remel

RPR CARD TEST

INTENDED USE

Remel RPR Card Test is a nontreponemal flocculation test intended for detection of reagin (antilipoidal antibodies) in human serum for presumptive serological diagnosis of syphilis when used in conjunction with a treponemal test.

SUMMARY AND PRINCIPLE

RPR Card Test is a nontreponemal test for serological detection of syphilis, recommended for use when venous blood is collected. RPR Antigen consists of antigens derived from sources not directly associated with treponemal microorganisms.[1-3] In this method, carbon-particle cardiolipin antigen detects reagin, a substance present in sera of syphilitic persons and occasionally in sera of persons with other acute or chronic conditions.[4] Reagin is an antibody-like substance produced from the reaction of treponemal microorganisms with body tissue. The detection of reagin in serum when used in conjunction with a treponemal serological test aids in the diagnosis of syphilis.[5,6] A fourfold decrease in titer following syphilis treatment indicates that treatment has been successful; a fourfold increase indicates either treatment failure or reinfection.[7]

In serum containing reagin, flocculation occurs with agglutination of the carbon particles in the RPR Antigen. Black clumps appear against a white background which can be read macroscopically. In contrast, nonreactive specimens appear to have a uniform light-gray color.

PRECAUTIONS

This product is for *In Vitro* diagnostic use and should be used by properly trained individuals. Precautions should be taken against the dangers of microbiological hazards by properly sterilizing specimens, containers, and test materials after use. Carefully read the entire procedure prior to performing any tests.

1. Potential Biohazardous Material: All human serum should be considered potentially infectious and handled accordingly. Refer to the current edition of *Biosafety in Microbiological and Biomedical Laboratories* for information on handling human specimens.[8]
2. RPR Antigen contains thimerosal as a preservative, which may be toxic if ingested.
3. Refer to Material Safety Data Sheet for detailed information on reagent chemicals.

STORAGE

Store product in its original container at 2-8°C until used. Once the kit is opened, store the RPR Antigen at 2-8°C and all other kit components in a dry place at room temperature. Once the RPR Antigen is placed in the plastic dispensing bottle it is stable for 3 months or until the expiration date on the kit, provided it is stored at 2-8°C. Do not freeze or expose to temperature extremes.

PRODUCT DETERIORATION

This product should not be used if (1) the appearance of the reagents has changed, (2) there is evidence of contamination, (3) the expiration date has passed, or (4) there are other signs of deterioration.

SPECIMEN COLLECTION AND HANDLING

Use serum prepared from whole blood collected without anticoagulant. Allow blood to fully clot before centrifuging. Serum should be clear and separated from cells as soon after collection as possible. Hemolyzed specimens are not acceptable for testing when printed matter cannot be read through them.[1]

MATERIALS AND REAGENTS SUPPLIED

Components:	Tests/Kit	
	150	500
RPR Antigen: 0.003% Cardiolipin, 0.09% Cholesterol, 0.021% Lecithin, 0.0125 M EDTA, 0.01 M Na_2HPO_4, 0.01 M KH_2PO_4, 0.01875% Charcoal, 0.1% Thimerosal (preservative), 10.0% Choline Chloride, w/v, Demineralized Water	1 x 3 ml	3 x 3 ml
Plastic Dispensing Bottle	1 each	1 each
20 Gauge, Galvanized Needle, Blunt Cut	1 each	1 each
White, 10 well Test Cards	15 each	50 each
Pipette/Stirrers, 50 µl	150 each	500 each
REF Number	R16302	R16303

MATERIALS REQUIRED BUT NOT SUPPLIED

(1) Mechanical rotator with fixed speed or adjusted to 100 rpm, circumscribing a ¾" circle, (2) Humidifier cover with moistened blotter or sponge, (3) 1 ml pipette, (4) High intensity incandescent lamp, (5) RPR Liquid Controls (REF R16307) or suitable alternative, (6) Calibrated pipette, 100 µl, 50 µl, (7) Test tubes (12 X 75), (8) Normal saline (0.85%), (9) Human serum, nonreactive for syphilis (for quantitative procedure).

