510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K151429

B. Purpose for Submission:

New device

C. Measurand:

Anti-Jo-1 IgG autoantibodies

D. Type of Test:

Semi-quantitative chemiluminescent immunoassay (CIA)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash® Jo-1
QUANTA Flash® Jo-1 Calibrators
QUANTA Flash® Jo-1 Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5100, Antinuclear Antibodies Immunological Test System
   21 CFR §862.1150, Calibrator
   21 CFR §862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:

   Class II, Assay and Calibrators
   Class I, Control

3. Product code:

   LLL – Extractable antinuclear antibody, antigen and control
   JIT – Calibrator, Secondary

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

4. Panel:

   Immunology (82) (Assay)
   Clinical Chemistry (75) (Calibrators and Controls)

H. Intended Use:

1. Intended use(s):

   QUANTA Flash® Jo-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Jo-1 antibodies in human serum. The presence of anti-Jo-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathies.

   QUANTA Flash® Jo-1 Calibrators are intended for use with the QUANTA Flash® Jo-1 Reagents for the determination of IgG anti-Jo-1 antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

   QUANTA Flash® Jo-1 Controls are intended for use with the QUANTA Flash® Jo-1 Reagents for quality control in the determination of IgG anti-Jo-1 antibodies in human serum.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use only

4. Special instrument requirements:

   BIO-FLASH® chemiluminescent analyzer (k083518)

I. Device Description:

The QUANTA Flash® Jo-1 Kit includes one QUANTA Flash® Jo-1 Reagent Cartridge with the following reagents for 50 determinations:

a. Jo-1 antigen coated paramagnetic beads, lyophilized
b. Assay Buffer
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer

The QUANTA Flash® Jo-1 Calibrators set is sold separately and contains:

a. Calibrator 1: Two barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrator 1 contains human antibodies to Jo-1 in buffer with concentration of 19 CU.
b. Calibrator 2: Two barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrator 2 contains human antibodies to Jo-1 in buffer with concentration of 320 CU.

The QUANTA Flash® Jo-1 Controls set contains is sold separately and contains:

a. Negative Control: Two barcode labeled tubes containing 0.5 mL, ready to use reagent. Negative control contains human antibodies to Jo-1 in buffer with concentration of 10 CU.
b. Positive Control: Two barcode labeled tubes containing 0.5 mL, ready to use reagent. Positive control contains human antibodies to Jo-1 in buffer with concentration of 50 CU.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   FIDIS Connective 10

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

   K102607

2. Comparison with predicate:

QUANTA Flash® Jo-1Reagent Kit:

Similarities		
Item	Device QUANTA Flash® Jo-1	Predicate FIDIS Connective 10
Intended Use	Semi-quantitative determination of anti-Jo-1 antibodies in human serum.	Same
Antigen	Recombinant Jo-1	Same
Sample Type	Serum	Same
Assay methodology	Solid phase immunoassay	Same
Traceability	International Reference preparation is not available. Results are traceable to in-house standards	Same
Shelf Life	One year at 2–8°C	Same

Differences		
Item	Device QUANTA Flash® Jo-1	Predicate FIDIS Connective 10
Detection	Chemiluminescent immunoassay	Multiplex bead-based flow cytometric fluorescent immunoassay
Solid Phase	Paramagnetic microparticles (beads)	Color-coded microspheres
Conjugate	Isoluminol conjugated anti-human IgG	Phycoerythrin conjugated anti-human IgG
Calibration	Lot specific Master Curve and two Calibrators (Sold separately)	Calibration system interpolates fluorescent intensity (included in the kit
Cut-off	Negative: < 20 CU Positive: ≥ 20 CU	Negative: < 30 AU/mL Borderline: 31–40 AU/mL Positive: > 40 AU/mL
Assay Measuring Range (AMR)	2.2–1147.2 CU	No claim for the reportable range

QUANTA Flash® Jo-1 Calibrators:

Similarities		
Item	Device QUANTA Flash® Jo-1 Calibrators	Predicate
Analyte	Anti-Jo-1 antibodies	Same
Matrix	Human serum, buffers, stabilizers and preservative	Same
Physico-chemical characteristics	Liquid, prediluted, ready to use	Same
Shelf Life/Storage	One year at 2–8°C	Same

