

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

A. 510(k) Number:

k113020

B. Purpose for Submission:

New Device

C. Measurand:

IgG, IgM, and IgA antibodies to Cardiolipin (aCL)

D. Type of Test:

Qualitative and semi-quantitative microwell-based ELISA assay

E. Applicant:

IMMCO Diagnostics, Inc.

F. Proprietary and Established Names:

ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA
ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA
ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA
ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA

G. Regulatory Information:

1. Regulation section:

21 CFR §866.5660 – Multiple autoantibodies immunological test system

2. Classification:

Class II

3. Product code:

MID – System, Test, Anti-Cardiolipin Immunological

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

ImmuLisa Enhanced™ Cardiophilin IgA Antibody (ACA) ELISA: Enzyme linked immunoassays (ELISAs) for the qualitative or semi-quantitative detection of Cardiophilin IgA antibodies in human serum to aid in the diagnosis of antiphospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

ImmuLisa Enhanced™ Cardiophilin IgG Antibody (ACA) ELISA: Enzyme linked immunoassays (ELISAs) for the qualitative or semi-quantitative detection of Cardiophilin IgG antibodies in human serum to aid in the diagnosis of antiphospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

ImmuLisa Enhanced™ Cardiophilin IgM Antibody (ACA) ELISA: Enzyme linked immunoassays (ELISAs) for the qualitative or semi-quantitative detection of Cardiophilin IgM antibodies in human serum to aid in the diagnosis of antiphospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

ImmuLisa Enhanced™ Cardiophilin Antibody IgA/IgG/IgM Antibody (ACA) ELISA: Enzyme linked immunoassay (ELISA) for the qualitative detection of Cardiophilin IgA, IgG and IgM antibodies in human serum to aid in the diagnosis of anti-phospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE) 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:

An ELISA microplate reader capable of reading absorbance values at 450nm. If a dual wavelength microplate reader is available, the reference filter should be set at 600-650 nm.

I. Device Description:

Each ImmuLisa Enhanced™ Cardioliipin Antibody IgA, IgG or IgM (ACA) ELISA kit contains: a microplate with individual breakaway wells coated with Cardioliipin antigen and bovine/human Beta-2 glycoprotien 1 (β 2GP1); Five levels of calibrators (120, 60, 30, 15, and 1 APL or GPL or MPL); antibody specific positive and negative controls; horseradish peroxidase (HRP) conjugated goat anti-human IgG or IgA or IgM; serum diluent; wash buffer concentrate; 3,3',5,5', tetramethylbenzidine (TMB); and stop solution.

Each ImmuLisa Enhanced™ Cardioliipin Antibody IgA/IgG/IgM Antibody (ACA) ELISA kit contains: a microplate with individual breakaway wells coated with Cardioliipin antigen and bovine/human Beta-2 glycoprotien 1 (β 2GP1); One level of calibrators (30 EU/mL); positive and negative controls; horseradish peroxidase (HRP) conjugated goat anti-human IgG/IgA/IgM; serum diluent; wash buffer concentrate; 3,3',5,5', tetramethylbenzidine (TMB); and stop solution.

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s)

QUANTA Lite™ ACA IgA ELISA, k953366
QUANTA Lite™ ACA IgG ELISA, k946034
QUANTA Lite™ ACA IgM ELISA, k946385
QUANTA Lite™ ACA Screen ELISA, k953291

2. Comparison with predicate:

ImmuLisa Enhanced™ Cardioliipin IgA Antibody (ACA) ELISA/ImmuLisa Enhanced™ Cardioliipin IgG Antibody (ACA) ELISA/ImmuLisa Enhanced™ Cardioliipin IgM Antibody (ACA) ELISA

Similarities		
Item	Device ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA	Predicate QUANTA Lite™ ACA IgA ELISA QUANTA Lite™ ACA IgA ELISA QUANTA Lite™ ACA IgM ELISA
Intended Use	An enzyme linked immunoassay (ELISA) for the qualitative or semi-quantitative detection of Cardiolipin antibodies in human serum to aid in the diagnosis of anti-phospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.	An enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of cardiolipin antibodies in human serum. The presence of cardiolipin antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in assessing the risk of thrombosis in individuals with systemic lupus erythematosus (SLE) or lupus-like disorders.
Methodology	ELISA	Same
Assay Format	Semi-quantitative and qualitative	Same
Sample Matrix	Serum	Same
Coating Antigens	Purified Cardiolipin antigen (1,1',2,2' Tetraoleoyl Cardiolipin (sodium salt)) and β2GP1 from human/bovine serum)	Same
Detection antibody	Goat anti-human IgA, IgG or IgM	Same
Conjugate	HRP	Same
Substrate/ Chromogen	TMB	Same
Controls	One Cardiolipin IgA or IgG or IgM Positive Control, one Negative Control	Same
Calibration	5-level Calibrators	Same
Stop solution	H ₂ SO ₄	Same
Dilution	1:101	Same
Reading	450 nm on spectrophotometer	Same

