DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

September 6, 2017

INOVA DIAGNOSTICS, INC.
Ronda Elliott
VP, Quality Systems and RA
9900 Old Grove Road
San Diego, CA 92131

Re: K163538
Trade/Device Name: Quanta Flash® Lkm-1 Reagents, Quanta Flash® Lkm-1 Calibrators, Quanta Flash® Lkm-1 Controls
Regulation Number: 21 CFR 866.5660
Regulation Name: Multiple autoantibodies immunological test system
Regulatory Class: II
Product Code: NBS, JIT, JJX
Dated: August 2, 2017
Received: August 7, 2017

Dear Ms. Elliott:

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 Parts 801 and 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.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. 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 the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Kelly Oliner -S

For
Leonthena Carrington, MBA, MS, MT (ASCP)
Division 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: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K163538

Device Name

QUANTA Flash LKM-1, QUANTA Flash LKM-1 Calibrators, QUANTA Flash LKM-1 Controls

Indications for Use *(Describe)*

QUANTA Flash LKM-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-liver/kidney microsome type 1 antibodies in human serum. The presence of anti-liver/kidney microsome type 1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of autoimmune hepatitis type 2.

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

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

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.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number."

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® LKM-1 Reagents
QUANTA Flash® LKM-1 Calibrators
QUANTA Flash® LKM-1 Controls

Scientific contact:
Roger Albesa, Supervisor, Research and Development
Inova Diagnostics, Inc.
9900 Old Grove Road, San Diego, CA, 92131
Phone: 858-586-9900 x1391
Fax: 858-863-0025
Email: ralbesa@inovadx.com

Quality Systems contact:
Ronda Elliott, VP, Quality Systems and RA
Inova Diagnostics, Inc
9900 Old Grove Road, San Diego, CA, 92131
Phone: 858-586-9900/1381
Fax: 858-863-0025
Email: relliot@inovadx.com

Device name (assay kit):

Proprietary name:	QUANTA Flash® LKM-1 Reagents
Common name:	Anti-LKM-1 Chemiluminescent Immunoassay
Classification name:	autoantibodies, lkm-1(liver/kidney microsome, type 1)

Regulation Description	Multiple autoantibodies immunological test system
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	NBS
Regulation Number	866.5660

Device Class	2	
Device name (Calibrators):	Proprietary name:	QUANTA Flash® LKM-1 Calibrators
	Common name:	LKM-1 Calibrators
	Classification name:	Calibrator, secondary
Regulation Description	Calibrator	
Regulation Medical Specialty	Clinical Chemistry	
Product Code	JIT	
Regulation Number	862.1150	
Device Class	2	
Device name (Controls):	Proprietary name:	QUANTA Flash® LKM-1 Controls
	Common name:	LKM-1 Controls
	Classification name:	single (specified) analyte controls (assayed and unassayed)
Regulation Description	Quality control material (assayed and unassayed)	
Regulation Medical Specialty	Clinical Chemistry	
Product Code	JJX	
Regulation Number	862.1660	
Device Class	1 (reserved)	

Predicate device

QUANTA Lite® LKM-1 ELISA, 510(k) number: k000535. Date declared: June 7, 2000.

Device description

The QUANTA Flash LKM-1 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 LKM-1 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Recombinant cytochrome P450 2D6 (LKM-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. Serum samples are diluted 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 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 (Fe(III)coproporphyrin in sodium hydroxide solution) and Trigger 2 (urea-hydrogen peroxide in sodium chloride solution) 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-LKM-1 antibodies bound to the corresponding beads.

For quantitation, the QUANTA Flash LKM-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 LKM-1 Calibrators. Based on the results obtained with the three Calibrators included in the Calibrator Set (sold separately), an instrument specific Working Curve is created, which is used to calculate chemiluminescent units (CU) from the instrument signal (RLU) obtained for each sample.

The QUANTA Flash LKM-1 Reagents kit contains the following materials:

One (1) QUANTA Flash LKM-1 Reagent Cartridge
One (1) vial of Resuspension buffer
One (1) Transfer pipette

The QUANTA Flash LKM-1 reagent cartridge contains the following reagents for 50 determinations:

a. LKM-1 coated paramagnetic beads, lyophilized.
b. Assay buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
c. Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.

