

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

K151559

B. Purpose for Submission:

New device

C. Measurand:

IgG autoantibodies specific for Centromere protein

D. Type of Test:

Immunoassay, qualitative and semi-quantitative

E. Applicant:

IMMCO Diagnostics Inc.

F. Proprietary and Established Names:

ImmuLisa™ Enhanced Centromere Antibody ELISA

G. Regulatory Information:

1. Regulation section:
21 CFR §866.5100, Antinuclear antibody immunological test system
2. Classification:
Class II
3. Product code:
LJM, antinuclear antibody (enzyme-labeled), antigen, controls
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
An enzyme linked immunoassay (ELISA) for the qualitative or semiquantitative detection of anti-centromere antibodies in human serum or plasma to aid in the diagnosis of limited cutaneous systemic sclerosis / CREST in conjunction with other laboratory tests and clinical findings.

2. Indication(s) for use:
Same as intended use
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 450 nm. If dual wavelength microplate reader is available, the reference filter should be set at 600–650 nm. An automatic microplate washer capable of accurately dispensing 200 µL of fluid is also required.

I. Device Description:

Each kit consists of 12- 1 x 8 antigen coated microwell strips, negative control, positive control, five assay calibrators, anti-human IgG-horse radish peroxidase conjugate, TMB substrate, stop solution, wash buffer and diluent.

J. Substantial Equivalence Information:

1. Predicate device name(s):
QUANTA Lite™ Centromere ELISA
2. Predicate 510(k) number(s):
K003959
3. Comparison with predicate:

Similarities		
Item	ImmuLisa™ Centromere	Predicate Device
Intended Use	An enzyme linked Immunoassay (ELISA) for the qualitative or semi-quantitative detection of anti-centromere antibodies in human serum or plasma to aid in the diagnosis of limited cutaneous systemic sclerosis / CREST in conjunction with other laboratory tests and clinical findings.	QUANTA Lite Centromere (CENP-A & CENP-B) is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Centromere antibodies, specifically reactive to the CENP-A and CENP-B antigens in human sera. Detection of these antibodies is an aid in diagnosis of certain connective tissue diseases such as the CREST variant of scleroderma.
Assay Type	ELISA	Same
Type of Test	Semi-quantitative and qualitative	Semi-quantitative

Similarities		
Item	ImmuLisa™ Centromere	Predicate Device
Capture Antigen	Recombinant purified CENP-A and CENP-B Centromere antigens	Same
Conjugate	HRP conjugated anti-human IgG	Same
Substrate	TMB	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Sample Type	Serum	Same
Screening Dilution	1:101	Same
Negative Cut-off	< 20 EU/mL	< 20 units
Conjugate	HRP conjugated anti-human IgG	Same
Instrumentation	Spectrophotometer 450 nm	Same
Signal	Optical density	Same

Differences		
Item	ImmuLisa™ Centromere	Predicate Device
Positive Cutoff	> 25 EU/mL	> 20 units
Indeterminate Region	20–25 EU/mL	Not applicable
Calibrators	Set of 5; values in EU/mL: 160,80 40, 20,1 and Cal D (20 EU/mL) for the single point calibration method	Single point calibrator
Linear Range	3.9 EU/mL–160 EU/mL	Not specified
Limit of Detection	3.9 EU/mL	Not specified

K. Standard/Guidance Document Referenced:

1. Evaluation of Precision Performance of Quantitative Measurement Methods (CLSI EP05-A2)

2. Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)
3. Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition (CLSI EP07-A2)
4. Method Comparison and Bias Estimation Using Patient Samples (CLSI EP9-A2)
5. User Protocol for Evaluation of Qualitative Test Performance (CLSI EP12-A2)
6. Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A)

L. Test Principle:

The test is performed as a solid phase immunoassay. Controls, calibrators and patient sera are incubated in the antigen coated wells to allow specific antibodies present in the serum to bind to the centromere antigen. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding an enzyme labeled anti-human IgG conjugate to the microwells. Unbound conjugate is removed by washing. Enzyme substrate (TMB) is then added to the wells and the presence of antibodies is detected by a color change produced by the conversion of TMB substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm.

Semi-quantitative results are determined from a series of five calibrators (160 EU/mL, 80 EU/mL, 40 EU/mL, 20 EU/mL, and 1 EU/mL). Values less than 20 EU/mL are considered negative results while values greater than 25 EU/mL are considered positive; results between 20 EU/mL and 25 EU/mL are considered 'indeterminate'. Qualitative results are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 EU/mL). The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. Values greater than 20 EU/mL are reported as positive.

