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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® RF IgM Reagents
QUANTA Flash® RF IgA Reagents

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Device name (assay kit):

Proprietary name:	QUANTA Flash® RF IgM Reagents QUANTA Flash® RF IgA Reagents
Common name:	Rheumatoid factor IgM, IgA Chemiluminescent Immunoassay
Classification name:	System, Test, Rheumatoid Factor

Regulation Description	Rheumatoid Factor immunological test system
Regulation Medical Specialty	Immunology
Review Panel	Immunology
Product Code	DHR
Regulation Number	866.5775
Device Class	2

Predicate device

QUANTA Lite® RF IgM ELISA, 510(k) number: k971614. Date declared: April 30, 1997.
QUANTA Lite® RF IgA ELISA, 510(k) number: k983084. Date declared: September 2, 1998.

Device description

The principle of the assays is chemiluminescent microparticle immunoassay, a variation of solid phase immunoassay. The QUANTA Flash® RF IgM and QUANTA Flash® RF IgA assays are 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® RF IgM and QUANTA Flash® RF IgA assays utilize a reagent cartridge format, which is compatible with the BIO-FLASH® instrument.

Rabbit polyclonal antibodies are coated onto paramagnetic beads, which are stored in the reagent cartridge under conditions that preserve the antibody in its reactive state. When the assay cartridge is ready to be used for the first time, the entire cartridge is inverted several times to thoroughly mix the reagents. The reagent cartridge is then loaded onto the BIO-FLASH instrument.

A patient serum sample is diluted 1:22.7 by the instrument using system rinse in a disposable plastic cuvette. An aliquot of the diluted patient serum, 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 IgM (QUANTA Flash® RF IgM) or anti-human IgA (QUANTA Flash® RF IgA) 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 RF antibodies bound to the antibodies on the beads.

The QUANTA Flash RF IgM and QUANTA Flash RF IgA assays utilize 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 international units per milliliter (IU/mL) (QUANTA Flash® RF IgM) or chemiluminescent units (CU) (QUANTA Flash® RF IgA) from the RLU value obtained for each sample.

QUANTA Flash RF IgM Calibrators, QUANTA Flash RF IgM Controls, QUANTA Flash RF IgA Calibrators and QUANTA Flash RF IgA Controls are sold separately.

The QUANTA Flash® RF IgM Reagents / QUANTA Flash® RF IgA Reagents kit contains the following materials:

One (1) QUANTA Flash RF IgM / RF IgA Reagent Cartridge

QUANTA Flash RF IgM Reagent Cartridge contains the following reagents for 100 determinations:

a. Rabbit pAb coated paramagnetic beads.
b. Assay buffer – colored pink, containing protein stabilizers and preservatives.
c. Tracer IgM – Isoluminol labeled anti-human IgM antibody, containing buffer, protein stabilizers and preservative.

QUANTA Flash RF IgA Reagent Cartridge contains the following reagents for 100 determinations:

a. Rabbit pAb coated paramagnetic beads.
b. Assay buffer – colored pink, containing protein stabilizers and preservatives.
c. Tracer IgA – Isoluminol labeled anti-human IgA antibody, containing buffer, protein stabilizers and preservative.

Intended use(s)

QUANTA Flash RF IgM is a chemiluminescent immunoassay for the quantitative determination of IgM rheumatoid factor (RF) antibodies in human serum. The presence of IgM RF antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis (RA).

QUANTA Flash RF IgA is a chemiluminescent immunoassay for the semi-quantitative determination of IgA rheumatoid factor (RF) antibodies in human serum. The presence of IgA RF antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis (RA).

Indications for use

Same as Intended use.

Substantial equivalence

The QUANTA Flash RF IgM Reagents and the QUANTA Flash RF IgA Reagents have the same intended use and assay principle as the predicate device.

