Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

anti-ß$_2$-Glycoprotein I IgG/IgM

Enzyme Immunoassay Test Kit

For *In Vitro* Diagnostic Use

Catalog No. 720-830 – 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 ß$_2$-glycoprotein-I in human serum by indirect enzyme immunoassay as an aid in the diagnosis of certain autoimmune disease thrombotic disorders 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. [1,2,3,4,5]

Recently ß$_2$-glycoprotein I has become well established as a co-factor required for the binding of anti-cardiolipin antibodies. ß$_2$-glycoprotein I, also known as apolipoprotein H, is a 50kDa ß$_2$-globulin occurring in plasma at a level of 200 ug/ml. It has been found that ß$_2$-glycoprotein I inhibits the intrinsic coagulation pathway and thus is involved in the regulation of blood coagulation. [7]

Detailed investigations about the nature of the cardiolipin ß$_2$-glycoprotein I complex have shown that epitopes on the fifth domain of ß$_2$-glycoprotein I are the real target of the anti-cardiolipin antibodies. ß$_2$-glycoprotein I is not only a pre-requisite for the binding of anti-cardiolipin antibodies; it has now been identified as the primary antigen for these antibodies. In addition, ELISA tests for ß$_2$-glycoprotein I antibodies appear to show better overall specificity since cross reactivity with other phospholipids, e.g. in syphilis positive samples, is eliminated. [8,9,10,11]

Anti-ß$_2$-glycoprotein I antibodies are found in the immunoglobulin classes IgG, IgM and/or IgA. The Diamedix *Is*-anti- ß$_2$-Glycoprotein I IgG /IgM Test Kit is an enzyme immunoassay intended to measure IgG and/or IgM antibodies to ß$_2$- glycoprotein I in human serum. The ß$_2$-glycoprotein I antibody test is a useful and specific assay to be used in conjunction with the traditionally used anti-cardiolipin kits for aiding in the diagnosis of thrombosis in at-risk patients.

PRINCIPLE OF THE PROCEDURE

Highly purified human ß$_2$-glycoprotein I is bound to microwells. Diluted patient sera, Standards, and Controls are placed in the microwells and incubated. Anti-ß$_2$-glycoprotein I 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 ß$_2$-glycoprotein I present in the sample.

REAGENTS

Each Is-anti-ß$_2$-Glycoprotein I IgG/IgM Test Kit contains reagents for 96 tests.

Reagent	Description
Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded red, coated with highly purified human ß$_2$-glycoprotein I.
Standard **A** (0 U/ml)	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** (6.3 U/ml)	One vial with green cap containing 1.8 ml of combined IgG and IgM Standard composed of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard **C** (12.5 U/ml)	One vial with brown cap containing 1.8 ml of combined IgG and IgM Standard composed of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard **D** (25 U/ml)	One vial with purple cap containing 1.8 ml of combined IgG and IgM Standard composed of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard **E** (50 U/ml)	One vial with white cap containing 1.8 ml of combined IgG and IgM Standard composed of pre-diluted human serum or defibrinated plasma in a PBS/BSA matrix. The assigned value is printed on the label.
Standard **F** (100 U/ml)	One vial with red cap containing 1.8 ml of combined IgG and IgM Standard composed 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 ß$_2$-glycoprotein I IgG and IgM antibodies. The assigned value 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 ß$_2$-glycoprotein I IgG and IgM antibodies. The assigned value is printed on the label.
Sample **F** Diluent	One bottle with a blue cap containing 60 ml phosphate buffer with protein stabilizers and 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 a 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 a 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 of 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, standards, controls and the materials that contact them as potential biohazards. Each donor unit in the standards 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-$ß_2$-glycoprotein I IgG and/or IgM 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 pre-maturely.
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.[12]

***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:

Standards and 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 Standards, 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. 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 U/ml) must be less than 0.200.
3. The absorbance of Standard F (100 U/ml) must be greater than 3 times the absorbance of Standard C (12.5 U/ml).
4. The absorbance of Standard C (12.5 U/ml) must be greater than the absorbance of Standard B (6.3 U/ml).
5. The absorbance of Standard D (25 U/ml) must be greater than the absorbance of Standard C (12.5 U/ml).
6. The absorbance of Standard E (50 U/ml) must be greater than the absorbance of Standard D (25 U/ml).