PROCEDURE

Preparation of Reagents:

Allow RPR Antigen to equilibrate to room temperature. Gently shake the bottle for 10-15 seconds to resuspend the antigen. Attach the needle (provided) to the tapered fitting on the empty plastic bottle. Withdraw the entire contents of one bottle of RPR Antigen (3 ml) by collapsing the plastic dispensing bottle and using it as a suction device. Label the dispensing bottle with the RPR Antigen lot number, expiration date, and the 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. Hold it in a vertical position and dispense 0.5 ml of suspension while simultaneously counting the drops. A needle delivery rate of 30 drops +/- 1 drop is acceptable. If the needle does not meet this specification it should not be used.

Upon completion of the daily tests, remove the needle from the dispensing bottle and rinse with demineralized water. This will help maintain clear passage of the suspension. Do not wipe the needle as this may affect the accuracy of the antigen drop as it is dispensed.

- Allow RPR Antigen, RPR Liquid Controls, and test specimens to equilibrate to room temperature prior to use.
- The temperature of the room and all test materials, including specimens, must be maintained in the range of 23-29°C. Temperatures below this range will cause false-negative reactions and lower titers, while temperatures above the range have the opposite effect.[7]
- Verify the speed of the mechanical rotator (100 +/- 2 rpm) to ensure reproducible results.
- Handle test cards by grasping the edge of the card; do not touch the surface of the test wells.
- RPR controls with established patterns of reactivity should be included in each test run. RPR Liquid Controls are available under a separate reference number (R16307).

Qualitative Test:

1. Label the test wells on the card with the specimen identification
2. Use a separate pipette/stirrer for each test specimen or control. Pre-squeeze the pipette/stirrer and draw up the specimen or control. Dispense 1 free-falling drop (50 µl) into the appropriate well.
3. Using the opposite, flattened end of the pipette/stirrer gently spread the specimen or control over the entire circle using a circular motion.
4. Gently shake RPR Antigen suspension in the dispensing bottle. Holding the bottle in a vertical position, dispense several drops into the cap to verify the needle passage is clear. Dispense 1 free-falling drop into each well containing specimen or control. Do not stir; mixing of the antigen suspension and the sample is accomplished during rotation.
5. Immediately place the test card on the mechanical rotator, cover with the humidifier cover, and rotate for 8 minutes at 100 rpm. **Note:** False-positive reactions may occur due to evaporation if samples are not properly covered during rotation.
6. Following the 8-minute rotation, briefly rotate and tilt the card back and forth by hand 3-4 x to aid in differentiating nonreactive from minimally reactive results. Immediately read the card macroscopically in the wet state under a high intensity incandescent lamp. Avoid glare when reading reactions.

Reading	Report
Small to large clumps (R) or slight but definite clumps (Rm)	Reactive (R)
No clumping or very slight roughness	Nonreactive (NR)

Note: All specimens with a reactive or rough reaction in the Qualitative Test should be tested according to the Quantitative Test procedure to provide a baseline from which changes can be determined, particularly for evaluating the efficiency of treatment. Initial reports should only be made on specimens that are nonreactive.[1,2]

Quantitative Test (**Note:** Perform only 2 titrations per card):

