Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

ANA ELISA Screen

Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-790 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of samples in Sample Diluent. Mix well.
2. Add 100 µl of 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 450/600-630 nm

INTENDED USE

The Diamedix Immunosimplicity® *Is*-ANA ELISA Screen is a qualitative enzyme immunoassay (EIA) intended to screen for the presence of antinuclear antibodies (ANAs) in human serum as an aid in the diagnosis of certain systemic rheumatic diseases. This assay collectively detects in one well, total ANAs against double stranded DNA (dsDNA, nDNA), Histones, SSA, SSB, Sm, Sm/RNP, Scl-70, Jo-1 and centromeric antigens along with sera positive for immunofluorescent (IF) HEp-2 ANAs.

SUMMARY AND EXPLANATION

Antinuclear antibodies (ANAs) directed against a variety of macromolecules occur in extraordinarily high frequency in systemic rheumatic diseases (1). Although these antibodies were first associated with systemic lupus erythematosus (SLE), the list of implicated diseases has expanded and many rheumatic diseases are characterized by the presence of one or more of these ANAs. For instance, anti-SSA and anti-SSB antibodies are associated with SLE and Sjogren's Syndrome, anti-dsDNA and anti-Sm antibodies with SLE, anti-histone antibodies with SLE and Drug Induced Lupus, anti-RNP antibodies with mixed connective tissue disease (MCTD) and SLE, anti-Scl-70 antibodies with scleroderma (progressive systemic sclerosis {PSS}), anti-Jo-1 with polymyositis and dermatomyositis and anti-centromere antibodies with CREST syndrome (2, 3, 4).

The Immunofluorescence assay (IFA) has been used as the standard method in the detection of ANAs (5). Although the IFA is a sensitive test, it is laborious when testing large numbers of patient samples and is subject to errors from human interpretation and from variability in fluorescent microscopes (1). The IFA-HEp-2 ANA test is also subject to the following concerns: it is sometimes insensitive to certain sera containing antibodies to SSA, SSB, Sm or dsDNA (6) and it tends to find sera positive in a large number of patients who do not develop systemic rheumatic disorders within a follow-up two year period (7). The enzyme immunoassay (EIA) test system is an excellent alternative to the IFA test system for screening patient's serum for the presence of ANAs of clinical significance. The EIA test system efficiently screens large numbers of patient samples and reduces human error.

The Diamedix *Is*-ANA ELISA Screen collectively detects, in one well, total ANAs against double-stranded DNA (dsDNA, nDNA), Histones, SSA, SSB, Sm, Sm/RNP, Scl-70, Jo-1 and centromeric antigens, along with sera positive for IFA-HEp-2 ANAs. Sera that are positive on the *Is*-ANA ELISA Screen should then be tested for specific autoantibodies indicative of the various systemic disorders.

PRINCIPLE OF THE PROCEDURE

Purified antigens (dsDNA, histones, SSA, SSB, Sm, Sm/RNP, Scl-70, Jo-1, centromere and other antigens extracted from the HEp-2 nucleus) are bound to microwells. Antibodies to these antigens, if present in diluted serum, will then bind to the microwells. Washing of the microwells removes unbound serum antibodies. Horseradish peroxidase (HRP) conjugated anti-human IgG immunologically binds to the bound patient antibodies forming a "conjugate-antibody-antigen" sandwich. Washing of the microwells removes unbound conjugate. An enzyme substrate is then added. In the presence of bound enzyme the substrate is converted to a blue colored end product. The addition of acid stops the reaction forming a yellow end-product. The intensity of this end-product can be read spectrophotometrically at 450 nm (reference 600-630 nm).

REAGENTS

Each Is-ANA ELISA Screen Test Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well clear microwell breakapart strips, coated with purified antigen.
Cut-Off Calibrator	One vial with blue cap containing 0.25 ml human serum, weakly reactive for ANA antibodies, preserved with 0.1% sodium azide.
Positive Control	One vial with white cap containing 0.25 ml human serum, reactive for ANA antibodies, preserved with 0.1% sodium azide.
Negative Control	One vial with black cap containing 0.25 ml human serum, non-reactive for ANA antibodies, preserved with 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.
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' Tetramethylbenz-idine).
Stop **O** Solution	One bottle with white cap containing 30 ml of 1 N 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.

NOTE: Once the antigen-coated well foil pouch has been opened, the wells are stable for 60 days.

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, Hepatitis C and HIV-I & 2 antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1 & 2, Hepatitis B virus, Hepatitis C 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 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 prematurely.

