Anti – Cardiolipin IgG

Enzyme Inmunoassay Test Kit

SUMMARY OF ASSAY PROCEDURE

Step	(20 - 25°C Room Temp)	Volume	Incubation time
1	Sample dilution 1:101 = 5µl / 500 µl		
2	Washing buffer (3 times)	350 µl	
3	Diluted samples, controls & calibrator	100 µl	30 minutes
4	Washing buffer (3 times)	350 µl	
5	Enzyme conjugate	100 µl	30 minutes
6	Washing buffer (3 times)	350 µl	
7	TMB Chromogenic Substrate	100 µl	15 minutes
8	Stop solution	100 µl	
9	Reading OD 450 nm		

NAME AND INTENDED USE

The Cardiolipin IgG Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection and semi-quantitative determination of IgG antibodies to Cardiolipin in human sera or plasma. The assay is to be used to detect IgG antibodies in a single specimen. The results of the assay are to be used as an aid in the diagnosis of the anti-phospholipid syndrome in patients with autoimmune disease.

SUMMARY AND EXPLANATION OF THE TEST

Anti- Cardiolipin autoantibodies (ACA) are frequently found in patients with systemic lupus erythematosus (SLE). They are also found in patients with other autoimmune diseases, as well as in some individuals with no apparent previous underlying diseases1,2. Elevated levels of ACA have been reported to be significantly associated with the presence of both venous and arterial thrombosis, thrombocytopenia, and recurrent fetal loss3,4. Anti-phospholipid syndrome has been used to describe patients who present these clinical manifestations, in association with ACA or lupus anticoagulant 5,6.

ACA are found in the immunoglobulin classes IgG, IgM and IgA. The determination of IgM antibodies is a valuable indicator in the diagnosis of beginning autoimmune disease, whereas IgG antibodies will be found in progressive stages of manifested autoimmune disorders. ACA IgG show a good correlation to the clinical status of the patient in thrombosis, thrombocytopenia, fetal loss, and some neurological disorders. ACA IgA are often associated with IgG antibodies. ACA IgA seem to have a greater validity in thrombosis and fetal loss4,5,6 .

Testing for ACA of various isotypes by ELISA aid in diagnosis of anti-phospholipid syndrome in patients with SLE and lupus-like disorders7,8,9,10.

PRINCIPLE OF THE TEST

Purified Cardiolipin antigens are coated on the surface of microwells. Diluted patient serum or plasma, and calibrators, are added to the wells. The Anticardiolipin specific antibodies, if present, bind to the antigens. All unbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibodies in the sample. The results are read by a microwell reader, and compared in a parallel manner with calibrators.

STORAGE AND STABILITY

1. Store the kit at 2 - 8o C.
2. After opening of pouch, the remaining coated wells must be carefully resealed inside the pouch with desiccants immediately. It is recommended to finish the whole coated wells within 30 days.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun, or strong light during storage or usage.

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2 - 8o C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum sample.

MATERIALS PROVIDED

1. Microwell strips: Cardiolipin antigen coated wells. 12 x 8 wells
2. Sample diluent: Yellow color solution. 50 ml / bottle
3. Washing concentrate 50x.15 ml / bottle
4. TMB Chromogenic Substrate: Amber bottle. 12 ml / bottle
5. Enzyme conjugate: Red color solution. 12 ml / bottle
6. Calibrator set (1:101 prediluted) : 5, 10, 20, 40, 80, 160 GPL. 1 ml / vial
7. Control set (1:101 prediluted) : Negative and Positive controls. Ranges are indicated on each label. 1 ml / vial
8. Stop solution. 12 ml / bottle

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
The calibrator and controls contain human source components, which have been tested and found nonreactive for Hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV,
Hepatitis B virus, or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control / National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
4. This product contains components preserved with sodium azide.
Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
5. To prevent injury and chemical burns, avoid contact with skin and eyes or inhalation and ingestion of the following reagents: Enzyme conjugate, TMB chromogenic substrate and Stop solution.

PREPARATION FOR ASSAY

1.Prepare 1x washing buffer.
Prepare washing buffer by adding distilled or deionized water to 50x wash concentrate to make a final volume of 750 ml.
2.Bring all specimens and kit reagents to room temperature (20- 25o C) and gently mix.

ASSAY PROCEDURE

1. Place the desired number of coated strips into the holder.

PRE-WASH Coated Wells - Repeat washing three times with washing buffer.

2. Prepare 1:101 dilution of test samples by adding 5 µl of the sample to 500 µl of sample diluent. Mix well.

Do not dilute 1:101 prediluted Calibrators & Controls.

