510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k063565

B. Purpose for Submission:
New device

C. Measurand:
Anti-SS-A 52 autoantibodies

D. Type of Test:
Semi-quantitative Enzyme-linked immunosorbent assay (ELISA)

E. Applicant:
INOVA Diagnostics, Inc.

F. Proprietary and Established Names:
QUANTA Lite™ SS-A 52 ELISA

G. Regulatory Information:
   1. Regulation section:
   CFR 866.5100 Antinuclear Antibody Immunological Test System
   2. Classification:
   Class II
   3. Product code:
   OBE Anti-SS-A 52 autoantibodies
   4. Panel:
   IM 82

H. Intended Use:
   1. Intended use:
   QUANTA Lite™ SS-A 52 ELISA is a semi-quantitative enzyme-linked immunosorbent assay (ELISA) for the detection of IgG anti-SS-A 52 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 lupus erythematosus, Sjögren's syndrome, systemic sclerosis, polymyositis and dermatomyositis.
   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 SS-A 52 ELISA consists of a polystyrene microwell ELISA plate coated with purified recombinant SS-A 52 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):
   INOVA QUANTA Plex Profile 6 (anti-SS-A antibodies parameter)
2. Predicate 510(k) number(s):
   k031450
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	SS-A 52 Antibody ELISA	**Quanta Plex Profile 6 (SS-A bead)**
Indications for Use	To aid in the diagnosis of systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis	To aid in the diagnosis of systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis
Analyte measured	Anti-SS-A 52	Anti-SS-A 52 <u>and</u> 60
Calculation of results	Compared to the low positive control	Same
Test matrix	Serum	Same
Controls	Negative, low positive, and high positive	Same
Sample diluent	Tris-buffered saline, Tween 20, protein stabilizers and preservative	Same

Differences		
Item	Device	Predicate
	SS-A 52 Antibody ELISA	**Quanta Plex Profile 6 (SS-A antibody)**
Indications for Use	Addition of *polymyositis and dermatomyositis*	Not included
Capture antigen(s)	Purified recombinant SS-A 52 antigen	Native bovine SS-A 60 and recombinant human SS-A 52 antigens
Units	Arbitrary ELISA Units	Luminex Units
Result interpretation	≤20 U/mL = negative; 20-39 = weak positive; 40-80 = mod. positive; >80 strong positive	≤20 LU/mL = negative; 20-49 = weak positive; 50-100 = mod. positive; >100 strong positive
Method	ELISA	Fluorescent immunoassay
Sample dilution	1:101	1:101, 5 μL sample is then diluted 1:10 for a final dilution of 1:1010

Differences		
Item	Device	Predicate
Solid phase	Polystyrene microwell plate coated with purified recombinant SS-A 52 antigen	Polystyrene microwell plate containing 7 different beads, one of which is a SS-A bead coated with a mixture of native bovine SS-A 60 and a recombinant human SS-A 52 antigens
Wash concentrate/buffer	Tris-buffered saline with Tween 20: 40X	No wash buffer
IgG conjugate	Horseradish peroxidase conjugated goat anti-human IgG	Fluorescently labeled goat anti-human IgG
Substrate	TMB Chromogen	None
Stop solution	0.344M sulfuric acid	None

K. Standard/Guidance Document Referenced (if applicable):
None referenced

L. Test Principle:
Purified recombinant SS-A 52 antigen is bound to the wells of a polystyrene microwell plate under conditions that will preserve it in an antigenic state. Pre-diluted controls and diluted patient sera are added to separate wells, allowing any anti-SS-A 52 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 7 times each on 3 lots of kits. The samples tested ranged from 7 to 107 Units with %CVs ranging from 1% to 4%.

	1	2	3	4	5	6	7	8	9
Mean units	20	22	38	107	53	34	51	7	8
SD	0.75	0.80	1.04	1.48	1.12	0.80	0.54	0.31	0.26
%CV	4%	4%	3%	1%	2%	2%	1%	4%	3%

Inter-assay variation was assessed by testing, in duplicate, a panel of 13 specimens twice in one day and once per day for 3 days, for a total of 5 different runs. The samples tested ranged from 2 to 134 U/mL. Percent CVs ranged from 2 to 47%. In the positive range including samples at the cut-off, percent CVs ranged from 2 to 5%.

	1	2	3	4	5	6	7	8	9	10	11	12	13
Mean units	2	9	9	6	9	22	24	24	45	134	65	42	59
SD	0	4	3	1	1	0	0	1	1	4	1	2	1
%CV	17%	47%	33%	21%	7%	2%	2%	5%	2%	3%	2%	4%	2%

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-SS-A 52 autoantibodies.

