RPR SYPHILIS TEST

**INTRODUCTION & PRINCIPLE**

Besides other antibodies, Treponema Pallidium produces non-Treponemal antibodies (reagin) in syphilitic persons. These antibodies can be detected by RPR antigen.

**PREPARING THE SPECIMEN**

SPECTRUM 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.

**MATERIALS PROVIDED**

1- SPECTRUM RPR carbon antigen reagent
2- Positive controls
3- Negative controls
4- RPR tests cards
5- 20 G dispensing needle (16 ul/drop)

**MATERIALS NEEDED BUT NOT PROVIDED**

Saline 0.9 %, rotator (100rpm), accurate pipette to deliver 50ul and timer.

**PRECAUTIONS**

1. The reagents in this kit should be stored in an upright position and refrigerated between 2 to 8. Never Freeze. Test cards need not to be refrigerated and can be kept at room temperature.
2. Reagents should be brought to room temperature and mixed well to obtain a uniform suspension of carbon particles.
3. After use, dispensing Dropper and needle should be washed well with distilled water then air dried.
4. Stability of the antigen may be reduced if stored in the plastic dispensing Dropper for a long time. It is highly recommended to return the antigen to the original glass Dropper at the end of the testing session.
5. Always use a fresh pipette tip for every test.

**QUALITATIVE PROCEDURES**

1. Bring reagents to room temperature.
2. Dispense 50ul of sample onto a single circle on the test card.
3. Repeat step 2 for the positive and negative controls.
4. Spread the sample of each test specimen over the entire test circle.
5. Mix the carbon antigen suspension well.
6. With the needle suck up reagent sufficient to the testing requirements.
7. Dispense one free-fall drop of the carbon antigen onto each test circle containing specimen. Do not mix the antigen with the sample.
8. Using the rotator, rotate the card at 100rpm for 8 minutes.
9. Read the results in good light immediately after 8 minutes.

**READING THE QUALITATIVE RESULTS**

**POSITIVE** - If large aggregates appear in the centre or the periphery of the test circle containing the sample. The aggregates are visible, but weak or small, then the test should be read as WEAK POSITIVE. 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.

**QUANTITATIVE PROCEDURES**

1. Dispense 50ul of 0.9 % saline onto 5 test circles numbered 2 to 5. Saline should not be spread. Dispense 50ul of specimen onto test circle 1.
2. Dispense 50ul 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 50ul from circle 2 to 3, to 4 and to 5. Dispose 50ul from circle 5 after mixing.
3. Starting from circle 5 and onto 4,3,2 and 1, mix and spread the serum over the entire area of each test circle.
4. Continue with steps 6 - 9 of the qualitative procedure.

**READING THE QUANTITATIVE RESULTS**

The dilutions 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 1:2: P P N N N
- Positive 1:4: P P P N N
- Positive 1:8: P P P P 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 quantitative procedure and steps 6-10 in qualitative procedure to obtain the required dilutions.

**PROCEDURE LIMITATION**

1. This test provides a presumptive diagnosis of syphilis. Physicians should evaluate all clinical and laboratory findings before making a definitive diagnosis.
2. In positive specimens, it is recommended to confirm the result by another serological test such as the TPHA.

**REFERENCES**

3. Larsen S.A., et. al., at: on file, Treponemal Research and Immunology lab, CDC.