Similarities		
Item	Device ImmuLisa Enhanced™ Cardioliipin IgA Antibody (ACA) ELISA ImmuLisa Enhanced™ Cardioliipin IgG Antibody (ACA) ELISA ImmuLisa Enhanced™ Cardioliipin IgM Antibody (ACA) ELISA	Predicate QUANTA Lite™ ACA IgA ELISA QUANTA Lite™ ACA IgA ELISA QUANTA Lite™ ACA IgM ELISA
Storage	2 – 8°C	Same
IgG Cutoff	15 GPL	Same

Differences		
Item	Device ImmuLisa Enhanced™ Cardioliipin IgA Antibody (ACA) ELISA ImmuLisa Enhanced™ Cardioliipin IgG Antibody (ACA) ELISA ImmuLisa Enhanced™ Cardioliipin IgM Antibody (ACA) ELISA	Predicate QUANTA Lite™ ACA IgA ELISA QUANTA Lite™ ACA IgA ELISA QUANTA Lite™ ACA IgM ELISA
Wash Buffer	Powdered or optional liquid concentrate	Liquid concentrate
IgA Cutoff	15 APL	12 APL
IgM Cutoff	15 MPL	12.5 MPL
Calibrators	Set of 5: 120, 60, 30, 15, 1 APL or GPL or MPL	Set of 5: 150, 75, 37.5, 18.75, 9.375 APL or GPL or MPL
Reportable Range	IgA: 1.8 APL – 120 APL IgG: 1.0 GPL – 120 GPL IgM: 2.8 MPL – 120 MPL	Not specified
Limit of Detection	IgA: 1.8 APL IgG: 1.0 GPL IgM: 2.8 MPL	Not specified
Results Interpretation	IgA: < 15 APL – negative 15 – 22.5 APL – indeterminant > 22.5 APL – positive IgG: < 15 GPL – negative 15 – 22.5 GPL – indeterminant > 22.5 GPL – positive IgM: < 15 MPL – negative 15 – 22.5 MPL – indeterminant > 22.5 MPL – positive	IgA: < 12 APL – negative 12 – 20 APL – indeterminant > 20 APL – positive IgG: < 15 GPL – negative 15 – 20 GPL – indeterminant > 20 GPL – positive IgM: < 15 MPL – negative 15 – 22.5 MPL – indeterminant > 20 MPL – positive

ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA

Similarities		
Item	Device: ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA	Predicate: QUANTA Lite™ ACA Screen ELISA
Intended Use	An enzyme linked immunoassay (ELISA) for the qualitative detection of Cardiolipin IgA, IgG and IgM antibodies in human serum to aid in the diagnosis of anti-phospholipid syndrome (APS) and APS associated with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.	An enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of cardiolipin antibodies in human serum. The presence of cardiolipin antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in assessing the risk of thrombosis in individuals with systemic lupus erythematosus (SLE) or lupus-like disorders.
Methodology	ELISA	Same
Sample Matrix	Serum	Same
Coating Antigens	Purified Cardiolipin antigen (1,1',2,2' Tetraoleoyl Cardiolipin (sodium salt)) and β2GP1 from human/bovine serum	Same
Detection Antibody	Goat anti-human IgA, IgG or IgM	Same
Assay Format	Qualitative	Same
Conjugate	HRP	Same
Substrate/ Chromogen	TMB	Same
Controls	One Cardiolipin IgA, IgG, and IgM Positive Control, one Negative Control	Same
Calibration	Single Calibrator	Same
Stop Solution	H ₂ SO ₄	Same
Dilution	1:101	Same
Reading	450nm on spectrophotometer	Same
Storage	2-8°C	Same

Differences		
Item	Device: ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA	Predicate: QUANTA Lite™ Cardiolipin Screen ELISA
Wash Buffer	Powdered or optional liquid concentrate	Liquid concentrate
Calibrator	Single Calibrator. 30 EU/mL	Single decision point calibrator. No units assigned.
Cut-off	20 EU/mL	Not specified (\geq decision point calibrator)
Limit of Detection	5.2 EU/mL	Not specified
Results Interpretation	< 20 EU/mL – negative \geq 20EU/mL – positive	All results with a mean absorbance greater than the decision point calibrator absorbance are considered positive < decision point calibrator – negative \geq decision point calibrator – positive

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

EN 13640: 2002, Stability Testing of *In Vitro* Diagnostic Reagents

CLSI EP06-A: Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition

CLSI EP09-A2: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition

CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantification; Approved Guideline

L. Test Principle:

Cardiolipin antigen and β 2GP1 are bound to the wells of a polystyrene microwell plate followed by blocking the unreacted sites to reduce non-specific binding.

