Cromatest

LINEAR

RPR-carbon CE

CONTENTS			
REF	2510010 2510025	RPR-carbon RPR-carbon	100 Tests 500 Tests
For *in vitro* diagnostic use only			

RPR-carbon

Determination of plasma reagins

SLIDE AND MICROPLATE TESTS

PRINCIPLE

The RPR-carbon antigen is a non-treponemal preparation specially developed for the rapid detection and semi-quantitation by coagglutination on a slide or microplate of plasma reagins, a group of antibodies directed against tissue components produced by almost every patient infected with *T. pallidum*. The assay also known as rapid plasma reagin (RPR) is performed by testing the antigen –an association of lipid complexes and particulate carbon- against unknown samples. The presence or absence of a visible agglutination indicates the presence or absence of circulating antibodies in the samples tested[1-4].

REAGENT COMPOSITION

R — **RPR-carbon AntIgen**. Stabilized suspension of 0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 10% choline chloride, 0.0125 mol/L EDTA, 0.01% particulate carbon, in phosphate buffer. Contains 0.95 g/L sodium azide.

CONTROL + — RPR-VDRL. Human serum. Contains 0.95 g/L sodium azide.

CONTROL - — RPR-VDRL-TPHA. Animal serum. Contains 0.95 g/L sodium azide.

Precautions: Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.

PACKAGING CONTENTS

REF 2510010, kit 100 tests. 1x2 mL RPR-carbon Antigen, 1x1 mL Positive control, 1x1 mL Negative control, 1 dispensing needle, 1 dispensing vial, 3 Test cards (Note 5) and 2x50 disposable stirrers.

REF 2510025, kit 500 tests. 2x5 mL RPR-carbon Antigen, 1x1 mL Positive control, 1x1 mL Negative control, 2 dispensing needles, 2 dispensing vials, 50 Test cards (Note 5) and 10x50 disposable stirrers.

STORAGE AND STABILITY

Store at 2-8ºC. Do not freeze. Frozen reagents could change the functionality of the test.
Antigen and Controls are stable until the expiry date stated on the label when stored tightly closed and contaminations are prevented.

Discard If appear signs of deterioration:

- RPR-Carbon: Visible agglutination.
- Controls: Presence of particles and turbidity.

REAGENT PREPARATION

- RPR-Carbon: Resuspend the Antigen gently to obtain a thorough mixing, attach the needle to the dispensing vial and aspirate the required amount of antigen from the glass vial to the plastic dispensing vial.
- Controls: Ready to use.

SAMPLES

Fresh and clear serum or plasma not inactivated, collected by standard procedures. Stable for 2 days at 2-10ºC. Or at –20ºC for longer periods.

MATERIAL REQUIRED

- Automatic pipettes.
- Saline solution 9 g/L, for semi-quantitation procedure.
- Mechanical rotator, adjustable at 100 r.p.m. and circumscribing a circle 2 cm in diameter on a horizontal plane.
- Laboratory alarm clock.
- General laboratory equipment

PROCEDURE

Qualitative Test

1. Bring the reagents and samples to room temperature (Note 1).
2. By means of an automatic pipette place 50 µL of each sample into a separate circle on the card. Use a separate tip for each sample. Dispense 1 drop of each of the two serum controls into two additional circles.
3. Gently shake the dispensing vial and holding the vial in vertical position, slightly press to remove air bubbles from the needle and the drop obtained is correct.
4. Place the needle in a vertical position perpendicular to the card (Note 2). Press gently the dispensing vial and deliver 1 drop of antigen to each circle next to the sample to be tested (Note 3).
5. Mix the contents of each circle with a disposable stirrer and spread over the entire area enclosed by the ring. Use separate applicators for each mixture.
6. Place the card on a mechanical rotator and rotate at 100 r.p.m. for **8 minutes**.
7. Observe macroscopically for agglutination within a minute after removing the card from the rotator.

Reading

Nonreactive Reaction: In a negative result the carbon particles remain in a smooth suspension with no visible aggregates, as shown by Negative control.
Positive Reaction: In a positive result slight but definite (W) to marked and intense visible aggregates (R) are seen (Note 4).

II. Quantitative Test

1. For each specimen to be tested place with an automatic pipette 50 µL of 9 g/L saline solution into each of 5 circles on the reaction card. Do not spread diluent.

