LABORATORY PROCEDURE

MACRO-VUE™
RPR CARD TESTS

18 MM CIRCLE
QUALITATIVE AND QUANTITATIVE
For the Serologic Detection of Syphilis

I. INTENDED USE

The **Macro-Vue**™ RPR (Rapid Plasma Reagin) 18 mm Circle Card Test is a nontreponemal testing procedure for the serologic detection of syphilis.[1, 2]

II. SUMMARY AND EXPLANATION

The **Macro-Vue™** RPR Teardrop Card Test (using finger puncture blood) was the original Card Test and was developed for field use where testing could be performed without laboratory equipment.[3, 4] By incorporating machine rotation, ringed test surfaces, and certain other technical changes, the RPR Circle Card Test was developed for use in large scale testing in public health and clinical laboratories.

The RPR 18 mm Circle Card Test is recommended when venous blood collection is employed and a large volume of serum is available, such as generally prevails in public health and clinical laboratories.[5-12] When a specimen contains antibody, flocculation occurs with a coagglutination of the carbon particles of the RPR Card antigen, which appear as black clumps against the white background of the plastic-coated card. By contrast, nonreactive specimens appear to have an even light-gray color.

In special situations when nontreponemal test results are needed rapidly and the specimen is collected as EDTA plasma, the RPR 18 mm Circle Card Test can be used if the test is performed within 24 hours.[20,21]

III. PRINCIPLES OF THE PROCEDURE

RPR Card antigen suspension is a carbon particle cardiolipin antigen[1] which detects "reagin", an antibody-like substance present in serum or plasma from syphilitic persons, and occasionally in serum or plasma of persons with other acute or chronic conditions. The reagin binds to the test antigen, which consists of cardiolipin-lecithin-coated cholesterol particles, causing macroscopic flocculation.

IV. REAGENTS

The ingredients* of the RPR Card antigen suspension are[1]: 0.003 % cardiolipin, 0.020-0.022% lecithin, 0.09% cholesterol, 0.0125 M EDTA, 0.01 M Na_2HPO_4, 0.01 M KH_2PO_4, 0.1% thimerosal (preservative), 0.02% charcoal (specially prepared, BD Diagnostic Systems), 10% choline chloride, w/v, and deionized/distilled water.

**Adjusted and/or supplemented as required to meet performance criteria.*

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Precautions: for *in vitro* Diagnostic use.

Antigen: Refrigeration is recommended for the RPR Card antigen suspension only. Storage in bright sunlight or temperatures above 30°C (86°F) should be avoided; such conditions may cause a rough appearance of the antigen when used with nonreactive sera. If the ampule of antigen is frozen during shipment, it can be reconstituted once by warming to room temperature; avoid repeated freezing and thawing. Immediate use of a refrigerated antigen may result in decreased sensitivity of the test. Therefore, upon removal from the refrigerator, allow the antigen to warm to room temperature (23 to 29°C) before use.

Do not use antigen beyond the expiration date.

Diagnostic Test Cards: Specially prepared, plastic-coated cards designed for use with the RPR Card antigen. In handling, take care not to finger-mark the card test areas, as this may result in an oily deposit and improper test results. When spreading specimen within confines of test areas, avoid scratching the card with the **Dispenstirs™** device or stirrer. If the specimen does not spread to the outer perimeter of test area, use another test area of card.

Dispenstirs™ and Capillaries: In performing the Card Tests, a **Dispenstirs** device (18 mm Circle qualitative test only) or capillary may be used to transfer the specimen to the card surface. A new **Dispenstirs** device or capillary must be used for each test specimen. When transferring from the collecting tube, the specimen must not be drawn up into the rubber bulb attached to the capillary, as this will cause incorrect readings on subsequent tests.

Needles: To maintain clear passage for accurate drop delivery, upon completion of the tests, remove the needle from the dispensing bottle and rinse the needle with deionized/distilled water. Do not wipe the needle since this will remove the silicone coating and may affect the accuracy of the drop of antigen being dispensed.

Reading of Card Test Results: Read immediately following rotation in the "wet" state under a high intensity incandescent lamp or strong daylight.