Comparison to predicate device

QUANTA Flash RF IgM Reagents

Similarities		
Item	**QUANTA Flash RF IgM Reagents**	**QUANTA Lite RF IgM ELISA**
Intended use	QUANTA Flash RF IgM is a chemiluminescent immunoassay for the quantitative determination of IgM rheumatoid factor (RF) antibodies in human serum. The presence of IgM RF antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis (RA).	QUANTA Lite RF IgM is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgM rheumatoid factor (RF) antibodies in patient sera. The presence of these antibodies, when considered in conjunction with other laboratory and clinical findings, is an aid in the diagnosis of rheumatoid arthritis (RA).
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Antigen	Rabbit polyclonal antibody	Rabbit polyclonal antibody
Sample type	Human serum	Human serum

Differences		
Item	**QUANTA Flash RF IgM Reagents**	**QUANTA Lite RF IgM ELISA**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well polystyrene plate
Conjugate	Isoluminol conjugated monoclonal anti-human IgM antibody	HRP conjugated monoclonal anti-human IgM antibody
Units	International Units per milliliter (IU/mL)	Units (U)
Cut-off	5.0 IU/mL	6.0 Units
Analytical Measuring Range	0.3 – 490.0 IU/mL	0.0 – 100.0 Units
Calibration	Lot specific Master Curve + Two calibrators (sold separately)	Five lot specific calibrators

QUANTA Flash RF IgA Reagents

Similarities		
Item	**QUANTA Flash RF IgA Reagents**	**QUANTA Lite RF IgA ELISA**
Intended use	QUANTA Flash RF IgA is a chemiluminescent immunoassay for the semi-quantitative determination of IgA rheumatoid factor (RF) antibodies in human serum. The presence of IgA RF antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of rheumatoid arthritis (RA).	QUANTA Lite RF IgA is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of IgA rheumatoid factor (RF) antibodies in patient sera. The presence of these antibodies, when considered in conjunction with other laboratory and clinical findings, is an aid in the diagnosis of rheumatoid arthritis (RA).
Assay methodology	Solid phase (heterogeneous) immunoassay	Solid phase (heterogeneous) immunoassay
Antigen	Rabbit polyclonal antibody	Rabbit polyclonal antibody
Sample type	Human serum	Human serum

Differences		
Item	**QUANTA Flash RF IgA Reagents**	**QUANTA Lite RF IgA ELISA**
Detection/ Operating principle	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Solid phase	Paramagnetic microparticles (beads)	96-well polystyrene plate
Conjugate	Isoluminol conjugated monoclonal anti-human IgA antibody	HRP conjugated monoclonal anti-human IgA antibody
Units	Chemiluminescent Units (CU)	Units (U)
Cut-off	20.0 CU	6.0 Units
Analytical Measuring Range	1.3 – 900.0 CU	0.0 – 100.0 Units
Calibration	Lot specific Master Curve + Two calibrators (sold separately)	Five lot specific calibrators

Analytical performance characteristics

Quantitation and units of measure

For quantitation, the QUANTA Flash RF IgM and QUANTA Flash RF IgA assays utilize a lot specific Master Curve that is uploaded onto the instrument through the reagent cartridge barcode. The Master Curve for QUANTA Flash RF IgM and QUANTA Flash RF IgA consist of 6 different Standards each. These Master Curve Standards are used to create the lot specific Master Curve during the manufacturing procedure.

List of QUANTA Flash RF IgM Standards:

Material	Assigned Value
RF IgM Master Curve Standard 1	0.0 IU/mL
RF IgM Master Curve Standard 2	1.9 IU/mL
RF IgM Master Curve Standard 3	7.8 IU/mL
RF IgM Master Curve Standard 4	31.0 IU/mL
RF IgM Master Curve Standard 5	124.1 IU/mL
RF IgM Master Curve Standard 6	496.5 IU/mL

List of QUANTA Flash RF IgA Standards:

Material	Assigned Value
RF IgA Master Curve Standard 1	0.0 CU
RF IgA Master Curve Standard 2	7.2 CU
RF IgA Master Curve Standard 3	14.4 CU
RF IgA Master Curve Standard 4	57.5 CU
RF IgA Master Curve Standard 5	230.0 CU
RF IgA Master Curve Standard 6	919.9 CU

Precision

The precision of the QUANTA Flash RF IgM and QUANTA Flash RF IgA assays was evaluated on 10 samples for the IgM subtype and 9 samples for the IgA one, containing various concentrations of RF 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 repeatability (within-run), between run, between day and within-laboratory precision (total precision) were calculated.
Acceptance criteria: Total %CV: < 12%

Results are summarized in the tables below.

