510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k122923

B. Purpose for Submission:

New device

C. Measurand:

Antinuclear IgG antibodies: Sm, RNP, Ro60 (SS-A), Ro52/TRIM21, SS-B (La), Scl-70 (topoisomerase I) and Jo-1

D. Type of Test:

Qualitative chemiluminescent immunoassay

E. Applicant:

INOVA Diagnostics Inc.

F. Proprietary and Established Names:

QUANTA Flash ENA7 Reagent Kit

QUANTA Flash ENA7 Calibrator Kit

QUANTA Flash ENA7 Control Kit

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5100 Antinuclear antibody immunological test system

   21 CFR §862.1150, Calibrator

   21 CFR §862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:

Class 2 (assay and calibrators)

Class 1 (Control)

3. Product code:

   LLL, Extractable Antinuclear Antibody, Antigen, Control

   JIX, Calibrator, Multi-Analyte Mixture

   JJX, Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

   Immunology (82)

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   QUANTA Flash® ENA7 Reagent Kit: QUANTA Flash ENA7 is a chemiluminescent immunoassay for the qualitative screening of IgG autoantibodies to Sm, RNP, Ro60 (SS-A), Ro52/TRIM21, SS-B (La), Scl-70 (topoisomerase I) and Jo-1 in human serum. The presence of these autoantibodies is used as an aid in the diagnosis of systemic lupus erythematosus (SLE), systemic sclerosis (SSc), polymyositis (PM), dermatomyositis (DM), Sjögren's syndrome (SjS) and mixed connective tissue disease (MCTD) in conjunction with clinical findings and other laboratory tests.

   QUANTA Flash® ENA7 Calibrator Kit: QUANTA Flash ENA7 Calibrators are intended for use with the QUANTA Flash ENA7 Reagents for the determination of IgG autoantibodies to Sm, RNP, Ro60 (SS-A), Ro52/TRIM21, SS-B (La), Scl-70 (topoisomerase I) and Jo-1 in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate the unit values.

   QUANTA Flash® ENA7 Control Kit: QUANTA Flash ENA7 Controls are intended for use with the QUANTA Flash ENA7 Reagents for quality control in the determination of IgG autoantibodies to Sm, RNP, Ro60 (SS-A), Ro52/TRIM21, SS-B (La), Scl-70 (topoisomerase I) and Jo-1 in human serum.

2. Indication(s) for use:

   See Intended Use.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   BIO-FLASH™ Instrument System (k083518)

I. Device Description:

The QUANTA Flash™ ENA7 reagent cartridge contains the following reagents for 50 determinations. Each cartridge has a barcode that contains the assay name, the assay ID number, the lot number and expiration date, the four parameters of the lot specific master curve, and the reagent cartridge specific serial number. Also included is a vial of resuspension buffer containing protein stabilizers and preservative.

- Recombinant Ro60, Scl-70, Jo-1, Ro52, SS-B (La), and native Sm and RNP coated paramagnetic beads preserved prior to first use.
- Assay buffer – containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
- Tracer IgG – isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

The QUANTA Flash™ ENA7 Calibrator set includes two calibrators (Calibrator 1 and Calibrator 2). These are barcoded tubes containing 0.3 mL pre-diluted, ready-to-use reagent. Calibrators contain human antibodies to ENA in buffer. They are sold separately.

The QUANTA Flash™ ENA7 Controls contain two vials (a Negative and a Positive) containing human antibodies to ENA in buffer, protein stabilizers and preservatives. They are sold separately.

