DRG® Anti-Cardiolipin IgG/IgM   (EIA-3587)

CE

Revised 27 June 2011 rm (Vers. 3.1)   IVD

Please use only the valid version of the package insert provided with the kit.

NAME AND INTENDED USE

Anti-Cardiolipin is an indirect solid phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG and IgM 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].These criteria have been updated in 2006 [3]. Accordingly, thrombophilic patients should be screened both by phospholipids 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 sup-posed 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 [4].Anti-Cardiolipin autoantibodies are found in the immunoglobulin classes IgG, IgM and/or IgA [5].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 [6].

Quantitative measurement of anti-cardiolipin antibodies, especially IgG, is an important parameter with high specificity in therapy-monitoring of SLE-secondary forms [7].

Indication for determination of anti-cardiolipin antibodies [8]:
- SLE   - Thrombosis
- Thrombocytopenia   - Cerebral Ischemia
- Chorea   - Epilepsy
- Recurrent Abortion   - Intrauterine Death

PRINCIPLE OF THE TEST

Highly purified cardiolipin is bound to microwells saturated with β2-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 and IgM immunologically detect 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 colour. The addition of an acid stops the reaction forming a yellow end-product. The intensity of this yellow colour is measured photometrically at 450 nm. The amount of colour is directly proportional to the concentration of IgG resp. IgM antibodies present in the original sample.

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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 acid. 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 cardiolipin and saturated with b2-Glycoprotein I. Ready to use.
6 vials, 1.5 ml each	Combined **calibrators** with IgG and IgM class Anti-Cardiolipin antibodies (A-F) in a serum/buffer matrix (PBS, BSA, NaN_3 <0,1% (w/w)) containing: **IgG:** 0; 7.5; 15;30; 60; and 120 GPL U/ml and **IgM:** 0;5; 10; 20; 40; 80 MPL U/ml. Ready to use.
2 vials, 1.5 ml each	Anti-Cardiolipin **controls** in a serum/buffer matrix (PBS, BSA, NaN_3 <0.1% (w/w)) positive (1) and negative (2), for the respective concentrations see the enclosed QC insert. 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**; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**Enzyme conjugate** solution (PBS, Proclin 300 <0.5% (v/v)), (light red) containing polyclonal rabbit **anti-human IgM**; labelled with horseradish peroxidase. Ready to use.
1 vial, 15 ml	**TMB substrate** solution. Ready to use.
1 vial, 15 ml	**Stop solution** (contains acid). Ready to use.

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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 pipette for 100 µl
– Vortex mixer
– Pipettes for 10 µl, 100 µl and 1000 µl
– 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-hemolysed. 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.
5. Avoid repetitive freezing and thawing of serum samples. This may result in variable loss of autoantibody activity.
6. Testing of heat-inactivated sera is not recommended.

PROCEDURAL NOTES
1. Do not use kit components beyond their expiration dates.
2. Do not interchange kit components from different lots.
3. All materials must be at room temperature (20-28 °C).

4. 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.
5. Perform the assay steps only in the order indicated.
6. Always use fresh sample dilutions.
7. Pipette all reagents and samples into the bottom of the wells.
8. To avoid carryover contamination, change the tip between samples and different kit controls.
9. It is important to wash microwells thoroughly and remove the last droplets of wash buffer to achieve best results.
10. All incubation steps must be accurately timed.
11. Control sera or pools should routinely be assayed as unknowns to check performance of the reagents and the assay.
12. 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
1. Prepare a sufficient number of microplate modules to accommodate controls and prediluted patient samples.
2. Pipette **100 µl** of calibrators, controls and prediluted patient samples in duplicate into the wells.

	1	2	3	4	5	6	
A	SA	SE	P1	P5			
B	SA	SE	P1	P5			
C	SB	SF	P2	P..			
D	SB	SF	P2	P..			SA-SF: standards A toF
E	SC	C1	P3				P1, P2...C: patient sample 1, 2 ...
F	SC	C1	P3				C1: positive control
G	SD	C2	P4				C2: negative control
H	SD	C2	P4				

3. Incubate for 30 minutes at room temperature (20-28 °C).
4. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
5. Dispense **100 µl** of enzyme conjugate into each well.
6. Incubate for 15 minutes at room temperature.
7. Discard the contents of the microwells and wash 3 times with **300 µl** of wash solution.
8. Dispense **100 µl** of TMB substrate solution into each well.
9. Incubate for 15 minutes at room temperature.
10. Add **100 µl** of stop solution to each well of the modules and incubate for 5 minutes at room temperature.
11. 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 Anti-Cardiolipin IgG/IgM 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 positive control (1) and negative control (2) as well as for the calibrator A and F 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.

