June 25, 2018

Inova Diagnostics, Inc.
Roger Albesa
Manager, Research and Development
9900 Old Grove Rd.
San Diego, California 92131-1638

Re: K180975
Trade/Device Name: QUANTA Flash HMGCR Reagents
Regulation Number: 21 CFR 866.5100
Regulation Name: Antinuclear antibody immunological test system
Regulatory Class: Class II
Product Code: LLL
Dated: April 12, 2018
Received: April 13, 2018

Dear Roger Albesa:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part

801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/) and CDRH Learn (http://www.fda.gov/Training/CDRHLearn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (http://www.fda.gov/DICE) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Kelly Oliner -S

For
Lea Carrington
Director
Division of Immunology
and Hematology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2020
See PRA Statement below.

510(k) Number *(if known)*
K180975

Device Name
QUANTA Flash HMGCR Reagents

Indications for Use *(Describe)*
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).

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Table of Contents

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

Administrative data

Submitter:
Inova Diagnostics, Inc
9900 Old Grove Road,
San Diego, CA, 92131

Purpose of submission: New device(s)

Devices in the submission: QUANTA Flash® HMGCR Reagents

Scientific contact:
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Device name (assay kit):

Proprietary name:	QUANTA Flash® HMGCR Reagents
Common name:	anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) Chemiluminescent Immunoassay
Classification name:	autoantibodies, HMGCR

Regulation Description	Extractable antinuclear antibody, antigen and control
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	LLL
Regulation Number	866.5100
Device Class	2

Predicate device

QUANTA Flash Jo-1, 510(k) number: k151429. Date declared: February 12, 2016.

Device description

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 (3-hydroxy-3-methylglutaryl-coenzyme A reductase) 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 resuspended 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.

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

The QUANTA Flash HMGCR Reagents kit contains the following materials:

a. One (1) QUANTA Flash HMGCR Reagent Cartridge
b. One (1) tube of Resuspension Buffer
c. One (1) 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.

Intended use(s)

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).

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash HMGCR Reagents has the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash HMGCR Reagents

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

Analytical performance characteristics

Quantitation and units of measure

For quantitation, the QUANTA Flash HMGCR assay utilizes a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash HMGCR consists of 6 Standards. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of HMGCR Standards:

Material	Assigned Value
HMGCR Master Curve Standard 1	0.0 CU
HMGCR Master Curve Standard 2	6.9 CU
HMGCR Master Curve Standard 3	20.8 CU
HMGCR Master Curve Standard 4	62.4 CU
HMGCR Master Curve Standard 5	187.2 CU
HMGCR Master Curve Standard 6	561.6 CU

Precision

The precision of the QUANTA Flash HMGCR assay was evaluated on 7 samples containing various concentrations of anti-HMGCR antibodies in accordance with CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Procedures - Approved Guideline. Samples were run in duplicates, twice a day, for 20 days.

Data were analyzed with the Analyse-it for Excel method evaluation software, and repeatability (within-run), between run, between day and within-laboratory precision (total precision) were calculated.

Acceptance criteria: Total %CV: < 12%

Results are summarized in the Table below.

QUANTA Flash HMGCR			Repeatability		Between-Run		Between-Day		Within-Laboratory Precision	
Sample ID	N	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	80	10.9	0.5	4.6	0.2	2.2	0.5	4.3	0.7	6.7
2	80	48.3	1.1	2.4	1.5	3.1	2.6	5.3	3.2	6.6
3	80	16.3	0.3	2.0	0.5	3.4	0.8	5.0	1.0	6.3
4	80	23.4	0.4	1.9	0.9	3.8	0.9	4.1	1.4	5.9
5	80	76.2	2.1	2.8	1.7	2.2	4.0	5.2	4.8	6.3
6	80	175.5	3.8	2.1	5.4	3.1	11.4	6.5	13.1	7.5
7	80	400.5	15.5	3.9	20.4	5.1	22.6	5.6	34.1	8.5

Reproducibility Studies

Reproducibility between sites (instruments)

Eight samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, at three different sites. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per site. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between site precision.

Acceptance criteria: Total %CV: < 12%

Results are summarized in the Table below.

