Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

anti-PR-3 (C-ANCA) IgG
Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-440 - 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:21 dilutions of 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 samples into the antigen wells.
3. Incubate at room temperature (18-30° C) for 30 ± 5 min.
4. Discard 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 (18-30° C) 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 (18-30° C) for 30 ± 5 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbances at 450 nm (reference at 600-630 nm).

INTENDED USE

For the semi-quantitative and qualitative detection of antibodies against the proteinase-3 (PR-3) antigen in serum as an aid in the diagnosis of Wegener's granulomatosis.

SUMMARY AND EXPLANATION

Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed at various lysosomal enzymes.1 Staining of alcohol-fixed neutrophils by indirect immunofluorescence (IFA) shows two main staining patterns: cytoplasmic (C-ANCA), and perinuclear (P-ANCA). The C-ANCA pattern is caused by autoantibodies to proteinase-3 (PR-3).[3,4] These autoantibodies are found in 84-100% of patients with Wegener's granulomatosis (WG).[1,5] The P-ANCA pattern is caused by autoantibodies to myeloperoxidase (MPO) and is strongly associated with other types of vasculitides.[3,4] Antibodies to MPO are found in 59% of patients with microscopic polyangiitis.[4] Autoantibodies to PR-3 and MPO are highly specific for WG and microscopic polyangiitis respectively, and antibody titer often correlates with disease activity.[1]

The Diamedix *Is*-anti-PR-3 IgG Test Kit is an EIA procedure intended for the qualitative detection and semi-quantitation of antibodies to proteinase-3 antigen. The results are reported in ELISA Units (EU) per ml and are determined by comparison to a standard curve.

PRINCIPLE OF THE PROCEDURE

Purified PR-3 antigen from human neutrophils is bound to microwells. Diluted patient sera, Standards and controls are placed in the microwells and incubated. Anti-PR-3 IgG 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) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgG antibodies to PR-3 present in the sample.

REAGENTS

Each Is-anti-PR-3 IgG Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded purple, coated with purified PR-3 antigen (from human neutrophils).
0 EU/ml Standard	One vial with yellow cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma, non-reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
5 EU/ml Standard	Two vials with green cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma, reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
12.5 EU/ml Standard	One vial with brown cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma, reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
25 EU/ml Standard	One vial with purple cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma, reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
50 EU/ml Standard	One vial with white cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma, reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
100 EU/ml Standard	One vial with red cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma, reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
Low Positive Control	One vial with blue cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma weakly reactive for PR-3 IgG antibodies, 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml range printed on label. The positive control is used to control the low range of the assay.
Negative Control	One vial with black cap containing 1.8 ml of pre-diluted human serum or defibrinated plasma non-reactive for PR-3 antibodies 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Assigned EU/ml range printed on the label. The Negative Control is used to control the negative range of the assay.
Sample **A** Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains 0.2% sodium azide and Proclin® 300, 90 ppm active ingredient. Color-coded blue.
Wash **T** Concentrate (20X)	Two bottles with clear caps containing 50 ml of Tris buffer with detergent and Proclin® 300, 15 ppm active ingredient. Each bottle is sufficient to make 1 liter of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with horseradish peroxidase. Also includes protein stabilizers and Proclin® 300, 30 ppm active ingredient. Color-coded pink.
Substrate **HRP**	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' Tetramethylbenzidine).
Stop **N** Solution	One bottle with white cap containing 30 ml of 1 N Sulfuric Acid. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

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.

PRECAUTIONS

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 and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B 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. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test reagents contain Proclin® 300 as a preservative. When disposing of reagents containing Proclin® 300, flush drains with copious amounts of water to dilute the active components below active levels.
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 prema-turely.
7. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **T** Concentrate, Substrate **HRP** and Stop **N** 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. Antigen 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.*
14. The reported concentration of anti-PR-3 IgG in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.

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, (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18A3).

***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 50 ml of Wash Concentrate (20X) to one liter of deionized or distilled H_2O.

MANUAL USERS

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

   For qualitative assays: The 5 EU/ml Standard is required and serves as the Cut-Off Standard for the assay. This Standard should be assayed in triplicate. In addition, a Blank (100 µl Sample Diluent only, in the first well of the strip) is required. This will ultimately be used to "zero" the photometer before reading the test result.