The QUANTA Flash LKM-1 Calibrators kit contains two vials each of Calibrator 1, Calibrator 2, and Calibrator 3:
QUANTA Flash LKM-1 Calibrators:

- QUANTA Flash LKM-1 Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to LKM-1 in stabilizers and preservatives.

- QUANTA Flash LKM-1 Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to LKM-1 in stabilizers and preservatives.
- QUANTA Flash LKM-1 Calibrator 3: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to LKM-1 in stabilizers and preservatives.

The QUANTA Flash LKM-1 Controls kit contains two vials of Negative Control and two vials of Positive Control:
QUANTA Flash LKM-1 Controls:

- QUANTA Flash LKM-1 Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to LKM-1 in stabilizers and preservatives.
- QUANTA Flash LKM-1 Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to LKM-1 in stabilizers, and preservatives.

Intended use(s)

QUANTA Flash LKM-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-liver/kidney microsome type 1 antibodies in human serum. The presence of anti-liver/kidney microsome type 1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of autoimmune hepatitis type 2.

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

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

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash LKM-1 Reagents, the QUANTA Flash LKM-1 Calibrators and the QUANTA Flash LKM-1 Controls have the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash LKM-1 Reagents

Similarities		
Item	**QUANTA Flash LKM-1**	**Predicate Device**
Intended use	QUANTA Flash LKM-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-liver/kidney microsome type 1 antibodies in human serum. The presence of anti-liver/kidney microsome type 1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of autoimmune hepatitis type 2	QUANTA Lite LKM-1 is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of LKM-1 antibodies in human serum. The presence of LKM-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of autoimmune hepatitis type 2.
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Antigen	recombinant	recombinant
Shelf life	One year	One year
Sample type	Serum	Serum

Differences		
Item	**QUANTA Flash LKM-1**	**Predicate Device**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well polystyrene plate
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Calibration	Lot specific Master Curve + three calibrators (sold separately)	LKM-1 ELISA Low Positive (single calibrator) - (Included in the kit)
Units	CU (Chemiluminescent units) (arbitrary)	Units (arbitrary)

QUANTA Flash LKM-1 Calibrators

Item	QUANTA Flash LKM-1 Calibrators	Predicate Device
Intended use	QUANTA Flash LKM-1 Calibrators are intended for use with the QUANTA Flash LKM-1 Reagents for the determination of IgG anti-LKM-1 autoantibodies 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.
Analyte	Anti-LKM-1 autoantibodies	Anti-LKM-1 autoantibodies
Method	QUANTA Flash LKM-1 chemiluminescent immunoassay	QUANTA Lite LKM-1 ELISA
Matrix	Human serum, stabilizer, and preservative	Human serum, buffer, protein stabilizer, and preservative
Units	CU (Chemiluminescent units) (arbitrary)	units (arbitrary)
Physico-chemical characteristics	Liquid, prediluted, ready to use	Liquid, prediluted, ready to use
Storage	2-8 ^{o}C	2-8 ^{o}C
Shelf life	One year	One year

QUANTA Flash LKM-1 Controls

Item	QUANTA Flash LKM-1 Controls	Predicate Device
Intended use	QUANTA Flash LKM-1 Controls are intended for use with the QUANTA Flash LKM-1 Reagents for quality control in the determination of IgG anti-LKM-1 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-LKM-1 autoantibodies	Anti-LKM-1 autoantibodies
Method	QUANTA Flash LKM-1 chemiluminescent immunoassay	QUANTA Lite LKM-1 ELISA
Matrix	Human serum, stabilizers, and preservative	Human serum, buffer, protein stabilizer, and preservative
Units	CU (Chemiluminescent units) (arbitrary)	units (arbitrary)
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (negative and positive)	3 (ELISA negative, low positive and high positive)
Storage	2-8 ^{o}C	2-8 ^{o}C
Shelf life	One year	One year

Analytical performance characteristics

Quantitation and units of measure

For quantitation, the QUANTA Flash LKM-1 assay utilizes a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash LKM-1 consists of 5 Standards. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of LKM-1 Standards:

