, neurological or gummatous lensions) reagin titres may rise. In some cases of neurosyphilis, reagin test in serum may be non-reactive but positive with CSF.
- Non-reactive RPR card test without clinical evidence of syphilis may suggests no current infection or an effectively treated infection .clinical evidence is seen in early stage of syphilis, in secondary syphilis as result of prozone reaction, and in some late syphilis.
- It doesn’t rule out an incubating syphilis infections.

Limitations of Rapid Plasma Reagin (RPR) Test

- The RPR card test cannot be used in spinal fluids.
- A prozone reaction may be encountered occasionally. In a prozone reaction, complete or partial inhibition of reactivity occurs with undiluted serum (maximum
reactivity is obtained only with diluted serum). The prozone phenomenon may be so pronounced that only a rough reading is produced in the qualitative test by a serum that will be strongly reactive when diluted. All test specimen producing any degree of roughness should be retested by using the qualitative procedure. In addition, a specimen should be tested for the prozone phenomenon when the clinician suspects syphilis, even if qualitative RPR test is non-reactive.

- Biological false positive reaction occurs as cardiolipin antigen mainly in specimen from persons with autoimmune disease (SLE, rheumatic disorder), leprosy, and malaria & in intravenous drug addiction.

- The RPR test may be positive (Reactive) in persons from areas where yaws, pinta or non-venerable syphilis in endemic. Generally, residual titres from these infection will be <1:8.

- RPR test cannot be performed in hot, dust field.

- Non treponemal test titres of persons who have been treated in latent or late stages of syphilis or who have become re-infected do not decreases as rapidly as do those of the persons in the early stages of their first infection.

References

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