Rotation: The recommended speed for mechanical rotation is 100 ± 2 rpm. The rotator should circumscribe a circle approximately two centimeters in diameter in the horizontal plane. A moistened humidifying cover should be used to prevent drying of test specimens during rotation.

Storage of Antigen: Refrigerate at 2 to 8°C. All other components of the kit should be stored in a dry place at room temperature in the original packaging. See “Precautions” for additional information.

Once placed in the *dispensing bottle* (provided in each kit) and refrigerated (2 to 8°C), the antigen reactivity remains satisfactory for approximately three months, or until the expiration date, if it occurs sooner.

Label the dispensing bottle with the antigen lot number, expiration date, and date antigen was placed in the bottle.

V. SPECIMEN COLLECTION

No special preparation of the patient is required prior to specimen collection.

Specimens derived from human blood may be infectious. Caution: Handle as if capable of transmitting disease.

To Test Unheated Serum: Collect blood by venipuncture into a clean, dry tube without anticoagulant and allow to clot. Centrifuge the specimen at a force sufficient to sediment cellular elements. Keep the serum in the original collecting tube or transfer the serum into a clean, dry test tube if testing is to be delayed. Serum, removed from the clot, may be frozen at -20°C or below in a Pyrex (or equivalent) vial or capped test tube.[1]

To Test Heated Serum: After collection and centrifugation, as for unheated serum, transfer to a clean dry tube and place in 56°C water bath, or a heat block for 30 min.

To Test Unheated Plasma: Collect blood by venipuncture into a tube containing anticoagulant such as EDTA, heparin, potassium oxalate, potassium sequestrene or sodium fluoride. EDTA and heparin have the advantage of not being critical with respect to concentration; as little as 1 mL of blood in a tube normally used to collect 7 mL of blood produces satisfactory results. With the other anticoagulants, it is advisable to collect no less than one half a tube of blood. Centrifuge as above. Keep plasma in the original collecting tube, and if stored, store the specimen at 2 to 8°C. Test specimen within 24 h of blood collection.

VI. PROCEDURES

Materials Provided: Various RPR Card Test kits are available (see "Availability") which contain sufficient card antigen suspension to perform the specified number of daily controls and card tests, and the required dispensing bottle, dispensing needle, cards and either capillaries, stirrers, or **Dispenstirs** devices.

Materials Required But Not Provided:

1. Controls with established patterns of graded reactivity should be included in each day's testing to confirm optimal reactivity of the antigen. See "Availability" for **Macro-Vue™** RPR 18 mm Circle Card Test Control Cards or **Macro-Vue™** RPR Card Test Liquid Controls.

2. A rotator, 100 ± 2 rpm, circumscribing a circle 2 cm in diameter, with automatic timer, friction drive, and a cover containing a moistened sponge or blotter. See "Availability" for **Macro-Vue™** Card Test Rotator and humidifying cover.

3. Saline (0.9%) for use in quantitative testing. Prepare by adding 900 mg dry sodium chloride, ACS to 100 mL deionized or distilled water.

4. Serum Nonreactive to syphilis in 0.9% saline; required for diluting test specimens giving a Reactive result at the 1:16 dilution.

Also required is the necessary equipment and glassware used in preparation, storage and handling of serologic specimens.

Preliminary Preparations: Review "Precautions" and "Specimen Collection" prior to performance of card tests. When tests are to be performed, the antigen suspension should be checked with controls of graded reactivity using the particular test procedure. Only those antigens that give the prescribed reaction should be used. Controls, RPR Card antigen suspension and test specimens should be at room temperature (23 to 29°C) when used.

Before use, vigorously shake the ampule for 10 to 15 seconds to resuspend the antigen and disperse any carbon particles lodged in the neck of the ampule. If any carbon should remain in the neck of the ampule after this shaking, no additional effort should be made to dislodge it, as this will only tend to produce a coarse antigen.

