Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

anti-Cardiolipin IgG/IgM
Enzyma Immunoassay Tast Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-400 - 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of patient samples in Sample Diluent. Mix well.
   Note that the Standards and Controls are pre-diluted.
   NO FURTHER DILUTION OF THESE IS REQUIRED.
2. Add 100 µl of Standards, Controls and diluted patient samples into the wells.
3. Incubate at room temperature (18-30° C) for 30 ± 5 min.
4. After incubation, discard the contents of the wells. Wash the wells 3 times with Wash Solution.
5. Add 100 µl of Conjugate to each well.
6. Incubate at room temperature for 30 ± 5 min.
7. Wash the wells as in #4 above.
8. Add 100 µl Substrate Solution to each well.
9. Incubate at room temperature for 30 ± 5 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbances at 450/600-630 nm.

INTENDED USE

For the semi-quantitative detection of IgG or IgM antibodies to cardiolipin in human serum by indirect enzyme immunoassay as an aid in assessing the risk of thrombosis in patients with systemic lupus erythematosus (SLE) or SLE-like disorders.

SUMMARY AND EXPLANATION

Anti-phospholipid antibodies are autoantibodies that react with most negatively-charged phospholipids including cardiolipin. Autoantibodies directed against phospholipids, and anti-cardiolipin in particular, have been associated with recurrent venous and arterial thrombosis, thrombocytopenia and spontaneous abortions. The term 'anti-phospholipid syndrome' is used to describe patients with these clinical manifestations. Autoantibodies to cardiolipin are also described in many autoimmune diseases. They are frequently found in patients with SLE as well as in other autoimmune diseases and can also be found in some individuals with no apparent underlying disease. For pregnant patients, in addition to spontaneous abortions, anti-cardiolipin antibodies have also been associated with pregnancy-induced hypertension, pre-eclampsia, and gestational diabetes. Anti-cardiolipin antibodies have also been detected in some non-thrombotic neurological disorders like cerebrovascular insufficiency, cerebral ischemia or chorea and in myocardial infarction (1,2,3,4,5).

Anti-cardiolipin antibodies are found in the immunoglobulin classes IgG, IgM and/or IgA. Anti-cardiolipin IgG antibodies show a good correlation to the clinical status of the patient in thrombosis, thrombocytopenia, fetal loss and some neurological disorders. The determination of IgM antibodies may be a valuable indicator in the diagnosis of early autoimmune diseases, whereas IgG antibodies will be found in progressive stages of manifested autoimmune disorders. Anti-IgA antibodies are often associated with IgG antibodies. Anti-cardiolipin IgA antibodies have also been found to be significantly higher in SLE patients with vascular complications than those without and correlated with a predisposition to thrombosis, thrombocytopenia and fetal loss (6,7,8,9).

The Diamedix *Is*-anti-Cardiolipin IgG/IgM Test Kit is an enzyme immunoassay intended to measure IgG and/or IgM antibodies to cardiolipin in human serum. The total assay time is less than 2 hours and results are reported in GPL or MPL Units per ml which are traceable to the reference sera from E. N. Harris (10).

PRINCIPLE OF THE PROCEDURE

Highly purified bovine cardiolipin is initially bound to microwells and then saturated with highly purified human β_2 glycoprotein I. β_2 glycoprotein I is known as a cofactor for the binding of anti-Cardiolipin antibodies (11). This coating procedure guarantees reproducible results independent of endogenous β_2-glycoprotein I. Diluted patient sera, Standards, and Controls are placed in the microwells and incubated. Anti-cardiolipin antibodies, if present, will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgG or IgM) is added and will bind to these complexes. Unbound conjugate is removed by aspirating and washing. Substrate is then added and incubated. In the presence of bound enzyme, the substrate is converted to a colored end product. Stop solution is added and the absorbance of this end product is then read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgG or IgM antibodies to cardiolipin present in the sample.