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 C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Second Edition (formerly NCCLS C24-A, Internal Quality Control Testing: Principles and Definitions).

RESULTS

1. Calculation

Semi-quantitative results may be obtained from the point to point curve fit or 4 parameter logistic curve fit using all six Standards. 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.

IgG U/ml Value	Interpretation
< 10.0	Negative, no detectable IgG antibodies to ß2-glycoprotein I
10.0 to 11.9	*Equivocal for IgG antibodies to ß2-glycoprotein I
≥ 12.0	Positive, IgG antibodies to ß2-glycoprotein I detected

IgM U/ml Value	Interpretation
< 5.0	Negative, no detectable IgM antibodies to ß2-glycoprotein I
5.0 to 6.9	*Equivocal for IgM antibodies to ß2-glycoprotein I
≥ 7.0	Positive, IgM antibodies to ß2-glycoprotein I detected

* Equivocal samples can be retested by this method, tested by another method or a new sample tested.

Samples which yield absorbances greater than that of Standard F (100 U/ml) may be reported as greater than 100 U/ml. Alternatively, such samples may be pre-diluted in Sample Diluent and retested. The resulting U/ml value must be multiplied by the dilution factor for reporting.

Example: If the specimen was pre-diluted 1:5 before testing, the resulting U/ml should be multiplied by 5.

CUT-OFF ESTABLISHMENT

The cut-off values were established to optimally discriminate those individuals with ß2-glycoprotein I antibodies from those individuals without ß2-glyco-protein-I antibodies. To determine the positive threshold for the *Is*-anti-ß2-Glycoprotein I IgG/IgM Test kit, two hundred and forty-seven normal sera were tested. The mean and standard deviation of the IgG U/ml values for these normal sera were 4.09 and 1.99 respectively. The mean and standard deviation for these normal sera of the IgM U/ml values were 1.22 and 1.45, respectively. The positive cut-off was determined as being the mean value plus four standard deviations. An equivocal zone, representing the difference between three and four standard deviations was included to account for the natural variations inherent in any serologic procedure. Thus, for *Is*-anti-ß2-Glycoprotein I IgG, the positive cut-off is 12.0, with 10.0 to 12.0 representing the equivocal zone. For *Is*-anti-ß2-Glycoprotein I IgM, the positive cut-off is 7.0, with 5.0 to 7.0 representing the equivocal zone. The appropriateness of the cut-off values was additionally verified by applying the principles from Receiver Operating Curves to over one hundred and sixty characterized sera tested in the *Is*-anti-ß2-Glycoprotein I IgG/IgM Test Kit as well as by another commercially available method. At the selected cut-off values, the *Is*-anti- ß2-Glycoprotein I IgG/IgM Test Kit has a relative sensitivity of 93% and a relative specificity of 95% for IgG antibodies and a relative sensitivity of 89% and a relative specificity of 100% for IgM antibodies. Similar values were obtained for both manual and MAGO® Plus testing.

LIMITATIONS

1. The results obtained with the *Is*-anti-ß2-Glycoprotein I IgG/IgM 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-ß2-Glycoprotein I antibody levels in diseases other than SLE is still under investigation.
3. When a normal anti-ß2-Glycoprotein antibody level is found in the presence of clinical manifestations, a lupus anticoagulant or other additional testing is indicated.
4. Some samples may be positive for anti-cardiolipin antibodies and negative for anti-ß2-glycoprotein antibodies. The *Is*-anti-ß2-Glycoprotein I IgG/IgM Test Kit is a more specific marker of thrombotic risk. Anti-cardiolipin tests can produce false positive results due to cross-reactions with dsDNA or certain infectious disease antibodies.
5. Assay performance characteristics have not been established for visual result determination or for spectrophotometry utilizing a single wave-length.
6. The test should be performed on serum. The use of whole blood or plasma has not been established.
7. Performance characteristics of the Diamedix *Is*-anti-ß2-Glycoprotein I IgG/IgM Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of anti-ß2-glycoprotein I IgG and/or IgM 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 ß2-glycoprotein I are generally absent, or have a very low incidence, in the normal healthy population. In a recently published study the distribution of anti-ß2-glycoprotein I antibodies in healthy controls was 3% (13). In patients with SLE the prevalence of ß2-glycoprotein IgG & IgM antibodies has been found to range from 20-30%. The prevalence of these antibodies in anti-phospholipid syndrome (APS) patients has been found to range from 40 to 65%.[13,14,15]