Controls, calibrators and diluted patient sera are added to separate wells, allowing any cardiolipin antibodies present to bind to the immobilized antigen. Unbound sample is washed away and an enzyme labeled anti-human IgG, IgA or IgM conjugate is added to each well. These enzyme conjugated antibodies bind specifically to the human immunoglobulin of the appropriate class. After washing away any unbound conjugate, specific enzyme substrate (TMB) is then added to the wells. After stopping the enzymatic reaction, the intensity of color change, which is proportional to the concentration of antibody, is read by a spectrophotometer at 450 nm. Results are expressed in units per milliliter (U/mL) according to the Harris classification.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Semi-Quantitative Precision:

Six samples from different parts of the claimed assay ranges were tested to establish between-day precision and within-run repeatability. Between-day imprecision was tested in replicates of six over thirteen assay runs (n = 78); Within-run repeatability was tested by 12 replicates performed in a single run. The total number of replicates was 90. Results are summarized below.

Immulin Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA

Sample	Mean (APL)	Within-Run (Repeatability)		Between-Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV
1	2.5	0.234	8.8%	0.366	14.6%	0.4	14.0%
2	12.4	0.915	7.4%	1.014	8.2%	1.0	8.0%
3	17.9	1.233	6.9%	1.470	8.2%	1.4	8.0%
4	29.1	1.466	5.2%	2.499	8.5%	2.4	8.2%
5	53.1	3.088	5.9%	4.182	7.9%	4.1	7.6%
6	103.7	5.847	5.5%	6.922	6.7%	6.8	6.5%

Immulin Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA

Sample	Mean (GPL)	Within-Run (Repeatability)		Between-Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV
1	3.8	0.207	9.6%	0.177	8.6%	0.183	9.2%
2	12.0	1.050	9.1%	1.118	9.3%	1.117	9.3%
3	18.0	1.383	7.7%	1.723	9.5%	1.676	9.3%
4	31.3	1.651	5.5%	2.805	8.9%	2.738	8.7%
5	80.4	3.317	4.1%	8.049	10.0%	7.586	9.4%
6	99.0	4.560	4.7%	7.011	7.0%	6.806	6.9%

ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA

Sample	Mean (MPL)	Within-Run (Repeatability)		Between-Day		Total Imprecision	
		SD	%CV	SD	%CV	SD	%CV
1	11.6	0.407	3.5%	0.930	8.0%	0.930	8.0%
2	13.0	0.904	7.0%	1.084	8.4%	1.084	8.4%
3	17.0	0.598	3.5%	1.257	7.4%	1.257	7.4%
4	45.7	1.305	2.9%	2.292	5.0%	2.292	5.0%
5	79.9	3.609	4.5%	5.580	7.0%	5.580	7.0%
6	114.8	4.811	4.2%	6.603	5.8%	6.603	5.8%

Lot-to-lot Reproducibility:

To assess lot-to-lot reproducibility, samples were selected through the linear range for ImmuLisa Enhanced™ Cardiolipin IgA, IgG and IgM Antibody (ACA) ELISAs. Three runs of each lot were performed. Results are summarized below.

ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA Lot-to-Lot Reproducibility

Sample	Mean (APL)	Lot 1		Lot 2		Lot 3		Lot-to-Lot Variation	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	8.2	0.9	11.6%	1.3	14.7%	0.8	9.8%	1.0	12.2%
2	14.3	0.8	5.5%	0.8	5.0%	0.6	4.1%	0.9	6.4%
3	19.0	1.7	9.2%	1.7	8.7%	1.1	5.9%	1.6	8.2%
4	36.1	2.0	5.7%	1.7	4.4%	2.7	7.7%	2.3	6.3%
5	56.7	1.8	3.3%	2.2	3.7%	2.1	3.9%	3.3	5.8%
6	77.1	3.1	4.0%	2.9	3.7%	3.3	4.3%	2.9	3.8%
7	104.2	4.0	3.8%	2.4	2.2%	2.2	2.2%	4.4	4.3%

ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA Lot-to-Lot Reproducibility

Sample	Mean (GPL)	Lot 1		Lot 2		Lot 3		Lot-to-Lot Variation	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	3.7	0.6	16.4%	0.6	13.5%	1.2	33.7%	0.8	21.2%
2	13.4	0.5	3.5%	0.8	5.3%	0.9	7.1%	0.9	6.7%
3	20.1	1.6	8.0%	1.8	8.6%	1.2	6.3%	1.9	9.6%
4	26.2	2.3	9.4%	1.2	4.5%	1.6	5.9%	2.0	7.5%
5	47.5	2.8	6.1%	3.0	6.0%	2.0	4.3%	3.4	7.2%
6	80.4	8.1	10.1%	3.6	4.2%	3.4	4.5%	6.6	8.2%
7	111.4	4.6	4.2%	5.1	4.4%	5.0	4.6%	5.1	4.6%

ImmULisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA Lot-to-Lot Reproducibility

Sample	Mean (MPL)	Lot 1		Lot 2		Lot 3		Lot-to-Lot Variation	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	10.1	1.0	8.8%	0.7	8.7%	1.3	12.2%	1.6	15.3%
2	15.0	0.5	3.4%	0.9	6.2%	1.0	6.6%	0.9	5.9%
3	19.1	1.0	5.1%	1.5	8.5%	1.5	7.3%	1.6	8.3%
4	28.4	1.6	5.3%	2.8	10.4%	2.3	7.9%	2.3	7.9%
5	43.8	3.1	7.0%	2.4	5.7%	3.0	6.5%	2.8	6.4%
6	82.1	8.1	9.9%	3.9	5.1%	5.4	6.1%	7.2	8.8%
7	96.6	3.2	3.3%	4.5	4.9%	4.1	4.0%	6.2	6.4%

Qualitative Reproducibility:

Five samples were tested in 90 runs; a low negative (but above limit of detection), a sample ~ 20% below cut-off, a sample at the cut-off concentration (15-APL, -GPL, or -MBL for IgA, IgG, or IgM kits respectively; 20 EU/mL for Screen), a sample ~ 20% above cut-off, and a moderate positive. Results are summarized below:

ImmULisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA

Sample	Test Range (APL)	Expected Result	# Samples with expected result	% Correct
Negative	2.2	Negative	90/90	100%
~ 20% below Cutoff	12.1	Negative	89/90	99%
~ Cutoff	15.3	Positive	47/90	52%
~ 20% above Cutoff	17.5	Positive	88/90	98%
Moderate Positive	27.7	Positive	90/90	100%

ImmULisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA

Sample	Test Range (GPL)	Expected Result	# Samples with expected result	% Correct
Negative	3.1	Negative	90/90	100%
~ 20% below Cutoff	11.5	Negative	90/90	100%
~ Cutoff	15.4	Positive	54/90	60%
~ 20% above Cutoff	18.6	Positive	89/90	99%
Moderate Positive	30.4	Positive	90/90	100%

ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA

Sample	Test Range (MPL)	Expected Result	# Samples with expected result	% Correct
Negative	11.5	Negative	90/90	100%
~ 20% below Cutoff	13.0	Negative	87/90	97%
~ Cutoff	15.1	Positive	54/90	60%
~ 20% above Cutoff	17.0	Positive	88/90	98%
Moderate Positive	46.1	Positive	90/90	100%

ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA

Sample	Test Range (EU/mL)	Expected Result	# Samples with expected result	% Correct
Negative	7	Negative	90/90	100%
~ 20% below Cutoff	17.4	Negative	87/90	97%
~ Cutoff	18.3	Negative	54/90	60%
~ 20% above Cutoff	24.6	Positive	90/90	100%
Moderate Positive	60	Positive	90/90	100%

b. Linearity/assay reportable range:

Linearity studies were performed according to CLSI EP-6A. Three serum samples for each of the isotype with values to cover the range of the assays were selected. Each sample was proportionally diluted with a known negative serum sample and tested. The observed values were graphed against the calculated values and a linear regression was performed. In cases where the high positive sample was above the highest calibrator (120 U/mL), the dilution points were truncated from the curve and recovery calculations. The results are summarized in the tables below:

ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA

Sample	Test Range (APL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery (obtained/expected)
1	2.6 – 47.6	0.999 (0.95 to 1.05)	-0.52 (-1.95 to 9.91)	1.00	97.7% to 114.0%
2	18.4 – 70.4	0.997 (0.85 to 1.15)	1.57 (-5.28 to 8.43)	0.98	85.1% to 101.9%
3	20.0 – 107.6	1.126 (0.995 to 1.257)	0.066 (-9.488 to 9.620)	0.99	84.4% to 100%

ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA

Sample	Test Range (GPL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery (obtained/expected)
1	2.4 to 98.1	0.989 (0.899 to 1.078)	-0.725 (-6.114 to 4.664)	0.99	95.9% to 114.6%
2	4.5 to 27.8	0.995 (0.938 to 1.052)	0.348 (-0.657 to 1.353)	1.00	90.8% to 101.1%
3	3.1 to 107.5	1.168 (0.940 to 1.396)	-4.734 (-20.637 to 11.170)	0.97	82.6% to 102.8%

ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA

Sample	Test Range (MPL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery (obtained/expected)
1	5.6 to 28.3	0.925 (0.830 to 1.021)	1.696 (-0.051 to 3.443)	0.99	83.0% to 102.3%
2	5.8 to 86.8	0.97 (0.87 to 1.07)	1.159 (-4.371 to 6.689)	0.99	93.4% to 106.9%
3	5.5 to 103.6	0.939 (0.764 to 1.113)	4.780 (-5.89 to 15.46)	0.99	81.6% to 105.2%