			Within-Run		Between-Day		Within-Site		Between-Site		Within-Laboratory Precision	
Sample ID	**N**	**Mean (CU)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**
Sample 1	75	16.1	0.6	3.4%	0.7	4.4%	0.9	5.6%	0.8	4.9%	1.2	7.5%
Sample 2	75	134.2	4.1	3.0%	7.7	5.7%	8.7	6.5%	0.4	0.3%	8.7	6.5%
Sample 3	75	123.9	3.6	2.9%	6.2	5.0%	7.2	5.8%	4.7	3.8%	8.6	6.9%
Sample 4	75	344.2	22.0	6.4%	18.3	5.3%	28.6	8.3%	14.8	4.3%	32.2	9.4%
Sample 5	75	22.8	0.8	3.4%	1.1	4.6%	1.3	5.7%	0.0	0.0%	1.3	5.7%
Sample 6	75	23.9	0.5	2.2%	1.0	4.2%	1.1	4.8%	0.5	1.9%	1.2	5.1%
Sample 7	75	10.6	0.3	3.0%	0.7	6.5%	0.8	7.2%	0.5	5.1%	0.9	8.8%
Sample 8	75	55.6	1.8	3.2%	3.0	5.5%	3.5	6.3%	0.0	0.0%	3.5	6.3%

Reproducibility between lots

Eight samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, using three different reagent lots. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per lot. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between lot precision.

Acceptance criteria: Total %CV: < 12%

Results are summarized in the Table below.

			Within-Run		Between-Day		Within-Lot		Between-Lot		Within-Laboratory Precision	
Sample ID	**N**	**Mean (CU)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**	**SD (CU)**	**CV (%)**
Sample 1	75	10.9	0.4	3.7%	0.8	7.7%	0.9	8.5%	0.2	1.8%	1.0	8.7%
Sample 2	75	15.1	0.3	2.2%	0.9	5.6%	0.9	6.0%	1.1	7.2%	1.4	9.4%
Sample 3	75	22.8	0.7	3.0%	1.3	5.8%	1.5	6.5%	0.8	3.6%	1.7	7.4%
Sample 4	75	22.3	0.7	3.0%	1.4	6.1%	1.5	6.8%	0.2	0.8%	1.5	6.8%
Sample 5	75	53.8	2.4	4.4%	2.9	5.3%	3.7	6.9%	1.7	3.1%	4.1	7.6%
Sample 6	75	122.6	3.3	2.7%	7.0	5.7%	7.8	6.3%	6.0	4.9%	9.8	8.0%
Sample 7	75	130.3	2.9	2.2%	9.3	7.1%	9.7	7.5%	2.6	2.0%	10.1	7.7%
Sample 8	75	349.8	13.5	3.8%	19.8	5.7%	24.0	6.8%	6.9	2.0%	24.9	7.1%

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ)

The LoD of the QUANTA Flash HMGCR assay is 0.2 CU, which is below the analytical measuring range of the assay. It was determined by using two reagent lots, consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 240 determinations, with 60 measurements on blank samples and 60 measurements of low level samples, per reagent lot. The LoB is 0.0 CU (354 RLU).

Four low level samples were tested in replicates of five on two reagent lots, once per day, for 3 days, obtaining 30 data points per sample to generate data used to calculate the LoQ for the QUANTA Flash HMGCR assay. The LoQ was determined by calculating the total imprecision of each sample.
Acceptance criteria: Total imprecision CV% <20%.
The LoQ for the assay has been found to be at 1.4 CU. Even though the LoQ has been found to be at 1.4 CU, the AMR of the QUANTA Flash HMGCR will start at 1.5 CU.

Analytical Measuring Range (AMR)

QUANTA Flash HMGCR: 1.5 CU – 550.0 CU

Auto-rerun function and reportable results

The BIO-FLASH software has an auto-rerun option available. If this option is selected, the instrument will automatically rerun any sample that has a result of >550.0 CU after further diluting it by 20 fold, thereby bringing the measured value within the AMR. The final result will be calculated by the software by taking into account the additional dilution factor. As the highest value that can be directly measured is 550.0 CU, the highest value that can be reported is 11,000.0 CU.

High concentration hook effect

To assess hook effect, measurement signal in relative light units (RLU) was examined by performing serial dilutions of two high positive samples (with results above the AMR when tested as neat samples). RLU values showed increase with increasing antibody concentrations above the AMR, thereby confirming that high positive specimens above the AMR do not show hook effect up to 5,193.6 CU (theoretical value calculated using the highest value in the AMR and its dilution factor) in the QUANTA Flash HMGCR assay.

