Rapid Plasma Reagin (RPR) Test: Principle, Procedure and Interpretations

The rapid plasma reagin (RPR) test is a macroscopic, nontreponemal flocculation card test used to screen for syphilis. **RPR Test card:** The anti lipoidal antibodies are antibodies that are produced not only as a consequence of syphilis and other treponemal diseases, but also in response to nontreponemal diseases of an acute and chronic nature in which tissue damage occurs.

**Antigen used in RPR Test:** RPR Antigen contains cardiolipin, lecithin, cholesterol, 10% choline chloride, EDTA, charcoal etc in Buffer. The antigen is prepared from a modified Venereal Disease Research Laboratory (VDRL) antigen suspension containing choline chloride to eliminate the need to heat inactivate serum, ethylenediaminetetraacetic acid (EDTA) to enhance the stability of the suspension, and finely divided charcoal particles as a visualizing agent. **Positive Control:** Prepared from human serum samples containing antibodies to *Treponema pallidum*. Serum is ready to use. Bring to room temp before use. The test kits can be purchased from many commercial sources.

**Basic Test Procedure:** RPR antigen is mixed with unheated or heated (to inactivate complement) serum or with unheated plasma on a plastic-coated card. If antibodies are present, they combine with the lipid particles of the antigen, causing them to agglutinate. The charcoal particles coagglutinate with the antibodies and show up as black clumps against the white card (macroscopically visible flocculation-type precipitation if the patient’s sera contains reagin). Without some other evidence for the diagnosis of syphilis, even a reactive nontreponemal test does not confirm *T. pallidum* infection and treponemal tests such as Treponema pallidum haemagglutination assay (TPHA) or Fluorescent Treponemal Absorption Test (FTAbs) must be done to confirm the syphilis. If antibodies are not present in the test serum, the test mixture is uniformly gray.

**PREPARATION FOR THE RPR ASSAY**

Bring RPR Antigen Suspension, Positive Control, Negative control and samples to room temperature (20–30°C) before use. All reagents are ready for use as supplied. Gently mix the reagents before use; avoid foaming.

**Qualitative RPR Test Procedure:**

1. Label a RPR card with patient and control information being careful not to interfere with the test areas of the card.
2. Using disposable serum dispensers or droppers, dispense one free-falling drop (0.05 ml) of serum or plasma sample onto a circle on the test card. When using droppers/dispensers, keep it in a vertical position to ensure accurate delivery.
3. Repeat by adding one free-falling drop of positive control and Negative control using a new dispenser for each sample.
4. Spread the sample smoothly across the circle area using the paddle side of the Dispenser as shown by instructor. Take care not to scratch the test area.
5. After mixing the antigen solution by swirling, add one drop of the antigen suspension to each sample / control testing area. Note: hold the antigen container upside down directly over the test area such that the drop falls directly onto the center of the circle. DO NOT STIR OR SPREAD THE ANTIGEN.
6. Place the card on an automatic rotator and cover to maintain humidity. Rotate at 100 ± 5 rpm for 8 minutes (7 minutes 50 seconds to 8 minutes 30 seconds). Following rotation, a brief hand rotation and tilting of the card (3–4 times) should be performed to aid in differentiating nonreactive from minimally reactive results.
7. Immediately read results macroscopically in the “wet” state under a high intensity light source.

**Interpretation of RPR Test**

1. Non-reactive (NR) – smooth suspension, no clumping or slight roughness
2. Reactive (R) – any degree of clumping