Check delivery of the needle by placing the needle firmly on a 1 mL pipet or syringe; fill the pipet or syringe with antigen suspension, and holding the pipet or syringe in a vertical position, count the number of drops delivered in 0.5 mL. The correct number of drops is given in the table, which follows:

Test Method	Color of Needle Hub	Number of Drops in 0.5 mL
18 mm Circle	Yellow, 20 G	30± 1 drop

Attach the needle to the tapered fitting on the dispensing bottle. Be sure the antigen is below the breakline; snap the ampule neck and withdraw all of the antigen into the dispensing bottle by collapsing the bottle and using it as a suction device. Shake the antigen-dispensing bottle gently before each series of antigen droppings.

The needle and dispensing bottle should be discarded when the kit is used up.

It is imperative techniques as described herein be followed in detail.

18 mm Qualitative Card Test Using Dispenstirs™ Devices:

1. Hold a **Dispenstirs** device between thumb and forefinger near the stirring or sealed end. Squeeze and do not release pressure until open end is below surface of specimen, holding

the specimen tube vertically to minimize stirring up of cellular elements when using original blood tube. Release finger pressure to draw up the sample.

2. Holding in a vertical position directly over the card test area to which the specimen is to be delivered (not touching card surface), squeeze **Dispenstirs** device allowing one drop to fall onto card (approx. 0.05 mL; *each **Dispenstirs** device is designed to expel slightly in excess of 0.05 mL to compensate for small amount of specimen retained by stirring end*).

3. Invert **Dispenstirs** device and with sealed stirring end, spread the specimen filling entire surface of circle. (If desired, sample remaining may be discharged into specimen tube from which it was drawn.) Discard **Dispenstirs** device. Repeat procedure for number of specimens to be tested.

4. Gently shake antigen dispensing bottle before use. Holding in a vertical position, dispense several drops in dispensing bottle cap to make sure the needle passage is clear. Place one "free falling" drop (20 G, yellow hub needle) onto each test area. *Do not restir; mixing of antigen and specimen is accomplished during rotation.* Pick up the pre-dropped antigen from bottle cap.

5. Rotate for 8 minutes (±30 sec), under humidifying cover, on mechanical rotator at 100 ± 2 rpm.

Following rotation, to help differentiate Nonreactive from Minimally Reactive results, a brief rotating and tilting of the card by hand (3 or 4 to-and-fro motions) must be made. Immediately read macroscopically in the "wet" state under a high intensity incandescent lamp or strong daylight.

Report as: Reactive - Showing characteristic clumping ranging from slight but definite (minimal-to-moderate) to marked and intense.

Nonreactive - Showing no clumping. See the Reading Guide.

Note: There are only two possible final reports with the Card Test -- Reactive or Nonreactive, regardless of the degree of reactivity. Reactivity minimal-to-moderate (showing slight, but definite clumping) is always reported as Reactive.

Slightly granular or "rough" reactions should be repeated using an alternative procedure. For donor screening, these tests may be reported as "Indeterminate" pending further evaluation. See "Limitations of the Procedure".

All reactive syphilis tests should be repeated using an alternative procedure.

18 mm Qualitative Card Test Using Capillaries:

1. Using a new capillary, attach rubber bulb to capillary and remove 0.05 mL of specimen from blood collecting tube by allowing specimen to rise to measuring line on capillary, taking care not to transfer cellular elements. (If desired, a serologic pipette may be used, but do not pipette by mouth.)

2. Place measured specimen onto circle of diagnostic test card, by compressing rubber bulb, while holding one finger over the hole in the bulb.

3. Using a new stirrer (broad end) for each specimen, spread to fill entire circle. Discard stirrer. Repeat procedure for number of specimens to be tested.

4. Gently shake antigen-dispensing bottle before use. Holding in vertical position, dispense several drops in dispensing bottle cap to make sure the needle passage is clear. Place one "free falling" drop (20 G, yellow hub needle) onto each test area. *Do not restir; mixing of antigen and specimen is accomplished during rotation.* Pick up the pre-dropped antigen from bottle cap.

5. Rotate for 8 minutes (±30 sec), under humidifying cover, on mechanical rotator at 100 ± 2 rpm.

Following rotation, to help differentiate Nonreactive from Minimally Reactive results, a brief rotation and tilting of the card by hand (3 or 4 to-and-fro motions) must be made. Immediately read macroscopically in the "wet" state under a high intensity incandescent lamp or strong daylight.

