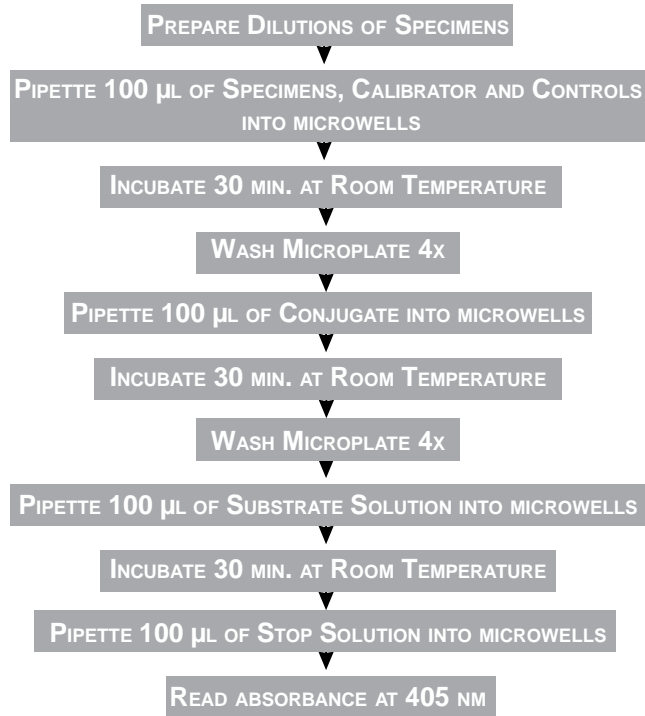


ImmuLisa™ PROCEDURE AT A GLANCE



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ImmuLisa™ Anti-Cardiolipin Antibody (ACA) Screen ELISA

For *in vitro* Diagnostic Use
CLIA Complexity: High
CDC Analyte Identification Code: 0434
CDC Test System Identification Code: 28515

PRODUCT INSERT

Catalog No. 1118S

96 Determinations

INTENDED USE

An enzyme linked immunoassay (ELISA) for the detection of IgG-, IgA-, IgM- class anti-cardiolipin antibodies, as an aid in assessing the risk of thrombosis in individuals with anti-phospholipid syndrome.

SUMMARY AND EXPLANATION

Antiphospholipid antibodies are a heterogeneous group of autoantibodies against negatively charged phospholipids¹. They are detected primarily by the anti-cardiolipin antibody (ACA) test, the biological false positive test for syphilis and the lupus anticoagulant test. These three tests detect related, but not necessarily identical antibodies. Thus, more than one of these tests is sometimes necessary to identify anti-phospholipid antibodies. Of the various methods for the detection of anti-phospholipid antibodies, the anti-cardiolipin antibody ELISA is the most sensitive². The presence of anti-cardiolipin antibodies helps identify patients at risk of venous and/or arterial thrombosis often accompanied by thrombocytopenia, a syndrome referred to as antiphospholipid syndrome¹⁻¹². Anti-phospholipid syndrome most commonly occurs in patients with systemic lupus erythematosus (SLE) or lupus-like disease where the criteria for SLE are not fulfilled⁵⁻⁷. High levels of anti-cardiolipin antibodies occur in thrombosis, fetal loss, thrombocytopenia and several other disorders¹⁻¹⁵. Low levels of anti-cardiolipin antibodies are found in a variety of clinical disorders which are unrelated to anti-phospholipid syndrome. Therefore, low levels of these antibodies are also of some, limited, significance. IgG and IgA class anti-cardiolipin antibodies appear to be more closely associated with anti-phospholipid syndrome than IgM class antibodies. However, IgM antibodies appear to be more influenced by treatment^{5,10}. Low levels of IgM antibodies can be identified in other autoimmune diseases such as rheumatoid arthritis, primary Sjögren's Syndrome, drug induced lupus erythematosus, Lyme disease, and syphilis^{8,10}.

