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

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

September 5, 2017

Re: K163525
Trade/Device Name: QUANTA Flash M2 (MIT3), QUANTA Flash M2 (MIT3) Calibrators, QUANTA Flash M2 (MIT3) Controls
Regulation Number: 21 CFR 866.5090
Regulation Name: Antimitochondrial antibody immunological test system
Regulatory Class: Class II
Product Code: DBM, JIT, JJX
Dated: December 14, 2016
Received: December 15, 2016

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.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and Part 809), please contact the Division of Industry and Consumer Education (DICE) 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 (DICE) 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,

Kelly Oliner -S

For
Lea Carrington, M.S., MBA, MT
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)*
k163525

Device Name

QUANTA Flash M2 (MIT3), QUANTA Flash M2 (MIT3) Calibrators, QUANTA Flash M2 (MIT3) Controls

Indications for Use *(Describe)*

QUANTA Flash M2 (MIT3) is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-mitochondrial antibodies in human serum. The presence of anti-mitochondrial antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of primary biliary cholangitis.

QUANTA Flash M2 (MIT3) Calibrators are intended for use with the QUANTA Flash M2 (MIT3) chemiluminescent immunoassay for the determination of IgG anti-mitochondrial antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash M2 (MIT3) Controls are intended for use with the QUANTA Flash M2 (MIT3) chemiluminescent immunoassay for quality control in the determination of IgG anti-mitochondrial antibodies 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.

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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® M2 (MIT3)
QUANTA Flash® M2 (MIT3) Calibrators
QUANTA Flash® M2 (MIT3) Controls

Scientific contact:
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Phone: 858-586-9900 x 1381
Fax: 858-863-0025
email: relliot@inovadx.com

Device name (assay kit):

Proprietary name:	QUANTA Flash® M2 (MIT3)
Common name:	Anti-M2 (MIT3) Chemiluminescent Immunoassay
Classification name:	antibodies, anti-mitochondrial

Regulation Description: Anti-mitochondrial antibody immunological test system

Regulation Medical Specialty: Immunology

Review Panel: Immunology

Product Code: DBM

Regulation Number:	866.5090	
Device Class:	2	
Device name (Calibrators):	Proprietary name:	QUANTA Flash® M2 (MIT3) Calibrators
	Common name:	M2 (MIT3) 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® M2 (MIT3) Controls
	Common name:	M2 (MIT3) 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® M2 EP (MIT3) ELISA, 510(k) number: k052262

Device description

The QUANTA Flash M2 (MIT3) 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 M2 (MIT3) assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Recombinant MIT3 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-M2 (MIT3) antibodies bound to the corresponding beads.

For quantitation, the QUANTA Flash M2 (MIT3) 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 M2 (MIT3) 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 M2 (MIT3) kit contains the following materials:

One (1) QUANTA Flash M2 (MIT3) Reagent Cartridge
One (1) vial of Resuspension buffer
One (1) Transfer pipette

The QUANTA Flash M2 (MIT3) reagent cartridge contains the following reagents for 50 determinations:

a. M2 (MIT3) 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 M2 (MIT3) Calibrators kit contains two vials each of Calibrator 1, Calibrator 2, and Calibrator 3:

QUANTA Flash M2 (MIT3) Calibrators:

- QUANTA Flash M2 (MIT3) Calibrator 1: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human anti-mitochondrial antibodies in stabilizer and preservative.
- QUANTA Flash M2 (MIT3) Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human anti-mitochondrial antibodies in stabilizer and preservative.
- QUANTA Flash M2 (MIT3) Calibrator 3: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human anti-mitochondrial antibodies in stabilizer and preservative.

The QUANTA Flash M2 (MIT3) Controls kit contains two vials of Negative Control and two vials of Positive Control:

QUANTA Flash M2 (MIT3) Controls:

- QUANTA Flash M2 (MIT3) Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human anti-mitochondrial antibodies in stabilizer and preservative.
- QUANTA Flash M2 (MIT3) Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human anti-mitochondrial antibodies in stabilizer and preservative.

Intended use(s)

QUANTA Flash M2 (MIT3) is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-mitochondrial antibodies in human serum. The presence of anti-mitochondrial antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of primary biliary cholangitis.

QUANTA Flash M2 (MIT3) Calibrators are intended for use with the QUANTA Flash M2 (MIT3) chemiluminescent immunoassay for the determination of IgG anti-mitochondrial antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

QUANTA Flash M2 (MIT3) Controls are intended for use with the QUANTA Flash M2 (MIT3) chemiluminescent immunoassay for quality control in the determination of IgG anti-mitochondrial antibodies in human serum.