Material	Assigned Value
LKM-1 Master Curve Standard 1	0.0 CU
LKM-1 Master Curve Standard 2	6.4 CU
LKM-1 Master Curve Standard 3	25.6 CU
LKM-1 Master Curve Standard 4	102.4 CU
LKM-1 Master Curve Standard 5	409.8 CU

Value assignment and traceability of Calibrators and Controls

The QUANTA Flash LKM-1 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-LKM-1 antibodies with stabilizer and preservative. The human serum is obtained from commercial sources and it is tested for markers of infectious substances.
The target CU is achieved through trial dilutions on small scale. Once a dilution is selected, the Calibrators and Control 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 5 to obtain a minimum of 10 data points to determine final value assignment.

Calibrator and Control values are directly traceable to the in-house Standards that are used to create the Master Curves for the QUANTA Flash LKM-1 assay.

LKM-1 Calibrators and Controls with target manufacturing values:

Material	Manufacturing Target Value	Manufacturing Target Range
LKM-1 Calibrator 1	10 CU	8 – 12 CU
LKM-1 Calibrator 2	100 CU	80 – 120 CU
LKM-1 Calibrator 3	325 CU	300 – 350 CU
LKM-1 Negative Control	10 CU	8 – 12 CU
LKM-1 Positive Control	50 CU	40 – 60 CU

Precision

The precision of the QUANTA Flash LKM-1 assay was evaluated on 8 samples containing various concentrations of anti-LKM-1 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 total imprecision (within-laboratory precision) were calculated.
Acceptance criteria: Total %CV: < 12%
Results are summarized in the Table below.

QUANTA Flash LKM-1			Repeatability		Between-Run		Between-Day		Total Imprecision	
Sample ID	N	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	80	9.2	0.59	6.4%	0.00	0.0%	0.12	1.3%	0.60	6.5%
2	80	44.7	1.07	2.4%	1.62	3.6%	0.71	1.6%	2.07	4.6%
3	80	16.5	0.52	3.2%	0.00	0.0%	0.38	2.3%	0.64	3.9%
4	80	17.5	0.42	2.4%	0.38	2.1%	0.26	1.5%	0.62	3.5%
5	80	14.7	0.29	2.0%	0.21	1.5%	0.55	3.8%	0.66	4.5%
6	80	101.1	2.01	2.0%	3.93	3.9%	2.23	2.2%	4.94	4.9%
7	80	201.4	7.21	3.6%	9.36	4.6%	12.4	6.2%	17.1	8.5%
8	80	353.7	16.6	4.7%	14.4	4.1%	17.9	5.1%	28.4	8.0%

Reproducibility Studies

Reproducibility between sites (instruments)

Five 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.

QUANTA Flash LKM-1			Between Site Reproducibility	
Sample ID	Number of replicates	Mean (CU)	SD (CU)	CV (%)
Sample 1	75	10.7	0.84	7.8%
Sample 2	75	22.0	1.06	4.8%
Sample 3	75	30.1	1.69	5.6%
Sample 4	75	108.9	8.86	8.1%
Sample 5	75	343.2	15.8	4.6%

Reproducibility between lots

Four samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, using three different lots. 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.

QUANTA Flash LKM-1			Between Lot Reproducibility	
Sample ID	**Number of replicates**	**Mean (CU)**	**SD (CU)**	**CV (%)**
Sample 1	75	20.9	0.81	3.9%
Sample 2	75	28.3	1.13	4.0%
Sample 3	75	102.3	3.73	3.7%
Sample 4	75	352.9	40.9	11.6%

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

The LoD of the QUANTA Flash LKM-1 assay is 0.26 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 0.14 CU.

The data generated from the LoD testing was used to calculate the LoQ for the QUANTA Flash LKM-1 assay. The LoQ was determined by calculating the total error (TE) of each sample per reagent lot using the Westgard model: TE = $|bias| + 2S$, where S= standard deviation.

The TE was then compared to the predefined accuracy goal (<25%). The LoQ for the assay is 1.6 CU, which has been set as the lower limit of the analytical measuring range.

Analytical Measuring Range (AMR)

QUANTA Flash LKM-1: 1.6 CU – 400.0 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 >400.0 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 400.0 CU, the highest value that can be reported is 8000.0 CU.

