

Enzyme Immunoassay for the Quantitative Determination of Anti-Streptolysin O in Human Serum and Plasma.

The serum titers of **anti-streptolysin O (ASLO)** may be of important diagnostic value in patients having a recent streptococcal group A infection, since the sequels of such infections include rheumatic fever, glomerulonephritis and erythema nodosum. Increased titers of ASLO develop after the second week of infection and reach a peak in 4 to 6 weeks: this peak usually occurs shortly after the onset of rheumatic fever.

ASLO is present in most individuals in low titers since streptococcal infections are common. Therefore, comparison of ASLO-titers at (bi-)weekly intervals yields more valuable information than a single determination. Elevated or increasing ASLO titers are indicative of recent infection.

The test is considered a valuable aid in the differential diagnosis of early rheumatic fever and rheumatoid arthritis (in which it is not elevated), when the clinical picture is not decisive^{1,2,3}.

The **ASLO ELISA manufactured by Advanced Practical Diagnostics BV** is an enzyme immunoassay for the quantitative determination of Anti-Streptolysin O in human serum and plasma.

PRINCIPLES OF THE ASLO ELISA

Microtiterstrips coated with antigen (streptolysin O) are incubated with diluted standard sera and patient samples. During this incubation step, ASLO is bound specifically to the coated wells. After removal of the unbound serum proteins by a washing procedure, the antigen-antibody complex in each well is detected with specific peroxidase-conjugated antibodies directed against human IgG.

After removal of the unbound conjugate, the strips are incubated with a substrate solution containing tetramethylbenzidin and hydrogen peroxide: a blue colour develops in proportion to the amount of immunocomplex bound to the wells of the strips. The enzymatic reaction is stopped by the addition of 0.5M H₂SO₄ and the absorbance values at 450 nm are determined.

A standard curve is obtained for each test by plotting the absorbance values versus the corresponding standard concentration values. The concentration of ASLO in patient samples is determined by interpolation from the standard curve.

REAGENTS

1. Specimen Dilution Buffer – [DIL] 5x

1 vial, containing 40 ml dilution buffer 5x concentrated. Contains 0.09 % NaN₃ and antimicrobial agents and an inert green dye.

2. Washing Solution - [WASH] 20x

1 vial containing 50 ml 20x concentrated phosphate buffered washing solution.

3. Chromogen Solution - [CHROM]

1 vial, containing 15 ml of a solution containing H₂O₂ and tetramethylbenzidin.

4. Stopping Solution - [STOP]

1 vial, containing 12 ml of 0.5M H₂SO₄.

5. Coated Microtiterstrips - [MTP]

12 x 8 well strips coated with Streptolysin O.

6a-6e. Standard Sera – [CAL] N

5 vials, each containing 1/10 prediluted ASLO standard solutions (0.5 ml), N having following values:

CAL 0: 0 IU/ml; CAL 100: 100 IU/ml; CAL 200: 200 IU/ml; CAL 400: 400 IU/ml; CAL 800: 800 IU/ml. Contain 0,09 % NaN₃.

7. Conjugate - [CONJ]

1 vial, containing peroxidase conjugated anti-human IgG antibodies (12 ml). Contains antimicrobial agents and an inert red dye.

8. Control Sample – [CTL]

1 vial, containing positive serum (0.1 ml). Target value is indicated on the label of the vial. Contains 0,09 % NaN₃.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Precision micropipettes and standard laboratory pipettes.
2. Clean standard laboratory volumetric glassware.
3. Clean glass or plastic tubes for the dilution of the samples.
4. A microtiterplate reader capable of measuring absorbances at 450 nm.

WARNINGS AND PRECAUTIONS FOR USERS

1. For *in vitro* diagnostic use only.
2. Human blood components used in the preparation of the standard sera have been tested and found to be non reactive for hepatitis B surface antigen, HCV antibodies and HIV I&II antibodies. Since no known method can ever offer complete assurance that products derived from human blood will not transmit hepatitis or other viral infections, it is recommended to handle these standard sera in the same way as potentially infectious material. Dispose of patient samples and all materials used to perform this test as if they contain infectious agents.
3. Do not mix reagents or coated microtiterstrips from kits with different lot numbers.
4. Chromogen Solution contains the hazardous ingredient N-Methyl-2-pyrrolidone at a concentration > 0,3 %. It is classified as a Reproductive Toxicant Category 1B. Following hazard statements are applicable: H360D: May damage the unborn child. Following precautionary statements are applicable: P280: Wear protective gloves/protective clothing/eye protection/face protection. P308+P313: IF exposed or concerned: Get medical advice/attention.
5. Some kit components contain sodium azide as a preservative. In order to prevent the formation of potentially explosive metal azides in laboratory plumbing, flush drains thoroughly after disposal of these solutions.

STORAGE CONDITIONS



1. Store the microtiterstrips in their original packaging with the desiccant until all the strips have been used.
2. Never use any kit components beyond the expiration date.
3. Do not expose Chromogen Solution (3) to strong light or high temperature during storage. This solution should be colourless; if not, it should be replaced.

SPECIMEN COLLECTION AND PREPARATION

Human serum and plasma may be used in this assay. Remove serum from clot as soon as possible to avoid hemolysis. Lipemic and/or hemolyzed samples can cause false results. Transfer the serum to a clean storage tube. Specimens may be stored at 2-8 °C for a few days, or they can be stored frozen for a longer period of time. Avoid repeated freezing and thawing.