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash M2 (MIT3) Reagent, the QUANTA Flash M2 (MIT3) Calibrators and the QUANTA Flash M2 (MIT3) Controls have the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash M2 (MIT3) reagent kit

Similarities		
Item	**QUANTA Flash M2 (MIT3)**	**Predicate Device**
Intended use	QUANTA Flash M2 (MIT3) is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-mitochondrial antibodies in human serum. The presence of anti-mitochondrial antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of primary biliary cholangitis.	QUANTA Lite M2 EP (MIT3) ELISA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of mitochondria antibodies in human serum. The presence of mitochondria antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of primary biliary cirrhosis.
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Antigen	recombinant	recombinant
Sample type	Serum	Serum
Shelf life	One year	One year

Differences		
Item	**QUANTA Flash M2 (MIT3)**	**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)	M2 EP (MIT3) ELISA Low Positive (single calibrator) - (Included in the kit)

QUANTA Flash M2 (MIT3) Calibrators

Item	**QUANTA Flash M2 (MIT3) Calibrators**	**Predicate Device**
Intended use	QUANTA Flash M2 (MIT3) Calibrators are intended for use with the QUANTA Flash M2 (MIT3) chemiluminescent immunoassay for the determination of IgG anti-mitochondrial antibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.	No separate intended use; calibrator is part of the kit.
Analyte	Anti-mitochondrial antibodies	Anti-mitochondrial antibodies
Method	QUANTA Flash M2 (MIT3) chemiluminescent immunoassay	QUANTA Lite M2 EP (MIT3) ELISA
Matrix	Human serum, stabilizer, and preservative	Human serum, buffer, protein stabilizer, and preservative
Unit	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 M2 (MIT3) Controls

Item	QUANTA Flash M2 (MIT3) Controls	Predicate Device
Intended use	QUANTA Flash M2 (MIT3) Controls are intended for use with the QUANTA Flash M2 (MIT3) chemiluminescent immunoassay for quality control in the determination of IgG anti-mitochondrial antibodies in human serum.	No separate intended use; controls are part of the kit.
Analyte	Anti-mitochondrial antibodies	Anti-mitochondrial antibodies
Method	QUANTA Flash M2 (MIT3) chemiluminescent immunoassay	QUANTA Lite M2 EP (MIT3) ELISA
Matrix	Human serum, stabilizers, and preservative	Human serum, buffer, protein stabilizer, and preservative
Unit	CU (Chemiluminescent units) (arbitrary)	units (arbitrary)
Physico-chemical characteristics	Liquid, ready to use	Liquid, prediluted, ready to use
Levels	2 (negative and positive)	2 (ELISA negative 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 M2 (MIT3) assay utilizes a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash M2 (MIT3) consists of 7 Standards. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of M2 (MIT3) Standards:

Material	Assigned Value
M2 (MIT3) Master Curve Standard 1	0.0 CU
M2 (MIT3) Master Curve Standard 2	6.7 CU
M2 (MIT3) Master Curve Standard 3	27.1 CU
M2 (MIT3) Master Curve Standard 4	108.6 CU
M2 (MIT3) Master Curve Standard 5	434.3 CU
M2 (MIT3) Master Curve Standard 6	1737.3 CU
M2 (MIT3) Master Curve Standard 7	3474.6 CU

Value assignment and traceability of Calibrators and Controls

The QUANTA Flash M2 (MIT3) Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-mitochondrial 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 manufactured to final scale, tested, and adjusted. Upon completion of the manufacturing process, the Calibrators and Controls are tested on at least two instruments, on at least two lots of reagent cartridge, in replicates of 10 to determine final value assignment.

Calibrator and Control values are directly traceable to the in-house Standards that are used to create the Master Curves for the QUANTA Flash M2 (MIT3) assay.

M2 (MIT3) Calibrators and Controls with target manufacturing values:

Material	Manufacturing Target Value	Manufacturing Target Range
M2 (MIT3) Calibrator 1	12 CU	10 – 14 CU
M2 (MIT3) Calibrator 2	400 CU	360 – 440 CU
M2 (MIT3) Calibrator 3	2550 CU	2450 – 2650 CU
M2 (MIT3) Negative Control	10 CU	8 – 12 CU
M2 (MIT3) Positive Control	50 CU	40 – 60 CU

Precision

The precision of the QUANTA Flash M2 (MIT3) assay was evaluated on 5 samples, along with negative and positive controls, containing various concentrations of anti-mitochondrial antibodies in accordance with CLSI EP05-A3, Evaluation of Precision 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 within run, between run, between day and total precision were calculated.