Report as: Reactive - showing characteristic clumping ranging from slight but definite (minimum-to-moderate) to marked and intense.

Nonreactive - Showing no clumping. See the Reading Guide.

Note: There are only two possible final reports with the Card Test -- Reactive or Nonreactive, regardless of the degree of reactivity. Reactive minimal-to-moderate (showing slight, but definite clumping) is always reported as Reactive.

Slightly granular or "rough" reactions should be repeated using an alternative procedure. For donor screening, these tests may be reported as "Indeterminate" pending further evaluation. See "Limitations of the Procedure".

All reactive syphilis tests should be repeated using an alternative procedure.

18 mm Circle Quantitative Card Test:

1. For each specimen to be tested, place 0.05 mL of 0.9% saline onto circles, numbered 2 to 5. A capillary (red line), or serological pipette, 1 mL or less, may be used. DO NOT SPREAD SALINE!

2. Using a capillary (red line graduated at 0.05 mL, to the tip) with rubber bulb attached, place 0.05 mL of specimen onto circle 1.

3. Refill capillary to red line with test specimen, and holding in a vertical position, prepare serial two-fold dilutions by drawing saline and test specimen mixture up and down capillary 5 to 6 times. Avoid formation of bubbles. Transfer 0.05 mL from circle 2, to 3, to 4, to 5, mixing after each transfer. Discard 0.05 mL after mixing contents in circle 5.

4. Using a new stirrer (broad end) for each specimen, start at highest dilution of serum (circle 5) and spread serum, filling the entire surface of circle. Proceed to circles 4, 3, 2 and 1 and accomplish similar spreading.

5. Gently shake antigen-dispensing bottle before use. Holding in vertical position, dispense several drops in dispensing bottle cap to make sure needle passage is clear. Place one "free falling" drop (20 G, yellow hub needle) onto each test area. *Do not restir; mixing of antigen and specimen is accomplished during rotation*. Pick up the pre-dropped antigen from bottle cap.

6. Rotate for 8 minutes (±30 sec), under humidifying cover, on mechanical rotator at 100 ± 2 rpm.

Following rotation, to help differentiate Nonreactive from Reactive minimal-to-moderate (RM) results, a brief rotating and tilting of the card by hand (3 or 4 to-and-fro motions) must be made. Immediately read macroscopically in the "wet" state under a high intensity incandescent lamp or strong daylight.

Report in terms of the highest dilution giving a Reactive including minimal-to-moderate reaction.

Unheated or Heated Serum: If the highest tested (1:16) is Reactive, proceed as follows:

1. Prepare a 1:50 dilution of Nonreactive serum in 0.9% saline. (This is to be used for making 1:32 and higher dilutions of specimens to be quantitated.)

2. Prepare a 1:16 dilution of the test specimen by adding 0.1 mL of serum to 1.5 mL of 0.9% saline. Mix thoroughly.

3. Place 0.05 ml of 1:50 Nonreactive serum in circles 2, 3, 4 and 5.

4. Using capillary, place 0.05 mL of 1:16 dilution of test specimen in circle 1.

5. Refill capillary, to red line, make serial two-fold dilutions and complete tests as described under steps 3 to 6. (See “18 mm Circle Quantitative Card Test”.)

Higher dilutions are prepared if necessary in 1:50 Nonreactive serum.

Plasma: If a baseline is to be established from which changes in titer can be determined, the test should be repeated on unheated serum (see Section "Unheated Serum").

Reading and Reporting the Macro-Vue RPR Card Tests

Individual reactions should be evaluated in the "wet" state, under a high intensity incandescent lamp or strong daylight. Immediately following rotation read and record as Reactive or Nonreactive.

VII. LIMITATIONS OF THE PROCEDURE

The diagnosis of syphilis should not be made on a single reactive result without the support of a positive history or clinical evidence. Therefore, as with any serological testing procedure, Reactive card test specimens should be subjected to further serologic study. Serum specimens which are Reactive in qualitative testing should be quantitated to establish a baseline from which changes in titer can be determined, particularly for evaluating treatment.[1] The use of plasma specimens to establish a baseline from which changes in titer can be determined has not been evaluated.

