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IMUCLONE™ aCL-HS IgM ELISA (High Specificity Anti-Cardiolipin IgM)

REF 649M



(CPT Code 86147)



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INTENDED USE

The IMUCLONE® aCL-HS IgM ELISA is a quantitative enzyme linked immunosorbent assay for use as an aid in diagnosing the Antiphospholipid Syndrome (APS) in patients presenting with thrombosis, pregnancy losses and/or thrombocytopenia. The assay measures IgM type anti-cardiolipin antibody levels in human serum or plasma.

EXPLANATION OF THE TEST

The anticardiolipin test¹ was devised for the diagnosis of patients with the Antiphospholipid Syndrome (APS).² The Antiphospholipid Syndrome is a disorder of recurrent venous thrombosis, pregnancy losses, and thrombocytopenia associated with positive anticardiolipin and/or lupus anticoagulant tests.³ Both the anticardiolipin and lupus anticoagulant tests detect antibodies that bind phospholipids.^{4,5} These antibodies are heterogeneous and the two tests do not necessarily identify the same antibodies.⁶⁻⁸ Therefore, both tests should be performed on individuals suspected of having APS.

A major drawback of anticardiolipin ELISAs has been false positive test results. *Non-specific* binding of plasma from patients with a variety of diseases other than APS is frequent.⁹⁻¹² This high specificity ELISA utilizes a more specific cardiolipin antigen to capture the IgM specific to APS.^{13,14}

The IMUCLONE aCL-HS IgM ELISA is calibrated using standardized anticardiolipin units (MPL units).¹⁵⁻²⁰ The ELISA includes an IgM calibrator to construct the standard curve, a positive control (with defined value and error range) and a Negative Control.

PRINCIPLE OF THE METHOD

An enzyme linked immunoassay (ELISA) technique has been employed in this assay. Calibrators, controls and patient samples are incubated in polystyrene microwell strips coated with cardiolipin antigen. This process allows IgM anticardiolipin antibodies in patient sera or plasma to react with this cardiolipin antigen. Washing removes any unbound protein. An antibody conjugate, comprised of an antibody specific for human IgM labeled with alkaline phosphatase is added. After an additional wash, a measurable color reaction ensues upon addition of alkaline phosphatase substrate.

REAGENTS

- R1 96 cardiolipin coated polystyrene microwells
- R2 2 bottles of Sample Diluent, ready-to-use with NaN₃ added, 30 mL
- R3 1 bottle of PBS Concentrate, 10 g
- R4-R9 6 vials of IgM Calibrator, 200, 100, 50, 25, 12.5, 6.25 MPL, 1 mL each
- R10 1 vial of IgM Negative Control, 40 µL
- R11 1 vial of IgM Positive Control, 40 µL
- R12 1 vial of Anti-Human IgM HRP Conjugate, ready-to-use with NaN₃ added, 15 mL
- R13 1 bottle of TMB Substrate Solution, ready-to-use, 15 mL
- R14 1 bottle of Stopping Solution, ready-to-use, 15 mL

WARNINGS AND PRECAUTIONS

The source material for several reagents in this kit are of human origin and were found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA Approved methods. As no known test method can provide complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious human specimen.

Sodium azide (NaN₃) under acidic conditions yields hydrazoic acid, a very toxic compound. Azide compounds should be discarded with running water to avoid deposit in the plumbing system.

The Stopping Solution is a dilute acid solution. Avoid contact with eyes, skin or mucous membranes. If accidental exposure occurs, rinse the affected area with water immediately and consult a physician.

This product should only be used by appropriately trained personnel.

The use of automated systems to perform the ELISA should be validated and compared to manual performance.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

5-1000 µL multi-channel pipette
1 Liter cylinder
Test tubes and racks
Distilled water
ELISA plate reader with a 450 nm filter
Automatic or semiautomatic ELISA plate washer (optional)
Magnetic stirrer

REAGENT STORAGE AND STABILITY

It is recommended that the IMUCLONE aCL-HS IgM ELISA be stored at 2-8°C until the expiration date, for both unopened reagents or unused reagents after opening. Do not freeze any of the components of the kit. Do not mix reagents between separate lots. Substitutions will result in unreliability. Do not use reagents beyond the expiration date.

