510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172078

B. Purpose for Submission:

New device

C. Measurand:

IgG autoantibodies specific for RNA polymerase III (RNA POL III)

D. Type of Test:

Immunoassay, qualitative and semi-quantitative

E. Applicant:

IMMCO Diagnostics Inc.

F. Proprietary and Established Names:

ImmuLisa™ Enhanced RNA POL III Antibody ELISA

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5100, Antinuclear antibody immunological test system

2. Classification:

   Class II

3. Product code:

   NYO – Autoantibody, anti-ribonucleic acid polymerase (RNAP) III

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   An enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of anti-RNA POL III IgG antibodies in human serum as an aid in the diagnosis of systemic sclerosis (scleroderma) in conjunction with other laboratory and clinical findings.

2. Indication for use:

   Same as Intended Use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   An ELISA microplate reader capable of reading absorbance values at 450 nm. If a dual wavelength microplate reader is available, the reference filter should be set at 600–650 nm. An automatic microplate washer capable of dispensing 200 µL of fluid is also required.

I. Device Description:

Each kit consists of 12 x 8-antigen coated microwell strips, negative control (1x 1.75 mL), positive control (1x 1.75 mL), five assay calibrators (5x 1.75 mL), horseradish peroxidase anti-human IgG conjugate (1x 15 mL), TMB enzyme substrate (1x 15 mL), stop solution (1x 15 mL), wash buffer (2 vials) and diluent (1x 60 mL).

J. Substantial Equivalence Information:

1. Predicate device name:

   QUANTA Lite RNA POL III ELISA

2. Predicate 510(k) number:

   K070066

3. Comparison with predicate:

Similarities		
Item	**New Device** ImmuLisa Enhanced RNA POL III ELISA	**Predicate** QUANTA Lite RNA POL III ELISA
Intended Use	An enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of anti-RNA POL III IgG antibodies in human serum to aid in the diagnosis of systemic sclerosis (scleroderma) in conjunction with other laboratory tests and clinical findings.	The QUANTA LITE RNA POL III ELISA is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-RNA Polymerase III antibodies in human serum. The presence of these antibodies, when considered in conjunction with other laboratory and clinical findings, is an aid in the diagnosis of systemic sclerosis (scleroderma) with increased incidence of skin involvement and renal crisis.
Assay Type	ELISA	Same
Capture Antigen	Recombinantly expressed and purified RNA POL III	Purified recombinant immunodominant fragment of RNA Pol III antigen
Sample Type	Serum	Same
Assay Format	Semi-quantitative and qualitative	Same
Labeled Detection Antibody (Conjugate)	Horseradish peroxidase conjugated to goat anti-human IgG	Same
Enzyme Substrate	Tetramethylbenzidene (TMB)	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Signal/Wavelength	Optical density/450nm	Same
Instrumentation	Microwell plate reader	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced RNA POL III IgG ELISA	**Predicate** QUANTA Lite RNA POL III ELISA
Calibrators	Set of five: Values in EU/mL: 1, 20, 40, 80,160	One calibrator Value in U/mL
Linear Range	3.2 EU/mL–160 EU/mL	Not specified

Differences		
Item	**New Device** ImmuLisa Enhanced RNA POL III IgG ELISA	**Predicate** QUANTA Lite RNA POL III ELISA
Limit of Detection	3.2 EU/mL	Not specified
Results Interpretation	Negative: < 20 EU/mL Indeterminate: 20–25 EU/mL Positive: > 25 EU/mL	Negative: ≤ 20 U/mL Positive: > 20 U/mL

K. Standard/Guidance Document Referenced:

CLSI guideline EP05-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline Second Edition"

CLSI guideline EP6-A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline"

CLSI guideline EP07-A2, "Interference Testing in Clinical Chemistry; Approved Guideline–Second Edition"

CLSI guideline EP09-A2, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline."

CLSI guideline EP12-A2, "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline–Second Edition"

CLSI guideline EP17-A2, "Evaluation of Detection Capability for Clinical. Laboratory Measurement Procedures; Approved Guideline–Second Edition"

CLSI EP12-A2 "User Protocol for Evaluation of Qualitative Test Performance."

