

Rapid Plasma Reagin (RPR)

Objectives

1. Evaluate appropriate specimens for syphilis antibody testing.
2. Perform the RPR test for the detection of syphilis antibodies with 100% accuracy.
3. Evaluate lecture notes, reagent package insert, and laboratory instructions 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.
4. Accurately record results of the controls and patients.

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. If treated early, syphilis is usually cured by penicillin. 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).

Syphilis is usually cured by penicillin. Patients that have been infected with syphilis will most likely produce an antibody-like protein called reagin. Several tests are noted here in this laboratory. RPR will be performed today on serum.

Laboratory Diagnosis

1. Direct detection of spirochetes

Darkfield microscopy – A specimen sample obtained from a lesion is evaluated using darkfield microscopy for characteristic corkscrew morphology and flexing motility. An experienced technician usually performs this type of testing because they must know how to exclude non-pathologic, morphologically similar organisms.

Fluorescent Antibody Testing of specimens

Fluorescent - labeled antibodies bind (direct or indirect methods) with *T. pallidum* organisms. Using a fluorescent microscope, a specimen is evaluated for fluorescence, which demonstrates the presence of an 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 slides during preparation.

2. Specific tests for treponemal antibodies require specific treponemal antigens and direct antibody directed against the *T. pallidum* organism.

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. The intensity of the fluorescence is graded 0-4+, with 2+ or greater indicating reactive.

The *Treponema pallidum* particle agglutination, TP-PA, test uses gel particles sensitized with *Treponema pallidum* of the Nichols strain (a pathogenic serotype). This test may not be as sensitive as ELISA. This test is a newer version of the hemagglutination test (MHA-TP) that utilizes red cells coated with antigens from the Nichols strain of *T. pallidum*. The TP-PA is an indirect agglutination assay. Gelatin particles are sensitized with the spirochete and the patient serum is added. The particles aggregate to form clumps when the patient serum is positive for syphilis.

3. **Non-specific reagin tests** (screening tests that include VDRL and RPR tests). Flocculation and precipitation tests are used to detect the presence of reagin, an antibody to cardiolipin. Appropriately reacting controls and good technique are 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).

The VDRL test procedure is used for CSF. Serum specimens used for the VDRL are heated to inactivate complement. CSF **cannot** be tested using the RPR 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 with plastic dispensing bottle
2. 20 -gauge needle without bevel
3. Syringe for calibrating 20-gauge needle
4. RPR plastic-coated cards
5. Dispensstirs, 0.05 mL per drop
6. 50 uL MLA pipettes for card serial dilution
7. 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. Patients and control serum specimens.

Procedures

Testing Accuracy of Delivery Needles

1. The 20 gauge needle without bevel should be checked daily.
2. Place the needle on a 1 mL syringe.
3. Fill the syringe with antigen solution and count the number of drops delivered in 0.5 mL when the needle and syringe are kept in a vertical position.
4. 30 drops +/- 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).
3. Using a timer, count the number of taps for one (1) minute.
4. Rotator should revolve 100 +/- 2 rotations per minute. Consult with the instructor if the rotator needs calibration.

Specimen Testing

1. 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,
4. Spread the sample smoothly across the circle area using the paddle side of the dispensstir as shown by the instructor. Take care not to scratch the card surface area.
5. After mixing the antigen solution by swirling, add one drop of the antigen suspension to each patient sample and 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.
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 by hand, if necessary, to assist in differentiating non-reactive from minimally reactive results.
9. Upon completion of tests, remove the needle, rinse with distilled water and air dry. Do not wipe the needle as it may remove the silicone coating. Recap the dispensing bottle and store in the refrigerator.

Interpretation

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

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

Expected Results

Non-reactive.

Limitations of the Procedure

1. Proper specimen collection, processing and testing procedure must be followed for reliable results.
2. Diseases related to syphilis (yaws, pinta, and nonvenereal endemic syphilis) 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 and the patient has not produced antibodies.
 - c. Treatment is underway.
 - d. Consumption of alcohol prior to testing.
 - e. The disease is latent, inactive, or the patient's body tolerates the organism.
 - f. Patient is immunocompromised and unable to respond.
 - g. Inferior technique.

Lab	SQ	Total
41	19	60

Rapid Plasma Reagin (RPR) Test Results and Study Questions

Name _____

Date _____

Kit Information		Points
Test Kit Name		1
Manufacturer		1
Lot Number		1
Expiration Date		1
Storage temperature		1

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

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

Test Completion Information		Points
Initials of individual performing test (Tech)		1
Date of completion		1
Time of completion		1

INSTRUCTOR USE ONLY		Points	Awarded
Student results matched instructor results.		5	
Organized, stayed on task, completed within an acceptable amount of time without clerical errors.		5	

MLAB 1335 RPR Study Questions

Name _____ Date _____

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

1. Based on the control results from the laboratory exercise, can the patient results be reported? (1 point)
(Circle one) **Yes** **No**
 - a. If not, explain why.
2. What are the two serologic screening tests for syphilis? (2 points)
 - a.
 - b.
3. What substance is being detected in the patient sample? (1 point)
4. State the composition of the RPR antigen. (4 points)
 - a.
 - b.
 - c.
 - d.
5. Why are charcoal particles incorporated into the RPR test? (1 point)
6. What is the genus and species of the causative organism of syphilis? (1 point)
7. Define "biological false positive" (BFP). (1 point)
8. State three (3) conditions or disease conditions, which could cause a BFP reaction to the RPR test. (3 points)
 - a.
 - b.
 - c.
9. According to your notes, what specimen is unsuitable for syphilis testing by RPR? (1 point)
10. A negative reagin serological test for syphilis does not prove that the patient does not have syphilis. Give at least four reasons that support this statement. (2 points)
11. Briefly state the principle of the Rapid Plasma Reagin (RPR) test for syphilis. (2 points)