SPECIMEN COLLECTION AND PREPARATION

Testing can be performed using human serum or plasma samples in EDTA, sodium citrate, or heparin. Heat inactivated samples (56°C for 30 minutes or more) should be avoided. Samples that are hemolyzed, lipemic or grossly contaminated should also be avoided. If patient samples will not be tested within 24 hours, they should be stored frozen at -20°C or colder.

ASSAY PROCEDURE

1. Phosphate Buffered Saline (PBS)

Empty the contents of the PBS Concentrate bottle into a 1 Liter cylinder. Add distilled water to one (1) Liter final volume. Stir with a magnetic stirrer until PBS is completely dissolved. Store any excess in the refrigerator.

2. Microwells

Remove the microwell plate from the pouch at least 10 minutes before use. If all of the microwells will not be used, select the strips to be used and cut the plastic cover with a sharp blade. Return unused strips to the pouch, reseal and place in the refrigerator. Make sure unused strips are returned to the frame and strip retainer before storage.

3. Dilution of Calibrators, Samples and Controls

- Calibrators are ready to use. No dilution is necessary.
- Dilute 10 µL of IgM Positive Control in 490 µL of Sample Diluent (1:50 dilution).
 - Dilute 10 µL of Negative Control in 490 µL of Sample Diluent (1:50 dilution).
- For each plasma sample, dilute 10 µL of plasma in 490 µL of Sample Diluent (1:50 dilution).
- Vortex after dilution is prepared (avoid excess foaming).

4. Addition of Calibrators, Controls, and Diluted Samples to the Microwells

- All Calibrators, Controls and diluted samples should be assayed in duplicate.
- Add 100 µL of Sample Diluent to the "blank" microwells.
- Add 100 µL of IgM Calibrators to microwells.
- Add 100 µL of IgM Positive Control to microwells.
 - Add 100 µL of Negative Control to microwells.
 - Add 100 µL of diluted sample to microwells.
- After addition, tap the microwells gently once or twice to ensure even distribution.
- Incubate the microwells for 30 minutes at room temperature.

5. Washing the Microwells

- After the incubation period, wash the microwells three (3) times with PBS using an automatic or semiautomatic plate washer or a multichannel pipette.
- Add 200 µL of PBS to each well for each wash.
- After each addition of PBS, tap the plate gently, then discard the buffer. Make sure the microwell strips remain in place.

ASSAY PROCEDURE (continued)

D. At the end of the third wash, invert the microwells and gently tap by turning face down on a flat area covered with blotting paper.

6. Adding the IgG HRP Conjugate

Add 100 μ L of IgM HRP Conjugate to each microwell. Incubate the microwells for 30 minutes at room temperature.

7. Adding the TMB Substrate

- Wash the microwells three (3) times with PBS as previously described.
- Add 100 μ L of Substrate solution per well using a multichannel pipette.
- Incubate the microwells for exactly 30 minutes at room temperature.

8. Stopping the Reaction

- Stop the color reaction by adding 100 μ L of Stopping Solution to each well using a multichannel pipette.
- Read the absorbances of the solutions in the microwells using a microwell plate reader at 450 nm.
- Use the data obtained to establish a calibration curve.

