510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k141328

B. Purpose for Submission:

New device

C. Measurand:

anti-Ro60 IgG autoantibodies

D. Type of Test:

Semi-quantitative chemiluminescent immunoassay

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash® Ro60

QUANTA Flash® Ro60 Calibrators

QUANTA Flash® Ro60 Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR§ 866.5100, Antinuclear antibody immunological test system

   21 CFR §862.1150, Calibrator

   21 CFR §862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:

   Class II (assay and calibrators)

Class I (controls)

3. Product code:

   LLL, Extractable antinuclear antibody, antigen and control

   JIT, Calibrator, Secondary

   JJX, Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

   Immunology (82)

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   QUANTA Flash Ro60 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro60 autoantibodies in human serum. The presence of anti-Ro60 autoantibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus and Sjögren's Syndrome.

   QUANTA Flash Ro60 Calibrators are intended for use with the QUANTA Flash Ro60 Reagents for the determination of IgG anti-Ro60 autoantibodies in human serum. Each calibrator establishes a point of reference for the working curve that is used to calculate unit values.

   QUANTA Flash Ro60 Controls are intended for use with the QUANTA Flash Ro60 Reagents for quality control in the determination of IgG anti-Ro60 autoantibodies in human serum.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   BIO-FLASH Instrument System (k083518)

I. Device Description:

The QUANTA Flash Ro60 Kit includes the following components:

- One (1) QUANTA Flash Ro60 Reagent Cartridge. The QUANTA Flash Ro60 reagent cartridge contains the following reagents for 50 determinations:
  - Ro60 antigen coated paramagnetic beads, lyophilized.
  - Assay buffer – colored pink, containing Tris-buffered saline, Tween 20, protein stabilizers and preservatives.
  - Tracer IgG – Isoluminol labeled anti-human IgG antibodies in buffer, containing protein stabilizers and preservative.
- One (1) vial of Resuspension buffer
- One (1) Transfer pipette

QUANTA Flash Ro60 Calibrators include the following components:

- QUANTA Flash Ro60 Calibrator 1: Two (2) barcode labeled vials containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to Ro60 in stabilizers and preservatives.
- QUANTA Flash Ro60 Calibrator 2: Two (2) barcode labeled tubes containing 0.3 mL prediluted, ready to use reagent. Calibrators contain human antibodies to Ro60 in stabilizers and preservatives.

QUANTA Flash Ro60 Controls include the following components:

- QUANTA Flash Ro60 Negative Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Ro60 in stabilizers and preservatives.
- QUANTA Flash Ro60 Positive Control: Two (2) barcode labeled tubes containing 0.5 mL, ready to use reagent. Controls contain human antibodies to Ro60 in stabilizers and preservatives.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   HYCOR AUTOSTAT™ Anti-SS-A Ro ELISA

2. Predicate 510(k) number(s):

   k962719

3. Comparison with predicate:

QUANTA Flash Ro60 Reagent Kit:

Similarities		
Item	Device QUANTA Flash Ro60	Predicate HYCOR AUTOSTAT Anti-SS-A Ro ELISA
Intended use	Semi-quantitative determination of anti-Ro60 IgG autoantibodies in human serum	Same
Assay methodology	Solid phase (heterogeneous) immunoassay	Same
Traceability	International Reference Preparation is not available; results are traceable to in-house standards	Same
Sample type	Serum	Same
Shelf life	12 months	Same

Differences		
Item	Device QUANTA Flash Ro60	Predicate HYCOR AUTOSTA Anti-SS-A Ro ELISA
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well plate
Antigen	Purified recombinant Ro60 antigen	Native Ro60 antigen, purified from bovine sources
Conjugate	Isoluminol conjugated anti-human IgG	HRP conjugated anti-human IgG
Indications for Use	Includes both SLE and Sjögren's syndrome	Sjögren's syndrome only
Assay format	Automatic	Manual
Calibration	Lot specific Master Curve + two Calibrators (Sold separately)	Single standard (Included in the kit)

QUANTA Flash Ro60 Calibrators:

Similarities		
Item	Device QUANTA Flash Ro60 Calibrators	Predicate HYCOR AUTOSTAT Anti-SS-A Ro ELISA
Analyte	Anti-Ro60 antibodies	Same
Physico-chemical characteristics	Liquid, prediluted, ready to use	Same
Storage	2−8°C	Same
Shelf life	12 months	Same

Differences		
Item	Device QUANTA Flash Ro60 Calibrators	Predicate HYCOR AUTOSTAT Anti-SS-A Ro ELISA
Intended use	QUANTA Flash Ro60 Calibrators are intended for use with the QUANTA Flash Ro60 Reagents for the determination of IgG anti-Ro60 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.
Method	QUANTA Flash Ro60 chemiluminescent immunoassay	HYCOR Anti-SS-A/Ro ELISA
Unit	CU (Chemiluminescent units) (arbitrary)	U/mL (arbitrary)
Matrix	Human serum, stabilizers, and preservative	Human serum with preservative

QUANTA Flash Ro60 Controls:

Similarities		
Item	Device QUANTA Flash Ro60 Controls	Predicate HYCOR AUTOSTAT Anti-SS-A Ro ELISA
Analyte	Anti-Ro60 antibodies	Same
Physico-chemical characteristics	Liquid, ready to use	Same
Levels	2 (negative and positive)	Same
Storage	2−8°C	Same
Shelf life	12 months	Same

Differences		
Item	Device QUANTA Flash Ro60 Controls	Predicate HYCOR AUTOSTAT Anti-SS-A Ro ELISA
Intended use	QUANTA Flash Ro60 Controls are intended for use with the QUANTA Flash Ro60 Reagents for quality control in the determination of IgG anti-Ro60 autoantibodies in human serum.	No separate intended use; controls are part of the kit.
Method	QUANTA Flash Ro60 chemiluminescent immunoassay	HYCOR Anti-SS-A/Ro ELISA
Unit	CU (Chemiluminescent units) (arbitrary)	U/mL (arbitrary)
Matrix	Human serum, stabilizers, and preservative	Human serum with preservative

K. Standard/Guidance Document Referenced (if applicable):

C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Addition.

EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition.

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

EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantification, Approved Guideline.

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

EP09-A2IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Second Edition (Interim Revision) (used for matrix comparison).

Guidance for Industry - Abbreviated 510(k) Submissions for In Vitro Diagnostic Calibrators; Final.

L. Test Principle:

The QUANTA Flash Ro60 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 Ro60 assay utilizes a reagent cartridge format, which is compatible with the BIO-FLASH instrument.

Purified recombinant Ro60 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. A patient serum sample is prediluted 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 all 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 and Trigger 2 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- Ro60 antibodies bound to the corresponding Ro60 on the beads**.**

For quantitation, the QUANTA Flash Ro60 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 Ro60 Calibrators. Based on the results obtained with the two 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision:

   The precision of the QUANTA Flash Ro60 assay was evaluated according to CLSI EP5-A2 guideline. Ten (10) samples obtained from a commercial vendor containing various concentrations of Ro60 antibodies were run in duplicate, twice a day, for 21 days for a total of 84 measurements per sample. Two production reagent lots were used in the study. Data were analyzed with the Analyse-it for Excel method evaluation software, and within-run, between-run, between-day and total precision were calculated and summarized in the Table below. No outliers were removed.

Total %CV values were within the manufacturer's pre-determined acceptance limit of ≤10%.