Differences		
Item	Device QUANTA Flash® Jo-1 Calibrators	Predicate
Intended Use	For use with QUANTA Flash® Jo-1 reagents for determination of IgG anti-Jo-1 antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.	No separate intended use; calibrator is part of the kit.
Unit	CU (Chemiluminescent Units)	AU/mL (arbitrary)s

QUANTA Flash® Jo-1 Controls:

Similarities		
Item	Device QUANTA Flash® Jo-1 Controls	Predicate
Analyte	Anti-Jo-1 antibodies	Same
Matrix	Human serum, buffer, stabilizer, and preservative	Same
Physico- chemical characteristics	Liquid, ready to use	Liquid, to be diluted
Levels	2 (negative and positive)	Same
Shelf Life/Storage	One year at 2–8°C	Same

Differences		
Item	Device QUANTA Flash® Jo-1 Controls	Predicate
Intended Use	For use with the QUANTA Flash® Jo-1 reagents for quality control in the determination of IgG anti-Jo-1 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Unit	CU (arbitrary)	AU/mL (arbitrary)

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

EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition.
EP06-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline, Second Edition.
EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition
EP09-A2-IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Second Edition (Interim Revision).
EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline, Second Edition
C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Addition

L. Test Principle:

The QUANTA Flash® Jo-1 assay is a microparticle chemiluminescent immunoassay designed for use on the BIO-FLASH® instrument. The instrument platform is a fully automated closed system with continuous load and random access capabilities that automatically processes the samples, runs the assay and reports the results. It includes liquid handling hardware, luminometer and computer with software-user interface. The QUANTA Flash® Jo-1 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH® instrument.

Purified recombinant Jo-1 antigen is coated onto paramagnetic beads. The bead suspension is lyophilized and stored in the bead tube. Prior to use in the BIO-FLASH® system, the sealed reagent tubes are pierced with the reagent cartridge lid and the beads are rehydrated and resuspended using resuspension buffer by pipetting up and down with a transfer pipette. The reagent cartridge is then loaded onto the BIO-FLASH® instrument. Samples are also loaded onto the instrument in sample racks. A patient serum sample is prediluted by the BIO-FLASH® with system rinse in a small disposable plastic cuvette. Small amounts of the diluted patient serum, the beads, and assay buffer are all combined into a second cuvette, and mixed. This cuvette is then incubated at 37°C. The beads are magnetized and washed several times. Isoluminol conjugated anti-human IgG antibodies are then added to the cuvette, and again incubated at 37°C. The beads are magnetized and washed repeatedly. The isoluminol conjugate is oxidized when Trigger 1 and Trigger 2 are added to the cuvette, and the flash of light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH® optical system. The RLU are proportional to the amount of isoluminol conjugate that is bound to the human IgG, which is in turn proportional to the amount of anti-Jo-1 antibodies bound to the corresponding Jo-1 on the beads.

For determining the amount of antibody in a sample, the QUANTA Flash® Jo-1 assay utilizes a predefined lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. Every new lot number of reagent cartridge must be calibrated before first use, with the QUANTA Flash® Jo-1 Calibrators. Based on the results obtained with the two Calibrators included in the Calibrator Set, an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the instrument signal (RLU) obtained for each sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision: The precision of the QUANTA Flash® Jo-1 assay was evaluated by testing nine serum samples prepared to contain various concentrations of anti-Jo-1 antibody. Each sample was run in duplicate, twice a day, for 20 days with one reagent lot (total of 80 replicates per sample). All %CV values were within the manufacturer's pre-determined acceptance limit (< 10%). The results are summarized in the table below.

Sample	Mean (CU)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	5.5	0.2	3.4	0.0	3.2	0.4	6.8	0.4	7.6
2	19.5	0.6	3.1	0.5	2.6	0.9	4.7	1.2	6.2
3	20.7	0.8	3.7	0.3	1.4	1.2	5.9	1.5	7.1
4	37.1	1.6	4.4	0.0	0.0	2.3	6.1	2.8	7.6

Sample	Mean (CU)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
5	42.0	1.2	2.9	1.0	2.3	2.4	5.6	2.8	6.7
6	97.5	3.1	3.2	2.4	2.4	6.0	6.1	7.1	7.3
7	288.2	10.0	3.5	8.5	3.0	19.1	6.6	23.2	8.0
8	445.6	18.7	4.2	0.0	0.0	29.4	6.6	34.8	7.8
9	879.3	43.6	5.0	13.2	1.5	43.8	5.0	63.2	7.2

Reproducibility: A total of five samples were tested at three different sites with one reagent lot to evaluate the site-to-site reproducibility. Each sample was run in replicates of five, once a day for five days, to generate 25 data points at each site (N=75 per sample for all sites combined). Data were analyzed for within-run, between-run, between-site, and total precision. The results are summarized in the tables below. All %CV values were within the manufacturer's pre-determined acceptance limit (< 15%).

