510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k040160

B. Analyte:
Anti-ENA antibodies (SSA, SSB, Sm, RNP, Scl-70, Jo-1, ribosomal P and chromatin)

C. Type of Test:
Flow Cytometry-based, homogeneous, multiplexed, microparticle fluorescent immunoassay, semi-quantitative

D. Applicant:
INOVA Diagnostics, Inc.

E. Proprietary and Established Names:
QUANTA Plex™ SLE Profile 8

F. Regulatory Information:
1. <u>Regulation section:</u>
   21 CFR 866.5100, Antinuclear Antibody Immunological Test System
2. <u>Classification:</u>
   Class II
3. <u>Product Code:</u>
   LLL, Extractable antinuclear antibody, antigen and control
   MQA, Anti-ribosomal P antibodies
4. <u>Panel:</u>
   IM (82)

G. Intended Use:
The QUANTA Plex™ SLE Profile 8 is a fluorescent immunosorbant assay for the semi-quantitative detection of Sm, RNP, SS-A, SS-B, Scl-70, Jo-1, ribosome-P and chromatin autoantibodies in human serum. The presence of these antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of systemic lupus erythematosus and related connective tissue diseases such as Sjögren's syndrome, scleroderma and polymyositis.

1. <u>Indication(s) for use:</u>
   As an aid in the diagnosis of systemic lupus erythematosus and related connective tissue diseases such as Sjögren's syndrome, scleroderma and polymyositis.
2. <u>Special condition for use statement(s):</u>
   For prescription use only.
3. <u>Special instrument Requirements:</u>
   Luminex 100™ System

H. Device Description:
The device consists of the following: a foil package containing 12 (1x8) microwell strips with holder (each microwell contains 9 different color beads and each color bead is coated with a different purified antigen (Sm, RNP, SSA, SS-B, Scl-70, Jo-1, ribosome-P, chromatin and an IgG Control), a negative control, a low positive

control, a high positive control, HRP sample diluent, PBS concentrate, PE-labeled goat anti-human IgG conjugate (Fc-specific)and conjugate diluent.

I. **Substantial Equivalence Information:**
1. Predicate device name(s):
   INOVA QUANTA Plex™ ENA Profile 6, INOVA QUANTA Lite™ Ribosome-P ELISA and INOVA QUANTA Lite™ Chromatin ELISA.
2. Predicate K number(s):
   K031450, K981237 and K982603
3. Comparison with predicate:
   INOVA QUANTA Plex™ ENA Profile 6 and INOVA QUANTA Plex™ SLE Profile 8 have the same technological characteristics except the analytes ribosome-P and chromatin are not included in the INOVA QUANTA Plex™ ENA Profile 6.

Quanta Plex™ ENA Profile 8	QUANTA Lite™ ELISA assays
A. Similarities	
Intended Use. - Semi-quantitative detection of Sm, RNP, SS-A, SS-B, Scl-70, Jo-1, ribosome-P and chromatin autoantibodies in human serum.	Same
Indications for Use - As an aid in the diagnosis of systemic lupus erythematosus and related connective tissue diseases such as Sjögren's syndrome, scleroderma and polymyositis.	Same
Sample Type – Serum	Same
Conjugate – Polyclonal goat anti-human IgG	Same
B. Differences	
Assay Method – Flow cytometry based	ELISA
Assay Format - Multiplexed	Individual analytes
Solid Phase – Differentially colored, carboxylated microspheres	Microtiter well
Conjugate label - Phycoerythrin	HR peroxidase
Detection Method - Fluorescence	Colorimetric
Instruments – Luminex 100™	ELISA reader
Dynamic Range – 20 to >20,000 FU	Not furnished