Precision Sample	Mean CU	Within Run		Between Run		Between Day		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
110685-1800	11.5	0.6	5.0%	0.0	0.0%	0.5	4.5%	0.8	6.8%
000674-800	16.7	0.9	5.4%	0.8	4.8%	0.7	4.4%	1.4	8.4%
110688-1000	20.7	1.4	6.9%	0.0	0.0%	0.9	4.3%	1.7	8.1%
110689-950	25.7	0.9	3.4%	0.8	3.3%	1.0	4.0%	1.6	6.2%
110687-1500	26.4	0.8	3.1%	0.6	2.4%	0.5	1.9%	1.1	4.4%
110687-200	142.1	7.7	5.4%	4.5	3.1%	6.2	4.3%	10.8	7.6%
110688-85	406.3	26.6	6.6%	0.0	0.0%	21.2	5.2%	34.0	8.4%
110684-28	807.5	25.9	3.2%	20.3	2.5%	18.6	2.3%	37.8	4.7%
000674-5.83	1181.0	45.3	3.8%	49.2	4.2%	30.6	2.6%	73.6	6.2%
110686-55	1246.3	67.9	5.4%	35.3	2.8%	60.8	4.9%	97.7	7.8%

Repeatability:

Three samples obtained from a commercial vendor were tested on two different reagent lots, using two different lots of calibrators, by two operators. Samples were run in quadruplicate, twice a day, for 10 days, to generate 80 data points per sample. Data were analyzed with the Analyse-it for Excel method evaluation software, and within-run, between-reagent lots, between-calibrator lots, between-operators and total precision were calculated and the results are summarized in the Tables below. All %CV values were within the manufacturer's pre-determined acceptance limit of ≤ 10%.

Sample	Mean (CU)	Within Run		Between Reagent Lots		Between Calibrator Lots		Between Operators		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)

1	11.2	0.2	1.9%	0.6	5.8%	0.6	5.4%	0.5	4.3%	0.6	5.3%
2	21.0	0.5	2.5%	1.6	7.4%	0.8	3.9%	0.7	3.1%	1.1	5.4%
3	100.0	2.7	2.7%	5.0	5.0%	5.3	5.3%	4.2	4.2%	5.1	5.1%

Lot to Lot:

A separate lot-to-lot comparison study was performed using 21samples from INOVA's serum bank covering the analytical measuring range of the assay and positive and negative controls (23 specimens total). Samples were tested in triplicate with three different reagent lots. All results were within the manufacturer's pre-defined acceptance criteria.

b. Linearity/assay reportable range:

The linearity of the analytical measuring range (4.9-1374.8 CU) was evaluated by a study according to CLSI EP6-A. Three serum samples with various Ro60 antibody concentrations were diluted in 10% increments (from 0% to 90% diluent) to obtain values that cover the AMR. The dilutions were assayed in duplicate. Percent recovery of obtained results was calculated compared to the expected results (based on the dilution factor). Obtained values were plotted against expected values, and linear regression analysis was performed using Analyse-it software. The slope and intercept of the regression line were calculated with 95% CI as well as the R2 values. All samples met the pre-defined acceptance criteria.

Sample Pool	Test Range (CU)	Regression equation	Slope (95% CI)	y-Intercept (95% CI)	R^2
1	148.3 to 137.2	y=1.05x+16.23	1.05 (1.02 to 1.08)	16.23 (-9.24 to 41.7)	1.00
2	15.3 to 178.6	y=1.00x+0.04	1.00 (0.96 to 1.04)	0.04 (-4.79 to 4.87)	0.99
3	7.5 to 45.6	y=1.02x-2.87	1.04 (1.00 to 1.08)	-2.87 (-4.11 to -1.63)	0.99

An additional linearity study was performed comparing the results of the assay when diluted with either serum or system rinse over a series of concentrations covering the AMR. The results of the study demonstrated that similar results were obtained from dilution of Ro60 in serum or system rinse.

The AMR is defined by the values of the lowest and highest Master Curve Standards. The QUANTA Flash Ro60 AMR is 4.9 CU to 1374.8 CU.

Hook effect/ Auto-dilution:

Auto-dilution:

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 >1374.8 by 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 measured is 1374.8 CU, the highest value that can be reported is 27496 CU.

