

### Rapid Plasma Reagin (RPR)

#### Objectives:

1. Accurately follow the written procedure to perform the RPR test for the detection of syphilis antibodies to obtain control and patient results that match instructor values with 100% accuracy.
2. State the substance being detected, the principle of the procedure, the expected value, significance of abnormal results, and limitations of the procedure.
3. State the composition and purpose of the ingredients in the RPR antigen.
4. State the number of drops for needle delivery, number of RPMs of rotator and temperature required for accurate results to be obtained.
5. Define “Biologic False Positive” and list four causes.
6. List four potential causes of a negative RPR test.
7. State one sample which cannot be used for the RPR test.
8. State the two broad types of serological tests used to detect antibodies to syphilis.
9. List and describe three confirmatory tests for syphilis.
10. List and describe four limitations of the RPR test.
11. Accurately record and report results as instructed.

#### Introduction:

Syphilis is a contagious venereal disease caused by the spirochete Treponema pallidum. The organism enters the body through a break in mucosa or epithelial layer. After a 10 to 60 day incubation period, a painless, inflammatory reaction producing a characteristic ulcerated lesion called a chancre usually appears at the site of entry by the treponeme. These lesions of *primary syphilis* usually heal spontaneously but the infection persists. Syphilis is usually cured by penicillin if treated early, but the spontaneous remission of the chancre may lull the affected individual into a false sense of security so that he/she fails to seek medical treatment. If left untreated, a generalized skin rash and other abnormalities will be seen six weeks to six months after the chancre disappears (*secondary* stage). Clinical symptoms then usually disappear (latent syphilis) for various periods. Latent syphilis may continue throughout life, it may terminate with spontaneous cure, or it may advance to *tertiary* syphilis. In its advanced states, syphilis can cause blindness, insanity, paralysis, vascular disease, bone and joint lesions, ulcers of skin and mucous membranes. Pregnant women with active syphilis (even primary syphilis) can transmit the organism to their unborn child (*congenital syphilis*).

#### Laboratory Diagnosis:

Direct detection of spirochetes by darkfield microscopy is a non-serologic method for diagnosis of syphilis. A specimen is obtained from the primary lesion (chancre). Treponemes may be visible using darkfield microscopy. The slide is observed for the characteristic corkscrew morphology and flexing motility of the organism, if present. Experience is required as non-pathologic, morphologically similar organisms must be excluded.

There are TWO types of **SEROLOGIC** tests for syphilis:

1. Tests for **non-specific reagin**.
2. **Tests for specific treponemal antibodies** of the IgG class.

Reagin, an antibody-like protein, is produced by most patients with syphilis (non-treponemal tests). This is a screening test only and there are many causes of false positive results. All positive reagin tests are followed by a confirmatory test for the detection of specific antibodies to *Treponema pallidum* (treponemal tests).

Two widely used reagin (non-treponemal) tests are the VDRL (Venereal Disease Research Laboratory) slide test and the RPR (rapid plasma reagin) card test. These tests are especially suited for screening and for monitoring therapy.

### **Reagin Tests**

Non-treponemal antibodies or reagin are formed in response to the tissue damaged by the action of the spirochetes. Syphilitic **reagin** is an IgM antibody produced by people who have been infected with treponemes. Because of the nonspecificity of reagin, non-treponemal assays are screening tests only. If a positive reaction is obtained, a more specific treponemal antibody test must be performed.

The VDRL antigen is an alcoholic extract of normal beef hearts, called cardiolipin, is the conventional source of antigen for detecting the presence of reagin in serum. Sensitivity of the reaction is enhanced by the addition of lecithin and cholesterol. Lecithin increases reproducibility, cholesterol provides larger antigen particles. For the RPR, choline chloride is added to inactivate inhibitors so that serum or plasma does not have to be heated.

### **VDRL Test**

A test developed by the Venereal Disease Research Laboratory, called the VDRL test, is frequently used on CSF for detecting tertiary syphilis. Less commonly, it is used on serum. It employs an alcoholic solution of the cardiolipin-lecithin-cholesterol antigen (VDRL antigen) and a special buffered saline.

Very rigid procedures are outlined for mixing the antigen and buffer, the size of the drops of antigen mixed with the patient's serum, and speed of rotation of the slides. When the VDRL antigen is mixed with buffered saline, it forms short rods of uniform size that can easily be seen at 100X magnification. When reagin combines with these antigen particles, they agglutinate and the clumps can be seen with the naked eye or a hand lens.

The VDRL test is a slide test performed as either a qualitative or quantitative procedure. All sera for use in VDRL tests must be heated at 56° for 30 minutes within four (4) hours of testing to inactivate heat-labile nonspecific inhibitors of the VDRL test reaction.

