〝IF〞 RPR (RPR Antigen)Test for Syphilis

IVD

INTENDED USE

The 〝IF〞 RPR (RPR Antigen)Test for Syphilis 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.

SUMMARY & PRINCIPLES

Treponema pallidum, the etiologic agent responsible for syphilis produces two kinds of antibodies in human infections. Treponemal antibodies can be detected by tests such as the Fluorescent Treponemal Antibody-Absorption (FTA-ABS) Test or MHA-TP[1] 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 light-grey homogenous suspension.

COMPONENT

Product number	A0216	A0218	A0225	A0200
UDI-DI	04717294530064	04717294530071	04717294530088	04717294530095
Item	100 T	500 T	2000 T	1000 mL/bottle
RPR reagent	2 mL x1	10 mL x1	10 mL x4	1000 mL x1
RPR reactive control	0.5 mL x1	2 mL x1	2 mL x2	——
RPR Non-reactive control	0.5 mL x1	2 mL x1	2 mL x2	——

MATERIALS SUPPLIED

1. **RPR Carbon Antigen :** Suspension of Carbon antigen containing approximately 0.003% Cardiolipin, 0.020~0.022% Lecithin,0.09% Cholesterol, 0.0125M EDTA, 0.01M Na2HPO4, 0.01M KH2PO4, 0.1% Thimerosal, 0.0188% Charcoal, and 10% choline chloride.
2. **Reactive Control :** Human serum reactive with RPR Carbon Antigen containing less than 0.1% sodium azide as preservative.
3. **Non-Reactive Control :** Human serum non-reactive with RPR Carbon Antigen containing less than 0.1% sodium azide as preservative.
4. Antigen Delivery System : 3mL Dropping bottle and Needle, which will deliver 60 +/- 2 drops/mL.
5. Sufficient Test Cards and Disposable Pipettes.
6. Additional Items Required :
   Mechanical rotator set at 100 +/- 2 r.p.m. with humidity cover, timer, automatic pipettes, test tubes, gloves, and light source.

STORAGE & STABILITY

When not in use, store reagents and controls at 2-8 ℃. **DO NOT FREEZE**. Prior to use, allow reagents and controls to warm up to room temperature. 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.

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 3 mL Dropping bottle.
2. Fill the Dropping bottle 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.

SPECIMEN COLLECTION

EDTA Plasma and serum may be used. Specimen should be free of bacterial contamination and hemolysis. Fresh, uncontaminated serum samples may be stored at 2-8 ℃ for 48 hours prior to testing. If the specimens can not be tested within 48 hours, the specimens should be stored at -20 ℃[3].

⚠ WARNING/NOTES

1. **All reagents of this kit are strictly intended for professional in vitro diagnostic use only.**
2. **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.**
3. **Wear disposable gloves while handling samples and wash hands thoroughly afterwards.**
4. **The preservative sodium azide may react with metal plumbing to form explosive metal azides. In disposal, flush with a large volume of water to prevent metal azide build up.**

PROCEDURE:

NOTE: All specimens, control serum samples and carbon antigen reagent should back to room temperature before using.

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, shaking gently by hands in the enough light will help to interpret the results

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.

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

Reactive

Non-Reactive

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Quantitative Card Test

1. Dispense 1 drop (0.05mL) 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

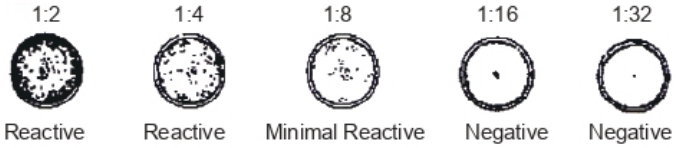
4. Using the flat end of the stirrer pipette, spread the diluted samples over the entire area of the test circles starting at circle no. 5 (highest dilution). Repeat this spreading procedure to circles 4,3,2, and 1.
5. Dispense 1 drop of carbon antigen from the dropping bottle to each circle. **DO NOT 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. 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

Quantitative



SENSITIVITY AND SPECIFICITY

Collect 100 known clinical samples (50 positive and 50 negative) from clinical hospitals and operate qualitative card testing.

Qualitative Results	Known Clinical Samples		Total
	Positive	Negative	
Positive	47	2	49
Negative	3	48	51
Total	50	50	100
Sensitivity	94.0%		
Specificity	96.0%		

INTERFERING SUBSTANCE

The sample contains the following interfering substances, which will not affect the test results below a certain concentration:

Hemoglobin	100 mg/dL
EDTA-K3	20 mg/mL

LIMIT OF DETECTION (LOD) AND PROZONE (HIGH-DOSE HOOK) EFFECT

Commercially available quality control solution (CDC Reactive Control Serum) was used for testing.

	CDC Reactive Control Serum					
IF RPR Antigen	1:1	1:2	1:4	1:8	1:16	1:32
	+	+	+	+	+	-

The end point (cut off) is 1/16 titer with the CDC Reactive Serum Control and no hook effect is observed.

QUALITY CONTROL PROCEDURE

The 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.

REFERENCES

(1) Treponema pallidum surface immunofluorescence assay for serologic diagnosis of syphilis. Clin Diagn Lab Immunol. 2000 May;7(3):417-21.

(2) Manual of Tests for Syphilis, PHS Publication No. 411(1969).

(3) Procedure for the handling and processing of blood spacemen;approved guideline-3rd edition, H18-A3, CLSI.

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