False-negative results can occur because of failure to recognize prozone reactions. Prozone reactions occur in 1% to 2% of patients with secondary syphilis. These specimens may exhibit a nonreactive pattern that is slightly granular or “rough”. Upon dilution, the reactivity will increase and then decrease as the endpoint titer is approached. All tests with a rough appearance should be further evaluated. False-negative nontreponemal test results are also seen in incubating primary and late syphilis.[1]

It is not necessary to perform the quantitative procedure on reactive donor samples.

The RPR Card Tests cannot be used for testing spinal fluids.

The ideal specimen for neonatal testing is the infant’s serum as obtained by heel stick procedure. However, cord blood may be used for baseline screening when no other specimen is available.[1]

With cardiolipin type antigens, biological false positive reactions have been reported in diseases such as infectious mononucleosis, leprosy[2] and malaria, lupus erythematosus, vaccinia and virus pneumonia. In leprosy, Portnoy[3] reported no false positives; Achimastos[13] reported 14 of 50 leprosy cases were Reactive and Scotti[14] reported 1 out of 208 cases was reactive with RPR Card which were nonreactive with the FTA-ABS and TPl tests. Dorwart[15] studied the incidence of chronic BFP reactions in various connective tissue disorders. Six out of 41 cases of systemic lupus erythematosus were reactive in the Card Test, whereas only 5 were reactive in the VDRL slide test. Only 1 out of 23 cases of rheumatoid arthritis was reactive with both RPR Card and VDRL slide tests. In pregnancy, several reports indicated the occurrence of false positive reactions.[11,16] Narcotic addiction and autoimmune diseases also may give false positive reactions.[17] Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test.[1]

Lipemia will not interfere with the card tests, however, if the degree of lipemia is so severe as to obscure the state of the antigen particles, the specimen should be considered unsatisfactory for testing.

Do not test specimens that are grossly hemolyzed, contaminated or extremely turbid; report as "Specimen unsatisfactory for testing."[1]

VIII. EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS

RPR Card antigen suspension is tested for the established pattern of reactivity against reference antigen suspensions and meets the U. S. Centers for Disease Control and Prevention (CDC) product specifications for performing the RPR 18 mm Circle Card Tests. These performance characteristics were established from a large number of papers which have appeared in the scientific literature, from routine daily test performances in syphilis serology testing laboratories and are in conformity with CDC specifications.

Reported studies show the RPR Card Tests have adequate sensitivity and specificity in relation to clinical diagnosis and a reactivity level similar to that of the VDRL slide test.[6-10,18,19]

Heating of serum specimens at 56°C for 30 minutes has been shown to have no effect on reactivity.[20]

A qualitative comparison of 1104 simultaneously collected serum and EDTA plasma specimens was conducted using the **Macro-Vue™** RPR 18 mm Circle Card Test. There was complete agreement in test results which included 134 reactive and 970 nonreactive pairs. In other studies comparable results were found between plasma and serum pairs (306 specimens) with RPR Card Tests both in qualitative and quantitative procedures.[21,22]

IX. AVAILABILITY

Macro-Vue™ RPR Card Tests

Kit No. 104: (300 qualitative tests), contains: two 3 mL ampules antigen, 20 G needle, dispensing bottle, 350 stirrers, 30 cards with ten 18 mm Circle spots ea. and 300-0.05 mL capillaries, Cat. No. 274449.

Kit No. 110: (500 qualitative tests), contains: three 3 mL ampules antigen, 20 G needle, dispensing bottle, 50 cards with ten 18 mm Circle spots ea. and 500-0.05 m **Dispenstirs®** devices**,** Cat. No. 275005.

Kit No. 112: (150 quantitative tests), contains: five 3 mL ampules antigen, 20 G needle, dispensing bottle, 200 stirrers, 50 cards with fifteen 18 mm Circle spots ea. and 150-0.05 mL capillaries, Cat. No. 275239.

