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Instruction for use
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ORG 515S Anti-Cardiolipin Screen

Immunometric Enzyme Immunoassay for the quantitative determination of Anti-Cardiolipin (IgG, IgM and IgA)

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NAME AND INTENDED USE

Anti-Cardiolipin is an indirect solid phase enzyme immunoassay (ELISA) for the simultaneous quantitative measurement of IgG, IgM and IgA class autoantibodies against cardiolipin in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of an increased risk of thrombosis in patients with Systemic Lupus Erythematosus (SLE) or lupus-like disorders.

SUMMARY AND EXPLANATION OF THE TEST

Antiphospholipid syndrome (APS) is a systemic autoimmune disease characterized by a thrombophilic state and by obstetrical complications [1]. The Scientific and Standardization Committee of the International Society on Thrombosis and Hemostasis has issued consensus criteria that may be used to help laboratory diagnosis [2]. Accordingly, thrombophilic patients should be screened both by phospholipid-dependent tests to detect lupus anti-coagulant (LA) and by assaying for phospholipid antibodies with solid phase ELISA tests to detect cardiolipin antibodies (aCl).

The presence of anti-cardiolipin antibodies in systemic lupus erythematosus (SLE) can be related to the development of thrombosis and thrombocytopenia, in gynaecology they are supposed to cause intrauterine death or recurrent abortion. Furthermore, anti-cardiolipin antibodies have been found in some non-thrombotic neurological disorders like cerebrovascular insufficiency, cerebral ischemia or chorea and in myocardial infarction [3].

Anti-Cardiolipin autoantibodies are found in the immunoglobulin classes IgG, IgM and/or IgA [4]. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune diseases, whereas IgG antibodies will be found in progressive stages of manifested autoimmune disorders. The determination of IgA antibodies seems to have a greater importance in the African-Caribbean population [5].

Quantitative measurements of anti-Cardiolipin antibodies, especially IgG, is an important parameter with high specificity in therapy-monitoring of SLE-secondary forms [6].

Indication for determination of anti-Cardiolipin antibodies [7]:

- | | |
|----------------------|----------------------|
| - SLE | - Thrombosis |
| - Thrombocytopenia | - Cerebral Ischemia |
| - Chorea | - Epilepsy |
| - Recurrent Abortion | - Intrauterine Death |

PRINCIPLE OF THE TEST

Highly purified cardiolipin is bound to microwells saturated with β 2-glycoprotein I. Antibodies against these antigens, if present in diluted serum or plasma, bind to the respective antigen. Washing of the microwells removes unspecific serum and plasma components. Horseradish peroxidase (HRP) conjugated anti-human IgG, IgM and IgA immunologically detects the bound patient antibodies forming a conjugate/antibody/antigen complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG, IgM resp. IgA antibodies present in the original sample.

WARNINGS AND PRECAUTIONS

1. All reagents of this kit are strictly intended for in vitro diagnostic use only.
2. Do not interchange kit components from different lots.
3. Components containing human serum were tested and found negative for HBsAg, HCV, HIV1 and HIV2 by FDA approved methods. No test can guarantee the absence of HBsAg, HCV, HIV1 or HIV2, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.
4. Avoid contact with the TMB (3,3',5,5'-Tetramethyl-benzidine). If TMB comes into contact with skin, wash thoroughly with water and soap.
5. Avoid contact with the Stop Solution which is hydrochloric acid (1 M). If it comes into contact with skin, wash thoroughly with water and seek medical attention.
6. Some kit components (i.e. Controls, Sample buffer and Buffered Wash Solution) contain Sodium Azide as preservative. Sodium Azide (NaN_3) is highly toxic and reactive in pure form. At the product concentrations (0.09%), though not hazardous. Despite the classification as non-hazardous, we strongly recommend using prudent laboratory practices (see 8., 9., 10.).
7. Some kit components contain Proclin 300 as preservative. When disposing reagents containing Proclin 300, flush drains with copious amounts of water to dilute the components below active levels.
8. Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.
9. Do not pipette by mouth.
10. Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.
11. Avoid contact between the buffered Peroxide Solution and easily oxidized materials; extreme temperature may initiate spontaneous combustion.

Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

CONTENTS OF THE KIT

Package size	96 determ.
Qty.1	Divisible microplate consisting of 12 modules of 8 wells each, coated with highly purified bovine cardioliplipin and saturated with β 2-Glycoprotein I. Ready to use.
4 vials, 1.5 ml each	combined Calibrators with IgG, IgA and IgM class Anti-Cardioliplipin antibodies in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) Negative Control (A) 3.3 U/ml, Cut-Off Control (B) 10 U/ml, Positive Control (C) 30 U/ml, Strong positive Control (D) 90 U/ml. Ready to use.

1 vial, 20 ml	Sample buffer (Tris, NaN_3 <0,1% (w/w)), yellow, concentrate (5x).
1 vial, 15 ml	Enzyme conjugate solution (PBS, PROCLIN 300 <0,5% (v/v)), (light red) containing polyclonal rabbit anti-human IgG, polyclonal rabbit anti-human IgM and polyclonal rabbit anti-human IgA; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	TMB substrate solution. Ready to use.
1 vial, 15 ml	Stop solution (1 M hydrochloric acid). Ready to use.
1 vial, 20 ml	Wash solution (PBS, NaN_3 <0,1% (w/w)), concentrate (50x).

STORAGE AND STABILITY

1. Store the kit at 2-8 °C.
2. Keep microplate wells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage and usage.
5. Diluted sample buffer and wash buffer are stable for at least 30 days when stored at 2-8 °C.

MATERIALS REQUIRED

Equipment

- Microplate reader capable of endpoint measurements at 450 nm
- Multi-Channel Dispenser or repeatable pipet for 100 μ l
- Vortex mixer
- Pipets for 10 μ l, 100 μ l and 1000 μ l
- Laboratory timing device
- Data reduction software

Preparation of reagents

- Distilled or deionized water
- Graduated cylinder for 100 and 1000 ml
- Plastic container for storage of the wash solution

SPECIMEN COLLECTION, STORAGE AND HANDLING

1. Collect whole blood specimens using acceptable medical techniques to avoid hemolysis.
2. Allow blood to clot and separate the serum by centrifugation.
3. Test serum should be clear and non-hemolyzed. Contamination by hemolysis or lipemia is best avoided, but does not interfere with this assay.
4. Specimens may be refrigerated at 2-8 °C for up to five days or stored at -20 °C up to six months.

- Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
- Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES

- Do not use kit components beyond their expiration dates.
- Do not interchange kit components from different lots.
- All materials must be at room temperature (20-28 °C).
- Have all reagents and samples ready before start of the assay. Once started, the test must be performed without interruption to get the most reliable and consistent results.
- Perform the assay steps only in the order indicated.
- Always use fresh sample dilutions.
- Pipette all reagents and samples into the bottom of the wells.
- To avoid carryover contamination change the tip between samples and different kit controls.
- It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
- All incubation steps must be accurately timed.
- Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
- Do not re-use microplate wells.

For all controls, the respective concentrations are provided on the labels of each vial. Using these concentrations a calibration curve may be calculated to read off the patient results semi-quantitatively.

PREPARATION OF REAGENTS

Preparation of sample buffer

Dilute the contents of each vial of the sample buffer concentrate (5x) with distilled or deionized water to a final volume of 100 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Preparation of wash solution

Dilute the contents of each vial of the buffered wash solution concentrate (50x) with distilled or deionized water to a final volume of 1000 ml prior to use. Store refrigerated: stable at 2-8 °C for at least 30 days after preparation or until the expiration date printed on the label.

Sample preparation

Dilute all patient samples 1:100 with sample buffer before assay. Therefore combine 10 µl of

sample with 990 µl of sample buffer in a polystyrene tube. Mix well. Controls are ready to use and need not be diluted.

TEST PROCEDURE

- Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
- Pipet **100 µl** of controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6
A	CA	P1	P..			
B	CA	P1	P..			
C	CB	P2				
D	CB	P2				
E	CC	P3				
F	CC	P3				
G	CD	P4				
H	CD	P4				

CA - CD: Controls A to D
P1, P2... patient sample 1, 2...

- Incubate for 30 minutes at room temperature (20-28 °C).
- Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
- Dispense **100 µl** of enzyme conjugate into each well.
- Incubate for 15 minutes at room temperature.
- Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
- Dispense **100 µl** of TMB substrate solution into each well.
- Incubate for 15 minutes at room temperature.
- Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
- Read the optical density at 450 nm and calculate the results. Bi-chromatic measurement with a reference at 600-690 nm is recommended.

The developed colour is stable for at least 30 minutes. Read optical densities during this time.