REAGENTS

Each Is-anti-Cardiolipin IgG/IgM Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded blue, coated with purified cardiolipin and saturated with β_2 glycoprotein I.
Standard A (0 GPL/MPL)	One vial with yellow cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard B (7.5 GPL/5 MPL)	One vial with green cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard C (15 GPL/10 MPL)	One vial with brown cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard D (30 GPL/20 MPL)	One vial with purple cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard E (60 GPL/40 MPL)	One vial with white cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard F (120 GPL/80 MPL)	One vial with red cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Negative Control	One vial with black cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix, negative for cardiolipin IgG and IgM antibodies. The assigned range is printed on the label.
Positive Control	One vial with blue cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix, moderately reactive for cardiolipin IgG and IgM antibodies. The assigned range is printed on the label.
Sample **F** Diluent	One bottle with blue cap containing 60 ml phosphate buffer with protein stabilizers. Color-coded blue.
Wash **X** Concentrate (50X)	Two bottles with clear caps containing 20 ml. Each bottle is sufficient to make 1020 ml of wash solution.
IgG Conjugate	One bottle with red cap containing 25 ml rabbit anti-human immunoglobulin G labeled with horseradish peroxidase, diluted in a PBS/BSA matrix. Color-coded pink.
IgM Conjugate	One bottle with red cap containing 25 ml rabbit anti-human immunoglobulin M labeled with horseradish peroxidase, diluted in a PBS/BSA matrix. Color-coded pink.
Substrate **H**	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).The substrate solution may develop a slight blue color upon storage.
Stop **P** Solution	One bottle with white cap containing 30 ml 1M Hydrochloric acid. CAUTION: Solution is corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water.

Store these reagents at 2 to 8º C.

OTHER MATERIALS REQUIRED

Manual Users:

Wash bottle or automated microplate washer

Pipettors capable of dispensing appropriate volumes

Timer

One liter graduated cylinder

One liter wash solution reservoir

Deionized or distilled water

Absorbent toweling

Tubes or microwell plate for sample dilution

Reader capable of reading absorbance at 450 nm, reference at 600-630 nm

Automated EIA Processor Users:

One liter graduated cylinder

Deionized or distilled water

Pre-dilution cups, strips or plates.

ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

WARNINGS AND PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use.

1. Handle samples, standards, controls and the materials that contact them as potential biohazards. Each donor unit in the calibrator and controls has been found negative for Hepatitis B surface antigen, HCV and HIV-1 and 2 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1 and 2, Hepatitis B virus or Hepatitis C virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual, "Biosafety in Microbiological and Biomedical Laboratories", 1993.
2. The concentrations of anti-cardiolipin IgG and/or IgM antibodies in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
3. Never pipette by mouth.
4. Avoid contact with open skin and mucous membranes.
5. Reagents containing Sodium Azide:
   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

   European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)

   H300 –Fatal if swallowed.
   H310 – Fatal if contact with skin.
   EUH032 – Contact with acids liberates very toxic gas.
   H410 – Very toxic to aquatic life with long lasting effect.
   P264 – Wash all exposed external body areas thoroughly after handling.
   P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
   P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
   P270 – Do not eat, drink or smoke when using this product.
   P501 – Dispose of contents/container as hazardous waste.
   P391 – Collect spillage.
   P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.

   (b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
6. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
7. Do not interchange reagents from different reagent lots except for Sample **F** Diluent, Wash **X** Concentrate, Substrate **H** and Stop **P** Solution.
8. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
9. Store unused reagents at 2 to 8º C.
10. Incubations above or below the recommended temperatures or times may give erroneous results.
11. The EIA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
12. Coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
13. (*Manual Procadura Only*) The washing procedure is very important and requires special attention. (Please refer to the Procedure section.)

NOTE: *Improparly washad walls may giva arronaous rasults.*

SPECIMEN COLLECTION

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22ºC for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2-8ºC). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -20ºC. Avoid multiple freeze-thaw cycles. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used. The CLSI, formerly NCCLS, provides recommendations for collecting and storing blood specimens (12).

