# ASO

*Latex Agglutination Method*

**Intended Use:**
a - Streptolysin-O (ASO) Test reagent/kit is a medical device intended for the screening of a-Streptolysin-O (ASO) in human specimen. It is intended for *in vitro* diagnostics use by trained healthcare professionals in IVD laboratory only and not near patients.

**Clinical Significance**
The first group of streptococci is called β-hemolytic streptococci which can be further subdivided into group (a), group (b), group (c) and group (d). It includes most of the species associated with primary streptococcal infections in humans.
The group (a) β-hemolytic streptococci produce various exotoxins such as streptolysin-O and streptolysin-S that can act as antigens. The affected individuals produce specific antibodies against streptolysin-O, namely Anti streptolysin-O.
Determination of these antibodies is very useful for the diagnosis of streptococcal infections and their relative effects such as rheumatic fever and acute glomerulonephritis. An elevated ASO titer of more than 200 IU/ml may indicate an acute streptococcal infection.

**Principle**
ASO slide test for detection of antibodies to streptolysin-O is based on the principle of agglutination. The test specimen (serum) is mixed with ASO latex reagent and allowed to react. If antibodies to streptolysin-O are present then a visible agglutination is observed. If antibodies to streptolysin-O are not present or are in concentration less than 200 IU/ml then no agglutination will be observed.

**Reagent Composition**

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|---|---|
| R1 ASO LATEX | Latex particles coated with streptolysin O, pH 8.2, preservative |
| R2 ASO Positive Control | Synthetic Positive control with , preservative |
| R3 Negative Control | Synthetic Negative control with , preservative |

**Reagent Preparation**
Reagentsare ready to use.

**Stability and Storage**
1. Store the reagent at 2-8$^0$C. **DO NOT FREEZE.**
2. The shelf life of the reagent is as per the expiry date mentioned on the reagent vial labels.

**Material provided with the kit**
**Reagents**
ASO latex reagent, Positive Control, Negative Control

**Accessories:**
Glass slide, plastic droppers, mixing sticks.

**Additional Material Required**
Stop watch, High intensity direct light source, Saline, Pipettes, Test tubes.

**Notes**
1. In vitro diagnostic reagent for laboratory and professional use only. Not for medicinal use.
2. All the reagents derived from human source have been tested for HBsAg and Anti HIV antibodies and are found to be non-reactive. However handle the material as if infectious.
3. Reagent contains 0.1% Sodium Azide as a preservative. Avoid contact with skin and mucosa. On disposal flush with large quantities of water.
4. The reagent can be damaged due to microbial contamination or on exposure to extreme temperatures. It is recommended that the Performance of the reagent be verified with the positive and negative controls supplied with the kit.
5. The Latex reagent should be shaken well prior to use, to ensure homogeneous suspension of latex
6. Only a clean and dry glass slide must be used. Clean the slide with distilled water and wipe dry.
7. Accessories provided with the kit only must be used for optimum results.

**Calibration**
The ASO-latex sensitivity is calibrated against the ASO International Standard from NIBSC ASO.

**Sample and Stability**
Only fresh serum must be used for testing. Should a delay in testing occur, store the sample at 2-8$^0$C.Samples can be stored for up to a week. Do not use hemolyzed serum.
Freshserum, Stablefor 7days at2-8ºC or3monthsat-20ºC.Sampleswithpresence of fibrin should becentrifuged beforetesting.Donotusehighlyhemolyzed or lipemic samples.

**Procedure**
Bring reagent and samples to room temperature before testing.

**Qualitative Method**
1. Pipette one drop of serum onto the glass slide using the disposable plastic droppers provided with kit.
2. Add one drop of ASO latex reagent to the drop of test sample on the slide.
3. Using a mixing stick, mix serum and the ASO latex reagent uniformly over the entire circle. Do not let the dropper tip touch the liquid on the slide.
4. Immediately start a stopwatch. Rock the slide gently, back and forth, observing for agglutination macroscopically at **two minutes**. Proceed similarly with each dilution.
5. Do not read the results beyond 2 minutes.

**Semi Quantitative Method**
Using normal saline prepare serial dilutions of the serum sample positive in the quantitative method 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and so on.
1. Pipette the diluted specimens onto the slide. Start with the 1:2 diluted test specimen.
2. Add a drop of ASO reagent to it. Mix well. Spread the mixture uniformly over the entire circle.
3. Immediately start a stopwatch. Rock the slide gently, back and forth, observing for agglutination macroscopically at **two minutes**. Proceed similarly with each dilution as test specimen.

**Interpretation of Results**

**Qualitative Method**
Agglutination is a positive test result and indicates the presence of detectable levels of Anti-streptolysin O in the test specimen.
No agglutination is a negative test result and indicates the absence of detectable levels of Anti-streptolysin O in the test specimen.

**Semi Quantitative Method**
Agglutination in the highest serum dilution corresponds to the amount of ASO in IU/ml present in the test specimen.

**Calculations**
The concentration of ASO can be calculated as follows:
ASO (IU/ml) = S X D
Where,
S = Sensitivity of the reagent i.e., 200 IU/ml
D = Highest dilution of serum showing Agglutination

**Reference Values**
Up to 200 IU/ml(adults) and 100 IU/ml (children < 5 years old)[6]. Each laboratory should establish its own reference range.

**Performance Characteristics**
1. *Analytical sensitivity*: 200 (± 50) IU/mL, under the described assay conditions
2. *Prozone effect*: No prozone effect was detected up to 1500IU/mL.
3. *Diagnostic sensitivity:* 98%.
4. *Diagnostic specificity:* 97%.

**Interferences**
Bilirubin (20 mg/dL), hemoglobin (10 g/L), lipids (10 g/L), rheumatoid factors (300 IU/mL) do not interfere. Other substances may interfere[7].

**Limitations of the procedure**
- False positive results may be obtained in conditions such as, rheumatoid arthritis, scarlet fever, tonsilitis, several streptococcal infections and healthycarriers.
- Early infections and children from 6 months to 2 years may cause false negativeresults.
- A single ASO determination does not produce much information about the actual state of the disease. Titrations at biweekly intervals during 4 or 6 weeks are advisable to follow the diseaseevolution.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratorydata.

**Remarks**
1. Lipemic, hemolysed and contaminated serum samples could produce non-specific results.
2. Serum samples having higher protein content may produce non-specific reagent aggregation.
3. Use of plasma rather than serum can lead to false positive results.
4. Do not read results beyond **two minutes**.
5. It is recommended that all positive test results should be further tested with methods enabling quantization of ASO titers.
6. It is recommended that results of the tests should be correlated with clinical findings to arrive at the final diagnosis.

**References**


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***For In vitro Diagnostic Use Only***