COD 36001 100 tests	COD 36002 500 tests
STORE AT 2-8ºC	
Reagents for measurement of RPR Only for *in vitro* use in the clinical laboratory	

RPR-CARBON

BioSystems

CE

RPR-CARBON
Agglutination

PRINCIPLE OF THE METHOD

Plasma reagins, antibodies directed against antigens derived from nontreponemal sources, aggregate with the antigen and coagglutinate with the carbon particles[1].

CONTENTS

	COD 36001	COD 36002
A. Reagent	1 x 2.2 mL	2 x 5.5 mL
C−. Negative Control	1 x 1 mL	1 x 1 mL
C+. Positive Control	1 x 1 mL	1 x 1 mL
Test Cards	10	50
Disposable stirrer sticks	1 x 100	5 x 100
Dispensing vial	1	2
Dispensing needle	1	2

COMPOSITION

A. Reagent: Stabilized suspension of lipids and carbon, sodium azide 0.95 g/L.

C−. Negative Control: Serum, sodium azide 0.95 g/L.

C+. Positive Control: Serum reactive against nontreponemal antigens, sodium azide 0.95 g/L.

Test Cards (Note 1).

Disposable stirrer sticks

Dispensing vial

Dispensing needle

Human sera used in the preparation of the positive and negative controls have been tested and found to be negative for the presence of antibodies anti-HIV and anti-HCV, as well as for HBs antigen. However, the controls should be handled cautiously as potentially infectious.

STORAGE

Store at 2-8ºC, except for the test cards, stirrer sticks, dispensing vial and dispensing needle, which may be kept at room temperature.

Reagent and Controls are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagent: Visible agglutination in the flask.
- Controls: Presence of particulate material.

REAGENT PREPARATION

A. Reagent. Mix the vial thoroughly until an homogeneous suspension is achieved. Fit the needle onto the dispensing vial and aspirate the required amount of Reagent.

Controls are ready to use.

ADDITIONAL EQUIPMENT

- Mechanical rotator adjustable to 100 r.p.m.

SAMPLES

Serum or plasma collected by standard procedures.

Stable for 2 days at 2-10ºC.

PROCEDURE

1. Bring test reagents and samples to room temperature (Note 2).
2. Place 50 µL of the sample and 1 drop of each Control into separate circles on the test card.
3. Gently shake the dispensing vial containing Reagent (A). Fit the dispensing needle onto the vial neck (Note 3). Invert the whole thing and slightly press to remove the air bubbles held inside the needle.
4. Place the needle in a vertical position on the test card and add one drop of Reagent (A) to each circle next to the sample.
5. Mix with a stirrer stick provided, and spread the mixture all over the surface within the circle. Use a different stirrer stick for each sample.
6. Rotate the test card at 100 r.p.m. for 8 minutes.

READING

Read the presence or absence of agglutination within the first minute after removing the test card from the rotator (Note 4). Results are scored according to the following criteria:

Agglutination	Reading	Result
Medium or large clumps	R (reactive)	Positive
Small clumps	W (weakly reactive)	Weak Positive
No clumps or slight roughness	N (non reactive)	Negative

R (reactive) **W (weakly reactive)** **N (non reactive)**

Positive serum may be tittered with serial two-fold dilutions in 9 g/L NaCl solution. The dilutions to be prepared in test tubes are summarised in the following table.

Titre	1:2	1:4	1:8	1:16	1:32	1:X
Sample volume (µL)	100	…	…	…	…	…
Saline volumen (9 g/L NaCl) (µL)	100	100	100	100	100	100
Previous dilution volume (uL)	…	100	100	100	100	100

Once the dilutions are prepared, add 50 µL of each one into separate circles on the test card and follow the instructions described in PROCEDURE section. The serum titre is defined as the highest dilution showing a positive result.

QUALITY CONTROL

Positive (C+) and Negative (C-) Controls provided should be tested toghether with the patients samples, in order to verify the assay performance.

Positive Control (C+) should cause a clear visible agglutination of the carbon particles.

Negative Control (C-) should not cause any agglutination of the carbon particles.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not meet the acceptable tolerances.

ASSAY CHARACTERISTICS

- Cut-off value: 0.25 IU/mL. Reference Material: WHO International Standard, 1st IS for human syphilitic plasma IgG and IgM, NIBSC code: 05/132.
- Interferences: Hemoglobin (500 mg/dL) and rheumatoid factor (300 IU/mL) do not interfere. Bilirubin (18 mg/dL) and lipemia (triglycerids 650 mg/dL) interfere. Other drugs and substances may interfere[2].
- Trueness: Results obtained with this reagent did not show differences when compared with reference reagents (Syphilis RPR test - HUMAN). Sample size (n)=100; Total agreement of 100 % (96 – 100 % confidence interval 95 %).

DIAGNOSTIC CHARACTERISTICS

The presence or absence of reagins in the sample analyzed aids in the diagnosis of syphilis. A positive result with nontreponemal tests as RPR, should be analyzed by means of treponemal tests as TPHA, prior to infection confirmation. False positives results have been reported in situations like drug addiction, other venereal diseases, pregnancy and postpartum, and autoimmune diseases[3].

False negatives may be seen in primary early syphillis and in late syphilis, and also as a result of the prozone reaction[4].

The sensitivity and specificity of the RPR-CARBON BioSystems kit for the syphilis was 100% in both cases (93 - 100 % confidence interval 95 %). The details of the study are available upon request.

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. The test cards are reusable, and must be washed out and thoroughly rinsed with distilled water free of all detergents.
2. Using fresh samples is recommended. If the assay is not to be performed the same day, store the samples at 2 - 10ºC or –20ºC for longer periods.
3. After each use, wash the needle with distilled water and air dry. Place the needle back in the plastic sleeve.
4. Reaction times longer than specified might cause false positive results due to drying effect.

BIBLIOGRAPHY

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4. Larsen SA, Creighton ET. Rapid plasma reagin (RPR) 18-mm circle card test. En: A manual of tests for syphilis. Larsen SA, Pope V, Johnson RE, Kennedy EJ Jr., eds., 9th edition, American Public Health Association, 1998.