



DIAGNOSTIC AUTOMATION, INC.

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IVD See external label 2°C-8°C
 Σ= tests REF cat. #1492-11

Anti-Cardiolipin IgM

NAME AND INTENDED USE

The DIAGNOSTIC AUTOMATION, INC. Anti-Cardiolipin IgM Enzyme-linked Immunosorbent Assay (ELISA), is intended for the detection and quantitative determination of IgM antibodies to Cardiolipin in human sera or plasma. The assay is to be used to detect IgM 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 antibodies (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 diseases^{1,2}. Elevated levels of ACA have been reported to be significantly associated with the presence of both venous and arterial thrombosis, thrombocytopenia, and recurrent fetal loss^{3,4}. Anti-phospholipid syndrome has been used to describe patients who present these clinical manifestations, in association with ACA or lupus anticoagulant^{5,6}.

Recent studies have shown that ACA require a plasma or serum co-factor for binding to Cardiolipin. The co-factor has been identified as a beta2 glycoprotein (beta2 GP1), a 50 KDa beta2-globulin that occurs in plasma at a level of 200 g/ml. ACA recognize Cardiolipin either by itself or as the complex of Cardiolipin-beta2GP1, or on induced conformational epitopes on beta2GP1 after immobilization^{7,8,9}.

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 loss^{4,5,6}.

Testing for ACA of various isotypes by ELISA aid in diagnosis of anti-phospholipid syndrome in patients with SLE and lupus-like disorders^{10,11,12,13}.

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 substrate and chromogen are added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgM 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 - 8° C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
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 - 8° 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:
50 ml / bottle
3. Washing concentrate 10x:
50 ml / bottle
4. Solution A: Substrate, buffer solution containing H₂O₂;
7 ml / bottle
5. Solution B: Chromogen, Tetramethylbenzidine;
7 ml / bottle
6. Solution C: Enzyme conjugate; Red color solution.
12 ml / bottle
7. Calibrator set: 0, 5, 10, 20, 40, 80 MPL / ml
150 I / vial
Calibrated against Louisville APL Diagnostics reference preparations #LAPL-GM-100
8. Stop solution: 2 N HCl;
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

- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- 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.

PREPARATION FOR ASSAY

- Prepare 1x washing buffer.
Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to make a final volume of 1 liter.
- Bring all specimens and kit reagents to room temperature (20- 25° C) and gently mix.

ASSAY PROCEDURE 1:100 Sample Dilution 100 / 100 / 50+50

30 / 30 / 30 RT

- Place the desired number of coated strips into the holder.
- Prepare 1:100 dilution of test samples, negative control, positive control, and calibrators by adding 5 μ l of the sample to 500 μ l of sample diluent. Mix well.
- Dispense 100 μ l of diluted sera, calibrators, and controls into the appropriate wells. For the reagent blank, dispense 100 μ l of sample diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
- Remove liquid from all wells. Repeat washing three times with washing buffer.
- Dispense 100 μ l of enzyme conjugate to each well and incubate for 30 minutes at room temperature.
- Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
- Dispense 50 μ l of solution A and 50 μ l of solution B and incubate for 30 minutes at room temperature.
- Add 100 μ l of 2 N HCl to stop reaction.

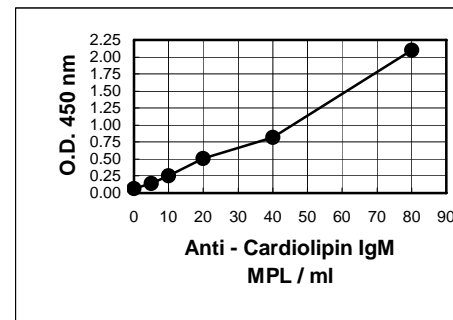
Make sure there are no air bubbles in each well before reading.

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

CALCULATION OF RESULTS

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

Standard Set	ACA IgM (MPL / ml)	O.D. 450 nm		O.D. 450 nm Mean	SD	CV %
		0.064	0.076			
Standard 1	0	0.064	0.076	0.070	0.008	12.12 2
Standard 2	5	0.136	0.149	0.143	0.009	6.451
Standard 3	10	0.248	0.254	0.251	0.004	1.690
Standard 4	20	0.455	0.570	0.513	0.081	15.86 7
Standard 5	40	0.832	0.801	0.817	0.022	2.685
Standard 6	80	2.030	2.163	2.097	0.094	4.486
Control 1-A	13.927	0.330	0.393	0.362	0.045	12.32 3
Control 2-B	35.223	0.713	0.800	0.757	0.062	8.132



QUALITY CONTROL

The test run may be considered valid provided the following criteria are met:

- The O.D. value of the reagent blank 0 MPL /ml against air should be less than 0.150.
- The O.D. value of 80 MPL / ml should be higher than 0.750.

INTERPRETATION

Negative: < 10 MPL / ml
 Low positive: 10 – 19 MPL / ml
 Moderate positive: 20 – 79 MPL / ml
 High positive: > 80 MPL / ml

EXPECTED VALUES

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^{14,15,16,17}.

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.
3. The range of normal ACA values may vary from population to population. The normal range shown in this insert is the range as recommended by the Louisville APL Diagnostics Laboratory.

PERFORMANCE CHARACTERISTICS

Sensitivity, specificity, and accuracy:

A total of 60 random samples from different sources were assayed with the DIAGNOSTIC AUTOMATION, INC. ELISA ACA IgM test and with another commercially available ELISA test kit.

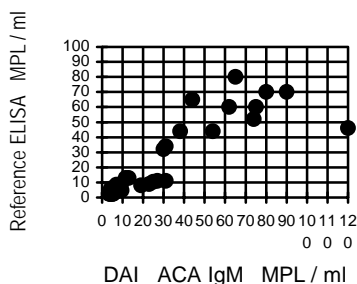
		Reference ELISA		
		N	P	Total
DIAGNOSTIC AUTOMATION , INC. ELISA ACA IgM	N	38 (D)		39
	P	1 (B)		21
		0 (C)		
		21 (A)		
	Total	38		60
		22		

$$\text{Sensitivity} = A / (A+B) = 21 / (21 + 1) = 95\%$$

$$\text{Specificity} = D / (C+D) = 38 / (0 + 38) = 100\%$$

$$\text{Accuracy} = (A+D) / (A+B+C+D) = (21 + 38) / (21 + 1 + 0 + 38) = 59 / 60 = 98\%$$

The correlation of quantitative values between two comparison methods was summarized:



Cross-reactivity:

A study was performed to determine the cross-reactivity of DIAGNOSTIC AUTOMATION, INC. ACA IgM with other IgM

antibodies. No cross-reactivity was found against the IgM positive samples of Rubella, CMV, HSV ½, EBV-VCA, Toxo and DS-DNA.

Precision:

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

Intra-assay	n	Mean MPL / ml	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 MPL / ml	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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