510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K180975

B. Purpose for Submission:

New device

C. Measurand:

Human anti-HMGCR (3-hydroxy-3-methylglutaryl-coenzyme A reductase) IgG autoantibodies

D. Type of Test:

Semi-quantitative chemiluminescent immunoassay (CIA)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash HMGCR Reagents

G. Regulatory Information:

1. Regulation sections:

   21 CFR §866.5100, Antinuclear Antibodies Immunological Test System

2. Classification:

   Class II

3. Product codes:

   LLL – Extractable antinuclear antibody, antigen and control

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   QUANTA Flash HMGCR is a chemiluminescent immunoassay for the semi-quantitative determination of IgG autoantibodies against HMGCR (3-hydroxy-3-methylglutaryl coenzyme A reductase) antigens in human serum. The presence of anti-HMGCR antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathy (IIM).

2. Indications for use:

   Same as Intended Use

3. Special conditions for use statement:

   For Prescription Use only

4. Special instrument requirements:

   BIO-FLASH® chemiluminescent analyzer (K083518)

I. Device Description:

The QUANTA Flash HMGCR Reagents kit contains the following materials:

a. One QUANTA Flash HMGCR Reagent Cartridge
b. One tube of Resuspension Buffer
c. One transfer pipette

The QUANTA Flash HMGCR reagent cartridge contains the following reagents for 50 determinations:

a. HMGCR coated paramagnetic beads, lyophilized
b. Assay buffer – colored pink, containing protein stabilizers and preservatives
c. Tracer IgG – Isoluminol labeled anti-human IgG antibody, containing buffer, protein stabilizers and preservative

QUANTA Flash HMGCR Calibrators and QUANTA Flash HMGCR Controls are sold separately.

The QUANTA Flash HMGCR Calibrators kit contains two vials each of Calibrator 1 and Calibrator 2:

a. QUANTA Flash HMGCR Calibrator 1: Two barcode labeled tubes containing 0.3 mL pre-diluted, ready to use reagent. Calibrators contain human antibodies to HMGCR in

stabilizers and preservatives.
b. QUANTA Flash HMGCR Calibrator 2: Two barcode labeled tubes containing 0.3 mL pre-diluted, ready to use reagent. Calibrators contain human antibodies to HMGCR in stabilizers and preservatives.

The QUANTA Flash HMGCR Controls kit contains two vials of Negative Control and two vials of Positive Control:

a. QUANTA Flash HMGCR Negative Control: Two barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to HMGCR in stabilizers and preservatives.
b. QUANTA Flash HMGCR Positive Control: Two barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to HMGCR in stabilizers, and preservatives.

J. Substantial Equivalence Information:

1. Predicate device name:

   QUANTA Flash Jo-1

2. Predicate 510(k) number:

   K151429

2. Comparison with predicate:

   QUANTA Flash HMGCR Reagents Kit:

Similarities		
Item	Device QUANTA Flash HMGCR Reagents	Predicate QUANTA Flash Jo-1 Reagents
Assay methodology	Solid phase (heterogenous) immunoassay	Same
Solid phase	Paramagnetic microparticles (beads)	Same
Detection/Operating principle	Chemiluminescent immunoassay	Same
Conjugate	Isoluminol conjugated anti-human IgG	Same
Shelf life	One year	Same
Sample type	Serum	Same
Calibration	Lot specific Master Curve with two calibrators (sold separately)	Same
Units	Chemiluminescent units (CU)	Same

Differences		
Item	Device QUANTA Flash HMGCR Reagents	Predicate QUANTA Flash Jo-1 Reagents
Intended Use	QUANTA Flash HMGCR is a chemiluminescent immunoassay for the semi quantitative determination of IgG autoantibodies against HMGCR (3- hydroxy-3 methylglutaryl-coenzyme A reductase) antigen in human serum. The presence of anti HMGCR antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathy (IIM).	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 myopathy.
Analytical Measuring Range	1.5 CU – 550.0 CU	2.2 – 1147.2 CU
Antigen	Recombinant HMGCR	Recombinant Jo-1

