

Antistreptolysin O Titer

Objectives:

1. Review information presented in the Streptozyme lab.
2. Follow instructions of the reagent package insert to select, and evaluate appropriate specimens for Streptolysin O testing.
3. Perform the ASO titer for the detection of antistreptolysin O antibodies to obtain control and patient results that match instructor values within ± 1 tube accuracy.
4. Evaluate control and patient results on instructor prepared ASO titers with 100% accuracy.
5. Review notes and product inserts 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.
6. Appropriately record and report results as instructed.
7. Utilize lecture notes, textbook and laboratory (including product insert) information to answer study questions.

Introduction:

The antistreptolysin O (ASO) test is the most widely used serological test for the detection of *Streptococcus A* sequelae. Since streptolysin is only one of several *Streptococcus A* exoenzymes, the ASO test will not detect the other antibodies to exoenzymes of *Streptococcus A*.

In the course of streptococcal infections, the extracellular products of the bacteria act as antigens to which the body responds by producing specific antibodies. Streptolysin O (SLO) is one of two hemolysins (the other being Streptolysin S) produced by virtually all strains of *Streptococcus pyogenes*. The letter "O" indicates that this toxin is oxygen labile. The SLO toxin has direct toxic effects on heart tissue. The toxic effects of SLO can be demonstrated in-vitro, as when added to a suspension of red blood cells, hemolysis will occur in minutes. In the course of a streptococcal infection, SLO stimulates the production of specific antistreptolysin antibodies, which in-vitro, neutralize the hemolytic properties of the antigen, SLO.

A titer of antistreptolysin O (ASO) is useful in the investigation of the disease processes related to streptococcal infections, such as acute poststreptococcal glomerulonephritis and rheumatic fever.

Principle:

This is a **neutralization** procedure. A specified quantity of streptolysin O (bacteria produced antigen) is added to progressively decreasing amounts of patient serum. In the tubes where patient antistreptolysin O antibody is present in sufficient amount to neutralize the antigen, no hemolysis will occur when indicator red cells are subsequently added. When the antigen exceeds the antibody, the excess streptolysin O will cause hemolysis of the indicator red blood cells. Put another way, in a test system, the patient's serum containing antistreptolysin O antibody is added to streptolysin O, an antigen-antibody reaction takes place, which, depending on the antibody level, completely or partially neutralizes the hemolytic action of the streptolysin O.

Materials:

1. Streptolysin O buffer
2. Streptolysin O reagent
3. ASO Standard
4. 5% sheep red cells (Or human group O, Rh negative red blood cells. 5 mL washed packed cells in 95 mL of SLO buffer.)
5. 37° C heat block or water bath
6. Four 2 mL serologic pipets, (can use 1 mL serologic pipets)
7. Test tube rack
8. Nineteen 12x75 mm tubes
9. Three 16x150 mm tubes
10. Patient serum specimen
11. Timer

Procedure:

See reagent product insert to verify the procedure.

1. Consecutively number fourteen (14) 12x75 mm tubes.
2. Maintain patient identity by placing their initials on the tubes.
3. Label five (5) 12x75 mm tubes S3 through S7. (These are the Standard's tubes and should be placed in the rack behind the patient's 3-7 tubes.
4. In the three 16x150 mm tubes, prepare dilutions of the patient's serum as follows: (be sure to mix each, before pipetting to the next)

Tube marked: 1:10 = 0.5 mL patient serum + 4.5 mL SLO buffer.

Tube marked: 1:100 = 1.0 mL of the 1:10 dilution + 9.0 mL SLO buffer.

Tube marked: 1:500 = 2.0 mL of the 1:100 dilution + 8.0 mL SLO buffer.

5. Set up and perform the test according to the product insert and according to instructor's directions. *The Standard (treated as a 1:100 dilution) is set up in tubes S3 - S7 only.*
6. Because it is oxygen labile and will lose its reactivity in a short time period, the Streptolysin O reagent must be added to tubes within 15 minutes after the reagent is prepared. Two students will use each bottle. Notify instructor when ready.

Interpretation:

The ASO antibody titer is the reciprocal of the highest serum dilution that **prevents hemolysis** of the cells and is reported out in Todd units. The last tube showing "no hemolysis" is the endpoint. For example, a serum showing "no hemolysis" in tubes 1-4, a trace of hemolysis in tube 5, and marked hemolysis in the remaining tubes is reported as containing 125 Todd units.

The ASO Standard (tubes S3 - S7) is expected to produce a titer of 166 Todd units (endpoint tube # 5, ± 1 tube).

Control tubes must give the expected results for the test to be valid.:

Tube number 13 is the cell control and must have “no hemolysis”.

Tube number 14 is the SLO control and must have complete “hemolysis”.

Expected Results:

Healthy people may have ASO titers as high as 125 Todd units as a result of previous exposure to streptococcal organisms and subsequent contact with streptolysin O. Therefore a single titer on a patient is of little value and clinical significance should only be attached to changes in ASO titers from specimens collected over a period of time.

Precautions:

1. Reagents and specimens must be at room temperature.
2. Streptolysin O Reagent should not be rehydrated until ready to be added to the tubes. Coordinate with the instructor and a classmate, as two students will be using each bottle of the SLO reagent.
3. ASO Standard (in tubes S3 - S7) has a target titer of 166. Consult with instructor if other results are obtained.
4. Control tubes must give expected results.
5. Test is very technique dependent. Check your tube volumes frequently as directed by instructor. A technician repeatedly performing this test should consistently produce results ± 1 tube dilution.

EXERCISE 11: ASO TITER

Name _____
 Test Kit Name _____
 Lot Number _____

Date _____
 Manufacturer _____
 Expiration Date _____

Fill in the following chart as follows:

For “Std (standard) endpoint” and “patient endpoint” write in the tube number of the endpoint of your standard and patient.
 For “Std Interp” and “patient interpretation” write the ASO titer using the correct units.
 For tubes #13 and #14 write “**H**” for hemolysis or “**NH**” for no hemolysis as your observation of the tube.

Patient Name and Number	Tube number Std Endpoint	Std Interp	#13 H/NH	#14 H/NH	Tube Number of Patient Endpoint	Patient Interpretation
1.						
2. Instructor Sample						
3. Instructor Sample						

Interpretation of validity of results:

For each of the following state whether the above results are valid; and write in “yes” or “no” in the appropriate space.

Patient Name and Number	Valid Standard Results “YES” or “NO”	Valid Hemolysin Control? “YES” or “NO”	Valid RBC Control? “YES” or “NO”	Can results be reported out? “YES” OR “NO”
1.				
2. Instructor Sample				
3. Instructor Sample				

ASO Study Questions

Name _____

Date _____

Using your textbook, lecture and lab results and notes, answer the following questions. Each question is worth one point, unless otherwise indicated.

1. What two toxins are produced by the Streptococcal organism? Which one is oxygen labile?

2. Explain in detail the 1) contents, 2) purpose and 3) expected results of tube #13.

3. Explain in detail the 1) contents, 2) purpose and 3) expected results of tube #14.

4. What is the purpose of performing testing on the standard? What are the expected results?

5. What occurs when SLO is released into the tissues of a patient?

6. Briefly describe the principle of the ASO titer.