Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

anti-Cardiolipin Screen

Enzyme Immunoassay Test Kit

For *In Vitro* Diagnostic Use

Catalog No. 720-480 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of patient samples in Sample Diluent. Mix well. Note that the Controls are pre-diluted.
   NO FURTHER DILUTION OF THESE IS REQUIRED.
2. Add 100 µl of 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 determination of IgG, IgM and IgA 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 described in many autoimmune diseases. They are frequently found in patients with SLE, in patients with other autoimmune diseases as well as in some individuals with no apparent underlying disease. For pregnant patients, in addition to spontaneous abortions, anti-cardiolipin antibodies have 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 antibody levels 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 Screen Test Kit is an enzyme immunoassay intended to measure IgG, IgM and IgA antibodies to cardiolipin in human serum. The assay system uses controls that are traceable to the reference sera from E. N. Harris.[10] The total assay time is less than 2 hours and results are reported in Units (U) per ml and represent the sum total of IgG, IgM and IgA antibodies present in the patient sample.

PRINCIPLE OF THE PROCEDURE

Highly purified bovine cardiolipin is initially bound to microwells and then saturated with highly purified human β_2-glycoprotein I which is known as a cofactor for the binding of anti-cardiolipin antibodies. This coating procedure guarantees reproducible results independent of endogenous β_2-glycoprotein I. Diluted patient sera 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, IgM and IgA) 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/IgM/IgA antibodies to cardiolipin present in the sample.

REAGENTS

Each Is-anti-Cardiolipin Screen 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 human β_2-glycoprotein I.
Negative Control 3.3 U/ml	One vial with black cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned U/ml value is printed on the label.
Cut-Off Control 10 U/ml	One vial with blue cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix containing IgG, IgM and IgA antibodies. The assigned U/ml value is printed on the label.
Positive Control 30 U/ml	One vial with white cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix containing IgG, IgM and IgA antibodies. The assigned U/ml value is printed on the label.
High Positive Control 90 U/ml	One vial with red cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix containing IgG, IgM and IgA antibodies. The assigned U/ml value 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 (50 X)	Two bottles with clear caps containing 20 ml. Each bottle is sufficient to make 1020 ml of wash solution.
Conjugate	One bottle with red cap containing 25 ml rabbit anti-human IgG, IgM and IgA, labeled with horseradish peroxidase, diluted in a PBS buffer with protein stabilizers. 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 8°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, 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 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 8°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 Procedure Only) The washing procedure is very important and requires special attention. (Please refer to the Procedure section.)

NOTE: *Improperly washed wells may give erroneous results.*

SPECIMEN COLLECTION

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2-8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -20°C. 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.[11]

CAUTION: *Serum samples must not be heat-inactivated prior to use.*

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 (50X) to one liter of deionized or distilled H_2O.

MANUAL USERS:

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

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 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: The developed color is stable for 30 minutes. Read the absorbances during this time.

Automated EIA Processor Users:

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

NOTE: *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

QUALITY CONTROL

1. All Controls must be included in each test run.
2. The absorbance of the Negative Control must be less than 0.350.
3. The absorbance of the Positive Control must be greater than the absorbance of the Cut-Off Control.
4. The absorbance of the High Positive Control must be greater than 3.0 times the absorbance of the Cut-Off Control.

If any of these criteria is not met, the results are invalid and the test should be repeated.

Note: Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices please refer to CLSI, formerly NCCLS, C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

Semi-quantitative results may be obtained from the point to point or 4 parameter logistic curve fit using all four Controls. For the BP-96 or Stat-Fax readers the point-to-point option should be selected and Control values entered accordingly. An Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor) will calculate and print results automatically.

2. Interpretation

The following is a guide to interpretation of results. Each laboratory is encouraged to establish its own "normal" ranges based on populations encountered.

U/ml Value	Interpretation
<12.0	Negative, no detectable IgG, IgM or IgA antibodies to cardiolipin.
12.0 to 14.9	* Equivocal for IgG, IgM or IgA antibodies to cardiolipin.
≥ 15.0	Positive, IgG, IgM or IgA antibodies to cardiolipin.

* Equivocal samples can be retested by this method, tested by another method or a new sample tested. Alternatively, equivocal samples can be tested on the specific IgG, IgM or IgA *Is*-anti-Cardiolipin kits.

