Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

anti-dsDNA

Enzyme Immunoassay Test Kit
For *In Vitro* Diagnostic Use
Catalog No. 720-700 – 96 Tests

SUMMARY OF PROCEDURE

1. Prepare a 1:101 dilution of Standards, controls and samples in Sample Diluent. Mix well.
2. Add 100 µl of dilutedStandards, controls and 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 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 absorbance at 450 nm (reference at 600 - 630 nm).

INTENDED USE

For the quantitative detection of IgG antibodies to double-stranded (ds)DNA in human serum by indirect enzyme immunoassay as an aid in the diagnosis of systemic lupus erythematosus (SLE).

SUMMARY AND EXPLANATION

A hallmark of the systemic rheumatic diseases has been the presence of circulating serum antibodies to various nuclear antigens.[1] Autoantibodies to nuclear antigens (ANA) include antibodies to all antigens present in the nucleus such as DNA, histones, non-histones and nucleoli. Autoantibodies to dsDNA are important and characteristic markers in patients with SLE and their appearance serves as an important tool in the diagnosis, prognosis, and monitoring of SLE patients. Antibodies to dsDNA occur in approximately 60-70% of SLE patients and there is considerable evidence to implicate immune complexes containing anti-dsDNA and DNA in the pathogenesis of SLE.[1,2]

Low levels of anti-dsDNA antibodies may occur in other rheumatic diseases [3] and may occur at a very low frequency (2-3%) in individuals without any symptoms of rheumatic disease.[4] It has also been reported that the appearance of anti-dsDNA in rheumatic patients can occur prior to the development of the complete clinical pattern.[5]

A number of techniques have been developed to detect anti-dsDNA antibodies. In the past, the most utilized tests have been the *Crithidia luciliae* immunofluorescent assay, and the Farr radioimmunoassay. However, the sensitivity, specificity, precision and ease of performance of these assays can vary considerably. The enzyme-linked immunosorbent assay (ELISA or EIA) offers advantages over these methods in terms of sensitivity, reproducibility, objectivity and potential for automation. The usefulness of EIAs in anti-DNA determinations is widely documented and accepted.[6,7,8]

The Diamedix *immunosimplicity*® anti-dsDNA Test Kit is an EIA procedure intended for the quantitation of IgG antibodies to dsDNA. The results are reported in IU/ml, traceable to the First International Standard for antibodies to double stranded DNA (Wo/80).[9]

PRINCIPLE OF THE PROCEDURE

Plasmid DNA (grown in *E. coli*, purified by alkaline lysis and chromatographic methods and treated to remove ssDNA) is bound to microwells. Diluted patient sera, Standards and controls are placed in the microwells and incubated. Anti-dsDNA 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 dsDNA present in the sample.

REAGENTS

Each Is-anti-dsDNA Kit contains reagents for 96 tests.

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded orange, coated with purified plasmid DNA.
0 IU/ml Standard	One vial with yellow cap containing 0.25 ml human serum or defibrinated plasma, non-reactive for dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml value printed on label.
12.5 IU/ml Standard	One vial with green cap containing 0.25 ml human serum or defibrinated plasma, with the assigned level of dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml value printed on label.
25 IU/ml Standard	One vial with brown cap containing 0.25 ml human serum or defibrinated plasma, with the assigned level of dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml value printed on label.
50 IU/ml Standard	One vial with purple cap containing 0.25 ml human serum or defibrinated plasma, with the assigned level of dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml value printed on label.
100 IU/ml Standard	One vial with white cap containing 0.25 ml human serum or defibrinated plasma, with the assigned level of dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml value printed on label.
200 IU/ml Standard	One vial with red cap containing 0.25 ml human serum or defibrinated plasma, with the assigned level of dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml value printed on label.
Positive Control	One vial with blue cap containing 0.25 ml human serum or defibrinated plasma reactive for dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml range printed on label. The Positive Control is used to control the lower to mid-range of the assay.
Negative Control	One vial with black cap containing 0.25 ml human serum or defibrinated plasma non-reactive for dsDNA IgG antibodies, and 0.1% sodium azide. Assigned IU/ml range printed on the label. The Negative Control is used to control the negative range of the assay.
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 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 **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.

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 serum 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, 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 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 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. 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-dsDNA 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.[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.