Automation

The ORGENTEC Anti-Cardiolipin Screen ELISA is suitable for use on open automated ELISA processors. The test procedure detailed above is appropriate for use with or without automation.

INTERPRETATION OF RESULTS

Quality Control

This test is only valid if the optical density at 450 nm for Negative Control (A), Cut-Off Control (B), Positive Control (C) and High Positive Control (D) complies with the respective range indicated on the Quality Control Certificate enclosed to each test kit! If any of these criteria is not fulfilled, the results are invalid and the test should be repeated.

Qualitative evaluation of ELISA

Evaluation of the Anti-Cardiolipin screen test is carried out by direct comparison of the optical density of each patient sample with the optical density of the controls.

Patient samples exhibiting optical densities higher than the optical density of the cut-off control are considered to be positive.

Negative: OD Patient < OD Cut-Off Control
 Positive: OD Patient > OD Cut-Off Control
 Strong Positive: OD Patient ≥ OD Strong Positive Control

Quantitative evaluation of ELISA

For quantitative calculation of the patients results the concentration of the controls may be used for creating a calibration curve. For Anti-Cardiolipin screen a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is recommended. The concentration of unknowns may be calculated from this calibration curve.

Interpretation of results

In a normal range study with serum samples from healthy blood donors the following ranges have been established with the Anti-Cardiolipin tests:

Anti-Cardiolipin Screen
 Cut-Off: 10 U/ml

Further differentiation and typing should be carried out using the fully quantitative Anti-Cardiolipin IgG, IgM and/or IgA kits. The Anti-Cardiolipin screen recognises the sum of IgG, IgM and IgA class anti-Cardiolipin autoantibodies. Due to additive effects, patient samples containing two or three Anti-Cardiolipin antibody classes with positive results in the Anti-Cardiolipin screen may be determined as negative using the single Anti-Cardiolipin IgG, IgM or IgA assays.

PERFORMANCE CHARACTERISTICS

Parallelism

Selected sera containing IgG, IgM and IgA-antibodies were diluted with sample buffer and assayed in the Anti-Cardiolipin screen kit.

Anti-Cardiolipin	Sample	Dilution	Observed [U/ml]	Expected [U/ml]	O/E
Screen	1	1:200	47.7		
		1:400	24.0	23.9	100 %
		1:800	11.5	11.9	97 %
		1:1600	6.0	6.0	100 %
		1:3200	2.7	3.0	90 %
screen	2	1:100	138.0		
		1:200	69.7	69.0	101 %
		1:400	33.5	34.5	97 %
		1:800	15.9	17.3	92 %
		1:1600	7.8	8.6	91 %
		1:3200	4.1	4.3	95 %

Precision (Reproducibility)

Statistics for coefficients of variation (CV) were calculated for each of four samples from the results of 22 determinations in a single run for Intra-Assay precision. Run-to-run precision was calculated from the results of 5 different runs with 6 determinations each:

Intra-Assay			Inter-Assay		
Sample No	Mean [U/ml]	CV [%]	Sample No	Mean [U/ml]	CV [%]
1	4.4	13.3	1	5.8	12.4
2	14.8	5.2	2	15.8	8.1
3	42.1	4.1	3	43.7	4.7
4	70.2	6.6	4	75.9	2.5

Specificity

The microplate is coated with highly purified bovine Cardiolipin and human β2-Glycoprotein I. Special coating processes, developed by the manufacturer guarantee for the native immunogenic structure of Cardiolipin after immobilisation on the solid phase. The Anti-Cardiolipin test kits are specific only for autoantibodies directed against Cardiolipin or to the complex of Cardiolipin and β2-Glycoprotein I. No crossreactivity was observed to anti-DNA antibodies and those types of antibodies occurring in Syphilis.

Calibration

The assay system is calibrated against the internationally recognised reference sera from E.N. Harris, Louisville, since no other international standards are available.

LIMITATIONS OF PROCEDURE

The Anti-Cardiolipin Screen ELISA is a diagnostic aid. A definite clinical diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical and laboratory findings have been evaluated.

INTERFERING SUBSTANCES

No interference has been observed with haemolytic (up to 1000 mg/dL), lipemic (up to 3 g/dL triglycerides) or bilirubin (up to 40 mg/dL) containing sera. Nor have any interfering effects been observed with the use of anticoagulants. However for practical reasons it is recommended that grossly hemolyzed or lipemic samples should be avoided.

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INCUBATION SCHEME

