

ASLO-Latex

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For <i>in vitro</i> diagnostic use only			

ASLO-Latex

Determination of anti-streptolysin O

SLIDE TEST

PRINCIPLE

ASLO-Latex Test is a rapid slide agglutination procedure, developed for the direct detection and semi-quantitation on a slide of clinically significant levels of anti-streptolysin O antibodies (ASLO) in serum.

The assay is performed by testing a suspension of latex particles coated with streptolysin O antigen against unknown serum. The presence or absence of a visible agglutination, indicates the presence or absence of ASLO in the samples tested.

REAGENT COMPOSITION

- R** **ASLO-Latex Antigen.** Suspension of polystyrene latex particles coated with stabilized streptolysin O in a buffered saline solution. Contains 0.95 g/L of sodium azide.
- CONTROL+** Human serum with an ASLO activity > 200 IU/mL. Contains 0.95 g/L of sodium azide.
- CONTROL-** Animal serum with an ASLO activity < 100 IU/mL. Contains 0.95 g/L of sodium azide.

Precautions: Components of different human origin have been tested and found to be negative for the presence of antibodies anti-HIV 1+2 and anti-HCV, as well as for HBsAg. However, the controls should be handled cautiously as potentially infectious.

Warning: The reagents in this kit contain sodium azide. Do not allow to contact with skin or mucous membranes.

PACKAGING CONTENTS

- REF** 2340005, kit 50 tests.
1 vial ASLO-Latex Antigen, 1x1 mL Positive control, 1x1 mL Negative control, 3 Test cards and 1x50 disposable stirrers.
- REF** 2340010, kit 100 tests.
2 vials ASLO-Latex Antigen, 1x1 mL Positive control, 1x1 mL Negative control, 3 Test cards and 2x50 disposable stirrers.

STORAGE AND STABILITY

Store at 2-8°C. Do not freeze. Frozen reagents could change the functionality of the test.
Antigen and Controls are stable until the expiry date stated on the label.

REAGENT PREPARATION

Antigen and Controls are ready to use.

SAMPLES

Fresh, clear serum.
After the clear serum has been separated it may be stored at 2-8°C for upto one week or longer periods at -20°C.

MATERIAL REQUIRED

- Automatic pipettes.
- Saline solution (0.9% NaCl, only for semi-quantitation procedure).
- Mechanical rotator, adjustable at 100 r.p.m.
- Laboratory alarm clock.

PROCEDURE

I. Qualitative Test

1. Bring the test reagents and samples to room temperature (Note 1).
2. Shake the latex vial gently. Aspirate dropper several times to obtain a thorough mixing.
3. Place 1 drop (50 µL) of the serum under test into one of the circles on the card. Dispense 1 drop of positive control serum and 1 drop of negative control into two additional circles.
4. Add 1 drop of ASLO-Latex Antigen to each circle next to the sample to be tested.
5. Mix the contents of each circle with a disposable stirrer while spreading over the entire area enclosed by the ring. Use separate stirrers for each mixture.
6. Rotate the slide by means of a mechanical rotator (100 r.p.m.) for a period of **2 minutes** (Note 2).
7. Observe immediately under a suitable light source for any degree of agglutination.

Reading

Nonreactive: Smooth suspension with no visible agglutination, as shown by negative control.

Reactive: Any degree of agglutination visible macroscopically.

II. Semi-quantitative Test

1. Dilute sample with CiNa 9 g/L as follow:

Dilution	1/2	1/3	1/4	1/5
Sample (µL)	50	50	50	50
CiNa 9 g/L (µL)	50	100	150	200
ASO (IU/mL) non-diluted sample	400	600	800	1000

2. Test each dilution as described in Qualitative Test.



Reading

Same as in Qualitative Test. The titer of the specimen is reported as the highest dilution that shows reactivity. The next higher dilution should be negative (Note 3).