1. *For Single Point Calibration assays:* The 200 IU/ml Standard only is required for Single Point Calibration. This Standard can be run singly or in duplicate. In addition, a Blank (100 µl of Sample Diluent only), in the first well of the first strip is required. This will ultimately be used to "zero" the photometer before reading the test result.

   For 6-Point Calibration assays: All six Standards are required to be assayed. No Blank is required. The 0 IU/ml Standard will function as the "zero" and will be placed in the first well of the first strip. Standards (from 0-200 IU/ml) can be run singly or in duplicate.

   * Positive and Negative Controls must be run for either assay option.

2 Prepare 1:101 dilutions of the Standard(s), controls and patient samples in Sample Diluent. (e.g., by addition of 5 µl sample to 500 µl Sample Diluent).

3 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 Standard(s), controls and patient samples to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

4. 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 5 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:

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 or the 0 IU/ml Standard must be < 0.200.
4. The absorbance of the Negative Control must be lower than that of the 25 IU/ml Standard.
5. The absorbance of the Positive Control must be higher than that of the 25 IU/ml Standard.
6. The absorbance of the 200 IU/ml Standard must be ≥ 3 times the absorbance of the 25 IU/ml Standard.

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 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

Since the dose response curve for the *Is*-anti-dsDNA Test Kit is sufficiently linear, results can be calculated using either a Single Point Calibrator only or, if the user wishes, a 6-Point Calibration system. The results obtained by the Single Point Calibration or 6-Point Calibration methods have been shown to have a correlation coefficient > 0.99 (data on file).

1. Single Point Calibration

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

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance Of 200 IU/ml Standard}} \times 200 = \text{IU/ml of sample}$$

2. 6-Point Calibration

Results are obtained from the best linear curve fit using all six Standards. For the Stat-Fax® 2100 readers (or equivalent) the Regression Mode should be selected and Standard values entered accordingly. An Automated EIA Processor (e.g. MAGO Plus® Automated EIA Processor) will calculate results automatically for either option.

3. 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-dsDNA Value	*Interpretation*
< 25.0 IU/ml	Negative for antibodies to dsDNA.
25.0 – 34.9 IU/ml	Equivocal for antibodies to dsDNA. 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**.
35.0 – 99.9 IU/ml	Weakly positive for dsDNA antibodies.
100 – 200 IU/ml	Moderately positive for dsDNA antibodies.
≥ 200 IU/ml	Strongly positive for dsDNA antibodies.

** 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.

Patient values, which contain high levels of antibody may produce absorbance values greater than the highest Standard absorbance. Patient sample results greater than the highest value can be reported as "Greater than 200 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 sample may be re-assayed simultaneously. Calculate the IU/ml for the dilution that is within the reportable range and multiply by the dilution factor to obtain estimated values.

LIMITATIONS

1. The results obtained with the *Is*-anti-dsDNA IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves but used in conjunction with clinical findings and other serological tests.
2. The test should be performed on serum. The use of whole blood or plasma has not been established.
3. Assay performance characteristics have not been established for visual result determination.
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 autoimmune disease being present. Testing should only be performed when clinical symptoms are present or disease suspected.
6. In approximately 30-40% of lupus patients, anti-dsDNA antibodies are undetectable.
7. The presence of low levels of antibodies to dsDNA may occur in other autoimmune diseases, may antedate the onset of clinical symptoms of SLE, may represent the period following exacerbation of symptoms or may reflect remission or control.
8. A small and variable percentage of people may have antibodies to dsDNA in the absence of disease.
9. The performance characteristics of the Diamedix *Is*-anti-dsDNA IgG Test Kit with automated equipment other than the MAGO Plus Automated EIA Processor have not been established.

EXPECTED VALUES

Antibodies to dsDNA are found in up to 60-70% of patients with SLE and are rarely present in normal populations. The expected values in the normal population were evaluated by assaying sera from 200 normal South Florida blood donors using the *Is*-anti-dsDNA Test Kit. FIGURES 1 and 2 show the distribution of anti-dsDNA in this normal population tested manually and by the MAGO Plus using the 6-point calibration method. The distribution of values of sera from 70 SLE patients tested manually and on MAGO Plus using the 6-point calibration method is shown in FIGURES 3 and 4.

