Wake Forest Baptist Medical Center	**BD Macro-Vue Rapid Plasma Reagin MS-9**	**Dept:**	705 Micro
		Effective Date:	Pre 1977
		Revised Date:	3/1/2019
		Contact:	Christy Hernandez
Name & Title: Dr. Gregory Pomper		**Date:**	
Signature:			

1) General Procedure Statement:

a. **Purpose:** This procedure is to serve as a guide for trained personnel in the Clinical Microbiology Laboratory to perform the test described herein. This procedure should be used in conjunction with proper training and only by qualified technologists.

b. **Responsible Department/Scope:**
   i. Procedure owner/implementer: Dr. Elizabeth Palavecino
   ii. Procedure prepared by: Louise Hester, MT(ASCP)
   iii. Who performs procedure: Clinical Microbiology Laboratory personnel

2) INTENDED USE:

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

3) SUMMARY AND EXPLANATION:

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.

4) PRINCIPLE:

RPR Card antigen suspension is a carbon particle cardiolipin antigen which detects reagin, an antibody like substance present in serum or plasma from syphilitic persons and, occasionally from 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.

5) SPECIMEN REQUIRED:

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 refrigerated at 2 - 8°C, for up to 5 days or frozen at -20°C or below. Avoid repeated freeze-thawing of specimens.

Plasma: Centrifuge the specimen at a force sufficient to sediment cellular elements. Keep plasma in the original collecting tube, and if stored, store the specimen at 2 - 8°C. **Test plasma specimen within 24 hours of blood collection.**

6) MATERIALS REQUIRED:

- RPR Kit (Becton Dickinson) (stored at 2 - 8°C)
- Syphilis controls – Impact RPR Liquid Control Kit by Alere (stored at 2 - 8°C)
- Saline (0.9%)
- Serum nonreactive for syphilis
- Eppendorf pipettors and tips, 100 µl and 50 µl
- 13 x 100 glass tubes
- 3ml syringe
- 5ml and 1ml sterile serological pipettes

7) EQUIPMENT REQUIRED:

- Rotator with humidifying cover

8) PROCEDURE: SCREENING TEST:

Preliminary Preparations:

1. Controls, RPR Card antigen suspension, and test specimens should be at room temperature when used.

2. If opening a new ampule, vigorously shake the ampule for 10-15 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.

3. With a 3ml syringe, transfer the antigen to the dispensing bottle. Label the dispensing bottle and the syringe with the antigen lot number, expiration date, and date antigen was placed in the bottle. **NOTE:** 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.

NOTE: The needle, dispensing bottle, and syringe should be discarded when the kit is used up.

Testing Accuracy of Delivery Needles:

To check the accuracy of the needle, place the needle on a 3 ml syringe. Fill the syringe with the antigen suspension and, holding in a vertical position, count the number of drops delivered in 1 ml. The needle is considered to be satisfactory if 60 drops ± 2 are obtained in 1 ml.

Preliminary Testing of Antigen Suspension with Controls:

When tests are to be performed, the antigen suspension should be checked with nonreactive, weakly reactive and reactive controls using the RPR Screening Procedure below. Only those antigens which give the prescribed reactions should be used.

RPR screening procedure:

Allow the antigen to warm to room temperature (23-29°C) before use.

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. 50µl; *each* Dispenstirs *device is designed to expel slightly in excess of 50µl 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 of antigen suspension on the top of the card 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 the card.

5. Rotate for 8 min (± 30 s) 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.

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

Note: Specimens giving any degree of clumping should be quantitated.

9) PROCEDURE: QUANTITATIVE RPR TEST:

A quantitative test will be performed on specimens found to be reactive in the screening test.

1. For each specimen to be tested, place 50 µl (using an Eppendorf 50 µl pipettor) of 0.9% saline onto circles numbered 2-5. Do not spread the saline.

2. Place 50 µl of specimen onto circle #1.

3. Prepare serial two-fold dilutions by placing 50 µl of specimen into circle #2 and draw mixture up and down 5-6 times. Avoid formation of bubbles. Transfer 50 µl from circle #2 to #3, to #4, to #5. Discard 50 µl after mixing contents in circle #5.

4. Using a new dispenstir (broad end) for each specimen, start at the 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. Use a new dispenstir for #1.

5. Gently shake antigen dispensing bottle before use. Holding in vertical position, dispense one or two drops on top of card to make sure needle passage is clear and delivering 1/60 ml of antigen. Then place one "free falling drop" onto each test area. Do not stir. Pick up the pre-dropped antigen from the top of the card.

6. Rotate for 8 minutes under humidifying cover, on mechanical rotator at 100 rpm.

Following rotation, to help differentiate non-reactive from weakly reactive results, a brief rotating and tilting of the card by hand (3-4 to and from motions must be made). Then immediately read macroscopically in the "wet" state under a high intensity lamp or strong daylight. **Report in terms of the highest dilution that gives a reactive or weakly reactive result.**

Examples:

1.1	1:2	1:4	1:8	1:16	Report
WR	N	N	N	N	Reac 1:1
R	R	R	N	N	Reac 1:4
R	R	R	WR	N	Reac 1:8

If the highest dilution tested (1:16) is Reactive, proceed as follows:

Quantitative RPR Test for Specimens 1:16:

1. Prepare a 1:50 dilution of nonreactive serum in 0.9% saline (4.9 ml of saline + 100 µl nonreactive serum). 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 50 µl of serum or plasma to 750 µl of 0.9% saline. Mix thoroughly.