To validate the Auto-rerun function, four high positive specimens with results above the analytical measuring range were selected. The samples were run with the Auto-rerun function enabled on the BIO-FLASH. All samples triggered the auto-rerun function. Then the specimens were manually diluted to the same level as the Auto-rerun function (20 fold) and tested on the BIO-FLASH. The results were within the analytical measuring range after auto-rerun or manual dilution for all specimens. The % recovery values for results obtained with the auto-rerun compared to the results obtained by manual dilution were between 87% and 101% (average 93%) (within the manufacturer's pre-determined ± 20% acceptance limit).

Hook effect:

To assess hook effect, the measurement signal (relative light units, RLU) was examined for four high positive samples (all results above the AMR) before and after automatic or manual dilution. All sera produced significantly higher RLU values (above the AMR) when used neat. Results were compared to the manually and automatically diluted samples (that were within the AMR), to demonstrate that high positive specimens above the analytical measuring range do not show hook effect up to 19,707 CU in the Ro60 assay.

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

Traceability:

There are currently no recognized international standards for the measurement of anti-Ro60 antibodies. The QUANTA Flash Ro60 Calibrators and Controls are manufactured by diluting human serum that contains high titer of anti-Ro60 antibodies with commercial antibody stabilizer, containing 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 10 to determine final value assignment.

Expected Values of QUANTA Flash Ro60 Calibrators and Controls:

Calibrator/Control Reagent	Target Value	Target Range
Ro60 Calibrator 1	12 CU	10 – 14 CU
Ro60 Calibrator 2	360 CU	324 – 396 CU
Ro60 Negative Control	10 CU	8 – 12 CU
Ro60 Positive Control	50 CU	40 – 60 CU

Stability:

Stability studies have been performed and the results support the following claims:

Sample Stability: A sample stability study was conducted using six (two negative, two positive and two around the cut-off) serum samples. All manufacturer pre-determined acceptance criteria were met. Study results supported the following sample stability claims:

- Storage at room temperature, 20–26°C: Samples can be stored at room temperature for up to 48 hours.
- Storage at 2–8°C: Samples can be stored at 2–8°C for up to 14 days.
- Freeze/thaw: Samples should be frozen at -20°C or lower. Samples may be frozen and thawed up to 3 times.

Shelf-Life/Un-Opened Stability: Both accelerated and real-time shelf life stability studies were performed for the Ro60 reagent, calibrators and controls. All manufacturer pre-determined acceptance criteria were met. Study results supported the following un-opened stability claims:

- Reagent: The reagent shelf-life stability claim is 12 months at 2–8°C.
- Calibrator and Control: The calibrator and control shelf-life stability claim of 12 months at 2–8°C.

On-board/Opened/In-Use Stability: On-board stability studies were performed for the Ro60 reagent, calibrators and controls. All manufacturer pre-determined acceptance criteria were met. Study results supported the following un-opened stability claims:

- Reagent: The Ro60 reagent cartridge is stable for up to 49 days on-board the instrument.
- Calibrator: The Ro60 calibrators are stable for up to 4 calibrations over 8 hour period of on-board instrument use.
- Control: The Ro60 controls are stable for up to 15 uses with an average of time of 10 minutes onboard the instrument per use.

d. Detection limit:

For the Ro60 assay, detection limits were determined according to CLSI EP17-A.

Limit of Blank (LoB):

LoB was determined using the assay specific sample dilution buffer (System Rinse) as "blank". Four (4) blank samples from two different lots were run in replicates of five, one time per day for three days for a total of 60 measurements per lot. LoB was calculated for each lot separately using the Reference Interval function of Analyse-it software at the 95th percentile, using the non-parametric method.

The resulting analysis provided a *p*-value indicating non-normal ($p < 0.0001$) distribution of the data points. The resulting LoBs for the two lots were 398 RLU and 452 RLU. The final LoB was determined to be 452 RL.

Limit of Detection (LoD):

To determine LoD, four low level samples were prepared by diluting anti-Ro60 positive samples. The low level samples were run in replicates of five on two reagent lots, one time per day for three days resulting in 60 measurements per lot. LoD was calculated based on formula that is published in the CLSI Guideline EP17-A: LoD = LoB + (cβ*SDS). The LoD was determined separately for each lot and was 519 RLU and 502 RLU. The final LoD for the QUANTA Flash Ro60 assay is 519 RLU, which is below the analytical measuring range of the assay e.g. < 4.9 CU.

e. Analytical specificity:

Interference:

The interference study was performed according to CLSI EP07-A2. Three specimens were tested (near-the cut-off negative: 15.2 CU; weak positive: 40.3 CU; high positive: 161.4 CU).