The ImmuLisa™ ACA Screen ELISA offers a convenient method of screening patient sera for the presence of ACA before further identifying positive samples for individual isotypes with the ImmuLisa™ ACA-IgG, -IgA and-IgM ELISA.

PRINCIPLES OF THE PROCEDURE

The ImmuLisa™ ACA-Screen test is performed as a solid phase immunoassay (ELISA). Microwells are coated with cardiolipin antigen followed by blocking the unreacted sites to reduce non-specific binding. Controls, calibrator and patient serum specimens are incubated in the antigen coated wells which allows anti-cardiolipin antibodies, present in the serum, to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding an enzyme labeled anti-human polyvalent conjugate to the wells.

Unbound enzyme-labeled conjugate is removed by washing. Specific enzyme substrate (pNPP) is then added to the wells and the presence of antibodies to cardiolipin is detected by a color change produced by the conversion of the pNPP substrate. The reaction is stopped and the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 405 nm. Results are expressed in enzyme units per milliliter (EU/ml).

REAGENTS

Storage and Preparation

Store all reagents at 2°-8°C. **Do not freeze.** Do not use if reagent is not clear or if a precipitate is present. All reagents must be brought to room temperature (20°-25°C) prior to use. When stored at 2°-8°C, the reconstituted wash buffer is stable until the kit expiration date. Reconstitute the wash buffer to 1 liter with distilled or deionized water. Coated microwell strips are for one time use only.

Precautions

All human derived components used have been tested for HBsAg, HCV, HIV-1 and 2 and HTLV-I and found negative by FDA required tests. However human blood derivatives and patient specimens should be considered potentially infectious. Follow good laboratory practices in storing, dispensing and disposing of these materials¹⁶.

WARNING - Sodium azide (NaN₃) may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal of liquids, flush with large volumes of water to prevent azide buildup. Sodium azide may be toxic if ingested. If ingested, report incident immediately to laboratory director or poison control center.

Instructions should be followed exactly as they appear in this kit insert to ensure valid results. Do not interchange kit components with those from other sources other than the same catalog number from IMMCO DIAGNOSTICS. Follow good laboratory practices to minimize microbial and cross contamination of reagents when handling. Do not use beyond expiration date on the label.

REFERENCES

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Comparison of ELISA Methods for the Detection of ACA

		ImmuLisa™ ACA-Screen		
		Positive	Negative	Total
ImmuLisa™ ACA-IgG/AM	Positive	31	0	31
	Negative	2	42	44
	Total	33	42	75

Relative Agreement: 97.3%
Relative Sensitivity: 100.0%
Relative Specificity: 95.5%

The ImmuLisa™ ACA Screen provides a high degree of reliability in detecting all isotypes of anti-cardiolipin antibodies. This is demonstrated by >95% relative agreement with individual ACA ELISA.

Materials provided

ImmuLisa™ ACA Screen Test System Catalog No. 1118S
Kit contains sufficient reagents to perform 96 determinations.

- 12 x 8** Ready to use **Microplate** with individual breakaway microwells coated with cardiolipin antigen. .
- 1 x 1.5 ml** *Ready to use **Positive Control** (*red cap*). Contains human serum positive for anti-ACA antibodies. The expected concentration range in EU/ml is printed on the label.
- 1 x 1.5 ml** *Ready to use **Negative Control** (*white cap*). Contains human serum.
- 1 x 1.5 ml** *Ready to use **Calibrator** (*green cap*). Human serum containing antibodies to cardiolipin antigen. Concentrations in EU/ml are printed on the label.
- 1 x 12 ml** *Ready to use **anti-human Alk. Phos. Conjugate**. Color coded pink.
- 2 x 60 ml** *Ready to use **Serum Diluent**. Color coded blue.
- 1 x 12 ml** *Ready to use **Enzyme Substrate**. Contains pNPP. **Protect from light.**
- 1 x 12 ml** Ready to use **Stop Solution**.
- 2 vials** Powder **Wash Buffer**. Reconstitute to one liter each.
- 1 x extra** Frame Holder
- 2 x** Protocol Sheets