Further confirmation of positive results should be carried out by testing samples using the specific IgG, IgM or IgA anti-Cardiolipin Test Kits.

NOTE: The *Is*-anti-Cardiolipin Screen Test Kit recognizes the sum of IgG, IgM and IgA-class antibodies. Due to additive effects, patient samples giving low positive results in the *Is*-anti-Cardiolipin Screen may be determined as negative using the specific anti-IgG, IgM or IgA assay.

CUT-OFF ESTABLISHMENT

To determine the positive threshold for the *Is*--Cardiolipin Screen Test kit, the results of two hundred and eight normal, negative sera tested on the kit were analyzed. The mean and standard deviation of the values for these normal sera were 5.148 and 2.405 respectively. The positive cut-off was determined as being the mean value plus four standard deviations, 5.148 + (4 x 2.405) = 14.77, appropriately rounded off. An equivocal zone, representing the difference between three and four standard deviations, 12-15, was also set to account for the natural variations inherent in any serologic procedure. These cut-off values were obtained using the kit controls, which are traceable to the available reference standards. The appropriateness of the cut-off values was additionally verified by applying the principles from Receiver Operator Curves to two hundred and three characterized sera tested in the *Is*-anti-Cardiolipin Screen Test Kit as well as by another commercially available method. At the selected cut-off value, the *Is*-anti-Cardiolipin Screen Test Kit has a relative sensitivity of 83% and a relative specificity of 100%. Note that comparable results were obtained for both manual and MAGO Plus testing.

LIMITATIONS

1. The results obtained with the *Is*-anti-Cardiolipin Screen Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. Results must be interpreted in conjunction with the patient history, clinical symptoms, physical findings as well as other diagnostic procedures.
2. The clinical significance of elevated anti-cardiolipin antibody levels in diseases other than SLE is still under investigation.
3. When a normal anti-cardiolipin antibody level is found in the presence of clinical manifestations, a lupus anticoagulant or other additional testing is indicated.
4. Treatment should not be initiated on the basis of a positive anti-cardiolipin level alone. Supportive clinical indications must also be present.
5. In published studies the prevalence of anti-cardiolipin antibodies in SLE generally ranges from approximately 20% to 60%.
6. Assay performance characteristics have not been established for visual result determination or for spectrophotometry utilizing a single wavelength.
7. The test should be performed on serum. The use of whole blood or plasma has not been established.
8. Performance characteristics of the Diamedix *Is*-anti-Cardiolipin Screen Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of anti-cardiolipin antibodies may vary depending on a number of factors such as age, gender, geographical location, race, type of test used and clinical history of individual patients. Antibodies to anti-cardiolipin are generally absent, or have a very low incidence, in the normal healthy population. Increased incidence can occur in the elderly population. A published study has shown a prevalence of 12% in the elderly population (mean age of 70 years) as opposed to 2% for a younger population. In addition, anti-cardiolipin antibodies were detected in 23% of elderly individuals who were also positive for anti-nuclear antibodies.[12]

In the present study, the expected values for a normal, healthy population were assessed by testing sera from one hundred and forty-eight S. Florida blood donors (ninety-eight males and fifty females) in the *Is*-anti-Cardiolipin Screen Test Kit. One hundred and forty sera (94.6%) were negative for antibodies, eight sera (5.4%) were positive and none were equivocal. The age distribution and antibody prevalence for this population are shown in TABLE 1.

TABLE 1
Age Distribution and Prevalence of anti-Cardiolipin IgG/IgM/IgA in a Normal S. Florida Population

	Number of donors	Prevalence
Total Number	148	
Geographic Location:	South Florida: 148	5.4%
Age		
10 – 19	7	14.3%
20 – 29	36	0.0%
30 – 39	73	8.2%
40 – 49	22	4.5%
50 – 59	8	0.0%
60 - 69	2	0.0%

The expected values for a clinical population were assessed by testing fifty-seven sera from patients with a diagnosis of anti-phospholipid syndrome (APS) in the *Is*-anti-Cardiolipin Screen Test Kit. Fifty-four (94.7%) were positive, three (5.3%) were negative and none were equivocal for IgG/IgM/IgA antibodies.

Histograms showing the distribution of values for these normal and clinical populations are shown in FIGURES 1 and 2.

