

#### PACKAGING

Ref.: 101-0426	Cont.: 250 tests
Ref.: 101-0220	Cont.: 500 tests
Ref.: 101-0431	Cont.: 250 tests (latex only)

Store at 2 - 8° C.

#### 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.

The assay is useful to follow the antibiotic therapy answer.

#### PRINCIPLE OF THE METHOD

The RPR-carbon is a non-treponemal slide agglutination test for the qualitative and semi-quantitative detection of plasma reagins in human serum. Carbon particles coated with a lipid complex are agglutinated when mixed with samples containing reagins of patient affected by syphilis.

#### REAGENTS

<b>RPR-carbon</b>	Carbon particles coated with a lipid complex, cardiolipin, lecithin and cholesterol in phosphate buffer 20 mmol/L. Preservative. pH, 7.0.
<b>Control + Red cap</b>	Artificial serum with reagin titer $\geq 1/4$ .
<b>Control - Blue cap</b>	Animal serum. Preservative

#### PRECAUTIONS

Control +: H319- Causes serious eye irritation.

Follow the precautionary statements given in MSDS and label of the product.

#### CALIBRATION

The sensitivity is calibrated against the International Reference WHO (1<sup>st</sup> Standard Human Syphilitic Serum, ref. 05/132).

#### PREPARATION

**RPR-carbon:** Homogenize the reagent before use. Place the needle to the plastic dispenser vial, open the RPR-carbon vial and aspirate the required amount of reagent. Once the test is finished, return the reagent to the original vial and rinse the needle and dispenser with distilled water.

#### STORAGE AND STABILITY

All the kit components will remain stable until the expiration date printed on the label, when stored tightly closed at 2 - 8° C and contaminations are prevented during their use. Mix reagents gently before use. Do not freeze: frozen reagents could change the functionality of the test.

**Reagents deterioration:** Presence of particles and turbidity.

#### ADDITIONAL EQUIPMENT

- Mechanical rotator with adjustable speed at 80 - 100 r.p.m.
- Humid store.
- Vortex mixer.
- Pipettes 50  $\mu$ L.

#### SAMPLES

Fresh serum or plasma. Stable 7 days at 2 - 8° C or 3 months at -20° C.

The samples with presence of fibrin should be centrifuged before testing. Do not use highly hemolyzed or lipemic samples.

#### PROCEDURE

##### Notes:

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.

#### Qualitative method

1. Allow the reagents and samples to reach room temperature. The sensitivity of the test may be reduced at low temperatures.

2. Place 50  $\mu$ L of the sample and one drop of each Positive and Negative controls into separate circles on the slide test.
3. Homogenize the reagent before using. Invert the dispenser vial and press lightly to remove air bubbles.
4. Place the dispenser vial together with the needle in a vertical position and perpendicular to the slide, and add one drop (20  $\mu$ L) of reagent together with each of the samples and controls.
5. Mix the drops with a stirrer, spreading them over the entire surface of the circle. Use different stirrers for each sample
6. Place the slide on a mechanical rotator at 80-100 r.p.m. for 8 min (Note 1). False positive results could appear if the test is read later than 8 minutes.

#### Semi-quantitative method

1. Make serial two fold dilutions of the sample in 9 g/L saline solution.
2. Proceed for each dilution as in the qualitative method.

#### Limitations of the procedure

RPR carbon test is non-specific for syphilis. All Reactive samples should be retested with treponemic methods such as TPHA and FTA-Abs to confirm the results.

A Non Reactive result by itself does not exclude a diagnosis of syphilis. Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

False positive results have been reported in diseases such as infectious mononucleosis, viral pneumonia, toxoplasmosis, pregnancy and autoimmune diseases.

#### NOTES

During the 8 minutes of reaction time do not expose the slide to a source of heat or intense light in order to reduce evaporation. Such evaporation could cause a false agglutination and therefore false positive results.

#### READING AND INTERPRETATION

Examine macroscopically the presence or absence of visible agglutination immediately after removing the slide test from the rotator. Rotate the slide twice by hand before reading.

#### Interpretation

Agglutination	Reading	Report
Medium or large clumps	R	Reactive
Small clumps	W	Weakly reactive
No clumping or very slight "roughness"	N	Non Reactive

The titer, in the semi-quantitative method, is defined as the highest dilution showing a positive result.

#### QUALITY CONTROL

Positive and Negative controls are recommended to monitor the performance of procedure, as well as a comparative pattern for a better result interpretation.

All result different from the negative control result, will be considered as a positive.

#### PERFORMANCE CHARACTERISTICS

**Analytical sensitivity:** Accurate titer determination of the Reference Material, under the described assay conditions (see calibration).

**Prozone effect:** No prozone effect was detected up to titers  $\geq 1/128$ .

**Diagnostic sensitivity:** 100%

**Diagnostic specificity:** 100 %.

#### INTERFERENCES

Bilirubin (20 mg/dL), hemoglobin (10 g/L) and lipids (10 g/L), do not interfere. Rheumatoid factors (300 IU/mL), interfere. Other substances may interfere<sup>5</sup>.

#### BIBLIOGRAPHY

1. George P. Schmid. Current Opinion in Infectious Diseases 1994; 7: 34-40.
2. Sandra A Larsen et al. Clinical Microbiology Reviews 1995; 8 (1): 1-21.
3. Sandra Larsen et al. A manual of Test for Syphilis American Public Health Association 1990: 1-192.
4. Joseph Earle Moore et al. Gastrointestinal Haemorrhage 1952; 150(5): 467-473.
5. Young DS. Effects of drugs on clinical laboratory test, 4th ed. AACC Press, 1995.