

5. The degree of agglutination observed in undiluted samples is not indicative of antibody levels since a prozone effect may limit agglutination.
6. The PULSE ASO TEST latex reagent vial must be kept tightly closed to prevent evaporation and subsequent flocculation.
7. Patients on therapy of penicillin or other antibiotics may suppress a rise in ASO titer.
8. Only serum specimens should be used. Do not use plasma samples as they could cause non-specific agglutination of the latex.

EXPECTED VALUES

A detectable level of 200 IU/ml ASO antibodies is usually regarded as the normal upper limit since less than 15-20% of healthy individuals demonstrate titers greater than 200 IU/ml when their sera are assayed. In most newborns the titer is initially greater than that of the mother due to maternally acquired IgG but the levels fall sharply during the first weeks of life.

Normal ASO levels for preschool children are generally less than 100 IU/ml but the levels rise with age, peaking in school age and decreasing in adulthood.

Increases in ASO titer generally occur one (1) to four (4) weeks after onset of infection with β -hemolytic streptococci Group A. As the infection subsides, the titer declines and returns to normal levels within six months. If the titer does not decrease, a recurrent of chronic infection may exist.

Elevated ASO titers may be associated with ankylosing spondylitis glomerulonephritis, scarlet fever, and tonsillitis. Increased ASO levels are generally not found in sera of patients with rheumatoid arthritis except during acute episodes.

Extremely low levels of ASO have been observed in the blood samples of patients with nephrotic syndrome and antibody deficiency syndromes

PERFORMANCE CHARACTERISTICS

The PULSE ASO TEST was compared to the ASO Latex Test manufactured by Behring. A total of 42 specimens were evaluated and a sensitivity of 95% and a specificity of 100% were obtained. The titres of the specimens were determined using both the PULSE ASO TEST, the Behring ASO Latex Test and the Behring Nephelometer Test. The PULSE ASO TEST titres gave a closer agreement to the Behring Nephelometer results.

<u>Expected Result</u>	<u>PULSE ASO TEST</u>	<u>Behring ASO Test</u>
Positive	19*/20	18*/20
Negative	22/22	22/22

*The negative specimen was determined to contain 199 IU/ml ASO using the Behring Nephelometer.

*The two negative specimens were determined to contain 239 and 242 IU/ ml ASO using the Behring Nephelometer.

BIBLIOGRAPHY

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Antistreptolysin - O (ASO) Latex Test

INTENDED USE

The PULSE ASO LATEX TEST (PULSE ASO TEST) is intended to be used for the qualitative screening and semi-quantitative determination of Antistreptolysin-O antibodies (ASO) in serum.

SUMMARY AND PRINCIPLES

In infections caused by B-hemolytic streptococci, Streptolysin-O is one of the two hemolytic exotoxins liberated from the bacteria that stimulates production of ASO antibodies in the human serum. The presence and the level of these antibodies in serum may reflect the nature and severity of infection.

The PULSE ASO TEST is a stabilized buffered suspension of polystyrene latex particles that have been coated with Streptolysin O. When the latex reagent is mixed with serum containing ASO, agglutination occurs. The sensitivity of the latex reagent has been adjusted to yield agglutination when the level of ASO is greater than 200 IU/ml, a level determined to be indicative of disease by epidemiological and clinical studies.

MATERIALS SUPPLIED

ASO Latex Reagent: Contains polystyrene latex particles coated with Streptolysin O in a stabilized buffer with less than 0.1% sodium azide as preservative.

ASO Positive Control: Human serum containing more than 200 IU/ml ASO with less than 0.1% sodium azide as preservative.

ASO Negative Control: Human serum that has been diluted and stabilized with buffer and contains less than 0.1% sodium azide as preservative.

Disposable pipettes and test slides

Additional Items Required:

Physiological saline, serological pipettes, 12 x 75 mm test tubes and timing device.

STORAGE & STABILITY

When not in use, store reagents and controls at 2 - 8 degrees Celsius. DO NOT FREEZE. Prior to use, allow reagents and controls to warm up to room temperature. Expiration date is specified on the kit label and on each vial. Biological indication of product instability is evidenced by inappropriate reaction of the latex reagent with the corresponding positive and negative control sera.

