Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

Rheumatoid Factor
Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-710 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of Calibrator, Controls and patient samples in Sample Diluent. Mix well.
2. Add 100 µl of diluted Calibrator, Controls and samples into the wells. Reserve one well for reagent blank (100 µl of Sample Diluent).
3. Incubate at room temperature (18 – 30°) 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°) 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°) 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 quantitative detection of RF IgM-class antibodies in human serum by indirect enzyme immunoassay as an aid in the diagnosis of rheumatoid arthritis (RA).

SUMMARY AND EXPLANATION

Rheumatoid arthritis (RA) is a chronic relapsing inflammatory arthritis of unknown etiology usually affecting multiple joints with a varying degree of systemic involvement. The disease has been estimated to occur in 1 to 2% of the general population. Females are affected more than males by a three to one margin and prevalence increases with age, peaking at 35-45 years of age.[1,2]

A characteristic of RA is the presence in the blood and synovial fluid of a reactive group of proteins collectively known as Rheumatoid Factors (RF). Rheumatoid Factors are immunoglobulins of any isotype with antibody activity directed against antigenic sites in the Fc region of human or animal immunoglobulin (IgG). Because of its pentavalent structure and ability to cross-link immunoglobulin G antigen, IgM-RF is the main isotype identified by clinically available diagnostic tests for RF detection.[3] RF has been reported to occur in approximately 70-80% of patients with confirmed RA.[4,5] The concentration of RF tends to be highest when the disease peaks and tends to decrease during prolonged remission. This high RF frequency in RA cases makes their detection useful as a diagnostic tool, however, these factors are not unique to RA. RF is found in approximately 4% of the general population. RF is present in 75% of adult patients, with the highest incidence of RF occurring in patients over 65 years of age and in nearly all patients with Felty and Sjogren's Syndrome. Increased titers may accompany a variety of acute immune responses, particularly viral infections and a number of other diseases such as infectious mononucleosis, tuberculosis, leprosy, various parasitic diseases, liver disease, sarcoidosis and systemic lupus erythematosus.[6,7]

Conventional methods for the measurement of RF-IgM have depended upon the agglutination of particles (latex, charcoal, bentonite or erythrocytes) coated with human or animal IgG. The latex agglutination test is sensitive but it can result in a fairly high number of false positives. Quantitative serological tests such as EIA provide an objective measurement on a single sample dilution.[8]

The Diamedix *immuno*simplicity® *Is*-Rheumatoid Factor Test Kit is an EIA procedure intended for the quantitation of IgM antibodies to IgG antigen. The results are reported in IU/ml, traceable to the WHO International Reference preparation.[9]

PRINCIPLE OF THE PROCEDURE

Diluted samples are incubated with purified RF antigen (human IgG) bound to the solid surface of a microtiter well. Any RF-IgM antibody present binds to the immobilized human IgG to form antigen-antibody complexes. Unbound antibody is washed from the wells and horseradish peroxidase-conjugated anti-human IgM is added. The enzyme conjugate binds to the antigen-antibody complex. Excess conjugate is washed away and a specific substrate added. Bound enzyme conjugate begins a hydrolytic reaction causing color development. After a specific time, the reaction is stopped. The intensity of the generated color is proportional to the amount of RF specific IgM antibody bound to the wells. The results are read on a spectrophotometer. The net absorbance is calculated by subtracting the absorbance values for the blank from the absorbance value for the sample.

A calibrator that is assayed with each run is then used to calculate the RF-IgM activity in IU/ml from the net absorbance value.

