510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k101319

B. Purpose for Submission:
New assay

C. Measurand:
Anti-double stranded DNA (dsDNA) antibodies

D. Type of Test:
Qualitative and semi-quantitative

E. Applicant:
IMMCO Diagnostics

F. Proprietary and Established Names:
ImmuLisa™ dsDNA Antibody ELISA

G. Regulatory Information:

1. Regulation section:
   21 CFR §866.5100, Antinuclear Antibody Immunological Test System
2. Classification:
   Class II
3. Product code:
   LRM, Anti-DNA antibody (enzyme-labeled), antigen, control
4. Panel:
   Immunology (82)

H. Intended Use:

1. Intended use(s):
   An enzyme-linked immunosorbent assay (ELISA) for the detection and semi-quantitation of IgG antibodies to double stranded DNA (dsDNA) in human serum, as an aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
   See Intended Use above.
3. Special conditions for use statement(s):
   For prescription use only
4. Special instrument requirements:
   An ELISA Microplate reader capable of reading absorbance values at 450nm. If dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm and an automatic microplate washer capable of accurately dispensing 200 µL of fluid.

I. Device Description:
The device consists of strips of microplate wells coated with recombinant dsDNA antigen, five levels of calibrator, a positive and a negative control, peroxidase-labeled goat anti-human IgG enzyme conjugate, serum diluent, wash buffer concentrate, TMB, and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Inova Quanta Lite™ dsDNA ELISA
2. Predicate K number(s):
   k903898
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	An enzyme linked immunosorbent assay (ELISA) for the *qualitative* and semi-qualitative determination of IgG antibodies to double stranded DNA (dsDNA) in human serum, as an aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings.	Same
Methodology	ELISA	Same
Detection of antibodies	dsDNA	Same
Component set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate	Same
Conjugate	HRP	Same
Substrate/Chromogen	TMB	Same
Positive control	dsDNA IgG Antibody	Same
Stop solution	H_2SO_4	Same
Screening dilution	1:101	Same
Reading	450 nm on spectrophotometer	Same
Storage	2-8°C	Same

Differences		
Item	Device	Predicate
Cutoff	50 IU/mL	200 IU/mL
Positive Control	Acceptance range printed on vial	No value/range assigned (IFU indicates value >= 1.0 OD for acceptance of assay)
Wash Buffer pH	7.4	7.2
Calibrators	Set of 5. Values in IU/mL: 1, 50, 100, 200, 400	Predicate Device Single. Value in WHO Units: 174
Linear Range	13.6 – 400 IU/mL	Not specified
Limit of Detection	13.6 IU/mL	Not specified

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP-6A - Evaluation of the Linearity of Quantitative Analytical Methods
CLSI EP9-A2 - Method Comparison and Bias Estimation using Patient Samples
CLSI EP12-A2 - User Protocol for Evaluation of Qualitative Test Performance
CLSI EP-17A - Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

Calibrators, controls, and diluted patient samples are added to the wells and autoantibodies recognizing the dsDNA antigen bind during the first incubation. After washing the wells to remove all unbound proteins, conjugate is added. The conjugate binds to the captured human autoantibody. Excess unbound conjugate is removed by another wash step. The bound conjugate is visualized with 3,3',5,5' tetramethylbenzidine (TMB) substrate. The intensity of color development is proportional to the concentration of autoantibody in the sample. Microtiter plates are read at 450nm. The controls and patient results are interpreted by comparing them as a ratio of one of the calibrators or to a 5 point calibration curve in the qualitative or quantitative method respectively

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility:*
   Precision:
   Six patient samples across the assay range were tested over a period of 12 days with six replicates of each sample tested per day. On the final day, a second run of 12 replicates was performed (total runs = 13, total replicates = 84). Assay results were calculated using the semi-quantitative method described in the package insert.

S #	Mean (IU/mL)	Total Imprecision		Between days		Within run (Repeatability)	
		SD (IU/mL)	CV%	SD (IU/mL)	CV%	SD (IU/mL)	CV%
1	33.6	3.87	11.5%	4.04	11.7%	2.71	7.9%
2	41.0	4.43	10.8%	4.03	10.1%	4.08	10.2%
3	59.8	3.81	6.4%	3.83	6.6%	3.68	6.3%
4	124.9	10.31	8.3%	10.76	8.5%	5.56	4.4%
5	270.7	16.12	6.0%	16.44	5.9%	14.39	5.2%
6	361.6	24.99	6.9%	26.38	7.3%	14.72	4.1%

Imprecision:
Samples 1 through 4 above were tested over a period of 12 days with six replicates of each sample tested per day. On the final day, a second run of 12 replicates was performed (total runs = 13, total replicates = 84). These samples represent a negative sample, a negative sample close to the cut-off, a positive sample close to the cut-off, and a moderately positive sample. Results were calculated using the qualitative method described in the package insert; essentially the absorbance of the test sample is compared to the absorbance of the cut-off calibrator to determine sample result.

