Diagnostic Automation / Cortez Diagnostics, Inc.
IMMUNO DIAGNOSTICS

AccuDiag™ Cardiolipin IgG/IgA/IgM ELISA Kit

Cat#1490-1

IVD See external Label 2°C–8°C ∇ 96 Tests
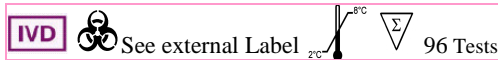
Test	Cardiolipin IgG/IgA/IgM ELISA
Method	Enzyme Linked Immunosorbent Assay
Principle	Indirect; Antigen Coated Plate
Detection Range	Semi-Quantitative/ Qualitative Positive, Negative Control
Sample	10 µL
Total Time	~ 90 min.
Shelf Life	12 Months from the manufacturing date
Specificity	98.5%
Sensitivity	1.0U/mL

INTENDED USE

The Diagnostic Automation Inc, Cardiolipin ELISA kit is a solid phase enzyme immunoassay employing highly purified cardiolipin plus native human ß2-glycoprotein I for the combined semi-quantitative and qualitative detection of IgA, IgG and IgM antibodies against cardiolipin in human serum. Anti-cardiolipin antibodies mainly recognize specific epitopes on a complex composed out of cardiolipin and ß2-glycoprotein I which are expressed only when ß2-glycoprotein I interacts with cardiolipin. The assay is an aid in the diagnosis of systemic lupus erythematosus (SLE), primary and secondary anti-phospholipid syndrome (APS) and should be used in conjuction with other serological tests and clinical findings.

SUMMARY AND EXPLANATION

Antibodies against cardiolipin belong to the group of anti-phospholipid antibodies specific for negatively charged phospholipids, components of biological membranes.Cardiolipin is an acidic phospholipid derived from glycerol and was namend because of its isolation from bovine heart in 1941. Anti-phospholipid antibodies are frequently found in sera of patients with systemic lupus erythematosus (SLE) and related diseases. The prevalence of anti-cardiolipin antibodies in SLE is 24-50 %.

The occurrence of anti-cardiolipin antibodies in patients with SLE and related diseases is typical for a secondary anti-phospholipid syndrome (APS). In contrast, anti-cardiolipin antibodies in patients with no other autoimmune diseases characterize the primary anti-phospholipide syndrome (APS). Many studies have shown a correlation between these autoantibodies and an enhanced incidence of thrombosis, thrombocytopenia and habitual abortions (as a consequence of placental infarct). The exact mechanism by which pathogenic anti-phospholipid antibodies induce thrombosis is not yet revealed fully.

TEST PRINCIPLE

Serum samples diluted 1:101 are incubated in the microplates coated with the specific antigen. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of the respective antibodies in the patient sample.

SPECIMEN COLLECTION AND PREPARATION

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements.

Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes. After separation, the serum samples should be used immediately, respectively stored tightly closed at 2-8°C/35-46°F up to three days, or frozen at -20°C/-4°F for longer periods.

MATERIALS AND COMPONENTS

Materials provided with the test kits

To be reconstituted:
5x Sample Buffer: 1 vial, 20 ml- 5x concentrated (capped white: yellow solution) containing: Tris, NaCl, BSA, sodium azide < 0.1% and thimerosal 0,01 % (preservative).
50x Wash Buffer: 1 vial, 20 ml-50x concentrated (capped white: green solution) containing: Tris, NaCl, Tween, sodium azide < 0.1% and thimerosal 0,01 % (preservative).

1. **Plate:** 12x8 well strips with breakaway microwells. Coating see paragraph 1.
2. **Stop Solution:** 1, vial 15 ml (capped white: colorless solution) Containing: 1M Hydrochloric Acid.
3. **TMB Substrate:** 1 vial, 15 ml (capped black) Containing: Stabilized TMB/H_2O_2.
4. **Conjugate:** 1 vial, 15 ml IgA/G/M (capped white: red solution) Containing: Anti-human immunoglobulins conjugated to horseradish Peroxidase and thimerosal 0,01% (preservative).
5. **Calibrator:** 6 vials, 1.5 ml each 0, 3,,10,30,100,300 U/ml. (color increasing with concentration: yellow solutions).Containing: Human sera (diluted), sodium azide < 0,1% (preservative).
6. **Cut-off Control:** 1 vial,1.5 ml (capped blue: yellow solution)Containing: Human serum (diluted), sodium Azide < 0,1 % (preservative)..
7. **Positive Control:** 1 vial, 1.5 ml (capped red: yellow solution) Containing: Human serum (diluted), sodium azide <0,1 % (preservative).
8. **Negative Control:** 1 vial, 1.5 ml (capped green: yellow solution) Containing: Human serum (diluted), sodium azide <0,1% (preservative).

Materials required but not provided
Microtiter plate reader 450 nm reading filter and optional 620 nm reference filter (600-690 nm). Glass ware, test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or multipi- pette. Microplate washing device (multichannel pipette or automated system), adsorbent paper.
Our tests are designed to be used with purified water according to the definition of the United States

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Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

ASSAY PROCEDURE

Preparations prior to pipetting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml). Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

Samples
Dilute serum samples 1:101 with sample buffer (1x)
e.g. 1000 µl sample buffer (1x) + 10 µl serum. Mix well !

Washing
Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells. e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:
Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:
Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8°C/ 35-46°F).

