

RPR SLIDE TEST

INTENDED USE

BIOLINE RPR Test is a nontreponemal flocculation test intended for detection of reagin (antilipoidal antibodies) in human serum for presumptive serological diagnosis of syphilis when used in conjunction with a treponemal test.

CLINICAL SIGNIFICANCE

Reagins are a group of antibodies against some components of the damage tissues from patients infected by *Treponema pallidum*, the agent which causes the syphilis. This microorganism produces some damage to the liver and heart, releasing some tissue fragments. Immunological patient system reacts producing reagins, antibodies against these fragments.

METHOD AND PRINCIPLE

In this method, carbon-particle cardiolipin antigen detects reagin, a substance present in sera of syphilitic persons and occasionally in sera of persons with other acute or chronic conditions. Reagin is an antibody-like substance produced from the reaction of treponemal microorganisms with body tissue. The detection of reagin in serum when used in conjunction with a treponemal serological test aids in the diagnosis of syphilis. A fourfold decrease in titer following syphilis treatment indicates that treatment has been successful; a fourfold increase indicates either treatment failure or reinfection. In serum containing reagin, flocculation occurs with agglutination of the carbon particles in the RPR Antigen. Black clumps appear against a white background which can be read macroscopically. In contrast, nonreactive specimens appear to have a uniform light-gray color.

REAGENT COMPOSITION

RPR Antigen: 0.003% Cardiolipin, 0.09% Cholesterol, 0.021% Lecithin, 0.0125 M EDTA, 0.01 M Na_2HPO_4 , 0.01 M KH_2PO_4 , 0.01875% Charcoal, 0.1% Thimerosal (preservative), 10.0% Choline Chloride, w/v, Demineralized Water.

WARNINGS AND PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and test materials after use. Carefully read the entire procedure prior to performing any tests.

REAGENT PREPARATION

Reagent and controls (Positive and Negative) are ready to use.

REAGENT STORAGE AND STABILITY

All reagents are stable till expiry date mentioned on the label when stored at 2-8 C away from direct light.

REAGENT DETERIORATION

This product should not be used if (1) the appearance of the reagents has changed, (2) there is evidence of contamination, (3) the expiration date has passed, or (4) there are other signs of deterioration

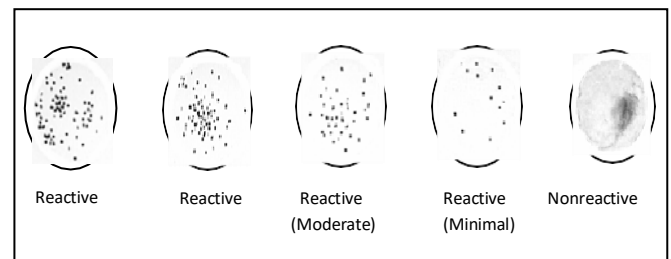
SPECIMEN COLLECTION AND STABILITY

Use serum prepared from whole blood collected without anticoagulant. Allow blood to fully clot before centrifuging. Serum should be clear and separated from cells as soon after collection as possible. Hemolyzed specimens are not acceptable for testing when printed matter cannot be read through them.

TESTING PROCEDURE :-Qualitative Test:

1. Label the test circle on the card with the specimen identification
2. Use a separate pipette tip/stirrer rod for each test specimen or control. Pre-squeeze the dropper and draw up the specimen or control. Dispense 1 free-falling drop (50 μl) into the appropriate well.
3. Gently spread the specimen or control over the entire circle using a circular motion.
4. Gently shake RPR Antigen suspension in the dispensing bottle. Holding the bottle in a vertical position, dispense several drops into the cap to verify the needle passage is clear. Dispense 1 free-falling drop into each well containing specimen or control. Do not stir; mixing of the antigen suspension and the sample is accomplished during rotation.
5. Immediately place the test card on the mechanical rotator, cover with the humidifier cover, and rotate for 6 minutes at 100 rpm. **Note:** False-positive reactions may occur due to evaporation if samples are not properly covered during rotation.
6. Following the 6-minute rotation, briefly rotate and tilt the card back and forth by hand 3-4 times to aid in differentiating nonreactive from minimally reactive results. Immediately read the card macroscopically in the wet state under a high intensity incandescent lamp. Avoid glare when reading reactions.

