

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM**

A. 510(k) Number:

K143736

B. Purpose for Submission:

New device

C. Measurand:

Rheumatoid Factors (RF) IgA, IgG, IgM, and Rheumatoid Factor Total

D. Type of Test:

Immunoassay, qualitative and semi-quantitative

E. Applicant:

IMMCO Diagnostics, Inc.

F. Proprietary and Established Names:

ImmuLisa Enhanced™ RF IgA Antibody ELISA
ImmuLisa Enhanced™ RF IgG Antibody ELISA
ImmuLisa Enhanced™ RF IgM Antibody ELISA
ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5775: Rheumatoid factor immunological test system

2. Classification:

Class II

3. Product code:

DHR: System, Test, Rheumatoid Factor

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

ImmuLisa Enhanced™ RF IgA Antibody ELISA:

Enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Rheumatoid Factor IgA antibodies in human serum to aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other laboratory tests and clinical findings.

ImmuLisa Enhanced™ RF IgG Antibody ELISA:

Enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Rheumatoid Factor IgG antibodies in human serum to aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other laboratory tests and clinical findings.

ImmuLisa Enhanced™ RF IgM Antibody ELISA:

Enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Rheumatoid Factor IgM antibodies in human serum to aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other laboratory tests and clinical findings.

ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA:

Enzyme linked immunoassay (ELISA) for the qualitative detection of Rheumatoid Factor IgA, IgG and IgM antibodies in human serum to aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other laboratory tests and clinical findings.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

A microplate reader capable of reading absorbance values at 450 nm. If a dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm. An automatic microplate washer capable of accurately dispensing 200 µL of fluid is also required.

I. Device Description:

Each kit consists of one 12 x8 microplate with individual breakaway microwells coated with purified rabbit IgG antigen, a five-level calibrator set for semi-quantitative analysis, a cut-off calibrator for qualitative analysis, a negative control, a positive control, Tetramethylbenzidine (TMB) chromogenic substrate, stop solution, wash buffer, and diluent.

The type of conjugate is specific for each kit: goat anti-human IgA, HRP (horseradish peroxidase) conjugate for the RF IgA Antibody ELISA; goat anti-human IgG HRP conjugate for the RF IgG Antibody ELISA; goat anti-human IgM HRP conjugate for the RF IgM Antibody ELISA; and a mixture of goat anti-human IgA, IgG, and IgM HRP conjugates for the RF IgA/IgG/IgM ELISA.

J. Substantial Equivalence Information:

1. Predicate device name(s) and numbers:

Inova QuantaLite[®] RF IgA ELISA, K983084
 Inova QuantaLite[®] RF IgG ELISA, K983083
 Inova QuantaLite[®] RF IgM ELISA, K971614

2. Comparison with predicates:

Similarities		
Item	New Devices: ImmuLisa Enhanced™ RF IgA/IgG/IgM Antibody ELISAs and RF IgA/IgG/IgM ELISA	Predicates: Inova QuantaLite[®] RF IgA/ IgG/IgM ELISA
Intended Use/Indication for Use	Enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Rheumatoid Factor IgA, IgG, or IgM antibodies in human serum to aid in the diagnosis of rheumatoid arthritis (RA) in conjunction with other laboratory tests and clinical findings.	Same
Test Principle	Enzyme-Linked Immunoassay (ELISA)	Same
Instrumentation	Spectrophotometer reading at 450 nm	Same
Analyte	RF IgA, IgG, or IgM antibodies	Same
Sample Type	Serum	Same
Sample Dilution	1:101	Same
Kit Components	Includes positive controls, negative controls, calibrators, conjugates, substrate, diluent, wash buffer, stop solution, microplates	Same
Capture Antigen	Rabbit IgG	Same
Detection Reagents	HRP conjugated to goat anti-human IgA, IgG, or IgM	Same
Substrate	TMB	Same
Incubation Times	Positive and negative controls and diluted patient samples: 30 min	Same

Similarities		
Item	New Devices: ImmuLisa Enhanced™ RF IgA/IgG/IgM Antibody ELISAs and RF IgA/IgG/IgM ELISA	Predicates: Inova QuantaLite® RF IgA/ IgG/IgM ELISA
	Conjugate: 30 min Substrate: 30 min (in dark)	
Stop Solution	Sulfuric acid (H ₂ SO ₄)	Same
Controls	Positive Controls: human sera positive for RF IgA, IgG, IgM, or total RF Negative Controls: human sera negative for RF IgA, IgG, IgM, or total RF	Same

