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See external label 2°C-8°C Σ=96 tests #2506

ENA PROFILE-6 ELISA TEST SYSTEM

An Enzyme-Linked Immunosorbent Assay (ELISA) for
Antibodies to Jo-1, Sm, Sm/RNP, SSA, SSB, and Scl-70

For *In Vitro* Diagnostic Use
Catalog No. 2506-2
Product Series: 2558-2,2557-2,2570-2,2559-2,2555-2,2556-2

INTENDED USE

The Diagnostic Automation, Inc. ENA Profile-6 ELISA test system is a semi-quantitative immunoassay for the detection of IgG antibodies to Jo-1, Sm, Sm/RNP, SSA(Ro), SSB(La), and Scl-70 in human sera. When performed according to these instructions, the results of this autoantibody profile may aid in the diagnosis and treatment of autoimmune connective tissue disorders. This device is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

In recent years it has become clear that autoantibodies to a number of nuclear constituents have proven to be useful in the diagnosis of various connective tissue diseases. The Jo-1 autoantibody is one of a family of characteristic autoantibodies seen in myositis patients (19). They are all specifically found in patients with myositis, and are all associated with a high incidence of accompanying interstitial lung disease (10). Antibodies directed against the Sm marker are highly specific for patients with SLE and are considered a diagnostic criterion for SLE (1,2). The presence of high level RNP antibodies alone are considered diagnostic of mixed connective tissue disease (MCTD) and are usually associated with a more benign disease course (3), while patients with low levels of RNP antibodies, together with other autoantibodies, may be observed in the serum of patients with progressive systemic sclerosis, Sjögren's Syndrome, and rheumatoid arthritis. The presence of RNP antibodies in the serum of SLE patients is usually associated with a lower incidence of renal involvement and a more benign disease course. To the contrary, patients with Sm antibodies experience a higher frequency of renal and central nervous system complications (4). Autoantibodies directed against SSA and SSB may be observed in patients with SLE (5-6) and Sjögren's disease (7-9). SSA antibodies are frequently present in the serum of ANA negative SLE patients, such as subacute cutaneous lupus erythematosus (12), a lupus-like syndrome associated with a homozygous C2 deficiency (13), and in a subset of patients who lack anti-dsDNA antibodies (11). Scl-70 antibodies are highly specific for scleroderma (11). They are also observed in a minority of SLE patients. Scl-70 positive scleroderma patients tend to have a more severe disease course, more internal organ involvement and diffuse rather than limited skin involvement (14). Scl-70 antibodies are rarely found in other autoimmune diseases, and thus, their detection in a patient with the recent onset of Raynaud's phenomenon is highly significant (15).

The relative frequency of these autoantibodies in association with SLE and other connective tissue diseases

either singly, or as multiple autoantibodies, requires an autoantibody profile assessment of each patient's serum in order to obtain the highest degree of clinical relevance in the laboratory workup of these types of patients. Until recently, autoantibodies were tested individually by indirect immunofluorescence, Ouchterlony gel diffusion, hemagglutination, radioimmunoassay, or enzyme-linked immunosorbent assay (ELISA). Although the exact etiology of autoimmune diseases is unknown, and the specific role played by autoantibodies in the onset of various autoimmune connective tissue diseases is obscure, the association and frequency of detection of these antibodies, particularly those of the IgG class, by the Diagnostic Automation, Inc. ENA Profile-6 ELISA test system, offers an efficient test procedure for the laboratory workup of patients with various connective tissue diseases.

The following table summarizes the various autoantibodies noted above with respect to disease association:

Table 1 (16)

Antibody	Disease State	Relative Frequency of Antibody Detection %
Anti-Jo-1	Myositis	25-44% (19)
Anti-Sm	SLE	30*
Anti-RNP	MCTD,SLE	100** and >40, respectively
Anti-SSA (Ro)	SLE, Sjögren's	15 and 30-40, respectively
Anti-SSB (La)	SLE, Sjögren's	15 and 60-70, respectively
Anti-Scl-70	Systemic sclerosis	20-28*
* Highly Specific		
* *Highly specific when present alone at high titer		

PRINCIPLE OF THE ELISA ASSAY

The Diagnostic Automation, Inc. ENA Profile-6 ELISA test system is designed to detect IgG class antibodies in human sera to a select group of six common extractable nuclear antigens. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in a series of microwells, each coated with a different autoantigen. If present in patient sera, specific antibodies will bind to the immobilized antigen(s). The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase conjugated goat anti-human IgG (γ chain specific) is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted conjugate.
3. The microtiter wells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After a period of time, the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the test sample.