FIGURE 1
Distribution of anti-Cardiolipin IgG/IgM/IgA Values in a Normal Population

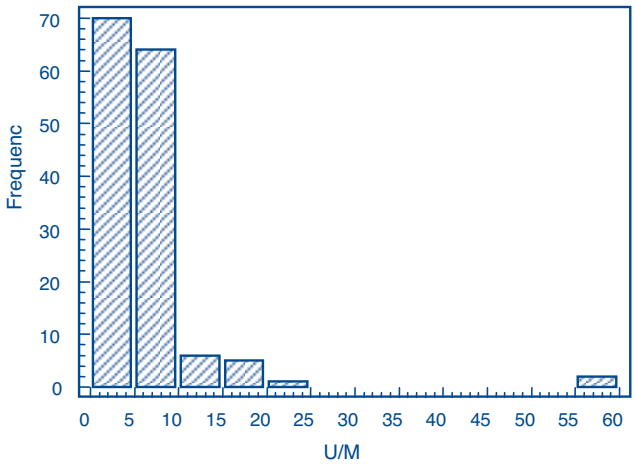
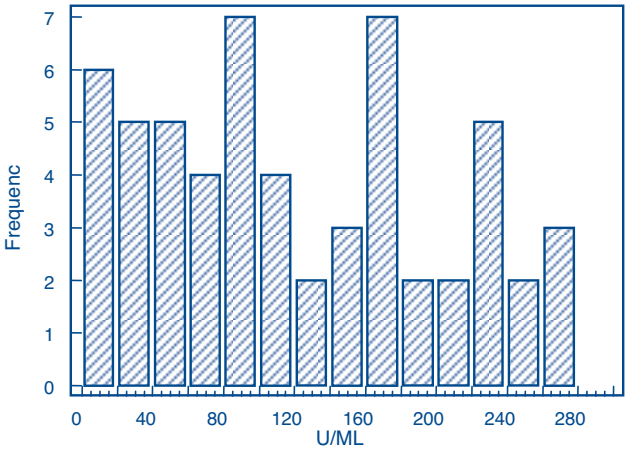


FIGURE 2
Distribution of anti-Cardiolipin IgG/IgM/IgA Values in a Clinical Population



PERFORMANCE CHARACTERISTICS

A. Relative Sensitivity and Specificity

Two hundred and three frozen, retrospective sera were tested for IgG/IgM/IgA cardiolipin antibodies using the *Is*-anti-Cardiolipin Screen Test Kit and a commercially available ELISA kit for detecting cardiolipin IgG/IgM/IgA antibodies. Based on the results of this testing the relative sensitivity, relative specificity and overall agreement were calculated. The results obtained are shown in TABLE 2. Further resolution of the discordant samples showed that seven samples that were negative in the *Is*-anti-Cardiolipin Screen and positive by the other EIA were negative by a referee EIA method. The remaining ten discordant samples were positive in the referee test.

TABLE 2
***Is*-anti-Cardiolipin Screen**

		Positive	Negative	*Equivocal
	Positive	83	18	3
Other EIA	Negative	0	99	0
	Equivocal*	0	0	0

				** 95% CI
Relative Sensitivity	83/101	=	82.2%	74.7 – 89.6%
Relativity Specificty	99/99	=	100.0%	96.3 – 100.0%
Overall Agreement	182/200	=	91.0%	86.2 – 94.6%

* Equivocal results were excluded from calculations .
** 95% Confidence Intervals (CI) calculated by the Exact Method.[13]

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.

B. Clinical Sensitivity and Specificity

A total of three hundred and forty-five frozen retrospective, clinically characterized sera were assayed using the *Is*-anti-Cardiolipin Screen Test Kit in order to assess both the clinical sensitivity and clinical specificity of the test system. These samples consisted of 215 normal sera, 57 sera from patients with diagnosed anti-phospholipid syndrome (APS), 34 sera from patients with systemic lupus erythematosus (SLE), 24 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 3.