PROCEDURAL NOTES

- Read and review the instruction booklet in its entirety prior to testing.
- Bring all reagents and samples to room temperature before use.
- Estimate the volume needed of each reagent for the run before starting based on the number of samples to be tested.
- Store all unused samples in the refrigerator as soon as possible after use.
- The IMUCLONE aCL-HS IgM ELISA Calibrators should only be used in the IMUCLONE aCL-HS IgM ELISA.
- Monitor incubation times carefully.
- Start the incubation time immediately after adding the last reagents.
- Use clean tips for each sample and reagent used.
- Do not substitute the components of the IMUCLONE aCL-HS IgM ELISA with other reagents.
- Do not use Tween or other detergents, and ensure glassware is free of this agent.
- Substrate and stopping solutions must be handled carefully. Avoid contact of these solutions with mucous surfaces.

CALCULATION OF RESULTS

A calibration curve should be constructed every time the assay is performed. Determine the mean optical density (OD) readings of the Calibrators, Positive Control, Negative Control and the reagent blank. Subtract the mean OD reading of the reagent blank (B) from all mean readings. The mean OD of the reagent blank should be less than 0.2.

Plot the mean OD of the Calibrators against their corresponding concentrations using a log-log (Figure 1) or a log-logit (Figure 2) calibration plot. This is best done using a computer with appropriate software. The concentration of each Calibrator is listed on each IgM Calibrator label.

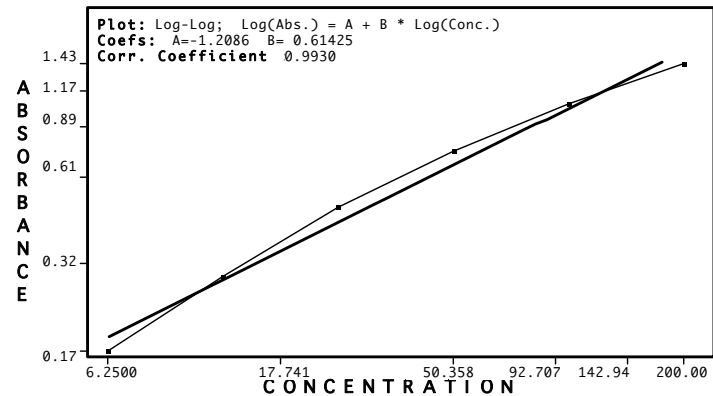


Figure 1. Example of a calibration curve for IgM aCL antibodies using a log-log plot.

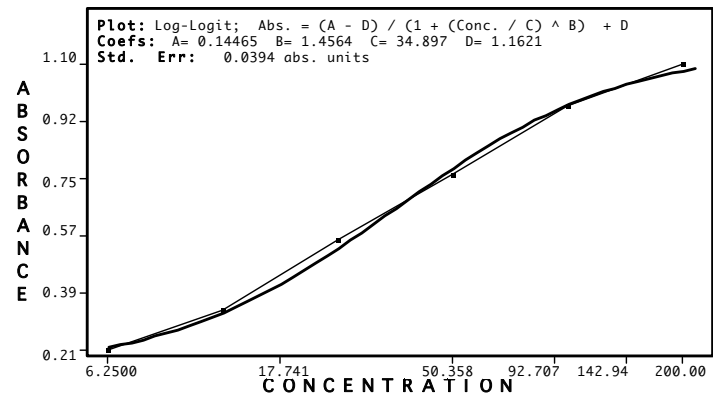


Figure 2. Example of a calibration curve for IgM aCL antibodies using a log-logit plot.

RESULTS

The O.D. readings obtained from a typical assay are found in Table 1. These values are given as an example only. Do not use these values to construct a calibration curve.

Table 1. IgM aCL-HS Calibration Curve Typical Values

Calibrator	Optical Density	MPL
1	1.608	200
2	1.070	100
3	0.841	50
4	0.481	25
5	0.294	12.5
6	0.154	6.25

MPL: 1 MPL unit is the cardiolipin binding activity of 1 µg/mL of an affinity purified IgM antibody.

EXPECTED VALUES

The range within which the IgM Positive Control should fall is indicated on its label. If the Positive Control falls outside the indicated range, the operator should review the calculations and procedure for errors. If there are no apparent errors, the assay should be repeated. The Negative Control should give values lower than the suggested cut-off point of 15 MPL units.

