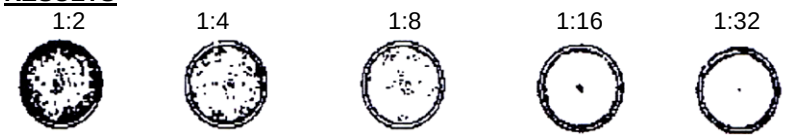
Circles 1 to 5 now represent a dilution series as follows:

| Circle | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Dilution | 1:16 | 1:32 | 1:64 | 1:128 | 1:256 |

d. Proceed with the test procedure described under STEPS 4 and 5 of the Quantitative Card Test.
e. Continue dilutions until an end-point titre is reached.

**RESULTS**




Reactive Reactive Minimal Reactive Negative Negative

**QUALITY CONTROL PROCEDURE**

The reactive, minimal reactive and non-reactive controls have been included with the test kit to monitor the performance of the reagent. If the expected results have not been observed, the reagent should not be used.

**LIMITATIONS OF THE PROCEDURE**

1. The results of this test SHOULD NOT be used as a single diagnostic tool to make a clinical diagnosis. Instead, the test results must be evaluated together with other clinical findings and observed symptoms to aid in the final diagnosis.
2. False positive reactions occur occasionally with the RPR Carbon Antigen Test. Such reactions sometimes occur in drug abuse and in such diseases as lupus erythematosus, mononucleosis, leprosy, viral pneumonia and after smallpox vaccinations.
3. Reactive RPR Test specimens should be subjected to further confirmation tests as recommended in the Manual of tests for Syphilis[2].
4. Temperature of the reagents and specimens is critical to test outcome.
5. Plasma specimens over 48 hours old may give erroneous results.

**PERFORMANCE CHARACTERISTICS**

The PULSE RPR Antigen has been tested according to Centers For Disease Control (CDC) RPR Card Test Procedure. The PULSE RPR Test was compared to the CDC RPR Antigen and to another commercially available RPR Antigen in a clinical evaluation. A total of 205 specimens were used in this study and the overall agreement of the results was 100%. All 109 Negative specimens gave negative results when tested by all 3 types of RPR Antigen. The 96 Positive specimens gave positive results when tested by all 3 types of RPR Antigen. Only 4 out of the 96 Positive specimens differed by 1 dilution difference in titre when quantitative tests were performed. No specific deviation trend has been observed.

| Specimens | Pulse Test | CDC Antigen | Competitor Test |
|---|---|---|---|
| Positive | 96 | 96 | 96 |
| Negative | 109 | 109 | 109 |

**REFERENCES**


1. Hunter, E.F. Deacon, W.E. and Meyer, P.E., An improved FTA Test for Syphilis, The Absorption Procedure (FTA-ABS). Public Health Reports, 79, 410-412, 1964.
2. Manual of Tests for Syphilis, Public Health Service Publication, No. 411, 1969.
3. Manual of Tests for Syphilis, Public Health Service Publication, No. 411, 1990.



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# RPR Screening Test for Syphilis

**INTENDED USE**

The Pulse Rapid Plasma Reagin (RPR) Test is a non-Treponemal Flocculation Test that is used to detect and quantify reagin, an antibody present in serum or plasma from persons with syphilis, or with other treponemal diseases. Occasionally individuals with other diseases or conditions may also be reactive in the non-Treponemal Tests.

**SUMMARY & PRINCIPLES**

*Treponema pallidum*, the etiologic agent responsible for syphilis produces at least two kinds of antibodies in human infections. Treponemal antibodies can be detected by tests such as the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test[1] or MHA-TP whereas the reagin antibody is detected by non-treponemal tests such as the RPR Antigen Card Test[2]. In the presence of the reagin antibody in the reactive sample, the RPR Antigen preparation will produce flocculation consisting of black clumps against the white background of the test card. By contrast, non-reactive samples will yield an even light-grey homogenous suspension.

**MATERIALS SUPPLIED**

**RPR Carbon Antigen:** *Suspension of Carbon containing approximately 0.003% Cardiolipin, 0.020% Lecithin, 0.09% Cholesterol and 0.2g/L Charcoal in buffer and less than 0.1% sodium azide as preservative.

**Reactive Control:** Human serum containing antibodies against *Treponema Pallidium* and less than 0.1% sodium azide as preservative.

**Minimal-Reactive Control:** Human serum containing antibodies against *Treponema Pallidium* and less than 0.1% sodium azide as preservative

**Non-Reactive Control:** Human serum free of antibodies against *Treponema Pallidium* and containing less than 0.1% sodium azide as preservative.

**Antigen Delivery System**: 3ml Dropping bottle and Needle, which will deliver 60 +/- 2 drops/ml.

**Sufficient Test Cards and Disposable Pipettes**.

**Additional Items Required:**

Mechanical rotator set at 100 +/- 2 r.p.m. with humidity cover, timing device, automatic pipettes, test tubes, gloves, and light source.

* Component concentrations may vary to maintain consistent reagent sensitivity.

## STORAGE & STABILITY

When not in use, store reagents and controls at 2-8 degree Celsius. DO NOT FREEZE. Prior to use, allow reagents and controls to warm up to 23-29 degree Celsius. The antigen should be agitated gently to ensure homogeneity before use. Remove only enough antigen from the bottle for the day's testing use. Expiration date is specified on the kit label and on each vial. Biological indication of product instability is evidenced by inappropriate reaction of the carbon antigen reagent with the corresponding reactive, minimal reactive and non-reactive control sera.