K. Standard/Guidance Document Referenced:

EP05-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical
    Laboratory; Approved Guideline, Third Edition

EP06-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline, Second
    Edition

EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition

EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement
    Procedures; Approved Guideline, Second Edition

EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical
    Laboratory; Approved Guideline, Third Addition

L. Test Principle:

The principle of the assay is chemiluminescent microparticle immunoassay, a variation of solid phase immunoassay. The QUANTA Flash HMGCR assay is designed to run on the BIO-FLASH instrument. This 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 HMGCR assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument. HMGCR antigen is coated on

to paramagnetic beads, which are stored in the reagent cartridge lyophilized. When the assay cartridge is ready to be used for the first time, a buffer solution is added to the tube containing the beads, and the beads are re-suspended with the buffer. The reagent cartridge is then loaded onto the BIO-FLASH instrument. A patient serum sample is diluted 1:17 by the instrument in a disposable plastic cuvette. An aliquot of the diluted patient serum, HMGCR-coupled beads, and assay buffer are combined into a second cuvette, and mixed. This cuvette is incubated at 37°C. The beads are then magnetized and washed several times. Isoluminol conjugated anti-human IgG antibody is then added to the cuvette, and incubated at 37°C. Again, the beads are magnetized and washed repeatedly. The isoluminol conjugate produces a luminescent reaction when "Trigger" reagents are added to the cuvette. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. RLU values are proportional to the amount of bound isoluminol conjugate, which in turn is proportional to the amount of anti-HMGCR antibodies bound to the antigen on the beads. The QUANTA Flash HMGCR assay utilizes a predefined lot specific Master Curve that is uploaded into the instrument through the reagent cartridge barcode. Based on the results obtained by running two calibrators, an instrument specific Working Curve is created, which is used by the software to calculate chemiluminescent units (CU) from the RLU value obtained for each sample.

M. Performance Characteristics:

1. Analytical performance:

   All the results below met the manufacturer's pre-specified acceptance criteria.

   a. *Precision/Reproducibility:*

   Precision: The precision of the QUANTA Flash HMGCR assay was evaluated by testing seven serum samples prepared to contain various concentrations of anti-HMGCR antibody. Each sample was run in duplicate, twice a day, for 20 days with one reagent lot (total of 80 replicates per sample). The results are summarized in the table below.

Sample	Mean (CU)	Within-Run SD	Within-Run CV (%)	Between-Run SD	Between-Run CV (%)	Between-Day SD	Between-Day CV (%)	Total SD	Total CV (%)
1	10.9	0.5	4.6	0.2	2.2	0.5	4.3	0.7	6.7
2	48.3	1.1	2.4	1.5	3.1	2.6	5.3	3.2	6.6
3	16.3	0.3	2.0	0.5	3.4	0.8	5.0	1.0	6.3
4	23.4	0.4	1.9	0.9	3.8	0.9	4.1	1.4	5.9
5	76.2	2.1	2.8	1.7	2.2	4.0	5.2	4.8	6.3
6	175.5	3.8	2.1	5.4	3.1	11.4	6.5	13.1	7.5
7	400.5	15.5	3.9	20.4	5.1	22.6	5.6	34.1	8.5

Reproducibility: A total of eight samples were tested according to CLSI EP05-A03 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 per sample at each site (N=75 per sample for all sites combined). Data were analyzed for within-run, between-day, between-site, and total imprecision. The results are summarized in the tables below.

Sample	Mean (CU)	Within-Run		Between-Day		Between-Site		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	10.6	0.6	3.4	0.7	6.5	0.5	5.1	0.9	8.8
2	16.1	1.0	3.1	0.7	4.4	0.8	4.9	1.2	7.5
3	22.8	0.8	3.4	1.1	4.6	0.0	0.0	1.3	5.7
4	23.9	0.5	2.2	1.0	4.2	0.5	1.9	1.2	5.1
5	55.6	1.8	3.2	3.0	5.5	0.0	0.0	3.5	6.3
6	123.9	3.6	2.9	6.2	5.0	4.7	3.8	8.6	6.9
7	134.2	4.1	3.0	7.7	5.7	0.4	0.3	8.7	6.5
8	344.2	22.0	6.4	18.3	5.3	14.8	4.3	32.2	9.4

To evaluate lot-to-lot variability, eight samples with anti-HMGCR antibody concentration at various levels across the measuring were tested. Each sample was tested in replicates of five, one run per day for five days using three difference reagent lots. The results are summarized below.

