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## RAPID PLASMA REAGIN (RPR) SET

**REF**

Catalog No. S20-714 100 Kit  
Catalog No. S20-715 500 Kit

**IVD**

*For In Vitro Diagnostic Use Only*

### INTENDED USE

Immunospec Rapid Plasma Reagin (RPR) set is a non-treponemal flocculation test that is used for the qualitative and semi-quantitative determination of reagin antibodies in serum or plasma from persons with syphilis.

### SUMMARY AND PRINCIPLE OF THE TEST

*Treponema pallidum*, the etiological agent of syphilis, produces at least two types of antibodies in human infections: treponemal antibodies that can be detected by Fluorescent Treponemal Antibody-Absorption (FTA-ABS) test<sup>1</sup> and non-treponemal antibody (reagin) that can be detected by RPR antigen card test.<sup>2</sup>

The antigen used in the kit is a modification of VDRL antigen, which contains micro-particulate charcoal to enhance the visual difference between a positive and negative result. If a specimen contains reagin, flocculation occurs with a coagulation of carbon particles contained in the antigen suspension, which appears as black clumps. Non-reactive specimens appear as an even light gray homogenous suspension.

### REAGENTS AND MATERIALS PROVIDED

1. *RPR Carbon Antigen Suspension*: 0.003% cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 0.0125M EDTA, 0.01M Na<sub>2</sub>HPO<sub>4</sub>, 0.1% thimerosal, 0.0188% charcoal, and 10% choline chloride.
2. *RPR Reactive Control*: human serum containing 0.1% sodium azide as preservative.
3. *RPR Non-reactive Control*: human serum containing 0.1% sodium azide as preservative. Stabilized liquid control, non-reactive with RPR antigen.
4. *Antigen Delivery System*: 3ml dropping bottle and needle.
5. Sufficient test cards and disposable pipette stirrers.

### MATERIALS REQUIRED BUT NOT PROVIDED

Timer, rotator (100 ± 2 rpm), saline (0.9%), test tubes, serological pipettes

### WARNING AND PRECAUTIONS

The RPR set is for *in vitro* diagnostic use. Normal precautions exercised in handling laboratory reagents should be followed. Reactive and Non-reactive controls used in this kit have been tested negative for HBsAg and HIV, however the material should still be regarded as potentially hazardous and be handled accordingly.

### REAGENT STORAGE AND STABILITY

When not in use, the antigen suspension and controls should always be refrigerated at 2-8°C. Do not store in bright sunlight. **DO NOT FREEZE**. If freezing occurs during shipment, it can be reconstituted once by warming to room temperature. Reagents are stable if unopened and stored at 2-8 °C until the expiration date on the label. Once the antigen has been placed in the dispensing bottle, the reactivity remains satisfactory for approximately three months, or until expiration date on the label whichever occurs first.

### PREPARATION

Prior to opening the bottles of RPR antigen suspension, shake the bottle for 10 - 15 seconds to resuspend the antigen. Attach the needle to the tapes fitting on the empty plastic bottle and withdraw the entire contents of the bottle by collapsing the bottle and using it as suction device. Shake the antigen in the dispensing bottle before each series of droppings. Label the dispensing bottle with the RPR antigen suspension lot number and date placed in bottle. The needle and dispensing bottle should be discarded

when the entire kit is used up. Check the delivery of the needle by placing it firmly on a 1 ml pipette. Fill the pipette with RPR antigen suspension and hold it in a vertical position, count the number delivered in 0.5 ml. The correct number of drops is 30 ± 1 drop.

### REAGENT DETERIORATION

Any sign of microbial contamination warrants discontinuance of use.

### SPECIMEN COLLECTION AND PREPARATION

Specimens, heated or unheated serum, or plasma collected with EDTA as anticoagulant may be used. Avoid hemolysis and bacterial contamination. Lipemia will not interfere with antigen suspension, however, if specimen is so severely lipemic that it obscures the state of the antigen particles, the specimen should not be used.

Fresh, uncontaminated serum samples are reportedly stable for 5 days prior to testing if stored at 2-8°C. Plasma collected with EDTA and stored at 2-8°C should be tested within 48 hours.

### INTERFERING SUBSTANCES

As with all cardiolipin type antigens, biological false positives may result. Diseases such as leprosy, lupus erythromatosus, infectious mononucleosis, malaria vaccina and vital pneumonia can cause these results. Several reports indicate the occurrence of false positives in pregnancy.<sup>3,4</sup> Autoimmune diseases and narcotic addiction also may give false positive reactions.<sup>5</sup>

### PROCEDURE

Note: All specimens, control serum samples, and reagents

should be at room temperature (25-30°C) before use.

### QUALITATIVE CARD TEST

1. Bring the RPR carbon antigen suspension, controls and samples to room temperature.
2. Hold pipette stirrer in vertical position and 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 pipette stirrer for each sample.
3. Use the flat end of the stirrer pipettes and spread the sample over the entire area of the test circle. Dispose of pipette stirrer. Repeat procedure for number of specimens tested.
4. Shake the carbon antigen suspension dispensing bottle prior to use. Hold in vertical position and dispense several drops into dispensing bottle cap to insure needle passage is clear. Place one (1) drop of "free falling" antigen suspension onto test circle containing the sample. DO NOT MIX the sample and the antigen.

- Place test card on an automatic rotator and rotate for 8 minutes at 100 r.p.m.
- Read results macroscopically under a high intensity incandescent lamp or strong daylight.