S #	Description	Expected result	# Samples with expected result	% Correct
1	LoD to Cutoff	negative	84/84	100 %
2	Cutoff -20%	negative	81/84	96.4 %
3	Cutoff +20%	positive	83/84	98.8 %
4	Moderate Pos	positive	84/84	100 %

b. Linearity/assay reportable range:
Six positive samples, with concentrations of antibodies known to be distributed throughout the calibrator range, were selected and assayed at ten (10) equidistant dilutions to determine linear range of the assay. A linear regression analysis of the samples falling within the assay range was performed:

Sample	Test Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	%Recovery
1	43.4 - 398.3	0.911 (0.87 to 0.95)	4.09 (-5.33 to 13.51)	0.998	100 to 112.3
2	41.7 - 332.2	0.98 (0.92 to 1.04)	9.74 (-3.52 to 22.99)	0.993	87.6 to 110.6
3	48.9 - 399.5	0.911 (0.87 to 0.95)	6.86 (-4.13 to 17.86)	0.997	97.6 to 110.8

Sample	Test Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	%Recovery
4	13.7 – 115.0	0.980 (0.94 to 1.02)	0.17 (-2.70 to 3.04)	1.00	96.3 to 107.1
5	13.7 – 225.3	1.00 (0.98 to 1.03)	0.12 (-3.68 to 3.92)	1.00	94.6 to 105.6
6	14.9 – 242.3	1.03 (0.98 to 1.08)	0.84 (-6.00 to 7.67)	1.00	91.2 to 106.3

The linear range of the assay was determined to be from the limit of detection to 400 IU/mL.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Traceability: Both the calibrators and the positive controls are traceable to the World Health Organization (WHO) Reference Reagent Wo/80. Based upon this standardization, results are reported in International Units (IU)/mL.

Kit stability: Real time stability studies support a claim of 18 months of an unopened device. The manufacturer demonstrated that opened kits were stable for 30 days when stored at the recommended conditions (2°-8°C). The sponsor recommends reseal plates and to close and return reagents to appropriate storage immediately after use.

Sample stability: The sponsor demonstrated that several serum samples ranging from negative to moderately positive were stable when stored for seven (7) days when stored at 4°C.

d. *Detection limit:*
The limit of blank (LoB) and limit of detection (LoD) were determined by following the study design recommended in CLSI EP17-A. Sixty replicates of the kit diluent were run; the LoB was determined sorting the results from low to high by OD and averaging the value of blanks in the 57th and 58th positions. The LoB was calculated in IU/mL by comparing this average to the standard curve; the resulting LoB was 12.9 IU/mL.

To determine the Limit of Detection (LoD) six normal serum samples were tested ten times each (a total of 60 determinations). These samples were used to calculate LoD according to CLSI EP17-A. The LoD of the assay is 13.6 IU/mL.

e. *Analytical specificity:*
Ten CDC ANA human reference sera from the Centers for Disease Control and Prevention and 10 sera of the AMLI Consensus Reference Panel 2002 were tested with the ImmuLisa™ dsDNA assay. As expected, the CDC sample specific for nDNA and the AMLI sample specific for DNA were positive by the assay; all other samples were negative. The other samples

represent other ANA-type antigens such as Scl-70, SS-A, SS-B, Jo-1, etc.

See clinical sensitivity and specificity discussion below for cross-reactivity with sera from patients with other autoimmune diseases.

Endogenous interferents: The following substances were spiked into serum in order to test for interference: hemoglobin (2 g/L), bilirubin (20 mg/dL), and rheumatoid factor (RF, 100 IU/mL). Five samples were evaluated for interference—a negative above the LoD, two samples in the indeterminate zone, and two strongly positive samples. Recoveries were all within ± 10% for all sample/substance combinations except for one; the recovery of the RF sample in the negative sample was 87% (approximately 3 IU/mL).