Additional IgA samples were tested to cover the lower range of the assay. Results are summarized below:

Test Range (APL)	Slope (95% CI)	Y-Intercept (95% CI)	R ²	% Recovery (obtained/expected)
9.2 – 36.1	0.953 (0.861 to 1.045)	1.319 (-1.049 to 3.687)	0.99	95.8% to 104.6%
3.6 – 34.2	1.041 (0.884 to 1.198)	-0.207 (-3.666 to 3.251)	0.99	87.7% to 104.4%
8.1 – 30.6	0.999 (0.941 – 1.057)	-0.235 (-1.450 – 0.980)	1	98.6% to 105.4%

The claimed reportable ranges of the ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISAs are as follows:

ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA: 1.8 – 120 APL;

ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA: 1.0 – 120 GPL;

ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA: 2.8 – 120 MPL.

Linearity was not assessed for the qualitative ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA.

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

Traceability:

There is no FDA recognized international reference material available for anti-cardiolipin antibodies. The calibrators and controls are assigned relative arbitrary units (U/mL -APL, -GPL, -MPL). Calibrator material (high positive patient sera) dilutions are compared to the Harris Standards to evaluate assay reference range.

Value Assignment:

Positive control and calibrators were derived from different pooled sera of cardiolipin/phospholipid antibody positive SLE/APS patients obtained from commercial sources. Antibody positivity and concentrations were confirmed using commercially available assays for cardiolipin IgA, IgG and IgM antibodies. Serial dilutions of calibrator are made to establish the 120 U/mL (Calibrator A), 60 U/mL (Calibrator B), 30 U/mL (Calibrator C), 15 U/mL (Calibrator D), and 1 U/mL (Calibrator E) calibrators. The samples are tested at various dilutions on at least two different lots of cardiolipin-coated plates. 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 as well as the Harris standards. Positive control lots are developed in the same manner as calibrators, but are derived from different sets of positive sera.

Device Stability:

Accelerated shelf life, real time shelf life, and open kit/reagent stability studies were performed as part of design control to assign expiration dating to components and as part of ongoing quality control/quality assurance analysis. All studies were performed on three lots of components/reagents.

Accelerated studies were conducted with materials incubated at 37°C. In these conditions, one day was considered equivalent to one month stored at 2°–8°C. Materials were removed from the incubator for testing at three day intervals for a minimum of 21 days. Based on these studies, an 18 month expiration date was established for this product.

Real time stability studies are ongoing and currently support device stability up to 12 months.

For open kit stability studies, materials were opened as required for bench-top usage and then assayed at designated intervals. The sponsor demonstrated

open kits were stable for 30 days when stored at the recommended conditions (2°–8°C).

Sample Stability

The sponsor presented a study that supports a claim that serum samples are stable at 2°–8°C for one week.

d. Detection limit:

The limit of blank (LoB) and limit of detection (LoD) studies were performed following the protocol in CLSI EP-17A.

To determine the LoB, sixty replicates of the blank (kit diluent) were run; the LoB was determined by sorting the results from low to high by OD and averaging the value of the 57th and 58th positions. The Limit of Blank for the ImmuLisa Enhanced™ Cardiolipin IgA, IgG and IgM Antibody (ACA) ELISAs was determined to be 1.8 APL for IgA, 1.0 GPL for IgG, 2.3 MPL for IgM and 4.7 EU/mL for the ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA .

To determine the LoD, six low negative samples were tested ten times each for a total of 60 measurements. The LoD was calculated using the equation $LoD = LoB + c_{\beta}SD_s$. EU/mL determined by plotting OD value vs. standard curve. The Limit of Detection for the ImmuLisa Enhanced™ Cardiolipin IgA, IgG and IgM Antibody (ACA) ELISAs was determined to be 1.8 APL for IgA, 1.0 GPL for IgG, 2.8 MPL for IgM and 5.2 EU/mL for the ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA.

e. Analytical specificity:

Interfering Substances:

Interference was studied by mixing sera with known cardiolipin antibody levels with potentially interfering serum samples and studying deviation from expected results. Five samples for IgA, IgG and IgM were evaluated for interference spanning the measuring range. There was no significant trend of interference observed. Results were within the expected CV ($\pm 15\%$) of the assay. The greatest impact is in lower negative samples. The instructions for use caution against the use of grossly hemolyzed or lipemic samples. The substances and the maximum levels tested are shown in the table below:

Substance	Final Concentration
Hemoglobin	2 g/L
Bilirubin	342 µmol/L
Cholesterol	13 mmol/L
Triglycerides	37 mmol/L
Rheumatoid Factor	100 EU/mL

Cross-Reactivity:

A set of potentially cross-reactive specimens from individuals with other connective tissue/autoimmune disorders were tested for cardiolipin antibodies using the ImmuLisa Enhanced™ Cardiolipin IgA, IgG, and IgM Antibody (ACA) ELISAs and ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA.