Values greater than 15 MPL and lower than 27 MPL are considered to be "indeterminate" (Grey Zone). Patient samples falling within this category should be retested to confirm positivity at a later date.²¹

If a patient sample has a higher O.D. reading than calibrator 1, the sample should be serially diluted and retested. The value obtained in MPL units should then be multiplied by the appropriate dilution factor.

QUALITY CONTROL

The IMUCLONE aCL-HS IgM Positive control and the IMUCLONE aCL-HS Negative Control have been provided to help ensure that the assay is performing correctly. The positive control has a defined IgM anticardiolipin level. The assay is considered to be performing correctly when the IgM anticardiolipin level of the positive control falls within the defined range.

TRACEABILITY OF CALIBRATORS AND CONTROL MATERIAL

Information on traceability of calibrators and control material is available upon request.

PERFORMANCE CHARACTERISTICS

Specificity

Normal

Samples from 50 normal healthy donors were tested with the IMUCLONE aCL-HS IgM ELISA. A cut-off value of 15 MPL units was determined based on 99% percentile.

Disease

Disease state plasmas were tested with the IMUCLONE aCL-HS IgM ELISA. The results are listed in the table below. Positive is defined as greater than 15 MPL for IgM.

<u>Disease</u>	<u>No. Tested</u>	<u>% Positive</u>
APS	39	39
Syphilis +	16	1
Other Autoimmune Disease	16	1

Sensitivity

Sera from 39 patients defined with the Antiphospholipid Syndrome were tested with the IMUCLONE aCL-HS IgM ELISA. All 39 patients tested positive for IgM anticardiolipin antibodies.

Precision

Intra-Assay Variation

Intra-assay variations were determined by testing 3 positive samples for IgM aCL antibodies in the IMUCLONE aCL-HS IgM ELISA, 10 times in the same plate. Statistics were calculated and shown in the following table:

<u>Sample</u>	<u>Mean</u>	<u>SD</u>	<u>% C.V.</u>
A	181.6	12.8	7.0
B	43.9	4.5	8.5
C	30.8	3.6	8.0

Inter-Assay Variation

Inter-assay variations were determined by assaying 3 positive samples (high, medium and low) for IgM aCL antibodies on 10 different tests. Statistics were calculated and are shown in the following table:

<u>Sample</u>	<u>Mean</u>	<u>SD</u>	<u>% C.V.</u>
A	178.5	6.5	8.0
B	45.6	5.0	6.8
C	11.2	2.0	4.5

Recovery

The IMUCLONE aCL-HS IgM Calibrator 1 was diluted with normal serum as indicated in the table below and the diluted samples tested in the assay. The expected values were calculated by dividing the concentration of the IgM Calibrator by the dilution factor, and the observed values determined from the calibration curve.

<u>Dilution</u>	<u>Observed MPL</u>	<u>Expected MPL</u>	<u>Recovery %</u>
Neat	200.7	200	100
1:2	86.8	100	87
1:4	53.6	50	106
1:8	19.2	25	77
1:16	12.6	12.5	100
1:32	7.9	6.25	126

LIMITATIONS OF THE PROCEDURE

Diagnosis of the Antiphospholipid Syndrome cannot be based solely on a positive anticardiolipin antibody test. Criteria for this diagnosis includes a history of one of the following clinical features: thrombosis, pregnancy loss or thrombocytopenia, combined with a positive anticardiolipin ELISA test and/or positive lupus anticoagulant test. Patients may have positive lupus anticoagulant but negative anticardiolipin tests, therefore both tests should be performed in patients suspected of having the Antiphospholipid Syndrome. Although the IMUCLONE aCL-HS IgM ELISA substantially reduces the frequency of syphilis samples giving positive tests, it is still possible that some samples may yield positive results. In addition, a variety of infectious states (including HIV positive patients) and drug-induced disorders may yield false positive tests.

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