In the present study, the expected values for a normal, healthy population were assessed by testing sera from one hundred and forty-eight South Florida blood donors in the *Is*-anti-ß2-Glycoprotein I IgG/IgM Test Kit for both IgG and IgM antibodies. Ninety-eight of these samples were from males and 50 were from females. For IgG antibodies, one hundred and forty-five sera (98.0%) were negative for antibodies, two sera (1.3%) were positive and one serum (0.7%) was equivocal. For IgM antibodies, one hundred and forty-three (96.6%) were negative, three sera (2.0%) were positive and two sera (1.4%) were equivocal. The gender, age distribution and antibody prevalences for this population are shown in TABLE 1.

TABLE 1
Age Distribution and Prevalence of anti-ß2-Glycoprotein I IgG and IgM in a Normal S. Florida Population

	Number of Donors			Prevalence	
Total Number	148			**IgG**	**IgM**
Geographic Location:	South Florida: 148			1.3%	2.0%
		Gender			
Age	**No.**	**M**	**F**		
10 – 19	7	3	4	14.3%	0.0%
20 – 29	36	20	16	0.0%	0.0%
30 – 39	73	53	20	1.4%	2.7%
40 – 49	22	15	7	0.0%	0.0%
50 – 59	8	6	2	0.0%	0.0%
60 - 69	2	1	1	0.0%	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-ß2-Glycoprotein I IgG/IgM test for both antibody types.

Forty-six (80.7%) were positive, ten (17.5%) were negative and one (1.8%) was equivocal for IgG antibodies. Twenty-five (43.8%) were positive, thirty (52.6%) were negative, and two (3.6%) were equivocal for IgM antibodies.

Histograms showing the distribution of values for the normal and clinical populations for both IgG and IgM antibodies are shown in FIGURES 1-4.