*Contains <0.1% NaN₃

Materials Required But Not Provided

- Deionized or distilled water
- Squeeze bottle to hold diluted wash buffer
- Pipettes capable of delivering 5 µl to 1000 µl
- Disposable pipette tips
- Clean test tubes 12 x 75 mm and test tube rack
- Timer
- Absorbent paper
- Microplate reader capable of reading absorbance values at 405 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.
- Automatic microplate washer capable of dispensing 300 µl

Other ACA ELISA available from IMMCO Diagnostics:

ImmuLisa™ ACA -IgG Test System	Catalog No. 1118G
ImmuLisa™ ACA -IgA Test System	Catalog No. 1118A
ImmuLisa™ ACA -IgM Test System	Catalog No. 1118M

SPECIMEN COLLECTION AND HANDLING

Only serum specimens should be used in this procedure. Grossly hemolyzed, lipemic or microbially contaminated specimens may interfere with the performance of the test and should not be used. Store specimens at 2°-8°C for no longer than one week. For longer storage, serum specimens should be frozen. Avoid repeated freezing and thawing of samples.

PROCEDURE

Procedural Notes

- Before starting with the assay read carefully the product insert.
- Let serum specimens and test reagents equilibrate at room temperature for at least 30 minutes before starting with the test procedure. Return all unused specimens and reagents to refrigerator immediately after use.
- All dilutions of the patient samples should be prepared prior to starting with the assay.
- Good washing technique is critical. If washing is performed manually, adequate washing is accomplished by directing a forceful stream of wash buffer with a wide tip wash bottle across the entire microplate. **An automated microplate washer is recommended.**
- Use a multichannel pipette capable of delivering 8 wells simultaneously. This speeds the process and provides for a more uniform incubation time.
- For all steps, careful control of timing is important. The start of all incubation periods begins with the completion of reagent addition.
- Addition of all samples and reagents should be performed at the same rate and in the same sequence.
- Remove required microwell strips from the pouch and carefully reseal the pouch to prevent condensation in the unused wells. Return pouch immediately to refrigerator.

PERFORMANCE CHARACTERISTICS

Precision:

Two samples with known concentrations of ACA were assayed with the Immulisa™ ACA Screen to determine inter- and intra-assay variations. Based upon these studies % CV in the inter- and intra-assay is approximately 6 and 10 respectively:

	inter-assay %CV	intra-assay %CV
Sample 1	5.9	7.6
Sample 2	6.3	10.4

Recovery:

Three samples with known ACA concentrations were mixed with another positive sample with known amounts of ACA. The ACA values of the spiked samples were then determined and the percent recovery calculated was greater than 95%.

	ACA conc. added (EU/ml)	ACA conc. obtained (EU/ml)	% Recovery
Sample 1	127.0	135.8	106.9
Sample 2	103.8	103.2	99.4
Sample 3	64.3	61.2	95.2

EXPECTED VALUES

The incidence of ACA in various disease conditions is summarized in the following tables:

Incidence of ACA in SLE^{15,17}

Antibody Isotype	% Incidence
IgG	39-44
IgA	17-57
IgM	5-33
Any isotype	53

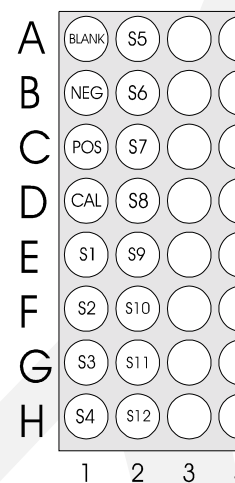
Disease Association of ACA

Condition	% Incidence
Recurrent Venous Thrombosis	28-71
Recurrent Fetal Loss	28-64
Transverse Myelitis	50
Hemolytic Anemia	38
Thrombocytopenia	27-33
Arterial Occlusions	25-31
Livedo Reticularis	25
Pulmonary Hypertension	20-40