Differences		
Item	New Devices: ImmuLisa Enhanced™ RF IgA/IgG/IgM Antibody ELISAs and RF IgA/IgG/IgM ELISA	Predicates: Inova QuantaLite® RF IgA/IgG/IgM ELISA
Measurement Type	RF IgA, IgG, and IgM Antibody ELISAs: Qualitative and semi-quantitative RF IgA/IgG/IgM ELISA: Qualitative only	Semi-Quantitative
Wash buffer	Powdered or optional liquid concentrate	Liquid concentrate
Calibrators	RF IgA and IgG Antibody ELISAs: Set of five vials with values in EU/ml: 1, 20, 40, 80, 160 RF IgM Antibody ELISA: Set of five vials with values in IU/mL: 1, 10, 20, 40, 80 RF IgA/IgG/IgM ELISA: 30 EU/mL (1- point calibration)	Set of five vials with value in units: 5, 12.5, 25, 50, 100
Reagent Stability	Reagents: until the expiration date at 2– 8°C Reconstituted wash buffer: until the kit expiration date at 2–8°C	Reagents: until the expiration date at 2–8°C Diluted wash buffer: 1 week at 2–8°C
Cut-offs	RF IgA/IgG and RF IgA/IgG/IgM ELISAs: 20 EU/mL RF IgM Antibody ELISA: 10 IU/mL	IgA, IgG, and IgM ELISAs: 6 Units
Linear Ranges	RF IgA Antibody ELISA: 3.7–160 EU/mL RF IgG Antibody ELISA: 2.2–160 EU/mL RF IgM Antibody ELISA: 1.3–80 IU/mL	Not Specified
Limits of Blank	RF IgA Antibody ELISA: 3.2 EU/mL RF IgG Antibody ELISA: 1.5 EU/mL RF IgM Antibody ELISA: 1.2 IU/mL RF IgA/IgG/IgM ELISA: 2.1 EU/mL	Not Specified
Limits of Detection	RF IgA Antibody ELISA: 3.7 EU/mL RF IgG Antibody ELISA: 2.2 EU/mL	Not Specified

Differences		
Item	New Devices: ImmuLisa Enhanced™ RF IgA/IgG/IgM Antibody ELISAs and RF IgA/IgG/IgM ELISA	Predicates: Inova QuantaLite® RF IgA/IgG/IgM ELISA
	RF IgM Antibody ELISA: 1.3 IU/mL RF IgA/IgG/IgM ELISA: 2.7 EU/mL	
Traceability	RF IgA and IgG Antibody ELISAs: Reference standard or method is not available. Results are traceable to in-house standards. RF IgM Antibody ELISA: traceable to the WHO RF IgM International Standard (NIBSC 64/2)	Not Specified

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

1. CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition
2. CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
3. CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
4. CLSI EP09-A3: Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition
5. CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline- Second Edition
6. CLSI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline - Second Edition

L. Test Principle:

All four ImmuLisa Enhanced RF Antibody tests are sandwich-type enzyme immunoassays. Serum samples diluted 1:101 are incubated in the microplates coated with purified rabbit IgG as the antigen. Patient antibodies, if present in the samples, bind to the antigen-coated wells. Unbound antibodies and other serum proteins are removed by washing the microwells. Bound antibodies are detected by adding anti-human immunoglobulins conjugated to horseradish peroxidase (conjugate) to the microwells. After incubation, excess conjugate is removed by washing. Specific enzyme substrate is then added to the microwells and the presence of antibodies to RF is detected by a color change produced by the conversion of the substrate to a colored reaction product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm.

Semi-quantitative results are determined from a standard curve generated from the results of each assay's five calibrators. Semi-quantitative results are reported as EU/mL for the IgA

and IgG assays and as IU/mL for the IgM assay. Results are reported as positive, negative, or indeterminate according to the following table. The manufacturer recommends that indeterminate results should be retested and evaluated along with other laboratory methods.

RF IgA or IgG Antibody ELISA Value (EU/mL)	RF IgM Antibody ELISA Value (IU/mL)	Interpretation
<20	<10	Negative
20–25	10–12.5	Indeterminate
>25	>12.5	Positive

Qualitative results for the RF IgA, IgG, and IgM Antibody ELISAs are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 EU/mL or 10 IU/mL). The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. It is recommended that qualitative results be reported as positive or negative. Values greater than or equal to the cut-off calibrator are considered positive.

Qualitative results for the RF IgA/IgG/IgM ELISA are determined using a ratio of the absorbance of the sample to the absorbance of the single calibrator at 30 EU/mL. The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. Values <20 EU/mL are reported as negative and values ≥ 20 EU/mL are reported as positive.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

Semi-quantitative precision:

The precision performance of each RF Antibody ELISA was evaluated in accordance with CLSI guideline EP05-A2. Seven serum samples from the intended use population that cover the measuring range of each ELISA were tested with six replicates in a single run per day over a period of 13 days. An additional 12 replicates were included on the final day (n = 90 replicates total). Assays were performed by two operators using one reagent lot and different instrumentation setups. The first instrumentation setup includes a multi-channel pipettor, microplate washer and microplate reader. The second instrumentation set was a liquid handling system that includes a microplate reader. The precision parameters were calculated and all values met the manufacturer’s pre-determined acceptance criteria. The data are presented in the tables below:

ImmULisa Enhanced™ RF IgA Antibody ELISA							
Sample	Mean (EU/mL)	Within-Run (Repeatability)		Between- Day		Total	
		SD	CV	SD	CV	SD	CV
1	9.2	0.39	4.2%	0.36	3.9%	0.54	5.8%
2	14.9	0.57	3.8%	0.58	3.9%	0.82	5.5%
3	21.2	0.97	4.6%	0.71	3.3%	1.20	5.7%
4	26.4	0.73	2.8%	1.21	4.6%	1.42	5.4%
5	74.6	2.21	3.0%	4.51	6.0%	5.02	6.7%
6	115.6	2.99	2.6%	4.34	3.8%	5.27	4.6%
7	158.1	4.99	3.2%	3.93	2.5%	6.35	4.0%