FIGURE 1
Distribution of anti-ß$_2$-Glycoprotein I IgG in a Normal Population

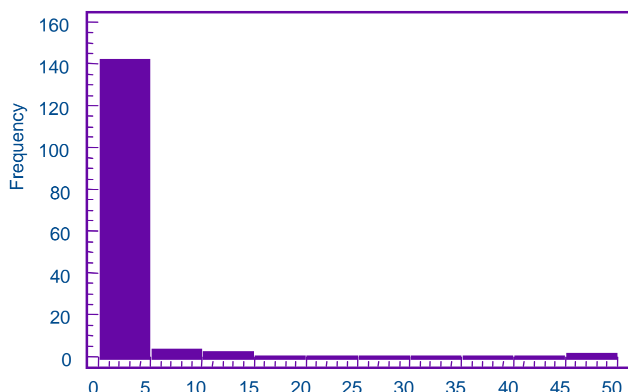


FIGURE 2
Distribution of anti-ß$_2$-Glycoprotein I IgM in a Normal Population

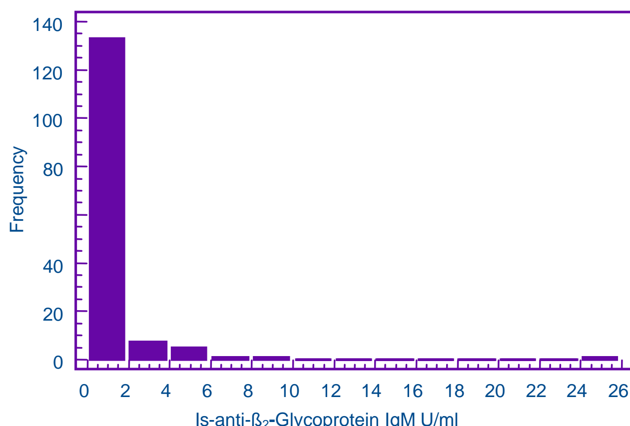


FIGURE 3
Distribution of anti-ß$_2$-Glycoprotein I IgG in a Clinical Population

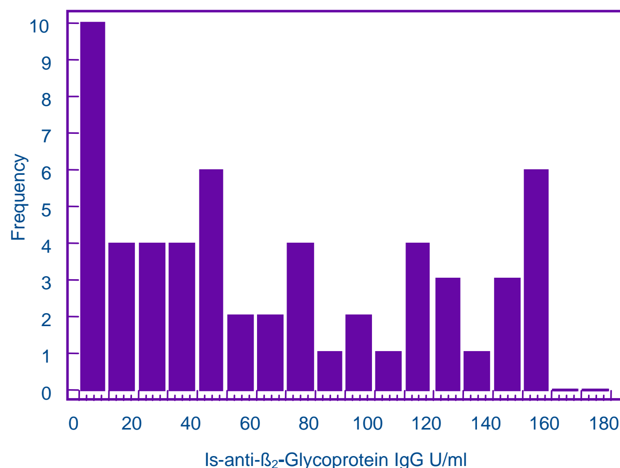
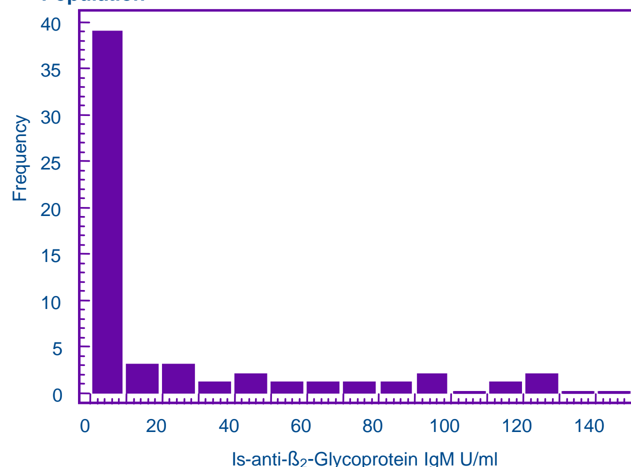


FIGURE 4
Distribution of anti-ß$_2$-Glycoprotein I IgM in a Clinical Population



PERFORMANCE CHARACTERISTICS

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

A. Relative Sensitivity and Specificity

One hundred and seventy-two frozen retrospective sera were tested for IgG antibodies and one hundred and sixty-one frozen retrospective sera were tested for IgM antibodies using the *Is*-anti-ß$_2$-Glycoprotein I IgG/IgM Test Kit and commercially available ELISA kits for detecting IgG and IgM ß$_2$-glycoprotein I antibodies. Based on the results of this testing the relative sensitivity, relative specificity and overall agreement were calculated. The results obtained are shown in TABLES 2 and 3. For anti-ß$_2$-Glycoprotein I IgG, further resolution of the discordant samples showed that the three samples that were negative in the *Is*-anti-ß$_2$-Glycoprotein I IgG and positive by the other EIA were also negative by a referee EIA method. Of the six samples positive in the *Is*-anti-ß$_2$-Glycoprotein I IgG and negative in the other EIA, one was positive and five were negative by a referee EIA method. For anti-ß$_2$-Glycoprotein I IgM, further resolution of the discordant samples showed that of the four samples that were negative in the *Is*-anti-ß$_2$-Glycoprotein I IgM but positive in the other EIA, two were positive and two were negative by a referee EIA method**.**

TABLE 2
***Is*-anti-ß$_2$-Glycoprotein I IgG**

		Positive	Negative	*Equivocal
Other EIA	Positive	38	3	0
	Negative	6	123	2
	*Equivocal	0	0	0

			**95% CI*
Relative Sensitivity	38/ 41	= 92.7%	80.1 - 98.5%
Relative Specificity	123/129	= 95.3%	90.2 - 98.3%
Overall Agreement	161/170	= 94.7%	90.2 - 97.6%

TABLE 3
***Is*-anti-ß$_2$-Glycoprotein I IgM**

		Positive	Negative	*Equivocal
Other EIA	Positive	31	5	2
	Negative	0	124	0
	*Equivocal	0	0	0

***95% CI*

Relative Sensitivity 31/ 35 = 88.6% 73.3 -
Relative Specificity 124/124 96.8% = 100.0% 97.1
Overall Agreement 155/159 -100.0% = 97.5%
93.7 - 99.3%

* Equivocal results were excluded from calculations.