1. Using a calibrated pipette, dispense 50 µl of 0.85% saline into wells 2, 3, 4, and 5. Do not spread saline.
2. Add 50 µl of patient specimen to wells 1 and 2.
3. Mix the contents of well 2 by aspirating and dispensing the mixture several times, using the same pipette tip. Avoid forming bubbles. Transfer 50 µl from well 2 to well 3.
4. Mix the contents of well 3 by aspirating and dispensing the mixture several times and transfer 50 µl from well 3 to well 4. Repeat in succession through well 5. Discard 50 µl from well 5.
5. Using a separate pipette/stirrer for each well mix the contents, spreading the mixture over the entire well.
6. Gently shake the RPR Antigen suspension in the dispensing bottle. Holding the bottle in a vertical position, dispense several drops into the cap to verify the needle passage is clear. Dispense 1 free-falling drop into each well containing specimen or control. Do not stir; mixing of the antigen suspension and the sample is accomplished during rotation.
7. Immediately place the test card on the mechanical rotator, cover with the humidifier cover, and rotate for 8 minutes at 100 rpm. **Note:** False-positive reactions may occur if samples are not properly covered during rotation.
8. Following the 8-minute rotation, briefly rotate and tilt the card back and forth by hand 3-4 x to aid in differentiating nonreactive from minimally reactive results. Immediately read the card macroscopically in the wet state under a high intensity incandescent lamp. Avoid glare when reading reactions.
9. Results are reported in terms of the highest dilution producing a reactive, moderately reactive, or minimally reactive result in accordance with the following example:

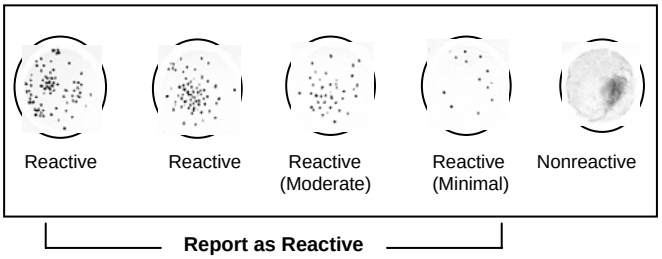
FOR EXAMPLE PURPOSE ONLY					
Undiluted Serum (1:1)	**1:2**	**1:4**	**1:8**	**1:16**	**Report**
Rm*	N	N	N	N	Reactive, 1:1 dilution
R	R	R	N	N	Reactive, 1:4 dilution
R	R	R	Rm	N	Reactive, 1:8 dilution

*Rm = Minimally to moderately reactive

10. If the 1:16 dilution is reactive, prepare further dilutions as follows:
    a. Prepare as diluent a 1:50 dilution of nonreactive serum in 0.85% saline. This will be used to prepare further dilutions of specimens reactive at a dilution of 1:16.
    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 0.85% saline. Mix thoroughly.
    c. Dispense 50 µl of the diluent (prepared in step 10.a.) into wells 2, 3, 4, and 5. Do not add diluent to well 1.
    d. Dispense 50 µl of the 1:16 dilution of patient serum (from step 10.b.) into wells 1 and 2.
    e. Mix the contents of well 2 by aspirating and dispensing the mixture several times using the same pipette tip. Transfer 50 µl from well 2 to well 3.
    f. Repeat in succession through well 5. Discard 50 µl from well 5.
    g. Proceed following Quantitative Test procedure, steps 5. through 8.

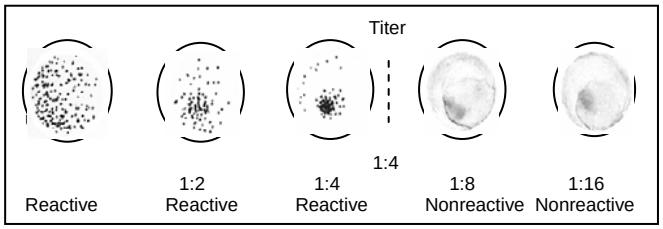
RPR 18 mm CIRCLE READING GUIDE

Qualitative Test (Screening)



Note: Regardless of the degree of reactivity, there are only 2 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 procedure described in this IFU.**

Quantitative Test (Titration)



INTERPRETATION OF RESULTS

Flocculation of RPR Antigen which appears as black clumps against the white background of the card 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.

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

QUALITY CONTROL

RPR controls with established patterns of reactivity should be included in each test run. A test run can be defined as a period of approximately 24 hours. Use reactive, minimally reactive, and nonreactive controls and test according to the Qualitative Test procedure. Remel RPR Liquid Controls can be obtained under a separate reference number (REF R16307).