FIGURE 1
Expected Values
Normal Samples - Manual

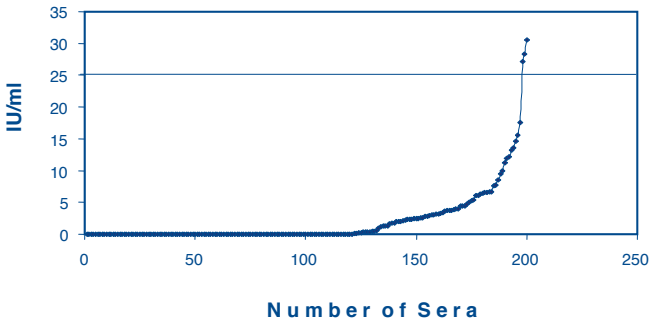


FIGURE 2
Expected Values
Normal Samples - MAGO Plus

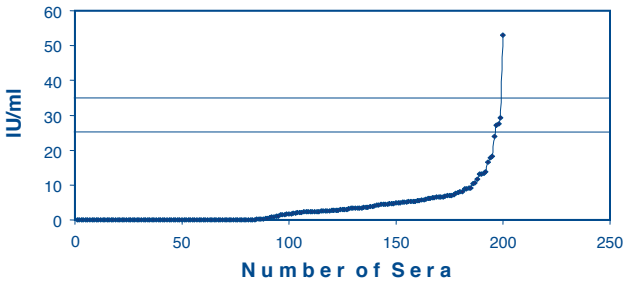


FIGURE 3
Expected Values
SLE Patients - Manual

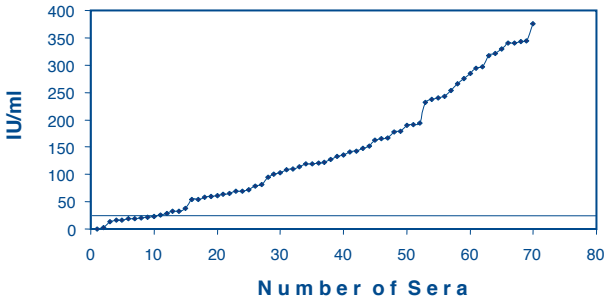
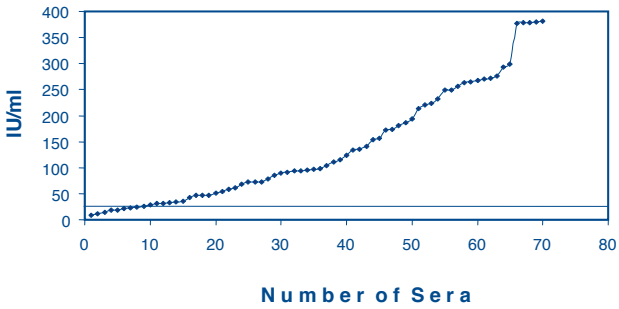


FIGURE 4
Expected Values
SLE Patients - MAGO Plus



PERFORMANCE CHARACTERISTICS

A. Comparison Studies : Relative Sensitivity and Specificity

The Diamedix *Is*-anti-dsDNA Test Kit was evaluated relative to another commercially available anti-dsDNA ELISA test with traceability to the WHO Standard. A total of 413 samples were tested by both methods. These samples were comprised of two hundred sera from normal blood donors, two hundred and nine sera from clinical patients with either a diagnosis of SLE or another autoimmune disease and four sera from patients whose status was unknown. Performance is summarized in TABLE 1 for both 6-Point and Single Point Calibration using the manual method of testing. Similar results were obtained using the MAGO Plus automated testing method.

TABLE 1

	6 Point Calibration			Single Point Calibration		
	# of Sera	%	95% CI	# of Sera	%	95% CI
Relative Sensitivity	104/108	96.3	90.8-99.0	111/112	99.1	95.1-100.0
Relative Specificity	264/275	96.0	93.0-98.0	251/271	92.6	88.8-95.4
Overall Agreement*	368/383	96.1	93.6-97.8	362/383	94.5	91.7-96.6

* Equivocal and QNS samples 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

Clinical sensitivity and specificity was assessed by evaluating the results from each patient group. The groups consisted of 200 normal samples, 70 sera from patients with a diagnosis of SLE and 138 sera from patients with suspected autoimmune disease. The results presented in TABLE 2 were obtained manually using the 6-Point calibration method.