Sample	Mean (CU)	Repeatability		Between-Day		Within-Lot		Between-Lot		Within Laboratory	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	10.9	0.4	3.7%	0.8	7.7%	0.9	8.5%	0.2	1.8%	1.0	8.7%
2	15.1	0.3	2.2%	0.9	5.6%	0.9	6.0%	1.1	7.2%	1.4	9.4%
3	22.8	0.7	3.0%	1.3	5.8%	1.5	6.5%	0.8	3.6%	1.7	7.4%
4	22.3	0.7	3.0%	1.4	6.1%	1.5	6.8%	0.2	0.8%	1.5	6.8%
5	53.8	2.4	4.4%	2.9	5.3%	3.7	6.9%	1.7	3.1%	4.1	7.6%
6	122.6	3.3	2.7%	7.0	5.7%	7.8	6.3%	6.0	4.9%	9.8	8.0%
7	130.3	2.9	2.2%	9.3	7.1%	9.7	7.5%	2.6	2.0%	10.1	7.7%
8	349.8	13.5	3.8%	19.8	5.7%	24.0	6.8%	6.9	2.0%	24.9	7.1%

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., 1.5–550 CU. The linearity across this range was evaluated by a study according to CLSI EP6-A. The linearity was evaluated using four human serum samples with various anti-HMGCR antibody concentrations which were combined with another human serum sample containing low levels of anti-HMGCR antibodies in 10% increments (from 0% to 90% of low sample) to obtain values that cover the entire AMR. Each dilution was tested in duplicate. Percentage recovery of obtained mean results was calculated compared to the expected results. Results were analyzed by performing linear regression analysis and identifying the best fitting polynomial. For all samples, it was found that the best fitting polynomial is linear, except for sample 3, where the best fitting polynomial was a second order polynomial. The deviation from linearity between the linear regression and the second order polynomial met the acceptance criteria. 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	61.0–610.2	0.97 (0.92–1.01)	3.9 (-3.5–11.3)	1.00	92.0–105.2%
2	13.8–138.0	0.98 (0.95–1.01)	-0.1 (-1.3–1.05)	1.00	90.8–100.0%
3	2.2–21.5	0.94 (0.91–0.98)	0.0 (-0.2–0.2)	1.00	90.2–100.0%
4	1.0–10.4	1.03 (0.99–1.06)	0.0 (-0.1–0.1)	1.00	98.6–106.7%
Combined	1.0–610.2	0.98 (0.96–0.99)	0.1 (0.0–0.2)	1.00	90.2–106.7%

Auto-rerun: To validate the auto-rerun function with 1:20 dilutions, three high positive specimens with anti-HMGCR antibody concentrations above assay measuring range 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 were used as reference values and tested with the concentration of 702.0, 1056.0 and 1272.0 CU, respectively. The percent (%) recovery values for results obtained with the auto-rerun results compared to the results obtained with the manual dilution were between 100% and 105.7%.

Hook effect: Two high positive samples with anti-HMGCR antibody concentrations above the assay measuring range (5193.6 CU and 744.0 CU) were tested to assess for a potential hook effect. No hook effect was observed up to 5193.6 CU.

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

Traceability: There is no international reference material for anti-HMGCR antibodies that allows for the standardization of anti-HMGCR antibodies detection assays. Calibrator and Control values are directly traceable to in-house Standards that are used to create the Master Curves for the QUANTA Flash HMGCR assay.

Value assignment: The QUANTA Flash HMGCR Calibrators and Controls are manufactured by diluting human serum that contains a high concentration of anti-HMGCR antibodies with a stabilizer and preservative solution. The human serum is obtained from commercial sources and it is tested for markers of infectious substances. Upon completion of the manufacturing process, the Calibrators and Controls are tested on at least two instruments, with at least two lots of reagent cartridge, and in replicates of five to obtain a minimum of 10 data points to determine final value assignment.