QUANTA Flash RF IgM			Repeatability		Between-Run		Between-Day		Within-Laboratory Precision	
Sample ID	N	Mean (IU/mL)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)
1	80	1.2	0.07	5.4%	0.04	3.1%	0.04	3.3%	0.08	7.0%
2	80	2.3	0.09	4.1%	0.12	5.2%	0.04	1.6%	0.15	6.8%
3	80	2.7	0.10	3.8%	0.10	3.6%	0.10	3.5%	0.17	6.3%
4	80	3.7	0.17	4.6%	0.12	3.2%	0.08	2.2%	0.22	6.0%
5	80	5.4	0.17	3.2%	0.17	3.2%	0.15	2.9%	0.29	5.4%
6	80	20.6	0.82	4.0%	0.38	1.8%	0.58	2.8%	1.07	5.2%
7	80	83.9	2.88	3.4%	2.71	3.2%	2.11	2.5%	4.49	5.3%
8	80	181.3	8.95	4.9%	8.62	4.8%	0.00	0.0%	12.43	6.9%
9	80	379.3	16.93	4.5%	22.47	5.9%	4.24	1.1%	28.45	7.5%
10	80	408.8	19.25	4.7%	21.63	5.3%	10.83	2.6%	30.91	7.6%

QUANTA Flash RF IgA			Repeatability		Between-Run		Between-Day		Within-Laboratory Precision	
Sample ID	N	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	80	10.6	0.37	3.5%	0.30	2.8%	0.40	3.7%	0.62	5.8%
2	80	11.2	0.59	5.3%	0.04	0.4%	0.22	1.9%	0.63	5.6%
3	80	20.9	0.84	4.0%	0.00	0.0%	0.67	3.2%	1.08	5.2%
4	80	21.6	0.72	3.3%	0.27	1.2%	0.54	2.5%	0.94	4.3%
5	80	48.7	2.08	4.3%	0.69	1.4%	0.55	1.1%	2.26	4.6%
6	80	107.0	2.53	2.4%	2.85	2.7%	2.25	2.1%	4.42	4.1%
7	80	415.8	11.17	2.7%	5.80	1.4%	8.78	2.1%	15.35	3.7%
8	80	721.1	31.70	4.4%	15.99	2.2%	30.78	4.3%	46.99	6.5%
9	80	749.3	25.30	3.4%	20.22	2.7%	21.92	2.9%	39.11	5.2%

Reproducibility Studies

Reproducibility between sites (instruments)

Eight samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, 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: Reproducibility %CV: < 12%

Results are summarized in the tables below.

QUANTA Flash RF IgM			Repeatability		Between-Day		Within-Site		Between-Site		Reproducibility	
Sample ID	N	Mean (IU/mL)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)
1	75	1.7	0.1	6.4%	0.1	4.4%	0.1	7.8%	0.1	2.7%	0.1	8.2%
2	75	3.0	0.1	4.0%	0.1	1.7%	0.1	4.4%	0.2	7.5%	0.3	8.7%
3	75	4.6	0.2	3.3%	0.2	4.0%	0.2	5.2%	0.1	2.2%	0.3	5.6%
4	75	6.3	0.2	2.9%	0.3	4.1%	0.3	5.0%	0.5	7.8%	0.6	9.3%
5	75	23.2	0.9	3.9%	0.7	2.9%	1.1	4.9%	0.2	1.1%	1.2	5.0%
6	75	92.8	2.4	2.6%	2.9	3.1%	3.7	4.0%	5.1	5.5%	6.3	6.8%
7	75	200.3	9.4	4.7%	10.7	5.4%	14.2	7.1%	4.9	2.4%	15.1	7.5%
8	75	412.9	15.5	3.8%	17.3	4.2%	23.3	5.6%	36.2	8.8%	43.0	10.4%

