

## LABORATORY PROCEDURE

### MACRO-VUE™ RPR CARD TEST CONTROL CARDS

#### I. INTENDED USE

The **Macro-Vue**™ RPR Card Test Control Cards are dehydrated control specimens of predetermined reactivity for quality-control testing of antigen before use in performing the **Macro-Vue** RPR Card Tests for the serologic detection of syphilis.

#### II. SUMMARY AND EXPLANATION

**Macro-Vue** RPR Card Test Control Cards were designed for routine, daily use as check-test specimens to determine the acceptable reactivity of RPR Card antigen suspension. RPR Card antigen is a cardiolipin type antigen, which detects “reagin,” an antibody-like substance present in serums from syphilitic persons, and occasionally in serums of persons with other acute and chronic conditions.<sup>1-4</sup>

#### III. PRINCIPLES OF THE PROCEDURE

The RPR 18 mm Circle Control Cards have labeled test areas containing dehydrated specimens with designated patterns of graded reactivity. Upon rehydration and proper execution of the Control Card procedure, the RPR Card antigen suspension should reproduce the established reactivity pattern (see “Procedure”) to confirm optimal reactivity of the antigen.

#### IV. REAGENTS

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 RPR Teardrop Control Card consists of two “teardrops” designated as: Reactive and Nonreactive. The specimen material is human plasma purchased from licensed blood banks as Reactive or Nonreactive for syphilis.

**Precautions:** For *in vitro* Diagnostic.

Before proceeding, review “Procedure” and “Precautions” in the package insert supplied in all **Macro-Vue** RPR Card Test Kits.

The plasma used on the control cards is derived from human blood tested by an FDA (U.S. Food and Drug Administration)-approved method for the presence of the antibody to HIV (human immunodeficiency virus) and HBsAg (hepatitis B surface antigen) and found to be nonreactive.

**WARNING:** Because no test method can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, SPECIMENS AND THESE REAGENTS SHOULD BE HANDLED AS THOUGH CAPABLE OF TRANSMITTING AN INFECTIOUS DISEASE. The FDA recommends such material be handled at a Biosafety Level 2. BSL2 is referenced in the Centers for Disease Control and Prevention/National Institutes of Health (CDC/NIH) manual, *Biosafety in Microbiological and Biomedical Laboratories*.

Controls and RPR Card antigen suspension should be at room temperature (23 to 29°C) when used.

If the established reactivity pattern is not obtained, delay routine testing until the optimal reactivity is obtained by rechecking antigen suspension, test procedures, room temperature, and equipment (including use of a rotator with humidifying cover). Non-acceptable results invalidate tests on individual specimens.

Once the dehydrated specimens on the control card are reconstituted, the specimen solutions should be tested immediately using the RPR Card Test procedure. Delay in testing may allow evaporation to affect the reactivity pattern.

Prolonged storage at cool room temperatures (21 to 25°C) and at elevated temperatures (above 27°C) causes deterioration of the specimens, showing a decrease in reactivity and difficulty in reconstituting the card. Once the sealed envelope is opened, the card should be used immediately.

**Reconstitution of Control Cards:** Each test area of the control card should be reconstituted with the following specified amount of *deionized/distilled water*, depending on the particular control card being used (see “Procedure”):

RPR Teardrop Control Card – 0.03 mL,  
RPR 18 mm Circle Control Card – 0.05 mL.

**Storage Instructions:** In the unopened foil-sealed envelopes, the control cards retain an acceptable reactivity pattern for the assigned expiration date when stored under refrigeration at 2 to 8°C, which is the recommended storage temperature.

## V. **PROCEDURE**

**Materials Provided:** RPR Card Test Control Cards as listed under “Availability.”

### **Materials Required But Not Provided :**

1. One of the following **Macro-Vue RPR Card Test Kits\***:  
18 mm Circle Test -----Kit No. 115, 110, 104, 510 or 532 (qualitative) or  
Kit No. 112 (quantitative)  
Teardrop Test-----Kit No. 100  
All kits contain sufficient overage of materials for performance of daily control card tests.
2. A micropipettor which dispenses 0.05 mL (50 µL) and 0.03 mL (30 µL).

3. A rotator,  $100 \pm 2$  rpm, 2 cm diameter, automatic timer, friction drive, with a humidifying cover containing a moistened blotter or sponge.

\*For availability of kits, the **Macro-Vue** Card Test Rotator (with humidifying cover), and micropipettor, refer to the package insert supplied with all **Macro-Vue** RPR Card Test Kits.

#### **Directions For Use of 18 mm Circle Control Card:**

1. Place 0.05 mL (50 $\mu$ l) deionized/distilled water onto the three circles, using an automatic micropipettor (0.05 mL capillary with rubber bulb attached). *Do not use **Dispenstirs**<sup>TM</sup> device to reconstitute a Control Card.*
2. Using the stirring end of an 0.05 mL **Dispenstirs** device (or broad end of a stirrer) for each circle, mix until dehydrated control specimen is dissolved. Spread specimen to the inside of the circle. *A new **Dispenstirs** device or stirrer must be used for each circle!*
3. Shake the antigen bottle before use. Holding in a vertical position, dispense one or two drops in the cap to make sure needle passage is clear. Place *one* “free-falling” drop of antigen (1/60 mL antigen -- [20 G yellow hub needle]) onto each test area. *Do not restir*; mixing of antigen and specimen is accomplished during rotation. Pick up pre-dropped antigen from the bottle cap.
4. Rotate for eight min, on a mechanical rotator at  $100 \pm 2$  rpm, using a moistened humidifying cover. Following rotation, to help differentiate Nonreactive from Reactive minimal-to-moderate results, a brief rotating and tilting of the card by hand (three or four to-and-fro motions) must be made.
5. *Immediately* read the card macroscopically in the “wet” state under a high intensity incandescent lamp or strong daylight.