Accelerated stability studies were conducted on 3 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, 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 two sera with know quantities of anti-SS-A 52. The results were compared to mixing the positive sera with normal sera.

	Normal sera	Positive sera #1	Recovery	Positive sera #2	Recovery
Substance added:		Units	%	Units	%
Mixed with normal sera	___	44		99	
Hemoglobin 1000 mg/dL	8 Units	40	91%	96	97%
Bilirubin 29.7 mg/dL	7 Units	42	95%	94	95%

	Normal sera	Positive sera #1	Recovery	Positive sera #2	Recovery
Cholesterol 354 mg/dL	5 Units	40	91%	98	99%
Triglycerides 2173 mg/dL	8 Units	40	91%	91	92%

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 136 sera with other autoimmune disease antibodies and infectious disease antibodies included the following: 6 samples with other autoimmune antibodies (RNP, Sm, Scl-70, SS-B, and Sm/RNP); 20 rheumatoid arthritis patients, 110 various infectious disease patient sera (HCV, HSV, CMV, toxo, rubella, and parvo). Nine (from the infectious disease group) of 136 (7%) were positive in the new assay.

f. *Assay cut-off:*
One hundred and nine normal blood donors and 20 patients with rheumatoid arthritis were tested for anti-SS-A 52 antibodies by the assay. Only one sample was positive at a value of 26 units meaning 99.2% were negative. The cut-off was also validated by ROC analysis. At the established cut-off of <20 Units = negative, the specificity was 95.8%.

2. Comparison studies:
   a. *Method comparison with predicate device:*
   One hundred and nine normal blood donors, 20 patients with rheumatoid arthritis (RA), 110 with high titers against infectious disease organisms, 31 with Sjögren's syndrome (SS), 149 with systemic lupus erythematosus (SLE), and 176 with systemic sclerosis (SSc) were tested internally on both the new assay and the predicate.

QUANTA Lite SS-A 52 ELISA	QUANTA Plex SS-A Bead		
	Positive	Negative	Total
Positive	78	36	114
Negative	13	468	481
Total	91	504	595

Positive percent agreement: 78/91 = 85.7%   (95% CI: 77%-92%)
Negative percent agreement: 468/504 = 92.9%   (95% CI: 90%-95%)
Overall agreement: 546/595 = 91.8%

b. *Matrix comparison:*
   Both assays use serum as the test matrix.

3. Clinical studies:
   a. *Clinical Sensitivity and Specificity:*
   Sera from a total of 763 subjects were tested. These samples included 114 normal blood donors, 20 patients with RA, 110 with high titers against infectious disease organisms, 71 with SS, 149 with SLE, 176 with SSc and 123 with polymyositis (PM) or dermatomyositis (DM).

Groups (N=763)	N	SS-A 52 +	% +	SS-A 52 -	% -
Normal	114			113/114	99.2
RA	20			20/20	100
Infectious diseases	110			101/110	91.8
Total disease neg.	244			234/244	95.9
SS	71	60/71	84.5		
SLE	149	46/149	30.9		
SSc	176	34/176	19.3		
PM (93)/DM (30)	123	90/123	73.2		
Total disease pos.	519	230/519	44.3		

Clinical sensitivity and specificity calculations:

	Disease positive	Disease negative	Total
SS-A 52 positive	230	10	240
SS-A 52 negative	289	234	523
Total	519	244	763

Clinical sensitivity: 230/519 = 44.3%   95% CI = 40%-49%
Clinical specificity: 234/244 = 95.9%   95% CI = 93%-98%

   b. *Other clinical supportive data:*
   In addition, 52 sera known to be positive for other myositis markers were tested. Seventy-five percent (39/52) were positive for SS-A 52 autoantibodies.

   Clinical study data versus literature data (based on labeling references # 2, 4, 7, 8, 9, and 10):

Disease	Literature			INOVA QUANTA Lite™ SS-A 52		
	N	SS-A 52+	Sensitivity	N	SS-A 52+	Sensitivity
SLE	123	41	33%	149	46	31%
SS	100	63	63%	71	60	85%
PM/DM***	262	98	37%	123	90	73%***
Jo-1	64	42	66%	32	25	78%
SSc*	263	31	12%	176	34	19%
Controls**	102	1	1%	129	1	1%

* includes 11 samples negative by Ouchterlony immunodiffusion.

** includes healthy volunteers and rheumatoid arthritis patients
***This population includes a high percentage of patients who are positive for myositis specific antibodies.

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 (≤ 20 Units).

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 acceptable and supports a substantial equivalence decision.