QUANTA Flash RF IgA			Repeatability		Between-Day		Within-Site		Between-Site		Reproducibility	
Sample ID	N	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	75	11.2	0.5	4.2%	0.4	3.3%	0.6	5.3%	0.4	3.1%	0.7	6.2%
2	75	11.3	0.5	4.0%	0.2	2.1%	0.5	4.5%	0.3	2.5%	0.6	5.2%
3	75	21.9	0.7	3.4%	0.1	0.4%	0.7	3.4%	0.6	2.9%	1.0	4.5%
4	75	23.2	0.9	3.9%	0.2	1.0%	0.9	4.0%	0.8	3.6%	1.3	5.4%
5	75	51.7	1.3	2.5%	0.7	1.2%	1.5	2.8%	0.5	0.9%	1.5	2.9%
6	75	112.6	3.2	2.9%	2.3	2.1%	4.0	3.5%	0.0	0.0%	4.0	3.5%
7	75	462.4	13.2	2.9%	10.9	2.4%	17.1	3.7%	19.8	4.3%	26.1	5.6%
8	75	738.4	27.3	3.7%	2.5	3.0%	35.3	4.8%	45.3	6.1%	57.4	7.8%

Reproducibility between lots

Eight samples were tested according to CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline, using three different lots. Samples were run in replicates of 5, once a day, for 5 days, to generate 25 data points per sample, per lot, 75 data points total for each sample. Data were analyzed with the Analyse-it for Excel method evaluation software to calculate between lot precision.
Acceptance criteria: Reproducibility %CV: < 12%

Results are summarized in the tables below.

QUANTA Flash RF IgM			Repeatability		Between-Day		Within-Lot		Between-Lot		Reproducibility	
Sample ID	N	Mean (IU/mL)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)	SD (IU/mL)	CV (%)
1	75	2.9	0.1	3.8%	0.0	1.6%	0.1	4.1%	0.0	1.0%	0.1	4.2%
2	75	3.3	0.1	3.1%	0.1	2.2%	0.1	3.8%	0.1	3.4%	0.2	5.1%
3	75	4.6	0.2	3.6%	0.1	3.0%	0.2	4.7%	0.0	0.0%	0.2	4.7%
4	75	23.2	0.8	3.4%	0.7	3.1%	1.1	4.6%	0.4	1.6%	1.1	4.8%
5	75	209.8	9.8	4.7%	14.0	6.7%	17.1	8.2%	9.8	4.7%	19.7	9.4%
6	75	1.6	0.1	5.2%	0.1	5.5%	0.1	7.6%	0.1	4.1%	0.1	8.7%
7	75	92.9	3.8	4.1%	2.4	2.6%	4.5	4.9%	4.8	5.2%	6.6	7.1%
8	75	6.0	0.2	3.8%	0.2	2.9%	0.3	4.8%	0.1	1.4%	0.3	5.0%

QUANTA Flash RF IgA			Repeatability		Between-Day		Within-Lot		Between-Lot		Reproducibility	
Sample ID	N	Mean (CU)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)	SD (CU)	CV (%)
1	75	11.7	0.5	4.3%	0.4	3.8%	0.7	5.7%	0.0	0.0%	0.7	5.7%
2	75	22.6	0.7	3.0%	0.6	2.7%	0.9	4.0%	0.2	0.9%	0.9	4.1%
3	75	22.5	0.6	2.9%	0.4	1.8%	0.8	3.4%	0.6	2.6%	1.0	4.3%
4	75	52.9	1.3	2.5%	0.8	1.6%	1.6	3.0%	0.4	0.8%	1.6	3.1%
5	75	487.0	13.6	2.8%	11.5	2.4%	17.8	3.7%	7.2	1.5%	19.2	3.9%
6	75	11.4	0.4	3.4%	0.2	2.0%	0.5	4.0%	0.0	0.3%	0.5	4.0%
7	75	113.2	3.3	2.9%	2.3	2.0%	4.0	3.5%	0.0	0.0%	4.0	3.5%
8	75	722.7	21.9	3.0%	23.7	3.3%	32.3	4.5%	18.7	2.6%	37.3	5.2%

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

The LoD of the QUANTA Flash RF IgM and QUANTA Flash RF IgA assays are 0.1 IU/mL and 0.5 CU respectively, which are below the analytical measuring range of the respective assays. The LoD of each assay was determined by using two reagent lots, consistent with CLSI EP17-A2 guideline with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%; based on 240 determinations, with 60 measurements on blank samples and 60 measurements of low level samples, per reagent lot. The LoB of the QUANTA Flash RF IgM is 0.0 IU/mL (450 RLU) while the LoB of the QUANTA Flash RF IgA is 0.3 CU (420 RLU).