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

K. **Test Principle:**
   The Quanta Plex™ SLE Profile 8 assay is a multiplexed, semi-quantitative, fluorescent immunoassay performed on the Luminex 100 system. Each of the antigens (Sm, RNP, SS-B, SS-A 52/SS-A 60, Scl-70, Jo-1, ribosome-P and chromatin) and the assay control (goat anti-human IgG) are coupled to sets of different colored polystyrene microspheres. Diluted patient serum is added to each well and if specific antibodies are present, they will bind to the immobilized antigens on the beads. Then β-phycoerytherin (PE)-conjugated goat anti-human IgG (Fc specific) is added to each well and incubated. The conjugate will react with the autoantibodies immobilized on the beads. No washing is required with this

procedure. The bead suspension is analyzed by the Luminex 100™ System. The bead sets are identified by their unique spectral addresses and the amount of PE conjugate is determined for each bead set. The fluorescence intensity is relative to the amount of antibody present in the patient serum. The median fluorescence of the PE conjugate is converted to Luminator Units (LU) by comparing to the fluorescence of the test sample to the fluorescence of the Low Positive for that specific analyte. The test principle and performance of the assay on the Luminex 100™ System were supported in k011140, k030681 and k031450.

L. Performance Characteristics (if/when applicable):

　　1.　Analytical performance:

　　　　a. *Precision/Reproducibility:*

To evaluate both intra-assay and inter-assay reproducibility, 12 patient sera were analyzed on the INOVA QUANTA Plex™ SLE Profile 8. These samples were selected to include low, moderate and high positives for each analyte. For intra-assay reproducibility, 8 samples were run 8 times and 4 samples 7 times on one plate in a single run. As expected the low positive samples had higher %CVs than the moderate and high positive samples. Results of the low, moderate and high positive samples are summarized below.

	>100 LU	50 LU – 100 LU	11 LU-49 LU
Mean CV%	4.8	5.6	8.1
Range (%)	1-9	2-11	4-15

For inter-assay reproducibility, 12 samples were run on 6 assays for 3 separate days. Similar to the intra-assay precision results, the %CVs for the low positive samples were higher than the moderate and high positive samples. The range and mean %CV for high, moderate and low positive samples are summarized below.

	>100 LU	50 LU – 100 LU	11 LU-49 LU
Mean CV%	7.7	9.7	9.4
Range (%)	2-13	5-15	4-17

The intra- and inter-assay reproducibility results performed by manual pipetting were compared to automated pipetting. The results for each analyte are illustrated below.

		SSA	SSB	Sm	RNP	Scl-70	Jo-1	Ribo-P	Chrom
Manual	Mean CV%	4.8	3.7	5.7	6.2	7.7	4.3	6.7	6.5
	Range (%)	1-10	3-4	4-7	5-9	5-12	4-5	5-8	3-11
Automated	Mean CV%	5.8	5.3	7.5	7.6	8.3	5.7	9.2	7.2
	Range (%)	2-13	3-7	3-12	4-13	4-15	3-9	7-12	6-11

		SSA	SSB	Sm	RNP	Scl-70	Jo-1	Ribo-P	Chrom
Manual	Mean CV%	6.8	6.6	8	8.4	12.7	8.3	9.2	115
	Range (%)	2-10	6-8	7-10	5-10	10-15	5-13	6-11	10-11
Automated	Mean CV%	6.6	10.7	8.2	8.6	8.3	10.7	9.7	11.2
	Range (%)	4-9	9-14	5-13	5-13	6-11	5-17	7-13	9-13

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b. *Linearity/assay reportable range:*
   Dynamic range of the assay is 20 to >20000 Fluorescent Units (FU). The amount of fluorescence is related to the quantity of autoantibody present on the bead but in a non-linear fashion. The antibody concentrations correspond to fluorescent signal changes but are not directly proportional.

c. *Traceability (controls, calibrators, or method):*
   No reference standards or method available. For each assay kit, negative, low positive and high positive controls are included.

d. *Detection limit (functional sensitivity):*
   Not relevant for this assay.

e. *Analytical specificity:*
   To assess potential cross-reactivity to other autoantibodies, 51 serum samples consisted of 30 RA and 21 infectious disease samples (herpes simplex virus (4), toxoplasmosis (4), hepatitis C (4), parvovirus (4) and cytomegalovirus (5)) were analyzed. Cross-reactivity was observed with one HCV (20 LU) for Sm; 1 RA (24 LU) for RNP and 3 RA (31, 598 and 108 LU) for chromatin.