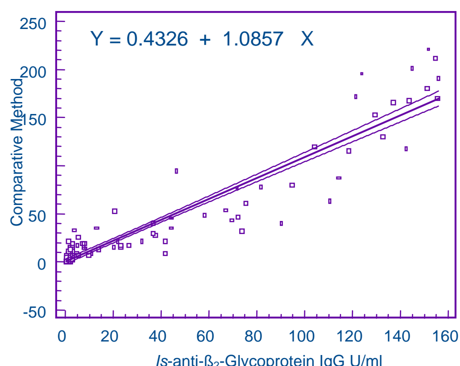
** 95% Confidence Intervals (CI) calculated by the Exact Method.[16]

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.

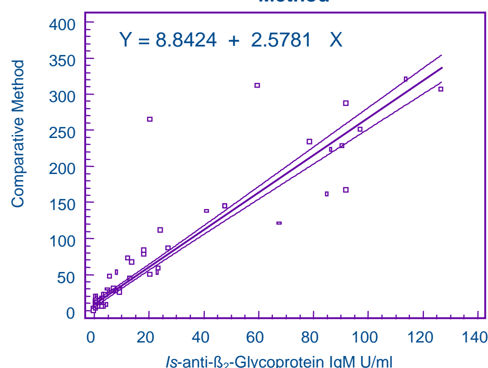
Linear regression analyses and scattergrams for the correlation studies with the comparative methods are shown in FIGURES 5 and 6.

FIGURE 5
***Is*-anti-ß$_2$-Glycoprotein I IgG**
Correlation with Comparative Method



Intercept: 0.4326 (95% CI -2.1954 to 3.0605) Slope: 1.0857 (95% CI 1.0307 to 1.1407) Coefficient of determination = 0.8994 Correlation coefficient **r** = 0.9484 95% CI for **r** = 0.9308 to 0.9616

FIGURE 6
***Is*-anti-ß$_2$-Glycoprotein I IgM**
Correlation with Comparative Method



Intercept: 8.8424 (95% CI 4.8121 to 12.8727) Slope: 2.5782 (95% CI 2.4199 to 2.7363) Coefficient of determination = 0.8669 Correlation coefficient **r** = 0.9311 95% CI for **r** = 0.9070 to 0.9491

B. Clinical Sensitivity and Specificity

A total of three hundred and eighty-eight frozen retrospective, clinically characterized sera were assayed using the *Is*-anti-ß$_2$-Glycoprotein I IgG/IgM Test Kit in order to assess both the clinical sensitivity and clinical specificity of the assay system. These samples consisted of 248 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, poly-myositis/dermatomyositis and rheumatoid arthritis and 15 samples from pa-tients with positive RPR titers. Results are summarized in TABLE 4.

TABLE 4

Patient Group	Total	*IgG* Positive	*IgG* Negative/Equiv.	*IgM* Positive	*IgM* Negative/Equiv.
Normals	**248**	**3**	**245**	**3**	**245**
APS	**57**	**46**	**11**	**25**	**32**
SLE	**33**	**7**	**2**	**7**	**26**
Other Autoimmune Diseases	**35**	**3**	**32**	**2**	**33**
RPR Positive	**15**	**0**	**15**	**1**	**14**

	IgG			*IgM*		
Clinical Specificity:	**#Neg or Equiv. /Total#**			**#Neg or Equiv./Total#**		
Normals	245/248	=	98.8%	245/248	=	98.8%
Other Autoimmune Diseases	32/35	=	91.4%	33/35	=	94.3%
RPR Positive	15/15	=	100.0%	14/15	=	93.3%
Clinical Sensitivity:	**#Pos/Total#**			**#Pos./Total#**		
APS	46/57	=	80.7%	25/57	=	43.9%
SLE	7/33	=	21.2%	7/33	=	21.2%

