RPR SYMPHILIS CARD TEST
A qualitative and Semi-quantitative rapid card test for the detection of Non-Treponema (reagin) in serum or plasma

INTENDED USE
For the qualitative and semi-quantitative detection of Non-Treponema in serum or plasma.

INTRODUCTION & PRINCIPLE
Besides other antibodies, Treponema Pallidium produces non-Treponemal antibodies (reagin) in syphilitic persons. These antibodies can be detected by RPR antigen. ATLAS RPR card test is a macroscopic screening test for the qualitative and Semi-quantitative detection of reagin antibodies in serum or plasma. The kit contains RPR antigen which is based on the easy to use VDRL carbon antigens. In the presence of the reagin, the antigen causes flocculation of the carbon particles, which appears as black clumps. The charcoal particles contained in the antigen suspension enhances the visual appearance of the coagglutination in positive samples.

MATERIALS
MATERIALS PROVIDED
- RPR carbon antigen reagent.
- Positive and negative controls.
- RPR test cards.
- Plastic sticks.

NOTE: This package insert is also used for individually packed reagent.

MATERIALS NEEDED BUT NOT PROVIDED
- Saline 0.9%.
- Rotator (100rpm).
- Accurate pipette to deliver 50 µl.
- Timer.

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 hemolized or lipemic samples.

STORAGE AND STABILITY
The reagents in this kit should be stored in an upright position and refrigerated between 2 to 8°C. Never Freeze. Test cards need not to be refrigerated and can be kept at room temperature.

Reagents should be brought to room temperature and mixed well to obtain a uniform suspension of carbon particles.

PREPARING THE SPECIMEN
- ATLAS RPR kit can be used with either unheated plasma or heated serum samples.
- Serum samples can stay stable for up to 5 days if stored at 2 to 8°C.
- Plasma samples collected with EDTA can stay stable up to 24 hours if stored at 2 to 8°C.

PROCEDURES
QUALITATIVE PROCEDURE
1. Mix well the RPR reagent before use.
2. Dispense 50 µl of sample onto a single circle on the test card.
3. Repeat step 2 for the positive and negative controls.

READING THE QUALITATIVE RESULTS
POSITIVE
- If large aggregates appear in the centre or the periphery of the test circle containing the sample, then the test should be read as positive (reactive).
- If the aggregates are visible, but weak or small, then the test should be read as weak positive (weakly reactive).
- If test is positive, then results should be confirmed by the quantitative procedure mentioned below.

NEGATIVE
If no aggregates appear and the specimen has smooth grey appearance (non-reactive)

SEMI-QUANTITATIVE PROCEDURE
- Mix well the RPR reagent before use.

1. Make serial two fold dilutions of the sample in 9 g/l saline solution.
2. Dispense 50 µl of 0.9% saline to test circles numbered 2 to 5. Saline should not be spread. Dispense 50 µl of specimen onto test circle 1.
3. Dispense 50 µl of specimen onto test circle 2. Prepare serial two-fold dilutions by drawing the mixture up and down the pipette 5-6 times avoid any bubble formation. Transfer 50 µl
from circle 2 to 3, to 4 and to 5. Dispose 50 µl from circle 5 after mixing.
4. Starting from circle 5 and onto 4,3,2 and 1, mix and spread the serum over the entire area of each test circle.
5. Continue with steps 6-9 of the qualitative procedure.

**READING THE SEMI-QUANTITATIVE RESULTS**
The dilution of the circles are as follows:

<table>
<thead>
<tr>
<th>Circle</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution</td>
<td>1:2</td>
<td>1:4</td>
<td>1:8</td>
<td>1:16</td>
<td></td>
</tr>
</tbody>
</table>

The titer of the sample is read as follows (P:Positive, N:Negative)

- Positive - P N N N N
- Positive 1:2 P P N N N
- Positive 1:4 P P P N N
- Positive 1:8 P P P N N
- Positive 1:16 P P P P P

Positive and negative results are read as in the reading qualitative results procedure.

If the result in circle 5 is positive, then further dilution to 1:32, 1:64, 1:128 and 1:256 is required. Use steps 3 in semi-quantitative procedure and steps 6-9 in qualitative procedure to obtain the required dilutions.

**Performance Characteristics**
1. Analytical sensitivity: Accurate titer determination of the Reference Material, under the described assay conditions (see calibration).
2. Prozone effect: No prozone effect was detected up to titers ≥1/128.
3. Diagnostic sensitivity: 100 %.
4. Diagnostic specificity: 100 %.

**Limitation**
- RPR carbon test is non-specific for syphilis. All Reactive samples should be retested with treponemic methods such as TPHA andFTA-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.

**References**
2. Larsen S.A., et. al., ata on file, Treponemal Research and Immunology lab, CDC.