ImmULisa Enhanced™ RF IgG Antibody ELISA							
Sample	Mean (EU/mL)	Within-Run (Repeatability)		Between- Day		Total	
		SD	CV	SD	CV	SD	CV
1	8.8	0.60	6.8%	0.47	5.3%	0.76	8.7%
2	16.4	0.72	4.4%	0.74	4.5%	1.03	6.3%
3	19.4	0.88	4.5%	0.60	3.1%	1.06	5.5%
4	22.7	0.94	4.1%	0.54	2.4%	1.08	4.8%
5	45.9	1.86	4.0%	1.12	2.4%	2.17	4.7%
6	79.7	2.64	3.3%	4.16	5.2%	4.93	6.2%
7	133.0	4.83	3.6%	4.15	3.1%	6.37	4.8%

ImmULisa Enhanced™ RF IgM Antibody ELISA							
Sample	Mean (IU/mL)	Within-Run (Repeatability)		Between- Day		Total	
		SD	CV	SD	CV	SD	CV
1	4.8	0.12	2.5%	0.13	2.7%	0.18	3.7%
2	8.5	0.28	3.3%	0.18	2.1%	0.34	3.9%
3	10.3	0.63	6.1%	0.48	4.6%	0.79	7.7%
4	11.9	0.45	3.8%	0.54	4.5%	0.70	5.9%
5	25.6	0.70	2.7%	0.82	3.2%	1.08	4.2%
6	45.6	1.16	2.6%	1.46	3.2%	1.87	4.1%
7	80.2	1.88	2.3%	3.12	3.9%	3.64	4.5%

Inter-Operator Precision Tables:

ImmuLisa Enhanced™ RF IgA Antibody ELISA									
Sample	Mean (EU/mL)	Within-Run (Repeatability)		Between- Day		Between- Operator		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	9.2	0.39	4.2%	0.37	4.0%	0.00	0.0%	0.54	5.8%
2	14.9	0.57	3.8%	0.52	3.5%	0.39	2.6%	0.86	5.8%
3	21.2	0.97	4.6%	0.60	2.8%	0.55	2.6%	1.26	6.0%
4	26.4	0.73	2.8%	1.08	4.1%	0.81	3.1%	1.53	5.8%
5	74.6	2.21	3.0%	3.88	5.2%	3.33	4.5%	5.57	7.5%
6	115.6	2.99	2.6%	3.43	3.0%	3.85	3.3%	5.96	5.2%
7	158.1	4.99	3.2%	3.94	2.5%	0.00	0.0%	6.35	4.0%

ImmuLisa Enhanced™ RF IgA Antibody ELISA							
Sample	Mean (EU/mL)	Operator/ Equipment 1		Operator/ Equipment 2		Total	
		SD	CV	SD	CV	SD	CV
1	9.2	0.59	6.3%	0.48	5.2%	0.54	5.8%
2	14.9	0.75	5.0%	0.74	5.0%	0.86	5.8%
3	21.2	0.94	4.4%	1.18	5.6%	1.26	6.0%
4	26.4	1.11	4.2%	1.29	4.9%	1.53	5.8%
5	74.6	3.32	4.5%	4.60	6.2%	5.57	7.5%
6	115.6	3.24	2.8%	4.81	4.2%	5.96	5.2%
7	158.1	3.87	2.4%	7.01	4.4%	6.35	4.0%

ImmuLisa Enhanced™ RF IgG Antibody ELISA									
Sample	Mean (EU/mL)	Within-Run (Repeatability)		Between- Day		Between- Operator		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	8.8	0.60	6.8%	0.33	3.7%	0.48	5.5%	0.83	9.5%
2	16.4	0.72	4.4%	0.72	4.4%	0.24	1.5%	1.04	6.4%
3	19.4	0.86	4.4%	0.58	3.0%	0.32	1.6%	1.08	5.6%
4	22.7	0.89	3.9%	0.57	2.5%	0.33	1.4%	1.11	4.9%
5	45.9	1.70	3.7%	1.40	3.1%	0.00	0.0%	2.20	4.8%
6	79.7	2.66	3.3%	3.94	4.9%	1.44	1.8%	4.97	6.2%
7	133.0	4.86	3.7%	3.18	2.4%	3.59	2.7%	6.83	5.1%

Immulin Enhanced™ RF IgG Antibody ELISA							
Sample	Mean (EU/mL)	Operator/ Equipment 1		Operator/ Equipment 2		Total	
		SD	CV	SD	CV	SD	CV
1	8.8	0.74	8.4%	0.62	7.1%	0.83	9.5%
2	16.4	0.93	5.7%	1.00	6.1%	1.04	6.4%
3	19.4	0.92	4.7%	1.05	5.4%	1.08	5.6%
4	22.7	1.20	5.3%	0.93	4.1%	1.11	4.9%
5	45.9	2.11	4.6%	2.14	4.7%	2.20	4.8%
6	79.7	3.76	4.7%	4.85	6.1%	4.97	6.2%
7	133.0	6.34	4.8%	5.26	4.0%	6.83	5.1%

Immulin Enhanced™ RF IgM Antibody ELISA									
Sample	Mean (IU/mL)	Within-Run (Repeatability)		Between-Day		Between-Operator		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	4.8	0.12	2.5%	0.13	2.7%	0.00	0.0%	0.18	3.7%
2	8.5	0.28	3.3%	0.16	1.9%	0.11	1.3%	0.34	4.0%
3	10.3	0.63	6.1%	0.50	4.8%	0.00	0.0%	0.81	7.8%
4	11.9	0.45	3.8%	0.55	4.6%	0.00	0.0%	0.71	6.0%
5	25.6	0.70	2.7%	0.85	3.3%	0.00	0.0%	1.10	4.3%
6	45.6	1.16	2.6%	1.44	3.2%	0.38	0.8%	1.89	4.1%
7	80.2	1.88	2.3%	2.56	3.2%	2.57	3.2%	4.09	5.1%