   Interference from serum components was evaluated by testing five serum samples negative for the eight autoantibodies. The serum samples consisted of 1 hemolyzed (hemoglobin 1000 mg/mL), 2 lipemic (cholesterol 329 mg/mL and triglycerides 1016 mg/mL), 1 icteric (bilirubin 29.7 mg/mL) and 1 high IgG (80 µg/mL). No false positive results were obtained. These samples were also mixed in equal volumes with 4 positive sera (one positive for SSA, SSB, Sm, RNPand ribosome-P, one for Scl-70, one for Jo-1 and SSA and the fourth one for Sm, RNP, ribosome-P and chromatin) to determine whether these substances would significantly decrease the antibody/antigen reactions on the microspheres and cause false negative results. Little or no interference was observed when compared to the same sera mixed with a normal sample.

f. *Assay cut-off:*
   The cut-off value was determined using 150 normal samples from a serum broker. Similar to the predicate devices, the cut-off was arbitrary set at 20 LU. The fluorescence unit that was assigned 20 units was based on non-parametric statistical analysis. The following table shows the cut-off values for each analyte.

Analyte	QUANTA Plex SLE Profile 8		
	Mean (LU)	SD (LU)	Cut-off (LU)
SM	4	5.9	4 ± 2.8SD
RNP	2	3.5	2 ± 5SD
SSA	3	3	3 ± 5.7SD
SSB	3	3.5	3 ± 5SD
Scl-70	5	4.5	5 ± 3.4SD
Jo-1	2	0.8	2 ± 24.6SD
Ribosome-P	1	1.0	1 ± 18.1SD
Chromatin	5	6.2	5 ± 2.4SD

2. Comparison studies:
   a. *Method comparison with predicate device:*
      Two hundred and seventy-four clinically defined patient samples (159 SLE, 44 scleroderma, 20 Jo-1, 30 RA and 21 infectious diseases) were tested with the QUANTA Plex™ SLE Profile 8 and the QUANTA Plex™ ENA Profile 6 for analytes found in both assays. Age and sex information were available for the SLE (61/159) and scleroderma patients. For the 61 SLE patients, there were 52 females and 7 males with a mean age of 35 years (13y-79y) and for the scleroderma group; there were 36 females and 8 males with a mean age of 50 years (31y-85y). Discrepant results were re-tested by both devices and the retest result was used as the final result. Discrepant samples were also tested with the INOVA QUANTA Lite™ ELISA assays. Majority of the discrepant results were borderline low positives. Results of the comparison study between QUANTA Plex™ SLE Profile 8 and QUANTA Plex™ ENA Profile 6 are summarized below.

	# samples	Both Neg	Both Pos.	Q6 Pos Q8 Neg	Q6 Neg Q8 Pos	%Positive Agreement	%Negative Agreement	%Total Agreement
Sm	274	212	51	4	7	92.7	96.8	96.0
RNP	274	198	69	3	4	95.8	98.0	97.4
SS-A	274	194	67	4	9	94.4	95.6	95.3
SS-B	274	248	19	3	4	86.4	98.4	97.4
Scl-70	274	226	37	5	6	88.1	97.4	96.0
Jo-1	274	251	18	0	5	100	98.0	98.2

For ribosome-P and chromatin, comparisons were made between QUANTA Plex™ SLE Profile 8 and INOVA QUANTA Lite™ ELISA assays since QUANTA Plex™ ENA Profile 6 does not have these two analytes. Discrepant results were retested by IFA on HEp-2 cells. For ribosome-P, all 9 discrepant samples were negative by IFA. For chromatin, 11 of the 29 samples were positive, 10 were negative and 3 were ambiguous.