REAGENTS

Each Is-Rheumatoid Factor Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded pink, coated with purified human IgG.
Calibrator	One vial with blue cap containing 0.25 ml of human serum, highly reactive for RF-IgM antibodies, 0.1% sodium azide. Assigned IU/ml value printed on label.
Positive Control	One vial with white cap containing 0.25 ml of human serum, moderately reactive for RF-IgM antibodies, 0.1% sodium azide. Assigned IU/ml range printed on label.
Negative Control	One vial with black cap containing 0.25 ml of human serum, non-reactive for RF-IgM antibodies, 0.1% sodium azide.
Sample **E** Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with Tween 20 and protein stabilizers. Contains Proclin® 300, 15 ppm active ingredient. Color-coded blue.
Wash **U** Concentrate (20X)	Two bottles with clear caps containing 50 ml of Phosphate buffer with detergent and Proclin® 300, 15 ppm active ingredient. Each bottle is sufficient to make 1050 ml of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin M labeled with horseradish peroxidase. Also includes protein stabilizers and preservatives. Color-coded pink.
Substrate **HRP**	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).
Stop **O** Solution	One bottle with white cap containing 30 ml of 1N Hydrochloric 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, calibrator, 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 & 2 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1 & 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 RF IgM-class 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. Certain of the test reagents contain Proclin® 300 as preservative. When disposing of reagents containing Proclin® 300 flush drains with copious amounts of water to dilute the active components below active levels.
7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants, which will cause the solution to change color prematurely.
8 The following components are interchangeable: Sample **E** Diluent, Wash **U** Concentrate, Substrate **HRP** and Stop **O** Solution.
9. Do not use reagents beyond their expiration date. Expiration dates are printed on the reagent labels.
10. Store unused reagents at 2 to 8°C.
11. Incubations above or below the recommended temperatures or times may give erroneous results.
12. 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.
13. RF-antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
14. *(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.[10]

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. Prepare 1:101 dilutions of the Calibrator, controls and patient samples in Sample Diluent. (e.g., by addition of 5 µl sample to 500 µl Sample Diluent).
2. Mix 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 diluted Calibrator, controls and patient samples to the coated wells. Avoid formation of bubbles when transferring diluted samples.
3. Allow the wells to incubate uncovered at room temperature (18 to 30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by inverting the plate and tapping firmly on paper toweling. Wash the wells by rinsing 3 times with 300 µl Wash Solution. Remove excess moisture from the wells after each 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 to 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 to 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 the wells at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 30 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 Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.200.
3. The Positive Control must be within its assigned range.
4. The Negative Control must be < 16 IU/ml.

If any one 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

Determine the IU/ml for each patient specimen or control using the following formula:

$$\frac{\text{IU/ml of Calibrator}}{\text{Absorbance of Calibrator}} \times \text{Absorbance of sample} = \text{IU/ml of sample}$$

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

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.

IU/ml	*Index Value*	*Interpretation*
< 16.0 IU/ml	< 0.80	Negative for RF.
16.0 – 19.9 IU/ml	0.8 – 0.99	Equivocal*
≥ 20.0 IU/ml	≥ 1.0	Positive for RF.

* If equivocal results are obtained, the sample may be retested, another sample obtained, or the sample may be tested by an alternate method.

The Diamedix *Is*-Rheumatoid Factor Test Kit has been developed using a single point calibrator. Patient values, which contain very high levels of RF may produce absorbance values greater than the Calibrator absorbance. Patient sample results greater than the Calibrator value should be reported as "Greater than 100 IU/ml ". If numerical results are required for such samples, pre-dilute the sample using Sample Diluent and re-assay. Several dilutions (for example 1/5, 1/10 and 1/20) of the pre-diluted sample may be re-assayed simultaneously. Select the dilution that has an absorbance reading about 50% of the absorbance reading of the Calibrator; calculate the IU/ml for this dilution and multiply by the dilution factor to obtain estimated values.

Example: If the specimen was pre-diluted 1:10 before testing, the resulting IU/ml value should be multiplied by 10.

CUT-OFF ESTABLISHMENT

The cut-off values were established to optimally discriminate those individuals with RF from those individuals without RF. To determine the positive threshold for the *Is*-Rheumatoid Factor Test Kit, two hundred and four sera were tested. The mean and standard deviation of the IU/ml values was 4.5093 and 3.9227 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 the *Is*-Rheumatoid Factor the positive cut-off is 20 with 16 to 20 representing the equivocal zone. The appropriateness of the cut-off was additionally verified by applying the principles from Receiver Operating Curves to one hundred and eighty-five sera tested in the *Is*-Rheumatoid Factor as well as by another commercially available method. At the selected cut-off, the *Is*-Rheumatoid Factor Test Kit has a relative sensitivity of 99% and a relative specificity of 98%.