Linearity

The linearity of the AMR of the QUANTA Flash HMGCR was evaluated by a study according to CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach;

Approved Guideline. 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. The dilutions were assayed in duplicates. Results were analyzed according to the guideline performing regression analysis and identifying the best fitting polynomial.
Acceptance criteria:
- Best fitting polynomial is a linear one, otherwise, the difference between the best-fitting nonlinear and linear polynomial is less than 15% or ±3 CU for negative samples (allowable nonlinearity).
All samples have been found that the best fitting polynomial is a linear one except Sample 3, where the best fitting polynomial found was a second order polynomial. The nonlinearity for Sample 3 ranged from -0.4 CU to 0.6 CU, fulfilling the acceptance criteria.

All four samples showed dilution linearity individually and in combination.

Serum Samples	Test Range (CU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	610.2 to 61.0	0.97 (0.92 to 1.01)	3.9 (-3.5 to 11.3)	1.00	98.5%
2	138.0 to 13.8	0.98 (0.95 to 1.01)	-0.1 (-1.3 to 1.05)	1.00	97.7%
3	21.5 to 2.2	0.94 (0.91 to 0.98)	0.0 (-0.2 to 0.2)	1.00	94.0%
4	10.4 to 1.0	1.03 (0.99 to 1.06)	0.0 (-0.1 to 0.1)	1.00	103.2%
Combined	610.2 to 1.0	0.98 (0.96 to 0.99)	0.1 (0.0 to 0.2)	1.00	98.4%

These data demonstrate the linearity of the analytical measuring range (1.5 CU – 550.0 CU) of the QUANTA Flash HMGCR assay.

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. A first set of four human serum specimens, one high positive, one moderately positive, one near the cutoff and one negative sample were tested using the following interfering substances (bilirubin, hemoglobin, triglycerides, cholesterol, human IgG and rheumatoid factor IgM). Additionally a second set of four human serum specimens, one moderately positive, one low positive, one near the cutoff and one negative sample were tested using the following interfering substances (atorvastatin, simvastatin, coenzyme Q, pyrroloquinoline quinone and methylprednisolone). All interferents were spiked at three different concentration levels into every specimen in 10% of total specimen volume, and 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 (10% of total sample volume). Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 15% of the cut-off (±3 CU) difference, whichever is greater.

No interference was detected with bilirubin up to 1 mg/mL (recovery: 95.5% to 106.7%), hemoglobin up to 2 mg/mL (recovery: 85.2% to 102.4%), triglycerides up to 1000 mg/dL (recovery: 87.9% to 99.5%), cholesterol up to 332.5 mg/dL (recovery: 90.1% to 101.1%), human IgG up to 35 mg/mL (recovery: 97.5% to 113.9%), rheumatoid factor IgM up to 153.4 IU/mL (recovery: 88.7% to 100.9%), atorvastatin up to 600 ng/mL (recovery: 100.0% to 105.2%), simvastatin up to 600 ng/mL (recovery: 94.4% to 104.7%), coenzyme Q up to 0.72 mg/mL (recovery: 105.0% to 110.8%), pyrroloquinoline quinone up to 24 µg/mL (recovery: 100.8% to 104.6%) and methylprednisolone up to 36 µg/mL (recovery: 100.4% to 111.8%).

Sample Stability and Handling

Four samples, encompassing negative, around the cut-off, moderate positive and high positive samples were tested in duplicates for up to 14 days while stored at 2-8°C, up to 48 hours while stored at room temperature, and after repeated freeze/thaw cycles up to 3 cycles. Results were compared to those obtained on control samples (day zero, stored at 2-8°C).

Acceptance criteria: 85-115% average recovery.

All samples fulfilled the acceptance criteria at each time point for each condition. Based on these result, we recommend that samples are stored up to 48 hours at room temperature, up to 14 days at 2-8°C, and can be subjected to up to 3 freeze/thaw cycles (when samples are stored at or below -20°C).

Reagent Stability

Shelf life

To establish the initial claim for shelf life, accelerated stability studies were performed for 3 weeks at 37 °C, where one week is equal to six months at 5 ± 3°C.
Accelerated stability testing was performed on each of the following sealed components which are exclusive of the QUANTA Flash HMGCR to establish initial stability claim:

- HMGCR beads (3 Lots)

Each week a new sealed component was placed in the incubator, and all components were tested at the end of the experiment together with the one that was stored at 5 ± 3°C. The recovery of the measured values was calculated for each time point (compared to those obtained with 5 ± 3°C stored reagent). All calculations were performed by comparing results of sealed components stored at 5 ± 3°C (control) to those stored at 37 ± 3°C (test) for 1, 2 and 3 weeks, where one week is equal to six months at 5 ± 3°C. Linear regression analysis was performed between recovery values and the number of days.

Acceptance criteria for one year preliminary expiration dating:

With regression analysis, the lower and upper 95% CI interval of the regression line is between 80% and 120% recovery at day 14.