TABLE 2

Patent Group:	Positive	Equivocal*	Negative	Total
Normals	0	3	197	200
SLE	56	4	10	70
Autoimmune Disease	63	5	70	138

Clinical Specificity:			**95% CI**
Normals	= 197/197	= 100.0%	98.1-100.0

Clinical Sensitivity:			**95% CI**
SLE patients	= 56/66	= 84.8%	73.9-92.5
Autoimmune disease patients	= 63/133	= 47.4%	38.9-55.9

* Equivocal results were excluded from calculations

C. Precision

The precision of the *Is*-anti-dsDNA Test Kit when performed either manually or on the MAGO Plus Automated EIA Processor using the 6-Point calibration method was determined by assaying six sera and the kit positive and negative controls in triplicate in two runs per day for three days. TABLES 3 and 4 show the intra-and interassay precision obtained. Comparable CVs for positive samples were also obtained when precision testing was performed using the Single Point calibration method (data on file).

TABLE 3
Intra-Assay and Interassay Precision- Manual Testing

	Intra-Assay Day 1			Intra-Assay Day 2			Intra-Assay Day 3			Interassay		
SERUM	MEAN IU/ml	SD	%CV	MEAN IU/ml	SD	%CV	MEAN IU/ml	SD	%CV	MEAN IU/ml	SD	%CV
A (NEG)	0.0	0.00	N/A	0.0	0.00	N/A	0.0	0.00	N/A	0.0	0.00	N/A
B (NEG)	0.0	0.00	N/A	0.0	0.00	N/A	0.0	0.00	N/A	0.0	0.00	N/A
C (POS)	49.3	5.17	10.5	48.5	7.12	14.7	45.1	0.95	2.1	47.6	5.17	10.9
D (POS)	68.5	6.88	10.0	62.3	5.45	8.8	66.9	1.95	2.9	65.9	5.59	8.5
E (POS)	96.6	5.89	6.1	90.5	8.16	9.0	79.2	4.51	5.7	88.7	9.53	10.7
F (POS)	152.4	4.06	2.7	156.9	8.61	5.5	150.3	1.59	1.1	153.2	5.95	3.9
POS CTRL	69.1	5.57	8.1	70.3	2.99	4.3	66.4	2.20	3.3	68.6	4.00	5.8
NEG CTRL	0.0	0.00	N/A	0.0	0.00	N/A	0.0	0.00	N/A	0.0	0.00	N/A

TABLE 4
Intra-Assay and Interassay Precision - MAGO Plus Testing

	Intra-Assay Day 1			Intra-Assay Day 2			Intra-Assay Day 3			Interassay		
SERUM	MEAN IU/ml	SD	%CV	MEAN IU/ml	SD	%CV	MEAN IU/ml	SD	%CV	MEAN IU/ml	SD	%CV
A (NEG)	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0
B (NEG)	0.0	0.00	0.0	2.1	2.90	0.0	0.0	0.00	0.0	0.0	0.00	0.0
C (POS)	52.9	3.55	6.7	51.3	2.80	5.5	52.6	3.26	6.2	52.3	3.11	5.9
D (POS)	76.0	5.02	6.6	76.8	3.14	4.1	72.9	5.19	7.1	75.2	4.61	6.1
E (POS)	80.0	9.54	11.9	81.6	6.58	8.1	89.7	7.36	8.2	83.8	8.63	10.3
F (POS)	164.6	7.49	4.6	158.1	11.40	7.2	162.0	11.40	7.0	161.6	10.00	6.2
POS CTRL	76.5	2.28	3.0	75.6	2.04	2.7	74.2	3.08	4.1	75.4	2.54	3.4
NEG CTRL	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0	0.0	0.00	0.0

D. Linearity

The dose response curve for the *Is*-anti-dsDNA Test Kit is sufficiently linear to allow for the use of either 6-Point or Single Point calibration systems. This linearity is illustrated in FIGURES 5 and 6. These figures depict samples that have been serially diluted in Sample Diluent and then each dilution tested and results determined using either calculation method. The samples selected were the WHO Reference preparation, the kit 200 IU/ml Standard and the in-house reference 200 IU/ml Standard. Recovered IU/ml values for each dilution were determined using either the 6-Point (Figure 5) or Single Point (Figure 6) calibration methods. The linearity data shown was obtained using the manual method of testing. Similar results were obtained using the MAGO® Plus.