KIT COMPONENTS

1. Twelve, 1 x 8-well ENA Profile strips. Each row of the plate is coated with a different ENA. The strips are packaged in a strip holder and sealed in an envelope with desiccant. Each plate is sufficient for twelve autoantibody profile determinations.
2. Horseradish peroxidase conjugated goat anti-human IgG (γ chain specific). One, 15mL vial with a white cap. Ready to use.
3. Human positive serum control. One, 0.35mL vial with a red cap.
4. Human serum calibrator. Two 1 ml vials with a red crimp (Lyophilized)..
5. Human negative serum control. One, 0.35mL vial with a green cap.
6. Sample diluent. One, 30mL bottle (green cap) containing Tween-20 bovine serum albumin, and phosphate-buffered-saline, pH 7.2 ± 0.2. Ready to use. Shake before use.

7. TMB : one, 15mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. Stop solution. One 15 ml vial (red cap) containing 8.0mL of 1.0M H_2SO_4,0.7M HCl. Ready to use. **Caution:** Do not allow the stop solution to contact skin or eyes.
9. Wash Buffer (10X) : dilute 1 part concentration + 9 parts deionized or distilled water. One 100mL bottle (clear cap) containing phosphate-buffered-saline and Tween-20. **NOTE:** 1X solution will have a pH of 7.2 ± 0.2
10. Package insert providing instructions.

PRECAUTIONS

1. Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

   WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

   Because no test method can offer complete assurance that human immunodeficiency virus hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1993, and FDA LABELING GUIDELINES FOR *IN VITRO* DIAGNOSTIC REAGENT MANUFACTURERS, DEC., 1985.
2. The microwell strips do not contain viable antigens. However, the strips should be considered potentially infectious and handled accordingly. Wash solutions should be collected in a disposable basin and treated with 0.5% sodium hypochlorite (10% household bleach) at the end of the days run.
3. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
4. Wipe bottom of plate free of residual liquid and/or fingerprints which can alter optical density (OD) readings.
5. Control sera, calibrator, conjugate, and wash buffer contain preservative which may be toxic if ingested. (Thimerosal at a concentration of 0.04%). The sample diluent contains sodium azide at a concentration of 0.1% (w/v).
6. Sodium azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing sodium azide.
7. Dilution or adulteration of these reagents may result in loss of sensitivity.
8. Do not substitute reagents from kits with different lot numbers or from other manufacturers.
9. For *in vitro* diagnostic use.
10. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin or mucous membranes.
11. Avoid microbial contamination of reagents. Incorrect results may occur.
12. Cross contamination of reagents and/or samples could cause false results.
13. Contamination of the TMB substrate solution with conjugate or other oxidants will cause the solution to change color prematurely. Do not use substrate solution if it has begun to turn blue. To help reduce the possibility of contamination, refer to Test Procedure, section D.1 to determine the amount of substrate solution to be used.
14. Reusable glassware must be washed out and thoroughly rinsed free of all detergents.
15. Strict adherence to the specified time and temperature of incubations is essential for accurate results. All reagents must be brought to 20-25ºC before starting the assay.
16. Improper washing will cause false positive or false negative results. Be sure to blot the plates free of any residual wash solution before adding conjugate or substrate solution. Do not allow the wells to dry out

between incubations.