Work flow

For pipetting scheme see Annex A, for the test procedure see Annex B
- Pipette 100 µl of each patient's diluted serum into the designated microwells.   - Pipette 100 µl calibrators OR cut-off control and negative and positive controls into the designated wells.   - Incubate for 30 minutes at room temperature (20-26°C/ 64-78, 8°F).   - Wash 3x with 300 µl washing buffer (diluted 1:50).   - Pipette 100 µl conjugate into each well.   - Incubate for 15 minutes at room temperature (20-26°C/ 64-78, 8°F).   - Wash 3x with 300 µl washing buffer (diluted 1:50).   - Pipette 100 µl TMB substrate into each well.   - Incubate for 15 minutes at room temperature (20-26°C/ 64-78, 8°F), in the dark.   - Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.   - Incubate 5 minutes minimum.   - Agitate plate carefully for 5 sec.   - Read absorbance at 450 nm (optionally 450/620 nm) within 30 minutes.

RESULTS

For **semi-quantitative interpretation** establish the standard curve by plotting the **optical density.(OD) of each calibrator (y-axis)** with respect to the corresponding concentration values in **U/ml. (x- axis)**. For best results we recommend log/lin coordinates and 4-Parameter Fit. From the OD of each sample, read the corresponding antibody concentrations expressed in **U/ml.**

Normal Range	Positive Results
≤ 20 U/ml	> 20 U/ml

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators IgA/G/M	OD 450/620 nm	CV % (Variation)
0 U/ml	0.056	2.5
3 U/ml	0.144	1.5
10 U/ml	0.311	2.4
30 U/ml	0.623	3.2
100 U/ml	1.228	3.1
300 U/ml	2.091	0.9

Example of calculation

Patient	Replicate (OD)	Mean (OD)	Result (U/ml)
P 01	1.357/1.334	1.346	116.2
P 02	0.790/0.781	0.785	45.7

For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house Quality Control by using own controls and/or internal pooled sera, as foreseen by EU regulations. ***Do not use this example for interpreting patients results!***

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

For **qualitative interpretation** read the optical density of the cut-off control and the patient samples. Compare patient´s OD with the OD of the cut-off control. All samples which are higher than cut-off are considered positive.

Negative: OD patient $<$ OD cut -off
Positive: OD patient $>$ OD cut -off

PERFORMANCE CHARACTERISTICS

1. Analytical sensitivity
The analytical sensitivity of this kit has been found at 1.0 U/ml.

2. Specificity and Sensitivity

The microplate is coated with highly purified **cardiolipin and native human ß2-Glycoprotein I.**

No crossreactivities to other autoantigens have been found. Cardiolipin antibodies are detected in up to 50% of SLE and 80-90% of APS patients (2).

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A study with 81 sera (25 SLE, 17 primary APS, 16 secondary APS, and various other autoimmune diseases) on the Cardiolipin is shown in the table below:

		Predicate device			
		+	-	Equivocal	Total
Cardiolipin	+	50	8	1	59
	-	0	21	1	22
	Total	50	29	2	81

Excluding the equivocal results, the agreement is 89.9%

Please be advised that agreement refers to the comparison of the assay's results to that of a similar assay. There was no attempt to correlate the assay's results with that of the disease presence or absence. No judgment can be made on the comparisons accuracy to predict disease.

3. Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the hetero- geneous nature of human autoantibodies there might be samples that do not follow this rule.

Sample No.	Dilution Factor	Measured concentration (U/ml)	Expected concentration (U/ml)	Recovery (%)
1	1 / 100	63.1	68.0	93.0
	1 / 200	33.7	34.0	99.1
	1 / 400	15.9	17.0	93.5
	1 / 800	9.0	8.5	105.9
2	1 / 100	138.6	141.8	97.7
	1 / 200	70.1	70.9	98.9
	1 / 400	33.2	35.5	93.5
	1 / 800	17.9	17.7	101.1

4. Precision

To determine the precision of the assay, the variability (intra and inter-assay) was assessed by examining its reproducibility on three serum samples selected to represent a range over the standard curve.

Intra-Assay			Inter-Assay		
Sample No.	Mean (U/ml)	CV (%)	Sample No.	Mean (U/ml)	CV (%)
1	586.2	1.5	1	499.8	0.9
2	67.4	3.4	2	68.9	1.7
3	34.5	7.6	3	40.7	4.6

5. Calibration

Because of the lack of WHO reference material, Cardiolipin is calibrated against reference sera from N.E. Harris, Louisville. The results are expressed in U/ml for IgA/G/M.

PRECAUTIONS

1. Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit.

Although this product is not considered particularly toxic or dangerous in conditions of normal use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.
Do not smoke, eat or drink when manipulating the kit.
Do not pipette by mouth.
All human source material used for some reagents of this kit (controls, standards e.g.) has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls, standards and patient samples as if capable of transmitting infectious diseases and according to national requirements.

2. General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.
Allow all components to reach room temperature (20-26°C/64-78.8°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/ 98, 6 °F. Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated. The diagnosis is to be verified using different diagnostic and medicinal methods if the patient has got infectious diseases accompanied by medication.

STORAGE

Store all reagents and the microplate at 2-8°C/ 35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for 1 month at 2-8°C/ 35-46°F, at least. *Reagents and the micro- plate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desic- cant, and seal tightly*

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Date Adopted	**Cat # 1490-1-FD**
2007-02-07	**AccuDiag™- Cardiolipin IgG/IgA/IgM ELISA -2013**
EC | REP	CEpartner4U, Esdoornlaan 13, 3951DB Maarn. The Netherlands. www.cepartner4u.eu
Revision C Date: 02-10-2014	

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