Test Method

- Step 1** Let all reagents and specimens equilibrate at room temperature.
- Step 2** Label protocol sheet to indicate sample placement and serial dilutions in the wells according to the following figure. The user has the option to run samples in duplicate.
- Step 3** Prepare with Serum Diluent a **1:201** dilution of patient serum samples by mixing **5 µl** of each of the above with **1.0 ml** of Serum Diluent.
- Step 4** Remove the required microwells from pouch and return unused strips in the sealed pouch to refrigerator. Securely place the

Sample Layout



- microwells into the extra provided holder.
- Step 5** Pipette **100 µl** of Ready to Use Calibrator, Positive and Negative controls and diluted patient samples to the appropriate microwells as per above sample layout.
- Note:** Include one well which contains **100 µl** of the Serum Diluent as a reagent blank. Zero the ELISA reader against the reagent blank. The absorbance of the reagent blank should not be more than 0.3 when read against air.

- Step 6** Incubate **30 minutes** (\pm 5 min) at room temperature.
- Step 7** Wash **4x** with wash buffer. For manual washing, fill each microwell with reconstituted wash buffer. Discard the fluid by inverting and tapping out the contents of each well or by aspirating the liquid from each well. To blot at the end of the last wash, invert strips and tap the wells vigorously on absorbent paper towels. Do not dry wells completely. For automatic washers, program the washer as per manufacturer's instructions.
- Step 8** Pipette **100 μ l** of Conjugate into microwells.
- Step 9** Incubate **30 minutes** (\pm 5 min) at room temperature.
- Step 10** Wash all microwells as in Step 7.
- Step 11** Pipette **100 μ l** of Enzyme Substrate into each microwell in the same order and timing as for the Conjugate.
- Step 12** Incubate **30 minutes** (\pm 5 min) at room temperature.
- Step 13** Pipette **100 μ l** of Stop Solution into each microwell using the same order and timing as for the addition of the Enzyme Substrate. Read absorbance values within 1 hour from adding Stop Solution.
- Step 14** Read absorbance of each microwell at **405 nm** using a single or dual wavelength microplate reader against the reagent blank set at zero absorbance.

Quality Control

Calibrator, Positive and Negative Controls and a reagent blank must be run with each assay to verify the integrity and accuracy of the test. The absorbance reading of the reagent blank should be <0.3 . The calibrator should have an absorbance reading of not less than 1.0, otherwise the test must be repeated. The negative control must be <20 EU/ml. If the test is run in duplicate, the mean of the two readings should be taken for determining EU/ml. The absorbance of the positive control should be greater than the negative control and lesser than that of the calibrator.

RESULTS

Calculations

The following method should be used to determine whether the specimen is positive or negative for ACA:

$$\frac{\text{Abs. of Test Sample}}{\text{Abs. of Calibrator}} \times \text{EU/ml of Calibrator} = \text{EU/ml Test Sample}$$

Interpretation

The following serves only as a guide in the interpretation of the laboratory results. Each laboratory must determine its own normal values.

ACA screen values	Interpretation
<20 EU/ml	Negative
20 - 25 EU/ml	Borderline
>25 EU/ml	Positive

The literature suggests that low positive anti-cardiolipin antibody levels may occur in a variety of clinical disorders unrelated to antiphospholipid antibody syndrome. Hence, according to the investigators recommendations the diagnosis of antiphospholipid antibody syndrome should be made only when the test results are moderately or highly positive¹⁴. Any test with borderline reactivity should be retested to confirm the result. It is also recommended that patients with borderline results should be retested in three month intervals to determine the status of the patient.

LIMITATIONS OF THE PROCEDURE

A diagnosis cannot be made on the basis of ACA results alone. The results of other laboratory tests and clinical findings must also be considered. Tests for rheumatoid factor (RF) should be performed because RF may interfere with this assay. When a negative ACA test result occurs in the presence of clinical indications, a lupus anticoagulant test or other additional testing is indicated. ACA occur transiently in a variety of infectious diseases. In these cases patients positive for ACA should be retested following an appropriate interval. Confirmed active or seropositive syphilis patients can have elevated ACA levels. To rule out syphilis, confirmatory tests should be performed.