

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

**A. 510(k) Number:**

k070066

**B. Purpose for Submission:**

New device

**C. Measurand:**

Anti-RNA polymerase III antibodies

**D. Type of Test:**

Semi-quantitative Enzyme-linked immunosorbent assay (ELISA)

**E. Applicant:**

INOVA Diagnostics, Inc.

**F. Proprietary and Established Names:**

QUANTA Lite™ RNA Polymerase III ELISA

**G. Regulatory Information:**

1. Regulation section:  
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:  
IM 82

**H. Intended Use:**

1. Intended use:  
QUANTA Lite™ RNA Polymerase III ELISA is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-RNA polymerase III antibodies in patient sera. 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.
2. Indication(s) for use:  
Same as intended use
3. Special conditions for use statement(s):  
For prescription use only
4. Special instrument requirements:  
Microwell plate reader capable of measuring OD at 450nm (and 620nm for dual wavelength readings)

**I. Device Description:**

The QUANTA Lite RNA Polymerase III ELISA consists of a polystyrene microwell ELISA plate coated with purified recombinant immunodominant fragment of RNA Pol III antigen; ELISA negative, low positive and high positive controls; sample diluent; wash concentrate; goat anti-human IgG horseradish peroxidase conjugate; TMB chromogen; and stop solution.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):  
MBL International Corp. RNA Polymerase III ELISA
2. Predicate 510(k) number(s):  
k060431
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	<b>QUANTA Lite RNA Polymerase III ELISA</b>	<b>MBL RNA Polymerase III ELISA</b>
Intended Use	The detection of anti-RNA Polymerase III antibodies	Same
Capture antigen	Purified recombinant fragment of RNA Pol III	Same
Sample matrix	Serum	Same
Units	Arbitrary ELISA Units	Same
Method	ELISA	Same
Solid phase	Polystyrene microwell strips	Same
Substrate	TMB Chromogen	Same
Stop solution	0.344M sulfuric acid	1N sulfuric acid

Differences		
Item	Device	Predicate
	<b>QUANTA Lite RNA Polymerase III ELISA</b>	<b>MBL RNA Polymerase III ELISA</b>
Indications for Use	To aid in the diagnosis of systemic sclerosis (scleroderma) <i>with increased incidence of skin involvement and renal crisis</i>	To aid in the diagnosis of systemic sclerosis
Calculation of results	Compared to a cut-off control	Compared to Calibrator 2
Result interpretation	≤20 U/mL = negative; 20-39 = weak positive; 40-80 = mod. positive; >80 strong positive	<28 U/mL = negative ≥28 U/mL = positive
Controls	Negative, low positive, and high positive	Not provided
Conjugate	Horseradish peroxidase labeled goat anti-human IgG	Horseradish peroxidase labeled goat anti-human IgG, IgA and IgM

Differences		
Item	Device	Predicate
Sample diluent	Tris-buffered saline, Tween 20, protein stabilizers and preservative	PBS buffer with Tween 20
Wash concentrate	Tris-buffered saline and Tween 20 at 40X	PBS buffer with Tween 20 at 10X
Incubation times	30-30-30 minutes	60-60-30 minutes

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

None referenced

**L. Test Principle:**

Purified recombinant immunodominant fragment of RNA Pol III antigen is bound to the wells of a polystyrene microwell plate. Pre-diluted controls 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 goat anti-human IgG antibody is added to each well. A second incubation allows the enzyme labeled anti-human IgG to bind to any patient antibodies which have become attached to the microwells. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity is measured by adding a chromogenic substrate and measuring the intensity of the color that develops. The assay is evaluated by spectrophotometrically measuring and comparing the color intensity that develops in the patient wells with the color in the control wells. Results determined with the assay are interpreted as negative or weak, moderate, or strong positive and are reported in arbitrary units.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay performance for the assay was evaluated by testing 9 specimens a total of 9 times each on 2 lots of kits. The samples tested ranged from 3 to 98 Units with %CVs ranging from 2% to 5%.