   For semi-quantitative assays: All six Standards are required. No Blank is required; the 0 EU/ml Standard will function as the "zero" and will be placed in the first well of the first strip. Standards (from 0-100 EU/ml) can be run singly or in duplicate.

   * Low Positive and Negative Controls should be run for either assay option.
2. Prepare 1:21 dilutions of the patient samples in Sample Diluent. (e.g., by addition of 10 µl sample to 200 µl Sample Diluent).
3. 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.

. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.

5. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300 µl of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
6. Place 100 µl of Conjugate into each well, avoiding bubble formation.
7. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
8. Wash the wells as described in Step 4 above.
9. Place 100 µl of Substrate into each well, avoiding bubble formation.
10. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
11. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
12. Read the absorbance of each well at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 minutes of adding Stop Solution.

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 Low Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank or the 0 EU/ml Standard must be < 0.250.
3. The absorbance of the Negative Control must be lower than that of the 5 EU/ml Standard.
4. The absorbance of the Low Positive Control must be higher than that of the 5 EU/ml Standard.
5. The absorbance of the 100 EU/ml Standard must be ≥ 3 times the absorbance of the 5 EU/ml Standard.
6. When using the test semi-quantitatively the Low Positive Control and the Negative Control must be within their assigned ranges.
7. When using the assay qualitatively the Low Positive Control Index Value must be ≥ 1.2 and the Negative Control Index Value must be < 0.8.

If any of these criteria are 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 or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to CLSI, formerly NCCLS, document C24-A2, Statistical Quality Control for Quantitative Measurements: Principles and Definitions.

RESULTS

1. Calculation

***Qualitative Assay*:** Qualitative results may be obtained using the 5 EU/ml Standard as a Cut-Off Standard, run in triplicate, following a single Blank well (100 µl Sample Diluent only). If performing the qualitative assay option, set the reader for absorbance mode and calculate the mean absorbance of the three 5 EU/ml Standard wells. Alternatively, a reader with a Cut-Off Control test option may be used.

Note: When calculating the mean absorbance value for the Cut-Off Standard exclude any absorbance value that deviates by more than 15% from the mean of the three absorbance values. Use the mean of the remaining two replicates in calculations. Exclusion of more than one of the three absorbance values invalidates the run.

Example: Absorbance values obtained for Cut-Off Standard: 0.276, 0.288, 0.258 (after subtraction of the blank)

Mean Absorbance of Cut-Off Standard = 0.274

Sample Absorbance = 1.150

Index Value =1.150 / 0.274 = 4.2

When using an Automated EIA Processor (e.g. MAGO ® Plus Automated EIA Processor), results are automatically calculated.

Semi-quantitative Assay: Semi-quantitative results may be obtained from the point-to-point curve fit using all six Standards. For the BP-96 or Stat-Fax readers the point-to-point option should be selected and Standard values entered accordingly.

An Automated EIA Processor (e.g. MAGO ® Plus Automated EIA Processor), will calculate 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.

Is-anti-PR3 Value	***Index Value****	***Interpretation***
< 4.0 EU/ml	< 0.80	Negative for antibodies to PR-3
4.0–5.9 EU/ml	0.80 - 1.19	Equivocal for antibodies to PR-3. Sample should be retested. If retest results are equivocal, the sample should be reported as equivocal, tested by another method, or a new sample should be tested.**
≥ 6.0 EU/ml	≥ 1.20	PR-3 IgG antibody detected.

* For Qualitative results only.

** Equivocal samples that give positive results on retest should be reported as positive. Equivocal results that give negative results on retest should be reported as negative.

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

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

CUT-OFF ESTABLISHMENT

To determine the positive threshold for the *Is*-anti-PR-3 IgG Test Kit, one hundred seventy-six normal sera were assayed on the kit. The mean and standard deviation of the absorbance values for these normal sera were 0.056 and 0.075 respectively. The cut-off was determined as being the mean absorbance value plus four standard deviations. A positive serum was titrated to yield an absorbance equivalent to the cut-off absorbance and was assigned an EU/ml value of 5. The positive serum was then diluted to obtain 100, 50, 25, 12.5, 5 and 0 EU/ml to create a set of six standards. An equivocal zone was included in the assay design to account for natural variations inherent in any serologic method. Sample values at or near the cut-off point may result in occasional false negative or false positive results. The equivocal zone in the *Is*-anti-PR-3 IgG Test Kit includes values of 4.0-5.9 EU/ml and indicates the probable presence of anti-PR-3 IgG antibodies. It is recommended that samples with equivocal results be re-tested. If re-test results remain equivocal they should be reported as equivocal and a fresh sample should be collected at a later date for testing.