7. Do not interchange reagents from different reagent lots except for Sample **E** Diluent, Wash **U** Concentrate, Substrate **HRP** and Stop **O** 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. Humidity affects the antigen-coated wells; do not open pouch until it reaches room temperature. Calculate the number of wells required for the current assay, remove them from the room temperature foil pouch, align them on the EIA Frame, then add samples immediately. Unused wells should be returned immediately to the resealed foil pouch with desiccant.

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 ANAs 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. Humidity affects the antigen-coated wells; do not open pouch until it reaches room temperature. Calculate the number of wells required for the current assay, remove them from the room temperature foil pouch, align them on the EIA Frame, then add samples immediately. Unused wells should be returned immediately to the resealed foil pouch with desiccant.

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

MANUAL USERS:

1. Prepare 1:101 dilutions of the Cut-Off Calibrator, controls and patient samples in Sample Diluent. (e.g., by addition of 5 µl sample to 500 µl Sample Diluent). The Cut-Off Calibrator must be run in triplicate.

2 Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of diluted Cut-Off Calibrator, controls and diluted patient samples, to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

   NOTE : Include one well which contains 100 µl of Sample Diluent only as a reagent blank. This will ultimately be used to "zero" the photometer before reading the test results.

3 Allow the wells to incubate uncovered 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 inverting the plate and tapping firmly 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.

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. 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:

If 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 Positive and Negative Controls must be within their assigned ranges.
3. The absorbance of the Blank must be less than 0.250.
4. The absorbance of the Cut-Off Calibrator must be $\geq$ 0.150.

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

NOTE: Each lot of *Is*-ANA ELISA Screen reagents is validated during quality control testing using all antibodies. All antibodies are not represented in the Cut-Off Calibrator and Positive Control materials. 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

Calculate the mean absorbance of the Cut-Off Calibrator. Note: When calculating the mean absorbance value for the Cut-Off Calibrator 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.

Determine the Index Value for each patient sample or control using the following formula:

$$\frac{\text{Absorbance of Sample}}{\text{Mean Absorbance of Cut-Off Calibrator}} = \text{Index Value}$$

An Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor) will calculate results using the above formula and will print them automatically.

Example : Absorbance values obtained for Cut-Off Calibrator : 0.289, 0.268, 0.275 (after subtraction of blank)

Mean Absorbance of Cut-Off Calibrator = 0.277

Sample Absorbance = 1.570

Index Value = 1.570 / 0.277 = 5.67

2. Interpretation

Index Value	*Interpretation*
< 0.90	Negative for ANA
0.90 to 1.09	Equivocal*
> 1.10	Positive for ANA

* When equivocal results are obtained samples can be reported as equivocal, retested, tested by another method or a new sample can be tested. Equivocal samples that give positive results upon retest should be reported as positive. Equivocal samples that give negative results upon retest should be reported as negative.

LIMITATIONS

1. The results obtained with the Diamedix *Is*-ANA ELISA Screen Test kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. The test should be performed on serum. The use of whole blood or plasma has not been established.
3. ANAs can be found in apparently healthy individuals.
4. Screening tests are used for testing entire populations or subsets of such populations for the presence of a characteristic. A negative screening result should infer that the individual has a high probability of being free of the characteristic, whereas a positive test may reflect only the need for further testing.
5. The Diamedix *Is*-ANA ELISA Screen will not identify the specific type of ANA present in a positive sample. Confirmatory testing for specific antibodies should be performed if a positive result is obtained.
6. The performance characteristics of the Diamedix *Is*-ANA ELISA Screen Test Kit with automated equipment other than the MAGO Plus Automated EIA Processor has not been established.

EXPECTED VALUES

The expected value for a normal patient is a negative result. However, positive ANA results may be found in apparently healthy individuals. In a recent study 12.4% of sera from normal healthy donors gave a detectable ANA result (8). Patient sera containing autoantibodies to those antigens represented in the *Is*-ANA ELISA Screen Test Kit will give positive results which can be further evaluated in specific tests. The number of positive samples detected is dependent upon the populations being tested.

The expected values in a normal S. Florida blood donor population were evaluated by assaying one hundred and forty-six sera both manually and using the MAGO Plus Automated EIA Processor. Figures 1 and 3 show the distribution of results in this normal population. For manual and MAGO PLUS testing 9.6% (14/146) gave positive results. Thirteen samples (8.9%) gave equivocal results manually and sixteen samples (11.4%) gave equivocal results on the MAGO Plus. The remainder of the samples gave negative results. Of the positive samples, three were found to contain specific autoantibodies and an additional two samples gave weakly positive IFA-ANA results.