	1	2	3	4	5	6	7	8	9
Mean units	22	31	26	90	39	34	58	2	8
SD	0.7	1.3	0.6	1.5	0.6	0.9	1.5	0.1	0.1
CV%	3%	4%	2%	2%	2%	3%	3%	5%	2%

Inter-assay variation was assessed by testing 12 specimens 6 separate times over 4 days, for a total of 5 different runs. This was done for 2 lots of kits. Percent CVs ranged from 2% to 5%.

	1	2	3	4	5	6	7	8	9	10	11	12
Mean Units	29	23	32	28	91	40	32	35	62	3	9	4
SD	0.5	0.5	1.6	1.2	3.5	1.8	0.9	1.7	1.4	0.2	0.4	0.2
CV%	2%	2%	5%	4%	4%	4%	3%	5%	2%	5%	4%	5%

*b. Linearity/assay reportable range:*

No claims were made regarding linearity for the assay. Reactivity is related to the quantity of antibody present in a non-linear fashion. While increases and decreases in patient antibody concentrations will be reflected in a corresponding rise or fall in reactivity, the change is not proportional (i.e. a doubling of the antibody concentration will not double the reactivity).

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

There is no recognized standard or reference material for anti-RNA Pol III antibodies.

Accelerated stability studies were conducted on 2 lots of antigen coated plates and 3 lots of low and high positive kit controls. Data supported a shelf life of one year.

*d. Detection limit:*

To determine the detection limit, a high positive sample was serially diluted until further dilutions did not cause a reduction in the O.D. reading. The lower limit was determined to be 2 Units.

*e. Analytical specificity:*

To demonstrate whether high levels of endogenous substances could cause a false positive result, sera with known quantities of potential interferant were tested. To determine if the presence of these substances could cause a false negative, the substances were added to three sera with known quantities of anti-RNA polymerase III. The results were compared to mixing the positive sera with normal sera.

Substance added:	Norm. sera Units	Pos. sera #1 Units	Recovery %	Pos. sera #2 Units	Recovery %	Pos. sera #3 Units	Recovery %
Mixed with normal sera	—	55		42		66	
Hemoglobin 1000 mg/dL	4	54	98%	39	93%	72	109%
Bilirubin 29.7 mg/dL	4	54	98%	40	94%	65	98%
Cholesterol 354 mg/dL	4	55	100%	39	93%	69	104%
Triglycerides 2173 mg/dL	5	58	105%	37	88%	67	100%
High level RF (OD 2.78)	4	57	104%	42	100%	67	100%

The package insert contains the following recommendations: “Microbially contaminated, heat-treated, samples with visible particulate should not be used. Grossly hemolyzed or lipemic specimens should be avoided.”

The testing of 102 sera with other autoimmune disease antibodies and infectious disease antibodies included the following: 60 samples with other autoimmune antibodies (RNP, Sm, Sm/RNP, Scl-70, SS-A, SS-B, Jo-1, Ribo P, and chromatin); 42 various infectious disease patient sera (HCV, HSV, CMV, toxoplasmosis, rubella, and parvovirus). Two (from the infectious disease group) of the 102 (2%) were positive in the new assay.

*f. Assay cut-off:*

The cut-off was established by testing 629 samples. Five-hundred and twenty-seven random blood donors were tested. Two samples were above the 20 Unit cut-off with values of 27 and 39 Units (99.6% were negative). The mean value of the blood donor samples was 3.4 Units and the standard deviation was 2.6 Units. The cut-off was validated by running disease controls which included other autoimmune disease (n=60, 30 RA and 30 SLE) and 42 sera from patients with antibodies to infectious disease organisms including herpes simplex, CMV, toxoplasmosis, rubella, parvovirus, hepatitis C. Two of the infectious disease sera showed low positive results (both at 22 Units). The specificity for all groups tested was 99.5%.

The cut-off was also validated by ROC analysis comparing the new assay to the laboratory radio-immunoprecipitation method with 1356 samples. At the established cut-off of <20 Units = negative, the specificity was 99.4%.