LIMITATIONS

1. The results obtained with the *Is*-anti-PR-3 IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.
2. Assay performance characteristics have not been established for visual result determination.
3. The test should be performed on serum. The use of whole blood or plasma has not been established.
4. The analysis of a single serum sample should not be used as the sole criteria for diagnosis of an autoimmune disease.
5. Screening of the general population should not be performed. The positive predictive value depends on the likelihood of Wegener's Granulomatosis being present. Testing should only be performed when clinical symptoms are present or disease is suspected.
6. Results from immunosuppressed patients should be interpreted with caution.
7. The performance characteristics of the *Is*-anti-PR-3 IgG Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.
8. Icteric, lipemic, hemolyzed, or heat inactivated sera may cause erroneous results and should be avoided.

EXPECTED VALUES

The prevalence of PR-3 IgG antibodies in the normal population can vary depending on a number of factors such as age, geographical location, race and testing method used.

In the present studies sera from 176 normal individuals of various ages and genders from different geographic locations were evaluated in the *Is*-anti-PR-3 IgG Test Kit. One hundred and sixty-eight samples (95.5%) were negative for IgG antibodies to PR-3 three samples (1.7%) were positive and five samples (2.8%) was equivocal for anti-PR-3 IgG antibodies. The age distribution and prevalence for this population is shown in Table 1.

Sera from 40 diagnosed Wegener's Granulomatosis (WG) patients were evaluated in the *Is*-anti-PR-3 IgG Test Kit. Two samples (5.0%) were negative for IgG antibodies to PR-3, 38 samples (95.0%) were positive and no samples (0.0%) were equivocal for PR-3 IgG antibodies. Histograms showing the distribution of EU/ml values for the normal and WG populations are shown in Figures 1 and 2.

Table 1

	Number of Donors	Prevalence
Total Number	176	1.7%
Geographic locations: Various	176	1.7%
Age		
4 – 10	9	0.0%
11 – 20	15	0.0%
21 – 40	49	0.0%
41 – 60	57	1.8%
61 – 80	33	6.1%
81 – 91	13	0.0%

FIGURE 1
Distribution of *Is*-anti-PR-3 IgG in a Normal Population

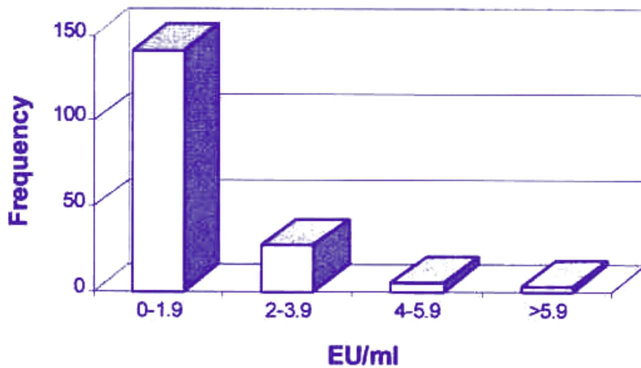
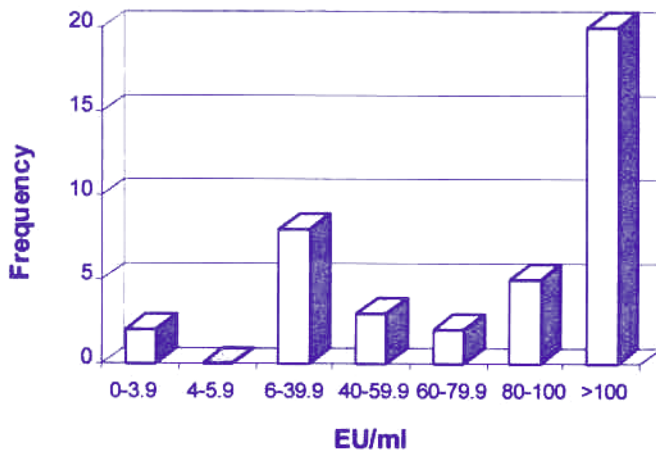