CAUTION: *Sarum samplas must not ba haat-inactivatad prior to usa.*

PROCEDURE

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 20 ml of Wash Concentrate (50**X**) to one liter of de-ionized or distilled H_2O.

MANUAL USERS:

Standards and Controls are provided ready to use: **DO NOT DILUTE FURTHER.**

The assay can be performed either using all six Standards and a 6-point Calibration system or by using three Standards, namely Stds. A, C and F, and a 3-point Calibration system. Positive and Negative Controls must be run for either assay option.

1. Prepare 1:101 dilutions of the patient samples in Sample Diluent (e.g., by addition of 5 µl sample to 500 µl Sample Diluent).
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of Standards (three or six), controls, and diluted patient samples to the antigen wells. Avoid formation of bubbles when transferring diluted samples.
3. Allow the wells to incubate at room temperature (18 - 30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling if necessary. Wash the wells by rinsing 3 times with at least 300 µl per well of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18 - 30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at room temperature (18 - 30°C) for 30 ± 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Mix well contents thoroughly.
12. Read the absorbance of each well at 450 nm. A suitable reference wavelength (e.g., 600-630 nm) reading should be used.

Note: *Tha davalopad color is stabla for 3O minutas. Raad tha absorbancas during this tima.*

Automated EIA Processor Users:

When using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

NOTE: *Automatad EIA Procassor usars must validata thair aquipmant to damonstrata that tha rasults obtainad ara aquivalant to thosa obtainad using manual assay.*

QUALITY CONTROL

1. The Positive and Negative Controls must be included in each test run and must be within their assigned ranges.
2. The absorbance of Standard A (0 GPL / 0 MPL U/ml) must be less than 0.200.
3. The absorbance of Standard F (120 GPL / 80 MPL U/ml) must be greater than 3 times the absorbance of Standard C (15 GPL / 10 MPL U/ml).
4. The absorbance of Standard C (15 GPL / 10 MPL U/ml) must be greater than the absorbance of Standard B (7.5 GPL / 5 MPL U/ml).
5. The absorbance of Standard D (30 GPL / 20 MPL U/ml) must be greater than the absorbance of Standard C (15 GPL / 10 MPL U/ml).
6. The absorbance of the Standard E (60 GPL / 40 MPL U/ml) must be greater than the absorbance of Standard D (30 GPL / 20 MPL U/ml).