17. Do not allow the stop solution to contact skin or eyes. If contact occurs, immediately flush with water.
18. Caution: Liquid waste at acidic pH should be neutralized before adding to sodium hypochlorite (bleach).
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. The calibrator must be fully reconstituted prior to performing the assay. Improper or inadequate reconstitution will produce erroneous results.
23. Do not allow the conjugate to come in contact with containers or instruments which may have previously contained a solution utilizing sodium azide as a preservative. Residual amounts of sodium azide may destroy the conjugate's enzymatic activity.
24. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this kit.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader capable of reading at a wavelength of 450nm.
- Microliter pipettes capable of accurately delivering 10 and 100 µL.
- Adjustable multichannel pipette (50-200µL) for dispensing conjugate, substrate, and stop solution.
- Reagent reservoirs for multichannel pipettes.
- Wash bottle or plate washing system.
- Distilled or deionized water.
- One liter graduated cylinder.
- Serological pipette: 1, and 10 or 25mL
- Disposable pipette tips.
- Paper towels.
- Timer with alarm capable of measuring to an accuracy of ± 1 second.
- Disposal basin and disinfectant, (Example: 0.5% sodium hypochlorite, 10% household bleach).
- Disposable microtubes.

SPECIMEN COLLECTION

Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (17,18). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterial contaminated sera. Sera should be stored at 2-8ºC for no longer than 5 days. If delay in testing is anticipated, store test sera at -20ºC or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8ºC.
2. ENA Profile-6 antigen coated microwell strips: Extra strips should be immediately resealed with desiccant and returned to storage at 2-8ºC. Strips are stable for 60 days after the envelope has been opened and properly resealed.
3. Peroxidase conjugated goat anti-human IgG: Store at 2-8ºC. DO NOT FREEZE.
4. Human positive and negative control sera: Store at 2-8ºC.
5. Human calibrator: Store at 2-8ºC. Following reconstitution, stable for 30 days at 2-8ºC.
6. TMB substrate solution: Store at 2-8ºC.
7. Wash buffer: Store at 2-25ºC. Stable for 30 days at 2-8ºC, or 7 days at room temperature after diluting to 1X.
8. Sample diluent: Store at 2-8ºC.

9. Stop solution: Store at 2-25ºC.

NOTE: All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date.

Preparation of Reagents:

1. Wash Buffer: Dilute the 100mL of 10X concentrate with 900mL of distilled or deionized water. Mix thoroughly to dissolve any crystals that may be present.
2. Human calibrator: Reconstitute the contents of the vial with 1.0 ml distilled or deionized water. After reconstitution the calibrator is ready ti use. Do not dilute additionally before use. Store unused reconstituted calibrator at 2-8 C for no longer than 30 days.
3. Sample diluent, conjugate, substrate solution, and stop solution are ready to use.

TEST PROCEDURE

A. Set-up of the Assay

Remove the individual kit components from storage and allow them to warm to room temperature (20-25ºC). Determine the total number of samples, standards, and controls to be tested. The low positive standard (LPS) must be run in triplicate. Also, a high positive control, negative control, and a reagent blank must be included each time the assay is performed. **NOTE:** When using Diagnostic Automation, Inc.'s software, controls must be run as follows: Blank (A1), Negative (B1), Low Positive Standard (C1-E1), High Positive (F1). Standards, controls, and samples should all be tested in duplicate until the laboratory becomes proficient with the test procedure. Each sample, standard, and control requires one antigen-coated microwell.

After the strips and holder have warmed to room temperature, cut open the protective envelope and remove the plate containing the antigen coated microwell strips. Strips that are not needed for the assay should be placed into the re-sealable pouch, sealed, and returned to storage at 2-8ºC.

Prepare the wash buffer according to the Preparation of Reagents section.

B. Serum Incubation

Prepare a 1:21 dilution of the high positive and negative controls, and each patient serum as follows:

1. Add 50µL of each sample, the positive control, and the negative control to a separate tube. Add 1000µL of sample diluent to each tube. Mix the contents of each tube thoroughly
2. Add 100 ul of sample diluent to every well in row A.
3. Using a multichannel pipette, load the controls, calibrator, and patient specimens as outlined below. Load 100 ul per well. The reconstituted calibrator is ready to load, do not dilute.