### QUALITATIVE RESULTS

Only two possible reports can be made with this test: *Reactive* or *Non-reactive*.

- Reactive:** Indicated by large or small aggregates in the center or the periphery of the test circle.
- Non-reactive:** Indicated by a smooth, even light gray appearance with no aggregates visible.

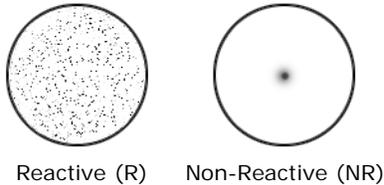


Figure 1.

Reactive results should be confirmed by re-testing the specimens using the quantitative procedure. Confirming serological tests such as TPHA is also recommended. The final diagnosis should be based on a correlation of test results with other clinical findings. (See Limitations).

### SEMI-QUANTITATIVE CARD TEST

- Bring the RPR carbon antigen suspension, controls and samples to room temperature.
- Place 50µl (0.05 ml) of specimen to be tested onto test circle 1.
- For each specimen to be tested, place 50 µl (0.05 ml) of 0.9% saline into test circles numbered 2 to 5. A calibrated capillary or serological pipette, 1ml or less, may be used. **DO NOT SPREAD SALINE.**
- Place 50µl (0.05 ml) of specimen onto test circle 2. Mix the mixture up and down the pipette at least 8 times. Avoid formation of bubbles. Transfer 50µl of mixed sample from circle 2 to 3. Repeat this serial dilution procedure in circle 4, then 5. Dispose 50µl from circle 5 after mixing. 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

- Using a new pipette stirrer (flat end) for each specimen, start at highest dilution of serum (circle 5) and spread serum over entire area of test circle. Proceed to circles 4, 3, 2, and 1.
- Shake the carbon antigen suspension dispensing bottle prior to use. Hold in vertical position and dispense several drops into dispensing bottle cap to insure needle passage is clear. Place one (1) drop of "free falling" antigen suspension onto each test circle containing the sample. **DO NOT MIX** the sample and the antigen.
- Place test card on an automatic rotator and rotate for 8 minutes at 100 RPM.
- Read results macroscopically under a high intensity incandescent lamp or strong daylight.
- If the sample is positive in the 1/16 dilution, the dilution series should be extended as follows:
  - 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 0.05ml of this diluent solution onto circles numbered 2 to 5.
  - Prepare a 1/16 dilution of test specimen by adding 0.1ml of undiluted serum to 1.5ml of 0.9% saline. Mix thoroughly.

Dispense 0.05ml of 1/16 dilution of test specimen onto circles 1 and 2.

- On circle 2, insert the tip of an automatic 0.05ml pipette into the resulting mixture (sample and diluent) and mix by drawing the mixture up and down the pipette approximately 8 times. Avoid bubble formation. Transfer 0.05ml of the mixed sample to the next circle. Repeat the mixing procedure. Continue this serial dilution to circle 5 and discard 0.05ml from this last circle. 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

- Proceed with the test procedure described under steps 5, 6, and 7 under Semi-Quantitative Card Test.
- Continue dilutions until an end-point titer is reached.

### SEMI-QUANTITATIVE RESULTS

Example:

Dilution	1 / 1	1 / 2	1 / 4	1 / 8	1 / 16
Results	R	R	R	RM	NR

R: Reactive      RM: Reactive Minimal      NR: Non-Reactive

The last dilution step that contains macroscopic aggregates indicates the titer of the sample.

Report	Circle 1 1/1 (Und)	Circle 2 1/2	Circle 3 1/4	Circle 4 1/8	Circle 5 1/16
Reactive 1/2	R	R	NR	NR	NR
Reactive 1/8	R	R	R	R	NR
Reactive 1/16	R	R	R	R	R

### QUALITY CONTROL

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

### LIMITATIONS OF THE PROCEDURE

False positive reactions occur occasionally with the RPR Carbon Antigen test. Such reactions sometimes occur in drug abuse and in such diseases as lupus erythematoaua, mononucleosis, leprosy, viral pneumonia and after smallpox vaccinations. Reactive RPR test specimens should be subjected to further confirmation testing. As with any other serological testing procedure, the diagnosis of syphilis should not be made on a single reactive result. Temperature of the reagents and specimens is critical to the test outcome. Plasma specimens over 48 hours old may give erroneous results.

### PERFORMANCE CHARACTERISTICS

One hundred samples were subjected to a comparison between our RPR Test and Stanbio's® Syphilis RPR Test, No. 1170 with the following results:

	<b>Immunospec</b>	<b>Panbio®</b>
Non-reactive	50	50

There was agreement on all samples, representing a 100% agreement.

Fifty of the samples testing reactive to both procedures were compared in the semi-quantitative procedure as described above. The titers ranged from 1/1 to 1/64. There was agreement to the final dilution in 47 cases. In the other three cases, there was agreement within one dilution.

#### REFERENCES

1. Hunter, E.F. et al, An Improved FTA Test for Syphilis, The Absorption Procedure (FTA-ABS). Public Health Reports 79: 410 (1964).
2. Manual of Tests for Syphilis, 1969, PHS Publication No. 411.
3. Walker, An A: Brit. Jr. Ve. Dis., 47:259-262, August (1971).
4. Garner, M.F. & Backhouse, J.L.: Med. Jr. Australia, 1:737-739, April (1973).
5. Kaufman, R.E., Weiss et al, Brit.Jr. Ven. Dis., 50: 350-353, (1974).

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