Reagin is not usually detected by the VDRL test until about four to five weeks after infection. Therefore, a negative test is no proof that an individual has not been infected. Once stimulated, reagin levels tend to climb to a maximum titer during the secondary stage of the disease. After that, the reagin titer tends to decrease to a fairly stable level during late syphilis.

### Rapid Plasma Reagin (RPR) Test

One of the most popular alternatives to the VDRL test and the most commonly used screening test for syphilis is the rapid plasma reagin (RPR) test. Advantages of the RPR include:

1. This test utilizes the VDRL cardiolipin antigen modified by the incorporation of choline chloride and inclusion of charcoal indicator particles. The choline chloride inactivates inhibitors so that serum or plasma does not have to be heated prior to testing.
2. The agglutination of the charcoal particles renders the serological reaction visible to the naked eye and therefore does not require reactions to be read under the microscope.
3. The antigen supplied with the RPR kit requires no preparation. This is a distinct advantage over the VDRL test where improper preparation of the antigen can lead to spurious results. The RPR test has not been approved for testing spinal fluids whereas the VDRL has been approved. Results on cordbloods may not be reliable. The RPR test has been found to be more specific and sensitive than the VDRL test but gives more false positives. All positive RPRs must be confirmed by testing for treponemal antibodies. ***RPRs cannot be performed on spinal fluid.***

### Principle

The RPR card antigen suspension is a cardiolipin antigen attached to charcoal particles which detects “reagin”, an antibody-like substance present in sera from syphilitic persons, and occasionally in serums of persons with other acute or chronic conditions.

When a specimen contains antibody (reagin), agglutination of the charcoal particles of the RPR Card antigen occurs, which appear as black clumps against the white background of the plastic coated card. This coagglutination can be read macroscopically

### Treponemal Antibody Test

The second group of antibodies for which tests have been developed are specific treponemal antibodies. This group of antibodies is directed against the treponeme and its components. In recent years, many treponemal tests have been described. The test of choice is the fluorescent treponemal antibody absorption (FTA-ABS) test. Indications for performing this test include: (1) distinguishing the biologic false positive reactions encountered in reagin tests from true positives, and (2) diagnosing late syphilis in which non-treponemal tests are often non-reactive.

Treponema Pallidum Immobilization Test (TPI) - measures ability of (patient produced) antibody and complement to immobilize live (reagent) treponemes.

Fluorescent Treponemal Antibody Absorption Test (FTA-Abs) - an indirect fluorescent antibody test requiring diluted heat-inactivated patient serum. The serum is mixed with non-pathogenic Reiter Strain treponemes to remove non-specific cross-reactive antibodies. The 'absorbed' serum is then tested with the Nichols Strain of *T. pallidum*, washed, stained with an antibody conjugate (anti-immunoglobulin with a fluorescein isothiocyanate label) and examined under a fluorescent microscope by an experienced tech. Sera containing antibody will result in fluorescent staining of the spirochetes. The intensity of the fluorescence is graded 0-4+, with 2+ or greater indicating reactive. This test will give positive results within two weeks into the primary stage.

Hemagglutination Tests (includes HATTS and MHA-TP) utilize red cells coated with antigens from the Nichols strain of *T. pallidum*. Serum is pre-treated to limit non-specific reactions. Agglutination as indicated by a rough jagged pattern is positive.

### **Biologic False Positives**

Biological false positives are caused by conditions which produce a false reaction for T. Pallidum in non-treponemal screening tests, but a negative reaction in the specific treponemal tests.

Biologic false positives (BFP) to reagin tests may occur in diseases such as leprosy, malaria, toxoplasmosis, infectious mononucleosis, tuberculosis, lupus erythematosus, and viral pneumonia. The presence of autoimmune or collagen-vascular disease, viral infection or hyperglobulinemia may also produce false positives. In addition, false positives may be attributed to pregnancy or aging (as many as 10% of persons above 70 years of age demonstrate BFP). BFP are found to occur at least 10 times more frequently in drug addicts than the general population.

### **Interpretation of Results**

In the interpretation of serological tests for syphilis, the following factors should be taken into account:

1. Geographical area or country of origin. Diseases related to syphilis, whose causative organisms are indistinguishable from Treponema pallidum, can result in misdiagnosis.
2. Ability of the patient to produce reagin or treponemal antibodies.
3. Stage of illness.
4. Previous antibiotic therapy.
5. Manner in which serological tests are performed.
6. Various conditions that may cause biological false positives

### **Expected Results:**

Patient who have not been exposed to treponemal organisms should not exhibit positive results.

