KETTERING Health Network

Macro-Vue RPR Card Test Procedure

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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.

PRINCIPLES OF THE PROCEDURE:

RPR Card antigen suspension is a carbon particle cardiolipin antigen1 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.

REAGENTS:

- **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.

Control Cards: The RPR 18 mm Circle Card Test Control Card consists of three circles designated as Reactive, Reactive minimal-to-moderate and Nonreactive. The labeled areas on this card contain graded reactivity specimens. The specimen material is human plasma purchased from licensed blood banks as Reactive and Nonreactive for syphilis.

RPR Card Liquid Controls (For Quantitative Test): Contains pooled human serum with 0.1% sodium azide as a preservative.

Control ++ 1.5mL Macro-Vue RPR Card Test Reactive Control Serum Positive.
Control + 1.5mL Macro-Vue RPR card Test Moderately Reactive Control Serum Weakly Positive.
Control – 1.5mL Macro-Vue RPR Card Test Negative Control Serum Negative Control Serum.

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 **3 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.

SPECIMEN COLLECTION:

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.

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.

CALIBRATION:

Not applicable

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.
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.
3. Saline (0.9%) for use in quantitative testing.
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.

5. A micropipettor which dispenses 50 uL

Quality Control:

- A RPR Control Card containing a Reactive, Reactive minimal to moderate and Nonreactive circles must be run each day of patient testing.
- The Reactive control should show characteristic strong clumping. The Nonreactive should show the smooth grayish appearance of unclamped particles. The Reactive minimal to moderate control should show minimal to moderate clumping.
- If these results are not obtained, the reagents may have been damaged and the kit should not be used. Control failures are documented in the departmental “Failure Log” and a new kit is opened. No patient results can be reported until the control issues have been resolved.

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 saline, 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 30 $\pm$ 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. A syringe may also be used to transfer the antigen from the vial to the dispensing bottle.
    - ❖ ***Note: 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.***

Directions for Use of 18mm Circle Control Card:

1. Place 50 uL of deionized/distilled water onto the 3 circles, using a MLA pipette. Do not use Dispenstirs device to reconstitute a Control Card.
2. Using the stirring end of a Dipenstirs device mix until the dehydrated control specimen is dissolved. Spread specimen to the inside of the circle. A new Dispenstirs device must be used for each circle.
3. Shake the antigen bottle before use. Holding in a vertical position, dispense 1 or 2 drops in the cap to make sure needle passage is clear. Place one “free-falling drop of antigen onto each test area. Do not re-stir, mixing of antigen and specimen is accomplished during rotation. Pick up pre-dropped antigen from the bottle cap.
4. Rotate for 8 minutes (±30 sec), under humidifying cover, on mechanical rotator at 100 ± 2 rpm.
5. 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.

18 mm Qualitative Card Test Using Dispenstirs™ Devices or Pipette:

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. A 50 uL pipette may also be used.

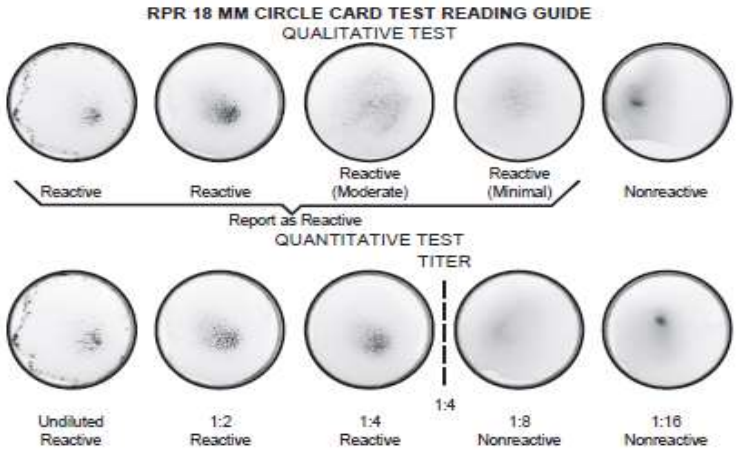
2. Holding in a vertical position directly over the card test area to which the specimen is to be delivered (not touching card surface), squeeze Dispenstirs device allowing one drop to fall onto card (approx. 50 uL; *each Dispenstirs device is designed to expel slightly in excess of 50uL to compensate for small amount of specimen retained by stirring end*). If using pipette dispense 50 uL onto card.

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.

6. 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.*
- Specimens that are NON-REACTIVE will automatically have a reflex test order that will be sent to a reference lab.
- Specimens that are reactive using the RPR Qualitative Card are tittered with serial dilutions.

18 mm Circle Quantitative Card Test:

1. For each specimen to be tested, place 50 uL of 0.9% saline onto circles, numbered 2 to 5. **DO NOT SPREAD SALINE**.
2. Using a 50 uL pipette place specimen onto circle 1.
3. Refill pipette with test specimen, and holding in a vertical position, prepare serial two-fold dilutions by drawing saline and test specimen mixture up and down pipette 5 to 6 times. Avoid formation of bubbles. Transfer 50 uL from circle 2, to 3, to 4, to 5, mixing after each transfer. Discard 50 uL 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.
7. 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.
8. Report in terms of the highest dilution giving a Reactive including minimal-to-moderate reaction.

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 50 uL of 1:50 Nonreactive serum in circles 2, 3, 4 and 5.
4. Using pipette place 50 uL of 1:16 dilution of test specimen in circle 1.
5. Refill pipette, make serial two-fold dilutions and complete tests as described under steps 3 to 8 above.

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

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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.
- 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.
- The RPR Card Tests cannot be used for testing spinal fluids.
- With cardiolipin type antigens, biological false positive reactions have been reported in diseases such as infectious mononucleosis, leprosy2 and malaria, lupus erythematosus, vaccinia and virus pneumonia. In leprosy, Portnoy3 reported no false positives; Achimastos13 reported 14 of 50 leprosy cases were Reactive and Scotti14 reported 1 out of 208 cases was reactive with RPR Card which were nonreactive with the FTA-ABS and TPl tests. Dorwart15 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.

- 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.

EXPECTED VALUES:

Qualitative test:

Valid checking: Non-Reactive, Weak Reactive, Reactive

Reference Range: Non-Reactive

Quantitative test:

Valid checking: 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256, >1:256

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