The following additional reagents are required for the test and supplied separately; BIO-FLASH system rinse, BIO-FLASH Triggers, and BIO-FLASH Cuvettes.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   QUANTA Lite ENA6 ELISA

2. Predicate 510(k) number(s):

   k961913

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	QUANTA Flash ENA7 is a chemiluminescent immunoassay for the qualitative screening of IgG autoantibodies to Sm, RNP, Ro60 (SS-A), Ro52/TRIM21, SS-B (La), Scl-70 (topoisomerase I) and Jo-1 in human serum. The presence of these autoantibodies is used as an aid in the diagnosis of systemic lupus erythematosus (SLE), systemic sclerosis (SSc), polymyositis (PM), dermatomyositis (DM), Sjögren's syndrome (SjS) and mixed connective tissue disease (MCTD) in conjunction with clinical findings and other laboratory tests.	QUANTA Lite ENA 6 is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Sm, RNP, SS-A (60kDa and 52kDa), SS-B, Scl-70 and Jo-1 antibodies 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 (SLE), and related connective tissue diseases, such as Sjögren's Syndrome.
Sample matrix	Serum	Same
Analyte detected	Human IgG, antibodies to extractable nuclear antigens	Same
Traceability	In-house standards	Same
Controls	Negative and Positive	Same

Differences		
Item	**Device**	**Predicate**
Assay methodology	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay (colorimetric)
Assay Type	Qualitative	Semi-quantitative
Instrument platform	BIO-FLASH® chemiluminescent analyzer	Manual
Antigens	recombinant Ro60, Scl-70, Jo-1, Ro52, SS-B (La), and native Sm and RNP purified from calf thymus	Native Sm, RNP, SS-A, SS-B, Scl-70 and Jo-1
Solid phase	Paramagnetic micro particles (beads)	96-well plate
Conjugate and detection antibody	Isoluminol conjugated anti-human IgG (monoclonal)	HRP conjugated anti-human IgG

Differences		
Item	**Device**	**Predicate**
		(polyclonal)
Calibration and unit calculation	Instrument specific working curve based off a 6-point lot specific master curve used for unit calculations; stored on the instrument for the life of the reagent lot.	Single point determination for unit calculations, run each time the assay is run.
Cutoff	20 CU (chemiluminescent units)	20 Units

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition

L. Test Principle:

The principles of the QUANTA Flash ENA7 assay are similar to other solid phase indirect immunosorbent assays. The assay is a chemiluminescent two-step immunoassay consisting of magnetic particles coated with the ENA antigens, which capture, if present, IgG antibodies from the patient sample.

The solid phase is paramagnetic beads and the detecting reagent is a mixture of isoluminol-conjugated monoclonal antibodies to human IgG. A patient's serum is diluted with sample dilution buffer in a disposable cuvette. A small amount of this patient dilution is combined with assay buffer and ENA7 beads in a second cuvette, and mixed. This reaction cuvette is incubated then exposed to a small magnet that holds the beads in place. The liquid is aspirated, and the beads are resuspended as system rinse is added to the cuvette and the magnet is removed. This wash cycle is repeated two more times. During the third wash, no system rinse is added after the aspiration step, rather the isoluminol conjugate (known as Tracer IgG) is added to the beads in the cuvette, and mixed. Again, the cuvette is incubated, and three wash steps, as described in the first wash step above, are performed on the beads. In the fourth wash step, no liquid is added to the beads after the aspiration.

The cuvette is then placed in a light-tight luminometer and the beads are exposed to a catalyst and an oxidizing agent. These two reagents, or "Triggers", cause the isoluminol to produce a flash of visible light. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. The RLUs are proportional to the amount of bound isoluminol conjugate, which in turn is proportional to the amount of IgG antibodies bound to the antigens on the ENA7 beads.

The calibration process utilizes the 2 calibrators included in the Calibrators set to adjust the predefined master curve into an instrument specific working curve. This working curve is used to calculate chemiluminescent unit (CU) values from the measured relative light units (RLU). The working curve is lot-specific, and is stored in the system for use with any reagent pack from that lot.

The QUANTA Flash ENA7 assay utilizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve from six calibrators. The Master Curve is predefined, lot dependent and it is uploaded to the instrument through the reagent cartridge barcode. With the measurement of calibrators, the predefined Master Curve is transformed to a new, instrument specific Working Curve. The concentration values of the calibrators are included in the calibrator tube barcodes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated according to CLSI EP05-A2. Eight patient serum samples were tested in duplicate, twice a day for 20 days (n = 80). Each patient sample had autoantibodies for two or more individual analytes.