LIMITATIONS

1. A negative result does not exclude rheumatoid arthritis. Approximately 25% of patients with a diagnosed case of rheumatoid arthritis may present a negative result for RF.
2. Certain non-rheumatoid conditions, connective tissue disorders and a variety of other diseases such as hepatitis may elicit a positive RF test.
3. RF exists in three major immunoglobulin classes: IgA, IgG and IgM. Most test systems for RF are designed to detect IgM-RF because the molecules are large and react more easily with human IgG coated on the solid phase of the test system. Consequently, these tests will only detect RF of the IgM class.
4. The test should be performed on serum. The use of whole blood or plasma has not been established.
5. The performance characteristics of the Diamedix *Is*-Rheumatoid Factor Test Kit with automated equipment other than the MAGO® Plus Automated EIA Processor have not been established.

EXPECTED VALUES

The prevalence of RF 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. The expected value in the normal population is negative. However, a small but variable percentage of apparently healthy asymptomatic individuals may have RF. These individuals usually have low titers. The incidence of false positives tends to increase with age and is similar in males and females.

In the present study the expected values for a normal healthy population were assessed by testing sera from one hundred and eighteen S. Florida blood donors in the *Is*-Rheumatoid Factor Test Kit. One hundred and twelve sera (94.9%) were negative, two sera (1.7%) were positive and four sera (3.4%) were equivocal. The age distribution and prevalences for this population are shown in TABLE 1. Note that similar results were obtained for both manual and MAGO Plus testing.

TABLE 1
Age Distribution and Prevalence of Rheumatoid Factor in a Normal S. Florida Population

	Number of Donors	Prevalence
Total Number	118	1.69%
Geographic location:	South Florida: 118	
Age		
10 – 19	5	0.0%
20 – 29	25	4.0%
30 – 39	61	1.6%
40 – 49	20	0.0%
50 – 59	7	0.0%

The expected values for a clinical population were assessed by testing ninety-three sera from patients with a diagnosis of rheumatoid arthritis in the *Is*-Rheumatoid Factor Test Kit. For this population eighty-seven sera (93.5%) were positive, four (4.3%) were negative and two (2.2%) were equivocal.

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

FIGURE 1
Distribution of IgM-RF Values in a Normal Population

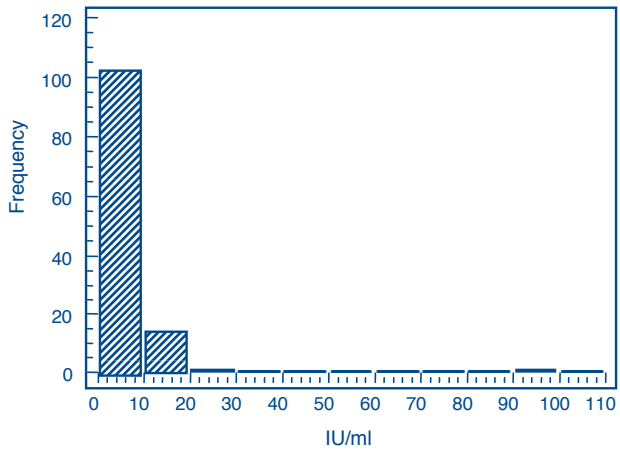
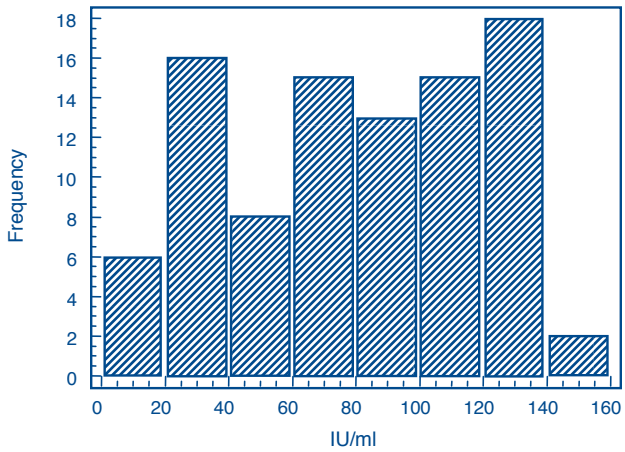


FIGURE 2
Distribution of IgM-RF Values in a Clinical Population



PERFORMANCE CHARACTERISTICS

Comparisons with Other Methods

A. Relative Sensitivity and Specificity versus Another EIA Test

One hundred and eighty-five frozen retrospective sera were tested using the *Is*-Rheumatoid Factor Test Kit and another commercially available EIA kit for detecting RF. Based upon the results of this testing the relative sensitivity, relative specificity and overall agreement were calculated. The results obtained are summarized in TABLE 2 and reveal excellent agreement with no discordant/discrepant sample results.