ASSAY PROCEDURE

General Remarks:

1. Use a separate disposable tip for each sample transfer to avoid cross-contamination.
2. All reagents must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
3. Once the assay has been started, all steps should be completed without interruption.
4. Absorbance is a function of the incubation time and temperature. Therefore the size of the assay run should be limited. It is suggested to run no more than 20 patient samples with one set of Reference Standards in duplicate.
5. If an ELISA Washer is used, adaptation of the washing step might be necessary to obtain optimal results.

Reconstitution of the Reagents:

Washing Solution: dilute 50 ml of concentrated Washing Solution (2) to 1000 ml with distilled water. Reconstituted solution can be stored at least 1 month, store at 2 – 8 °C.

At higher temperatures, the concentrated Washing Solution (2) may appear cloudy without affecting its performance. Upon dilution, the solution will be clear.

Specimen Dilution Buffer

Dilute 40 ml of the concentrated Specimen Dilution Buffer (1) to 200 ml with distilled water. Reconstituted solution can be stored at least 3 months or as long as solution remains clear.

Store at 2 – 8 °C.

Assay Procedure:

1. The 10x prediluted standard sera (6a-6e) are diluted 1:100 as follows : pipette 10 µl of each calibrator into separate glass or plastic dilution tubes. Add 990 µl of diluted Specimen Dilution Buffer and mix carefully.

2. The patient samples and Control Sample (8) are diluted 1:1000 in two consecutive steps: pipette 10 µl of each patient sample and the Control Sample into separate glass or plastic dilution tubes and add 990 µl of diluted Specimen Dilution Buffer. Mix thoroughly. Add 450 µl of Specimen Dilution Buffer to 50 µl of these 100x prediluted samples. Mix thoroughly.

WARNING: ALWAYS MAKE THE DILUTIONS IMMEDIATELY BEFORE PERFORMING THE TEST. DO NOT LEAVE THE DILUTIONS TO STAND FOR LONGER THAN 30 MINUTES.

3. Pipette 100 µl of the diluted calibrators and samples into each of a pair of adjacent wells of the Coated Microtiterstrips (5).

4. Incubate the covered microtiterstrips for 30 ± 2 min at room temperature.

5. Wash the microtiterstrips three times with Washing Solution. This can either be performed with a suitable microtiterplate washer or by briskly shaking out the contents of the strips and immersing them in washing solution. During the third step, the washing solution is left in the strips for 2-3 min. Finally empty the microtiterstrips and remove excess fluid by blotting the inverted strips on adsorbent paper.

6. Add 100 µl of Conjugate Solution (7) and incubate the covered microtiterstrips for 30 ± 2 min at room temperature.

7. Repeat the washing cycle as described in 5.

8. Add 100 µl of Chromogen Solution (3) to each well.

9. Incubate for 10 ± 2 min at room temperature. Avoid light exposure during this step.

10. Add 50 µl of 0.5M H₂SO₄ (4) to each well.

11. Determine the absorbance of each well at 450 nm or at 450 nm with reference filter 600-650 nm within 30 min following the addition of acid.

RESULTS

The average absorbance value of each calibrator is plotted against the corresponding ASLO-value and the best calibration curve (e.g. log/linear) is constructed.

Use the average absorbance of each patient sample obtained in the ASLO ELISA to determine the corresponding value by simple interpolation from the corresponding curve.

Depending on the experience and/or availability of computer capability, other methods of data reduction may be used.

EXPECTED VALUES

Example of typical OD values:

CALIBRATOR IU/ml	OD value
0	0.019
100	0.488
200	0.870
400	1.475
800	2.144

The positive control sample must meet the specification as indicated on the label of the vial.

Using the ASLO ELISA from Advanced Practical Diagnostics BV, 75 % of a population of Belgian blood donors showed values < 300 IU/ml.

Precision

Intra Assay (n=10)	Level 1	Level 2	Level 3
Mean (IU/ml)	90.5	258.8	508.7
SD (IU/ml)	8.3	16.23	52.18
%CV	9.2	6.3	10.3
Inter Assay (n=7)	Level 1	Level 2	Level 3
Mean (IU/ml)	88.5	278.6	538.4
SD (IU/ml)	6.3	29.2	106.0
%CV	7.1	10.5	19.7

MINIMAL DETECTABLE CONCENTRATION

The minimal detectable concentration is approx. 8 IU/ml.

TEST VALIDITY

The following specifications must be met for each run to be valid:

OD value for the zero calibrator: < 0.080

OD value for the highest value calibrator: > 1.000

If one of the specifications is not met, the test run should be repeated.

TROUBLE SHOOTING

In case of high background signal, the washing was insufficient. Repeat the test with more vigorous washing (increased number of cycles, soak time).

REFERENCES

¹ GRENIER B. Le dosage des antistreptolysines O. Interêt. Conduite pratique. Rev. du Prat., 35, 31-34 (1985)

² PICARD B., PICARD M. and GOULLET P. Etude des taux critiques de trois anticorps antistreptococciques par l'analyse des données. La Presse Médicale, 12, 1467-1471 (1983)

³ SPAUN J., BENTZON M. V., OLESEN LARSEN S. and HEWITT L. F. Bull. Wld. Hlth. Org., 24, 271 (1961)

ASLO 11-21

Version history

Version number	Description
ASLO 11-18	Previous version
ASLO 11-21	Writing in full the manufacturer name, according to its legal status: Advanced Practical Diagnostics BV instead of the abbreviation apDia. Assay procedure: reading of OD values at 450nm may equally well be done using reference filter 600-650 nm. Correction of typo.



In vitro diagnostic kit

ASLO ELISA



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