Patient Sample	Mean (CU)	Within Run		Between Run		Between Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	7.9	0.3	3.2	0	0	0.3	3.8	0.4	4.9
2	19.1	0.4	2.2	0.5	2.6	1.2	6.3	1.4	7.1
3	19.8	0.4	1.8	0.3	1.5	0.7	3.3	0.8	4.1
4	30.1	1.2	4.1	0.7	2.2	1.4	4.8	2.0	6.7
5	42.9	1.1	2.6	0	0	1.3	3.1	1.7	4.0
6	100.8	4.4	4.3	0	0	3.8	3.7	5.8	5.7
7	207.0	5.1	2.5	3.9	1.9	5.3	2.6	8.4	4.0
8	386.8	11.9	3.1	7.4	1.9	10.8	2.8	17.7	4.6

The qualitative reproducibility for each sample was assessed:

Sample	Mean (CU)	Expected Result	% Expected Result	Range of Samples (CU)
1	7.9	Negative	100	7.0 – 8.8
2	19.1	Negative	62.5	16.1 – 21.4
3	19.8	Negative	60	17.9 – 21.6
4	30.1	Positive	100	20.6 – 33.1
5	42.9	Positive	100	38.9 – 47.7
6	100.8	Positive	100	91.2 – 115.2
7	207.0	Positive	100	184.8 – 226.1

Sample	Mean (CU)	Expected Result	% Expected Result	Range of Samples (CU)
8	386.8	Positive	100	352.9 – 429.4

To assess the lot-to-lot reproducibility of the assay, 19 patient samples spanning the assay range were tested in duplicate on two different lots. The resulting weighted linear regression analysis yielded the following: y = 0.97x – 0.32 CU. The constant bias is -0.32 CU (95% CI -1.96 – 1.33) and the proportional bias is 0.97 (95% CI 0.90 – 1.04). The weighted $r^2 = 0.991$, and the predicted bias at the cut-off of 20 CU is -0.91 CU (95% CI -2.21 – 0.39).

To further assess the performance of the assay around the cut-off, a set of well-characterized samples known to be reactive to only one of the analytes in the assay were diluted with normal serum to about ± 25% of the cut-off, then tested twice a day for 10 days (n = 40):

Sample	Sample Specificity	Mean (CU)	Expected Result	% Expected Result	Range of Samples (CU)
1	anti-Sm Abs	20.8	Positive	67.5	18.4 – 24.9
2	anti-RNP Abs	25.3	Positive	100	22.8 – 28.6
3	anti-Ro60 Abs	24.9	Positive	100	23.3 – 27.4
4	anti-Ro52 Abs	27.8	Positive	100	25.7 – 30.3
5	anti-SS-B Abs	18.1	Negative	97.5	17.2 – 20.2
6	anti-Scl-70 Abs	18.7	Negative	92.5	17.5 – 20.5
7	anti-Jo-1 Abs	26.1	Positive	100	23.6 – 30.3

b. *Linearity/assay reportable range:*

Not applicable.

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

Traceability: There are no recognized international standards for the measurement of the IgG autoantibodies detected by this kit. Calibrators and controls are traceable to in-house standards that are used to create the master curve for the QUANTA Flash ENA7 assay.

Value assignment:
Calibrators and controls are manufactured by diluting human serum that contains high titer of IgG autoantibodies to Sm, RNP, Ro60 (SS-A), Ro52/TRIM21, SS-B (La), Scl-70 (topoisomerase 1), and Jo-1with a buffer, stabilizers, and preservative. The human serum is obtained from commercial sources and it is tested for markers of infectious substances. The target CU value is achieved through trial dilutions on a small scale. Once a dilution is selected, the calibrators and controls are bulked, tested, and adjusted. Upon completion of the manufacturing process, the calibrators and controls are tested on at least two instruments, on at least two lots, of reagent cartridge in replicates of 10 to determine final value assignment.