All components tested fulfilled the acceptance criteria above, so one year expiration dating was assigned to each component

In-use (onboard) stability

Reagent Cartridge

To establish the in-use stability of the QUANTA Flash HMGCR reagent cartridge, two lots of reagent cartridge were tested with 4 to 6 serum specimens (with different reactivity levels). The specimens were tested periodically for a minimum of 61 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting percent recovery against the number of days. The claim was established using the following criteria (using the one that is fulfilled first):

- The stability claim is established at the actual measurement day proceeding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- At the actual measurement day preceding the day when ≥2% of the recovery data, (3 data points) is ≤75% or ≥125% recovery.

The onboard stability results are as follows:

Lot RP0004: 62 days

Lot 171325: 61 days

Using these criteria, the in-use (onboard) stability of the QUANTA Flash HMGCR reagent cartridge was set at 60 days.

Real time stability

Real time stability testing has been scheduled to be performed every six months on the QUANTA Flash HMGCR Reagents kit, to verify the one year expiration that was assigned based on accelerated stability studies. At the time of the submission, results were available up to 13 months.

For reagent cartridge, a negative sample (Negative Control), a low positive sample (Positive Control) and a high positive sample were tested in replicates of 6 (replicates of 9 at time zero) at each time point.

- Acceptance criteria: results should fall within their respective ranges.

All results to date were within the acceptance limits.

Cut-off, reference range

QUANTA Flash HMGCR:

Negative	<20 CU
Positive	≥20 CU

The reference population for establishing the reference interval for the QUANTA Flash HMGCR assay consisted of 145 subjects:

Sample Group	N
Apparently healthy donors	23
Infectious Disease Controls (HBV, HCV, Syphilis)	22
Scleroderma Controls	18
Systemic Lupus Erythematosus Controls	20
End Stage Renal Disease Controls	48
False Positive Cohort (high reactivity samples)	14

All specimens were the same matrix (human serum) as specified in the Intended Use. All specimens were unaltered. The cut-off was established in accordance to CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition. The Analyse-it for Excel software was used to make the calculations. The distribution of the results was non-normal (Shapiro-Wilk $p<0.0001$), so the non-parametric percentile method was used.

Additionally, twelve diagnosed idiopathic inflammatory myopathy (IIM) patient specimens were assayed to aid in the determination of the cutoff. Based on the distribution of result values in these (known) positive samples, the cutoff was established at 30,000 RLU to ensure optimal differentiation between negatives and positives samples. The cut-off was established at a value greater than the 99th percentile of the control results (30,000 RLU) and it was assigned a value of 20 CU.

Clinical performance characteristics

Clinical sensitivity, specificity

Based on the revised EULAR/ACR classification criteria[1], immune mediated necrotizing myopathies (IMNM) represent a subgroup of patients with idiopathic inflammatory myopathies (IIM). Further sub-classification defined IMNM as subform of polymyositis (PM). Due to the low number of cases, IMNM could not be further separated into a distinct category. Consequently, we used IIM as target population to calculate sensitivity of anti-HMCGR antibodies. However, we also provide prevalence data of anti-HMGCR antibodies in the different subforms, including IMNM as defined by the European Neuromuscular Center criteria (ENMC) 2003 for IMNM. As inclusion criteria for IIM, we followed the EULAR/ACR classification criteria and applied a score of 5.5 (not considering biopsy).

A cohort of characterized samples, none of which were used for establishing the reference range, was used to validate the clinical performance of the QUANTA Flash HMGCR. A total of 723 characterized samples were included in the Validation Set for the QUANTA Flash HMGCR. All samples were run on the QUANTA Flash HMGCR.

The distribution of the cohort and the 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 of the QUANTA Flash HMGCR assay using IIM samples as target population.

Clinical Performance N=723		QUANTA Flash HMGCR		
		Positive	Negative	Total
Diagnosis	IIM	65	192	257
	Controls	1	465	466
	Total	66	657	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%)

Expected values

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.

Comparison with predicate device

The predicate device used for this submission is the QUANTA Flash Jo-1, which was selected to have an equivalent intended use to the QUANTA Flash HMGCR.

Despite the fact that the two assays aid in the diagnosis of idiopathic inflammatory myopathies (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.

References

1. Bottai M, Tjarnlund A, Santoni G, Werth VP, Pilkington C, de VM *et al*.: **EULAR/ACR classification criteria for adult and juvenile idiopathic inflammatory myopathies and their major subgroups: a methodology report.** *RMD Open* 2017, **3:** e000507.