2. Comparison studies:

*a. Method comparison with predicate device:*

A total of 51 samples were tested in two clinical laboratories on both the INOVA assay and the predicate assay.

		MBL Anti-RNA Polymerase III		
		Positive	Negative	Total
<b>QUANTA Lite RNA Pol III ELISA</b>	Positive	32	0	32
	Negative	2	17	19
	Total	34	17	51

Positive percent agreement: 32/34 = 94.1% (95% CI: 80-99%)  
 Negative percent agreement: 17/17 = 100% (95% CI: 80-100%)  
 Overall agreement: 49/51 = 96.1%

*b. Additional comparison study:*

In this study 1319 patients with systemic sclerosis as well as 37 normal blood donors were tested by a radio-immunoprecipitation (RIP) assay and the new device.

		Radio-immunoprecipitation (RIP)		
		Positive	Negative	Total
<b>QUANTA Lite RNA Pol III ELISA</b>	Positive	320	20	340
	Negative	14	1002	1016
	Total	334	1022	1356

Positive percent agreement:  $320/354 = 95\%$  (95% CI: 93-98%)  
 Negative percent agreement:  $1002/1022 = 98\%$  (95% CI: 80-100%)  
 Overall agreement:  $1350/1356 = 97\%$

- c. *Matrix comparison:*  
 Both assays use serum as the test matrix.
3. Clinical studies:
- a. *Clinical Sensitivity and Specificity:*  
 A total of 1737 patients with systemic sclerosis and 666 blood donors or subjects with infectious or autoimmune diseases were tested on the new assay to calculate the clinical sensitivity and specificity.

		Disease		
		positive	negative	Total
<b>RNA pol III</b>	<b>positive</b>	394	4	398
	<b>negative</b>	1343	662	2005
	Total	1737	666	2403

Clinical sensitivity:  $394/1737 = 22.7\%$       95% CI = 20.7-24.7%  
 Clinical specificity:  $662/666 = 99.4\%$       95% CI = 98.5-99.8%

- b. *Other clinical supportive data:*

#### Prevalence of anti-RNA Pol III antibodies in various diseases

Disease	Literature			INOVA RNA Pol III ELISA		
	# Total	# anti-RNA Pol III Pos	% Pos	# Total	# anti-RNA Pol III Pos	% Pos
All SSc	2323	356	15%	1737	394	23%
SLE	177	3	2%	30	0	0%
MCTD	49	0	0%	ND		
PM/DM	50	0	0%	ND		
RA	84	1	1%	30	0	0%
Primary	32	1	3%	ND		
UCTD	243	0	0%	ND		
NBD	360	4	1%	564	2	0.4%
Nucleolar	200	3	2%	ND		
ID	ND			42	2	5%
All controls	968	12	1%	666	4	1%

Abbreviations: Pos=Positive; SSc=systemic sclerosis; SLE=systemic lupus erythematosus; MCTD=mixed connective tissue disease; PM/DM=polymyositis/dermatomyositis; RA=rheumatoid arthritis; UCTD=unspecified connective tissue disorder; NBD=normal blood donor; Nucleolar=nucleolar immunofluorescent pattern; ID=infectious disease. References 1 and 5 used ELISA, 2 used Western blot, and 6, 7 and 8 used radio-immuno precipitation.

**Anti-RNA Pol III antibodies in Diffuse versus Limited SSc\***

# anti-RNA Pol III Pos	# Diffuse Cutaneous	%	# Limited or overlap	%	# Renal Crisis	%
319	264	83%	55	17%	86	27%

\* based on literature

4. Clinical cut-off:

See assay cut-off and expected values.

5. Expected values/Reference range:

The expected result in the normal population is negative ( $\leq 20$  Units). At the established cut-off of 20 Units, 99.4% of the non-target populations were negative. Refer to section M.3.b. for the incidence of anti-RNA Pol III antibodies in other disease groups and the normal population as found in the literature.

**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.