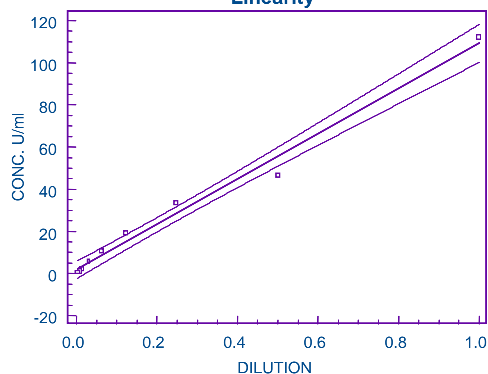
C. Cross-Reactivity

To assess the potential for positive results due to cross reactive antibodies, 50 samples which were reactive to various autoantibodies (SSA/SSB, Scl-70. Jo-1, dsDNA, RF and RPR positive) were tested using the *Is*-anti-ß$_2$-Glycoprotein I IgG/IgM Test Kit. One sample positive for dsDNA was positive in both the *Is*-anti-ß$_2$-Glycoprotein I IgG and IgM tests. One sample positive for Scl-70 antibodies was positive in the *Is*-anti-ß$_2$-Glycoprotein I IgG test. One sample positive for Jo-1 antibodies was positive in both the *Is*-anti-ß$_2$-Glycoprotein I IgG and IgM tests and one RPR positive sample was positive in the *Is*-anti-ß$_2$-Glycoprotein I IgM test. The remaining samples were negative.

D. Linearity

To assess the linearity of the *Is*-anti-ß$_2$-Glycoprotein 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 7 and 8.

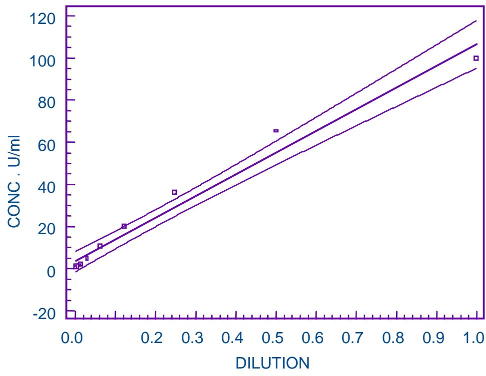
FIGURE 7
***Is*-anti-ß$_2$-Glycoprotein I IgG**
Linearity



Regression Equation
Y = 1.6732 + 107.5806 X

Intercept 1.67318 Slope 107.58059
Coefficient of Determination = 0.9876 Correlation Coefficient r = 0.9938 95% CI for **r** = 0.9695 to 0.9987

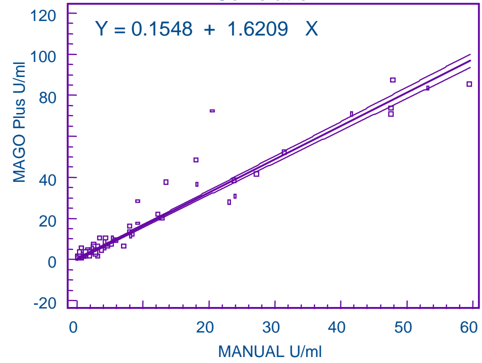
FIGURE 8
***Is*-anti-ß$_2$-Glycoprotein I IgM**



Regression Equation
Y = 3.3897 + 103.2019 X

Intercept: 3.3897 Slope: 103.2019
Coefficient of Determination = 0.9760 Correlation Coefficient r = 0.9879 95% CI for r = 0.9479 to 0.9972

FIGURE 10
***Is*-anti-ß$_2$-Glycoprotein I IgM**
Manual vs. MAGO Plus
Correlation



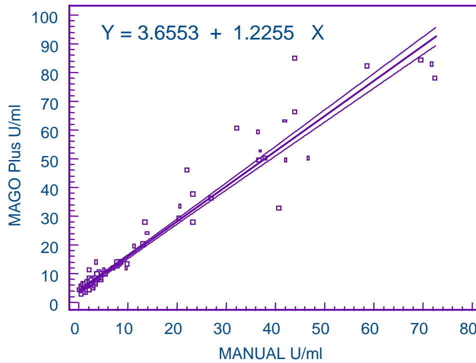
Intercept 0.1548 (95% CI -0.4904 to 0.7800) Slope 1.6209 (95% CI 1.5616 to 1.6802) Coefficient of determination = 0.9418 Correlation coefficient **r** = 0.9704