TABLE 2
***Is*- Rheumatoid Factor**

		Positive	Negative	*Equivocal
Other EIA	Positive	89	0	2
	Negative	0	91	1
	*Equivocal	2	0	0

		***95% CI*
Relative Sensitivity	89/89 = 100%	95.9-100.0%
Relative Specificity	91/91 = 100%	96.0.- 100%
Overall Agreement	190/190 = 100%	98.1- 100%

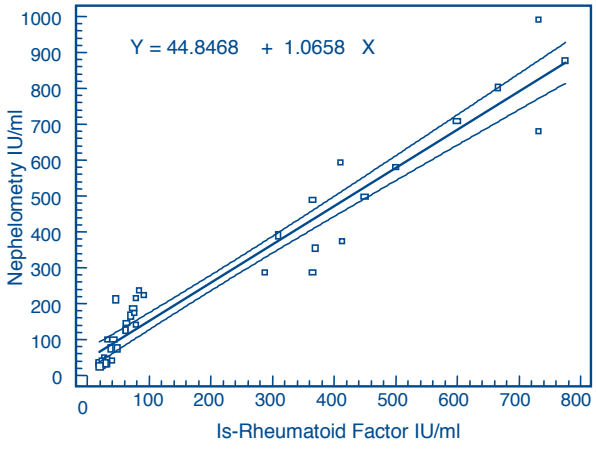
* Equivocal results were excluded from calculations

** 95% Confidence Intervals (CI) calculated by the Exact Method.[11]

B. Correlation with Nephelometry Results

Forty samples containing varying levels of RF as determined by nephelometry were tested using the *Is*-Rheumatoid Factor Test Kit. Samples whose results exceeded the Calibrator value were diluted and results obtained were then multiplied by the dilution factor. IU/ml values determined by both methods were then subjected to linear regression analysis. The correlation between IU/ml values determined by both methods is shown below.

FIGURE 3
Correlation with Nephelometry



Intercept 44.8468
Slope 1.0658
Coefficient of determination = 0.9355
Correlation Coefficient r = 0.9672
95% CI for r = 0.9826

C. Comparison with Latex Agglutination

The performance of the *Is*-Rheumatoid Factor Test Kit was also compared to that of the latex agglutination test which is another commonly used method for detecting RF. A total of 71 sera were tested by both methods. These consisted of 40 normal samples, 18 known clinical samples and 13 samples containing other autoantibodies with or without RF. The results are summarized in TABLE 3 below.

Table 3
Comparison with a Latex Agglutination Test

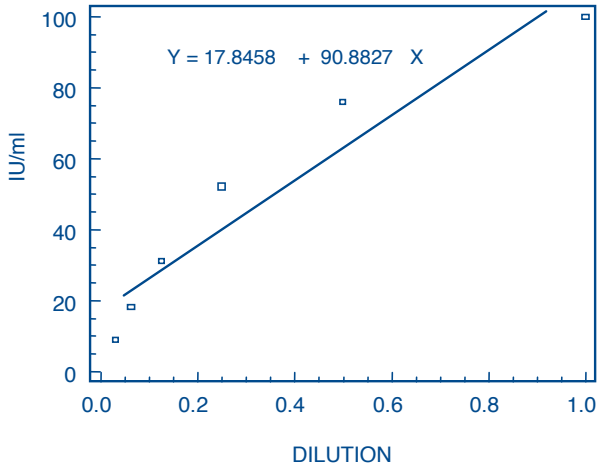
Sample Type	#	Latex Results	*Is*-Results	Comments
Normal Sera	40	40/40 Negative	40/40 Negative	Specificity: Latex & *Is*-100%
Clinical Sera	18	9/18 Positive	18/18 Positive	Sensitivity: Latex 50% Sensitivity: *Is*- 100%
Other Sera	13	4/13 Positive	10/13 Positive	Other ELISA 9/13 Positive

It should be noted that the screening dilution for the latex is 1:20. A positive result at this dilution is considered equivalent to 60 IU/ml. Therefore, all samples less than 60 IU/ml by either ELISA or nephelometry were negative by latex.

