

SEROLOGICAL TESTS

Exercise 24

Introduction

Serological tests are based on antibody/antigen reactions and because antibodies are so specific for their antigen, these tests continue to become more and more useful as diagnostic tools in medicine. Serological tests can be direct or indirect. **Direct tests** use a known antibody from the lab to directly identify an unknown antigen (microbe) from the patient. **Indirect tests**, on the other hand, diagnose disease by an indirect route. Rather than taking the actual microbe from the patient, these tests check to see what antibodies the patient is producing. Serum from the patient is mixed with an antigen from the lab. If a reaction occurs the patient has the specific antibody for that disease. In this lab exercise you will have an opportunity to play a computer game to learn more about serological tests. You will also perform a real serological test for syphilis diagnosis (without real patients of course).

RPR Test for Syphilis

Treponema pallidum, the etiologic agent for syphilis, is difficult to isolate from patients and cannot be cultured on microbiological media. Therefore a large number of indirect serological tests have been developed for the diagnosis of syphilis. We will look at one example, the RPR test, in today's lab.

In some diseases patients develop non-specific antibodies that will react with other antigens besides the microbe causing the disease. The antibodies are called **heterophile antibodies**, and the lab antigens they react with are called **heterophile antigens**. Syphilis patients produce heterophile antibodies, called **reagin**, which cross-react with other antigens besides *Treponema pallidum*. One of these antigens is **cardiolipin** (from cow hearts), and this is the heterophile antigen used in the RPR tests. The cardiolipin is attached to black charcoal particles. When the antibodies attach to the cardiolipin antigens, the particles are clumped together as visible, black aggregates. On the other hand, if the patient does not have antibodies for syphilis, there will be no clumping.

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Materials

Per Class

1. *Serologic* computer program on the CD-ROM disk
2. RPR antigen (cardiolipin) in dropper bottle
3. Reactive, Weak and Non-reactive serum samples
4. 0.1 ml. pipets for serum samples
5. RPR typing cards
6. Slide rotating machine

Methods

SeroLogic Computer Program

1. *SeroLogic* is a pair of computer games on your CD-ROM that give you a chance to use your powers of logic and critical thinking to figure out the mechanism for two serological tests: ELISA and Complement Fixation.
2. Each game begins with a brief introduction about one of the tests. Read each page (screen of information) carefully, and study the labeled drawings and animations. This information will provide you with valuable clues for the game. Continue clicking the Forward button until you have read all the pages of background explanation. You can click on the Backup button to review.
3. When you are finished with the introductory information, you are ready to try the challenge game. Click on the Start Game button. In this simulation you will practice your critical thinking skills to discover for yourself the mechanism for a serological test.
4. The bottom half of the screen (see Fig. 24.1) contains the reaction chambers where we will compare a positive reaction (person who has the disease) with a negative sample (healthy patient).
5. Along the top of the screen are various test components and reagents available to you. Use your mouse to drag a component into the test chamber area. To get more information on any of the test components, point the cursor at that object and hold down the Control (Ctrl) Key.
6. The idea is to place the correct test components into the reaction chamber in the correct order. So you are making, testing and modifying hypotheses. Don't be afraid to make mistakes, even on purpose. Mistakes will provide you with more clues to solve the puzzle. Have fun.