L. Test Principle:

Recombinantly expressed and purified RNA POL III antigen is bound to the wells of a polystyrene microwell plate followed by blocking of the unreacted sites to reduce non-specific binding. Controls, calibrators and diluted patient sera are added to separate wells, allowing any anti-RNA POL III antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG conjugate is added to each well. After washing away any unbound conjugate, specific enzyme substrate (TMB) is then added to the wells. After stopping the enzymatic reaction, the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL).

Semi-quantitative results are determined from a series of five calibrators (1 EU/mL, 20 EU/mL, 40 EU/mL, 80 EU/mL, and 160 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/borderline'. The sponsor states the

following recommendations in the Package Insert: "*Indeterminate/borderline results should be retested and evaluated along with other laboratory methods detection of antibodies to RNA POL III such as indirect immunofluorescence assays (IFA) using a HEp-2 substrate.*".

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 or equal to 20 EU/mL are considered positive and values less than 20 EU/mL values are considered negative.

M. Performance Characteristics:

1. Analytical performance:

   The results of all the studies met the manufacturer's pre-determined acceptance criteria.

   a. *Precision/Reproducibility:*
   Precision performance was evaluated in accordance with CLSI guideline EP05-A2. A panel consisting of seven patient sera with levels of RNA POL III antibodies that cover the analytical measuring range was assayed in duplicate, twice a day, for 20 days with one reagent lot (a total of 80 replicates per sample). Testing was performed by two operators using different equipment sets. The first operator performed the assay with a multi-channel pipettor, microplate washer and microplate reader. The second operator used different instruments with the same type of equipment. The results are summarized in the table below:

   ImmuLisa Enhanced RNA POL III ELISA:

Specimen	**Mean** (EU/mL)	**Within-Run (Repeatability)**		**Between-Run**		**Between-Day**		**Total**	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	10.0	0.7	7.3%	0.7	0.7	0.5	5.1%	0.7	7.3%
2	18.2	0.8	4.4%	0.8	0.8	0.4	2.2%	0.8	4.4%
3	19.6	0.9	4.7%	0.9	0.9	0.6	2.8%	0.9	4.8%
4	22.0	1.2	5.4%	1.3	1.3	1.1	4.9%	1.3	5.8%
5	46.2	3.4	7.3%	3.6	3.6	2.4	5.3%	3.6	7.8%
6	82.8	5.0	6.1%	5.2	5.2	3.8	4.6%	5.2	6.2%
7	159.0	9.3	5.8%	9.4	9.4	5.6	3.5%	9.4	5.9%

   Qualitative Reproducibility:

   Studies were performed in accordance with CLSI guideline EP12-A2 "User Protocol for Evaluation of Qualitative Test Performance." Eighty replicates of each patient serum with concentrations of analyte in the negative range, ~20% below cut-off, near

the cut-off, ~20% above cut-off and in the moderate positive range were performed to determine qualitative within-laboratory imprecision. Results were calculated using single-point (qualitative) analysis as indicated in the product insert. The results are summarized in the table below:

ImmuLisa Enhanced RNA POL III ELISA:

Specimen	Mean (EU/mL)	Total replicates	Qualitative Agreement	
			Positive/Negative	% Correct
Negative	9.9	80	0/80	100
Cut-off −20%	17.2	80	0/80	100
Cut-off	19.7	80	50/30	40
Cut-off +20%	21.9	80	2/78	97.5
Moderate Positive	39.0	80	80/0	100
Moderate to High Positive	65.8	80	80/0	100
High Positive	116.9	80	80/0	100

Lot-to-lot Reproducibility:
To evaluate lot-to-lot reproducibility, a panel of five samples with levels of anti-RNA POL III antibodies spanning the assay range was tested with five replicates per day on three different lots over the course of five days. The results are summarized in the table below:

ImmuLisa Enhanced RNA POL III ELISA:

Specimen	Mean (EU/mL)	Within-Run		Within-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	9.0	0.3	3.6%	0.8	8.6%	0.8	8.6%
2	19.3	0.6	3.2%	0.8	4.2%	0.9	4.5%
3	46.0	1.5	3.3%	1.9	4.2%	2.1	4.6%
4	87.5	5.9	6.8%	8.4	9.6%	8.4	9.6%
5	145.0	7.2	5.0%	8.0	5.5%	9.1	6.3%