Immulin Enhanced™ RF IgM Antibody ELISA							
Sample	Mean (IU/mL)	Operator/ Equipment 1		Operator/ Equipment 2		Total	
		SD	CV	SD	CV	SD	CV
1	4.8	0.15	3.2%	0.18	3.7%	0.18	3.7%
2	8.5	0.24	2.8%	0.35	4.1%	0.34	4.0%
3	10.3	0.73	7.1%	0.80	7.8%	0.81	7.8%
4	11.9	0.59	4.9%	0.72	6.0%	0.71	6.0%
5	25.6	1.11	4.3%	1.02	4.0%	1.10	4.3%
6	45.6	2.08	4.6%	1.59	3.5%	1.89	4.1%
7	80.2	2.93	3.7%	3.07	3.8%	4.09	5.1%

Lot-to-lot reproducibility:

Seven serum samples from the intended use population that cover the measuring range of each ELISA were measured with five replicates and one run per day for five

days using three lots of reagents on one instrument and one operator at one site (n = 75 replicates total). For Lot 1, the %CV for total precision for the seven samples across all four ELISAs ranged from 2.4%–8.2%. For Lot 2, the %CV for total precision for the seven samples across all four ELISAs ranged from 2.2%–11.6%. For Lot 3, the % CV for total precision for the seven samples across all four ELISAs ranged from 2.8%–9.4%. The combined lot-to-lot reproducibility parameters for each ELISA type are detailed in the tables below. All values met the manufacturer’s acceptance criteria.

Immulin Enhanced™ RF IgA Antibody ELISA									
Sample	Mean (EU/mL)	Within-Run (Repeatability)		Between- Day		Between-Lot		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	8.48	0.33	3.9%	0.00	0.0%	0.10	1.2%	0.34	4.1%
2	13.67	1.07	7.8%	0.00	0.0%	0.00	0.0%	1.07	7.8%
3	20.52	1.36	6.6%	0.00	0.0%	0.00	0.0%	1.36	6.6%
4	24.86	1.01	4.1%	0.00	0.0%	0.06	0.2%	1.02	4.1%
5	72.09	4.99	6.9%	0.00	0.0%	0.00	0.0%	4.99	6.9%
6	109.74	3.95	3.6%	0.00	0.0%	0.28	0.3%	3.96	3.6%
7	148.24	5.93	4.0%	0.00	0.0%	1.91	1.3%	6.23	4.2%

Immulin Enhanced™ RF IgG Antibody ELISA									
Sample	Mean (EU/mL)	Within-Run (Repeatability)		Between- Day		Between-Lot		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	8.36	0.82	9.8%	0.06	0.8%	0.00	0.0%	0.82	9.8%
2	15.80	0.79	5.0%	0.00	0.0%	0.30	1.9%	0.84	5.3%
3	19.74	1.27	6.4%	0.00	0.0%	0.34	1.7%	1.31	6.7%
4	22.11	0.83	3.8%	0.00	0.0%	0.22	1.0%	0.86	3.9%
5	44.11	1.24	2.8%	0.00	0.0%	0.30	0.7%	1.28	2.9%
6	77.03	4.71	6.1%	0.00	0.0%	0.00	0.0%	4.71	6.1%
7	127.59	5.72	4.5%	1.10	0.9%	0.00	0.0%	5.82	4.6%

ImmuLisa Enhanced™ RF IgM Antibody ELISA									
Sample	Mean (IU/mL)	Within-Run (Repeatability)		Between-Day		Between-Lot		Total	
		SD	CV	SD	CV	SD	CV	SD	CV
1	4.76	0.39	8.2%	0.00	0.0%	0.10	2.1%	0.40	8.5%
2	8.39	0.38	4.6%	0.15	1.8%	0.11	1.4%	0.43	5.1%
3	9.87	0.82	8.3%	0.00	0.0%	0.00	0.0%	0.82	8.3%
4	0.00	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.70	5.8%
5	25.81	0.72	2.8%	0.22	0.9%	0.00	0.0%	0.75	2.9%
6	47.64	1.68	3.5%	0.00	0.0%	0.87	1.8%	1.89	4.0%
7	82.68	2.94	3.6%	1.42	1.7%	0.00	0.0%	3.27	4.0%

Qualitative reproducibility:

Because the ImmuLisa Enhanced™ RF Antibody ELISAs can be used in either a semi-quantitative or a qualitative mode, a separate qualitative analysis using six human serum samples was performed according to CLSI EP12-A2. Ninety replicates were measured for sera in the negative range, ~20% below the cut-off, at ~cut-off, ~20% above the cut-off and in the moderate positive and high positive range of the assays. All values met the manufacturer's acceptance criteria and are summarized in the tables below.