Sample	Mean (CU)	Within-Day		Between-Day		Between-Site		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	9.8	0.5	4.7	0.5	4.6	0.8	8.0	1.0	10.4
2	32.4	1.0	3.1	1.9	5.9	2.3	7.0	3.1	9.6
3	77.3	3.1	4.0	3.2	4.2	6.4	8.3	7.8	10.1
4	168.3	6.6	3.9	8.1	4.8	14.7	8.8	18.0	10.7
5	854.6	34.4	4.0	29.7	4.0	0.4	6.8	100.0	11.7

To evaluate lot-to-lot reproducibility, five samples with anti-Jo-1 antibody concentration at various levels across the measuring range (18.6, 23.3, 73.3, 382.5, and 911.9 CU) were tested. Each sample was tested in replicates of five, one run per day for five days using three difference reagent lots. Mean and %CV for each sample were calculated and %CV values were from 3.6% to 7.9% for all samples.

b. Linearity/assay reportable range:

Linearity: The analytical measuring range (AMR) of the assay is defined by the lowest and highest points on the master curve, e.g., 2.2–1147.2 CU. The linearity across this range was evaluated by a study according to CLSI EP6-A. Serially diluted samples with anti-Jo-1 antibody concentrations ranging from 1.7 to 1226.6 CU were prepared by diluting each of four high positive serum samples with analyte free (stripped) serum in 10% increments. Each dilution was tested in duplicate. Percentage recovery of obtained mean results was calculated compared to the expected results with the acceptant criteria of recovery between 80 and 120%, or ± 4 CU, whichever is greater. The linear regression analysis was performed using the samples falling within the master curve and the results of samples within AMR are

summarized as follows:

Sample	Test Range (CU)	Slope (95% CI)	Y-intercept (95% CI)	R^2	% Recovery
1	3.0–14.4	0.99 (0.96–1.02)	0.22 (-0.09–0.52)	1.00	97.1–107.2%
2	4.4–44.3	0.99 (0.95–1.02)	0.27 (-0.77–1.32)	0.99	90.4–113.0%
3	11.0–110.5	0.99 (0.98–1.01)	-1.12 (-2.08– -0.16)	1.00	90.5–100.5%
4	68.7–686.8	1.00 (0.95–1.04)	-11.28 (-29.25–6.68)	0.99	85.7–106.6%
5	125.2–1126.4	0.92 (0.89–0.96)	31.38 (8.50–54.26)	1.00	95.0–111.5%

Auto-rerun: To validate the auto-rerun function with 1:20 dilutions, two high positive specimens with anti-Jo-1 antibody concentrations above assay measuring range (8967.5 and 19914.0 CU) were run with the auto-rerun function enabled on the BIO-FLASH®. The same set of samples prepared manually with a 1:20 fold dilution was used as reference and tested with the concentration of 9815.2 and 19343.6 CU, respectively. The % recovery values for results obtained with the auto-rerun results compared to results obtained with the manual dilution were 91% and 103%, respectively.

Hook effect: Two high positive samples having anti-Jo-1 antibody concentration above assay measuring range (31783.1 CU and 65625.5 CU) were examined to assess potential hook effect. No hook effect was observed up to 65625.5 CU.

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

Traceability: There is no recognized standard or reference material for anti-Jo-1 autoantibodies. The calibrator and control values are directly traceable to in-house standards that are used to create the master curves for the QUANTA Flash® Jo-1.