The table below shows the number (n) of samples containing potential cross reactants as disease state evaluated by the ImmuLisa Enhanced™ Cardiolipin IgA, IgG, and IgM Antibody (ACA) ELISAs and the ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA. The cross reactivity of ImmuLisa™ Cardiolipin assays was obtained as the positivity rate (%pos) among the total number (n) of cross reactant samples evaluated.

Cross Reactive Disease State	IgA		IgG		IgM		IgA/IgG/IgM	
	n	% pos	n	% pos	n	% pos	n	% pos
SLE	77	16.5%	79	24.1%	86	9.3%	69	32.4%
Thrombocytopenia	15	6.7%	15	13.3%	15	0.0%	15	13.3%
Pre-eclampsia	15	6.7%	15	20.0%	15	0.0%	15	20.0%
Celiac Disease	21	0.0%	22	0.0%	24	0.0%	18	0.0%
Mixed Connective Tissue Disease	24	4.2%	23	4.3%	29	10.3%	15	6.7%
Myositis	3	0.0%	3	33.3%	4	0.0%	2	0.0%
Rheumatoid Arthritis	89	2.2%	89	6.7%	95	7.4%	85	10.6%
Sjögren's Syndrome	25	8.0%	25	8.0%	27	11.1%	20	10.0%
Syphilis*	40	15.0%	40	82.5%	40	35.0%	40	97.5%
Systemic Sclerosis	44	2.3%	44	0.0%	45	4.4%	36	5.6%
Thyroiditis	8	0.0%	8	0.0%	7	0.0%	8	0.0%
Vasculitis	0	not tested	0	not tested	0	not tested	8	0.0%

* High percent of seropositive syphilis specimens were tested positive in

particular for IgG and the combined screening tests. A warning is included in the product insert which states that “Confirmed active or seropositive syphilis patients can have elevated anti-cardiolipin antibody (ACA) levels. To rule out syphilis, confirmatory tests should be performed”.

High dose hook effect:

To assess high dose hook effect, four to five high APLS specimens all above the highest calibrator for IgA, IgG and IgM Cardiolipin were tested in 1:100, 1:200, 1:400, 1:800 dilutions. These dilution series did not demonstrate hook effect. Results above the upper limit of the measuring range are reported as “over”. No recommendations are made for dilution of samples outside the measuring range in the package insert.

f. Assay cut-off:

The assay cut-off for each assay was established using the mean OD plus optimal standard deviations of ODs obtained by testing a set of normal samples and disease control sera. Based on internal comparison with the Harris cardiolipin IgA, IgG and IgM standards, the cut-offs for the Immulisa Enhanced™ Cardiolipin IgA, IgG and IgM Antibody (ACA) ELISA was assigned a value of 15 U/mL -APL, -GPL, and -MBL respectively. The indeterminate/borderline range was set within 50% of the cutoff. For Immulisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA, cutoff was assigned a value of 20 EU/mL. There is no indeterminate/borderline range for the screen assay.

2. Comparison studies:

a. Method comparison with predicate device:

Immulisa Enhanced™ Cardiolipin IgA, IgG, and IgM Antibody (ACA) ELISAs and the Immulisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA were compared to predicate ELISA kits using sera of APS and APS with SLE subjects and disease controls. Only samples in the linear range of the IMMCO assay are included in this method comparison. The number (n) and disease state of specimens tested is indicated in the table below:

Disease Category	IgA	IgG	IgM	IgA/IgG /IgM
	n	n	N	n
APS	71	58	70	100
APS with SLE	49	74	55	90
Suspected APS*	167	180	149	150
Celiac Disease	12	18	16	8

Disease Category	IgA	IgG	IgM	IgA/IgG /IgM
	n	n	N	n
Mixed Connective Tissue Disease	6	20	15	4
Polymyositis/Dermatomyositis	1	1	1	0
Pre-Eclampsia	15	13	12	10
Rheumatoid Arthritis	81	80	76	57
Systemic Sclerosis (Scleroderma)	34	40	28	20
Sjögren's Syndrome	25	23	22	16
SLE	65	68	55	56
Submitted SLE	80	84	79	73
Thrombocytopenia	14	15	9	8
Hashimoto's Thyroiditis (TPO)	1	7	3	7
Vasculitis	0	0	0	6
Total samples tested:	621	681	590	605

* Suspected APS were samples from patients suspected of having APS but did not have a definitive diagnosis available.

ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA

		Predicate ACA IgA ELISA			
		Positive	Borderline (12– 20 APL)	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA	Positive	70	19	8	97
	Borderline (15 – 22.5 APL)	6	10	7	23
	Negative	17	22	462	501
	Total	93	51	477	621

Borderline Samples Considered Positive		Predicate ACA IgA ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA	Positive	105	15	120
	Negative	39	462	501
	Total	144	477	621

Positive Percent Agreement: 72.9% (95% CI: 64.8% – 79.8%)

Negative Percent Agreement: 96.9% (95% CI: 95.7% – 98.2%)

Overall Percent Agreement: 91.3% (95% CI: 88.7% – 93.3%)

Borderline Samples Considered Negative		Predicate ACA IgA ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA	Positive	70	27	97
	Negative	23	501	524
	Total	93	528	621

Positive Percent Agreement: 75.3% (95% CI: 65.0% – 83.4%)
 Negative Percent Agreement: 94.9% (95% CI: 92.6% – 96.5%)
 Overall Percent Agreement: 91.9% (95% CI: 89.5% – 93.9%)

Borderline Samples Excluded		Predicate ACA IgA ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgA Antibody (ACA) ELISA	Positive	70	8	78
	Negative	17	462	479
	Total	87	470	557

Positive Percent Agreement: 80.5% (95% CI: 70.3% – 87.9%)
 Negative Percent Agreement: 98.3% (95% CI: 96.5% – 99.2%)
 Overall Percent Agreement: 95.5% (95% CI: 93.5% – 96.9%)

ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA

		Predicate ACA IgG ELISA			
		Positive	Borderline (15 – 20 GPL)	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA	Positive	140	5	19	164
	Borderline (15 – 22.5 GPL)	5	5	13	23
	Negative	9	16	469	494
	Total	154	26	501	681

Borderline Samples Considered Positive		Predicate ACA IgG ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA	Positive	155	32	187
	Negative	25	469	494
	Total	180	501	681

Positive Percent Agreement: 86.1% (95% CI: 80.0% – 90.6%)
 Negative Percent Agreement: 93.6% (95% CI: 91.0% – 95.5%)
 Overall Percent Agreement: 91.6% (95% CI: 89.2% – 93.5%)

Borderline Samples Considered Negative		Predicate ACA IgG ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA	Positive	140	24	164
	Negative	14	503	517
	Total	154	527	681

Positive Percent Agreement: 90.9% (95% CI 84.9% – 94.8%)

Negative Percent Agreement: 95.4% (95% CI 93.2% – 97.0%)

Overall Percent Agreement: 94.4% (95% CI 92.3% – 96.0%)

Borderline Samples Excluded		Predicate ACA IgG ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgG Antibody (ACA) ELISA	Positive	140	19	159
	Negative	9	469	478
	Total	149	488	637

Positive Percent Agreement: 94.0% (95% CI 88.5% – 97.0%)

Negative Percent Agreement: 96.1% (95% CI 93.9% – 97.6%)

Overall Percent Agreement: 95.6% (95% CI 93.7% – 96.9%)

ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA

		Predicate ACA IgM ELISA			
		Positive	Borderline (12.5–20 MPL)	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA	Positive	102	4	1	107
	Borderline (15 – 22.5 MPL)	8	5	1	14
	Negative	20	30	419	469
	Total	130	39	421	590

Borderline Samples considered Positive		Predicate ACA IgM ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA	Positive	119	2	121
	Negative	50	419	469
	Total	169	421	590

Positive Percent Agreement: 70.4% (95% CI: 62.8% – 77.1%)

Negative Percent Agreement: 99.5% (95% CI: 98.1% – 99.9%)

Overall Percent Agreement: 91.2% (95% CI: 88.5% – 93.3%)

Borderline Samples Considered Negative		Predicate ACA IgM ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA	Positive	102	5	107
	Negative	28	455	483
	Total	130	460	590

Positive Percent Agreement: 78.5% (95% CI: 70.2% – 85.0%)

Negative Percent Agreement: 98.9% (95% CI: 97.3% – 99.6%)

Overall Percent Agreement: 94.4% (95% CI: 92.2% – 96.1%)

Borderline Samples Excluded		Predicate ACA IgM ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgM Antibody (ACA) ELISA	Positive	102	1	103
	Negative	20	419	439
	Total	122	420	542

Positive Percent Agreement: 83.6% (95% CI: 75.6% – 89.5%)

Negative Percent Agreement: 99.8% (95% CI: 98.5% – 100%)

Overall Percent Agreement: 96.1% (95% CI: 94.2% – 97.5%)

ImmuLisa Enhanced™ Cardiolipin IgA/IgG/IgM Antibody (ACA) ELISA

≥ Cutoff (20 EU/mL) = Positive		Predicate ACA Antibody Screen ELISA		
		Positive	Negative	Total
ImmuLisa Enhanced™ Cardiolipin IgA/IgG/ IgM Antibody (ACA) ELISA	Positive	243	27	270
	Negative	36	299	335
	Total	279	326	605

Positive Percent Agreement: 87.1% (95% CI: 80.5% – 90.7%)

Negative Percent Agreement: 91.7% (95% CI: 88.0% – 94.4%)

Overall Percent Agreement: 89.6% (95% CI: 86.8% – 91.9%)

b. *Matrix comparison:*

Not applicable.

