510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K163538

B. Purpose for Submission:

New assay device on previously cleared instrument

C. Measurand:

Anti-liver/kidney microsome type 1 antibodies (IgG)

D. Type of Test:

Chemiluminescence, semi-quantitative

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

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

G. Regulatory Information:

1. Regulation section:

   21 CFR § 866.5660, Multiple Autoantibodies Immunological Test System
   21 CFR § 862.1150, Calibrator
   21 CFR § 862.1660, Quality Control Material (assayed and unassayed)

2. Classification:

   Class II
   Class I (reserved)

3. Product code:

   NBS – Autoantibodies, LKM-1 (liver/kidney microsome type 1)

JIT – Calibrator, Secondary
JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

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

H. Intended Use:

1. Intended uses:

   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.

2. Indications for use:

   Same as Intended Use.

3. Special conditions for use statements:

   The device is for prescription use only.

4. Special instrument requirements:

   BIO-FLASH® Chemiluminescent Analyzer (K083518, K094060)

I. Device Description:

The QUANTA Flash LKM-1 kit includes one QUANTA Flash LKM-1 Reagent Cartridge, one vial of resuspension buffer, and one transfer pipette. The QUANTA Flash LKM-1 reagent cartridge contains the following reagents for 50 determinations:

- LKM-1 coated paramagnetic beads, lyophilized
- Assay buffer

- Tracer IgG, isoluminol labeled anti-human IgG antibodies in buffer containing protein stabilizers and preservative

The QUANTA Flash LKM-1 Calibrators are sold separately and contain two vials each of Calibrator 1, 2, and 3. These calibrators contain human antibodies to LKM-1 in stabilizers and preservatives. Each calibrator has two barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent.

The QUANTA Flash LKM-1 Controls are sold separately and contain two vials each of Negative Control and Positive Control. These controls contain human antibodies to LKM-1 in stabilizers and preservatives. Each control has two barcode labeled tubes containing 0.5 mL ready to use reagent.

J. Substantial Equivalence Information:

1. Predicate device name:

   QUANTA Lite LKM-1 ELISA

2. Predicate 510(k) number:

   K000535

3. Comparison with predicate:

QUANTA Flash® LKM-1 Reagents

Similarities		
Item	Device QUANTA Flash LKM-1	Predicate QUANTA Lite LKM-1 ELISA
Intended Use	QUANTA Flash LKM-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-liver/kidney microsome type 1antibodies 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	Same
Antigen	Recombinant LKM-1	Same
Shelf Life	One year at 2–8 ^{o}C	Same

Similarities		
Item	Device QUANTA Flash LKM-1	Predicate QUANTA Lite LKM-1 ELISA
Sample Type	Serum	Same

Differences		
Item	Device QUANTA Flash LKM-1	Predicate QUANTA Lite LKM-1 ELISA
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)	Units (arbitrary)
Measuring range	1.6 – 400.0 CU	0 – 100 Units
Cut-off	Negative: <20 CU Positive: ≥20 CU	Negative: 0.0–20.0 Units Equivocal: 20.1–24.9 Units Positive: >25 Units

QUANTA Flash LKM-1 Calibrators

Similarities		
Item	Device QUANTA Flash LKM-1 Calibrators	Predicate
Analyte	Anti-LKM-1 autoantibodies	Same
Matrix	Human serum, stabilizer, and preservative	Same
Physico-chemical characteristics	Liquid, prediluted, ready to use	Same
Shelf life	One year at 2–8°C	Same

Differences		
Item	Device QUANTA Flash LKM-1 Calibrators	Predicate
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	No separate intended use; calibrator is part of the kit.

Differences		
Item	Device QUANTA Flash LKM-1 Calibrators	Predicate
	used to calculate unit values.	
Method	QUANTA Flash LKM-1 chemiluminescent immunoassay	QUANTA Lite LKM-1 ELISA
Units	CU (arbitrary)	Units (arbitrary)

QUANTA Flash LKM-1 Controls

Similarities		
Item	Device QUANTA Flash LKM-1 Controls	Predicate
Analyte	Anti-LKM-1 autoantibodies	Same
Physico-chemical characteristics	Liquid, prediluted, ready to use	Same
Shelf life	One year at 2-8°C	Same