95% CI for **r** = 0.9606 to 0.9779

E. Correlation of Manual and MAGO Plus Results

The *Is*-anti-ß$_2$-glycoprotein I 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 153 serum samples tested for anti-ß$_2$-glycoprotein I IgG antibodies and 163 sera tested for anti-ß$_2$-glycoprotein I IgM tested 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 9 and 10. The data indicate good correlation with Correlation Coefficients (r) of 0.9720 for anti-ß$_2$-glycoprotein I IgG and 0.9704 for anti-ß$_2$-glycoprotein I IgM.

FIGURE 9
***Is*-anti-ß$_2$-Glycoprotein I IgG**
Manual vs. MAGO Plus
Correlation



Intercept 3.6553 (95% CI 2.8680 to 4.4426) Slope 1.2255 (95% CI 1.1778 to 1.2731) Coefficient of determination = 0.9447 Correlation coefficient **r** = 0.9720

95% CI for **r** = 0.9616 to 0.9796

F. Precision

To assess the precision of the *Is*-anti-ß$_2$-Glycoprotein I IgG/IgM Test Kit six serum samples of varying reactivity were tested in triplicate in three seperate 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-ß$_2$-Glycoprotein I IgG

SERUM	INTRA-ASSAY RUN 1			INTRA-ASSAY RUN 2			INTRA-ASSAY RUN 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	1.8	0.208	11.35	1.7	0.153	8.81	2.0	0.153	7.77	1.8	0.181	9.82
B	3.0	0.208	7.02	3.1	0.300	9.68	3.3	0.173	5.25	3.1	0.249	7.97
C	25.9	0.252	0.97	25.7	0.058	0.22	26.8	0.557	2.08	26.1	0.589	2.26
D	33.6	0.850	2.53	32.0	1.193	3.73	31.7	1.662	5.25	32.4	1.439	4.44
E	46.7	0.700	1.50	44.6	0.608	1.36	45.5	0.643	1.41	45.6	1.074	2.36
F	57.4	8.088	14.09	59.1	1.332	2.25	59.4	3.675	6.19	58.6	4.587	7.82

TABLE 6
MAGO Plus Intra-Assay and Interassay Precision
for *Is*-anti-ß$_2$-Glycoprotein I IgG

SERUM	INTRA-ASSAY RUN 1			INTRA-ASSAY RUN 2			INTRA-ASSAY RUN 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	6.2	0.929	15.07	5.2	0.058	1.12	4.5	0.458	10.18	5.3	0.893	16.91
B	8.6	1.930	22.35	7.1	0.306	4.32	5.5	0.503	9.21	7.1	1.702	24.13
C	34.3	1.916	5.59	33.3	0.643	1.93	32.2	1.930	5.99	33.3	1.659	4.99
D	51.7	2.330	4.51	42.7	3.857	9.03	48.8	3.009	6.17	47.7	4.811	10.08
E	59.3	1.716	2.89	60.1	3.650	6.07	58.3	3.764	6.46	59.2	2.867	4.84
F	89.6	4.521	5.05	88.1	2.409	2.74	88.6	3.119	3.52	88.8	3.074	3.46

TABLE 7
Manual Intra-Assay and Interassay Precision
for *Is*-anti-ß$_2$-Glycoprotein I IgM

SERUM	INTRA-ASSAY RUN 1			INTRA-ASSAY RUN 2			INTRA-ASSAY RUN 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	1.2	0.208	17.84	1.1	0.208	19.52	0.9	0.058	6.19	1.1	0.181	17.15
B	4.8	0.208	4.37	4.9	0.000	0.00	4.6	0.208	4.49	4.8	0.187	3.92
C	13.2	1.940	14.73	12.9	1.375	10.66	12.2	0.100	0.82	12.8	1.266	9.93
D	19.3	1.790	9.26	18.3	2.003	10.97	15.2	1.480	9.74	17.6	2.409	13.69
E	37.8	3.499	9.26	39.4	4.451	11.30	36.7	0.153	0.42	38.0	3.062	8.06
F	51.7	2.330	4.51	54.2	3.318	6.12	55.5	0.781	1.41	53.8	2.657	4.94