	Target Value (CU)	Target Range (CU)
QUANTA Flash HMGCR Calibrators		
Calibrator 1	10	8–12
Calibrator 2	200	180–220
QUANTA Flash HMGCR Controls		
Negative control	10	8–12
Positive control	50	40–60

Stability:

Kit stability (unopened): Kit stability studies were performed. Accelerated stability study was performed for three weeks at 37°C using three lots and six samples run in duplicate at the following concentrations: 10.6 CU, 19.4 CU, 49.3 CU, 62.7 CU, 139.0 CU, and 317.2 CU. The accelerated stability study supports a claim for a 12-month shelf life. Real-time stability study has been performed at six-month intervals on the QUANTA Flash HMGCR reagents, to verify the 12-month expiration date assigned based on the accelerated stability study. A negative sample (Negative Control), a low positive sample (Positive Control) and a high positive patient sample were tested in replicates of six (replicates of nine at time zero). At the time of the submission, results were available up to 12 months for the Reagent Cartridge. The results of the real-time stability study performed to date support a claim of 12 months stability for unopened reagent cartridge.

On-board (In-use) stability: On-board stability study was performed for the 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 61 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.

The results support on-board stability claim of 60 days.

Sample stability: The study was performed with four samples (negative, around the cut-off, moderate positive and high positive samples) for 14 days while stored at 2–8°C, and up to 48 hours while stored at room temperature (RT). In addition, the samples were tested for stability for three repeated freeze/thaw cycles. The results support sample stability for 48 hours of storage at RT, for 14 days of storage at 2–8°C, and for 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 for each lot. LoB was calculated as the 95th percentile using the parametric method for one lot, and non-parametric method for the other lot, as the dataset showed non-normal distribution. The LoB was determined to be 0.0 CU for each lot.

The Limit of Detection (LoD) was determined by assaying four low level samples prepared by mixing human serum samples with high and low levels of anti-HGMCR antibodies. Each sample was tested in five replicates over three days with two reagent lots. LoD was calculated as the LoB + 1.652 x SD (standard deviation) of the replicates for the low level samples. The LoD of the QUANTA Flash HMGCR assay was determined to be 0.2 CU, which is below the AMR of the assay.

The Limit of Quantitation (LoQ) was determined by assaying four low level samples prepared by mixing human serum samples with high and low levels of anti-HGMCR antibodies that were run in five replicates over three days with two reagent lots. There were 30 data points per sample. The LoQ of the QUANTA Flash HMGCR assay was determined to be 1.5 CU, which has been set as the lower limit of the analytical measuring range.

e. *Analytical specificity:*

Endogenous Interference: Four human serum specimens, one high positive (257.4 CU), one moderately positive (125.2 CU), one around the cutoff (18.0 CU) and one negative sample (7.4 CU) were tested. Interfering substances were spiked into every specimen in 10% of total specimen volume at the following concentrations: bilirubin (1 mg/ml, 0.5 mg/ml, 0.25 mg/ml); hemoglobin (2mg/ml, 1mg/ml, 0.5 mg/ml); triglycerides (1000 mg/dl, 500 mg/dl, 250 mg/dl); cholesterol (332.5 mg/dl, 166.25 mg/dl, 83.125 mg/dl); human IgG (70 mg/dl, 35 mg/dl, 17.5 mg/dl); Rheumatoid Factor IgM (153.4 IU/ml, 76.7 IU/ml, 38.35 IU/mL). The resulting samples were tested in triplicates with the QUANTA Flash HMGCR assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of

diluents only. No interferences were detected with the aforementioned interferents at the tested concentrations.