ImmuLisa Enhanced™ RF IgA Antibody ELISA			
Sample	Mean (EU/mL)	%Negative	%Positive
Low Negative	9.4	100%	0%
Cut-off -20%	14.9	100%	0%
Cut-off	21.0	13.3%	86.7%
Cut-off +20%	25.4	0%	100%
Moderate Positive	76.8	0%	100%
High Positive	117.9	0%	100%

ImmuLisa Enhanced™ RF IgG Antibody ELISA			
Sample	Mean (EU/mL)	%Negative	%Positive
Low Negative	7.7	100%	0%
Cut-off -20%	16.1	100%	0%
Cut-off	19.4	77.8%	22.2%
Cut-off +20%	23.2	0%	100%
Moderate Positive	48.8	0%	100%
High Positive	76.6	0%	100%

ImmuLisa Enhanced™ RF IgM Antibody ELISA			
Sample	Mean (IU/mL)	%Negative	%Positive
Low Negative	4.8	100%	0%
Cut-off -20%	8.5	100%	0%
Cut-off	10.2	30%	70%
Cut-off +20%	11.5	0%	100%
Moderate Positive	19.1	0%	100%
High Positive	24.8	0%	100%

ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA			
Sample	Mean (EU/mL)	%Negative	%Positive
Low Negative	12.5	100%	0%
Cut-off -20%	17.8	100%	0%
Cut-off	19.8	59%	41%
Cut-off +20%	24.4	0%	100%
Moderate Positive	55.4	0%	100%
High Positive	97.2	0%	100%

b. *Linearity/assay reportable range:*

i. *Linearity:*

The linear ranges for the ImmuLisa Enhanced™ RF Antibody ELISAs were determined in accordance with CLSI EP06-A. Three human serum samples were diluted with the assay kit sample diluent to cover the entire calibration range and tested in duplicate. The measured values were graphed against the expected values and a linear regression was performed. Results are summarized below and met the manufacturer's pre-determined acceptance criteria.

ImmuLisa Enhanced™ RF IgA Antibody ELISA					
Sample	Dilution range (EU/mL)	Slope (95% CI)	Intercept (95% CI)	R²	% Recovery Range
1	4.8–13.7	0.96 (0.86–1.06)	0.83 (-0.12–1.77)	1.00	91.2%–100%
2	10.5–64.9	0.96 (0.84–1.09)	3.66 (-1.16–8.48)	0.98	83.4%–101.3%
3	17.3–155.0	1.00 (0.88–1.14)	2.55 (-9.4–14.5)	0.99	88%–104.1%

ImmuLisa Enhanced™ RF IgG Antibody ELISA					
Sample	Dilution range (EU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²	% Recovery Range
1	3.6–13.8	1.00 (0.92–1.08)	0.33 (-0.40–1.07)	1.00	92.2%–100.9%
2	7.0–68.7	1.01 (0.89–1.12)	1.81 (-2.85–6.47)	0.99	87.5%–100.1%
3	16.1–154.7	1.07 (0.95–1.20)	-1.59 (-13.3–10.2)	1.00	88.2%–105.6%

ImmuLisa Enhanced™ RF IgM Antibody ELISA					
Sample	Dilution range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²	% Recovery Range
1	2.0–8.1	0.97 (0.82–1.26)	-0.50 (-2.60–1.25)	1.00	88.2%–105.6%
2	8.1–61.4	0.96 (0.91–1.01)	1.43 (-0.56–3.43)	1.00	87.6%–103.1%
3	13.0–73.3	1.00 (0.84–1.15)	2.24 (-4.88–9.36)	1.00	89.4%–100.0%

The claimed reportable ranges for the RF Antibody ELISAs are:

IgA: 3.7–160 EU/mL

IgG: 2.2–160 EU/mL

IgM: 1.3–80 IU/mL

ii. High dose hook effect:

High antibody concentrations specimens were serially diluted to assess the presence of decreases in assay signal associated with antigen excess. No hook effect was seen above the analytical measuring range.

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

i. Traceability:

There are no reference standards or methods for RF IgA and IgG. The calibrators and controls for the ImmuLisa Enhanced™ RF IgA and RF IgG Antibody ELISAs are traceable to in-house standards. The ImmuLisa Enhanced™ RF IgM Antibody ELISA is traceable directly to the International Rheumatoid Arthritis Standard, NIBSC 64/2.

ii. Value Assignment:

Calibrators and Positive Controls are dilutions of pooled RF antibody positive sera. The manufacturer formulates new calibrator and control lots from an array of antibody positive sera obtained from various commercial plasma centers stored frozen at -70°C. The calibrators and controls are taken from different pooled

sera. Manufactured calibrator sets are stored in aliquots frozen at -70°C . As new lots of calibrators are developed, comparison studies are performed to calibrate values against original calibrators. Each lot of calibrator is also tested in comparison with normal human sera, clinical samples and internal standards.

iii. Kit Stability:

Shelf-life stability: Accelerated and real-time stability studies were performed on three reagent lots of each ImmuLisa Enhanced™ RF IgA, IgG, and IgM Antibody ELISAs and the RF IgA/IgG/IgM ELISA. Data from the accelerated stability study support a shelf-life of 18 months. Currently available data from the ongoing real-time stability study support a stability claim of 9 months for ImmuLisa Enhanced™ RF IgA and RF IgG Antibody ELISAs and 12 months for ImmuLisa Enhanced™ RF IgM Antibody and RF IgA/IgG/IgM ELISAs.

Open-kit stability: Each ImmuLisa Enhanced™ RF Antibody and RF IgA/IgG/IgM ELISA kit was tested after first opening and determined to be stable at 45 days, but the sponsor chose a more restrictive 30-day open-kit stability claim.

Sample Stability: The manufacturer recommends storing specimens at $2^{\circ}\text{--}8^{\circ}\text{C}$ for no longer than one week. For longer storage, serum specimens should be frozen. It is further recommended that frozen specimens be tested within one year. Avoid repeated freezing and thawing of samples.