3. Dispense 100 µl of diluted sera and prediluted calibrators & controls into the appropriate wells. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 100 µl of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Dispense 100 µl of TMB Chromogenic Substrate into each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of Stop solution to stop reaction.

Make sure there are no air bubbles in each well before reading.

9.Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1.Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of calibrator GPL values on the x-axis on a log-log graph paper or log-lin graph.
2.Using the O.D. value of each specimen, determine the concentration from the standard curve.
3.A typical example:

PRESENTACIÓN:

CONT. CODIGO: RSET055-2

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Calibrator Set	Cardiolipin IgG (GPL)	O.D. 450 nm		O.D. 450 nm Mean	SD	CV %
Calibrator 1	5	0.267	0.240	0.254	0.019	7.531
Calibrator 2	10	0.443	0.456	0.450	0.009	2.045
Calibrator 3	20	0.826	0.835	0.831	0.006	0.766
Calibrator 4	40	1.220	1.159	1.190	0.043	3.626
Calibrator 5	80	1.612	1.650	1.631	0.027	1.647
Calibrator 6	160	2.021	2.035	2.028	0.010	0.488

QUALITY CONTROL

1. The negative control and positive control should be run with every batch of samples tested, and the concentration must be within the range stated on its label.
2. The O.D. value of Sample Diluent (0 GPL) must be lower than 0.150 and the O.D. value of calibrator 160 GPL must be greater than 0.750.

Additional controls may be prepared from human serum specimens and kept under -20o C.

INTERPRETATION OF RESULTS

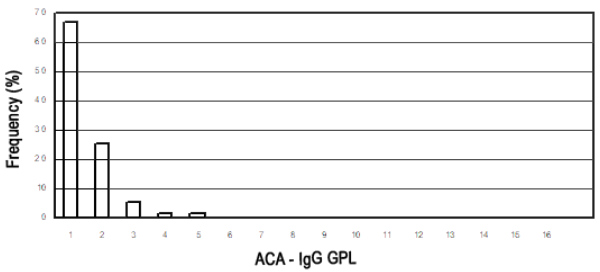
Negative: < 10 GPL
Low positive: 10 - 19 GPL
Moderate positive: 20 - 79 GPL
High positive: > 80 GPL

EXPECTED VALUE:

Elevated levels of ACA are occasionally, though infrequently, observed in the normal population. However, several autoimmune and infectious diseases can result in transient or chronic increases in ACA.
Elevated ACA levels have been reported in SLA, rheumatoid arthritis, tuberculosis, Behcet's syndrome, and other illnesses [11,12,13,14].
The range of normal ACA values may vary from population to population.

Histogram
60 random normal samples are determined with Anti-Cardiolipin IgG ELISA.
The results of obtained mean values is 1.4 GPL. SD = 0.736.

Histogram of ACA IgG Total normal samples n=60



LIMITATIONS OF THE TEST

1. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.
2. Although ACA has been associated with certain SLE subsets, the clinical significance of ACA in SLE and other diseases remains under investigation.

PERFORMANCE CHARACTERISTICS

Sensitivity, specificity, and accuracy:

A total of 72 samples from different sources were assayed with the ACA IgG test and with another commercially available ELISA test kit.

ELISA		REFERENCE ELISA N	REFERENCE ELISA P	REFERENCE ELISA TOTAL
Anti-caridiolipin IgG	N	27 (D)	18 (B)	45
	P	0 (C)	27 (A)	27
	TOTAL	27	45	72

Relative sensitivity = A / (A+B) = 27 / (27 + 18) = 60 %

Relative specificity = D / (C+D) = 27 / (0 + 27) = 100 %

Agreement = (A+D) / (A+B+C+D) = (27 + 27) / (27 + 18 + 0 + 27) = 54 / 72 = 75 %

Cross-reactivity:

A study was performed to determine the cross-reactivity of ACA IgG with other IgG antibodies. No cross-reactivity was found against the IgG positive samples of Rubella, CMV, HSV, EBV-VCA, Toxo, DS-DNA, Chlamydia trachomatis, ANA, Dengue and RF IgM.

Precision:

The mean, SD, and % CV were calculated inter- and intra-assay:

Intra-assay	n	Mean GPL	SD	%CV
Serum 1	8	16.3	1.17	7.17
Serum 2	8	33.8	1.25	3.68
Serum 3	8	67.1	4.55	6.78

Inter-assay	n	Mean GPL	SD	%CV
Serum 1	8	16.5	1.39	7.94
Serum 2	8	35.9	2.17	6.04
Serum 3	8	69.4	2.83	4.07

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