Site-to-site Reproducibility:

To evaluate site-to-site reproducibility, a panel of five samples with levels of anti-RNA POL III antibodies spanning the assay range was tested in replicates of five at three different sites over the course of five days using one lot of reagent. The results are summarized in the table below:

ImmuLisa Enhanced RNA POL III ELISA**:**

Specimen	Mean (EU/mL)	Within Run		Between Sites		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)
1	10.0	0.4	4.4	0.8	7.6	0.8	8.3
2	22.7	0.6	2.7	1.4	6.2	2.1	9.4
3	47.7	1.4	2.9	2.9	6.0	4.1	8.6
4	88.4	4.7	5.3	7.8	8.8	8.5	9.6
5	146.2	5.1	3.5	12.0	8.2	12.8	8.8

b. Linearity/assay reportable range:

Linearity and recovery were tested by diluting positive specimens across the measuring range in equidistant dilutions with negative patient sera. The observed values were graphed against the calculated values and a linear regression was performed. The linear range was determined to be 3.2–160 EU/mL for the assay. The results are summarized in the table below:

ImmuLisa Enhanced RNA POL III ELISA:

Sample	Test Range (EU/ml)	Slope (95% CI)	Y-intercept (95% CI)	R^2	% Recovery
1	2.5 to 33.5	1.02 (0.96 to 1.10)	-0.15 (-1.72 to 1.43)	0.994	96% to 117%
2	8.0 to 66.1	0.98 (0.95 to 1.01)	1.56 (0.13 to 2.98)	0.999	97% to 109%
3	31.8 to 169.5	0.99 (0.88 to 1.09)	-2.68 (-14.56 to 9.20)	0.989	91% to 103%

High dose hook effect: Four high concentrations specimens were serially diluted to assess the presence of any artifactual decrease in assay signal associated with anti-RNA POL III antibody excess (hook effect). No hook effect was demonstrated in sample levels up to 2092.3 EU/mL.

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

i. Traceability:

There are currently no recognized international standards for the measurement of RNA POL III antibodies. Calibrator and Control values for both assays are directly traceable to in-house standards.

ii. Value Assignment:

Calibrators and positive controls are dilutions of pooled anti-RNA POL III

antibody positive sera obtained from various commercial plasma centers. The calibrators and controls are taken from different pooled sera. In order to have traceability with newly made calibrators, each new lot of calibrators is assayed on an already cleared kit and compared to the existing calibrators as reference. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards. Five calibrator levels (Cal A-E) with assigned values from 0–160 EU/mL are included to provide semi-quantitative results and must be used with each run. The cut-off calibrator for the qualitative analysis is derived from Cal D. Test results greater than or equal to Cal D are considered positive.

For semi-quantitative determinations, the positive controls must give values in the range stated on the vial. The negative control contains negative human serum in buffer. The negative control must be <10 EU/mL.

iii. Stability:

Shelf-life Stability:
Accelerated and real-time stability studies were conducted on three lots of components/reagents. The accelerated stability study was conducted with materials incubated at 37°C. Data from the accelerated stability study support a shelf-life stability of 18 months. A real-time stability study is on-going and currently supports a real-time claim of stability claim at this writing.

Open-Kit Stability:
For the open-kit stability study, materials in each ImmuLisa Enhanced RNA POL III Antibody ELISA were opened and stored in a dark environment as required for bench-top usage, then assayed at 15, 45 and 90 day intervals. Although data from the open-kit stability study demonstrate that opened reagents are stable at 45 days, the sponsor chose a more restrictive one month open-kit stability claim.