M. Performance Characteristics:

1. Analytical performance:

a. Precision/Reproducibility:

Semi-Quantitative Precision:

Sera from seven patients from the Intended Use population were selected to cover the analytical measuring range of the assay and included samples in the negative range, ~20% below cut-off, around the cut-off, ~20% above cut-off and in the moderate positive range of the assays. Samples were run in duplicate, twice per day for 20 days (n = 80 replicates per sample). Assays were run by two operators on two different sets of equipment, each using a multichannel pipettor, microplate washer and microplate reader. The manufacturer's pre-determined acceptance criteria met for all measures.

Semi-Quantitative Precision:

	EU/mL Mean	Repeatability		Between Days		Inter-operator		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	8.5	0.3	4.1	0.3	3.3	0.2	2.8	0.4	5.2
2	16.4	0.5	3.0	0.8	5.2	0.6	3.6	1.0	6.0
3	20.4	0.9	4.4	0.5	2.3	0.5	2.5	1.0	5.0
4	23.8	0.5	1.9	0.8	3.5	0.5	2.2	0.9	4.0
5	49.0	1.5	3.0	2.2	4.5	1.5	3.1	2.6	5.4
6	105.1	1.7	1.6	2.7	2.6	2.4	2.3	3.2	3.0
7	150.6	4.7	3.1	2.7	1.8	0.0	0.0	5.4	3.6

Operator-to-Operator Reproducibility:

The sponsor provided operator-to-operator reproducibility for the manual method. This study incorporated three operators running five replicates per assay over three days. The data are summarized in the following table:

Sample	Mean EU/mL	Operator 1 %CV	Operator 2 %CV	Operator 3 %CV	Total %CV
Low Negative	7.9	10.1	12.4	10.5	10.8
Cut-off - 20%	16.3	7.5	6.6	5.1	6.5
Cut-off	20.6	6.1	6.3	4.5	5.8
Cut-off + 20%	23.5	5.4	5.0	5.4	5.3
Moderate Positive	43.0	5.9	5.6	4.2	5.2
High Positive	95.8	3.2	3.2	3.7	3.8
High Positive	154.0	3.9	3.8	2.8	3.5

The manufacturer’s pre-determined acceptance criteria were met for all measures.

Qualitative Reproducibility:

Studies were performed under the guidance of CLSI EP12-A2 “User Protocol for Evaluation of Qualitative Test Performance.” Eighty replicates each of sera in the negative range: 20% below the cut-off, at the cut-off, 20% above the cut-off and in the moderate positive range of the assays were tested to evaluate the reproducibility of the qualitative method of analysis. The results were calculated using single-point (qualitative) analysis as indicated in the product insert. The manufacturer’s pre-determined acceptance criteria were met. Results are summarized below.

Sample	Mean EU/mL	% Negative	% Positive
Low Negative	7.9	100	0
Cut-off -20%	16.2	100	0
Cut-off	20.4	37	63

Sample	Mean EU/mL	% Negative	% Positive
Cut-off +20%	23.4	1	99
Moderate Positive	44.5	0	100
High Positive	79.3	0	100
High Positive	149.4	0	100

Lot-to-Lot Reproducibility:

Inter-lot reproducibility was tested by using samples spanning the assay range. Three lots of material were used in the study. Seven samples were tested in duplicate on three different lots of the kit. Assays were run by two operators on two different sets of equipment, each using a multichannel pipettor, microplate washer and microplate reader. There was no recalibration of this equipment over the course of this study. The manufacturer's pre-determined acceptance criteria were met for all measures.

Lot-to-Lot Reproducibility:

Sample	EU/mL	Lot 1 %CV	Lot 2 %CV	Lot 3 %CV	Between Lots %CV
1	8.0	9.2	8.7	8.2	9.0
2	16.2	7.1	6.6	7.0	6.9
3	20.2	6.3	5.2	7.2	6.7
4	23.0	6.2	5.1	5.8	6.2
5	43.3	5.0	5.7	6.4	6.1
6	96.5	3.6	3.1	3.1	3.7
7	154.0	4.2	4.6	3.8	4.4

Control Material Precision Studies:

Positive and negative control materials were tested in duplicate, twice per day for 20 days for a total of 80 replicates per sample. Assays were run by two operators on two different sets of equipment.

	Mean	Repeatability		Between Runs		Total	
		SD	%CV	SD	%CV	SD	%CV
Negative Control	5.3	0.1	2.2	0.4	7.6	0.4	7.3
Positive Control	82.6	2.4	2.9	5.1	6.2	5.1	6.2

b. Linearity/assay reportable range:

Positive samples with values throughout the measuring range were selected and assayed in duplicate, at equidistant dilutions to determine linear range of the assays.