D. Linearity

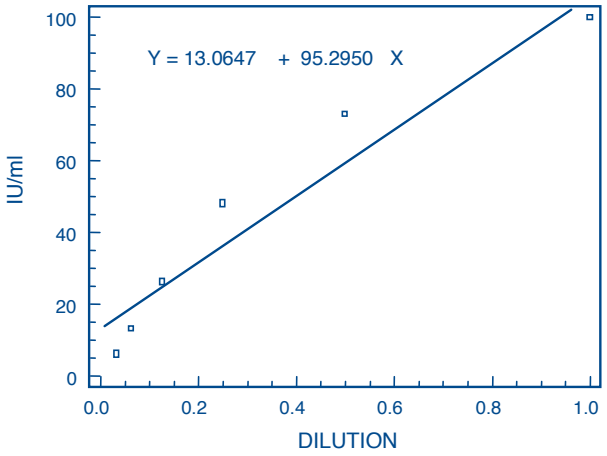
To assess the linearity of the *Is*-Rheumatoid Factor Test Kit several highly positive samples were serially diluted using Sample Diluent and each dilution was then tested in the assay system. In addition to this testing, the WHO Standard and the Diamedix in-house Reference Standard, both assigned values of 100 IU/ml, were also serially diluted and each dilution then tested with the assay system. FIGURES 4 and 5 show the titration of these materials. The Correlation Coefficients of the other samples were in close agreement with those shown below.

FIGURE 4
Linearity of WHO Standard



Intercept 17.8458
Slope 90.8827
Coefficient of determination = 0.9133
Correlation Coefficient r = 0.9557

FIGURE 5
Linearity of In-House Standard



Intercept 13.0647
Slope 95.2950
Coefficient of determination = 0.9282
Correlation Coefficient r = 0.9634

E. Lack of Cross-reactivity with Other Antinuclear Antibodies

Antinuclear antibodies (ANA) have been found in 14 to 28% of patients with RA and are usually found in patients with more advanced disease.[1] Several RF-negative samples (as determined by testing in a commercially available RF kit) containing various ANA were evaluated to ensure lack of interference from these antibodies in RF-negative sera. These results are shown in TABLE 4 and show that only one sample containing anti-SSB gave a very low positive result.

TABLE 4
Cross-reactivity Results

# of Samples	Primary ANA Specificity	*Is*-Rheumatoid Factor IU/ml Values	Interpretation
5	SSA	1.8, 1.9, 4.4, 1.4, 1.4	5/5 NEG
4	Sm	9.1, 1.7, 1.6, 1.3	4/4 NEG
5	RNP	4.0, 0.9, 1.2, 0.8, 1.6	5/5 NEG
3	Scl-70	0.8, 1.6, 3.2	3/3 NEG
4	Jo-1	1.9, 2.6, 2.7, 1.8	3/3 NEG
3	dsDNA	1.6, 3.8, 3.7	3/4 NEG
1	SSB	21.2	1/1 POS

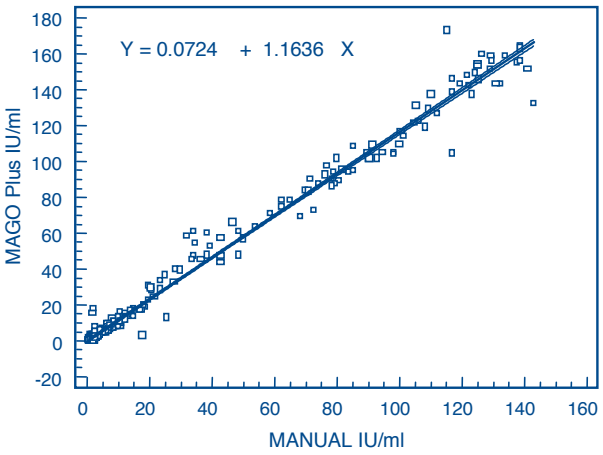
F. Lack of Prozone/High-Dose Hook Effects

The lack of interference from prozone/high-dose hook effects was determined by testing several sera, serially diluted and undiluted in the *Is*-Rheumatoid Factor test Kit. A total of 8 sera were evaluated, 4 contained the highest concentrations available as established by nephelometry, 2 contained levels in the mid range and 2 were in the negative range. No prozone or high-dose hook effects were evidenced by any of the results obtained from the samples tested in the assay system.