Sample Stability and Storage:
The package insert recommends that sera be assayed soon after separation or stored at 2–8°C for no longer than one week. For longer storage, serum specimens should be frozen. Users should avoid repeated freezing and thawing of samples. The sponsor recommends that frozen specimens be tested within one year.

d. Detection limit:

The analytical sensitivity was determined in accordance with CLSI guideline EP17-A2. The Limit of Blank (LoB) was determined by assaying a blank sample in 60 replicates. The limit of detection (LoD) was determined by assaying six low negative sera in 10 replicates over three days using two reagent lots of each ImmuLisa Enhanced RNA POL III Antibody ELISAs. The LoB and LoD for the assay was 1.7 and 3.2 EU/mL, respectively. The Limit of Quantitation (LoQ) was determined by testing four low level samples in replicates of four over three days using two reagent lots and was determined to be 3.3 EU/mL.

e. Analytical specificity:

i. Endogenous Interference:

Interference studies studies were performed according to CLSI guideline EP07-A2 by testing five serum samples with anti-RNA POL III antibody levels corresponding to the negative range, near the assay cut-off, and in the low, moderate and high positive range. Each sample was mixed with known quantities of potentially interfering substances and analyzed in one assay run with two replicates with one lot of reagents. The recovery was calculated by comparing to control samples spiked with the same volume of diluents. No significant interference was detected for the following substances up to the concentrations listed in the table below:

Potential Interfering Compound	**Test Concentration**
Hemoglobin	2 g/L
Bilirubin	342 µmol/L
Triglycerides	37 mmol/L
Rheumatoid Factor	100 EU/mL
Total cholesterol	13 mmol/L
Prednisone	0.84 µmol/L
Naproxen	25 mg/mL

The 'Limitations of Procedure' section of the Package Insert states "*This test should not be performed on grossly hemolyzed, microbially contaminated or lipemic samples*".

ii. Exogenous Interference:

Interference by selected exogenous substances was evaluated according to CLSI guideline EP07-A2 by testing five serum samples with anti-RNA POL III antibody levels corresponding to the negative range, near the assay cut-off, and in the low, moderate and high positive range. Each sample was mixed with known quantities of potentially interfering substances and analyzed in one assay run with one lot of reagenrs. The recovery was calculated by comparing to control samples spiked with the same volume of diluents. No significant interference was detected for the following substances at the concentrations listed in the table below:

Potential Interfering Compound	Test Concentration
Methotrexate	2 mmol/L
Enalapril	0.86 umol/L
Sildenafil	12.9 pmol/L
Amlodipine	245 nmol/L
Cyclophosphamide	1437 µmol/L
Omeprazole	17.4 µmol/L
Metoclopramide	1.5 µmol/L
Doxycycline	67.5 µmol/L
Bosetan	3 µg/mL
Tocilizumab	549 µg/mL
Mycophenolate mofetil	14.01µg/mL
Heparin	3000 U/L

ii. Cross-reactivity:

Numerous potentially cross-reactive autoimmune and infectious disease sera were tested for anti-RNA POL III antibody levels. Refer to test results for 521 serum samples from patients in the Non-Target Disease Group in the table presented in the section below on Clinical studies. The number of serum samples tested positive is nine (1.7%) for the assay.

f. Assay cut-off:

The assay cut-offs for the ImmuLisa Enhanced RNA Pol III Antibody ELISA was determined by testing specimens from healthy blood donors. The optical density of the 97.5th percentile value of the results was defined as a value of 20 EU/mL and assigned for the cut-off to establish the following result interpretations:

RNA Pol III antibody value	Result interpretation
<20 EU/mL	Negative
20–25 EU/mL	Indeterminate (Borderline)
>25 EU/mL	Positive

The cut-off using the 20 EU/mL was validated in a separate study using 123 samples from healthy blood donors and samples from patients with autoimmune, infectious, and other conditions expected to be found in the differential diagnosis of SSc; 2.4% (3/123) of the samples from were positive for RNA Pol III.

