

EXERCISE 2: Hemolysin Titer

Textbook: None

Skills: 10 points

Objectives:

1. Using provided serum and diluent, carefully prepare a series of dilutions according to the instructions provided.
2. Following instructions, evaluate tubes to determine the presence or absence of hemolysis.
3. After following all instructions, determine the endpoint of the procedure obtaining results falling within ± 1 tube of instructor's value.
4. Appropriately record and report results as instructed.
5. Utilize lecture notes, textbook and laboratory information to answer study questions.

Introduction:

Red blood cells are occasionally used in serological testing as indicators of antigen-antibody reactions. The agglutination of red cells is the more common approach, but red cell hemolysis is also used. The hemolysis of the red blood cell membrane can result as the consequence of the interaction of an antigen on the red cell and an antibody (hemolysin). The term hemolysis is applied when the red blood cell membrane is broken and hemoglobin is released from the cell. The supernatant of a hemolyzed specimen will appear clear red, while the supernatant of non-hemolyzed specimens will be colorless.

Principle:

A hemolytic antibody has the ability to bind to a specific antigen and cause destruction of the red blood cell. In a serial dilution the amount of hemolytic antibody present in the test system decreases

EXERCISE 2: Hemolysis

Procedure:

Materials:

1. Patient serum
2. Hemolysin antibody
3. 5% Red blood cell suspension
4. Five 12 x 75 test tubes and test tube rack
5. Three 1 mL serological pipets and pipet bulb
6. 37°C heat block or waterbath
7. Gloves
8. Timer
9. Sharpie marker
10. Serofuge

Procedure:

1. Label five tubes with patient information and numbers 1-5
2. Prepare the dilutions according to the following table.

Tube Number:	1	2	3	4	5
Serum amount:	1.0 mL	0.8 mL	0.6 mL	0.4 mL	0.3 mL
Hemolysin amount:	none	0.2 mL	0.4 mL	0.6 mL	0.7 mL

3. Shake to mix well and show your tubes to the instructor.
4. Add 0.5 mL of a 5% red blood cell suspension to each tube and note the appearance of the tubes.
5. Shake gently to mix and incubate at 37°C for 5 minutes.
6. Centrifuge all tubes for 2 minutes and examine supernatant for hemolysis.

Interpretation:

The last tube / lowest numbered tube with no hemolysis is the *endpoint* of the test.

EXERCISE 2: Hemolysis

Recording/Interpreting Results

Name _____

Date _____

1. Record your observations in the following chart by putting “H” in the appropriate place(s) for tubes showing hemolysis and “NH” for those without hemolysis.
2. According to the instructions, identify the tube that is the endpoint of the test and record it in the appropriate place.

Patient Identification: _____				
Tube 1	Tube 2	Tube 3	Tube 4	Tube 5

Endpoint is tube # _____ .