

**Rapid Plasma Reagin (RPR)**  
for detection of syphilis antibodies

**Objectives:**

1. Follow instructions of the reagent package inserts / instructor's directions to select, and evaluate appropriate specimens for syphilis antibodies testing.
2. Perform the RPR test for the detection of syphilis antibodies to obtain control and patient results that match instructor values with 100% accuracy.
3. Evaluate lecture notes, reagent package insert, lab instructions, and instructor's directions to determine the substance being analyzed, the principle of the procedure, the expected value, significance of abnormal results, limitations of the procedure, and troubleshooting procedures to follow if / when control results are unacceptable.
4. Appropriately record and report results as instructed.
5. Utilize lecture notes, textbook and laboratory (including product insert) information to answer study questions.

**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 10-60 day incubation, a painless inflammatory reaction producing a characteristic ulcerated lesion called a chancre usually appears at the site of entry. These lesions of *primary syphilis* usually heal spontaneously although the infection persists. Syphilis is usually cured by penicillin, if treated early. If untreated, a generalized skin rash and other abnormalities will begin appearing six weeks to six months following the disappearance of the chancre (*secondary stage syphilis*). Again, the clinical symptoms may disappear (*latent stage syphilis*). The 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, and ulcers of skin and mucous membranes. Pregnant women with active syphilis (even primary stage) can transmit the organism to the unborn child (*congenital syphilis*).

**Laboratory Diagnosis:**

Methods of laboratory diagnosis of syphilis:

1. **Direct detection of spirochetes:**

Darkfield microscopy - Specimen obtained from lesion is evaluated using darkfield microscopy for characteristic corkscrew morphology and flexing motility. Well experienced technician required and non-pathologic morphologically similar organisms must be excluded.

Fluorescent Antibody Testing of specimen - Fluorescent - labeled antibodies bind (direct or indirect methods) with *T. pallidum* organism. Using a fluorescent microscope, specimen evaluated for fluorescence which demonstrates presence of organism. Use of monoclonal antibodies has increased specificity, but subspecies of *T. pallidum* may react. Care must be taken to prevent organisms from washing off slide during preparation.

2. Specific tests for treponemal antibodies. Requires specific treponemal antigens and direct antibody directed against the *T. pallidum* organism.
  - 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-pathologic 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. The intensity of the fluorescence is graded 0-4+, with 2+ or greater indicating reactive.
  - 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.
3. Non-specific Reagin tests (includes VDRL and RPR tests). Flocculation and precipitation tests to detect the presence of reagin, an antibody to cardiolipin. Serum specimens may need to be heated to inactivate complement. Appropriately reacting controls and good technique required.

The RPR test utilizes the VDRL cardiolipin antigen modified by the incorporation of choline chloride to inactivate inhibitors (eliminating need for heat treating specimen) and includes charcoal indicator particles to improve reaction visibility (eliminating need to read test microscopically). Antigen supplied comes pre-prepared (an advantage over the VDRL procedure).

### **Principle: Rapid Plasma Reagin (RPR) test**

Patient sera mixed with a fine particle cardiolipin antigen which has been enhanced with cholesterol, lecithin, and charcoal will result in a macroscopically visible flocculation-type precipitation if the patient's sera contains reagin - an antibody formed against cardiolipin.

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

1. RPR Card Test Antigen
2. 20 -gauge needle without bevel
3. Plastic dispensing bottle
4. RPR plastic-coated cards
5. Dispensstirs, 0.05 mL per drop
6. Capillary pipets, 0.05 mL capacity
7. Rubber bulbs, stirrers, and a timer
8. Mechanical rotator calibrated to rotate at 100 rpm/min
9. Humidifier cover: Any convenient cover containing a moistened pad may be used to appropriately cover cards during rotation.
10. Patient and control serum specimens.

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

1. The 20 gauge needle without bevel should be checked daily.
2. Place needle on a 1 ml serologic pipet.
3. Fill the pipet with antigen solution and count the number of drops delivered in 0.5 mL when the needle and pipet are kept in a vertical position.
4. 30 drops  $\pm$  1 drop is considered satisfactory.

**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). ( Instructor will demonstrate this procedure.)
3. Using a timer, count the number of taps for one (1) minute.
4. Rotator should revolve 100  $\pm$ 2 times per minute. Consult with instructor if rotator needs calibration.

**Specimen Testing** (all reagents, controls, and specimens must be at room temperature prior to testing)

1. Label a RPR card with patient and control information being careful not to interfere with the test areas of the card.
2. 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,
3. 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.
4. After mixing the antigen solution by swirling, add one drop of the antigen suspension to each sample / control 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.**
5. Carefully place the card on the rotator and cover with the dampened humidifier cover.
6. Rotate for 8 minutes at 100 RPM.

7. 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 differentiate non reactive from minimally reactive results.
8. Upon completion of tests, 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 and store in refrigerator.

**Interpretation:**

- ⇒ Non-reactive (NR) - smooth suspension, no clumping or slight roughness
- ⇒ Reactive (R) - any degree of clumping

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 & 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.

**Expected Results:**

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

**Limitations:**

1. Proper specimen collection, processing and testing procedure must be followed for reliable results.
2. Diseases related to syphilis (yaws, pinta, & nonvenereal 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. **Negative** serological reactions may indicate any of the following:
  - a. The patient does not have syphilis.
  - b. The infection is too recent, patient has not produced antibodies.
  - c. Treatment is underway.
  - d. Consumption of alcohol prior to testing.
  - e. The disease is latent, inactive, or patient's body tolerates the organism.
  - f. Patient is immunocompromised and unable to respond.
  - g. Inferior technique.

**Laboratory 9: Rapid Plasma Reagin (RPR) Test**  
Results and Study Questions

Name \_\_\_\_\_

Date \_\_\_\_\_

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

Manufacturer \_\_\_\_\_

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

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

State the interpretation (i.e. "R" for reactive or "NR" for non-reactive).

Delivery needle (drops / mL)	
Rotator check (RPMs)	
Patient Name and Identification Number	Result
1.	
2.	
3.	
4.	
Controls	
Weakly Reactive	
Reactive	
Non-reactive	

Using your textbook, lecture and lab results and notes, answer the following questions.

1. Based on the control results, can these patient results be reported? (circle one) **Yes** **No**  
If “no”, explain why.
2. What are the two *major* kinds of **serologic** tests for syphilis?
3. What substance is being detected in the patient sample?
4. State the composition of the RPR antigen.
5. What component of the RPR antigen is involved with a reactive patient’s sample?
6. Why are charcoal particles incorporated into the RPR test?
7. What is the genus and species of the causative organism of syphilis?
8. Define “biological false positive” (BFP).
- (1.5 pts) 9. State three (3) conditions / disease conditions which could cause a BFP reaction to the RPR test.
10. According to your notes, what specimen is unsuitable for syphilis testing by RPR?
- (2 pts) 11. 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.
12. Briefly state the principle of the Rapid Plasma Reagin (RPR) test for syphilis.