Each interfering substance was tested at three concentrations (low, medium, and high) in three different specimens (15.2, 40.3, and 161.4 CU). The resulting samples were assessed in triplicate with the Ro60 assay. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluent (10% of total).

No interference ($\leq \pm 15\%$) was detected up to the following concentrations tested:

Substance	Concentration	% recoveries
Triglyceride	1000 mg/dL	89% to 109%
Cholesterol	224.3 mg/dL	89% to 109%
Bilirubin	10 mg/dL	99% to 109%
Hemoglobin	200 mg/dL	100% to 108%
Rheumatoid Factor	500 IU/mL	90% to 107%

Cross reactivity:

Cross reactivity was investigated using international reference sera from the Center of Disease Control and Prevention (CDC). All 12 sera were tested. The CDC ANA reference sera #2 (reference serum for human antibodies to SS-B/La), #3 (reference serum, fluorescence antinuclear antibody, speckled pattern) and #7 (reference serum for human antibodies to SS-A/Ro) were positive for Ro60 by this assay and produced the following results: CDC ANA #2: >1374.8 CU; CDC ANA #3: 920.0 CU and CDC ANA #7 >1374.8 CU. The other 9 reference sera in the panel were analyzed and found to be negative for Ro60 by this assay.

f. Assay cut-off:

The QUANTA Flash Ro60 cut-off was established using unaltered samples in the same matrix (serum) from 156 subjects indicated in the table below:

Diagnosis	#
Apparently healthy blood donors	115
Viral hepatitis positive samples	8
Syphilis positive samples	5
Rheumatoid arthritis	23
HIV positive samples	5

The cut-off was established in accordance to CLSI C28-A3c. The distribution of the results was non-normal (Shapiro-Wilk $p<0.0001$), so the non-parametric percentile method was used. The 99^{th} percentile of the obtained values calculated as 8193.6 RLU.

Additionally, 12 proficiency testing samples from the College of American Pathologists (CAP) and United Kingdom National External Quality Assessment Service (UKNEQAS) with known consensus/target results were tested to aid in the determination of the cut-off. Taking into account the target results of the proficiency testing samples, the cut-off was increased to 12,000 RLU to ensure optimal differentiation between negatives and positives, and a 20 CU value was assigned to this RLU value. No reference sample (156 serum and 12 proficiency) tested positive at this cut-off level.

	Positive	Negative
QUANTA Flash Ro60	≥ 20 CU	< 20 CU

2. Comparison studies:

a. Method comparison with predicate device:

The QUANTA Flash Ro60 assay was compared with the predicate using available samples from the clinical validation set presented below. Because many were outside the measuring range of the assay, only results within the analytical measuring range of the assay are presented. The 143 sample cohort consisted of SLE (n=27), Sjogren's Syndrome (SS; n=19), and relevant disease controls (n=97).

The predicate has an equivocal region, so the data were analyzed with the equivocal results as either positive or negative. Of the 143 available samples, 63 were within the AMR. These samples contained 25 SLE, 18 SS, and 20 disease controls.

ELISA equivocal as negative		Predicate Ro60 ELISA		
		Positive	Negative	Total
QUANTA Flash Ro60 CIA	Positive	40	4	44
	Negative	3	16	19
	Total	43	20	63

Positive Agreement = 93.0% (40/43) (95% CI: 80.9 – 98.5%)
Negative Agreement = 80.0% (16/20) (95% CI: 56.3 – 94.4%)
Overall Agreement = 88.9% (56/63) (95% CI: 78.5 – 95.4%)

ELISA equivocal as positive		Predicate Ro60 ELISA		
		Positive	Negative	Total
QUANTA Flash Ro60 CIA	Positive	40	4	44
	Negative	6	13	19
	Total	46	17	63