Reading	Report
Small to large clumps (R) or slight but definite clumps (Rm)	Reactive (R)
No clumping or very slight roughness	Nonreactive (NR)



REPORT AS REACTIVE

Semi quantitative test:

1. Place 50 μl of 0.9 % saline solution in 2, 3, 4 and 5 circles of the card by using micropipette. Do not spread the saline solution.
2. Using micropipette, add 50 μL sample in 1 and 2 circle.
3. Mix sample with saline in 2 circle by drawing the mixture up and down for 5 times in the micropipette. Avoid bubble formation.
4. Aspirate 50 μL from 2 circle and transfer to 3 circle. Repeat the same successively upto 5 circle. Aspirate 50 μL from the 5 circle and discard it.
6. After gently mixing RPR antigen suspension place one drop (15 to 20 μL) by antigen dropper in each diluted sample drop.

7. Mix well and spread out the liquid on entire area of circle by using disposable mixing stick.

8. Rock the slide gently for 6 minutes and observe under good light source for appearance of carbon particle clumping.

9. If the highest dilution tested (1:16) is reactive, continue as follows :

a) Prepare a 1: 50 dilution of non reactive serum in 0.9% saline to be used for making 1:32 and higher dilutions of the specimen to be tested.

b) Prepare 1:16 dilution of the test specimen by adding 0.1 ml of serum to 1.5 ml of 0.9% normal saline. Mix it thoroughly.

c) Place 50 µL of the 1:50 non reactive serum diluent in circles 2 to 5 of an RPR card.

d) Using a safety pipetting device with disposable tip place 50 µL of the 1:16 dilution of the test specimen in circle 1 and 50 µL in circle 2.

e) Using the same pipette and tip, make two fold dilutions.

f) After gently mixing RPR antigen suspension place one drop (15 to 20 µL) by antigen dropper in each diluted sample drop.

g) Mix well and spread out the liquid on entire area of circle by using disposable mixing stick.

h) Rock the slide gently for 6 minutes and observe under good light source for appearance of carbon particle clumping.

i) Use a clean tip for each specimen tested. Prepare higher dilutions if necessary in 1:50 non reactive serum diluent.

10. The end point is the highest dilution showing visible black clumps.

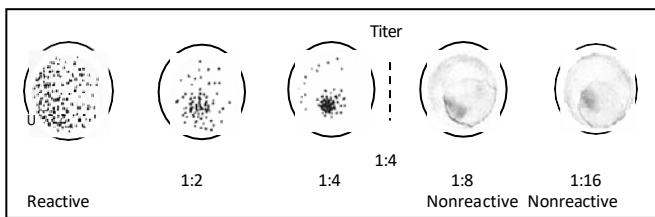
specimens appear to have a uniform gray color.

Results	Report	Interpretation
Reactive	Positive for reagin antibody	A reactive result may indicate past or present infection with <i>Treponema pallidum</i> or may be a false-positive. A fourfold rise in titer on a repeat specimen may indicate infection, reinfection, or treatment failure; a fourfold decrease in titer usually indicates adequate therapy when testing is performed with the same nontreponemal test.
Nonreactive	Negative for reagin antibody	A nonreactive result may indicate no current infection or an effectively treated infection. This may occur in patients with primary, secondary, or late syphilis. Further serodiagnostic testing is recommended if clinical diagnosis of syphilis cannot be excluded or if an incubating syphilis infection is suspected.

QUALITY CONTROL

RPR controls with established patterns of reactivity should be included in each test run. A test run can be defined as a period of approximately 24 hours. Use reactive, minimally reactive, and nonreactive controls and test according to the Qualitative Test procedure.

Each laboratory should establish endpoint titers for the quantitative controls used. If controls do not perform as expected, patient results should not be reported. Quality control testing should be performed according to established laboratory quality control procedures following the guidelines and recommendations of applicable federal, state, and local regulatory agencies.



READING AND INTERPRETATION

Agglutination with Positive Control and no agglutination with Normal Saline validate test results. No agglutination up to one minute is a negative test, and indicates the absence of corresponding antibodies.

Agglutination within **one minute** is a positive test, and indicates presence of corresponding antibodies. Then proceed for semi-quantitative slide or tube technique for determination of antibody titre.

INTERPRETATION OF RESULTS:

Flocculation of RPR Antigen which appears as black clumps against the white background of the card indicates a Reactive (R) specimen. Slight but definite clumping indicates a Minimally Reactive (Rm) specimen. In contrast, Nonreactive (N)