Acceptance criteria: Total %CV: < 10%

Results are summarized in the Table below.

QUANTA Flash M2 (MIT3)			Within Run		Between Runs		Between Days		Total	
Sample ID	Number of replicates	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
N	80	8.5	0.4	4.2	0.2	2.4	0.0	0.3	0.4	4.8
P	80	45.4	1.2	2.7	1.0	2.2	0.6	1.3	1.7	3.7
1	80	21.2	0.7	3.1	0.5	2.3	0.5	2.3	1.0	4.5
2	80	18.3	0.5	2.6	0.2	1.1	0.2	0.8	0.5	2.9
3	80	277.3	7.4	2.7	5.3	1.9	0.0	0.0	9.1	3.3
4	80	1007.5	39.3	3.9	25.3	2.5	15.0	1.5	49.1	4.9
5	80	2183.7	119.6	5.5	78.7	3.6	85.2	3.9	166.6	7.6

Reproducibility

Reproducibility between sites (instruments)

Nine 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: < 10%

Results are summarized in the Table below.

QUANTA Flash M2 (MIT3)			Between Site Precision (Reproducibility)	
Sample ID	Number of replicates	Mean (CU)	SD (CU)	CV (%)
1	75	9.3	0.8	9.1
2	75	11.1	0.4	3.4
3	75	22.9	1.4	6.2
4	75	30.5	1.1	3.5
5	75	53.7	2.8	5.2
6	75	404.3	31.4	7.8
7	75	617.8	50.4	8.2
8	75	939.4	59.1	6.3
9	75	2182.7	44.9	2.1

Lot to lot comparison

Reproducibility between lots

Lot to lot reproducibility study was performed according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures, by testing six samples with three different lots of reagents in five replicates for 5 days, to generate 25 data points per lot, 75 data points total for each sample.

Data were analyzed with the Analyse-it for Excel method evaluation software, between lots imprecision was calculated, and the results are summarized in the Table below. All %CV values were within the acceptance limit, 10%.

Results are summarized in the Table below.

QUANTA Flash M2 (MIT3)			Between Lot Precision (Reproducibility)	
Sample ID	N	Mean (CU)	SD (CU)	CV (%)
1	75	19.7	0.9	4.7
2	75	18.9	1.2	6.4
3	75	869.4	37.9	4.4
4	75	2186.7	191.0	8.7
5	75	8.3	0.5	6.5
6	75	42.4	1.7	4.0

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

The LoD of the QUANTA Flash M2 (MIT3) assay is 0.61 CU, which is below the analytical measuring range (AMR) 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; 60 measurements on blank samples and 60 measurements of low level samples with 2 reagent lots. The LoB is 0.43 CU.

The data generated from the LoD testing was used to calculate the LoQ for the QUANTA Flash M2 (MIT3) 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 1.2 CU, which has been set as the lower limit of the AMR.

Analytical Measuring Range (AMR)

QUANTA Flash M2 (MIT3): 1.2 CU – 3000.0 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 >3000.0 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 3000.0 CU, the highest value that can be reported is 60000.0 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 256,113.1 CU (theoretical value calculated using the highest value in the Working Curve and its dilution factor) in the QUANTA Flash M2 (MIT3) 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	Calculated Result	RLU	Calculated Result
Dilution	Average	CU	Average	CU
1:1	799996	256113.1	732727	147096.3
1:2	715723	128056.6	710978	73548.1
1:4	618923	64028.3	699656	36774.1
1:8	511752	4622.2	677796	18387.0
1:16	384064	1811.4	631528	9193.5
1:32	244576	797.7	511180	4596.8
1:64	152935	429.0	394228	1928.0
1:128	79728	208.5	251350	831.0
1:256	40556	105.8	149460	417.4
1:512	19995	53.6	94047	248.1
1:1024	9743	27.1	44318	115.4
1:2048	5152	14.6	24173	64.3
1:4096	2618	7.4	10961	30.3