FIGURE 2
Distribution of *Is*-anti-PR-3 IgG in WG Patients



PERFORMANCE CHARACTERISTICS

A. Relative Sensitivity and Specificity

Frozen retrospective sera from two hundred and fifty-six patients were tested on the *Is*-anti-PR-3 IgG Test Kit and a commercially available ELISA for anti-PR-3 antibodies. Based on the results of this testing, the relative sensitivity and specificity were calculated. The results obtained are shown in Table 2:

TABLE 2
Is-anti-PR-3 IgG

Other ELISA		POSITIVE	*EQUIVOCAL	NEGATIVE
	POSITIVE	57	0	0
	* EQUIVOCAL	1	0	0
	NEGATIVE	4	7	187

		95% CI
Relative Sensitivity	57/57 =100.0%	93.7-100.0%
Relative Specificity	187/191 =97.9%	94.7-99.4%
Overall Agreement	244/248 =98.4%	95.9-99.6%

* Equivocal results were excluded from calculations.

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 judgment can be made on the comparison's accuracy to predict disease.

B. Clinical Sensitivity and Specificity Using Characterized Sera

A total of 256 frozen retrospective, clinically characterized sera were assayed using the *Is*-anti-PR-3 IgG Test Kit. The results obtained are shown in Table 3.

TABLE 3

Patient Group:	Positive	Equivocal*	Negative	Total
Normals	3	5	168	176
Wegener's Granulomatosis	38	0	2	40
Microscopic Polyangiitis	21	2	17	40

Clinical Specificity:				95% CI
Normals	168/171	=	98.3%	95.0-99.6

Clinical Sensitivity:				95% CI
Wegener's Granulomatosis	38/40	=	95.0%	83.1-91.4
Microscopic Polyangiitis	21/38	=	55.3%	38.3-71.4

* Equivocal results were excluded from calculations.

C. Precision

To determine the precision of the *Is*-anti-PR-3 IgG Test Kit, four positive and two negative sera were assayed ten times each in three different runs at two different sites. The intra- and interassay precision obtained at each site is shown in Tables 4 and 5.

TABLE 4
Site #1- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY RUN 1		INTRA-ASSAY RUN 2		INTRA-ASSAY RUN 3		INTERASSAY	
	MEAN EU/ml	CV%	MEAN EU/ml	CV%	MEAN EU/ml	CV%	MEAN EU/ml	CV%
A (POS)	43.3	12.60	43.7	7.34	35.5	9.71	40.8	13.65
B (POS)	65.1	12.86	55.5	8.10	48.8	9.35	56.5	15.86
C (POS)	20.9	14.19	17.8	11.46	13.2	12.18	17.3	22.56
D (POS)	30.6	5.94	27.7	8.11	27.1	6.74	28.4	8.60
E (NEG)	0.5	20.18	0.3	33.99	0.2	57.92	0.3	50.84
F (NEG)	0.5	15.17	0.4	38.33	0.3	43.90	0.4	38.89

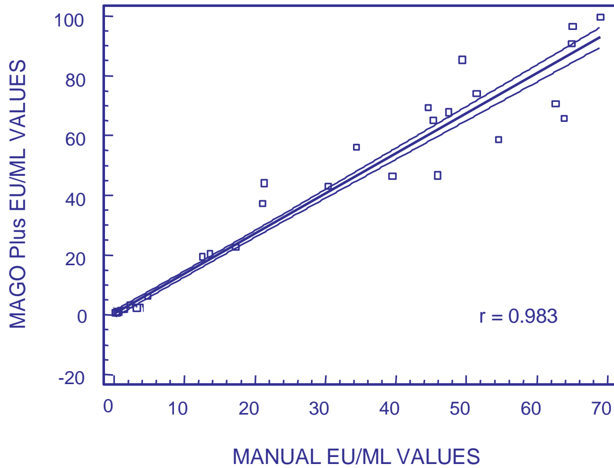
TABLE 5
Site #2- Intra-Assay and Interassay Precision