FIGURE 1
Distribution of anti-Cardiolipin IgG in a Normal Population

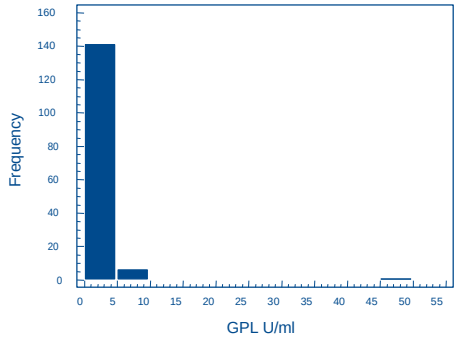


FIGURE 2
Distribution of anti-Cardiolipin IgM in a Normal Population

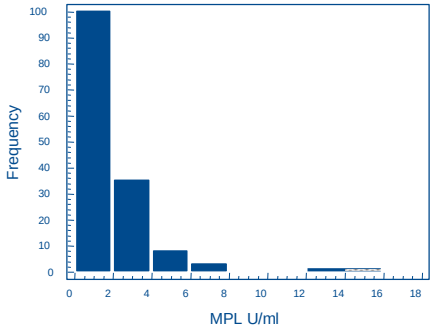


FIGURE 3
Distribution of anti-Cardiolipin IgG in a Clinical Population

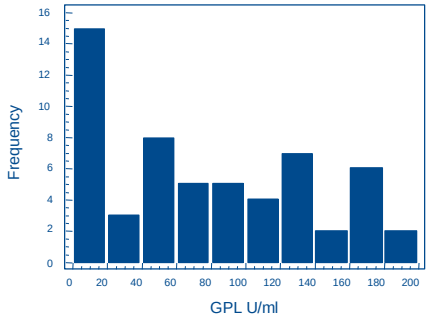
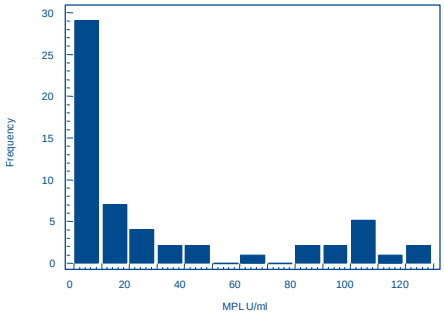


FIGURE 4
Distribution of anti-Cardiolipin IgM in a Clinical Population



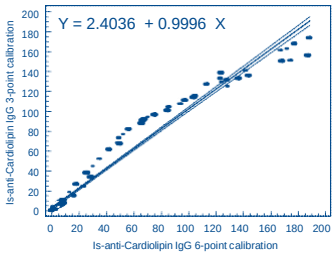
PERFORMANCE CHARACTERISTICS

All non-clinical studies were performed using the manual method and 6-point calibration unless otherwise indicated.

A. 3-point vs. 6-point Calibration

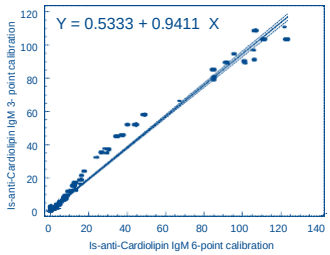
To demonstrate the equivalence of both calibration methods the results of 172 samples tested using the Is-anti-Cardiolipin IgG and 162 samples tested using the Is-anti-Cardiolipin IgM calculated using either the 3-point or 6-point calibration systems were subjected to linear regression analysis. Scattergrams and regression lines of the results obtained with 95% confidence intervals are shown in FIGURES 5 and 6. Also included are the regression statistics.

FIGURE 5: Is-anti-Cardiolipin IgG 3-point vs 6-point Result Correlation



Intercept: 2.4036 (95% CI 1.0456 to 3.7616)
Slope: 0.9996 (95% CI 0.9740 to 1.0251)
Sample Size: 172
Coefficient of determination = 0.9723
Correlation coefficient **r** = 0.9861
95% CI for **r** = 0.9812 to 0.9897

FIGURE 6: Is-anti-Cardiolipin IgM 3-point vs 6-point Result Correlation



Intercept: 0.5333 (95% CI -0.03251 to 1.0991)
Slope: 0.9411 (95% CI 0.9230 to 0.9592)
Sample Size: 162
Coefficient of determination = 0.9850
Correlation coefficient **r** = 0.9925
95% CI for **r** = 0.9898 to 0.9945

B. Relative Sensitivity and Specificity

One hundred and seventy-two frozen retrospective sera were tested for IgG antibodies and one hundred and ninety-four frozen retrospective sera were tested for IgM antibodies using the *Is*-anti-Cardiolipin IgG/IgM Test Kit and a commercially available ELISA kit for detecting IgG and/or IgM cardiolipin antibodies. Based on the results of this testing the relative sensitivity, specificity and overall agreement were calculated. The results obtained are shown in TABLES 2 and 3. For anti-cardiolipin IgG, further resolution of the discordant samples showed that the four samples that were negative in the *Is*-anti-Cardiolipin IgG and positive by the other EIA were also negative by a referee EIA method. For anti-cardiolipin IgM, further resolution of the discordant samples showed that of the 16 samples negative in the *Is*-anti-Cardiolipin IgM and positive in the other EIA, thirteen were negative and three were positive by a referee method.