Linearity

The linearity of the AMR was evaluated by a study according to CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Six serum samples with various mitochondrial 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).
All six 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
1	3373.5 to 481.9	1.08 (0.92 to 1.24)	-146.8 (-486.0 to 192.5)	0.98	98.5%
2	1496.5 to 149.7	1.01 (0.96 to 1.06)	26.0 (-21.7 to 73.7)	1.00	105.2%
3	281.5 to 28.1	1.03 (1.00 to 1.06)	-1.6 (-7.2 to 4.0)	1.00	100.8%
4	52.5 to 5.3	1.00 (0.98 to 1.03)	0.4 (-0.5 to 1.2)	1.00	102.0%
5	22.7 to 2.3	0.98 (0.94 to 1.02)	1.0 (0.4 to 1.6)	1.00	109.2%
6	11.8 to 1.2	0.96 (0.92 to 1.00)	0.1 (-0.2 to 0.4)	1.00	100.3%
All samples	3373.5 to 1.2	1.02 (1.00 to 1.04)	-2.2 (-18.7 to 14.4)	1.00	102.9%

These data demonstrate the linearity of the analytical measuring range of the QUANTA Flash M2 (MIT3) assay.

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Five specimens, one high positive (1422.5 CU), one moderately positive (392.4 CU), two near the cutoff (13.9 and 22.1 CU), and one negative (9.9 CU) sample were tested. Interfering substances (hemoglobin, bilirubin, triglycerides, cholesterol, and human IgG) 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 M2 (MIT3) assay. Additionally, four samples, one negative (11.8 CU), two near the cutoff (18.3 and 23.8 CU) and one positive (195.8 CU) samples were tested for interference with Ursodeoxycholic acid, corticosteroids (Prednisone), methotrexate, cholestyramine, colestipol, hydroxyzine, Aminotransferases (Glutamate Oxacatate Transaminase) and HDL cholesterol (high density lipoprotein). Moreover, 6 additional samples were tested for RF interference by combining them with different concentrations of a high positive RF IgM serum sample (1534 IU/mL). 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: 93% to 100%), hemoglobin up to 2 mg/mL (recovery: 94% to 101%), triglycerides up to 1000 mg/dL (recovery: 94% to 102%), cholesterol up to 332.5 mg/dL (recovery: 97% to 104%), human IgG up to 35 mg/mL (recovery: 91% to 115%, or 1.8 CU to 3.7 CU), Ursodeoxycholic acid up to 0.75 mg/mL (recovery: 97% to 104%), Prednisone up to 0.3

mg/mL (recovery: 100% to 105%), methotrexate up to 9.1 mg/mL (recovery: 96% to 103%), cholestyramine up to 18 mg/mL (recovery: 97% to 100%), colestipol up to 18 mg/mL (recovery: 98% to 104%), Hydroxyzine up to 1 mg/mL (recovery 100% to 108%), Glutamate Oxacatate Transaminase up to 0.12 IU/mL (recovery: 98% to 115%), HDL Cholesterol up to 3.5 mg/mL (recovery: 98% to 105%) and rheumatoid factor up to 153.4 IU/mL (recovery: 96% to 111%).

Cross-reactivity

To test potential cross-reactivity with autoantibodies and infection-induced antibodies, results obtained on all 417 control samples plus 14 ISSc 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. The composition of the cohort and the anti-mitochondrial positivity rate is shown in the Table below:

Patient group	N	Number positive	% positive
Autoimmune Hepatitis type 1 (AIH1)	41	2	4.9%
LKM-1 antibody positive Autoimmune Hepatitis 2 (AIH2)	28	0	0.0%
Primary Sclerosing Cholangitis (PSC)	21	0	0.0%
Celiac Disease	19	0	0.0%
Hepatitis B virus (HBV)	10	0	0.0%
Hepatitis C virus (HCV)	25	0	0.0%
Syphilis	10	0	0.0%
Ulcerative Colitis	26	0	0.0%
Crohn's Disease	10	0	0.0%
Liver Cancer (LC)	10	0	0.0%
Alcoholic liver disease (ALD)	20	0	0.0%
Idiopathic inflammatory myopathies (IIM)	8	0	0.0%
Systemic lupus erythematosus (SLE)	8	0	0.0%
Sjögren's syndrome (SS)	4	0	0.0%
Sicca syndrome	20	0	0.0 %
Type 1 Diabetes	30	0	0.0 %
Osteoporosis	28	1	3.6 %
Chronic fatigue	30	1	3.3 %
Skin	30	0	0.0 %
Drug-induced hepatotoxicity	9	0	0.0 %
Hypothyroidism	30	0	0.0 %
Limited cutaneous systemic sclerosis (lSSc)*	14	5	35.7%
Total	**431**	**9**	**2.1%**

* Literature indicates a relatively high prevalence of PBC in SSc patients, as compared to the general population.