Antigen:	Row:	1	2	3	4	5
Ag Mixture	A	Dil	Dil	Dil	Dil	Dil
Jo-1	B	NC	PC	Cal	Test 1	Test 2
Sm	C	NC	PC	Cal	Test 1	Test 2
Sm/RNP	D	NC	PC	Cal	Test 1	Test 2
SSA	E	NC	PC	Cal	Test 1	Test 2
SSB	F	NC	PC	Cal	Test 1	Test 2
Scl-70	G	NC	PC	Cal	Test 1	Test 2
Control Ag	H	NC	PC	Cal	Test 1	Test 2

4. Cover the wells with a plate sealer and incubate the plate at room temperature (20-25ºC) for 20 to 25 minutes.
5. Wash the microwell strips 5X:
   a. Vigorously shake out the liquid from the wells.
   b. Fill each well with wash buffer. Make sure no air bubbles are trapped in the wells.
   c. Repeat steps **a.** and **b.** for a total of five washes.
   d. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with 0.5% sodium hypochlorite (bleach) at the end of the days run.

   Note: Auto-wash: If using an automated wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. Remove microtiter plate from washer, invert plate over a paper towel, and tap firmly to remove any residual wash solution from the wells.

C. Conjugate Incubation

1. Add 100µL of conjugate solution to each well at the same rate and in the same order as the specimens were added.
2. Cover the plate with the plate sealer and incubate at room temperature for 20 to 22 minutes.
3. Wash the plate by following the procedure in Step B.4, **a.** through **d.**

D. Substrate Incubation

1. Add 100µL of TMB substrate solution to each well at the same rate and in the same order as the specimens were added. (1mL of TMB substrate is sufficient for 8 wells).
2. Incubate the plate at room temperature (20-25ºC) for 10 to 15 minutes.
3. Add 50µL of stop solution to each well at the same rate and in the same order as the TMB substrate solution was added. Positive samples will turn from blue to yellow. After adding the stop solution, tap the plate several times to ensure that the samples are thoroughly mixed.
4. Set the microplate reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. The plate should be read within 30 minutes after addition of the stop solution.

INTERPRETATION OF RESULTS

A. Calculations

1. Determining Autoantigen-Specific Optical Density (OD):

Controls, calibrator, and test specimens are all loaded on the control antigen well (Row H). The control antigen well is prepared by coating and blocking the plastic with solutions which **do not** contain any autoantigens. Therefore, the control antigen well provides measurement of **non-specific absorbance** in each sample. Autoantigen-specific absorbance may be represented as the difference in optical density between the antigen coated well and the control antigen well.

Example:
Test Specimen A, Control Well OD (Row H) = 0.175
Test Specimen A, SSB Well OD (Row F) = 1.563

SSB Specific OD = 1.563 - 0.175 = 1.388

Using the respective control antigen well for each calibrator, control, and sample, determine the autoantigen-specific OD for each autoantigen. Do not subtract the control antigen OD from the conjugate control well OD.

2. Calibrator

Based upon testing of normal and disease-state specimens, a maximum normal autoantibody unit (AAU) value has been determined by Diagnostic Automation, Inc. and correlated to the calibrator. The calibrator will allow you to determine the unit value of test samples for each of the autoantigens, and to correct for slight day-to-day variations in test results. The unit value is determined for each autoantibody antigen for each lot of kit components and is printed on the Master Lot Specification Sheet.

3. Conversion of Optical Density to AAU/mL:

The conversion of autoantigen-specific OD to unit value (AAU/mL) can be represented by the following equation:

Test Specimen AAU/mL = (A x B)/C

Where:

AAU/mL = Unknown unit value to be determined

A = OD of test specimen in question.

B = Unit value of calibrator for autoantigen in question (AAU/mL).

C = OD of calibrator.

Example:

Test specimen specific OD for SSA = 0.946

Calibrator specific OD for SSA = 0.435

Calibrator unit value for SSA = 155 AAU/mL

Test Specimen AAU/mL = (0.946 x 155)/0.435

Test Specimen = 337 AAU/mL for anti-SSA

B. Quality Control

1. Each time the assay is run, the positive control, negative control, and calibrator must be included. For each profile strip, it is necessary to include a diluent blank (Row A), and a control antigen blank (Row H). The diluent blank measures the nonspecific interaction between the conjugate and the autoantigens. The control antigen well provides a measurement of non-specific interaction between patient antibody and a control antigen.
2. Refer to the Master Lot QC Specification Sheet included with each kit. This sheet describes the lot specific specifications for the calibrator. If the calibrator is out of range, the results are considered invalid, and patient results may not be reported.
3. The positive and negative control must meet the following specifications:

    Positive Control must be > 180 AAU/mL

    Negative Control must be < 150 AAU/mL

    Positive Control/Negative Control must be $\geq$ 2.00.