Each laboratory should establish endpoint titers for the quantitative controls used. If controls do not perform as expected, patient results should not be reported. Quality control testing should be performed according to established laboratory quality control procedures following the guidelines and recommendations of applicable federal, state, and local regulatory agencies.

LIMITATIONS OF PROCEDURE

1. A diagnosis of syphilis should not be based on a single reactive serologic test. All historical information, clinical findings, and laboratory results should be taken into consideration.[7,9]
2. Biologic false-positive (BFP) reactions occurring with nontreponemal tests may be acute or chronic.[10-13] Acute false-positives (titers lasting <6 months) have been associated with hepatitis, infectious mononucleosis, viral pneumonia, chicken pox, measles, malaria, antiviral immunizations, and pregnancy.[7] Chronic false-positives (titers lasting >6 months) have been observed with connective tissue diseases (e.g., systemic lupus erythematosus), narcotic addiction, aging, leprosy, and malignancy. Unusually high false-positive titers may be seen in patients diagnosed with lymphoma.
3. The RPR test cannot be used to test cerebrospinal fluid.[7,9]
4. A prozone reaction may be encountered in specimens with high titers of reagin. Prozone is demonstrated by complete or partial inhibition of reactivity occurring with undiluted serum (maximum reactivity is obtained only with diluted serum) and may be so pronounced that only a rough reading is produced in the qualitative test with strong reactions in the dilutions.
5. The predictive value of RPR Card Test, within a population, decreases when the prevalence of syphilis decreases. When a low-risk population is being tested all reactive results should be confirmed with a treponemal test. The predictive value of RPR Card Test is increased when combined with a reactive treponemal test, such as FTA-ABS or MHA-TP.[5,6]

EXPECTED VALUES

1. Studies have demonstrated the RPR test has adequate sensitivity and specificity in relation to clinical diagnosis.[13,14]
2. High RPR titers may be seen with concurrent HIV-1 infection.[6,9]
3. Individuals in areas where yaws, pinta, or nonvenereal syphilis is endemic may have positive RPR tests.[5,6] Residual titers from these infections will, as a general rule, be <1:8.[9]
4. Some persons with syphilis who have been treated may retain low-level reactive titers for life (i.e., remain serofast).[6,7,9]
5. This product was tested in a U.S. state public health laboratory and an urban general hospital in the southwestern U.S. Reactive sera exhibited titers from 1:1 (undiluted) to 1:1024 (two-fold dilutions). Of 1,354 specimens tested at the two sites a total of 409 were reactive: 85 at 1:1, 60 at 1:2, 38 at 1:4, 28 at 1:8, 16 at 1:16, 16 at 1:32, 17 at 1:64, 2 at 1:128, 1 at 1:512, and 1 at 1:1,024 as determined by the reference method. Of the total, 145 were reactive but not titered to endpoint.

PERFORMANCE CHARACTERISTICS

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

Treated	RPR Card Test		Other RPR test	
	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 RPR Card Test. Six samples were tested at two different sites, 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.

These results demonstrate the performance of RPR Card Test is comparable to other commercial RPR tests, CDC carbon particle cardiolipin antigen suspensions, and a generally accepted reference treponemal method (MHA-TP).

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PACKAGING

REF R16302, RPR Card Test Kit..... 150 Tests/Kit
REF R16303, RPR Card Test Kit..... 500 Tests/Kit
REF R16305, RPR Antigen ..... 3 Vials/Pack
REF R16307, RPR Liquid Controls..... 3 Vials/Pack
REF R16308, RPR 10-Well Test Card..... 300/Pack
REF R16309, RPR Pipette/Stirrer..... 500/Pack
REF R16310, RPR 20 Guage Needle..... 10/Pack
REF R16314, RPR Dispensing Bottle..... 10/Pack

Symbol Legend

REF	Catalog Number
IVD	In Vitro Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use (IFU)
	Temperature Limitation (Storage Temp.)
LOT	Batch Code (Lot Number)
	Use By (Expiration Date)

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