In the present studies one-hundred and eighty-five clinical sera obtained from patients with an autoimmune disease or with a known autoantibody reactivity were also evaluated in the *Is*-ANA ELISA Screen Test Kit (for MAGO Plus testing one hundred and seventy-nine were available, six being QNS). Figures 2 and 4 show the distribution of results for this positive population. For manual testing 97.2% (180/185) of samples were positive. For MAGO Plus testing 98.9% (177/179) were positive.

FIGURE 1. Expected Values
Normal Samples – Manual

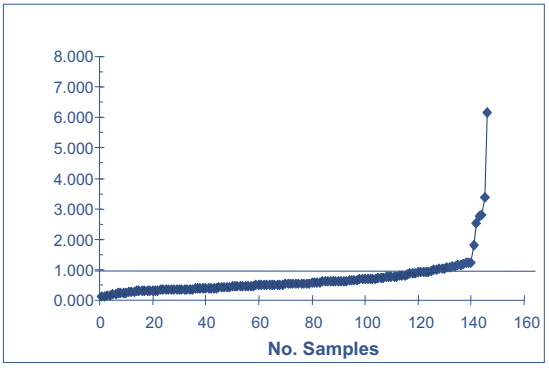


FIGURE 2. Expected Values
Clinical Samples – Manual

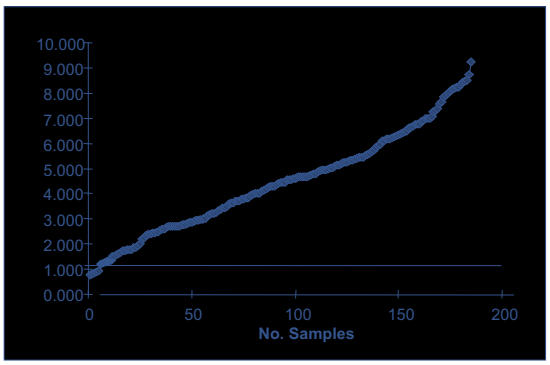


FIGURE 3. Expected Values
Normal Samples – MAGO Plus

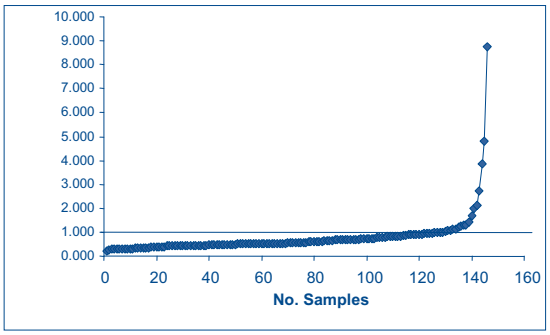
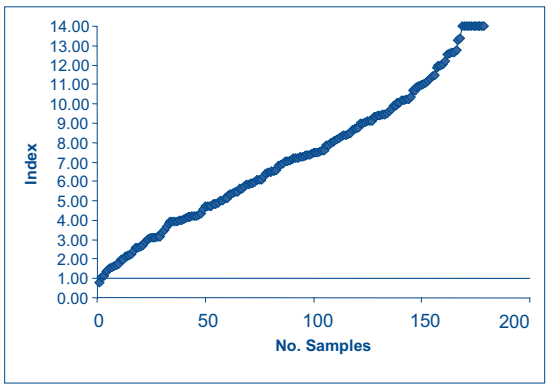


FIGURE 4. Expected Values
Clinical Samples – MAGO Plus



PERFORMANCE CHARACTERISTICS

A. Comparison Testing: Relative Sensitivity and Specificity

The Diamedix *Is*-ANA ELISA Screen Test Kit was evaluated relative to another commercially available ANA Screen test. One hundred and forty-six sera from normal blood donors and one hundred and eighty-five sera from clinical patients were tested by the *Is*-ANA Screen ELISA Test Kit and the comparative method. Testing by both methods was performed both manually and using the MAGO PLUS Automated EIA Processor. The results shown in Table 1 are the comparison of the *Is*-ANA ELISA Screen performed manually compared to the comparative method, both manual and automated. The results shown in Table 2 are the comparison of the *Is*-ANA ELISA Screen performed on the MAGO Plus compared to the comparative method, both manual and automated.

