remel

RPR CARD TEST

Cat.No. 16-302, 16-303, 16-304 **TI No. 16302**

INTENDED USE

The REMEL RPR Card Test is a flocculation test intended to detect reagin (antilipid antibodies) in human serum and is indicated for the presumptive serological diagnosis of syphilis, when used in conjunction with a treponemal test.

SUMMARY AND PRINCIPLE

RPR test reagent consists of antigens derived from non-treponemal sources (sources not directly associated with treponemal organisms) used in serological testing to detect reagin, an antibody-like agent, which is produced from the reaction of treponema microorganisms with body tissue.[1] The detection of reagin when used in conjunction with a treponemal serological test aids in the diagnosis of syphillis. In the quantitative test, a fourfold decrease in titer in early syphillis usually indicates adequate syphillis therapy.[1]

REMEL RPR Card Test is a non-treponemal test for the serologic detection of syphilis and is recommended when venous blood collection is employed.[2,3,4,5,6,7,8,9] In this method, carbon-particle cardiolipin antigen detects "reagin", a substance present in serum from syphilitic persons and occasionally in sera of persons with other acute or chronic conditions. In specimens which contain reagin, flocculation occurs with a clumping of the carbon particles in the RPR Card Antigen Suspension, appearing as black clumps against a white background. The clumping can be read macroscopically. In contrast, nonreactive specimens appear to have a uniform light-gray color.

REAGENTS (KIT CONTENTS)

	150 Test Kit	500 Test Kit	5000 Test Kit
RPR Card Antigen Suspension: Each bottle contains 0.003% cardiolipin, 0.09% Cholesterol, 0.021% Lecithin, 0.0125M EDTA, 0.01M Na_2HPO_4, 0.01M KH_2PO_4, 0.01875% charcoal, 0.1% thimerosal (preservative), 10.0% choline chloride, w/v, and demineralized water.	1 x 3 ml	3 x 3 ml	30 x 3 ml
Plastic Dispensing Bottle	1 each	1 each	10 each
20 Gauge, Galvanized Needle, Blunt Cut	1 each	1 each	10 each
White, 10 Well Test Cards	15 each	50 each	500 each
Pipet/Stirrers, 50µl	150 each	500 each	5000 each

PREPARATION OF REAGENTS – Prior to opening a bottle of RPR Card Antigen Suspension, allow it to equilibrate to room temperature (23-29°C), then gently shake the bottle for 10-15 seconds to resuspend the antigen. Attach the needle to the tapered fitting on the empty plastic bottle and withdraw the entire contents of one bottle of RPR Card Antigen Suspension (3 ml) by collapsing the plastic dispensing bottle and using it as a suction device. Shake the RPR Card Antigen Suspension in the dispensing bottle gently before each series of reagent droppings. Label the dispensing bottle with the RPR Card Antigen Suspension lot number, expiration date 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 pipet. Fill the pipet with the antigen suspension and holding it in a vertical position, count the number of drops delivered in 0.5 ml. The needle is considered to be satisfactory if 30 drops ± 1 drop is obtained in 0.5 ml. A needle not meeting this specification should be replaced with another needle that does meet this specification. To maintain clear passage of the needle for accurate drop delivery, upon completion of the daily tests, remove the neecle from dispensing bottle and rinse the needle with distilled or deionized water. Do not wipe the needle since this may affect the accuracy of the drop of antigen being dispensed.

STORAGE INSTRUCTIONS – Store the unopened kit as packaged at 2-8°C, the kit is stable until the expiration date printed on the label. Once opened, store the RPR Card Antigen Suspension at 2-8°C. All other kit components should be stored in a dry place at room temperature. Once placed in the plastic dispensing bottle, the RPR Card Antigen Suspension is stable for 3 months when stored at 2-8°C or until the expiration date, whichever occurs sooner. DO NOT FREEZE or use beyond the expiration date printed on the label.

INDICATIONS OF DETERIORATION – Any sign of microbial contamination warrants discontinuance of use.

SPECIMEN COLLECTION AND HANDLING

SERUM – Collect blood in tubes without anticoagulant. Use clear serum that has been separated from the blood cells as soon after collection as possible.