TABLE 2: *Is*-anti-Cardiolipin IgG

Other EIA	Positive	Negative	*Equivocal
Positive	43	4	0
Negative	0	107	0
*Equivocal	3	15	0

		*´95% CI
Relative Sensitivity	43/47 = 91.5%	79.6 - 97.6%
Relative Specificity	107/107 = 100.0%	96.6 -100.0%
Overall Agreement	150/154 = 97.4%	93.5 - 99.3%

TABLE 3: *Is*-anti-Cardiolipin IgM

	Positive	Negative	*Equivocal
Positive	58	16	6
Negative	0	87	0
*Equivocal	0	27	0

		*´95% CI
Relative Sensitivity	58/74 = 78.4%	67.3 - 87.1%
Relative Specificity	87/87 = 100.0%	95.8 -100.0%
Overall Agreement	145/161 = 90.1%	84.4 - 94.2%

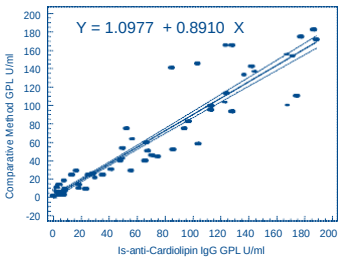
* Equivocal results were excluded from calculations.

** 95% Confidence Intervals (CI) calculated by the Exact Method (14).

NOTE: Please be advised that 'relative' refers to the comparison of the assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison's accuracy to predict disease.

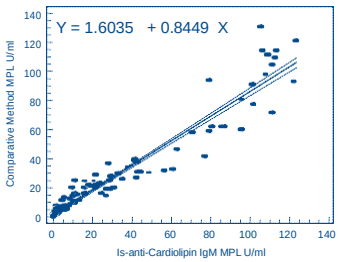
Linear regression analyses and scattergrams for the correlation studies with the comparative method are shown in FIGURES 7 and 8.

FIGURE 7: Is-anti-Cardiolipin IgG Correlation with Comparative Method



Intercept: 1.09769 (95% CI -0.9942 to 3.1896)
Slope: 0.89103 (95% CI 0.8517 to 0.9304)
Coefficient of determination = 0.9216
Correlation Coefficient **r** = 0.9600
95% CI for **r** = 0.9463 to 0.9703

FIGURE 8: Is-anti-Cardiolipin IgM Correlation with Comparative Method



Intercept: 1.6035 (95% CI 0.4892 to 2.7177)
Slope: 0.8449 (95% CI 0.8141 to 0.8758)
Coefficient of determination = 0.9383
Correlation Coefficient **r** = 0.9687
95% CI for **r** = 0.9586 to 0.9763

C. Clinical Sensitivity and Specificity

A total of three hundred and fifty-four frozen retrospective, clinically characterized sera were assayed using the *Is*-anti-Cardiolipin IgG/IgM Test Kit in order to assess both the clinical sensitivity and clinical specificity of the assay system. These samples consisted of 214 normal sera, 57 sera from patients with diagnosed anti-phospholipid syndrome (APS), 33 sera from patients with systemic lupus erythematosus (SLE), 35 sera from patients with other autoimmune diseases such as Sjogren's Syndrome, scleroderma, polymyositis/dermatomyositis and rheumatoid arthritis and 15 samples from patients with positive RPR titers. Results are summarized in TABLE 4.

Note that the analytical sensitivity, or limit of detection, calculated by assaying Standard A 20 times and taking the mean of these values plus 2 Standard Deviations was determined as being 0.4 GPL or MPL U/ml.

TABLE 4

		IgG		IgM	
Patient Group	Total	Positive	Negative/Equiv.	Positive	Negative/Equiv.
Normals	214	1	213	3	211
APS	57	47	10	27	30
SLE	33	7	26	7	26
Other Autoimmune Diseases	35	3	32	5	30
RPR Positive	15	4	11	4	11

	IgG	**IgM**
Clinical Specificity:	**#Neg. or Equiv. /Total#**	**#Neg. or Equiv. /Total#**
Normals	213/214 = 99.5%	211/214 = 98.5%
RPR Positive	11/15 = 73.3%	11/15 = 73.3%
	IgG	**IgM**
Clinical Sensitivity:	**#Pos./Total#**	**#Pos./Total#**
APS	47/57 = 82.5%	27/57 = 47.4%
SLE	7/33 = 21.2%	7/33 = 21.2%
Other Autoimmune Diseases	3/35 = 8.6%	5/35 = 14.2%