High concentration hook effect

To assess hook effect, measurement signal (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 analyte concentrations, thereby confirming that high positive specimens above the AMR do not show hook effect up to 13,812.8 CU (theoretical value calculated using the highest value in the Working Curve and its dilution factor) in the QUANTA Flash LKM-1 assay. It is worth to remark that when a sample is run using the auto-rerun function it is diluted 1:20 prior to be in contact with the bead or the tracer, so at that point it is not different from another sample yielding results in the AMR, except that at the end the result is multiplied by 20 before reported.

	Sample 1			Sample 2		
	RLU	Reported Result	Calculated Result	RLU	Reported Result	Calculated Result
Dilution	Average	CU	CU	Average	CU	CU
1:1	465801	>409.8	13812.8	210638	>409.8	743.2
1:2	442021	>409.8	6906.4	164386	392.9	392.9
1:4	410251	>409.8	3453.2	101984	172.7	172.7
1:8	312917	>409.8	1726.6	60402	86.8	86.8
1:16	220136	>409.8	863.3	34402	45.7	45.7
1:32	159396	368.5	368.5	18926	24.2	24.2
1:64	103137	175.6	175.6	8949	11.2	11.2
1:128	57650	82.1	82.1	5002	6.2	6.2
1:256	26765	34.8	34.8	3269	3.9	3.9

Linearity

The linearity of the AMR was evaluated by a study according to CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Six serum samples with various anti-LKM-1 antibody concentrations were diluted with negative serum in 10% increments (from 0% to 90% negative serum) to obtain values that cover the 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 20%, or ± 4 CU, whichever is greater (allowable nonlinearity).

Sample 1 only had 5/10 dilutions within the AMR of the assay and did not fulfill acceptance criteria.

The remaining five specimens fulfilled the acceptance criteria, showing good linearity in their respective ranges.

Sample	Test Range (CU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
2	158.2 to 15.8	0.97 (0.94 to 1.00)	4.8 (1.5 to 8.1)	1.00	104.4%
3	20.9 to 2.1	0.98 (0.95 to 1.01)	0.3 (-0.2 to 0.7)	1.00	102.3%
4	16.6 to 1.7	1.08 (1.03 to 1.13)	-0.8 (-1.3 to -0.3)	1.00	93.8%
5	229.0 to 22.9	1.00 (0.93 to 1.06)	-5.9 (-15.0 to 3.3)	0.99	93.8%
6	411.2 to 41.1	0.96 (0.91 to 1.02)	-4.7 (-19.6 to 10.2)	0.99	94.2%
All samples	411.2 to 1.7	0.95 (0.94 to 0.97)	1.0 (-1.2 to 3.2)	1.00	97.7%

These data demonstrate the linearity of the analytical measuring range (1.6 CU – 400.0 CU) of the QUANTA Flash LKM-1 assay.

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Six specimens, one high positive (290.9 CU), one moderately positive (124.8 CU), one low positive (28.9 CU), two near the cutoff (17.0 and 25.1 CU), and one negative (12.3 CU) samples were tested. Interfering substances (hemoglobin, bilirubin, triglycerides, cholesterol, human IgG and RF IgM) were spiked into every specimen at three different concentrations in 10% of total specimen volume, and the resulting samples were assessed in triplicates with the QUANTA Flash LKM-1 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 ± 4 CU difference, whichever is greater.

No interference was detected with bilirubin up to 1 mg/mL (recovery: 95% to 105%), hemoglobin up to 2 mg/mL (recovery: 94% to 101%), triglycerides up to 1000 mg/dL (recovery: 94% to 104%), cholesterol up to 332.5 mg/dL (recovery: 94% to 109%), human IgG up to 35 mg/mL (recovery: 94% to 105%), and rheumatoid factor IgM up to 153.4 IU/mL (recovery: 98% to 109%).

Additionally, four samples, one negative (2.3 CU), two around the cutoff (19.9 and 21.1 CU) and one low positive (31.1 CU) were tested to assess the interference caused by corticosteroids (Prednisone), Azathioprine and interferon alpha by using the very same methodology described above.