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined in accordance with CLSI EP17-A2. For LoB determinations, 60 samples of kit diluent were measured with one reagent lot of each ImmuLisa Enhanced™ RF Antibody kit. For LoD determination, ten replicates of each of six low-level serum samples were tested with one reagent lot of ImmuLisa Enhanced™ RF Antibody kit. The results are presented in the table below:

ImmuLisa Enhanced™ RF Antibody	LoB	LoD
IgA	3.2 EU/mL	3.7 EU/mL
IgG	1.5 EU/mL	2.2 EU/mL
IgM	1.2 IU/mL	1.3 IU/mL

e. Analytical specificity:

i. Endogenous interference:

Interference studies were performed according to CLSI EP07-A2 by testing five samples: one negative, two with RF concentrations near the assay cutoff, and two

moderate to high positive samples. Each sample was mixed with potentially interfering substances; hemoglobin (2 g/L), bilirubin (342 μ mol/L), triglycerides (37 mmol/L) and cholesterol (13 mmol/L). The deviation from the expected result was calculated for each sample tested. Triglycerides and bilirubin interfered ($\geq 15\%$ in at least two of five samples) with the RF IgM assay at the concentrations tested. The sponsor has added a caution that icteric, lipemic and hemolysed samples should be avoided for all assays.

f. Assay Cut-Off:

The assay cutoff is >20 EU/mL for the Immulisa Enhanced™ RF IgA and IgG Antibody and RF IgA/IgG/IgM ELISAs. The assay cut-off is >10 IU/mL for the Immulisa Enhanced™ RF IgM Antibody ELISA.

2. Comparison studies:

a. Method comparison with predicate device:

The Immulisa Enhanced™ RF Antibody and RF IgA/IgG/IgM ELISAs were tested in comparison to the Inova QuantaLite® ELISA kits using sera from a total of 377–427 patients with rheumatoid arthritis or infectious diseases and other autoimmune conditions (disease controls). The number of specimens tested varies and is indicated in the respective tables. A direct comparison was made between the device and the predicate with the same isotype specificity: IgA vs. IgA, IgG vs. IgG, IgM vs. IgM. The Immulisa Enhanced™ IgA/IgG/IgM ELISA was compared to all predicate devices individually and in aggregate, where a sample was declared positive for the predicate if a single isotype was positive, and negative if all predicate isotype-specific devices were negative.

Positive, negative, and overall percent agreements were calculated by grouping Immulisa Enhanced™ RF IgA, IgG, and IgM Antibody semi-quantitative ELISA indeterminate results with both the positive and the negative results separately. The data presented in the tables below represent results from the semi-quantitative Immulisa Enhanced™ RF Antibody IgA, IgG, and IgM ELISAs. Results were also calculated using the qualitative IgA, IgG, and IgM ELISAs and found to be identical to the results from the semi-quantitative ELISAs.

Immulin Enhanced™ RF IgA Antibody ELISA vs QuantaLite® Inova RF IgA				
Indeterminate Samples Called Positive		QuantaLite® Inova RF IgA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgA Antibody ELISA	Positive: >20 EU/mL	113	9	122
	Negative: ≤20 EU/mL	5	250	255
	Total	118	259	377
Positive Percent Agreement	95.8%	95% CI: 90.5%–98.2%		
Negative Percent Agreement	96.5%	95% CI: 93.5%–98.2%		
Overall Percent Agreement	96.3%	95% CI: 93.9%–97.8%		
n = 147 Rheumatoid Arthritis Samples, n = 230 Disease Control Samples				

Immulin Enhanced™ RF IgA Antibody ELISA vs QuantaLite® Inova RF IgA				
Indeterminate Samples Called Negative		QuantaLite® RF IgA ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgA Antibody ELISA	Positive: ≥25 EU/mL	99	5	104
	Negative: <25 EU/mL	19	254	273
	Total	118	259	377
Positive Percent Agreement	83.9%	95% CI: 76.2%–89.4%		
Negative Percent Agreement	98.1%	95% CI: 95.6%–99.2%		
Overall Percent Agreement	93.6%	95% CI: 90.7%–95.7%		
n = 147 Rheumatoid Arthritis Samples, n = 230 Disease Control Samples				

Immulin Enhanced™ RF IgG Antibody ELISA vs QuantaLite® Inova RF IgG				
Indeterminate Samples Called Positive		QuantaLite® RF IgG ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgG Antibody ELISA	Positive: >20 EU/mL	88	24	112
	Negative: ≤20 EU/mL	7	271	278
	Total	95	295	390
Positive Percent Agreement	92.6%	95% CI, 85.6%–96.4%		
Negative Percent Agreement	91.9%	95% CI, 88.2%–94.5%		
Overall Percent Agreement	92.1%	95% CI, 88.9%–94.3%		
n = 154 Rheumatoid Arthritis Samples, n = 236 Disease Control Samples				

Immulin Enhanced™ RF IgG Antibody ELISA vs QuantaLite® Inova RF IgG				
Indeterminate Samples Called Negative		QuantaLite® RF IgG ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgG Antibody ELISA	Positive: ≥25 EU/mL	78	16	94
	Negative: <25 EU/mL	17	279	296
	Total	95	295	390
Positive Percent Agreement	82.1%	95% CI, 73.2%–88.5%		
Negative Percent Agreement	94.6%	95% CI, 91.4%–96.6%		
Overall Percent Agreement	91.5%	95% CI, 88.4%–93.9%		
n = 154 Rheumatoid Arthritis Samples, n = 236 Disease Control Samples				