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

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2. To circle one add 50 µL of specimen next to the saline solution and, using the same tip, mix the saline solution with the sample by repeated aspiration and expulsion of the fluid. Transfer 50 µL of the mixture to the saline solution in the second circle.
3. Continue with the 2-fold serial dilutions in a similar manner up to the fifth circle, and discard 50 µL from this circle. Final sample dilutions will be: 1:2, 1:4, 1:8, 1:16, 1:32.
4. Test each dilution as described in steps 3-7 for the Qualitative Test.

Reading
Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilution should be negative.
If the highest dilution tested is reactive repeat the test starting with a preliminary 1:16 dilution. Use a 1:50 dilution of Negative control in 9 g/L saline solution as diluent.

II. Qualitative Test in microplate (flat bottom)

1. By means of an automatic pipette place 50 µL of each sample into a separate well on the microplate. Use a separate tip for each sample and discard after use. Dispense 1 drop of each of the two serum controls into two additional wells.
2. Dispense 1 drop of antigen in each well of the microplate that contain the samples to be tested.
3. Place the microplate on a mechanical rotator and rotate at 200 ± 50 r.p.m., during **20 minutes**.
4. Observe macroscopically for agglutination under a high intensity lamp over a white surface, within a minute after removing the microplate from the rotator.

Reading
Same as in Qualitative Test.

QUALITY CONTROL

Positive and negative controls should be run daily following the steps outlined in the Qualitative Test, in order to check the optimal reactivity of the antigen.
Positive control should produce clear agglutination.
Negative Control should not cause any agglutination.
If the expected result is not obtained, do not use the kit.
Each laboratory should establish its own Internal Quality Control and procedures for the corrective action if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE[5]

Syphilis is caused by infection with the bacterium *Treponema pallidum* which can be transmitted congenitally or by sexual contact. The test permits a rapid screening of large numbers of persons so that reactors can be given treatment.
RPR-carbon test has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings. But, all positive samples should be confirmed performing treponemal tests such as TPHA or FTA-ABS.

PERFORMANCE CHARACTERISTICS

- Analytical sensitivity is equivalent to that observed when using a Human Reactive Serum from Center of Disease Control (CDC), Atlanta, GA, USA.
- Diagnostic specificity: 98%.
- Diagnostic sensitivity: 86% (primary syphilis) and 100% (secondary syphilis).
- Results obtained with this reagent did not show significant differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Hemoglobin (<10 g/L), bilirubin (<20 mg/dL) y lipemia (<10 g/L) do not interfere. Rheumatoid factors (>300 IU/mL) interfere. Other substances may interfere[7].

LIMITATIONS OF PROCEDURE

- False negatives may be seen in primary early syphilis and in late syphilis, and also as a result of the prozone reaction. A negative result for a patient strongly suspected of having syphilis, should be tested by semi-quantitative method in order eliminate the possibility of this effect.
- With cardiolipin type antigens, biological false positive reactions have been reported in diseases such as infectious mononucleosis, hepatitis, brucellosis, leprosy, malaria, measles, lupus erythematosus, virus pneumonia and other virus infections. Pregnancy, malignancy, narcotic addiction and autoimmune diseases also may give false positive reactions.
- Do not use on spinal fluid.

NOTES

1. The sensitivity of the test may be reduced at low temperatures. The best results are achieved between 20-25ºC.
2. It is extremely important to maintain the dispensing needle vertically at 90º to the reaction card. If this is not adhered to, it is possible to dispense an insufficient amount of antigen due to splattering resulting from air in the needle.
3. At the end of each day's testing, the needle should be removed, rinsed with distilled water and air dried. Place the needle back in the plastic sleeve.
4. Some samples may show a nonreactive roughness, which tends to be a graininess around the periphery with a homogeneous suspension in the center of the circle. A brief rotating and tilting of the slide by hand can help to differentiate this from minimal types of reaction.
5. Test cards are reusable, and must be washed out and thoroughly rinsed with distilled water free of all detergents.

SOURCES OF ERROR

- Plasma containing excessive concentrations of anticoagulants may yield unreliable results.
- The circles of the test card should never be touched with the fingers since the oil on the fingers may prevent an even spreading of the sample.
- Do not perform the test near heating systems or air conditioners to avoid false positive reactions, high temperature may cause test components to dry on the slide giving an agglutination aspect that can be interpreted as false positive results. It is recommended to place the slide under a humidifying cover.
- Rotator malfunction, excess of sample, cold reagents (antigen, specimen or saline solution), cold room temperature, and outdated antigen may lead to false negative results.
- Reading times longer than specified might cause false positive results due to drying effect.

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