TABLE 8
MAGO Plus Intra-Assay and Interassay Precision for *Is*-anti-ß$_2$-Glycoprotein I IgM

SERUM	INTRA-ASSAY RUN 1			INTRA-ASSAY RUN 2			INTRA-ASSAY RUN 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	2.1	0.361	17.17	1.6	0.173	10.83	2.0	0.265	13.23	1.9	0.332	17.46
B	6.2	0.306	4.90	6.5	0.529	8.14	7.3	0.709	9.76	6.7	0.660	9.89
C	16.8	2.454	14.64	19.2	3.453	18.02	22.0	4.466	20.33	19.3	3.815	19.77
D	21.5	2.369	11.00	26.4	1.823	6.90	25.8	1.069	4.15	24.6	2.796	11.38
E	43.4	2.754	6.35	48.9	2.646	5.41	53.0	3.958	7.46	48.4	5.020	10.37
F	65.3	9.794	15.07	77.6	1.501	1.93	75.7	4.130	5.45	72.9	7.878	10.81

REFERENCES

1. Harris, E. N., Gharavi, A. E. and Hughes, G. R. V. 1985. Antiphos-pholipid Antibodies. Clin Rheum Dis 11(3): 591-609.
2. Love, P. E., *et al.* 1990. Antiphospholipid antibodies: Anticardiolipin and the lupus anti-coagulant 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.* 1986. Thrombosis recurrent fetal loss, and thrombo-cytopenia:Predictive value of the anticardiolipin antibody test. Arch. Intern. Med. 146:2153-2156.
5. Harris, E. N. 1992. Serological Detection of antiphospholipid antibodies. Stroke. 23 (2 Suppl):I3-6.
6. Galli, M. P. *et al.* 1990. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet. 335:1544-1547.
7. McNeill, H. P. *et al.* 1990 Anti-phospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation: ß$_2$-glycoprotein I (apolipo-protein H). Proc. Natl. Acad. Sci USA 87: 4120-4124.
8. Matsuura, E. *et al.* 1995. Molecular studies on phospholipid-binding sites and cryptic epitopes appearing on ß$_2$-glycoprotein I structure recognized by anti-cardiolipin antibodies. Lupus. Vol. 4 suppl. S13-S17.
9. Matsuda, J. *et al.* 1995. Prevalence of ß$_2$-glycoprotein I antibody in systemic lupus erythematosus patients with ß$_2$-glycoprotein I dependent antiphospholipid antibodies. Ann. Rheum. Dis. 54:73-75.
10. Koike, T. 1994. Anticardiolipin antibodies and ß$_2$-glycoprotein I. Clin. Immunol and Immunopathol. 72: 187-192.
11. Ichikawa, K. *et al.* 1994. ß$_2$-Glycoprotein I reactivity of monoclonal anticardiolipin antibodies from patients with the antiphospholipid syn-drome. Arth. and Rheumat. 37: 1453-1461.
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. Bruce, I. N. *et al.* 2000. Prevalence of antibodies to ß$_2$-glycoprotein I in systemic lupus erythematosus and their association with antiphos-pholipid antibody syndrome criteria: a single center study and literature review. J. Rheumatol. 27 (12): 2833-2837.
14. Guerin, J. *et al.* 1999. Anti-Beta 2-glycoprotein I antibody isotype and IgG subclass in anti-phospholipid syndrome patients. Autoimmunity 31(2): 109-116.
15. Sanmarco, M. *et al.* 1997. Prevalence and clinical significance of IgG isotype anti-beta 2-glycoprotein I antibodies in antiphospholipid syn-drome: a comparative study with anti-cardiolipin antibodies. J Lab Clin Med 129 (5) 499-506.
16. Gardner, M. J. and Altman, D. G. 1986. Confidence Intervals Rather than Hypothesis Testing.
17. 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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