G. Correlation of Manual and MAGO Plus Results

The *Is*-Rheumatoid Factor Test Kit has been developed for both automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus procedures, the results of a total of 303 normal and clinical serum samples tested for RF by both the manual and MAGO Plus methods were plotted. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in FIGURE 6. The data indicate excellent correlation with a Correlation Coefficient (r) = 0.9933.

FIGURE 6
Manual vs. MAGO Plus Correlation



Intercept = 0.0724
Slope = 1.1636
Coefficient of determination = 0.9866
Correlation Coefficient r = 0.9933
95% CI for r = 0.9916 to 0.9946

H. Precision

To assess the precision of the *Is*-Rheumatoid Factor Test Kit six serum samples of varying reactivity as well as the kit Calibrator and controls were tested in triplicate in two runs per day for three days. Precision was assessed both manually and using the MAGO® Plus Automated EIA Processor. Results are summarized in TABLES 5 and 6.

TABLE 5
Manual Intra-Assay and Interassay Precision for *Is*-Rheumatoid Factor

	Intra-assay (n=6)									Interassay (n=18)		
SERUM	DAY 1			DAY 2			DAY 3					
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
A (Neg)	0.7	0.21	30.00	1.0	0.10	10.00	0.8	0.05	6.25	0.8	0.16	20.00
B (Neg)	0.4	0.33	>50.00	0.7	0.09	12.86	0.5	0.05	10.00	0.5	0.23	46.00
C (Pos)	34.1	1.25	3.67	35.1	2.94	8.38	34.2	1.94	5.67	34.5	2.08	6.03
D (Pos)	57.6	1.80	3.13	60.0	1.50	2.50	57.9	2.05	3.54	58.5	2.01	3.44
E (Pos)	82.2	1.93	2.35	84.9	3.57	4.20	79.6	1.57	1.97	82.2	3.23	3.93
F (Pos)	98.4	2.18	2.22	102.3	2.07	2.02	96.7	1.86	1.92	99.1	3.10	3.13
Cal.	98.8	2.03	2.05	101.6	1.25	1.23	98.4	1.16	1.18	101.0	3.98	3.94
Pos.	41.6	1.43	3.44	46.5	0.44	0.95	43.2	1.44	3.33	43.8	2.39	5.46
Neg.	0.7	0.18	25.71	0.9	0.05	5.78	0.6	0.09	15.00	0.7	0.16	22.86

TABLE 6
MAGO Plus Intra-Assay and Interassay Precision for *Is*-Rheumatoid Factor

	Intra-assay (n=6)									Interassay (n=18)		
SERUM	DAY 1			DAY 2			DAY 3					
	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%	Mean	SD	CV%
A (Neg)	0.9	0.26	28.89	0.9	0.95	>50.00	0.2	0.18	>50.00	0.6	0.65	>50.00
B (Neg)	0.3	0.25	>50.00	0.4	0.40	>50.00	0.4	0.63	>50.00	0.4	0.43	>50.00
C (Pos)	33.7	3.75	11.13	34.9	1.67	4.79	35.1	2.00	5.70	34.5	2.56	7.42
D (Pos)	69.6	1.18	1.70	70.3	3.05	4.34	69.9	1.96	2.80	69.9	2.09	2.99
E (Pos)	87.0	4.42	5.08	85.0	1.91	2.25	85.7	3.26	3.80	85.9	3.27	3.81
F (Pos)	102.0	1.84	1.80	101.2	2.52	2.49	101.6	2.91	2.86	101.6	2.33	2.29
Cal.	108.1	3.38	3.13	102.0	2.76	2.71	103.9	4.24	4.08	104.7	4.21	4.02
Pos.	43.3	7.29	16.84	40.5	2.54	6.27	40.2	2.02	5.02	41.3	4.56	11.04
Neg.	0.4	0.40	>50.00	0.7	0.60	>50.00	0.2	0.48	>50.00	0.4	0.50	>50.00

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