In a separate study the interference by triglycerides (3000 mg/dL) and cholesterol (500 mg/dL) were investigated in a similar panel of samples. Recoveries for these samples ranged from 87 – 113%. The instructions for use caution against the use of lipemic, hemolyzed, or contaminated samples. The sponsor states that the assay does not react to anti-ssDNA antibodies.

f. Assay cut-off:
Testing was performed to evaluate expected values in the normal population and confirm the defined cut-off. Samples from 120 normal blood donors and non-SLE disease control specimens, equally distributed by age and gender, were obtained from a commercial source. The mean value plus 3 SD of these samples was established as the cut off between normal and abnormal results. This value was assigned a unit value of 50 IU/mL based upon studies performed against World Health Organization (WHO) Reference Reagent Wo/80. Of the 120 samples, 4 were positive: 1 sample from a Rheumatoid Arthritis patient and 3 samples that were ANA-positive.

2. Comparison studies:
   a. Method comparison with predicate device:
   Testing was performed on 173 samples within the measuring range of the assay. The samples were from clinically defined patients (87 clinical samples, 23 other autoimmune disease controls, 58 normal sera). Disease controls in each study were taken from patients with Graves disease, Hashimoto's thyroiditis, rheumatoid arthritis and non-SLE subjects positive for certain autoantibodies including anti-nuclear antibodies (ANA) and anti-Hsp-70 antibodies. Only specimens in the linear range of the assay were included in the method comparison. These results are summarized below.

   Indeterminate Samples (samples 50 – 60 IU/mL) considered positive:

		SLE Diagnosis		
		Positive	**Negative**	**Total**
IMMCO dsDNA	**Positive**	71	10	81
	Negative	6	86	92
	Total	77	96	173

Positive % Agreement: 92.2% (95% CI 83.2% - 96.8%)
Negative % Agreement: 89.6% (95% CI 81.3% - 94.6%)
Overall % Agreement: 90.8% (95% CI 85.2- 94.5)

Indeterminate Samples (samples 50 – 60 IU/mL) considered negative:

		SLE Diagnosis		
		Positive	**Negative**	**Total**
IMMCO dsDNA	**Positive**	69	6	75
	Negative	8	90	98
	Total	77	96	173

Positive % Agreement: 89.6% (95% CI 80.0% - 95.1%)
Negative % Agreement: 93.8% (95% CI 86.4% - 97.4%)
Overall % Agreement: 91.9% (95% CI 86.5% - 95.3%)

b. Matrix comparison:
Not applicable; this assay is only used with serum.

3. Clinical studies:
   a. Clinical Sensitivity and Specificity:
   The clinical sensitivity and specificity of a set of 540 clinically characterized sera (see below for diagnoses) was evaluated with the assay.

Indeterminate Samples (samples 50 – 60 IU/mL) considered positive:

		SLE Diagnosis		
		Positive	**Negative**	**Total**
IMMCO dsDNA	**Positive**	155	14	169
	Negative	94	277	371
	Total	249	291	540

Sensitivity 62.2% (95% CI 55.9% - 68.2%)
Specificity 95.2% (95% CI 91.9% - 97.2%)
Agreement 80.0% (95% CI 76.3% - 83.2%)

Indeterminate Samples (samples 50 – 60 IU/mL) considered negative:

		SLE Diagnosis		
		Positive	**Negative**	**Total**
IMMCO dsDNA	**Positive**	143	9	152
	Negative	106	282	388
	Total	249	291	540

Sensitivity	57.4%	(95% CI 51.0% - 63.6%)
Specificity	96.9%	(95% CI 94.0% - 98.5%)
Agreement	78.7%	(95% CI 75.0% - 82.0%)

Anti-dsDNA Assay Results by Clinical Diagnosis:

Clinical Diagnosis	Tested	# Positive*	% Positive
Systemic Lupus Erythematosus	249	155	62.2
Other Diseases			
Primary APS	25	0	0
Primary Myositis	20	0	0
Scleroderma	4	1	25.0
Rheumatoid Arthritis	43	5	11.6
Autoimmune Thyroid Disease			
Graves' disease	9	0	0
Hashimoto's thyroiditis	14	1	7.1
Undifferentiated	9	1	11.1
Autoimmune Hearing Loss	8	0	0
Healthy Normals	159	6	3.8

***** Equivocal samples considered positive.

4. Clinical cut-off**:**
   See assay cut-off section above.

5. Expected values/Reference range:
   The expected value in the normal population is negative although a small portion of the population may have anti-dsDNA autoantibodies without clinical disease.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.