Immulin Enhanced™ RF IgM Antibody ELISA vs QuantaLite® Inova RF IgM				
Indeterminate Samples Called Positive		QuantaLite® RF IgM ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgM Antibody ELISA	Positive: >20 EU/mL	124	16	140
	Negative: ≤20 EU/mL	4	235	239
	Total	128	251	379
Positive Percent Agreement	96.9%	95% CI: 92.2%–98.8%		
Negative Percent Agreement	93.6%	95% CI: 89.9%–96.0%		
Overall Percent Agreement	94.7%	95% CI: 92.0%–96.6%		
n = 139 Rheumatoid Arthritis Samples, n = 240 Disease Control Samples				

Immulin Enhanced™ RF IgM Antibody ELISA vs QuantaLite® Inova RF IgM				
Indeterminate Samples Called Negative		QuantaLite® RF IgM ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgM Antibody ELISA	Positive: ≥25 EU/mL	120	12	132
	Negative: <25 EU/mL	8	239	247
	Total	128	251	379
Positive Percent Agreement	93.8%	95% CI: 88.2%–96.8%		
Negative Percent Agreement	95.2%	95% CI: 91.8%–97.2%		
Overall Percent Agreement	94.7%	95% CI: 92.0%–96.6%		
n = 139 Rheumatoid Arthritis Samples, n = 240 Disease Control Samples				

Immulin Enhanced™ RF IgA/IgG/IgM ELISA vs QuantaLite® Inova RF IgA				
		QuantaLite® RF IgA ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgA/IgG/IgM ELISA	Positive	115	53	168
	Negative	10	216	226
	Total	125	269	394
Positive Percent Agreement	92.0%	95% CI: 85.9%–95.6%		
Negative Percent Agreement	80.3%	95% CI: 75.1%–84.6%		
Overall Percent Agreement	84.0%	95% CI: 80.1%–87.3%		
n = 152 Rheumatoid Arthritis Samples, n = 242 Disease Control Samples				

Immulin Enhanced™ RF IgA/IgG/IgM ELISA vs QuantaLite® Inova RF IgG				
		QuantaLite® RF IgG ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgA/IgG/IgM ELISA	Positive	95	78	173
	Negative	3	223	226
	Total	98	301	399
Positive Percent Agreement	96.9%	95% CI: 91.4%–99.0%		
Negative Percent Agreement	74.1%	95% CI: 68.9%–78.7%		
Overall Percent Agreement	79.7%	95% CI: 75.5%–83.4%		
n = 157 Rheumatoid Arthritis Samples, n = 242 Disease Control Samples				

Immulin Enhanced™ RF IgA/IgG/IgM ELISA vs QuantaLite® Inova RF IgM				
		QuantaLite® RF IgM ELISA		
		Positive	Negative	Total
Immulin Enhanced™ RF IgA/IgG/IgM ELISA	Positive	161	35	196
	Negative	11	215	226
	Total	172	250	422
Positive Percent Agreement	93.6%	95% CI: 88.9%–96.4%		
Negative Percent Agreement	86.0%	95% CI: 81.2%–89.8%		
Overall Percent Agreement	89.1%	95% CI: 85.8%–91.7%		
n = 180 Rheumatoid Arthritis Samples, n = 242 Disease Control Samples				

ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA vs QuantaLite® Inova RF IgA/IgG/IgM				
		QuantaLite® RF IgA/IgG/IgM ELISA Aggregate Data		
		At Least One Positive	All Negative	Total
ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA	Positive	183	18	201
	Negative	21	205	226
	Total	204	223	427
Positive Percent Agreement		89.7%	95% CI: 84.8%–93.2%	
Negative Percent Agreement		91.9%	95% CI: 87.6%–94.8%	
Overall Percent Agreement		90.9%	95% CI: 87.8%–93.2%	
n = 184 Rheumatoid Arthritis Samples, n = 243 Disease Control Samples				

b. Matrix comparison:

Not applicable since human serum is the only claimed specimen matrix.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The performances of the ImmuLisa Enhanced™ RF Antibody and RF IgA/IgG/IgM ELISAs were compared to a clinical diagnosis of RA. The validation set consisted of 179–249 clinically characterized sera from RA and 310 disease controls. Clinical sensitivity and specificity were calculated with 95% confidence intervals (95% CI).

Clinical sensitivity and specificity were calculated by grouping ImmuLisa Enhanced™ RF IgA, IgG, and IgM Antibody semi-quantitative ELISA indeterminate results with both the positive and the negative results separately. The data presented in the tables below represent results from the semi-quantitative ImmuLisa Enhanced™ RF Antibody IgA, IgG, and IgM ELISAs. Results were also calculated using the qualitative IgA, IgG, and IgM ELISAs and found to be identical to the results from the semi-quantitative ELISAs.