The approximate ASLO level (IU/mL) present in the sample may be obtained multiplying the titer of the last positive dilution by the minimum detectable unit (analytical sensitivity).

e.g. titer 1/3

ASO concentration = 3 x 200 = 600 IU/mL

QUALITY CONTROL

Positive and negative controls should be run daily following the steps outlined in the Qualitative Test, in order to check the optimal reactivity of the antigen.

The positive control should produce clear agglutination. If the expected result is not obtained, do not use the kit.

EXPECTED VALUES^{1,2}

Ninety-five per cent of healthy adults have ASLO titers of 200 IU/mL or less, the highest titers been found in school children with titers up to 250 IU/mL. Since a single ASLO determination does not provide much information unless it is high, titrations at bi-weekly intervals for 4 to 6 weeks of the doubtful cases are advisable to follow the evolution of the disease. The ASLO titers resulting from ordinary streptococcal infections and acute rheumatic fever differ in that the titer of the later condition is usually much higher and persists for a longer period of time.

CLINICAL SIGNIFICANCE³⁻⁵

Elevated ASLO serum titers occur in response to infection with hemolytic streptococci of Group A, C y G, producers of streptolysin O, an extracellular protein of enzymatic character with strong antigenic properties. Immunochemical assay of these specific antibodies to streptococcal metabolites provide valuable information to establish a diagnosis of streptococcal infections (acute rheumatic fever, glomerulonephritis).

ASLO testing has a high diagnostic value on a tentative diagnosis made on the basis of case history and clinical findings.

ANALYTICAL PERFORMANCE

- The minimum detectable unit (analytical sensitivity) is of approximately 200 IU/mL (\pm 50 IU/mL), tested against a ASO International Calibrator (WHO).
- Diagnostic specificity: 97%.
- Prozone effect: No prozone effect was detected up to 1500 IU/mL.
- Results obtained with this reagent did not show significant differences when compared with reference reagents. Details of the comparison experiments are available on request.
- Hemoglobin (<10 g/L), bilirubin (<20 mg/dL) and lipemia (<10 g/L) do not interfere. Other substances may interfere⁶.

LIMITATIONS OF PROCEDURE

- Positive reactions do occur in conditions other than rheumatic fever and glomerulonephritis in which the production of ASLO is especially high. In scarlet fever, early and acute periods of rheumatoid arthritis, healthy carriers, complicated and no complicated tonsillitis and various streptococcal infections increased ASLO levels have been found.
- Biologically false negative reactions can occur in early primary infections and during the early years of life (from six months to 2 years).

NOTES

1. The sensitivity of the test may be reduced at low temperatures. The best results are achieved at 15-25°C.
2. Delays in reading the results may result in over-estimation of the antibody present.
3. Titers obtained with the latex test compare favourably with those obtained by the SHA within the range of precision of the two methods⁷.

SOURCES OF ERROR

- Bacterial contamination of controls and specimens as well as freezing and thawing of the antigen may lead to false positive results.
- Traces of detergent in the test cards may give false positive results. Wash used cards first under tap water until all reactants are removed and then with distilled water. Allow to air dry, avoiding the use of organic solvents as they may impair the special finish on the slide.
- The ASLO-Latex Antigen must not be used beyond its expiry date because prolonged storage can affect the sensitivity of the suspension.

REFERENCES

1. Klein, G.C. Manual of Clinical Immunology, chapter 33, American Society for Microbiology, Washington D.C. (1976).
2. Klein, G.C., Baker, C.N. and Jones, W.L. Applied Microbiology. 21: 999 (1971).
3. Halbert, S.P. Ann. NY Acad. Sci. 103 (1963).
4. Alouf, J.E. and Raynaud, M. Biochimie. 56 (1973).
5. Bisno, A.L. Principles and Practice of Infectious Diseases, 3rd ed. (Mandell GL et al. eds.), Churchill Livingstone, NY, 176-177 (1990).
6. Young, D.S. Effects of Drugs on Clinical Laboratory Tests. 4th Edition. AACC Press (1995).
7. Schmidt, K., Mueller-Eckardt, Ch. and Beckmann, A. Rheumatol. 29 (1970).