SERUM	INTRA-ASSAY RUN 1		INTRA-ASSAY RUN 2		INTRA-ASSAY RUN 3		INTERASSAY	
	MEAN EU/ml	CV%	MEAN EU/ml	CV%	MEAN EU/ml	CV%	MEAN EU/ml	CV%
A (POS)	43.8	8.70	46.4	5.91	48.5	8.01	46.6	8.07
B (POS)	67.3	5.27	66.1	7.52	68.7	8.15	67.4	7.04
C (POS)	15.2	12.28	27.4	8.18	16.4	10.52	19.7	29.87
D (POS)	26.1	10.71	35.1	5.10	28.2	7.80	29.8	15.11
E (NEG)	0.5	29.22	0.4	16.64	0.4	20.20	0.4	31.44
F (NEG)	0.5	21.74	0.5	17.89	0.5	12.95	0.5	17.26

D. Correlation of Manual and MAGO Plus Results

The *Is*-anti-PR-3 IgG 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 90 serum samples tested by both methods were plotted. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in Figure 3. The data indicate good correlation with a Pearson Correlation Coefficient of 0.983.

FIGURE 3
Manual and MAGO Plus Result Correlation



E. MAGO Plus Precision

The precision of the *Is*-anti-PR-3 IgG Test Kit when performed on the MAGO ® Plus Automated EIA Processor was determined by assaying six sera ten times each in three different runs. Table 6 shows the intra-and interassay precision obtained using the MAGO ® Plus Automated EIA Processor.

TABLE 6
Site #2- Intra-Assay and Interassay Precision - MAGO Plus

SERUM	INTRA-ASSAY RUN 1		INTRA-ASSAY RUN 2		INTRA-ASSAY RUN 3		INTERASSAY	
	MEAN EU/ml	CV%	MEAN EU/ml	CV%	MEAN EU/ml	CV%	MEAN EU/ml	CV%
A (POS)	34.9	15.47	41.2	11.44	51.8	13.68	42.9	20.55
B (POS)	53.9	17.30	71.9	23.20	80.8	11.19	69.4	23.50
C (POS)	19.5	25.46	25.2	7.59	25.1	7.66	23.3	17.82
D (POS)	32.1	13.83	36.0	5.40	32.7	8.37	33.6	10.61
E (NEG)	0.4	78.26	0.5	27.43	0.5	24.00	0.4	44.80
F (NEG)	0.7	74.05	0.7	23.22	0.7	22.81	0.7	44.89

REFERENCES

1. Peter, J. B. 1991. Neutrophil cytoplasmic antibodies, In: J. B. Peter (Ed.), *Use and Interpretation of Tests in Clinical Immunology*, Eighth Edition. Specialty Laboratories, Inc., Santa Monica, CA. pp. 174-177.
2. Hagen, E. C., et al. 1996. Development and standardization of solid phase assays for the detection of anti-neutrophil cytoplasmic antibodies (ANCA). *J. Immunol. Methods.* 196:1-15.
3. Savage, C. O. S., L. Harper, and D. Adu. 1997. Primary systemic vasculitis. *Lancet.* 349:553-558.
4. Hagen, E. C., et al. 1992. Anti-neutrophil cytoplasmic antibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenetic consequences. *Blood.* 91: 1996-2002.
5. Zhao, M. H., and C. M. Lockwood. 1996. A comprehensive method to purify three major ANCA antigens: proteinase-3, myeloperoxidase, and bactericidal/permeability increasing protein from human neutrophil granule acid extract. *J. Immunol. Methods.* 197:121-130.
6. 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.

Proclin® 300 is a registered trademark of Rohm and Haas Corp. Philadelphia, PA.

Diamedix Corporation • A Subsidiary of ERBA Diagnostics, Inc. 14100 NW 57th Court – Miami Lakes, Florida 33014 - USA
(305) 324-2300 / (800) 327-4565
www.erbadiagnostics.com

Delta Biologicals S.r.l., Via Nicaragua 12/14, 00071 - Pomezia, Rome
Italy Telephone #: +39-06-91190.1 Fax #: +39-069105244

I-720-440
Rev. 6 – June 15

THIS PAGE HAS INTENTIONALLY BEEN LEFT BLANK.