Value assignment: The QUANTA Flash® Jo-1 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-Jo-1 antibodies. The target CU is achieved through trial dilutions on 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 target values and ranges for the Calibrators and Controls are listed below:

	Target Value (CU)	Target Range (CU)
QUANTA Flash® Jo-1 Calibrators		
Calibrator 1	19	17–21
Calibrator 2	320	280–360
QUANTA Flash® Jo-1 Controls		
Negative control	10	8–12
Positive control	50	40–60

Stability:

Kit stability (unopened): The accelerated stability study was performed using three lots of Jo-1 coupled beads, calibrators, and controls. Real-time stability is on-going; the results to date support a claim of 12 months stability for unopened reagent cartridge, up to 15 months on calibrators, and up to 16 months on controls stored at 2–8°C.

On-board (In-use) stability: On-board stability study was performed for calibrators, controls and reagent cartridge:

i. Calibrators: Calibrators were placed uncapped, onboard the instrument, and calibration was performed five times over 9.5 hours. Controls and a panel of characterized patient specimens were run on each calibration curve. Each calibrator is measured in triplicate during calibration.
ii. Controls: Two vials of each control were assayed twice a day for a total of 21 runs. The first run was used to establish baseline value, and then an additional 19 runs were performed. During runs, the Controls were left uncapped, onboard the instrument for 15 minutes per run. When not in use, the controls were capped, and stored at 5ºC ± 3°C.
iii. Reagent Cartridge: Two lots of cartridges were tested with four serum specimens (with different reactivity levels) along with the Negative and Positive Controls. The specimens were tested periodically up to 92 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting % recovery against the number of days.

All results met the manufacturer's acceptance criteria and support the following on-board stability claim:

Calibrators	8 hours on-board; up to 4 calibrations
Controls	Up to 15 uses with 10 min on-board per use
Reagent Cartridge	71 days on-board

Sample stability: The study was performed with seven samples (two negatives, three positives, and two around the cut-off), tested at 2–8°C, and room temperature (RT). In addition, the samples were tested for the stability after up to three repeated

freeze/thaw cycles. The results support sample stability up to 48 hours of storage at RT, up to 14 days of storage at 2–8°C, and up to three freeze/thaw cycles when samples are stored at or below -20°C.

d. Detection limit:

Limit of Blank (LoB) was determined by assaying four blank samples in five replicates per sample over three days with two reagent lots. Sixty data points were generated. LoB was calculated at the 95th percentile using the non-parametric method, as the dataset showed non-normal distribution. The LoB was determined to be 337 RLU.

The Limit of Detection (LoD) was determined by assaying four samples with anti-Jo-1 antibody concentration between LoB and approximately four times of LoB. Each sample was tested in five replicates over three days with two reagent lots. LoD value was calculated as the LoB + 1.645 x SD of the replicates for the low level samples. The LoD of the QUANTA Flash® Jo-1 assay was determined to be 409 RLU, which is below the value of the lowest QUANTA Flash® Jo-1 Master Curve standard (2.2 CU), and therefore below the AMR of the assay.

e. Analytical specificity:

Endogenous Interference: Three serum samples with antibody concentrations at 11.3 CU (negative), 21.1 CU (around the cut-off), and 117.0 CU (positive) were spiked with known quantities of bilirubin (10, 5.0, or 2.5 mg/dL), hemoglobin (200, 100, or 50 mg/dL), or triglycerides/cholesterol (1000/224.3, 500/112.2, or 250/56.1 mg/dL). Each sample was tested in triplicate and the recovery was calculated by comparing to control samples spiked with the same volume of diluents. For rheumatoid factor (RF) interference, three samples with antibody concentrations at 7.3 CU (negative), 32.5 CU, and 123 CU were tested by spiking with different proportions of a high positive RF IgM serum sample (1894 IU/mL). Each sample was tested triplicate and the recovery was calculated by comparing to control samples spiked with the same proportions of a negative serum. No interference (85%–115% recovery for samples above the cutoff, and ± 4 CU difference for samples below the cutoff) was detected in the samples up to the concentrations listed in the table below:

Potential Interfering Substances	**Maximum Concentration**
Bilirubin	10 mg/dL
Hemoglobin	200 mg/dL
Triglycerides	1000 mg/dL
Cholesterol	224.3 mg/dL
RF	947 IU/mL

Analytical cross-reactivity: Cross reactivity of the QUANTA Flash® Jo-1 was investigated using 12 reference sera from the Center of Disease Control and

Prevention (CDC) with three lots of QUANTA Flash Jo-1 reagents. The ANA human reference serum #10 (for human antibodies to Jo-1) tested positive on all three lots with an average of 935.9 CU. The other reference sera in the panel were below 2.2 CU for all three lots tested.