The Reactive control should show characteristic strong clumping; the Nonreactive control should show the smooth, grayish appearance of unclumped particles. The Reactive minimal-to-moderate control should show minimal-to-moderate clumping. See the Reading Guide in the package insert provided in all RPR Card Test Kits.

#### **Directions For Use of Teardrop Control Card:**

1. Using an 0.03 mL capillary (or automatic micropipettor), place 0.03 mL of deionized/distilled water onto the Nonreactive and Reactive areas.
2. Using a new stirrer (broad end) for each “teardrop,” mix until dehydrated control specimen is dissolved. *A new stirrer must be used for each specimen!*
3. Shake the antigen bottle before use. Holding in a vertical position, dispense one or two drops in the bottle cap to make sure needle passage is clear. Then place one

“free-falling” drop of antigen (21 *G green hub needle*) just below the reconstituted specimen. Pick up pre-dropped antigen from the bottle cap.

4. Using a new stirrer (broad end) for each “teardrop,” mix RPR Card antigen suspension with test specimen and spread over entire “teardrop” surface.
5. Rocking of the control card is extremely important. Rock the card slowly (approximately 20 to-and-fro motions per min.) for four min., allowing time for the mixture alternately to flow into the apex (so that particles will be in close proximity) and then to spread out as it flows away from the apex.
6. Read macroscopically in the wet state. Reactive controls should show characteristic clumping during the rocking period as evidenced by the appearance of a front of agglutinated particles which move out from the apex of the “teardrop” area. As the clumped particles reach the outer limit of the “teardrop,” they tend to deposit at the periphery. Nonreactive controls should show the smooth, grayish appearance of unclumped particles. See the Reading Guide provided in all **Macro-Vue** RPR Card Test Kits.

## **VI. LIMITATIONS OF THE PROCEDURE**

If the specimens do not dissolve satisfactorily when reconstituted with deionized/distilled water, the card should not be used. Delay in performance of the test procedure following reconstitution, or performing the test procedure in an environment of low humidity (particularly without a humidifying cover on the rotator) may cause an undesirable reactivity pattern (a granular appearance in the Nonreactive specimen). Since the dehydrated specimens are quantitatively controlled for reactivity, the proper execution of the Control Card and RPR Card Test procedures are essential for the determination of the expected reactivity pattern.

The intensity of clumping observed should not be used as a reading standard or guide in interpreting reactions observed with unknown specimens. The Reading Guide contained in the package insert provided with all RPR Card Test Kits is intended for this purpose.

## **VII. EXPECTED VALUES AND PERFORMANCE CHARACTERISTICS**

Each lot of RPR Teardrop and 18 mm Circle Control Cards is tested and compared for the designated pattern of reactivity against reference antigen suspensions and meets the U.S. Centers for Disease Control and Prevention (CDC) product specifications.

The expected patterns of reactivity on the control cards following the performance of RPR Card Test procedures are shown in the Reading Guides contained in the package insert provided in all **Macro-Vue** RPR Card Test Kits.

### **18 mm Circle Card:**

Reactive Circle – Similar to undiluted Reactive titer spot shown under the RPR Card quantitative test.

Reactive minimal-to-moderate Circle – Similar to Reactive minimal spot of RPR Card qualitative test and to Reactive (1:4) spot of RPR Card quantitative test.

Nonreactive Circle – Similar to RPR Card Nonreactive spot.

**Teardrop Cards:**

Reactive spot – Similar to Reactive (moderate) spot of RPR Card Test.

Nonreactive spot – Similar to Nonreactive spot of RPR Card qualitative test.

**VIII. AVAILABILITY**

**Macro-Vue®** RPR Card Test Control Cards, Box of ten individual foil-sealed cards; 18 mm Circle (Reactive, Nonreactive and Reactive Minimal-to-Moderate circles), Box of 10, Cat. No. 276709.  
Teardrop (Reactive and Nonreactive teardrops), Box of 10, Cat. No. 276509.

**Macro-Vue®** Card Test Rotator (with humidifying cover),  $100 \pm 2$  rpm, automatic timer, friction drive, Model 51-II (110 V), Cat. No. 4978051 and Model 54 (220 V), Cat. No. 278054.

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

**IX. REFERENCES**

1. Creighton, E.T. 1990. Rapid plasma reagin (RPR) 18 mm circle card test, p. 99 - 108. *In* S.A. Larsen, E.F. Hunter, and S.J. Kraus (ed.), *A manual of tests for syphilis*, 8th ed. American Public Health Association, Washington, D.C.
2. Portnoy, J., J.H. Brewer, and A. Harris. 1962. Rapid plasma reagin card test for syphilis and other treponematoses. *Public Health Rep.* 77:645-652.
3. Portnoy, J. 1963. Modifications of the rapid plasma reagin (RPR) card test for syphilis, for use in large scale testing. *Am. J. Clin. Pathol.* 40:473-479.
4. Falcone, V.H., G.W. Stout, and M.B. Moore, Jr. 1964. Evaluation of rapid plasma reagin (circle) card test. *Public Health Rep.* 79:491-495.

**Technical information:** In the United States telephone B D Diagnostic Systems  
Technical Services, toll free (800) 638-8663, Prompt 2.

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