---

**Negative Reactions**

1. The patient does not have syphilis.
2. The infection is too recent, patient has not produced antibodies.
3. The test is temporarily non-reactive because of treatment.
4. The test has been rendered temporarily non-reactive by consumption of alcoholic fluids prior to testing.
5. The disease is latent, inactive, or patient's body tolerates the organism.
6. Patient is immunocompromised and unable to respond.
7. The patient's body tolerates the organism.
8. Inferior technique.

**Weakly Reactive Results**

1. Very early infection.
2. Lessening of the activity of the disease after treatment.
3. Biological false-positive reaction.
4. Inferior technique.

**Positive Results**

If BFP and inferior technique are ruled out, positive results usually indicate that the patient has syphilis.

**Control of Serological Tests for Quality Control**

Control sera of graded reactivity should be included with each serological testing procedure performed. For the non-treponemal flocculation tests the antigen suspension should be controlled daily. The results obtained should produce the expected reactivity pattern. Patient results should be considered invalid if controls are unacceptable

Three control serums are supplied and should be used prior to performing the RPR Card Test. Follow directions for Qualitative Card Test in evaluating control serums. Holding control vials vertically, deliver a free-falling drop of each control serum onto a separate circle of the card. Sufficient volumes of each control serum are provided for use with every two test cards. The expected reactivities of each serum after the 8 minute rotation period are:

1. Reactive = Produces distinct flocculation of charcoal particles.
2. Reactive – Moderate-to-Minimal = Produces slight but definite flocculation.
3. Non-reactive = A gray, homogeneous suspension or only slight roughness is observed.

The reactive control serums are standardized for their reactivity with each RPR Test antigen. If the antigen tested does not produce the expected and described results, the control tests should be repeated. Only those RPR Card Test antigen which produce the expected reactivities should be used. **The controls supplied with this kit are not to be used as reading standards. They are to be used only for determining the sensitivity of the antigen and test system to assure proper test performance.**

**Limitations:**

1. Proper specimen collection, processing and testing procedure must be followed for reliable results.
2. Diseases related to syphilis (yaws, pinta, and non-venereal endemic syphilis) whose causative organisms are nearly indistinguishable from *T. pallidum*, can cause positive reactions.
3. Biological false positives (BFP), to reagin tests may occur in diseases such as leprosy, malaria, toxoplasmosis, infectious mononucleosis, tuberculosis, lupus erythematosus, and viral pneumonia. The presence of autoimmune or collagen-vascular disease, viral infection or hyperglobulinemia may also produce false positives. IV drug users, pregnant women and the elderly may have false positive reactions.
4. Blood collected in siliconized tubes, bacterially contaminated specimens or grossly hemolyzed specimens may produce false positive results

**Materials: Rapid Plasma Reagin (RPR) card test**

1. RPR Card Test Antigen - Contains a suspension of specially prepared charcoal particles. The antigen should be stored according to the manufacturer's directions (usually 2 to 8°C) in which case an unopened ampule will have a shelf life of at least 12 months from the date of manufacture. Once the ampule has been opened, it usually remains stable about three months.
2. 20-gauge needle without bevel
3. Plastic dispensing bottle
4. RPR plastic-coated cards
5. Dispenstirs, 0.05 mL per drop
8. Mechanical rotator calibrated to rotate at 100 rpm/min
9. Humidifier cover
10. Patient and control serum specimens.

**Directions for Use of Dispenstirs®**

To avoid the risk of mouth pipetting of possibly infectious serums, the 0.05 ml Dispenstirs® are recommended by the U.S. Department of HEW, Center for Disease Control for performing the RPR 18mm Circle Card Test.

1. Hold 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. Release finger pressure to draw up the sample.
2. Holding in a perpendicular position directly over the surface to which specimen is to be delivered, squeeze, allowing **one free falling drop** (approximately 0.05 ml) undiluted plasma or serum to be dispensed. Each Dispenstirs® device is designed to expel slightly in excess of 0.05 ml to compensate for a small amount of specimen retained by the stirring end.
3. Invert Dispenstirs® device, and with sealed stirring end spread the specimen. (If desired, sample remaining in Dispenstirs® device may be discharged into specimen tube from which it was drawn.) Each device should be used once and discarded.