Positive Agreement = 87.0% (40/46) (95% CI: 73.7 – 95.1%)
Negative Agreement = 76.5% (13/17) (95% CI: 50.1 – 93.2%)
Overall Agreement = 84.1% (56/63) (95% CI: 72.7 – 92.1%)

b. Matrix comparison:

Not applicable

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

A total of 475 samples were included in the clinical validation study for the QUANTA Flash Ro60 as shown in the table below:

Patient group	N	Number positive	% positive
Graves' Disease	10	0	0.0%
Hashimoto Thyroiditis	10	0	0.0%
Celiac Disease	11	0	0.0%
Crohn's Disease	20	1	5.0%
Ulcerative Colitis	20	0	0.0%
HCV	9	0	0.0%
HBV	9	0	0.0%
HIV	5	0	0.0%
Syphilis	10	0	0.0%
Osteoarthritis	20	1	5.0%
Primary Anti-phospholipid Syndrome (PAPS)	15	1	6.7%
Secondary Antiphospholipid Syndrome (SAPS)*	15	4	26.7%
Other rheumatic diseases	38	1	2.6%
Vasculitis	1	0	0.0%
Systemic sclerosis	48	1	2.1%
Autoimmune myositis	1	0	0.0%
Rheumatoid arthritis	20	1	5.0%
Autoimmune liver disease**	24	1	4.2%
Sjögren's Syndrome (SS)	39	26	66.7%
Systemic Lupus Erythematosus (SLE)	150	36	24.0%
Total	**475**		

* Patients may have SLE

** Samples contain autoimmune liver disease specific antibodies (SLA, F-actin, M2)

All samples were run on the QUANTA Flash Ro60 CIA. The results were analyzed to calculate sensitivity and specificity for SLE (n=150) and SS (n=39) together and separately. The SAPS (n=15) group was excluded from all calculations because the samples might have SLE.

SLE + SS Clinical Analysis		SLE and SS Clinical Diagnosis		
		Positive	Negative	Total
QUANTA Flash Ro60	Positive	62	7	69
	Negative	127	264	391
	Total	189	271	460

QUANTA Flash® Ro60 Sensitivity = 32.8% (62/189) (95% CI: 26.2 – 40.0%)
QUANTA Flash® Ro60 Specificity = 97.4% (264/271) (95% CI: 94.8 – 99.0%)

SLE Clinical Analysis		SLE Clinical Diagnosis		
		Positive	Negative	Total
QUANTA Flash Ro60	Positive	36	7	43
	Negative	114	264	378
	Total	150	271	421

QUANTA Flash® Ro60 Sensitivity = 24.0% (36/150) (95% CI: 17.4 – 31.6%)
QUANTA Flash® Ro60 Specificity = 97.4% (264/271) (95% CI: 94.8 – 99.0%)

SS Clinical Analysis		SS Clinical Diagnosis		
		Positive	Negative	Total
QUANTA Flash® Ro60	Positive	26	7	33
	Negative	13	264	277
	Total	39	271	310

QUANTA Flash® Ro60 Sensitivity = 66.7% (26/39) (95% CI: 49.8 – 80.9%)
QUANTA Flash® Ro60 Specificity = 97.4% (264/271) (95% CI: 94.8 – 99.0%)

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

Not applicable.

4. Clinical cut-off**:**

See assay cut-off.

5. Expected values/Reference range:

The expected value in the normal population is "negative". Anti-Ro60 autoantibody levels were analyzed in a cohort of 98 apparently healthy blood donors (52 females and 46 males, ages 17 to 60 years, with an average age of 33.2 years and median age of 29 years) using the QUANTA Flash Ro60. This patient population was different from the one that was used to establish the cut-off, and was only used to assess expected values. At the cut-off of 20 CU, 2 (2 %) of the samples were positive on the QUANTA Flash Ro60. The mean concentration was 6 CU, and the values ranged from <4.9 to 84.8 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.