PRECAUTIONS

This product is for In Vitro Diagnostic Use Only.

Each donor unit used in the preparation of this product has been tested by an FDA approved method and found non-reactive for the presence of HbsAg and antibody to HIV Virus. Because no known test method can offer complete assurance that hepatitis B virus, HIV Virus, or other infectious agents are absent, all human blood based products should be handled in accordance with good laboratory practices. The preservative sodium azide may react with metal plumbing to form explosive metal oxides. In disposal, flush with a large volume of water to prevent metal azide build up.

SPECIMEN COLLECTION

The test should be performed on fresh serum specimens only. Plasma must not be used since fibrinogen may cause non-specific agglutination of the latex. Fresh specimens (less than 24 hours) should be used in performing the test. Serum samples may be stored at 2 - 8 degree Celsius for up to 48 hours prior to testing. If longer storage is necessary, sera should be frozen at -20 degree Celsius. Bacterial contamination may cause false positive agglutination.

PROCEDURE

A. Method I (Qualitative)

1. Bring all test reagents and serum specimens to room temperature.
2. Gently shake the ASO latex vial to disperse and suspend latex particles.
3. Positive and negative controls should be tested with each series of test.
4. Using the disposable pipette provided, place one drop of test serum onto a circle on the slide. Use a separate disposable pipette for each test serum. Important: The Pulse ASO Latex Reagent must be agitated well for about 10 seconds prior to using on each day's testing. Do not use a vortex mixer. Deliver one drop of ASO Latex to each circle that contains specimens on the slide. Spread the resulting mixture by using the paddle end of the pipette. Do not use the same paddle end to mix each test serum or control as this will cause cross-contamination.
5. Gently tilt and rotate slide by hand for two (2) minutes.
6. Observe for macroscopic clumping under a high intensity light.
7. Compare the reaction of the test serum to the ASO positive and negative control sera.

B. Method II (Semi-Quantitative)

1. For each test serum to be titrated, set up a least 6 test tubes (12 x 75 mm) and label 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, etc.
2. To each tube add 0.2 ml of physiological saline.
3. To Tube No. 1 add 0.2 ml of undiluted test serum.
4. Serially make two-fold dilutions by mixing contents of Tube No. 1 with pipette and transferring 0.2 ml to Tube No. 2. Repeat serial transfers for each tube. For the

tubes, the dilutions range from 1:2 to 1:64. If required, additional serum dilutions can be added.

5. Repeat steps 3 to 7 as given in Method I (Qualitative).

RESULTS

Qualitative

Positive Result: Agglutination

Negative Result: Smooth milky suspension

Since negative results may be caused by antigen excess, the test should be repeated using a diluted serum sample in case prozone effect is suspected.

Semi-Quantitative

Sera that are positive in the screening test should be retested in the titration test to provide verification for borderline interpretations. The greatest dilution of test sample showing agglutination is considered the endpoint. Multiplication of the dilution factor by 200 IU/ml will yield the approximate level of ASO present. The following table is only shown as an example for the determination of ASO concentration in specimen. Actual specimen will have ASO concentrations higher or lower than the levels indicated in this table.

<u>DILUTION</u>	<u>CONCENTRATION (IU/ml)</u>
1:1 (neat specimen)	200
1:2	400
1:4	800
1:8	1600
1:16	3200
1:32	6400

QUALITY CONTROL PROCEDURE

Positive and Negative controls should be included in each test series. The Positive control should produce visible agglutination; and Negative control should produce no agglutination.

LIMITATIONS OF THE PROCEDURE

1. The results of this test SHOULD NOT be used as a single diagnostic tool to make a clinical diagnosis. Instead, the test results must be evaluated together with other clinical findings and observed symptoms to aid in the final diagnosis.
2. This test is designed to be performed by hand rotation. The use of a mechanical rotator could yield false positive/negative results.
3. Serum samples showing gross hemolysis, lipemia, turbidity, or contamination should not be used since false positive results may occur. Both elevated Beta-lipoprotein and cholesterol level may suppress a rise in ASO titer.
4. The test reaction must be read immediately following the two (2) minutes rotation. Delayed readings may result in false positive results.