2. Comparison studies:

a. Method comparison with predicate device:

For analysis of agreement with the predicate device, clinically defined serum samples from the target disease, systemic sclerosis (SSc), and non-target disease group

(defined in Section 3.a, below) were tested with both the predicate and the new device. A total of 413 samples were tested by the RNA Pol III assay; 209 SSc samples and 204 non-target disease samples were included. Only specimens in the linear range of both the predicate device and the new device were included in the method comparison. The semi-quantitative results for each assay are summarized below:

ImmuLisa Enhanced RNA Pol III ELISA:

		Predicate ELISA		
		Pos	Neg	Total
ImmuLisa Enhanced RNA Pol III	Pos	41	1	42
	Equiv	9	1	10
	Neg	2	359	361
	Total	52	361	413

Borderline samples considered positive:
Positive Percent Agreement: 96.2% (95% CI 85.7% - 99.3%)
Negative Percent Agreement: 99.4% (95% CI 97.8% - 99.9%)
Overall Agreement: 99.0% (95% CI 97.5% - 99.6%)

Borderline samples considered negative:
Positive Percent Agreement: 78.8% (95% CI 64.9% - 88.4%)
Negative Percent Agreement: 99.7% (95% CI 98.2% - 100.0%)
Overall Agreement: 97.1% (95% CI 95.0% - 98.3%)

The qualitative agreement is the same as the Borderline samples considered positive analysis above.

b. Matrix comparison:

Serum is the only matrix indicated for these assays.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A total of 830 serum samples were included in the clinical validation for the ImmuLisa Enhanced RNA POL III IgG ELISA. The validation set of samples included the target disease group of 281 systemic sclerosis subjects, and a non-target disease group of 549 samples from subjects with autoimmune, infectious, and other conditions expected to be found in the differential diagnosis of systemic sclerosis. The 2013 ACR/EULAR Classification Criteria for Systemic Sclerosis1 were used for the diagnosis of SSc samples. Test results for each individual disease and both disease groups are shown below:

	RNA POL III IgG		
Condition	n	n Pos*	% Pos
Target Disease Group:			
Systemic sclerosis (SSc)	281	65	23.1
Diffuse cutaneous SSc (dcSSc)	105	61	58.1
Limited cutaneous SSc (lcSSc)	176	4	2.3
Non-Target Disease Group:			
Systemic lupus erythematosus	40	0	0
Sjögren's syndrome	41	0	0
Myositis	40	1	2.5
Rheumatoid arthritis	40	1	2.5
Mixed Connective Tissue Disorder	19	1	5.3
Raynaud's	13	0	0
Vitiligo	1	0	0
Autoimmune hepatitis (AIH)	13	0	0
Acute renal failure	29	0	0
Colorectal cancer	10	0	0
Ovarian cancer	10	0	0
Pancreatic cancer	10	0	0
Breast cancer	7	1	14.3
Esophageal reflux	31	1	3.2
Hypertension	27	1	3.7
Pulmonary hypertension	29	0	0
Psoriasis	27	3	11.1
Morphea	2	0	0
CMV	20	0	0
Hepatitis C	20	1	5.0
HSV 1	20	0	0
HSV 2	20	0	0
Lyme's disease	20	0	0
Rubella	20	0	0
Syphilis	20	0	0
Toxoplasmosis	20	0	0
Non-Target Disease Group Total	*549*	*10*	*1.8%*
Total Samples	830		

The performance of the ImmuLisa Enhanced RNA POL III IgG Antibody ELISA was evaluated against the clinical diagnosis of systemic sclerosis. The clinical sensitivity and specificity were calculated by grouping the indeterminate results with test-negative results, and then sensitivity and specificity were calculated again by grouping the equivocal results with test-positive results:

ImmuLisa Enhanced RNA POL III IgG ELISA:

		Systemic Sclerosis Diagnosis		
		Positive	Negative	Total
ImmuLisa Enhanced RNA POL III IgG	Positive	52	8	60
	Equivocal	13	2	15
	Negative	216	539	755
	Total	281	549	830

Equivocal samples considered positive:
Clinical Sensitivity: 23.1% 65/281 (95%CI: 18.6 – 28.4)
Clinical Specificity: 98.2% 539/549 (95%CI: 96.7 – 99.0)

Equivocal samples considered negative:
Clinical Sensitivity 18.5% 52/281 (95%CI: 14.4 – 23.5)
Clinical Specificity 98.5% 541/549 (95%CI: 97.2 – 99.3)

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   A total of 239 normal human sera tested with the ImmuLisa™ Enhanced RNA Pol III Antibody ELISAs yielded six positive (2.5%) results by the qualitative analysis and five indeterminate (2.1%) results and one positive (0.4%) result by the semi-quantitative analysis.

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.