The QUANTA Flash® ENA7Calibrators are designed to adjust the predefined Master Curve into an instrument specific working curve.

Calibrators and controls are required to use the assay but are sold separately from the assay kit. The table below summarizes the controls and calibrators target values and target ranges:

	ENA7
Calibrators	
Calibrator 1	13 ± 3 CU
Calibrator 2	110 ± 30 CU
Controls	
Control 1	10 CU
Control 2	50 CU

Stability: Accelerated stability studies (37°C for 14 days) suggest that the controls, calibrators, and unopened kit and beads are stable for one year at 2 – 8°C. Real time studies are underway; to date they support a three month real-time stability claim for the kit and components. The BIO-FLASH software monitors the expiration dates of the onboard cartridges, as well as the reagent cartridge lots.

Opened kit components are stable as follows:

Reagent Cartridge and beads	36 days
Calibrators	8 hours total use time onboard; up to 4 uses
Controls	10 min per use, up to 2.5 hours total onboard or up to 15 uses (whichever is fulfilled first)

d. Detection limit:

Not applicable.

e. Analytical specificity:

Endogenous interferents: Three specimens, a positive (92.9 CU), a sample close to the cut-off (23.0 CU), and a negative (10.7 CU) were tested. Interfering substances were spiked into every specimen at three different concentrations and the spiked specimens were analyzed in triplicate. Recovery of the unit values was calculated by comparing to control samples spiked with the same volume of diluents. No interference (≤ 10%) was detected with bilirubin up to 10 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 1,000 mg/dL, cholesterol up to 224.3 mg/dL. The three specimens spiked with Rheumatoid Factor IgM up to 500 IU/mL showed recovery from 107% to 114%; the samples with more RF IgM had better recoveries than the samples spiked with lower amounts of RF IgM.

Analytical Specificity: Twelve CDC ANA human reference sera from the Centers for Disease Control and Prevention were tested with the Quanta Flash ENA7. As expected, the seven CDC samples known to contain autoantibodies against the assay's component antigens tested strongly positive. The other five samples containing other ANA-type antigens such as dsDNA, anti-PM/Scl, etc. were tested negative.

f. Assay cut-off:

The assay cut-off is 20 CU. Results <20 CU should be reported as negative and results ≥20 CU should be reported as positive.

2. Comparison studies:

a. Method comparison with predicate device:

Serum specimens were obtained from 747 subjects including patients with various connective tissue diseases (SLE, systemic sclerosis, MCTD, etc.) and patients with non-connective tissue diseases were tested on the QUANTA Flash™ ENA7 Screen assay and the predicate.

Of the 29 discrepant samples positive by ENA7, 18 were negative by the predicate and by individual mono-specific assays, 8 were positive or equivocal by an assay that detects autoantibodies against SS-A 60 (which is not included in the QUANTA Lite ENA6 screen) and 3 were positive or equivocal by the SS-A 60 assay and other individual mono-specific ELISA assays. Of the 23 samples negative by the ENA7 assay and positive by the predicate, four were negative by all mono-specific assays, four were positive or equivocal by an assay specific for SS-A 60, and the remaining 15 were positive or equivocal by two or more mono-specific ELISA assays.

		QUANTA Lite ENA6		
		Positive	Negative	Total
QUANTA Flash™ ENA7 Screen	Positive	235	29	264
	Negative	23	460	483
	Total	258	489	747

Positive agreement (235/258) = 91.1% (95% C.I. = 86.9 – 94.3%)
Negative agreement (460/489) = 94.1% (95% C.I. = 91.6 – 96.0%)
Overall agreement (695/747) = 93.0% (95% C.I. = 91.0 – 94.8%)

Comparison studies were performed with different monospecific autoantibody ELISAs and clinical and contrived samples to ensure that all targets are recognized by the QUANTA Flash ENA7 assay.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and specificity:

Clinical sensitivity was assessed by testing 552 samples from various connective tissue diseases (CTD) including Systemic Lupus Erythematosus (SLE), systemic sclerosis (SSc), polymyositis (PM), dermatomyositis (DM), Sjögren's syndrome (SjS) and mixed connective tissue disease (MCTD) All CTD patients were classified according to the American College of Rheumatology criteria for each disease. No demographic information was available for the samples. The INOVA ENA7 Assay showed an overall sensitivity of 63.2% (95% CI: 59.0% - 67.3%). The results are shown in the table below.