Kit No. 115: (150 qualitative tests), contains: one 3 mL ampule antigen, 20 G needle, dispensing bottle, 15 cards with ten 18 mm Circle spots ea. and 150-0.05 mL **Dispenstirs**® devices, Cat. No. 275539.

Bulk Kit No. 510: (5000 qualitative tests), Cat. No. 275110.

Bulk Kit No. 532: (10,000 qualitative tests), Cat. No. 275692.

Macro-Vue™ RPR Card Test Control Cards containing graded reactivity specimens, (R, RM & NR 18 mm circles), Box of 10, Cat. No. 276709.

Macro-Vue™ RPR Card Test Liquid Controls, containing graded reactivity specimens, (Control ++, Control +, and Control -), Carton of 3 vials, Cat. No. 276909.

Macro-Vue™ Card Test Rotator (with humidifying cover), 100 ± 2 rpm, with automatic timer, friction drive, Model 51-II (110V), Cat. No. 278051 and Model 54 (220V), Cat. No. 278054 (or equivalent).

Macro-Vue™ Card Test Rotator Accessories Package, containing one 15" x 7" extension top and two humidifying covers, Cat. No. 277979.

Dispenstirs™ (single use, plastic pipettes), 0.05 ml, Box of 500, Cat. No. 272905.

X. REFERENCES

1. Larsen, S.A., E.F. Hunter, and S.J. Kraus (ed.). 1990. A manual of tests for syphilis, 8th ed. American Public Health Association, Washington, D.C.
2. Larsen, S.A., V. Pope, and T.J. Quan. 1992 Immunologic methods for the diagnosis of spirochetal diseases, p. 467-4481. *In* N.R. Rose, E.C. de Macario, J.L. Fahey, H. Friedman, and G.M. Penn (ed.), Manual of clinical laboratory immunology, 4th ed. American Society for Microbiology, Washington, D.C.
3. Portnoy, J., Brewer, J.H. and Harris, A.: Public Health Rep., **77**:645-642.
4. Portnoy, J. 1963. Military Med. **128**:414-147
5. Portnoy, J. 1965. Public Health Lab. **23**:43.
6. Reed, E.L. 1965. Public Health Lab. **23**:96-103.
7. Reed, E.L. 1966. Public Health Lab. **24**:203-206.
8. Reed, E.L. 1968. Public Health Lab. **26**:123-133.
9. Reed, E.L. October 22, 1968. Presented at FTA-ABS Test Seminar, co-sponsored by Md. State Dept. of Public Health, Bureau of Laboratories, and the NCDC, VDRL.
10. Reed, E.L. 1969. J. Conf. Public Health Lab. Dir. **27**:8-14.
11. Walker, A.N. 1971. Br. J. Vener. Dis. **47**:259-262.
12. Croix, J.C. 1975. Feuillets de Biologie. **16**:61-65.
13. Archimastos, A., G. Tolis, G., Papadopoulos, and K. Kouzoutzakoglou. 1970. Public Health Rep. **85**:66-68.
14. Scotti, A.T., D.M. Mackey, and J.R Trautman. 1970. Arch.Dermatol. **101**:328-330.
15. Dorwart, B.B., and A.R. Myers. 1974. Br. J. Vener. Dis. **50**:435-436.
16. Garner, M.F. and J.L. Backhouse. 1973. Med. J. Aust. **1**:737-739.
17. Kaufman, R.E., S. Weiss, J.D. Moore, V. Falcone, and P.J. Weisner. 1974. Br. J. Vener. Dis. **50**:350-353.
18. Falcone, V.H., G.W. Stout, and M.B. Moore, Jr. 1964. Public Health Rep. **79**:491-495.
19. Portnoy, J. 1963. Am. J. Clin. Pathol. **40**:473-479.

20. Larsen, S.A., B.T. Craig, M.E. Shepherd, and B. McLaurin. 1988. Data on file. Treponema Research Branch, CDC.
21. Warner, G.S., et al. 1980. Data on file. BD Diagnostic Systems.
22. Larsen, S.A., D.E. Pettit, M.W. Perryman, E.A. Hambie, R. Mullally, and W. Whittington. 1983. J. Clin. Microbiol. **17**:341-345.

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