Hemolyzed specimens are unacceptable for testing when printed matter cannot be read through them.[1]

PRECAUTIONS

1. *For In Vitro Diagnostic Use.*
2. Always handle test cards by grasping the edge of the card. Do not touch surface of test wells with fingers.
3. Verify speed of mechanical rotator (100 ± 2 rpm) to ensure reproducible results.
4. Avoid glare when reading under high intensity lamp.
5. False reactives may occur if specimens are not properly covered during rotation.
6. Treat all test sera as if they are potentially infectious, information on handling human sera is provided in the CDC/NIH manual, *Biosafety in Microbiology and Biochemical Laboratories.*

MATERIALS PROVIDED

RPR Card Antigen Suspension
Galvanized Needle (20 gauge)
Pipet/Stirrers (50ul)
Plastic Dispensing Bottle
10 Well Test Cards

MATERIALS REQUIRED BUT NOT PROVIDED

Mechanical rotor fixed speed, or adjusted to 100 rpm circumscribing 3/4" circle
Hudifier cover, moistened blotter or sponge.
REMEL RPR Liquid Controls (Cat. No. 16-307)
Pipet (1 ml)
High intensity incandescent lamp

For TITRATION (in addition to above):
Human serum non-reactive in tests for syphilis.
Semi-automatic pipettor capable of dispensing 0.05 and 0.10 ml
0.9% Saline (900 mg NaCl/100 ml water)

TEST PROCEDURE 18 mm QUALITATIVE CARD TEST

NOTE: Read the entire procedure prior to performing any tests.

NOTE: Slide flocculation tests for syphilis are affected by room temperature. For reliable and reproducible results, RPR Card Antigen Suspension, Controls, and test specimens should be at room temperature (23–29°C) when tests are performed.

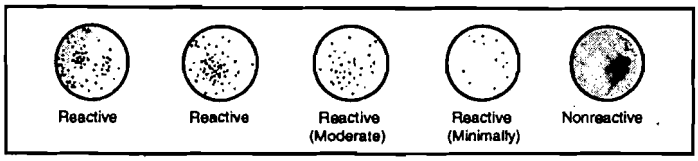
1. Label the test well(s) on the card(s) with the appropriate sample identification.
2. Using an individual Pipet/Stirrer for each test specimen or sample or control, presqueeze and draw up sample. Dispense one (1) free falling drop (50µl) into appropriate well(s).
3. Using the opposite, flattened end of the Pipet/Stirrer used in step 2, gently mix the contents of each well using a circular motion, spreading the sample over the entire area of the well.
4. Gently shake the antigen dispensing bottle before use. Holding in a vertical position, dispense several drops into the cap to make sure the needle passage is clear. Then, dispense one (1) free falling drop into the appropriate well(s). DO NOT STIR – mixing of the antigen suspension and the sample is accomplished during rotation.
5. Immediately place the test card on the mechanical rotor, cover with the humidifier cover, and rotate for eight (8) minutes at 100 rpm.
6. Following rotation, a brief rotating and tilting of the card by hand (3-4 to and fro motions) must be made to aid in differentiating nonreactive from minimally reactive results. Immediately read the card macroscopically in the wet state under a high intensity incandescent lamp.

7. Results:

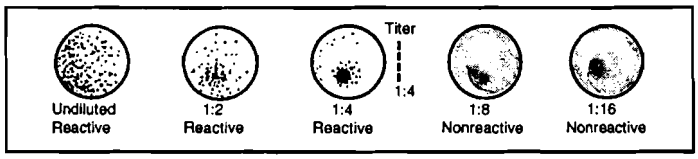
Reading	Report
Small to large clumps	Reactive (R)
No clumping or very slight roughness	Nonreactive (N)

RPR 18 mm CIRCLE READING GUIDE

Screening (Qualitative)
Qualitative Test



Titration (Quantitative)
Quantitative Test



NOTE: Regardless of the degree of reactivity, there are only two possible reports, R or N. Minimal to Moderate Reactivity (slight but definite clumping) is always reported as Reactive (R). ANY SPECIMEN EXHIBITING ANY DEGREE OF CLUMPING SHOULD BE RETESTED USING THE QUANTITATIVE PROCEDURES DESCRIBED IN THIS INSERT.

QUALITY CONTROL

RPR controls with established patterns of reactivity should be included in each day's testing.

If liquid RPR controls are used, use a Reactive, Minimally Reactive and Nonreactive control as described under "Test Procedures". REMEL RPR Liquid Controls can be used. See Technical Information Insert No. 16307.

For quantitative controls, each laboratory should establish endpoint titers for controls used. If controls do not behave as expected, patient results should not be reported. Additional controls may be warranted in accordance with appropriate regulatory and accrediting requirements.

QUANTITATIVE PROCEDURE (USING SEMI-AUTOMATIC PIPETTOR)

IMPORTANT: SEE PRECAUTIONS AND NOTES ABOVE (2 titrations per card).