The linear range of the assay was determined to be 3.9 –160 EU/mL.

Sample	Dilution Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery
1	3.2–46.0	1.05 (0.97 –1.13)	-0.67 (-2.88 –1.53)	0.994	92 –116
2	6.3–123.1	1.02 (0.95 –1.10)	-0.18 (-5.64 –5.29)	0.995	93 –104
3	4.9–188.1	1.03 (0.95 –1.11)	1.42 (-7.15 –9.98)	0.984	87 –102

High dose hook effect: High concentrations specimens were serially diluted (from approximated 407 EU/mL) to assess the presence of a decrease in assay signal associated with antigen excess (hook effect). A hook effect at concentrations up to 407 EU/mL was not seen.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

i) *Traceability:*

There are currently no recognized international standards for the measurement of anti-centromere antibodies. Calibrator and Control values are directly traceable to in-house standards.

ii) *Value Assignment:*

Calibrators and Positive Controls are dilutions of pooled Centromere antibody-positive sera. The sponsor formulates new calibrator and control lots from an array of antibody positive sera obtained from commercial plasma centers stored frozen at -70°C. The calibrators and controls are taken from different pooled sera. All source sera has been tested and found negative for infectious disease as stated in the product insert. Manufactured calibrator sets are stored in aliquots frozen at -70°C. As new lots of calibrators are developed, comparison studies are performed to calibrate values against original calibrators. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards.

iii) *Stability:*

Shelf life stability:

Accelerated and open kit studies for each device were performed on three lots of components/reagents. Accelerated studies were conducted with materials incubated at 37°C. In these conditions, one day is considered equivalent to one month stored at 2°–8°C. Materials are removed from the incubator for testing at three-day intervals for a minimum of 18 days.

Real time stability of three kit lots was tested using five specimens with reactivities across the analytical measuring range; current results support a six month shelf life stability claim.

Open Kit Stability:

For open kit stability studies, materials are opened and stored as required for bench-top usage, then assayed at 15, 45 and 90 day intervals. Open vial stability studies demonstrate opened reagents are stable at 45 days, but the sponsor chose a more restrictive one month open kit stability claim.

d. Detection limit:

The Limit of Blank (LoB) and the Limit of Detection (LoD) were determined using 60 replicates of the blank and 10 replicates each of 6 low-level (normal human) samples by following CLSI EP17-A. Sixty samples of diluent were run as blank samples and six different normal human sera were each assayed 10 times. LoB was determined to be 3.5 and LoD was determined to be 3.9 EU/ml.

e. Analytical specificity:

Interference:

The studies were performed according to CLSI EP07-A2, *Interference Testing in Clinical Chemistry, Approved Guideline- Second Edition*. Interference was studied by mixing sera with known Centromere antibody levels with serum samples containing potentially interfering substances and studying deviation from expected results. Samples were selected from the negative range, near the assay cutoff and in the low, moderate and high positive range less than the value of the top calibrator. No significant interference (defined as > 15% deviation from expected) was demonstrated in the Centromere assay for the following substances at the levels indicated: Hemoglobin (2 g/L), Bilirubin (342 µmol/L), Rheumatoid Factor (100 EU/mL), Triglycerides (37 mmol/L) and Cholesterol (13 mmol/L).

Comparison to Reference Sera:

Twelve ANA human reference sera from the Centers for Disease Control and Prevention were tested with the ImmuLisa Centromere Antibody ELISA. As expected, the CDC sample known to contain anti-Centromere antibodies tested strongly positive. The eleven other samples were negative. The other samples represent other ANA-type antigens such as SS-A, SS-B, Jo-1, etc.

Ten ANA human reference sera from the Association of Medical Laboratory Immunologists were tested with the ImmuLisa Centromere Antibody ELISA. As expected, the AMLI sample known to contain anti-Centromere antibodies tested strongly positive. The nine other samples were negative. The other samples represent other ANA-type antigens such as SS-A, SS-B, Jo-1, etc.

f. Assay Cut-off:

Positive assay cut-offs were assigned a unit values of 25 EU/mL for the semi-quantitative method and 20 EU/mL for the qualitative method, based on the standardized method used by other IMMCO products. Indeterminate/borderline results (defined as 20–25 EU/mL) for the semi-quantitative method, are

recommended to be retested and evaluated along with other laboratory methods for detection of antibodies to ENA.