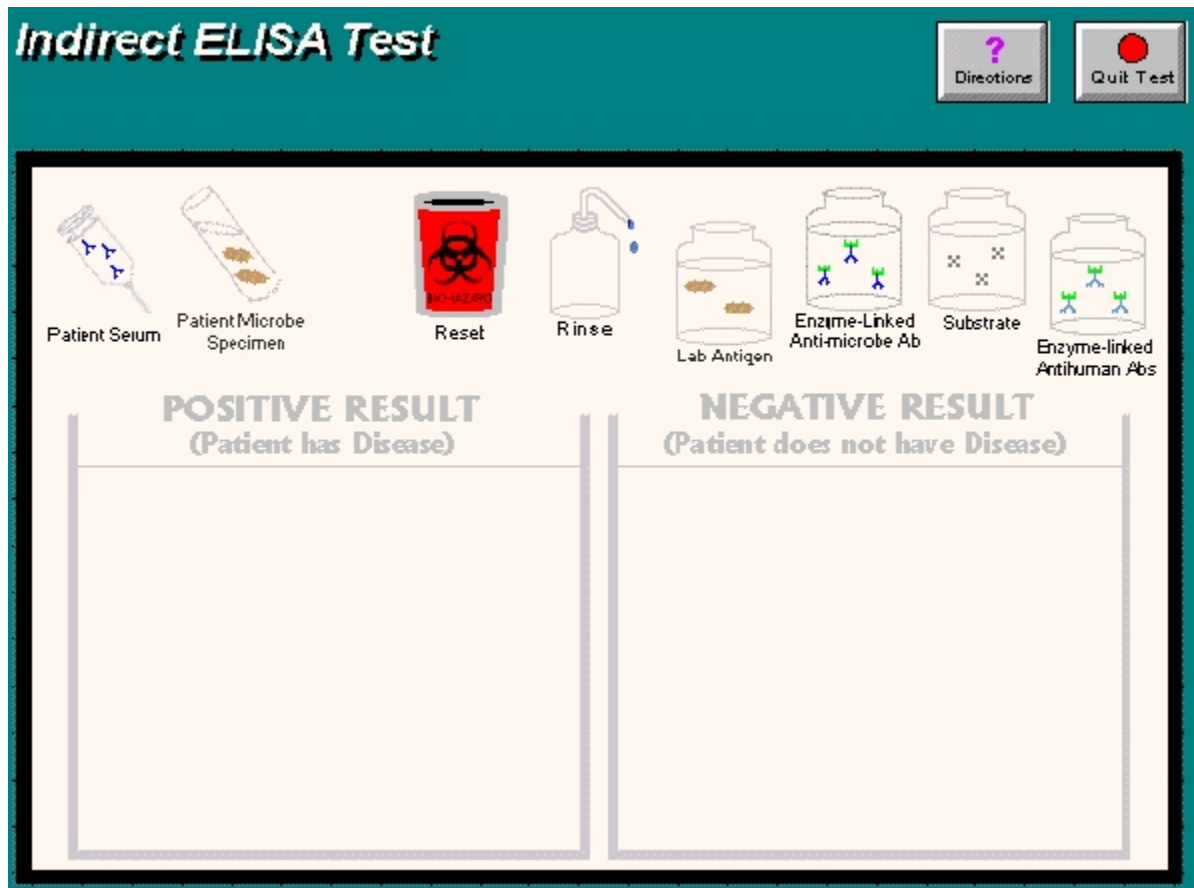


Figure 24.1 Serologic Computer Simulation

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RPR Test for Syphilis

1. Label three rings of an RPR typing card "R" (reactive), "W" (weakly reactive), and "N" (negative).
2. Shake the RPR dropper bottle to thoroughly suspend the cardiolipin antigens. Hold the dropper vertically and release one drop of RPR antigen (cardiolipin) onto each of the labeled circles on your card.
3. Small plastic dispensing tubes or dropper bottles are provided for measuring the serum samples. Place one drop of the serum sample onto the appropriately labeled circle on the card. (If using dispensing tubes, use a separate tube for each serum sample.) Use a toothpick or the flattened end of the dispensing tube to mix the sera and antigen, and spread the mixture to fill the circle.
4. Place the RPR card on the rotating machine (100 rpm) for eight minutes.
5. Following rotation, tilt the card back and forth 3-4 times.
6. Observe the results while they are still wet under bright lighting. If available, a dissecting microscope can be used to observe the reaction more closely.