Differences		
Item	Device QUANTA Flash LKM-1 Controls	Predicate
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.
Levels	2 (negative and positive)	3 (negative, low positive and high positive)
Units	CU (arbitrary)	Units (arbitrary)

K. Standard/Guidance Document Referenced:

- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline–Third Edition
- CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
- CLSI EP07-A2, Interference Testing in Clinical Chemistry
- CLSI EP09-A3, Measurement Procedure Comparison & Bias estimation using patient samples
- CLSI EP17-A2, Protocols for Determination of Limits of Detection and Limits of Quantitation
- CLSI C28-A3c, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

L. Test Principle:

Recombinant cytochrome P450 2D6 (LKM-1) 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 by the instrument using system rinse in a disposable plastic cuvette. An aliquot of the diluted patient serum, LKM-1 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-LKM-1 antibodies bound to the antigen on the beads.

The QUANTA Flash LKM-1 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 the Calibrators, an instrument specific Working Curve is created, which is used by the software to calculate chemiluminescent units (CU) from the RLU value obtained for each sample.

M. Performance Characteristics:

1. Analytical performance: The results presented below were within the sponsor's pre-determined acceptance criteria for each study.

   a. Precision/Reproducibility:

   Precision:

   The precision of the QUANTA Flash LKM-1 assay was evaluated on eight samples containing various concentrations of anti-LKM-1 antibodies in accordance with CLSI EP05-A3. Samples were run in duplicates, twice a day, for 20 days. Within-run, between-run, between-day and total imprecision were calculated and are summarized in the table below.

Sample	N	Mean (CU)	Within-Run		Between-Run		Between-Day		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	80	9.2	0.6	6.4	0.0	0.0	0.1	1.3	0.6	6.5
2	80	14.7	0.3	2.0	0.2	1.5	0.6	3.8	0.7	4.5
3	80	16.5	0.5	3.2	0.0	0.0	0.4	2.3	0.6	3.9
4	80	17.5	0.4	2.4	0.4	2.1	0.3	1.5	0.6	3.5
5	80	44.7	1.1	2.4	1.6	3.6	0.7	1.6	2.1	4.6
6	80	101.1	2.0	2.0	3.9	3.9	2.2	2.2	4.9	4.9
7	80	201.4	7.2	3.6	9.4	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

Site-to-Site Reproducibility:

Five samples containing various concentrations of anti-LKM-1 antibodies were tested at three different sites. The samples were run in replicates of five, once a day, for five days, to generate 25 data points per sample, per site. Between site precision was calculated and summarized in the table below.

Sample	N	Mean (CU)	Within-Run		Between-Day		Within-Site		Between-Site		Total	
			SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	75	10.7	0.9	8.7	0.0	0.0	0.4	3.8	0.8	7.8	0.9	8.7
2	75	22.0	1.2	5.2	0.0	0.0	0.4	2.0	1.1	4.8	1.2	5.2
3	75	30.1	1.8	6.0	0.0	0.0	0.7	2.2	1.7	5.6	1.8	6.0
4	75	108.9	9.4	8.7	0.0	0.0	3.3	3.0	8.9	8.1	9.4	8.7
5	75	343.2	21.6	6.3	8.7	2.5	14.7	4.3	15.8	4.6	23.3	6.8

Lot-to-Lot Reproducibility:

Four samples were tested using three different reagent lots at one site. Samples were run in replicates of five, once a day, for five days, to generate 25 data points per sample, per lot (total 75 replicates). Results are summarized in the table below.

Sample	Mean (CU)	Between-Lot	
		SD	CV (%)
1	20.9	0.8	3.9%
2	28.3	1.1	4.0%
3	102.3	3.7	3.7%
4	352.9	40.9	11.6%

b. *Linearity/assay reportable range:*

Linearity:

The analytical measuring range (AMR) of the assay is 1.6 CU to 400.0 CU. The linearity of the AMR was evaluated by a study designed according to CLSI EP6-A. Five serum samples with various anti-LKM-1 antibody concentrations were serially diluted in analyte free serum to obtain values that cover the AMR. Each dilution was tested in duplicate. The linear regression analysis with samples falling within the AMR resulted in the following equation:

Sample	Test Range (CU)	Slope (95% CI)	Y-intercept (95% CI)	R^2	Average % Recovery
1	1.7–16.6	1.08 (1.03–1.13)	-0.8 (-1.3– -0.3)	1.00	93.8%
2	2.1–20.9	0.98 (0.95–1.01)	0.3 (-0.2–0.7)	1.00	102.3%
3	15.8–158.2	0.97 (0.94–1.00)	4.8 (1.5–8.1)	1.00	104.4%
4	22.9–229.0	1.00 (0.93–1.06)	-5.9 (-15.0–3.3)	0.99	93.8%
5	41.1–411.2	0.96 (0.91–1.02)	-4.7 (-19.6–10.2)	0.99	94.2%
All samples	1.7–411.2	0.95 (0.94–0.97)	1.0 (-1.2–3.2)	1.00	97.7%

Hook effect:

Two high positive samples having anti-LKM-1 antibody concentration above assay measuring range (743.2 CU and 13,812.8 CU) were examed to assess potential hook effect. No hook effect was observed up to 13,812.8 CU.

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

Traceability:

There is no recognized standard or reference material for anti-LKM-1 antibodies. Calibrators and controls values are directly traceable to the in-house standards.

Value Assignment:

The QUANTA Flash LKM-1 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-LKM-1 antibodies. The target CU is achieved through trial dilutions on a small scale. Once a dilution is selected, the Calibrators and Controls are bulked, tested, and adjusted. Upon completion of the manufacturing process, the Calibrators and Controls are tested on at least two instruments, on at least two lots of reagent cartridge, in replicates of five to obtain a minilum of 10 data points to determine final value assignment. The target values and ranges for the Calibrators and Controls are listed below:

	Target Value (CU)	Target Range (CU)
QUANTA Flash® LKM -1 Calibrators		
Calibrator 1	10	8–12
Calibrator 2	100	80–120
Calibrator 3	325	300–350
QUANTA Flash® LKM-1 Controls		
Negative control	10	8–12
Positive control	50	40–60

Stability:

Kit stability (unopened): The accelerated stability study was performed using three lots of LKM-1 coupled beads, resuspension buffer 7, calibrators, and controls. Real-time stability is on-going; the results to date support a claim of 12 months stability for unopened reagent cartridge and up to 6 months on calibrators and controls stored at 2–8°C.

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

i. Calibrators: Calibrators were placed uncapped, onboard the instrument, and calibration was performed five times over 9 hours. Controls and a panel of characterized patient specimens were run on each calibration curve.
ii. Controls: Two vials of each control were assayed once a day for a total of 20 runs. The first run was used to establish baseline value, and then an additional 19 runs were performed. During runs, the Controls were left uncapped, onboard the instrument for 15 minutes per run. When not in use, the controls were capped, and stored at 5ºC ± 3°C.
iii. Reagent Cartridge: Two lots of cartridges were tested with five serum specimens (with different reactivity levels) along with the Negative and Positive Controls. The specimens were tested periodically up to 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.

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

Calibrators	8 hours on-board; up to 4 calibrations
Controls	Up to 15 uses with 10 min on-board per use
Reagent Cartridge	60 days on-board

Sample stability: The study was performed with four samples (one negative, one positive, and two around the cut-off), tested at 2–8°C, and room temperature (RT). In addition, the samples were tested for the stability after up to three repeated freeze/thaw cycles. The results support sample stability up to 48 hours of storage at RT, up to 14 days of storage at 2–8°C, and up to three freeze/thaw cycles when

samples are stored at or below -20°C.

d. Detection limit:

The Limit of Blank (LoB) was determined by assaying four blank samples in five replicates per sample over three days with two reagent lots. Sixty data points were generated. LoB was calculated at the 95th percentile using the parametric method, as the dataset showed non-normal distribution. The LoB of both two lots was below the measuring range and was determined to be 0.00 CU and 0.14 CU. The claimed LoB was determined to be 0.14 CU.

The Limit of Detection (LoD) was determined by assaying four low-level samples with anti-LKM-1 antibody concentration tested in five replicates over three days on two reagent lots (60 replicates per lot). The LoD of the QUANTA Flash LKM-1 assay for the two lots were below the measuring range and was determined to be 0.24 CU and 0.26 CU. The claimed LoD is 0.26 CU.

