

## **Serial Dilution to Detect Cold Reacting Antibodies**

### **Objectives:**

1. Perform a serial dilution to determine the amount of cold reacting antibody present in a patient specimen with the results obtained falling within  $\pm 1$  tube of instructor's value.
2. Properly dispense the correct amounts of diluent and red blood cells and transfer the necessary amount of serum from tube to tube, using great precision and care.
3. Calculate the dilution of each tube in the serial dilution once all reagents and patient sample have been added.
4. Recognize the clumping of red blood cells as agglutination and properly interpret and record each tube as being positive or negative for agglutination according to the criteria in the procedure.
5. Recognize the endpoint for the test and correctly interpret and record the titer.
6. List 2 limitations of the procedure and describe how the results will be affected.

### **Principle:**

Antibodies may be produced in response to disease producing microorganisms or to other structures recognized as foreign by the human body. Antigens are found on the surface of red blood cells and their presence can be detected by adding a known antibody specific for the antigen on a red blood cell sample. If the antigen to which the antibody is directed is present then agglutination (clumping) of the red blood cells will occur. This type of reaction is called hemagglutination (agglutination of red blood cells).

Serial dilutions are performed to determine the *amount* of antibody present in a patient sample. The serum is diluted out several times and the highest dilution to give a positive reaction is the end point, or titer, of the sample. For the lab being performed today a dye has been added to the antibody containing sample to be tested. As the procedure is performed notice how the color of the antibody solution and strength of the red cell agglutination weakens as the solution is diluted out. This is indicative of fewer number of antibody molecules being present in the test system. This is one popular method used to quantitate the amount of antibody present in a patient specimen and is called a **titration** procedure. The following titration procedure is a serial dilution. In a serial dilution each succeeding tube in the procedure will contain one-half the amount of substance being detected as the previous tube. This allows an easy mathematical calculation of the titer.

### **Limitations of the Procedure:**

1. This procedure must be performed with great precision and care. Dispensing incorrect quantities of diluent or red blood cell solution or transferring more or less than the required amount of diluted serum will adversely affect the outcome of this test, resulting in a falsely increased or decreased titer.
2. The temperature of incubation is critical to the ability of the antigen-antibody reaction to occur in the tube. Warmer temperatures for the cold agglutinin test will prevent or decrease the amount of reactivity resulting in a falsely negative or decreased titer. Too cold of a temperature may cause hemolysis of the red blood cells, making the test invalid.
3. The technique for shaking the tubes to detect agglutination is critical. Harsh shaking may cause weak or fragile agglutinates to break apart, resulting in a false negative result in the tube and a false decrease in the reported titer.

**Procedure:**

- Materials:**
1. Antibody A
  2. 3% Red blood cell suspension
  3. 0.85% Saline
  4. Five 12 x 75 Test tubes
  5. Test tube rack
  6. Three serological pipets
  7. Refrigerator
  8. Sharpie or water proof marker

- Procedure:**
1. Label five tubes 1 - 5.
  2. Place 0.25 ml of saline in each of the five tubes.
  3. Use a clean serological pipet to draw up 0.25 ml of antibody A. Add the antibody to tube #1 by carefully lowering and raising the solution into the pipet three times to mix, being careful to avoid creating bubbles in the mixture.
  4. Draw up 0.25 ml from tube #1 and transfer to tube #2. Again raise and lower to solution into the pipet three times to mix.
  5. Repeat step 4, transferring 0.3 ml tube #2 to tube #3, then #4, then #5, etc. Discard 0.25 ml from tube #5.
  6. Use a clean serological pipet to add 0.25 ml of 3% red blood cell suspension to each tube.
  7. Mix well and show your instructor your tubes.
  8. Place in refrigerator for 30 minutes.
  9. Remove from refrigerator, centrifuge for 20 seconds. Read immediately for agglutination by *gently shaking the tube to dislodge the red blood cell button*. If the tubes are shaken too roughly false negative reactions can occur. Clumping of the red blood cells is positive. A smooth, uniform appearance of red blood cell suspension is negative.

**Interpretation:** The last tube showing agglutination is the *endpoint* of the test. The *titer* is reported out as the reciprocal of the last dilution showing a positive result.

Name \_\_\_\_\_

Date \_\_\_\_\_

**Laboratory 1: Serial Dilution**  
Recording/Interpreting Results

1. After you have set up your tubes for the dilution procedure show them to your instructor *BEFORE* placing them in the refrigerator for incubation (2 points).
2. In the chart below record the reactions on the “Observed Result” row for your visual observation of each tube after centrifugation and shaking has been performed. Record “*aggt*” for agglutination and “*no aggt*” for no agglutination. *Please remember that only results written in ink will be accepted, pencils are not allowed for recording results in the clinical laboratory.* (1 point)

*Fill in the chart according to the directions given below*

Tube Number	1	2	3	4	5	Titer
<b>Instructor Check</b>						
<b>Observed Result</b>						
<b>Dilution</b>						
<b>Titer</b>						

3. Directions for chart:
  - a. In the first tube you added 0.25 mL of patient serum, 0.25 mL diluent, and 0.25 mL of red blood cells. Calculate the dilution value for tube number 1 and *record it in the “Dilution” row in the chart above* (1 point)
  - b. Based on the value of tube 1, determine the dilution values for tubes 2-5 and *record them in the chart above in the “Dilution” row* (0.5 points each).
  - c. Convert each of the dilutions to titers and record in the appropriate column of the “**Titer**” row. (2 points)
  - d. The end point for this test is the last tube which shows agglutination. For example, if tube 4 has agglutination and tube 5 does not, then tube 4 is your endpoint. Record in the space provided the number of the tube which was your *endpoint?* (1 point)  
Endpoint \_\_\_\_\_
  - e. A titer is reported out as the reciprocal of the dilution of the endpoint tube. For example, an endpoint tube had a dilution factor of 1:64, the titer is reported out as 64. *Record the titer of your endpoint in the appropriate column above* (2 points).
  - f. List 2 limitations of this procedure *AND* describe how this would affect the results of the test.(2 points)