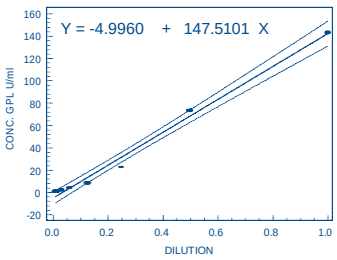
D. Cross Reactivity

To assess the potential for positive results due to cross reactive antibodies, 36 samples which were reactive to various autoantibodies (SSA/SSB, Scl-70, Jo-1, dsDNA and RF) were tested using the *Is*-anti-Cardiolipin IgG/IgM Test Kit. One sample positive for Jo-1 antibodies and one sample positive for dsDNA antibodies were positive in both the *Is*-anti-Cardiolipin IgG and IgM Tests. The remaining 34 samples were negative.

E. Linearity

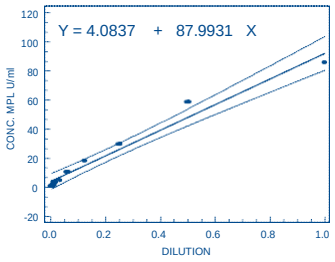
To assess the linearity of the *Is*-anti-Cardiolipin IgG/IgM Test Kit several highly positive samples were serially diluted using Sample Diluent and each dilution was then tested in the respective IgG or IgM assay systems. Representative linear regression graphs and scattergrams with 95% confidence intervals are presented in FIGURES 9 and 10.

FIGURE 9: *Is*-anti-Cardiolipin IgG Linearity



Intercept: -4.99597 Slope: 147.51010
Coefficient of determination = 0.9915
Correlation Coefficient r = 0.9957

FIGURE 10: *Is*-anti-Cardiolipin IgM Linearity

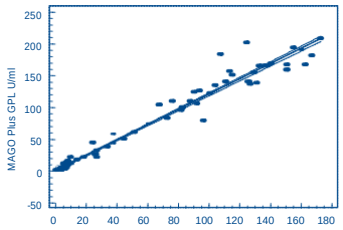


Intercept: 4.08374 Slope: 87.99309
Coefficient of determination = 0.9701
Correlation Coefficient **r** = 0.9849
95% CI for **r** = 0.9275 to 0.9969

F. Correlation of Manual and MAGO Plus results

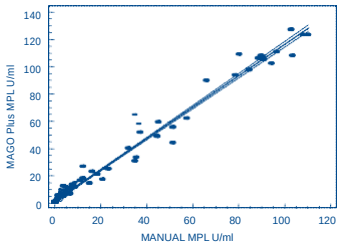
The Is-anti-Cardiolipin IgG/IgM Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus procedures, the results of 172 serum samples tested for anti-cardiolipin IgG antibodies and 162 sera tested for anti-cardiolipin IgM by both the manual and automated methods were plotted. Scattergrams and regression lines of the results obtained with 95% confidence intervals are shown in FIGURES 11 and 12. The data indicate good correlation with Correlation Coefficients (r) of 0.9883 for anti-cardiolipin IgG and 0.9917 for anti-cardiolipin IgM.