Four low level samples were tested in replicates of five on two reagent lots, once per day, for 3 days, obtaining 30 data points per sample to generate data used to calculate the LoQ for the QUANTA Flash RF IgM and QUANTA Flash RF IgA assays. The LoQ was determined in each case by calculating the total imprecision of each sample.
Acceptance criteria: Total imprecision CV% <20%.

The LoQ for the QUANTA Flash RF IgM assay has been found to be at 0.3 IU/mL.
The LoQ for the QUANTA Flash RF IgA assay has been found to be at 1.2 CU.
Even though the LoQ for the QUANTA Flash RF IgA has been found to be at 1.2 CU, the AMR of the QUANTA Flash RF IgA will start at 1.3 CU.

Analytical Measuring Range (AMR)

QUANTA Flash RF IgM:	0.3 IU/mL – 490.0 IU/mL
QUANTA Flash RF IgA:	1.3 CU – 900.0 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 >490.0 IU/mL, for the QUANTA Flash RF IgM, or >900.0 CU, for the QUANTA Flash RF IgA, 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.
For the QUANTA Flash RF IgM, the highest value that can be directly measured is 490.0 IU/mL, so the highest value that can be reported is 9,800.0 IU/mL.
For the QUANTA Flash RF IgA, the highest value that can be directly measured is 900.0 CU, so the highest value that can be reported is 18,000.0 CU.

High concentration hook effect

To assess hook effect, measurement signal in relative light units (RLU) was examined by performing serial dilutions of two high positive samples (with results above the AMR of each assay when tested as neat samples). RLU values showed increase with increasing antibody concentrations above the AMR for both assays, thereby confirming that high positive specimens above the AMR do not show hook effect up to 1,830.6 IU/mL for the QUANTA Flash RF IgM assay and 42,710.4 CU for the QUANTA Flash RF IgA assay (theoretical value calculated using the highest value in the AMR and its dilution factor).

Linearity

The linearity of the AMR of the QUANTA Flash RF IgM and QUANTA Flash RF IgA was evaluated by a study according to CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. The linearity was evaluated using four human serum samples for the QUANTA Flash RF IgM and three human serum samples for the QUANTA Flash RF IgA with various RF antibody concentrations which were combined with another human serum sample containing low levels of RF antibodies in 10% increments (from 0% to 90% of low sample) to obtain values that cover the entire AMR of the assays. The dilutions were assayed in duplicates. Results were analyzed according to the guideline performing regression analysis and identifying the best fitting polynomial.

Acceptance criteria:
- Best fitting polynomial is a linear one, otherwise, the difference between the best-fitting nonlinear and linear polynomial is less than 15% or ±0.75 IU/mL / ±3 CU for negative samples (allowable nonlinearity).

For the QUANTA Flash RF IgM, all samples have been found that the best fitting polynomial is a linear one except Sample 3, where the best fitting polynomial found was a second order polynomial. The nonlinearity for Sample 3 ranged from -5.2% to 3.8% and from -0.1 IU/mL to 0.6 IU/mL, fulfilling the acceptance criteria.

All four samples showed dilution linearity individually and in combination.

Serum Samples	Test Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	60.5 – 544.1	0.92 (0.88 – 0.96)	25.9 (11.9 – 39.9)	0.99	104.0%
2	10.8 – 108.3	1.04 (0.99 - 1.10)	0.6 (-2.8 – 4.0)	0.99	105.6%
3	1.5 – 15.3	1.00 (0.95 – 1.05)	-0.6 (-1.1 – -0.1)	0.99	91.2%
4	0.2 – 1.5	1.07 (0.99 – 1.14)	-0.1 (-0.1 – 0.0)	0.98	96.7%
Combined	0.2 – 544.1	0.98 (0.97 – 0.99)	2.7 (0.5 – 4.9)	1.00	99.3%

For the QUANTA Flash RF IgA, all samples have been found that the best fitting polynomial is a linear one except Sample 1, where the best fitting polynomial found was a third order polynomial. The nonlinearity for Sample 1 ranged from -10.8% to 5.6%, fulfilling the acceptance criteria.

All three samples showed dilution linearity individually and in combination.