## PRECAUTIONS

This product is for In Vitro Diagnostic Use Only. The bottle dispenser should be thoroughly washed and the needle should be rinsed with distilled water and air dried after use. The accuracy of the needle can be checked by the following procedure:

1. Attach needle to a 2 ml syringe.
2. Fill the syringe with antigen and eliminate air bubbles, and count the number of drops delivered in 0.5ml by holding the needle in a vertical position.
3. The needle is considered satisfactory if it delivers 30 +/- 1 drop in 0.5 ml.

Each donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HbsAg and antibody to HIV Virus. Because no known test method can offer complete assurance that hepatitis B virus, HIV Virus, or other infectious agents are absent, all human serum products and patient specimens should be handled in accordance with good laboratory practices. The preservative sodium azide may react with metal plumbing to form explosive metal oxides. In disposal, flush with a large volume of water to prevent metal azide build up.

## SPECIMEN COLLECTION

EDTA Plasma and unheated or heated serum may be used. The test sera and controls should not be heat inactivated. Specimen should be free of bacterial contamination and hemolysis. Fresh, uncontaminated serum samples may be stored at 2-8 degree Celsius up to 5 days prior to testing. Otherwise, the serum samples should be kept frozen. Plasma specimens should be tested within 48 hours[3], after that time the specimen should be discarded.

## PROCEDURE:

NOTE: All specimens, control serum samples and carbon antigen reagent should be at 23-29 degree Celsius before use.

**Qualitative Card Test**

1. The person performing this test should refer to the RESULTS section to become familiar with the expected results before performing test. Otherwise, perform test with the controls supplied to become familiar with the expected results. Dispense 1 drop of EACH control onto separate circles of the test card and follow STEPS 3 to 5 below.
2. Dispense one drop of serum or plasma sample onto a separate circle on the test card with the disposable stirrer pipettes supplied. Use a fresh stirrer pipette for each sample. When using the stirrer pipette, hold it in a vertical position to ensure accurate delivery.
3. Using the flat end of the stirrer pipettes, spread the sample over the entire area of the test circle.
4. Mix the carbon antigen reagent well. Attach needle to the dropping bottle. Squeeze the dropping bottle to release air and draw sufficient reagent into the bottle. Discard the first few drops and then dispense 1 drop (17μL) of the antigen (while holding the bottle in a vertical position) to a test circle containing the sample. DO NOT MIX the sample and the antigen.
5. Place the card on an automatic rotator and place a humidity cover over card. Rotate at 100 r.p.m. for 8 minutes. Following rotation, a brief hand rotation and tilting of the card (3 to 4 times) should be made to aid in differentiating non-reactive from minimally reactive results. Read results macroscopically in the "wet" state under a high intensity incandescent lamp.

## RESULTS

**Qualitative**

**Positive (Reactive) Result:** A reactive result is indicated by the presence of large aggregates in the centre or periphery of the test circle. All specimens showing any degree of reactivity or roughness should be quantitated (follow Quantitative Test Procedure). Roughness is sometimes an indication of a sample with a prozone.

**Minimal Reactive Result:** Minimal reactive samples are indicated by the presence of small or fine aggregates.

**Negative (Non-Reactive) Result:** A non-reactive result will display a smooth grey appearance.

Reactive

Non-Reactive

**Quantitative Card Test**

1. Dispense 1 drop (0.05 ml) of specimen using stirrer pipette onto circle 1.
2. Using an automatic 0.05 ml pipette (or stirrer pipette), dispense 1 drop of 0.9% saline onto circles to be numbered 2 to 5. DO NOT SPREAD.
3. Using an accurate volumetric pipette, dispense 0.05 ml of the test sample onto circle 2. Insert the tip of the pipette into the resulting mixture and mix them by drawing the mixture up and down the pipette approximately 8 times. Avoid any bubble formation and transfer 0.05 ml of the mixed sample to the third circle. Repeat this serial dilution procedure to circle 5 and discard 0.05 ml from the last circle. Circles 1 to 5 now represent a dilution series as follows:

| Circle | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Dilution | 1:1 | 1:2 | 1:4 | 1:8 | 1:16 |

4. Using the flat end of the stirrer pipette, spread the diluted samples over the entire area of the test circles starting at circle no. 5 (highest dilution). Repeat this spreading procedure to circles 4,3,2, and 1.
5. Dispense 1 drop of carbon antigen from the dropping bottle to each circle. DO NOT MIX. Place the card onto the automatic rotator and rotate for 8 minutes.
6. Immediately after 8 minutes of rotation, read results macroscopically in the "wet" state under a high intensity incandescent lamp. The titre of the sample is the reciprocal of the highest dilution to show macroscopic aggregates (see diagram in the RESULTS section).
7. If the sample is positive in the 1:16 dilution, the dilution series should be extended as follows:
   a. Prepare a 1:50 dilution of non-reactive serum in 0.9% saline. This is to be used for making 1:32 and higher dilutions of specimens to be tested. Dispense 0.05 ml of this diluent solution onto circles numbered 2 to 5.
   b. Prepare a 1:16 dilution of test specimen by adding 0.1 ml of serum to 1.5 ml of 0.9% saline. Mix thoroughly. Dispense 0.05 ml of 1:16 dilution of test specimen onto circles 1 and 2.
   c. On circle 2, insert the tip of an automatic 0.05 ml pipette into the resulting mixture (sample and diluent) and mix by drawing the mixture up and down the pipette approximately 8 times. Avoid any bubble formation. Transfer 0.05 ml of the mixed sample to the next circle. Repeat the mixing procedure. Continue this serial dilution to circle no. 5 and discard 0.05 ml from this last circle.