FIGURE 11: *Is*-anti-Cardiolipin IgG Manual vs MAGO Plus Correlation



Intercept 1.1058 (95% CI -0.4306 to 2.6422)
Slope: 1.1976 (95% CI 1.1696 to 1.2256)
Sample Size: 172
Coefficient of determination = 0.9768
Correlation Coefficient **r** = 0.9883
95% CI for **r** = 0.9842 to 0.9913

FIGURE 12: *Is*-anti-Cardiolipin IgM Manual vs MAGO Plus Correlation



Intercept: 1.3732 (95% CI 0.6787 to 2.0676)
Slope: 1.1510 (95% CI 1.1276 to 1.1743)
Sample Size: 162
Coefficient of determination = 0.9835
Correlation Coefficient **r** = 0.9917
95% CI for **r** = 0.9887 to 0.9939

With the 6-point calibration, linear regression of the IgG results showed (automated) = 1.0696 (manual) + 4.0821; r = 0.9517. 95% CI for the slope and intercept are 1.0174 to 1.1218 and 1.3042 to 6.8600 respectively. For IgM results (automated) = 1.0169 (manual) + 2.2121; r = 0.9772. 95% CI for the slope and the intercept are 0.9824 to 1.0514 and 1.1343 to 3.2899 respectively.

G. Precision

To assess the precision of the *Is*-anti-Cardiolipin IgG/IgM Test Kit six serum samples of varying reactivity were tested in triplicate in three separate runs. Precision was assessed both manually and using the MAGO Plus Automated EIA Processor. Precision was assessed for both IgG and IgM antibody types. The results obtained using 6-point Calibration are shown in TABLES 5-8.

TABLE 5: Manual Intra-Assay and Interassay Precision for Is-anti-Cardiolipin IgG

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN GPL	SD	CV%	MEAN GPL	SD	CV%	MEAN GPL	SD	CV%	MEAN GPL	SD	CV%
A	1.6	0.00	0.00	1.6	0.06	3.69	1.6	0.12	7.07	1.6	0.07	4.42
B	1.1	0.06	5.41	1.1	0.06	5.41	1.1	0.06	5.09	1.1	0.06	5.52
C	17.4	0.45	2.59	17.6	1.69	9.63	18.6	1.53	8.23	17.9	1.28	7.17
D	25.4	2.11	8.30	24.8	1.39	5.60	27.2	1.21	4.46	25.8	1.78	6.89
E	35.3	0.72	2.05	29.0	0.58	1.99	31.2	1.59	5.10	31.8	2.92	9.17
F	58.1	2.19	3.77	74.6	2	2.68	73.5	10.15	13.82	68.7	9.57	13.93

TABLE 6: MAGO Plus Intra-Assay and Interassay Precision for *Is*-anti-Cardiolipin IgG

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN GPL	SD	CV%	MEAN GPL	SD	CV%	MEAN GPL	SD	CV%	MEAN GPL	SD	CV%
A	2.5	0.47	18.90	3.2	0.58	17.86	3.9	1.23	31.51	3.2	0.95	29.69
B	1.3	0.15	11.75	2.0	0.12	5.87	1.9	0.1	5.26	1.7	0.35	20.59
C	29.1	1.15	3.96	31.8	1.06	3.33	40.0	6.32	15.79	33.6	5.90	17.56
D	39.7	7.45	18.76	42.9	1.18	2.76	49.2	4.51	9.16	44.0	6.07	13.80
E	41.5	0.90	2.16	45.2	2.68	5.92	51.5	1.76	3.41	46.1	4.69	10.17
F	95.3	2.30	2.42	81.1	5.00	6.16	71.3	7.04	9.87	82.6	11.34	13.73

TABLE 7: Manual Intra-Assay and Interassay Precision for *Is*-anti-Cardiolipin IgM

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN MPL	SD	CV%	MEAN MPL	SD	CV%	MEAN MPL	SD	CV%	MEAN MPL	SD	CV%
A	0.80	0.15	18.33	1.1	0.00	0.00	0.8	0.12	15.06	0.9	0.18	20.03
B	1.10	0.12	10.19	1.4	0.00	0.00	0.9	0.06	6.19	1.2	0.21	18.41
C	26.00	0.35	1.35	26.2	0.61	2.32	21.2	2.18	10.29	24.5	2.73	11.15
D	35.30	0.53	1.50	36.1	1.50	4.17	31.5	0.85	2.70	34.3	2.29	6.67
E	61.40	2.60	4.23	65.6	1.86	2.83	55.5	0.35	0.62	60.8	4.67	7.67
F	63.30	3.87	6.11	65.3	3.23	4.96	62.6	2.60	4.16	63.7	3.08	4.84