Serum Samples	Test Range (CU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	Average % Recovery
1	104.5 – 1045.0	1.00 (0.96 – 1.04)	12.8 (-12.4 – 38.1)	0.99	102.9%
2	10.8 – 107.7	0.94 (0.90 – 0.98)	5.1 (2.7 – 7.6)	0.99	106.6%
3	1.1 – 11.1	0.97 (0.92 – 1.02)	0.3 (-0.1 – 0.6)	0.99	104.9%
Combined	1.1 – 1045.0	1.01 (1.00 – 1.02)	1.7 (-2.8 – 6.2)	1.00	104.8%

These data demonstrate the linearity of the analytical measuring range (0.3 IU/mL – 490.0 IU/mL) of the QUANTA Flash RF IgM assay and the analytical measuring range (1.3 CU – 900.0 CU) of the QUANTA Flash RF IgA assay.

Interference

The interference study was performed according to CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. A set of four human serum specimens, one high positive, one low positive, one near the cutoff and one negative sample were tested using the following interfering substances (bilirubin, hemoglobin, triglycerides, cholesterol, human IgG, ascorbic acid, methotrexate and prednisone). All interferents were spiked into every specimen in 10% of total specimen volume, and the resulting samples were assessed in triplicates with the QUANTA Flash RF IgM and the QUANTA Flash RF IgA assays. Recovery of the unit values was calculated compared to control samples spiked with the same volume of diluents (10% of total sample volume). Acceptance criteria for the interference studies were 85% - 115% recovery, or ± 15% of the cut-off (±0.75 IU/mL or ±3 CU) difference, whichever is greater.
No interference was detected with any of the assays with bilirubin up to 1 mg/mL (recovery: from 89.0% to 98.2% and from 92.5 to 93.1 for the IgM and IgA subtypes respectively), hemoglobin up to 2 mg/mL (recovery: from 94.5% to 95.0% and from 90.9 to 94.7 for the IgM and IgA subtypes respectively), triglycerides up to 1000 mg/dL (recovery: from 91.2% to 112.2% and from 97.8 to 105.4 for the IgM and IgA subtypes respectively), cholesterol up to 332.5 mg/dL (recovery: from 89.7% to 91.6% and from 91.0 to 91.0 for the IgM and IgA subtypes respectively), human IgG up to 70 mg/mL (recovery: from 86.8% to 89.3% and from 91.6 to 95.7 for the IgM and IgA subtypes respectively), ascorbic acid up to 60.1 mg/L (recovery: from 95.6% to 101.7% and from 98.1 to 102.6 for the IgM and IgA subtypes respectively), methotrexate up to 9.1 mg/mL (recovery: from 96.1% to 102.8% and from 92.9 to 105.8 for the IgM and IgA subtypes respectively), and prednisone up to 0.3 mg/L (recovery: from 101.9% to 109.5% and from 93.0 to 94.9 for the IgM and IgA subtypes respectively).

Sample Stability and Handling

For the QUANTA Flash RF IgM, five human serum samples, encompassing negative (n=1), around the cut-off (n=1), and positive samples (n=3), and for the QUANTA Flash RF IgA four human serum samples, encompassing negative (n=1), around the cut-off (n=1), and positive samples (n=2), 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 (time zero / zero cycles).

Acceptance criteria: recovery is between 85-115% for positive samples, and between 80-120% for negative samples (<5 IU/mL or <20 CU).

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 3 weeks at 37 °C, where one week is equal to six months at 5 ± 3°C.
Accelerated stability testing was performed on each of the following sealed components to establish initial stability claim:

- RF IgM beads (3 Lots)
- Anti-IgM tracer (3 Lots)
- RF IgA beads (3 Lots)

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

Acceptance criteria for one year preliminary expiration dating:

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

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

In-use (onboard) stability

Reagent Cartridge

To establish the in-use stability of the QUANTA Flash RF IgM and QUANTA Flash RF IgA reagent cartridges, two lots of reagent cartridge were tested using up to 10 human serum samples (with different reactivity levels). The specimens were tested periodically for of 91 days. Percent recoveries were calculated compared to the day zero average values, and linear regression analysis was performed by plotting percent recovery against the number of days. The claim was established using the following criteria (using the one that is fulfilled first):
- The stability claim is established at the actual measurement day proceeding the day when the 95% confidence interval of the regression line reaches 85% or 115% recovery, or
- At the actual measurement day preceding the day when ≥2% of the recovery data, (3 data points) is ≤75% or ≥125% recovery.