No interference was detected with corticosteroids (Prednisone) up to 0.3 mg/L (recovery: 95.0% to 99.5%), Azathioprine up to 2.99 mg/L (recovery: 98.2% to 102.5%) and interferon alpha up to 0.33 mg/L (recovery: 97.2% to 102.1%).

Sample Stability and Handling

Four samples, encompassing negative, around the cut-off, and 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 of the QUANTA Flash LKM-1 to establish initial stability claim:

- LKM-1 beads (3 Lots)
- Resuspension Buffer 7 (3 Lots)
- Calibrators 1, 2 and 3 (3 Lots)
- Negative and Positive controls (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, 3, and (optional) 4 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:

- Beads and Resuspension Buffer:

With regression analysis, the lower and upper 95% CI interval of the regression line is between 85% and 115% recovery at day 14, and no individual data point has ≤75% or ≥125% recovery at day 14.

- Controls and Calibrators:
With regression analysis, the lower and upper 95% CI interval of the regression line is between 90% and 110% recovery at day 14, and no individual data point has ≤80% or ≥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

Calibrators

Onboard stability claim: 4 calibrations, or 8 hours onboard
During assessing onboard stability, Calibrators were placed uncapped, onboard the instrument, and calibration was performed altogether five times over 9 hours. Controls and a panel of characterized patient specimens were run on each calibration curve.
Calibrators are considered stable if all four calibrations performed in the 8 hour period are successful, mean Calibrator RLU recovery values for the first 4 calibrations are between 90% and 110% compared to the first use, and Control/patient panel CU recovery values are between 85% and 115% of those obtained on the first calibration curve.
The first four calibrations performed in the 8 hour period were considered valid by the software. The calibrators yielded average RLU recovery values ranging from 100.0% to 107.1%. The Control/patient panel CU recovery ranged from 91.0% 100.0%. This supports the claim that calibrators can be used for up to 4 calibrations over an 8 hour period.

Controls

Onboard stability claim: up to 15 uses, at 10 minutes onboard per use
During assessing onboard stability, 2 vials of each Control were assayed once a day for a total of 20 runs. The first run was used to establish baseline value, by running each vial in duplicate, and then additional 19 runs were performed, by running each vial in singleton. During runs, the Controls were left uncapped, onboard the instrument for 15±1 minutes per run. When not in use, the controls were capped, and stored at 5 ± 3°C.
Percent recovery of each value was calculated compared to the baseline value. Controls are considered stable when all values run within their established range, and the linear regression line obtained by plotting %recovery values against the number of runs stays between 85% and 115% at run 15.
All controls ran within their respective acceptable ranges for all runs. Moreover, the regression line remained between 85% and 115% at run 15 for both Controls. These results support the claim that controls can be used for up to 15 times, at 10 minutes per use.

Reagent Cartridge

To establish the in-use stability of the QUANTA Flash LKM-1 reagent cartridge, two lots of cartridges were tested with up to 5 serum specimens (with different reactivity levels). The specimens were tested periodically a minimum of 62 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 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 data points or ≥2% of the recovery data (whichever is greater) is ≤ 75% or ≥ 125% recovery.

The onboard stability results of the two lots are as follows:
RP0002: 62 days
151001: 64 days
Using these criteria, the in-use (onboard) stability of LKM-1 reagent cartridge was set at 60 days.

Real time stability

Real time stability testing has been scheduled to be performed every three or six months on the reagent cartridge, Calibrators and Controls, 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 12 months for reagent cartridge, and up to 6 months on Calibrators and Controls.

For reagent cartridge, a negative sample (Negative Control), a low positive sample (Positive Control) and a high positive sample (a sample which is part of the QC panel) were tested in replicates of 6 (replicates of 9 at time zero) at each time point. The QC panel is a group of characterized patient samples with target values, used by the QC Department for reagent release and QC.
- Acceptance criteria: results should fall within their respective ranges.

Calibrators were tested in triplicates on a calibrated cartridge at each time point. Averages of the triplicates were compared to the value that was assigned to the Calibrators at release.
- Acceptance criteria: % recovery of the average of the triplicates is between 85% and 115%, and %CV of the triplicates is < 10%.