    If the assay controls do not meet the above specifications, then the assay is considered invalid, and the patient results may not be reported.
4. Calculate the mean of the conjugate control wells (Row A). This value should be less than 0.200. A high mean OD for the conjugate control could indicate inadequate washing, poor water quality, or contaminated reagents. Variability from well-to-well indicates poor pipetting and/or inconsistency of washing, well-to-well.

C. Interpretation of Results

Using 152 normal healthy donor specimens, and 185 disease-state specimens, Diagnostic Automation, Inc. has established the following guidelines for the interpretation of patient results:

< 150 AAU/mL	-	Negative
150 - 180 AAU/mL	-	Equivocal
> 180 AAU/mL	-	Positive

Use the above guidelines when evaluating or interpreting patient specimens. Equivocal specimens should be repeated. Specimens which are repeatedly equivocal should be evaluated using an alternate serological method. Elevated autoantibody levels to any of the profile autoantigens may be indicative of a specific rheumatic disorder. The SIGNIFICANCE AND BACKGROUND section of this package insert describes some of the more common diseases associated with elevated autoantibody levels.

NOTE: When interpreting the anti-Sm/RNP result to determine potential anti-RNP (only) activity, one must consider the anti-Sm and the anti-Sm/RNP result simultaneously.

For example, below are three likely scenarios:

A. Anti-Sm result = 80, and anti-Sm/RNP result = 986 AAU/mL. Patient expresses a significant amount of anti-RNP.
B. Anti-Sm result = 493 AAU/mL, and anti-Sm/RNP result = 1139 AAU/mL. Patient expresses significant amounts of both autoantibodies.
C. Anti-Sm result = 37 AAU/mL, and anti-Sm/RNP result = 63 AAU/mL. Patient is negative for both autoantibodies.

LIMITATION OF THE ASSAY

1. A diagnosis should not be made solely on the basis of the ENA Profile-6 ELISA test results.
2. Test results should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.

EXPECTED VALUES

The expected value for a normal patient is a negative result. The number of reactives, and the degree of reactivity is dependent upon parameters such as population type being tested, treatment, etc. Each laboratory should establish their own expected values based upon the specimens typically being tested.

With respect to disease-state and percent reactivity, Table 1 in the SIGNIFICANCE AND BACKGROUND section of this package insert shows the relative frequency of autoantibody activity for various rheumatic disorders.

PERFORMANCE CHARACTERISTICS

Comparative Study:

A comparative study was performed to demonstrate the equivalence of the Diagnostic Automation, Inc. ENA Profile-6 ELISA to several other commercially available autoantibody ELISA test systems. The performance of the ENA Profile-6 ELISA was evaluated using 337* serum specimens; 152 normal donor samples from the northeastern and southeastern United States, and 185 disease-state repository samples previously characterized with respect to autoantibody activity. The results of the investigation have been summarized in Tables 1 and 2 below:

* The total population tested for anti-Jo-1 was 126; 64 normal donor samples, and 62 of the disease-state repository samples.

Table 1: Relative Sensitivity; Disease-State Specimens

Autoantigen	A	B	C	D	Sensitivity
Jo-1	8	8	0	8	8/8 = 100.0%
Sm	13	16	3	13	13/13 = 100.0%
Sm/RNP	46	58	11	50	46/50 = 92.0%
SSA	56	74	18	57	56/57 = 98.2%
SSB	28	34	6	29	28/29 = 96.6%
Scl-70	8	17	9	8	8/8 = 100.0%

A - Number of specimens reactive on Diagnostic Automation, Inc. Test System.
B - Number of specimens reactive on Commercial ELISA Test System.
C - Number of discrepant specimens.
D - Number of positive specimens in the population after resolution of the discrepant specimens using alternate methodology such as gel immunodiffusion (GID), IFA, and third-party ELISA tests.