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison study was performed with the QUANTA Lite™ Centromere ELISA kit, using 119 samples from patients with limited system sclerosis (LcSSc)/ CREST Syndrome (SSc)] and 288 disease controls. Sample values were within the analytical measuring range for the device. Performance relative to the predicate was determined by calculating agreement where borderline values were considered positive or negative. Qualitative results were the same as those obtained when semi-quantitative indeterminate values are considered positive.

Borderline values considered positive		Quanta Lite Centromere Antibody ELISA		
		Positive	Negative	Total
IMMCO Centromere Ab ELISA	Positive	55	16	71
	Negative	4	332	336
	Total	59	348	407

Positive Percent Agreement: 93.2% (95% CI 88.7–97.8%)
 Negative Percent Agreement: 95.4% (95% CI 92.5 –97.3%)
 Total Agreement: 95.1% (95% CI 92.4–96.9%)

Borderline values considered negative		Quanta Lite Centromere Antibody ELISA		
		Positive	Negative	Total
IMMCO Centromere Ab ELISA	Positive	51	13	64
	Negative	8	335	343
	Total	59	348	407

Positive Percent Agreement: 86.4% (95% CI 74.5–93.6%)
 Negative Percent Agreement: 96.3% (95% CI 93.5–97.9%)
 Total Agreement: 94.8% (95% CI 92.2–96.6%)

The manufacturer’s pre-determined acceptance criteria were met for all measures.

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The clinical sensitivity and specificity of the IMMCO Centromere assay was evaluated in 989 serum samples representing various autoimmune and infectious conditions; the performance was assessed against the clinical diagnosis. Borderline results were considered positive and negative for these analyses. When results were calculated by the qualitative method, sensitivity and specificity are identical to the semi-quantitative results when borderline results are considered positive.

Borderline values considered positive		Diagnosis –IcSSC		
		IcSSC/CREST	Controls	Total
IMMCO Centromere Ab ELISA	Positive	66	39	105
	Negative	58	826	884
	Total	124	865	989

Sensitivity = 53.2% (95% C.I. = 44.1 – 62.2%)

Specificity = 95.5% (95% C.I. = 93.8 – 96.7%)

Borderline values considered negative		Diagnosis –IcSSC		
		IcSSC/CREST	Controls	Total
IMMCO Centromere Ab ELISA	Positive	61	35	96
	Negative	63	830	893
	Total	124	865	989

Sensitivity = 49.2% (95% C.I. = 40.2 – 58.3%)

Specificity = 96.0% (95% C.I. = 94.4–97.2 %)

The distribution of the Centromere positivity rate in the cohort is in the Table below:

Cohort	n	# Pos¹	% Pos¹
Limited cutaneous systemic sclerosis	124	66	53.2%
Diffuse cutaneous scleroderma	39	1	2.6%
Anti-phospholipid syndrome	36	4	11.1%
Anti-phospholipid syndrome with SLE	20	0	0.0%
Celiac Disease	35	2	11.4%
Churg-Strauss syndrome	27	0	0.0%
Crohn's disease	25	0	0.0%
Granulomatosis with polyangiitis	27	0	0.0%
Graves' disease	20	0	0.0%
Hashimoto's thyroiditis	20	0	0.0%
Osteoarthritis	20	0	0.0%
Polymyositis/Dermatomyositis	30	1	3.3%
Rheumatoid Arthritis	24	1	4.2%
Sjögren's Syndrome	37	2	5.4%
Systemic Lupus Erythematosus	92	1	1.1%
Thrombocytopenia	15	2	13.3%
Ulcerative Colitis	25	0	0.0%
Autoimmune hepatitis	30	4	13.3%
Mixed Connective Tissue Disease	30	2	6.7%
Primary Biliary Cirrhosis	30	6	20.0%
IDDM	30	1	3.3%
CMV	20	0	0.0%
Hepatitis C	20	0	0.0%
HSV-1	20	3	15.0%
HSV-2	20	0	0.0%
Lyme disease	20	0	0.0%
Mononucleosis	20	1	5.0%
Rubella	20	0	0.0%
Syphilis	17	0	0.0%
Toxoplasmosis	20	0	0.0%
Advanced chronic renal failure	30	3	10.0%
Cancers ²	46	3	6.5%

1. Indeterminate results were considered positive
2. Cancers included breast, colorectal, lung, ovarian and pancreatic.

The manufacturer's pre-determined acceptance criteria were met for all measures.

Clinical cut-off:

Not applicable

4. Expected values/Reference range:

Test results in a normal population are expected to be negative. A study of 117 normal, apparently disease-free samples tested with the anti-Centromere assay yielded no borderline results (0.0%) and no positive results (0.0%).

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.