TABLE 1

Is-ANA ELISA Screen	Other ELISA : Manual			Other ELISA : MAGO Plus		
Manual	**# of Sera**	**%**	**95%CI**	**# of Sera**	**%**	**95%CI**
Relative Sensitivity	188/196	95.9	92.1-98.2	185/191	96.9	93.3-98.8
Relative Specificity	107/112	95.5	89.9-98.5	110/115	95.7	90.1-98.6
Overall Agreement	295/308*	95.8	92.7-97.7	295/306**	96.4	93.7-98.2

*Twenty-two samples equivocal in either or both methods were excluded from calculations; ** Twenty-one samples, equivocal in either or both methods were excluded from calculations.

TABLE 2

Is-ANA ELISA Screen	Other ELISA : Manual			Other ELISA : MAGO Plus		
MAGO Plus	**# of Sera**	**%**	**95%CI**	**# of Sera**	**%**	**95%CI**
Relative Sensitivity	183/188	97.3	93.9-99.1	183/187	97.9	94.6-99.4
Relative Specificity	105/112	93.8	87.5-97.5	109/116	94.0	88.0-97.5
Overall Agreement	288/300*	96.0	93.1-97.9	292/303**	96.4	93.6-98.2

*Twenty-two samples equivocal in either or both methods were excluded from calculations; ** Twenty-one samples, equivocal in either or both methods were excluded from calculations.

B. Clinical Sensitivity and Specificity using Characterized Sera

A total of three hundred and thirty-one characterized sera were assayed using the *Is*-ANA ELISA Screen Test Kit. These consisted of a number of sera of known ANA reactivity and a number of samples with no known apparent ANA reactivity. Samples tested were as follows:

a. NORMAL BLOOD DONOR SERA

One hundred and forty-six samples from normal blood donors were tested in the *Is*-ANA ELISA Screen. Results are shown in Table 3.

b. MONOSPECIFIC SERA

Forty-five sera obtained from a variety of sources and shown to contain monospecific antibodies of clinical significance were tested in the *Is*-ANA ELISA Screen Test Kit both manually and using the MAGO Plus Automated Processor. The results are summarized in Table 3.

c. IFA-ANA POSITIVE SERA

Seventy sera shown to be positive by the IFA- ANA method were tested in the *Is*-ANA ELISA Screen Test Kit. These seventy sera consisted of thirty-five sera with IFA-ANA titers between 1:40 and 1:320 and thirty-five sera with titers in excess of 1: 320. The results obtained are summarized in Table 3.

d. AUTOIMMUNE DISEASE STATE SERA

Seventy sera from a variety of sources from patients diagnosed with an autoimmune disorder were tested both manually and on the MAGO Plus using the *Is*-ANA ELISA Screen. Results are shown in Table 3.

TABLE 3

Patient group		Positive	Negative	Equivocal*	Total
a. *Normal Sera*	Manual	14	119	13	146
	MAGO Plus	14	116	16	146
b. *Monospecific*	Manual	45	0	0	45
Sera	MAGO Plus	44	0	0	44**
c. *IFA-ANA*					
Low Titer Sera	Manual	30	4	1	35
	MAGO Plus	34	0	1	35
High Titer Sera	Manual	35	0	0	35
	MAGO Plus	35	0	0	35
d. *Autoimmune*	Manual	70	0	0	70
Disease Sera	MAGO Plus	64	1	0	65***

* Equivocal results were excluded from calculations
** One sample QNS for MAGO Plus
*** Five samples QNS for MAGO Plus

Clinical Specificity	Manual	95% CI	MAGO Plus	95% CI
Normals	119/133 = 89.5%	84.3-94.7	116/130 = 89.2%	83.9-94.6

Clinical Sensitivity	Manual	95% CI	MAGO Plus	95% CI
Low Titer ANA+ Sera	30/34 = 88.2%	72.6-96.7	34/34 = 100.0%	89.7-100.0
High Titer ANA+ Sera	35/35 = 100.0%	90.0-100.0	35/35 = 100.0%	90.0-100.0
Monospecific Sera	45/45 = 100.0%	92.1-100.0	44/44 = 100.0%	92.0-100.0
Autoimmune Disease Sera	70/70 = 100.0%	94.9-100.0	64/65 = 98.5%	91.7-100.0

C. Precision

The precision of the *Is*- ANA ELISA Screen Test Kit was determined by testing six different sera (2 negative and 4 positive) plus the kit calibrator and controls in triplicate in two different runs on three different days. Precision was evaluated manually and using the MAGO PLUS Processor. The intra- and interassay precision for the manual procedure is shown in Table 4 and for the MAGO Plus Automated EIA Processor in Table 5.