**Procedure:****Testing Accuracy of Delivery Needles**

4. The 20 gauge needle without bevel should be checked daily.
5. Place needle on a 1 ml serologic pipette.
6. Fill the pipette with water and count the number of drops delivered in 0.5 mL when the needle and pipette are kept in a vertical position.
7. 30 drops  $\pm$  1 drop is considered satisfactory.
8. **RECORD YOUR RESULTS ON YOUR FORM.**

**Checking Calibration of Rotator**

1. Turn the rotator on.
2. Take a pencil or other similar device and hold it perpendicularly next to the rotator such that the rotator table touches it as it rotates around (it should make a light tapping sound).
3. Using a timer, count the number of taps for one (1) minute.
4. Rotator should revolve  $100 \pm 2$  times per minute. If it is more or less than 100 per minute adjust the rpms and repeat until it is set at 100 rpms. Consult with instructor if rotator needs calibration.
5. **RECORD YOUR RESULTS ON YOUR FORM.**

**Room Temperature Check**

Room temperature must be checked ( $23^{\circ}\text{C}$ - $29^{\circ}\text{C}$ ) and recorded each day RPRs are performed. A standard bench top thermometer is sufficient. **RECORD YOUR RESULTS ON YOUR FORM.**

**CAP regulations require needle delivery, rotator RPM, and room temperature check to be done each day RPRs are performed.**

**Specimen Testing**

1. Bring all reagents, controls, and specimens must be at room temperature prior to testing)
2. Label a RPR card with patient and control information being careful not to interfere with the test areas of the card.
3. Place 0.05 mL of patient serum or control sample on the 18 mm circle of the plastic coated test card using a new 0.05 Dispensstirs for each sample. **THE DROP MUST BE FREE FALLING, DO NOT TOUCH DROP TO SLIDE.**
4. Spread the sample smoothly across the circle area using the paddle side of the Dispensstir as shown by instructor. Take care not to scratch the card surface area.
5. Thoroughly mix antigen solution by inverting the bottle several times, add one drop of the antigen suspension to each testing area. Note: hold the antigen container upside down directly over the test area such that the drop falls directly onto the center of the circle. **DO NOT STIR OR SPREAD THE ANTIGEN.**
6. Carefully place the card on the rotator and cover with the dampened humidifier cover.
7. Rotate for 8 minutes at 100 RPM.
8. Read macroscopically immediately after rotation under a high-intensity incandescent lamp or strong daylight. The card may be briefly rotated or tilted to and fro by hand, if necessary, to assist in differentiating non-reactive from minimally reactive results.
9. Upon completion of testing, remove the needle, rinse with distilled water and air dry. **Do not wipe needle** as it may remove the silicone coating. Recap the dispensing bottle. Place all reagents and dispensing needle back in the box and return to the supply counter.

**Interpretation:**

⇒ **Non-reactive** (NR) - a gray, homogeneous suspension showing no clumping or only slight roughness.

⇒ **Reactive** (R) - showing characteristic black clumping of charcoal ranging from slight (minimal-to-moderate) to intense.

**Note:** There are only two possible reports with this card test – reactive (R) or non-reactive (NR) – regardless of degree of reactivity. Slight but definite flocculation should always be reported as reactive. All reactive results should be confirmed by the quantitative procedure.

If the test is negative, but the physician still suspects the infection is present, the more specific treponemal tests should be ordered.

Reagin tests (VDRL and RPR) are considered screening tests. If positive results are obtained, the more specific treponemal testing (FTA-ABS, MHA-TP, etc.) should be performed. Specimens giving any degree of clumping should be subjected to further serological study.

Laboratory 9: Rapid Plasma Reagin (RPR) Test

Name \_\_\_\_\_

Date \_\_\_\_\_

Test Kit Name \_\_\_\_\_

Manufacturer \_\_\_\_\_

Lot Number \_\_\_\_\_

Expiration Date \_\_\_\_\_

	Acceptable Range	Results	Check ONE	
			Acceptable	Not Acceptable
Delivery needle (drops / mL)				
Rotator check (RPMs)				
Room Temperature				

**REPORT RESULTS AS “R” for reactive or “NR” for non-reactive**

Patient Name	Identification Number	Result
1.		
2.		
3.		
4.		
<b>Controls</b>		
<b>Weakly Reactive</b>		
<b>Reactive</b>		
<b>Non-reactive</b>		

Name \_\_\_\_\_

Date \_\_\_\_\_

Using your textbook, lecture and lab results and notes, answer the following questions. Each question is worth one point, unless otherwise indicated.

1. Based on the control results, can these patient results valid and reportable?  
**Yes No** (circle one)  
If not, explain why.
2. What is the genus and species of the causative organism of syphilis?
3. What are the two *major* types of **SEROLOGIC** tests for syphilis?
4. What substance is being detected in the patient sample?
5. State the composition of the RPR antigen.
6. Why are charcoal particles incorporated into the RPR test?
7. Define “biological false positive” (BFP).
8. State three (3) conditions or diseases which may cause a BFP reaction to the RPR test. (3 points)
9. What specimen is unsuitable for syphilis testing by RPR?
10. A negative reagin serological test for syphilis does not prove that the patient does not have syphilis. Give at least 4 reasons that support this statement.