FIGURE 5
6-Point Calibration

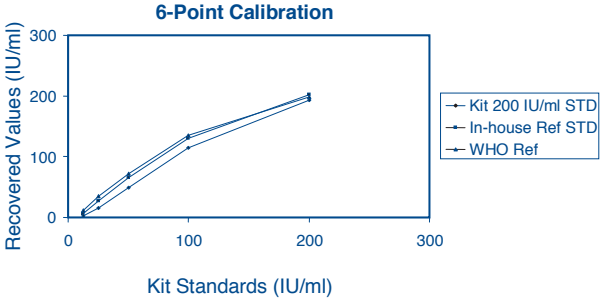
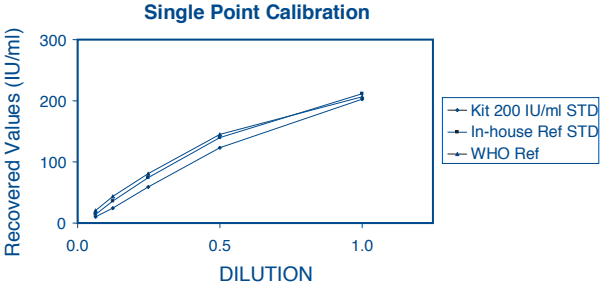


FIGURE 6
Single Point Calibration



E. Lack of Cross-Reactivity

A potential advantage in using a plasmid-based DNA ELISA system is its specificity for detecting antibodies to double-stranded DNA only. This was illustrated by testing forty-six samples positive for antibodies to single-stranded DNA but negative for antibodies to double-stranded DNA. Testing was performed manually. These samples were also tested on another commercially available plasmid-based DNA ELISA test. Of the 46 samples tested, five were positive and one was equivocal in the *Is*-anti-dsDNA Test Kit. Of the 5 samples that were positive, three were also positive by the other method. Of the remaining 2 samples, one was equivocal and one was negative in the other ELISA test.

The specificity of the *Is*-anti-dsDNA test was further verified by testing 21 serum samples containing a variety of autoantibodies such as SSA, SSB, Sm, Sm/RNP, Jo-1, Scl-70, Histone, and centromere but devoid of antibodies to dsDNA. Of these samples one was positive in the Is-anti-dsDNA Test Kit. This sample was also positive in the other anti-dsDNA ELISA test. This sample was identified as containing anti-histone antibodies.

F. Correlation of Manual and MAGO Plus Results

The *Is*-anti-dsDNA 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 385 serum samples tested by both methods using the Single Point calibration system and the 6-Point calibration system were plotted. Scattergrams and regression lines of the results obtained with 95% confidence intervals are shown in FIGURES 7 and 8. The correlation coefficient (r) for the Single Point Calibration method was 0.9858. The correlation coefficient for the 6-Point calibration method was 0.9810.

FIGURE 7
Manual and MAGO Plus Correlation -
Single Point Calibration

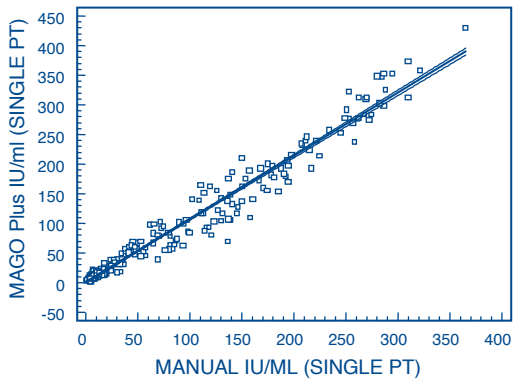
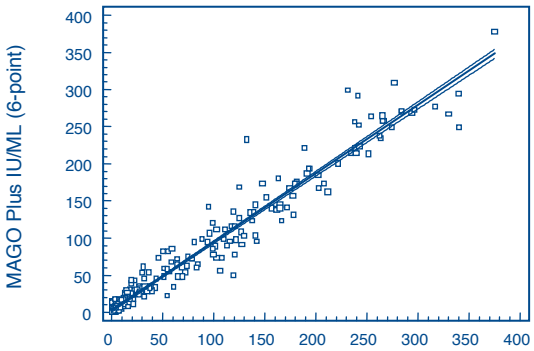


FIGURE 8
Manual and MAGO Plus Correlation -
6-Point Calibration



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