The Limit of Quantitation (LoQ) was determined based on the data generated from the LoD testing with a total error (TE) goal of 25%. The claimed LoQ is 1.6 CU.

e. Analytical specificity:

The interference study was performed according to CLSI EP07-A2 using 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). Each interfering substance (hemoglobin, conjugated bilirubin, triglycerides, cholesterol, human IgG and RF IgM) was spiked into every specimen at three different concentrations in 10% of total specimen volume. Each resulting sample was 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. No interference was detected with conjugated bilirubin up to 1 mg/mL, hemoglobin up to 2 mg/mL, triglycerides up to 1000 mg/dL, cholesterol up to 332.5 mg/dL, human IgG up to 35 mg/mL, and rheumatoid factor IgM up to 153.4 IU/mL.

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 same methodology described above. No interference was detected with corticosteroids (prednisone) up to 0.3 mg/L, azathioprine up to 2.99 mg/L and interferon alpha up to 0.33 mg/L.

f. Assay cut-off:

The assay cut-off was determined using 242 samples from reference subjects as shown in the table below. The cut-off was established as 20 CU based on the 99th

percentile of the results obtained on the reference subjects.

Sample Group	N
Apparently healthy blood donors	120
Celiac Disease	20
Rheumatoid Arthritis	31
Infectious Disease (HBV, HCV, HIV)	30
Liver Diseases (AIH-1, PBC)	39
Myositis	2
Total	**242**

2. Comparison studies:

a. Method comparison with predicate device:

Samples for the method comparison analysis included 334 samples from the clinical validation study, along with additional 10 pooled samples that yield results around the cut-off. All samples were tested on both the QUANTA Flash LKM-1 and on the predicate device. Among 334 samples, a total of 119 samples within the assay measuring ranges of both assays were included in the method comparison analysis. The results are summarized below:

Equivocal Range of Predicate as Positive		**QUANTA Lite LKM-1 ELISA**		
		Positive	Negative	Total
QUANTA Flash LKM-1	Positive	27	1	28
	Negative	5	86	91
	Total	32	87	119
Positive agreement: 84.4% (95% CI: 68.2% – 93.1%) Negative agreement: 98.9% (95% CI: 93.8% – 99.8%) Total Agreement: 95.0% (95% CI: 89.4% – 97.7%)				

Equivocal Range of Predicate as Negative		**QUANTA Lite LKM-1 ELISA**		
		Positive	Negative	Total
QUANTA Flash LKM-1	Positive	25	3	28
	Negative	1	90	91
	Total	26	93	119
Positive agreement: 96.2% (95% CI: 81.1% – 99.3%) Negative agreement: 96.8% (95% CI: 90.9% – 98.9%) Total Agreement: 96.6% (95% CI: 91.7% – 98.7%)				

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A total of 633 samples were included in the clinical validation study for the QUANTA Flash LKM-1 assay. This validation set included 26 samples from Autoimmune Hepatitis Type 2 (AIH-2) patients, and 607 control samples from patients with various types of liver and gastroenterological diseases, other autoimmune syndromes, and various infectious diseases. Clinical sensitivity and specificity summary of the QUANTA Flash LKM-1 are shown in the table below:

		Clinical Diagnosis of AIH-2		
		Positive	Negative	Total
QUANTA Flash LKM-1	Positive	20	12	32
	Negative	6	595	601
	Total	26	607	621

Clinical Sensitivity: 76.9% (95% CI: 56.4 – 91.0%)
Clinical Specificity: 98.0% (95% CI: 96.6 – 99.0%)

Distribution of samples and anti-LKM-1 antibody positivity rate in the validation study are tabulated as follows:

	N	N of Positive	% Positive
Target Disease			
Autoimmune Hepatitis type 2 (AIH-2)	26	20	76.9%
Control Disease			
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%

	N	N of Positive	% Positive
Polymyositis	1	0	0.0%
Autoimmune Endocrine Disease	60	0	0.0 %
Rheumatoid Arthritis	30	0	0.0 %
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 of Controls	607	12	2.0%

* *LKM-1 antibody positivity has been described in patients with Hepatitis C virus infection. These are also stated in the Summary and Explanation of the Test, and Limitations of the Procedure sections of the Package Insert..*

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

Not applicable

4. Clinical cut-off**:**

Same as assay cut-off

5. Expected values/Reference range:

The expected value in the normal population is "negative". Anti-LKM-1 antibody levels were verified 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 with the values ranging from <1.6 to 3.6 CU.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.