Controls were tested in replicates of 6 (replicates of 9 at time zero) on a calibrated cartridge at each time point. Individual values were compared to the values that were assigned to the Controls at release.
- Acceptance criteria: results should fall within their acceptable ranges as were established at the release of the Controls.

All results to date were within the acceptance limits.

Cut-off, reference range

QUANTA Flash LKM-1:

Negative	<20 CU
Positive	≥20 CU

The reference population for establishing the reference interval for the LKM-1 assay consisted of 242 subjects:

Sample Group	N
Apparently healthy blood donors	120
Celiac disease	20
Rheumatoid arthritis	31

Infectious disease (HBV, HCV and HIV)	30
Liver diseases (AIH-1 and PBC)	39
Myositis	2

All specimens were the same matrix (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. The 99th percentile of the remaining obtained values was calculated as 5,098 RLU.

Additionally, diagnosed AIH-2 patient specimens were assayed to aid in the determination of the cutoff. Based on the distribution of RLU values in these (known) positive samples, the cutoff was increased to 25,000 RLU to ensure optimal differentiation between negatives and positives and minimize risk of false positive samples, and a 20 CU value was assigned to this RLU value. It was found that none of the AIH-2 samples reported results <70,000 RLU.

Clinical performance characteristics

Clinical sensitivity, specificity

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 LKM-1. A total of 633 characterized samples were included in the Validation Set for the QUANTA Flash LKM-1. All samples were run on the QUANTA Flash LKM-1. The distribution of the cohort and the LKM-1 positivity rate is in the Table below:

Patient Group	N	N Positive	% Positive
Autoimmune Hepatitis type 1 (AIH-1)	51	0	0.0%
Primary Biliary Cirrhosis	75	0	0.0%
Primary Sclerosing Cholangitis	33	0	0.0%
Liver Cancer	10	0	0.0%
Alcoholic Liver Disease	35	0	0.0%
Celiac Disease	43	0	0.0%
Hepatitis B virus	31	0	0.0%
Hepatitis C virus*	30	12	40.0%
Syphilis	10	0	0.0%
Ulcerative Colitis	26	0	0.0%
Crohn's Disease	14	0	0.0%
Limited scleroderma	15	0	0.0%
Dermatomyositis	7	0	0.0%
Systemic Lupus Erythematosus	33	0	0.0%
Sjogren’s Syndrome	4	0	0.0%
Polymyositis	1	0	0.0%
Autoimmune Endocrine Disease	60	0	0.0 %
Rheumatoid Arthritis	30	0	0.0 %

Patient Group	**N**	**N Positive**	**% Positive**
Non-Alcoholic Fatty Liver Disease	30	0	0.0 %
Genetic Diseases with Hepatic Involvement	30	0	0.0 %
Skin Lesions	30	0	0.0 %
Drug-induced Liver Injuries	9	0	0.0 %
Total Controls	**607**	**12**	**2.0%**
Autoimmune Hepatitis type 2 (AIH-2)	26	20	76.9%
Total	**633**	-	-

**LKM-1 antibody positivity has been described in patients with Hepatitis C virus infection. See also Cross-Reactivity section.*

Clinical sensitivity and specificity of the QUANTA Flash LKM-1.

Clinical Analysis (N=633)		QUANTA Flash LKM-1			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	AIH-2	20	6	26	Sensitivity: 76.9% (56.4 - 91.0%)
	Controls	12	595	607	Specificity: 98.0% (96.6 – 99.0%)
	Total	32	601	633	

Expected values

The expected value in the normal population is "negative". Anti-LKM-1 antibody levels were analyzed using the QUANTA Flash LKM-1 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 LKM-1. The mean concentration was < 1.6 CU, and the values ranged from <1.6 to 3.6 CU.