Based on the results, the QUANTA Flash M2 (MIT3) assay does not show cross-reactivity with autoantibodies that are present in various autoimmune diseases, or with antibodies against infectious agents.

Sample Stability and Handling

Four samples, encompassing negative, around the cut-off, and positive samples were tested in duplicates for up to 21 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: 90-110% average recovery.
All samples fulfilled the acceptance criteria at each time point for each condition. Based on these results, 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 up to 4 weeks at 37°C ± 3°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 M2 (MIT3) to establish initial stability claim: the beads, the two Calibrators, and the Negative and Positive Controls. 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:
With regression analysis, the lower 95% CI interval of the regression line is ≥85% and the upper 95% CI interval is at ≤115% at day 14, and no individual data point has ≤75% or ≥125% recovery at day 14.

- Controls and Calibrators:
With regression analysis, the lower 95% CI interval of the regression line is ≥90% and the upper 95% CI interval is ≤110% at day 14, and no individual data point has ≤80% or ≥120% recovery at day 14.

Beads

Testing was performed on three lots of M2 (MIT3) coupled beads using up to 9 characterized samples with various reactivity levels.
All three lots of beads retained between 85% and 115% reactivity (considering the 95% CI) after two weeks at 37 ± 3°C, and therefore pass the acceptance criteria for one year expiration date.

Calibrators and Controls

Testing was performed on three lots of M2 (MIT3) Calibrators and Controls. All Calibrators and Controls maintained between 90% and 110% reactivity (considering the 95% CI) when stored at 37 ± 3°C for 2 weeks, and therefore pass the acceptance criteria for one year expiration dating.

In-use (onboard) stability

Calibrators

Onboard stability claim: 4 calibrations, or 8 hours onboard

During assessing on-board 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% to 110%. The Control/patient panel CU recovery ranged from 87.8% to 96.6%. 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 on-board 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 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 M2 (MIT3) reagent cartridge, two lots of cartridges were tested with up to 4 serum specimens (with different reactivity levels). The specimens were tested periodically up to 70 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:
RP0006: 70 days
151003: 60 days
Using these criteria, the in-use (onboard) stability of M2 (MIT3) reagent cartridge was set at 60 days.

Real time stability

Real time stability testing has been scheduled to be performed every three 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 9 months for reagent cartridge and Controls, while 3 months data for Calibrators will be available in January 2017.

For reagent cartridge, negative and positive controls, along with one additional sample, were tested in replicates of 9 at 0 and 6 month time points, and replicates of 3 at 3 and 9 month time points.
- Acceptance criteria: compared to the average baseline value, the percent recovery of each sample is between 80-120%. The % CV of replicates is <15% at each time point.

Calibrators will be tested in triplicates on a calibrated cartridge at each time point. Averages of the triplicates will be 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 triplicates on a calibrated cartridge at each time point. Individual values, and averages of the triplicates 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 M2 (MIT3):

Negative	<20 CU
Positive	≥20 CU

The reference population for establishing the reference interval for the M2 (MIT3) assay consisted of 180 subjects:

Sample Group	N
Apparently healthy blood donors	100
Infectious disease	30
Rheumatoid arthritis	30
Celiac disease	20

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 (Saphiro-Wilk $p<0.0001$), so the non-parametric percentile method was used. One high positive sample was excluded from the calculations as an outlier. This sample was confirmed to be true positive with the predicate device. The 99th percentile of the remaining obtained values was calculated as 3835 RLU.

Additionally, three diagnosed PBC patient specimens were assayed to aid in the determination of the cut-off. Based on the distribution of RLU values in these (known) positive samples, the cutoff was increased to 6000 RLU to ensure optimal differentiation between negatives and positives, and a 20 CU value was assigned to this RLU value. No reference patients tested positive at this cutoff level.

