

Anti Streptolysin O (ASO) Test:

Principle, Procedure and Interpretation.

What is Anti-Streptolysin O (ASO)?

Anti-Streptolysin O (ASO or ASLO) is the antibody made against streptolysin O, an immunogenic, oxygen-labile hemolytic toxin produced by most strains of group A and many strains of groups C and G streptococci.

The ASO titer test is a blood test that checks for a strep infection. When you come into contact with harmful bacteria, your body produces antibodies to defend itself against these bacteria and produces antibodies specific to the bacteria they fight. Usually, when you have a strep infection like strep throat, you receive antibiotics that kill the strep bacteria. But some people don't have any symptoms during a strep infection and may not know they need treatment. When this happens, an untreated infection can lead to future complications. These complications are known as post-streptococcal complications. The letter "O" indicates that this toxin is oxygen labile. The SLO toxin has direct toxic effects on heart tissue. In the course of a streptococcal infection, SLO stimulates the production of specific anti streptolysin (ASO) antibodies, which in-vitro; neutralize the hemolytic properties of the antigen.

Principle of the Test

The ASO LATEX REAGENT is a stabilized and buffered suspension of polystyrene latex particles that have been coated with streptolysin-O. When the LATEX REAGENT is mixed with serum containing antibodies to streptolysin-O, agglutination occurs. The LATEX REAGENT has been adjusted so that agglutination will take place only when the level of antibodies to streptolysin-O is greater than 200 IU/ml, a level determined by epidemiological and clinical studies to be indicative of disease.

Clinical Significance of ASO Test

- An anti streptolysin titer **greater than (>200 IU)** is considered a positive test in adults, while The normal value for adults is less than **(>200 IU)** , which indicates a negative test. Measurement of ASO antibody titer is important in the investigation of post-streptococcal diseases, particularly **acute post-streptococcal glomerulonephritis** and **rheumatic fever**.
- Over 80% of patients with acute rheumatic fever and 95% of patients with acute glomerulonephritis have elevated titers of ASO.
- The antibodies level starts to rise in 1-3 weeks after streptococcal infection, peaks in 3-5 weeks, and then goes back to insignificant level over 6-12 months, so a positive test can indicate current but more recent group A, C, and G streptococcal infection.

TEST PROCEDURE

PREPARATION FOR THE ASSAY

1. Allow all reagents and samples to warm to room temperature (20-30°C) before use. Remove reagents from foam holders. Do not heat reagents in a water bath.
2. All reagents are ready for use as supplied. Gently mix the reagents before use; avoid foaming.
3. Gently mix the LATEX REAGENT before each use to ensure homogeneity.

ASSAY PROTOCOL - QUALITATIVE

1. Bring all reagents and specimens to room temperature.
2. Place one drop (50 µl) of the positive control and 50 µl of the patient serum into separate circles on the glass slide.
3. Shake the ASO latex reagent gently and add one drop (45 µl) on each circle next to the sample to be tested and control.
4. Mix well using disposable stirrer spreading the mixture over the whole test area and tilt the slide gently. Agitate for about 2 minutes with rotator or by hand and observe for the presence or absence of agglutination.



ASSAY PROTOCOL - SEMI QUANTITATIVE

1. Prepare serial dilutions of patient serum, in saline, in test tubes as follows:

<u>Tube</u>	<u>Dilution</u>	<u>Composition</u>
1	1:2	0.25 ml of serum + 0.25 ml of saline. Mix.
2	1:4	0.25 ml from tube 1 + 0.25 ml of saline. Mix.
3	1:8	0.25 ml from tube 2 + 0.25 ml of saline. Mix.
4	1:16	0.25 ml from tube 3 + 0.25 ml of saline. Mix.
5	1:32	0.25 ml from tube 4 + 0.25 ml of saline. Mix.

Testing on additional dilutions should be performed as needed.

2. Using each dilution as a separate test specimen, apply the samples to the card as described in Step 3 of the Qualitative Assay Protocol and proceed through 4 of the Qualitative Assay Protocol. Include undiluted sample if not tested previously on that day with the same lot of LATEX REAGENT.

INTERPRETATION OF RESULTS- QUALITATIVE

Agglutination indicates an ASO concentration of greater than or equal to 200 IU/ml in the serum sample. Sera that elicit a positive result should be retested and titered using the Semiquantitative Assay Protocol.

INTERPRETATION OF RESULTS- SEMIQUANTITATIVE

The highest dilution in which visible agglutination occurs is considered the endpoint titer. The corresponding ASO concentration (in IU/ml) is calculated as the product of the endpoint dilution factor and the assay cut-off value as shown in the following table. For example, if the endpoint dilution is 1:8, the corresponding ASO serum concentration would be 8 x 200, or 1600 IU/ml.

Dilution	ASO IU/ml (in NEAT specimen)
NEAT	200
1:2	400
1:4	800
1:8	1600
1:16	3200
1:32	6400