The onboard stability results for the QUANTA Flash RF IgM are as follows:
Lot RP0005: 83 days
Lot RP0006: 90 days
Using these criteria, the in-use (onboard) stability of the QUANTA Flash RF IgM reagent cartridge was set at 80 days.

The onboard stability results for the QUANTA Flash RF IgA are as follows:
Lot RP0003: 90 days
Lot RP0004: 84 days
Using these criteria, the in-use (onboard) stability of the QUANTA Flash RF IgA reagent cartridge was set at 80 days.

Real time stability

Real time stability testing has been scheduled to be performed every three or six months on the QUANTA Flash RF IgM and QUANTA Flash RF IgA Reagents kits, to verify the one year expiration that was assigned based on accelerated stability studies. At the time of the submission, results were available up to 13 months for QUANTA Flash RF IgM Reagent kits and for QUANTA Flash RF IgA Reagent kits.

A negative sample (Negative Control), a low positive sample (Positive Control), and a high positive sample were tested in replicates of 6 (replicates of 9 at time zero) at each time point.
- Acceptance criteria: results should fall within their respective ranges.

All results to date were within the acceptance limits.

Cut-off, reference range

QUANTA Flash RF IgM:

Negative	<5 IU/mL
Positive	≥5 IU/mL

QUANTA Flash RF IgA:

Negative	<20 CU
Positive	≥20 CU

The reference population for establishing the reference interval for the QUANTA Flash RF IgM and QUANTA Flash RF IgA assays consisted of 191 subjects:

Sample Group	N
Apparently healthy donors	117
Infectious Disease Controls (HBV, HCV, HIV, Syphilis)	27
False Positive Cohort (high reactivity samples)	13
Antiphospholipid syndrome (APS)	12
Systemic Lupus Erythematosus (SLE)	9
Celiac Disease	8
Vasculitis	5

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

Additionally, 42 diagnosed rheumatoid arthritis (RA) patient specimens were assayed to aid in the determination of the cutoff values.

For the QUANTA Flash RF IgM, based on the distribution of result values and in these (known) positive samples, a cutoff of 5 IU/mL (16,300 RLU) has been set to ensure optimal differentiation between negatives and positives samples.

For the QUANTA Flash RF IgA, based on the distribution of result values and in these (known) positive samples, the cutoff was established at 6,000 RLU to ensure optimal differentiation between negatives and positives samples. The cut-off was established at a value greater than the 95th percentile of the control results (3,767 RLU) and it was assigned a value of 20 CU.

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 RF IgM and the QUANTA Flash RF IgA assays. A total of 706 characterized samples were included in this Validation Set. All samples were run on the QUANTA Flash RF IgM and the QUANTA Flash RF IgA assays. The distribution of the cohort and the RF positivity rate is in the Table below:

Patient Group	N	RF IgM N Positive	RF IgM % Positive	RF IgA N Positive	RF IgA % Positive
Ulcerative Colitis	50	8	16.0%	10	20.0%
Crohn's Disease	30	3	10.0%	2	6.7%
Osteoarthritis	30	3	10.0%	0	0.0%
Autoimmune Hepatitis	30	0	0.0%	1	3.3%
Sjögren's Syndrome	30	7	23.3%	6	20.0%
Systemic Sclerosis	30	9	30.0%	10	33.3%
Celiac Disease	29	0	0.0%	0	0.0%
Systemic Lupus Erythematosus	28	4	14.3%	4	14.3%
Hepatitis B Virus	20	1	5.0%	0	0.0%
Hepatitis C Virus	19	0	0.0%	1	5.3%
Polymyalgia Rheumatica	15	2	13.3%	0	0.0%
Psoriatic Arthritis	12	0	0.0%	0	0.0%
Syphilis	12	1	8.3%	0	0.0%
Parvo virus infection	10	2	20.0%	2	20.0%
Lyme disease	10	0	0.0%	0	0.0%
Fibromyalgia	10	1	10.0%	0	0.0%
Ankylosing Spondylitis	8	0	0.0%	0	0.0%
Mixed connective tissue disease	8	1	12.5%	1	12.5%
Hashimoto's disease	8	0	0.0%	0	0.0%
Grave's disease	6	1	16.7%	0	0.0%
Polymyositis	10	3	30.0%	2	20.0%
Dermatomyositis	5	2	40.0%	0	0.0%
Total Controls	**410**	**48**	**11.7%**	**39**	**9.5%**
Rheumatoid Arthritis	296	206	69.6%	168	56.8%
Total	**706**	**-**	**-**	**-**	**-**