TABLE 3

Patient Group	Total	# Positive	# Negative	Equivocal
Normals	215	9	205	1
APS	57	54	3	0
SLE	34	10	19	5
Other Autoimmune Diseases	24	6	18	0
RPR Positive	15	5	10	0

Clinical Specificity:	**#Neg/Total#**		
Normals	205/215	=	95.3%
RPR Positive	10/15	=	66.7%

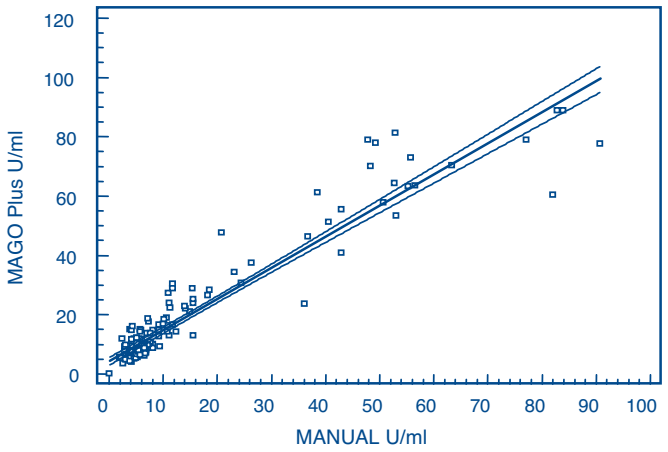
(Note that the positive samples in these groups were also positive in another ELISA)

Clinical Sensitivity:	**#Pos/Total#**		
APS	54/57	=	94.7%
SLE	10/34	=	29.4%
Other Autoimmune	6/24	=	25.0%

C. Correlation of Manual and MAGO Plus results

The *Is*-anti-Cardiolipin Screen 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 152 serum samples tested for anti-Cardiolipin IgG/IgM/IgA antibodies by both the manual and automated methods, and whose values were within the reportable range of the assay, were plotted. Scattergrams and regression lines of the results obtained with 95% confidence intervals are shown in FIGURE 3. The data indicate good correlation with a Correlation Coefficient (r) of 0.9497.

FIGURE 3
***Is*- anti-Cardiolipin Screen**
Manual vs MAGO Plus Correlation



D. Precision

To assess the precision of the *Is*-anti-Cardiolipin Screen Test Kit six serum samples of varying reactivity (two negative and four positive) were tested in triplicate in three separate runs. Precision was assessed both manually and using the MAGO® Plus Automated EIA Processor. The results obtained are shown in TABLES 4 and 5.

TABLE 4
Manual Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN U/ml	SD	CV%	MEAN U/ml	SD	CV%	MEAN U/ml	SD	CV%	MEAN U/ml	SD	CV%
A	3.3	0.265	8.02	3.3	0.173	5.25	3.7	0.115	3.09	3.4	0.274	7.97
B	2.8	0.404	14.61	3.3	0.058	1.77	3.8	0.252	6.57	3.3	0.521	15.83
C	21.4	0.945	4.41	21.9	0.950	4.33	24.6	1.443	5.86	22.7	1.787	7.88
D	33.4	3.317	9.94	36.0	0.700	1.94	34.7	1.411	4.07	34.7	2.161	6.23
E	52.9	2.957	5.59	58.8	6.851	11.65	72.5	2.207	3.04	61.4	9.527	15.51
F	40.1	1.528	3.81	44.8	0.954	5.83	45.0	1.473	3.27	43.3	2.654	6.13

for *Is*-anti-Cardiolipin Screen

TABLE 5
MAGO® Plus Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY DAY 1			INTRA-ASSAY DAY 2			INTRA-ASSAY DAY 3			INTERASSAY (n=9)		
	MEAN U/ml	SD	CV%	MEAN U/ml	SD	CV%	MEAN U/ml	SD	CV%	MEAN U/ml	SD	CV%
A	4.6	0.058	1.25	6.8	0.503	7.44	9.8	1.762	17.91	7.1	2.442	34.51
B	4.2	0.289	6.82	6.5	0.300	4.62	8.7	0.306	3.50	6.5	1.966	30.29
C	27.0	1.115	4.13	36.6	2.600	7.10	36.2	5.101	14.08	33.3	5.555	16.70
D	42.5	1.686	3.97	63.4	3.509	5.53	53.2	6.934	13.03	53.0	9.899	18.67
E	79.3	7.927	10.00	92.2	19.931	21.62	74.9	11.243	15.00	82.1	14.390	17.52
F	46.6	3.287	7.06	66.4	5.179	7.80	63.0	6.933	11.01	58.7	10.290	17.54

for *Is*-anti-Cardiolipin Screen

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