TABLE 4: Manual Precision

SERUM	INTRA-ASSAY		DAY 1	INTRA-ASSAY		DAY 2	INTRA-ASSAY		DAY 3	INTERASSAY		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A (NEG)	0.262	0.007	**2.7**	0.223	0.024	**10.8**	0.280	0.019	**6.8**	0.255	0.030	**11.6**
B (NEG)	0.292	0.009	**3.1**	0.220	0.049	**22.3**	0.247	0.019	**7.7**	0.253	0.042	**16.7**
C (POS)	1.677	0.221	**13.2**	1.731	0.148	**8.5**	1.682	0.030	**1.8**	1.697	0.147	**8.7**
D (POS)	1.696	0.098	**5.8**	1.521	0.093	**6.1**	1.586	0.050	**3.2**	1.601	0.108	**6.7**
E (POS)	3.310	0.192	**5.8**	3.232	0.338	**10.5**	3.179	0.114	**3.6**	3.305	0.261	**7.9**
F (POS)	3.482	0.299	**8.6**	3.365	0.294	**8.7**	2.963	0.132	**4.5**	3.270	0.330	**10.1**
c/o CAL	1.022	0.068	**6.7**	0.992	0.110	**11.1**	0.999	0.121	**12.1**	1.004	0.097	**9.6**
POS	2.938	0.176	**6.0**	3.022	0.256	**8.5**	2.766	0.122	**4.4**	2.909	0.211	**7.3**
NEG	0.247	0.039	**15.8**	0.210	0.036	**17.1**	0.264	0.030	**11.4**	0.240	0.041	**16.9**

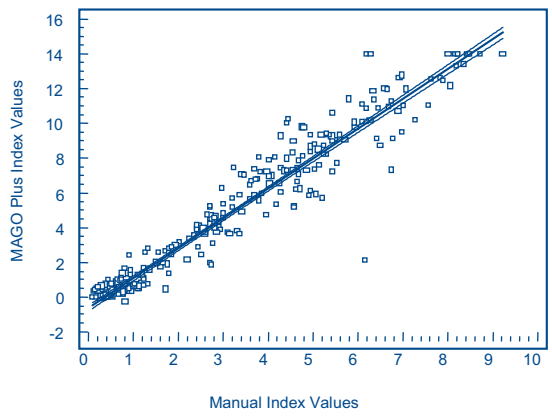
TABLE 5: MAGO Plus Precision

SERUM	INTRA-ASSAY		DAY 1	INTRA-ASSAY		DAY 2	INTRA-ASSAY		DAY 3	INTERASSAY		
	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%	MEAN INDEX	SD	CV%
A (NEG)	0.377	0.028	**7.4**	0.407	0.052	**12.8**	0.395	0.068	**17.2**	0.39	0.051	**12.9**
B (NEG)	0.322	0.008	**2.5**	0.320	0.021	**6.6**	0.322	0.025	**7.8**	0.32	0.018	**5.6**
C (POS)	2.402	0.059	**2.5**	2.540	0.239	**9.4**	2.625	0.100	**3.8**	2.52	0.173	**6.8**
D (POS)	2.215	0.106	**4.8**	2.218	0.120	**5.4**	2.437	0.158	**6.5**	2.29	0.163	**7.1**
E (POS)	4.382	0.171	**3.9**	4.528	0.183	**4.0**	4.835	0.305	**6.3**	4.58	0.291	**6.4**
F (POS)	5.385	0.071	**1.3**	5.470	0.137	**2.5**	5.903	0.246	**4.2**	5.59	0.281	**5.0**
c/o CAL	0.968	0.053	**5.5**	1.025	0.078	**7.6**	1.113	0.055	**4.9**	1.04	0.085	**8.2**
POS	4.407	0.238	**5.4**	4.310	0.270	**6.3**	5.050	0.167	**3.3**	4.59	0.401	**8.7**
NEG	0.350	0.031	**8.9**	0.343	0.035	**10.2**	0.388	0.030	**7.7**	0.36	0.040	**11.1**

D. Correlation of Manual and MAGO PLUS Results

The ANA ELISA Screen Test Kit has been developed for automated as well as manual use. To demonstrate the equivalence of the manual and MAGO Plus procedures, the results of three hundred and twenty-five 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 5. The data indicate a good correlation with a correlation coefficient (r) of 0.9712.

FIGURE 5. Manual vs MAGO PLUS Correlation



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