Exogenous Interference: Four human serum specimens, one moderately positive (99.5 CU), one low positive (42.6 CU), one around the cutoff (24.1 CU), and one negative sample (9.8 CU) were tested. Interfering substances were spiked into every specimen in 10% of total specimen volume at the following concentrations: atorvastatin (600 mg/ml); PQQ (24 µg/mL); simvastatin (600 ng/ml); methylprednisolone (36 µg/mL); coenzyme Q (0.72 mg/ml). The resulting samples were assessed in triplicates with the QUANTA Flash HMGCR assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents only. No interferences were detected with the aforementioned interferents at the tested concentrations.

f. *Assay cut-off:*

The QUANTA HMGCR cut-off was determined by testing a set of samples from a reference population of 157 subjects [23 apparently healthy donors, 22 infectious disease controls (HBV, HCV, syphilis), 18 scleroderma controls, 20 systemic lupus erythematosus controls, 48 end stage renal disease controls, 14 false positive cohort (high reactivity samples) and 12 diagnosed idiopathic inflammatory myopathy (IIM) patient specimens]. The cut-off was established as 20 CU (30,000 RLU) based on the 99th percentile of the results obtained.

	Positive	Negative
QUANTA Flash HMGCR	≥ 20 CU	< 20 CU

2. Comparison studies:

a. *Method comparison with predicate device:*

Despite the fact that the two assays aid in the diagnosis of IIM, there is no apparent overlap between the two types of autoantibodies in IIM diagnosed subjects. Consequently, a method comparison study between the QUANTA Flash HMGCR and the QUANTA Flash Jo-1 is not applicable.

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Clinical Specificity:*

A total of 723 characterized samples, none of which were used for establishing the reference range, were included in the clinical validation for the QUANTA Flash

HMGCR. The distribution of the samples and the anti-HMGCR positivity rate is in the table below:

Patient Group	N	N Positive	% Positive
Infectious (HBV)	14	0	0.0%
Infectious (HCV)	13	0	0.0%
Infectious (HIV)	13	0	0.0%
Infectious (Syphilis)	12	0	0.0%
Systemic lupus erythematosus (SLE)	80	0	0.0%
Sjögren's syndrome (SS)	44	0	0.0%
Scleroderma (SSc)	59	1	1.7%
Mixed Connective Tissue Disease (MCTD)	36	0	0.0%
Celiac Disease (CD)	25	0	0.0%
Rheumatoid Arthritis (RA)	39	0	0.0%
Fibromyalgia	13	0	0.0%
Hypothyroidism	14	0	0.0%
Lyme Disease	15	0	0.0%
Polymyalgia Rheumatica	13	0	0.0%
Primary Raynaud's Syndrome	15	0	0.0%
Sarcoidosis	15	0	0.0%
Breast cancer	10	0	0.0%
Colorectal cancer	10	0	0.0%
Lung cancer	10	0	0.0%
Ovarian cancer	10	0	0.0%
Paraneoplastic syndrome	6	0	0.0%
Total Controls	**466**	**1**	**0.2%**
Dermatomyositis (DM)	67	0	0.0%
Amyopathic Dermatomyositis	8	0	0.0%
Juvenile Dermatomyositis	13	1	7.7%
Polymyositis (PM)	88	9	10.2%
Inclusion Body Myositis	13	0	0.0%
Overlap	1	0	0.0%
Immune Mediated Necrotizing Myopathy (IMNM)	67	55	82.1%
Total idiopathic inflammatory myopathy (IIM)	**257**	**65**	**25.3%**
Total	**723**	**-**	**-**

Clinical performance data, including sensitivity and specificity, are summarized in the following tables:

Clinical Performance N = 723	Diagnosis		
	IIM	Controls	Total
Positive	65	1	66
Negative	192	465	657
Total	257	466	723

QUANTA Flash HMGCR	Clinical Performance Characteristics (95% Confidence Interval)
Sensitivity	25.3% (20.4% – 30.9%)
Specificity	99.8% (98.8% – 100.0%)
PPV	98.5% (90.1% – 99.8%)
NPV	70.8% (69.3% – 72.2%)

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-HMGCR antibody levels were analyzed using the QUANTA Flash HMGCR on a panel of 100 apparently healthy blood donors (50 females/50 males, ages 17 to 57 years, with an average and median age of 34 years). With a cut-off of 20 CU, all samples were negative with the QUANTA Flash HMGCR. The mean concentration was < 1.8 CU, and the values ranged from <1.5 to 8.0 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.