Comparison with predicate device

Samples for the method comparison analysis included 334 samples from the clinical validation study, plus additional 10 pooled samples that yield results around the cutoff on the QUANTA Flash LKM-1 assay. These 334 samples were tested on both the QUANTA Flash LKM-1 and on the predicate ELISA. The data are presented in six tables showing the predicate equivocal results calculated as either negative or positive: the first and second tables shows all 334 samples; the third and fourth tables shows only the samples within the AMR (auto rerun function enabled); the fifth and sixth tables shows only the samples within the AMR (auto rerun function disabled):

Table 1: Method Comparison, all samples, samples in the equivocal range of the predicate device treated as negative samples:

Method Comparison – All Samples (N=344) Equi. Range PD = Neg		QUANTA Flash LKM-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	311	3	314	Neg. Agreement: 99.0% (97.2% – 99.7%)
	Positive	1	29	30	Pos. Agreement: 96.7% (83.3% – 99.4%)
	Total	312	32	344	Total Agreement: 98.8% (97.0% – 99.5%)

Table 2: Method Comparison, all samples, samples in the equivocal range of the predicate device treated as positive samples:

Method Comparison – All Samples (N=344) Equi. Range PD = Pos		QUANTA Flash LKM-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	307	1	308	Neg. Agreement: 99.7% (98.2% – 99.9%)
	Positive	5	31	36	Pos. Agreement: 86.1% (71.3% – 93.9%)
	Total	312	32	344	Total Agreement: 98.3% (96.2% – 99.2%)

Table 3: Method Comparison, samples within the AMR, Auto Rerun function enabled, samples in the equivocal range of the predicate device treated as negative samples:

Method Comparison – Within AMR (N=119) Equi. Range PD = Neg		QUANTA Flash LKM-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	90	3	93	Neg. Agreement: 96.8% (90.9% – 98.9%)
	Positive	1	25	26	Pos. Agreement: 96.2% (81.1% – 99.3%)
	Total	91	28	119	Total Agreement: 96.6% (91.7% – 98.7%)

Table 4: Method Comparison, samples within the AMR, Auto Rerun function enabled, samples in the equivocal range of the predicate device treated as positive samples:

Method Comparison – Within AMR (N=119) Equi. Range PD = Pos		QUANTA Flash LKM-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	86	1	87	Neg. Agreement: 98.9% (93.8% – 99.8%)
	Positive	5	27	32	Pos. Agreement: 84.4% (68.2% – 93.1%)
	Total	91	28	119	Total Agreement: 95.0% (89.4% – 97.7%)

Table 5: Method Comparison, samples with the AMR, Auto Rerun function disabled, samples in the equivocal range of the predicate device treated as negative samples:

Method Comparison – Within AMR (N=109) Equi. Range PD = Neg		QUANTA Flash LKM-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	90	3	93	Neg. Agreement: 96.8% (90.9% – 98.9%)
	Positive	1	15	16	Pos. Agreement: 93.8% (71.7% – 98.9%)
	Total	91	18	109	Total Agreement: 96.3% (90.9% – 98.6%)

Table 6: Method Comparison, samples with the AMR, Auto Rerun function disabled, samples in the equivocal range of the predicate device treated as positive samples:

Method Comparison – Within AMR (N=109) Equi. Range PD = Pos		QUANTA Flash LKM-1			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	86	1	87	Neg. Agreement: 98.9% (93.8% – 99.8%)
	Positive	5	17	22	Pos. Agreement: 77.3% (56.6% – 89.9%)
	Total	91	18	109	Total Agreement: 94.5% (88.5% – 97.5%)

Cross-reactivity

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on the 607 control samples that were included in the clinical validation study were assessed. These samples were from patients with autoimmune diseases that are characterized with disease specific autoantibodies, or from patients with positive infectious disease serology.

LKM-1 antibodies have been reported in patients with chronic hepatitis C virus (HCV) infection. Viral hepatitis infection, in particular HCV must be excluded for the diagnosis of AIH-2.

Out of 30 HCV samples tested, 12 samples yielded positive results for QUANTA Flash LKM-1 (40.0%). This is not new information as relevant association was already found between anti-LKM-1 antibodies and HCV. Although anti-LKM-1 autoantibodies are considered to be a specific serological marker of type 2 autoimmune hepatitis (AIH-2), patients with chronic HCV hepatitis have been described to be also LKM-1 positive. This QUANTA Flash LKM-1 assay limitation will be stated in the *Limitations of the Procedure* section of the assay's Direction Insert. Based on these results, the QUANTA Flash LKM-1 assay does not show cross-reactivity with autoantibodies other than anti-HCV antibodies.