1. Using a semi-automatic pipettor, add 0.05 ml 0.9% saline to well #'s 2,3,4, and 5. DO NOT SPREAD SALINE.
2. Using the semi-automatic pipet, add 0.05 ml of patient specimen to well #'s 1 and 2.
3. Mix the contents of well #2 by drawing the mixture up and down in the semi-automatic pipettor 5 to 6 times. Avoid the formation of bubbles.
4. Using the semi-automatic pipettor, transfer 0.05 ml from well #2 to well #3. Mix the contents of well #3 as per step 3, then transfer 0.05 ml from well #3 to well #4. Mix contents of well #4 as per step 3. Then, transfer 0.05 ml from well #4 to well #5. Mix contents of well #5 as per step 3. Then, discard 0.05 ml of the contents of well #5.
5. Using a separate Pipet/Stirrer for each well, mix the contents of each well over the entire surface area of the well.
6. Gently shake the antigen dispensing bottle prior to use. Holding in a vertical position, dispense several drops into the cap to make sure the needle passage is clear. Then, dispense one (1) free falling drop into the appropriate well(s). DO NOT STIR – mixing of the antigen suspension and sample is accomplished during rotation.
7. Immediately place the test card on the mechanical rotor, cover with the humidifier cover, and rotate for eight (8) minutes at 100 rpm.
8. Following rotation, a brief rotating and tilting of the card by hand (3-4 to and fro motions) must be made to aid in differentiating non-reactive from minimally reactive results. Immediately read the card macroscopically in the wet state under a high intensity incandescent lamp.
9. Results are reported in terms of the highest dilution giving a reactive or minimal to moderate reaction in accordance with the following example:

FOR EXAMPLE PURPOSE ONLY					
Undiluted **Serum (1:1)**	**1:2**	**1:4**	**1:8**	**1:16**	**Report**
Rm	N	N	N	N	Reactive, 1:1 dilution
R	R	R	N	N	Reactive, 1:4 dilution
R	R	R	Rm	N	Reactive, 1:8 dilution

10. If the highest dilution tested (1:16) is reactive, proceed as follows:
    a. Prepare as diluent a 1:50 dilution of non-reactive serum in 0.9% saline which will be used to prepare a 1:32 and higher dilutions of the specimen to be quantitated.
    b. In a small test tube (12 x 75 mm), prepare a 1:16 dilution of the patient's serum by adding 0.1 ml of serum to 1.5 ml of 0.9% saline. Mix thoroughly.
    c. Using a semi-automatic pipettor, add 0.05 ml of the 1:50 nonreactive serum (from step a) to well #'s 2,3,4, and 5. DO NOT ADD THIS SERUM TO WELL #1.
    d. Using the semi-automatic pipettor, add 0.05 ml of the 1:16 dilution of patient sample (from step b) to well #'s 1 and 2.
    e. Using the semi-automatic pipettor, mix and transfer 0.05 ml aliquots from well #2 to well #3, from well #3 to well #4, and from well #4 to well #5, discarding 0.05 ml from well #5. Refer to steps 3 and 4 above.
    f. Perform Steps 5,6,7, and 8 as outlined above. Report results as per examples in Step 9.

INTERPRETATION OF RESULTS

Flocculation of the RPR Card Antigen Suspension which appears as black clumps against the white background indicates a Reactive (R) specimen. Slight but definite clumping indicates a Minimally Reactive (Rm) specimen. In contrast, Nonreactive (N) specimens appear to have a uniform gray color.

Any specimen exhibiting reactive or rough reactions should be quantitated. Initial reports should only be made on specimens that are nonreactive.

Results	Report	Interpretation
Reactive/titer	Positive for reagin antibody	May indicate past or present infection with *T. pallidum* or a false positive reaction. A 4X rise in titer on a repeat specimen may indicate infection, reinfection or treatment failure; a 4X decrease usually indicates adequate therapy when testing is performed with the same nontreponemal test.
Nonreactive	Negative for reagin antibody	May indicate no current infection or an effectively treated infection. A nonreactive result can be seen in some patients with early primary syphilis, and also secondary and late syphilis. Further serodiagnostic testing is recommended if clinical diagnosis of syphilis cannot be excluded or incubating syphilis infection is suspected.