TABLE 8: MAGO Plus Intra-Assay and Interassay Precision for *Is*-anti-Cardiolipin IgM

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN MPL	SD	CV%	MEAN MPL	SD	CV%	MEAN MPL	SD	CV%	MEAN MPL	SD	CV%
A	2.6	0.25	9.68	1.9	0.06	2.99	1.8	0.06	3.15	2.1	0.37	17.62
B	2.8	0.21	7.43	2.2	0.15	6.84	2.0	0.12	5.87	2.3	0.38	16.52
C	29.4	2.05	6.98	30.8	1.30	4.22	25.0	0.95	3.79	28.4	2.95	10.39
D	37.7	1.60	4.24	40.6	2.31	5.69	36.7	2.53	6.91	38.3	2.60	6.79
E	65.8	3.25	4.94	70.4	0.65	0.92	59.5	2.81	4.73	65.2	5.22	8.01
F	85.9	9.30	10.83	80.2	2.00	2.49	66.7	2.87	4.30	77.6	9.88	12.73

REFERENCES

1. Harris, E.N., Gharavi, A. E. and Hughes, G. R. V. 1985. Anti-phospholipid Antibodies. Clin Rheum Dis 11(3): 591-609.
2. Love, P.E., et al. 1990. Antiphospholipid antibodies: Anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann. Intern. Med. 112:682-698.
3. Mackworth-Young, C. 1990. Antiphospholipid Antibodies: more than just a disease marker? Immunology Today, 2:60-65.
4. Harris E.N., et al. Thrombosis recurrent fetal loss, and thrombocytopenia: Predictive value of the anticardiolipin antibody test. Arch. Intern. Med. 1986. 146:2153-2156.
5. Harris, E. N. 1992. Serological Detection of antiphospholipid antibodies. Stroke. 23 (2 Suppl) : I3-6.
6. Kalunian, K. C. et al. 1988. Clinical Significance of a Single Test for Anti-Cardiolipin Antibodies in Patients with Systemic Lupus Erythema-tosus. Am. J. Med. 85:602-608.
7. Wilson, W.A. et al. 1998. Significance of IgA antiphopholipid antibodies. Lupus 7 Suppl. 2 : S110-113.
8. Al-Momen, A.-K., et al. 1991. IgA Antiphospholipid and Adrenal Insufficiency: Is there a link? Thrombosis Research 64: 571-578.
9. Tikkainen, U., et al. 1991. Subclasses of IgG anticardiolipin antibodies in patients with systemic lupus erythematosus. Scand. J. Immunol. 34:265-271.
10. Harris, E. N et al. 1987. Evaluation of the anti-cardiolipin antibody test: report of an International Workshop held April 4, 1986. Clin. and Exper. Immunol. 68: 215-222
11. McNeil, H. P. et al. 1990. Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: β_2 Glycoprotein I (apolipoprotein H). Proc. Natl. Acad. Sci. 87: 4120-4124.
12. Procedures for the Handling and Processing of Blood Specimens: Approved Guideline - Third Edition CLSI (formerly NCCLS) Document H18-A3, Vol. 24, No. 38. 2004.
13. Fields, R.A, Toubbeth, H., Searles, R.P. and Bankhurst, A.D. 1989. The Prevalence of Anti-cardiolipin Antibodies in a Healthy Elderly Popu-lation and its Association with Antinuclear Antibodies. J. Rheumatol. 16:623-5.
14. Gardner, M.J. and Altman, D.G. 1986. Confidence Intervals Rather than HypothesisTesting. Brit. Med. J. 292: 746-750.
15. Manual Guide – Safety Management No. CDC – 22, “Decontamination of Laboratory Sink Drains to Remove Azide Salts”, Centers for Disease Control and Prevention, Atlanta, GA, April 30, 1976.

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