Table 2: Relative Specificity; Normal Donor Specimens

Autoantigen	E	F	G	H	Specificity
Jo-1	64	64	0	64	64/64 = 100.0%
Sm	136	137	1	137	136/137 = 99.3%
Sm/RNP	141	144	3	144	141/144 = 97.9%
SSA	146	146	0	146	146/146 = 100.0%
SSB	147	147	0	147	147/147 = 100.0%
Scl-70	151	151	0	151	151/151 = 100.0%

E - Number of specimens non-reactive on Diagnostic Automation, Inc. Test System.
F - Number of specimens non-reactive on Commercial ELISA Test System.
G - Number of discrepant specimens.
H - Number of non-reactive specimens in the population after resolution of the discrepant specimens using alternate methodology such as gel immunodiffusion (GID), IFA, and third-party ELISA tests.

REPRODUCIBILITY

To assess the intra-assay and inter-assay variability of the test procedure, a strong positive, a low positive, and a negative sample for all of the autoantigens were tested eleven times on each of three days. The mean unit value, the standard deviation, and the percent CV were calculated for each sample. The results of this study are depicted in Tables 3 - 6 below:

Table 3: Intra-Assay Reproducibility, "High Positive" Specimen: Diagnostic Automation, Inc. ENA Profile-6 IgG ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	459	15	3	391	22	6	385	18	5
Sm	576	71	12	690	71	10	702	29	4
Sm/RNP	535	73	14	426	73	17	608	76	12
SSA	818	62	7	652	68	10	779	52	7
SSB	1022	120	12	881	65	7	987	67	7
Scl-70	669	95	14	626	65	10	726	93	3

Table 4: Intra-Assay Reproducibility, "Low Positive Specimen; Diagnostic Automation, Inc. ENA Profile-6 IgG ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	232	11	5	189	9	4	189	8	4
Sm	460	43	9	587	52	9	392	28	7
Sm/RNP	184	34	18	246	34	14	216	29	13
SSA	199	26	13	231	38	17	189	22	12
SSB	178	29	16	167	20	12	210	25	12
Scl-70	231	21	9	214	10	5	270	21	8

Table 5: Intra-Assay Reproducibility, Negative Specimen; Diagnostic Automation, Inc. ENA Profile-6 IgG ELISA

	Day 1			Day 2			Day 3		
Antigen	Mean	SD	%CV	Mean	SD	%CV	Mean	SD	%CV
Jo-1	5	2	N/A	5	1	N/A	4	1	N/A
Sm	12	3	N/A	8	3	N/A	7	1	N/A
Sm/RNP	26	4	N/A	29	9	N/A	22	6	N/A
SSA	27	4	N/A	14	6	N/A	13	5	N/A
SSB	2	2	N/A	1	1	N/A	1	1	N/A
Scl-70	5	2	N/A	5	3	N/A	3	2	NA/

Table 6: Intra-Assay Reproducibility;
Diagnostic Automation, Inc. ENA Profile-6 ENA ELISA

	Day 1			Day 2			Day 3		
Antigen	**Mean**	**SD**	**%CV**	**Mean**	**SD**	**%CV**	**Mean**	**SD**	**%CV**
Jo-1	412	38	9	203	23	11	5	2	N/A
Sm	656	85	13	479	93	19	9	3	N/A
Sm/RNP	532	97	18	216	42	19	26	7	N/A
SSA	750	95	13	207	35	17	18	9	N/A
SSB	963	108	11	185	32	17	1	1	N/A
Scl-70	674	97	14	238	30	13	5	2	N/A

CROSS REACTIVITY

Specimens negative for ANA by HEp-2 IFA and positive for IgG antibody to various antigens such as EBV-VCA, EBNA, HSV-1, HSV-2, CMV, Rubella, and/or Toxo, were tested for potential cross-reactivity using the Diagnostic Automation, Inc. ENA Profile-6 ELISA Test System. All specimens tested were negative on the ELISA, indicating that the potential for cross reactivity with such antibodies is not likely, and therefore, should not interfere with the results obtained.

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