	# samples	Both Neg	Both Pos.	E Pos Q8 Neg	E Neg Q8 Pos	%Positive Agreement	%Negative Agreement	%Total Agreement
Ribosome-P	274	244	21	7	2	72.4	99.2	96.7
Chromatin	274	174	71	29	0	88.7	100	89.4

In addition to the above in-house study, 40 patient samples (36 ribosome-P positive and 4 negative) from Germany were tested by an external site on the QUANTA Plex™ SLE Profile 8. These samples have been previously analyzed in Germany, on 4 commercial ribosome-P ELISAs and 4 experimental ELISAs. The positive agreement between QUANTA Plex™ SLE Profile 8 to QUANTA Lite™ Ribosome-P ELISA was 94.4% (34/36), the negative agreement was 100% (4/4) and total agreement was 95% (38/40).

The following table compiles results of the 424 serum samples (150 normal, 159 SLE, 44 scleroderma, 20 Jo-1, 30 RA and 21 infectious diseases) analyzed with the Quanta Plex™ SLE Profile 8, the Quanta Plex™ ENA Profile 6 and the predicate ELISA devices for ribosome-P and chromatin. For ribosome-P, 40 additional sera were tested.

Analyte (# samples)	Both Neg	Both Pos	Q6 Pos Q8 Neg	Q6 Neg Q8 Pos	%Positive Agreement	%Negative Agreement	%Total Agreement
Sm (424)	359	55	7	3	95	98	97.6
RNP (424)	343	71	5	5	93	99	97.6
SS-A (424)	341	68	9	6	92	97	96.5
SS-B (424)	397	19	5	3	86	99	98.1
Scl-70 (424)	373	39	6	6	87	98	96.9
Jo-1 (424)	401	18	5	0	100	99	98.6
Analyte (# samples)	Both Neg	Both Pos	E Pos Q8 Neg	E Neg Q8 Pos	%Positive Agreement	%Negative Agreement	%Total Agreement
Ribosome-P (464)	398	55	2	9	86	99	97.6
Chromatin (424)	319	73	2	30	71	99	92.4

      b. *Matrix comparison:*
         Both devices use serum.
3. Clinical studies:
      a. *Clinical sensitivity:*
         Since anti-ribosome-P and anti-chromatin autoantibodies are primarily found in SLE, clinical sensitivity was determined using SLE patient data only. For this group of SLE patients, the clinical sensitivity was 13.8% (22/159) for ribosome-P and 42.8% (68/159) for chromatin. These results were comparable to those in published literature. Clinical sensitivities for the other analytes were studied in previous QUANTA Plex™ Profile assays and were not addressed in this submission.
      b. *Clinical specificity:*
         Clinical specificity was determined using all non-SLE patient data. The clinical specificity was 99.6% (1/265) for ribosome-P and 97.4% (7/265) for chromatin. These results were comparable to those in published literature. Clinical specificities for the other analytes were studied in previous QUANTA Plex™ Profile assays and were not addressed in this submission
      c. *Other clinical information*
         Not applicable
4. Clinical cut-off:
    See assay cut-off.
5. Expected values/Reference range:
    The normal range for each analyte was determined by testing 150 normal samples from a serum broker. The samples were from 89 males and 61 females with an average age of 33 years, ranging from 17 years to 74 years. The normal range results are shown in table below.

Analyte	QUANTA Plex SLE Profile 8		
	% Negative	Range LU	Mean ± SD (LU)
SM	96.5	0-41	4.0 ± 5.9
RNP	98.7	0-21	2.0 ± 3.5
SSA	99.3	0-27	3.0 ± 3.0
SSB	99.3	0-24	3.0 ± 3.5
Scl-70	98.7	0-24	5.0 ± 4.5
Jo-1	100	0-4	2.0 ± 0.8
Ribosome-P	100	0-8	1.0 ± 1.0
Chromatin	97.3	0-51	5.0 ± 6.2

M. Conclusion:

Based on the review of the information provided in this 510 (k), QUANTA Plex™ SLE Profile 8 kit is Substantially Equivalent to the QUANTA Plex™ ENA Profile 6 kit for Sm, RNP, SS-A, SS-B, Scl-70 and Jo-1 and to the QUANTA Lite™, ELISA assays for ribosome-P and chromatin, product code LLL, Extractable Antinuclear Antibody, Antigen and Control and MQA, Anti-ribosomal P antibodies, Immunology Device Panel 82, Class II.