ImmuLisa Enhanced™ RF IgA Antibody ELISA				
Indeterminate Samples Called Positive		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgA Antibody ELISA	Positive: >20 EU/mL	131	33	164
	Negative: ≤20 EU/mL	118	277	395
	Total	249	310	559
Sensitivity		52.6%	95% CI: 46.4%–58.7%	
Specificity		89.4%	95% CI: 85.4%–92.3%	

ImmuLisa Enhanced™ RF IgA Antibody ELISA				
Indeterminate Samples Called Negative		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgA Antibody ELISA	Positive: ≥25 EU/mL	115	23	138
	Negative: <25 EU/mL	134	287	421
	Total	249	310	559
Sensitivity		46.2%	95% CI: 40.1%–52.4%	
Specificity		92.6%	95% CI: 89.1%–95.0%	

ImmuLisa Enhanced™ RF IgG Antibody ELISA				
Indeterminate Samples Called Positive		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgG Antibody ELISA	Positive: >20 EU/mL	126	23	149
	Negative: ≤20 EU/mL	123	287	410
	Total	249	310	559
Sensitivity		50.6%	95% CI: 44.4%–56.8%	
Specificity		92.6%	95% CI: 89.1%–95.0%	

ImmuLisa Enhanced™ RF IgG Antibody ELISA				
Indeterminate Samples Called Negative		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgG Antibody ELISA	Positive: ≥ 25 EU/mL	98	16	114
	Negative: < 25 EU/mL	151	294	445
	Total	249	310	559
Sensitivity		39.4%	95% CI: 33.5%–45.5%	
Specificity		94.8%	95% CI: 91.8%–96.8%	

ImmuLisa Enhanced™ RF IgM Antibody ELISA				
Indeterminate Samples Called Positive		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgM Antibody ELISA	Positive: > 20 EU/mL	176	39	215
	Negative: ≤ 20 EU/mL	73	271	344
	Total	249	310	559
Sensitivity		70.7%	95% CI: 64.7%–76.0%	
Specificity		87.4%	95% CI: 83.3%–90.7%	

ImmuLisa Enhanced™ RF IgM Antibody ELISA				
Indeterminate Samples Called Negative		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgM Antibody ELISA	Positive: ≥ 25 EU/mL	159	36	195
	Negative: < 25 EU/mL	90	274	364
	Total	249	310	559
Sensitivity		63.9%	95% CI: 57.7%–69.6%	
Specificity		88.4%	95% CI: 84.3%–91.5%	

ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA				
		RA Diagnosis		Total
		Positive	Negative	
ImmuLisa Enhanced™ RF IgA/IgG/IgM ELISA	Positive: ≥25 EU/mL	152	45	197
	Negative: <25 EU/mL	27	265	292
	Total	179	310	489
Sensitivity		84.9%	95% CI: 78.9%–89.4%	
Specificity		85.5%	95% CI: 81.1%–89.0%	

b. Incidence of cross-reactivity in related autoimmune and infectious diseases

A total of 310 samples from related autoimmune and infectious diseases were tested with each ImmuLisa Enhanced™ RF Antibody ELISA and the ImmuLisa Enhanced™ IgA/IgG/IgM ELISA to determine incidence of positive results. Results are presented in the table below:

Clinical Diagnosis	# Samples	% Positive			
		RF IgA	RF IgG	RF IgM	RF IgA/IgG/IgM
Juvenile Arthritis	10	0%	10%	10%	10%
Osteoarthritis	30	0%	0%	3%	0%
Psoriatic Arthritis	33	6%	9%	6%	6%
Spondyloarthritis	33	3%	0%	3%	3%
Mixed Connective Tissue Disease	10	10%	20%	40%	30%
Churg-Strauss	10	10%	10%	10%	20%
Sjögren's Syndrome	20	45%	10%	55%	45%
Systemic Lupus Erythematosus	30	37%	20%	17%	40%
Systemic Sclerosis	20	15%	20%	25%	25%
Wegener's	8	13%	13%	13%	13%
Celiac Disease	8	0%	0%	0%	0%
Graves' Disease	10	0%	10%	10%	10%
Hashimoto's Disease	8	0%	0%	0%	0%
Ulcerative Colitis	10	0%	10%	0%	10%
Syphilis	10	0%	0%	0%	0%
Rubella	10	0%	0%	10%	10%
Mononucleosis	5	20%	0%	0%	0%

Clinical Diagnosis	# Samples	% Positive			
		RF IgA	RF IgG	RF IgM	RF IgA/IgG/IgM
Lyme	5	0%	0%	0%	0%
HCV	10	10%	10%	20%	30%
CMV	10	0%	0%	0%	10%
HSV	10	10%	0%	20%	10%
Toxoplasmosis	10	10%	0%	10%	10%

4. Clinical cut-off:

See Assay Cut-Off

5. Expected values/Reference range:

A reference range study was performed with 62–80 normal human serum samples, and a verification study with 121–137 samples that were a combination of normal human sera and sera from patients with infectious diseases and other autoimmune conditions that were not RA (disease controls). The upper limit of the optical density (OD) results from the normal samples was calculated using the average plus 2 standard deviations for ImmuLisa Enhanced™ RF IgA and IgG ELISAs, and the average plus 2.5 standard deviations for ImmuLisa Enhanced™ RF IgM and IgA/IgG/IgM ELISAs. This OD was set to 20 EU/mL for the IgA, IgG, and IgA/IgG/IgM ELISA, and 10 IU/mL for the IgM ELISA. The data from the verification study are summarized in the table below:

ImmuLisa Enhanced™ RF Antibody	# of Samples	% Samples Negative	% Samples Indeterminate	% Samples Positive
IgA	127	94.5%	3.9%	1.6%
IgG	121	99.0%	1.0%	0.0%
IgM	137	99.0%	1.0%	0.0%
IgA/IgG/IgM	137	100.0%	0.0%	0.0%

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.