LIMITATIONS OF PROCEDURE

1. The diagnosis of syphilis should not be made on the basis of a single reactive serologic test without taking historical, clinical, and other laboratory information into consideration. Therefore, all specimens which are reactive in the qualitative (screening) test should be quantitated (titration test) to provide a baseline from which changes can be determined, particularly for evaluating efficiency of treatment.[1]

2. Biologic false positive (BFP) may occur with cardiolipin suspensions due to the presence of substances that react like reagin in the serum of persons having a variety of acute or chronic conditions. Acute BFP may be caused by such conditions as viral and bacterial infections, infectious mononucleosis, pregnancy, and atypical pneumonia.[2,8,9,10,11] The reactivity is usually transient, i.e. less than 6 months in duration, the titer is usually low, e.g. less than 1:8, and tends to drop with time. Chronic BFP may be caused by such conditions as SLE, rheumatoid arthritis, leprosy, malaria, lymphoma, myeloma, and narcotic addiction.[11,12] The titer is usually fixed at a low level; however, it may be high and remain constant.

3. RPR card tests cannot be used to test spinal fluids.[1]

4. A prozone reaction may be encountered occasionally. In a prozone reaction, complete or partial inhibition of reactivity occurs with undiluted serum (maximum reactivity is obtained only with diluted serum). The prozone phenomenon may be so pronounced that only a rough reading is produced in the qualitative test by a serum that will be strongly reactive when diluted. All test specimens producing any degree of roughness or reactivity with the RPR Card Test antigen in the qualitative test should be retested by using the quantitative procedure.[1]

5. Titers of some individuals will not decrease and these individuals may remain serofast retaining a low level reactive titer for life.[1]

6. Persons from areas where yaws, pinta, or nonvenereal syphilis is endemic may have reactive specimens.[1]

7. The predictive value will decrease when prevalence decreases. The predictive value of a reactive RPR Card Test in the serologic diagnosis of syphillis is increased when combined with a reactive treponemal test, such as FTA-ABS or MHA-TP.[1]

EXPECTED VALUES

1. Numerous studies have shown that the RPR test has adequate sensitivity and specificity in relation to clinical diagnosis.[5,6,7,12]

2. Usually high RPR test titers can be seen with concurrent HIV-1 infection.[1]

3. Unusually high false positive titers may be seen in patients with lymphomas. Residual titers from other treponemal infections will be <1:8.

4. Low level reactive titers may persist over a lifetime in individuals who have been treated.

5. This product was tested in a US state public health laboratory and an urban southwestern US general hospital. Reactive specimens tested in this study exhibited titers between 1:1 (undiluted) and 1:1024, two-fold dilutions. Of 1354 specimens tested at the two sites a total of 409 were reactive: 85 were reactive at 1:1, 60 were reactive at 1:2, 38 were reactive at 1:4, 28 were reactive at 1:8, 16 were reactive at 1:16, 16 were reactive at 1:32, 17 were reactive at 1:64, 2 were reactive at 1:128, one was reactive at 1:512, and one was reactive at 1:1024 as determined by the reference method. 145 of the specimens in the study were reactive but not titered to endpoint.

PERFORMANCE CHARACTERISTICS

Seven hundred ten (710) patient samples were tested in blind duplicate using REMEL RPR Card Test versus a commercially available RPR card test. The results are summarized below. An additional 664 different patient samples were tested in blind duplicate using the REMEL RPR Card Test versus a commercially available Unheated Serum Reagin (USR) Test. The results are summarized below. All reactive samples from both studies were tested against a treponemal (micro-hemagglutination test *Treponema pallidum*: MHA-TP) to rule out biological false positives. A good correlation resulted when testing with the RPR and MHA-TP and USR and MHA-TP methods.

RPR Card	NT*–	NT+/T**–	NT+/T+	NT–/T+	NT–/T–
Site #1 (+)	0	2	189	2	0
(–)	476	2	9	0	0
Site #2 (+)	0	35	161	6	1
(–)	436	6	2	3	0

*NT = nontreponemal **T = treponemal
Relative Sensitivity: 97% (95% confidence limits = 95-99%)
Relative Specificity: 99% (95% confidence limits = 98-100%)

Additionally, both sites tested documented syphilis patient samples with the following distribution:

	REMEL RPR		Other RPR	
Treated	**Reactive**	**Nonreactive**	**Reactive**	**Nonreactive**
primary	5	0	5	0
secondary	22	4	23	3
early latent	50	0	49	1
late latent	23	0	23	0
Untreated	15	1	16	0
Total	115	5	116	4

Reproducibility testing was performed with the REMEL RPR Card Test. Six samples were sent to two different sites and were tested once per day for five consecutive days. There was 100% correlation of the results within one doubling dilution for each specimen on each of the five days tested at both sites.

The above studies demonstrate that the REMEL RPR Card Test performs in the same manner as competitive and CDC carbon particle cardiolipin antigen suspensions; and compares with a generally accepted reference treponemal method (MHA-TP).

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