3. Place 50 µl of 1:50 nonreactive serum in circles #2, #3, #4, and #5.

4. Place 50 µl of 1:16 dilution of test specimen in circle #1.

5. Make serial two-fold dilutions and complete tests as described under steps 3-6 for Quantitative Test. Higher dilutions are prepared if necessary in 1:50 nonreactive serum.

10) QUALITY CONTROL:

Controls are ordered in Beaker using Batch Editor. Create using Batch Type SEROLOGY-RPR-MANUAL. This Batch orders the following:

- SERO RPR REAC (reactive control)
- SERO RPR WR (weak reactive control)
- SERO RPR NR (nonreactive control)
- SERO NEEDLE DROPS
- SERO RPMS

Three controls (Reactive, Weak Reactive, and Nonreactive) should be included in each day of testing to confirm optimal reactivity of the antigen. These results are then recorded in Beaker. **If controls do not yield the expected response, the assay results should be considered invalid and patient results should not be reported**. Repeat the assay. If control failures are repeated, contact Becton Dickinson's Technical Services Department. Note corrective action in Beaker.

Needle accuracy should be checked each day of testing and should be 60±2 drops per 1 ml. If not, obtain and calibrate a new needle. In addition, when control patterns cannot be reproduced, or when a drop of antigen does not drop cleanly from the tip of the needle, that needle should be replaced with another needle that meets specification. Record number of needle drops in Beaker.

The rotator must be set at 100±2 rpms. Rotator is calibrated monthly. If out of range, contact Trimedx. Record results in Beaker.

Each new lot or new shipment of RPR antigen should be tested with 3 levels of reactivity. Antigenic strength will be tested in parallel with the RPR controls currently in use in the lab. Thus a successive comparison of antigen reactivity shall be maintained with the old vs the new vial of antigen. These results are logged in the Serology Quality Control Manual.

New Lot information is updated in the Beaker system under the function QC Material. This information includes lot number, expiration date and reference ranges if appropriate.

11) INTERPRETATION OF RESULTS:

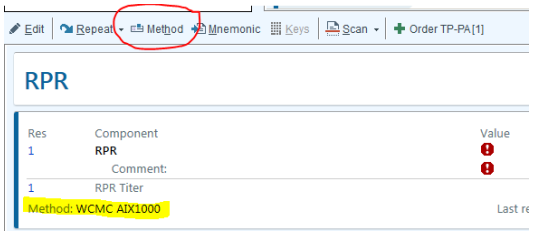
Reactive (Abnormal patient result): Showing characteristic clumping ranging from slight but definite to marked and intense. The **titer** is reported in terms of the highest dilution that gives a reactive or weakly reactive result.

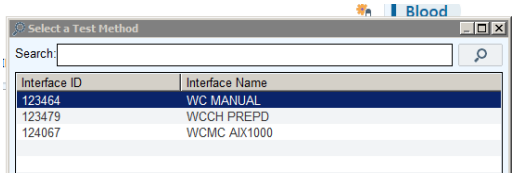
Nonreactive (Normal patient result): Showing slight roughness or no clumping.

NOTE: Reactive RPR with titer ≥ 16 must be reported to the Health Department (See Infectious Disease Reporting.)

Reporting controls and patient results in Beaker:

The test method must be changed from WCMC AIX1000 to WC MANUAL. Before the Edit button is selected, hit Method. A "Select a Test Method " box will appear. Click the magnifier to open the drop down box. Select WC MANUAL. This same process must be done for each control and patient.





12) PROCEDURAL NOTES:

1. A reactive RPR must be confirmed by ordering a TP-PA. However, if a patient has been reactive in the past, the TP-PA result may be referred and not retest.

2. Before use, gently shake antigen dispensing bottle.

3. Before testing, dispense several drops of antigen suspension on the top of the card to make sure the needle passage is clear.

4. Store reagents as recommended by the manufacturer.

5. The proper use of reagents and controls in the kit procedures are followed as recommended by the manufacturer.

6. Do not mix reagents from different lots.

7. **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.

13) LIMITATIONS:

1. The diagnosis of syphilis should not be made on a single reactive result without the support of a positive history or clinical evidence, 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. The use of plasma specimens to establish a baseline from which changes in titer can be determined has not been evaluated.

2. 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.

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

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

5. 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.

6. With cardiolipin type antigens, biological false positive reactions have been reported in diseases such as infectious mononucleosis, leprosy, malaria, lupus erythematosus, vaccinia and virus pneumonia. In pregnancy, several reports indicated the occurrence of false positive reactions. Narcotic addiction and autoimmune diseases also may give false positive reactions. Pinta, yaws, bejel and other treponemal diseases produce positive reactions in this test.

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

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

14) REFERENCE:

Becton Dickinson Microbiology Systems, Cockeysville, Md., Macro-Vue RPR Card Tests 18mm Circle. Product Insert, July 2015.

15) Review/Revision/Implementation:

All procedures must be reviewed at least every 2 years.

- All new and procedures that have major revisions must be signed by the CLIA Director.

- All reviewed procedures and procedures with minor revisions can be signed by the designated section medical director.

16) Revised/Reviewed Dates and Signatures: Adopted pre 1977 (BLW), Reviewed 3/18/2008 (EP), Reviewed 3/27/2009 (EP), Reviewed 3/20/2010 (EP), Reviewed 3/21/2011 (EP), Reviewed 3/23/2012 (EP), Reviewed 3/20/2013 (EP), Reviewed 3/31/2015 (EP), Reviewed 3/6/2017 (EP), Revised 2/5/2019 (LH)

Review/Revision Date	Signature