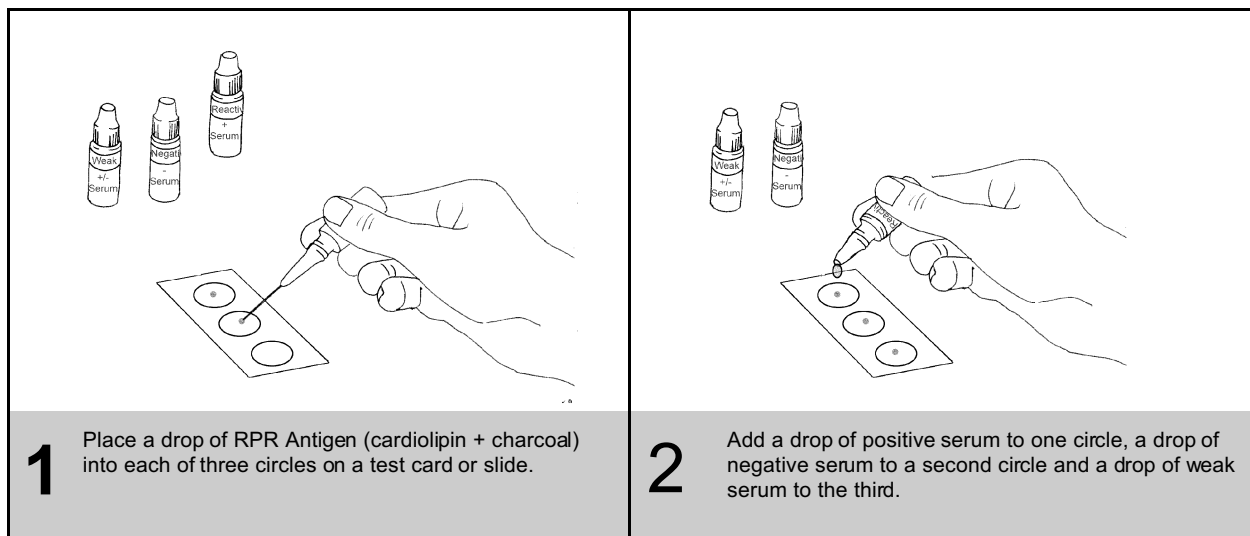


Figure 24.2 RPR Test

Results

- The RPR cardiolipin antigen is an emulsion that appears as rod-like particles. A positive reaction for patient antibodies is indicated by the formation of clumped, black aggregates or flocs. Fill in the following table with 0 to ++++ for your results, where 0 indicates separate rod-like particles without clumping and 4+ indicates a strongly positive reaction with very large flocs.

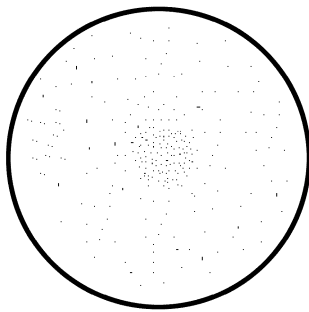


Figure 24.2 Negative Results

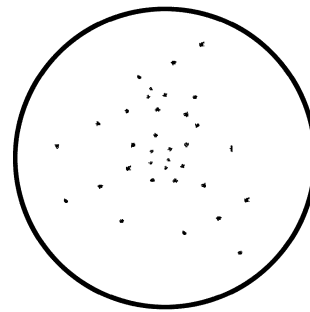


Figure 24.3 Positive Results

SERUM SAMPLE	REACTION
Reactive	
Weak	
Negative	

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Discussion

1. Define the following terms:

a. direct test

b. indirect test

c. heterophile antigen

d. reagin

e. agglutination

f. precipitation

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2. List the steps of the ELISA test (using HIV as an example). Include diagrams for each step, contrasting the results for a positive sample versus a negative sample.

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3. List the steps of the Complement Fixation Test. Include diagrams for each step, contrasting the results for a positive sample versus a negative sample.

Scientific Inquiry

- Survey the class results and based on this data, determine the specificity and sensitivity of the RPR test.