Connective Tissue Diseases	**n**	**QUANTA Flash ENA7 Positive (Sensitivity %)**	**95% CI**
Systemic Lupus Erythematosus (SLE)	261	166 (63.6%)	57.6 – 69.2
Systemic Sclerosis (SSc)	68	39 (57.4%)	45.5 – 68.4
Polymyositis (PM)	31	16 (51.6%)	34.8 – 68.0
Dermatomyositis (DM)	13	7 (53.8%)	29.1 – 76.8
PM or DM Overlap Syndrome	19	11 (57.9%)	36.3 – 76.9
Myositis	9	2 (22.2%)	5.7 – 47.7
MCTD	12	10 (83.3%)	55.2 – 95.3
Undifferentiated CTD (UCTD)	74	43 (58.1%)	46.7 – 68.7
Sjögren's Syndrome (SjS)	65	55 (84.6%)	74.0 – 91.4

Connective Tissue Diseases	n	QUANTA Flash ENA7 Positive (Sensitivity %)	95% CI
Total CTD	**552**	**349 (63.2%)**	59.0 – 67.3

b. Clinical Specificity:
Clinical specificity was assessed by testing 698 samples from other non-CTD samples with the ENA7 assay. The overall specificity of the INOVA ENA7 assay was 91.8% (95% CI: 89.5% - 93.8%). The results are listed below.

Non-Connective Tissue Diseases	n	QUANTA Flash ENA7 Negative (Specificity %)	95% CI
Rheumatoid Arthritis (RA)	132	122 (92.4%)	86.6 – 95.8
Osteoarthritis	50	48 (96.0%)	86.5 – 98.9
Other arthritides*	48	47 (97.9%)	89.1 – 99.6
Primary Biliary Cirrhosis (PBC)	52	42 (80.8%)	68.1 – 89.2
Autoimmune Hepatitis (AIH)	50	45 (90.0%)	78.6 – 95.6
Hashimoto's Thyroiditis	21	20 (95.2%)	77.3 – 99.2
Grave's Disease	21	21 (100%)	84.5 – 100
Crohn's Disease	20	19 (95.0%)	76.4 – 99.1
Ulcerative Colitis	20	18 (90.0%)	69.9 – 97.2
Celiac Disease	63	58 (92.3%)	82.7 – 96.6
Primary Anti-phospholipid Syndrome (PAPS)	52	41 (78.8%)	66.0 – 87.8
Granulomatosis with polyangiitis (Wegener's)	75	66 (88.0%)	78.7 – 93.6
Infectious Diseases†	94	94 (100%)	96.1 - 100
Total (non-CTD)	**698**	**57 (91.8%)**	89.5 – 93.8

***** Other arthritides: Polymyalgia Rheumatica (21), Psoriatic Arthritis (14), and Ankylosing Spondylitis (13)
† Infectious Diseases: Hepatitis B (36), Hepatitis C (44), Human Immunodeficiency Virus (HIV) (7), Syphilis (7)

c. Other clinical supportive data (when a. and b. are not applicable):

4. Clinical cut-off**:**

   Not applicable.

5. Expected values/Reference range:

   Samples from apparently normal healthy individuals (n = 196) were tested with the ENA7. With the cutoff of 20 CU, 10 samples (5.1%) were positive. The mean concentration was 9.1 CU (95% CI: 5.7 – 12.5 CU).

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.