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 M2 (MIT3). A total of 586 characterized samples were included in the Validation Set for the QUANTA Flash M2 (MIT3). All samples were run on the QUANTA Flash M2 (MIT3). The distribution of the cohort and the M2 (MIT3) positivity rate is in the Table below:

Patient group	N	Number positive	% positive
Autoimmune Hepatitis type 1 (AIH1)	41	2	4.9%
LKM1 antibody positive Autoimmune Hepatitis 2 (AIH2)	28	0	0.0%
Primary Sclerosing Cholangitis (PSC)	21	0	0.0%
Liver Cancer (LC)	10	0	0.0%
Celiac Disease	19	0	0.0%
Hepatitis B virus (HBV)	10	0	0.0%
Hepatitis C virus (HCV)	25	0	0.0%
Syphilis	10	0	0.0%
Ulcerative Colitis	26	0	0.0%
Crohn's Disease	10	0	0.0%
Alcoholic liver disease (ALD)	20	0	0.0%
Idiopathic inflammatory myopathies (IIM)	8	0	0.0%
Systemic lupus erythematosus (SLE)	8	0	0.0%
Sjögren's syndrome (SS)	4	0	0.0%
Sicca syndrome	20	0	0.0 %
Type 1 Diabetes	30	0	0.0 %
Osteoporosis	28	1	3.6 %
Chronic fatigue	30	1	3.3 %
Skin conditions	30	0	0.0 %
Drug-induced hepatotoxicity	9	0	0.0 %
Hypothyroidism	30	0	0.0 %
Controls used for Specificity Calculations	**417**	**4**	**1.0%**
Other: Limited cutaneous systemic sclerosis (lSSc)*	14	5	35.7%
Other: AIH/PBC overlap**	4	4	100.0%
PBC	151	127	84.1%
Total	**586**		

* Literature indicates a relatively high prevalence of PBC in SSc patients, as compared to the general population. lSSc samples were therefore excluded from sensitivity and specificity calculations

** Four samples with AIH/PBC overlap were excluded for all sensitivity and specificity calculations.

Clinical sensitivity and specificity of the QUANTA Flash M2 (MIT3) were analyzed in the table below:

Clinical Analysis including all PBC (N=568)		QUANTA Flash M2 (MIT3)			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	PBC	127	24	151	Sensitivity: 84.1% (77.4 -89.1%)
	Controls	4	413	417	Specificity: 99.0% (97.6 - 99.6%)
	Total	131	437	568	

Expected values

The expected value in the normal population is "negative". Anti-mitochondrial antibody levels were analyzed in a cohort of 100 apparently healthy blood donors (50 females and 50 males, ages 17 to 57 years, with an average and median age of 34 years) using the QUANTA Flash M2 (MIT3). This patient population was different from the one that was used to establish the cutoff, and was only used to assess expected values. With the cut-off of 20 CU, two samples were positive on the QUANTA Flash M2 (MIT3). The mean concentration was 2.8 CU, and the values ranged from <1.2 to 91.4 CU.

Comparison with predicate device

Samples for method comparison analysis include 409 samples from the clinical validation study, plus 8 additional PBC samples that yield results around the cutoff of the assay to increase their percentage to 11.5% of the samples within the AMR, for a total of 417 samples. These samples were tested on both the QUANTA Flash M2 (MIT3) and on the predicate ELISA. Negative percent agreement (NPA), positive percent agreement (PPA), and total percent agreement (TPA) were calculated. The data are presented in four ways; first using all samples (EIA equivocal presented as positive, then negative), and next using only the 174 samples within the AMR (EIA equivocal presented as positive, then negative).

Method Comparison, all samples:

Method Comparison – all samples (N=417) ELISA equivocal=pos		QUANTA Flash M2 (MIT3)			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	250	7	257	NPA: 97.3% (94.5% – 98.7%)
	Positive	24	136	160	PPA: 85.0% (78.7% – 89.7%)
	Total	274	143	417	TPA: 92.6% (89.6% – 94.7%)

Method Comparison – all samples (N=417) ELISA equivocal=neg		QUANTA Flash M2 (MIT3)			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	255	12	267	NPA: 95.5% (92.3% – 97.4%)
	Positive	19	131	150	PPA: 87.3% (81.1% – 91.7%)
	Total	274	143	417	TPA: 92.6% (89.6% – 94.7%)

Method Comparison, samples within the AMR:

Method Comparison - Within AMR (N=174) ELISA equivocal=pos		QUANTA Flash M2 (MIT3)			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	71	7	78	NPA: 91.0% (82.6% – 95.6%)
	Positive	13	83	96	PPA: 86.5% (78.2% – 91.9%)
	Total	84	90	174	TPA: 88.5% (82.9% – 92.4%)

Method Comparison - Within AMR (N=174) ELISA equivocal=neg		QUANTA Flash M2 (MIT3)			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	73	12	85	NPA: 85.9% (76.9% – 91.7%)
	Positive	11	78	89	PPA: 87.6% (79.2% – 93.0%)
	Total	84	90	174	TPA: 86.8% (80.9% – 91.0%)