f. Assay cut-off:

The QUANTA Flash® Jo-1 cut-off was determined by testing a set of samples from a reference population of 207 subjects (31 systemic sclerosis samples, 30 systemic lupus erythematosus samples, 21 Crohn's disease samples, 19 multiple sclerosis, 19 hepatitis C positive samples, 18 ulcerative colitis samples, 13 psoriatic arthritis samples, 10 syphilis positive samples, 10 healthy individuals, 9 polymyalgia rheumatica samples, 8 rheumatoid arthritis samples, 5 spondylarthritis samples, 3 Sjögren's syndrome samples and 11 other disease control samples). The cut-off was established as 20 CU (10000 RLU) based on the 99th percentile of the results obtained.

	Positive	**Negative**
QUANTA Flash® Jo-1	≥ 20 CU	< 20 CU

2. Comparison studies:

a. Method comparison with predicate device:

Samples for method comparison analysis included 487 samples from the clinical validation study (see below) along with 26 additional samples contrived by diluting Jo-1 positive samples with negative serum to cover the reportable range of assay. These samples were tested on both the QUANTA Flash® Jo-1 and on the predicate FIDIS Connective 10. From the total of 513 samples, results for 105 samples were within the reportable range of the assay. The results are summarized below:

		FIDIS Connective 10			
		Positive	Borderline	Negative	Total
QUANTA Flash® Jo-1	Positive	20	5	6	31
	Negative	2	2	70	74
	Total	22	7	76	105

Borderline as negative:
Positive agreement: 90.9% (95% CI: 72.2–97.5%)
Negative agreement: 86.7% (95% CI: 77.8–92.4%)
Overall agreement: 87.6% (95% CI: 80.0–92.6%)

Borderline as positive:
Positive agreement: 86.2% (95% CI: 69.4–94.5%)
Negative agreement: 92.1% (95% CI: 83.8–96.3%)
Overall agreement: 90.5% (95% CI: 83.4–94.7%)

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Clinical Specificity:

A total of 487 samples were included in the clinical validation for the QUANTA Flash® Jo-1. The validation set of samples includes 206 samples from patients diagnosed with idiopathic inflammatory myopathy (IIM) and 281 samples from patients with other autoimmune diseases. Clinical sensitivity and specificity in this sample cohort are summarized in the following tables:

		Clinical Diagnosis of IIM		
		Positive	Negative	Total
QUANTA Flash® Jo-1	Positive	24	2	26
	Negative	182	279	461
	Total	206	281	487

Sensitivity: 11.7% (95% CI: 8.0–16.7%)
Specificity: 99.3% (95% CI: 97.4–99.8%)

The distribution of the cohort and the Jo-1 positivity rate for each clinical subgroup are summarized below:

Disease category	**QUANTA Flash® Jo-1**		
	N	# of positive	% Positive
Target Diseases (IIM):			
Dermatomyositis (DM)	95	11	11.7%
Polymyositis (PM)	71	7	9.9%
Juvenile Dermatomycotic	7	1	14.3%
Others (IMNM, OM, UM)*	5	1	20.0%
IIM not further specified	28	4	14.3%
Total of IIM	**206**	**24**	**11.7%**
Control Diseases:			
Sjögren's syndrome	15	0	0.0%
SLE	41	0	0.0%
Systemic Sclerosis	44	0	0.0%
Rheumatoid arthritis	59	1	1.7%
MCTD	103	1	0.9%
Septicemia	19	0	0.0%
Total of controls	**281**	**2**	**0.7%**

* IMNM: Immune mediated necrotizing myopathy
OM: overlap myositis
UN: undifferentiated myositis

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

Not applicable

4. Clinical cut-off**:**

Not applicable

5. Expected values/Reference range:

The expected value in the normal population is "negative". Anti-Jo-1 antibody levels were analyzed in a cohort of 400 apparently healthy blood donors (246 females and 154 males, ages 17 to 60 years, with an average age of 34.7 years and median age of 34 years) using the QUANTA Flash® Jo-1. The results showed a mean concentration of 2.3 CU with the values ranging from < 2.2 to 16.3 CU. None of the samples were positive on the QUANTA Flash® Jo-1.

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.