Clinical sensitivity and specificity of the QUANTA Flash RF IgM were analyzed in the table below:

Clinical Analysis (N=706)		QUANTA Flash RF IgM			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	RA	206	90	296	Sensitivity: 69.6% (64.1 – 74.6%)
	Controls	48	362	410	Specificity: 88.3% (84.8 – 91.1%)
	Total	254	452	706	

Clinical sensitivity and specificity of the QUANTA Flash RF IgA were analyzed in the table below:

Clinical Analysis (N=706)		QUANTA Flash RF IgA			Analysis (95% confidence)
		Positive	Negative	Total	
Diagnosis	RA	168	128	296	Sensitivity: 56.8% (51.1 – 62.3%)
	Controls	39	371	410	Specificity: 90.5% (87.3 – 93.0%)
	Total	207	499	706	

Expected values

QUANTA Flash RF IgM

The expected value in the normal population is “negative”. RF IgM antibody levels were analyzed using the QUANTA Flash RF IgM assay on a panel of 100 apparently healthy blood donors (50 females/50 males, ages 24 to 70 years, with an average and median age of 50 and 51 years respectively). With a cut-off of 5 IU/mL, six samples (6.0%) were positive on the QUANTA Flash RF IgM. The mean and median concentration were 3.6 and 0.5 IU/mL respectively, and the values ranged from <0.3 to 219.1 IU/mL.

QUANTA Flash RF IgA

The expected value in the normal population is “negative”. RF IgA antibody levels were analyzed using the QUANTA Flash RF IgA assay on a panel of 100 apparently healthy blood donors (50 females/50 males, ages 24 to 70 years, with an average and median age of 50 and 51 years respectively). With a cut-off of 20 CU, six samples (6.0%) were positive on the QUANTA Flash RF IgA. The mean and median concentration were 8.2 and 2.9 CU respectively, and the values ranged from <1.3 to 324.9 CU.

Comparison with predicate device

Samples for method comparison analysis included all samples from the clinical validation study. These samples were tested on both the QUANTA Flash RF assays and on their predicate ELISA.
Of the 706 samples tested, 577 samples fell within the AMR of the QUANTA Flash RF IgM assay, while 612 fell within the AMR of the QUANTA Flash RF IgA assay.

Method comparison of the QUANTA Flash RF IgM with the predicate device using samples within the AMR.

Method Comparison Within AMR (N=577)		QUANTA Flash RF IgM			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	291	11	302	NPA: 96.4 (93.6– 98.0)
	Positive	52	223	275	PPA: 81.1 (76.0 – 85.3)
	Total	343	234	577	TPA: 89.1 (86.3 – 91.4)

PPA= Positive Percent Agreement; NPA= Negative Percent Agreement; TPA= Total Percent Agreement

Additionally, a quantitative comparison has been performed on the 577 samples in the AMR of the QUANTA Flash RF IgM assay utilizing a Spearman correlation. The results revealed a Spearman's rs of 0.85 (95% CI, 0.82 – 0.87).

Method comparison of the QUANTA Flash RF IgA with the predicate device using samples within the AMR.

Method Comparison Within AMR (N=612)		QUANTA Flash RF IgA			Percent Agreement (95% Confidence)
		Negative	Positive	Total	
Predicate ELISA	Negative	385	10	395	NPA: 97.5 (95.4– 98.6)
	Positive	27	190	217	PPA: 87.6 (82.5 – 91.3)
	Total	412	200	612	TPA: 94.0 (91.8 – 95.6)

PPA= Positive Percent Agreement; NPA= Negative Percent Agreement; TPA= Total Percent Agreement