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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 Anti-streptolysin-O antibodies (ASO) in serum.

SUMMARY AND PRINCIPLES
In infections 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 ASO antibody level in serum may reflect the nature and severity of infection. The Pulse ASO TEST is a suspension of latex particles coated with Streptolysin O to show agglutination in serum containing > 200 IU/ml of ASO (sensitivity = 200IU.ml). ASO levels at > 200IU/ml is indicative of disease by epidemiological and clinical studies.

MATERIALS SUPPLIED
ASO Latex Reagent: Latex particles coated with Streptolysin O in buffer with < 0.1% sodium azide.
ASO Positive Control: Human serum (> 200 IU/ml ASO) with < 0.1% sodium azide.
ASO Negative Control: Human serum with < 0.1% sodium azide.
Disposable pipettes and test slides.
Additional Items Required: Serological pipettes, 12 x 75 mm test tubes and timing device.

STORAGE & STABILITY
Store product at 2 - 8 degree Celsius. Do not freeze. Before use, warm reagent and controls to room temperature. Product instability is shown by improper reaction between latex reagent with the positive and negative control sera.

PRECAUTIONS
This product is for In Vitro Diagnostic Use Only. The positive and negative controls have been tested by an FDA approved method and found to be 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. In disposal, flush with a lot of water as sodium azide may react with metal plumbing to form explosive metal oxides.

SPECIMEN COLLECTION
Only fresh serum specimens (<24 hours) should be used. Plasma must not be used since fibrinogen may cause non-specific agglutination of the latex. Specimens can be stored at 2-8C for up to 48 hours if testing is delayed. 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. Positive and negative controls should be tested with each series of test.
3. Place one drop of specimen onto a circle on the slide. Use new pipette each time. Deliver one drop of ASO Latex to each circle that contains specimens or controls. Use paddle end of pipette to spread the mixture on each circle. Use new pipet each time avoid cross-contamination.
4. Gently tilt and rotate slide by hand for two (2) minutes. Observe for macroscopic clumping using the indirect oblique light source.

B. Method II (Semi-Quantitative)
1. For each tested serum, set up 6 test tubes 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 4 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 undiluted serum sample in case prozone effect is suspected.
Semi-Quantitative
Positive sera should be retested to provide verification for borderline interpretations. The highest dilution of sample showing agglutination is the endpoint. Multiplication of the dilution factor by 200 IU/ml will yield the approximate ASO level.
Dilutions/Concentration (IU/ml)
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
The results of this test should not be used as a single diagnostic tool to make clinical diagnosis. Other clinical findings and observed symptoms must be evaluated to aid in the final diagnosis. This test is to be performed by hand rotation. The use of a mechanical rotator could yield false positive/negative results. 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. Do not use plasma samples as they could cause non-specific agglutination of the latex. Delayed result readings (longer than two minutes) may lead to false positive results. Patients on therapy of penicillinor other antibiotics may suppress a rise in ASO titer. The degree of agglutination observed in undiluted samples is not indicative of ASO levels since a prozone effect may limit agglutination.

EXPECTED VALUES
ASO antibody level of 200 IU/ml is regarded as the normal upper limit since less than 15-20% sera of healthy individuals demonstrate titers > 200 IU/ml. In most newborns the titer is initially greater than that of the mother (maternally acquired IgG) but the levels fall sharply during the first weeks of life. Normal ASO levels for preschool children are less than 100 IU/ml. 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 β-hemolytic streptococci Group A infection. When 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 from patients with rheumatoid arthritis except during acute episodes. Low ASO levels may be detected in patient samples with nephrotic syndrome and antibody deficiency syndromes.

PERFORMANCE CHARACTERISTICS
The Pulse ASO TEST was compared to the ASO Latex Test from Behring. A total of 42 specimens were evaluated and a sensitivity of 95% and a specificity of 100% were obtained. Specimen titres were determined using ASO Test from both suppliers. including the Behring Nephelometer. The Pulse ASO TEST titres gave a closer agreement to the Behring Nephelometer results.

Expected Result	PULSE ASO TEST	Behring ASO Test
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 Behring Nephelometer yielded 239 and 242 IU/ml of ASO for the two negative specimens.

BIBLIOGRAPHY
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