Calculation of results
For Anti-Cardiolipin IgG and IgM a 4-Parameter-Fit with lin-log coordinates for optical density and concentration is the data reduction method of choice.

Recommended Lin-Log Plot
First calculate the averaged optical densities for each calibrator well. Use lin-log graph paper and plot the averaged optical density of each calibrator versus the concentration. Draw the best fitting curve approximating the path of all calibrator points. The calibrator points may also be connected with straight line segments. The concentration of unknowns may then be estimated from the calibration curve by interpolation.

Calculation example
The figures below show typical results for Anti-Cardiolipin IgG/IgM ELISA. These data are intended for illustration only and should not be used to calculate results from another run.

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No	Position	OD 1	OD 2	Mean	Conc. 1	Conc. 2	Mean	decl. Conc.	CV %
STA	A 1/B 1	0.050	0.050	0.050	0.1	0.1	0.1	0.0	0
STB	C 1/D 1	0.322	0.300	0.311	7.8	7.0	7.4	7.5	7
STC	E 1/F 1	0.520	0.519	0.520	15.2	15.2	15.2	15.0	0
STD	G 1/H 1	0.803	0.824	0.814	29.2	30.5	29.9	30.0	3
STE	A 2/B 2	1.226	1.191	1.209	61.6	58.2	59.9	60.0	3
STF	C 2/D 2	1.640	1.637	1.638	120.5	119.9	120.2	120.0	0

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 test:

	Anti-Cardiolipin-Ab	
	IgG [GPL U/ml]	**IgM [MPL U/ml]**
Normal:	< 10	< 7
Positive:	≥10	≥ 7

Positive results should be verified concerning the entire clinical status of the patient. Also every decision for therapy should be taken individually.

It is recommended that each laboratory establishes its own normal and pathological ranges of serum Anti-Cardiolipin. The values below should be regarded as guidelines only.

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PERFORMANCE CHARACTERISTICS

Parallelism

In dilution experiments sera with high antibody concentrations were diluted with sample buffer and assayed in the Anti-Cardiolipin kit.

Anti-Cardiolipin	Sample	Dilution	Observed [U/ml]	Expected [U/ml]	O/E
IgG	1	1:200	126.7		
		1:400	63.7	63.4	100 %
		1:800	32.9	31.7	104 %
		1:1600	14.1	15.8	89 %
		1:3200	7.2	7.9	91 %
IgG	2	1:100	112.3		
		1:200	56.1	56.2	100 %
		1:400	25.0	28.1	89 %
		1:800	12.0	14.0	86 %
		1:1600	6.0	7.0	86 %
IgM	3	1:100	55.0		
		1:200	27.0	27.5	98 %
		1:400	13.0	13.8	94 %
		1:800	6.4	6.9	93 %
IgM	4	1:200	46.5		
		1:400	23.2	23.3	100 %
		1:800	10.9	11.6	94 %
		1:1600	5.2	5.8	90 %
		1:3200	2.8	2.9	97 %

Precision

Statistics for coefficients of variation (CV) were calculated for each of three samples from the results of 24 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:

anti-Cardiolipin (IgG) Intra-Assay		
Sample No	Mean [GPL U/ml]	CV [%]
1	29.1	5.4
2	62.5	5.8
3	109.4	4.1

anti-Cardiolipin (IgM) Intra-Assay		
Sample No	Mean [MPL U/ml]	CV [%]
1	8.4	3.7
2	40.1	4.5
3	58.6	5.3

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Inter-Assay				Inter-Assay		
Sample No	Mean [GPL U/ml]	CV [%]		Sample No	Mean [MPL U/ml]	CV [%]
1	32.9	3.8		1	10.3	3.4
2	70.9	2.5		2	47.0	3.3
3	118.3	2.7		3	79.1	2.5

Sensitivity

The lower detection limit for Anti-Cardiolipin IgG was determined at 1.0 GPL U/ml.
Anti-Cardiolipin IgM yielded a sensitivity of 0.5 MPL U/ml.

Specificity

The microplate is coated with highly purified 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 against Cardiolipin or to the complex of Cardiolipin and β2-Glycoprotein I. No cross reactivity 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 and the IgG specific reference material IRP 97/656 and HCAL/EY2C9.

LIMITATIONS OF PROCEDURE

The Anti-Cardiolipin IgG/IgM 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 hemolysed or lipemic samples should be avoided.

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