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

The study used a set of clinically characterized sera from APS, APS with SLE and non-APS diseased controls. The study was designed to be reflective of the intended use population. For that reason, the study did not include any samples from diagnosed APS patients with known anti-cardiolipin antibody status. The results of the ImmuLisa Enhanced™ Cardiolipin IgA, IgG, and IgM Antibody (ACA) ELISAs and the ImmuLisa Enhanced™ Cardiolipin IgA/IgG/ IgM Antibody (ACA) ELISA in each of disease category are shown below:

Disease Category	IgA			IgG			IgM			IgA/IgG/ IgM		
	n	pos	%	n	pos	%	n	pos	%	n	pos	%
APS	127	11	8.7%	99	56	56.6%	108	38	35.2%	90	56	62.2%
APSwSLE	53	30	56.6%	95	47	49.5%	80	17	21.3%	66	60	90.9%
Total Diagnosed APS	180	41	22.8%	194	103	53.1%	188	55	29.3%	156	116	74.4%
Other disease sera (included in non-APS diagnosis population)												
Submitted for APS testing/suspected APS	188	14	7.4%	184	45	24.5%	188	13	6.9%	185	38	20.5%
Submitted SLE testing/suspected SLE	90	5	5.6%	88	13	14.8%	90	5	5.6%	88	16	18.2%
Thrombocytopenia	15	1	6.7%	15	2	13.3%	15	0	0.0%	15	2	13.3%
Pre-eclampsia	15	1	6.7%	15	3	20.0%	15	0	0.0%	15	3	20.0%
Celiac Disease	21	0	0.0%	22	0	0.0%	24	0	0.0%	18	0	0.0%
Mixed Connective Tissue Disease	24	1	4.2%	23	1	4.3%	29	3	10.3%	15	1	6.7%
Myositis	3	0	0.0%	3	1	33.3%	4	0	0.0%	2	0	0.0%
Rheumatoid Arthritis	89	2	2.2%	89	6	6.7%	95	7	7.4%	85	9	10.6%
Sjögren's Syndrome	25	2	8.0%	25	2	8.0%	27	3	11.1%	20	2	10.0%
Systemic Lupus Erythematosus	77	13	16.5%	79	20	24.1%	86	8	9.3%	69	24	32.4%
Systemic Sclerosis	44	1	2.3%	44	0	0.0%	45	2	4.4%	36	2	5.6%
Thyroiditis	8	0	0.0%	8	0	0.0%	7	0	0.0%	8	0	0.0%
Vasculitis	0	0	not tested	0	0	not tested	0	0	not tested	8	0	0.0%
Total Diseased Samples:	779			789			813			719		

The following table summarizes the clinical sensitivity and specificity of the ImmuLisa Enhanced™ Cardiolipin IgA, IgG, and IgM Antibody (ACA) ELISAs and the ImmuLisa Enhanced™ Cardiolipin IgA/IgG/ IgM Antibody (ACA) ELISA:

Disease	IgA		IgG		IgM		IgA/IgG/IgM	
	Clin Sen (95% CI)	Clin Spec (95% CI)						
APS	8.7% (4.6%, 15.3%)	93.7% (91.3%, 95.4%)	56.6% (46.2%, 66.4%)	85.0% (81.9%, 87.8%)	35.2% (26.4%, 45.0%)	93.4% (91.1%, 95.2%)	62.2% (50.7%, 71.4%)	83.8% (80.4%, 86.7%)
APS with SLE	56.6% (42.4%, 69.9%)	93.7% (91.3%, 95.4%)	49.5% (39.1%, 59.9%)	85.0% (81.9%, 87.8%)	21.3% (13.2%, 32.1%)	93.4% (91.1%, 95.2%)	90.9% (80.4%, 86.7%)	83.8% (80.4%, 86.7%)

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

Not applicable.

4. Clinical cut-off:

See Assay Cutoff

5. Expected values/Reference range:

The expected value in the general population is negative. The normal range was established by testing a population of normal blood donor specimens on the assays. Sets of normal human sera were tested on the Immulisa Enhanced™ Cardiolipin IgA, IgG, and IgM Antibody (ACA) ELISAs and the Immulisa Enhanced™ Cardiolipin IgA/IgG/ IgM Antibody (ACA) ELISA. Results demonstrating incidence in the apparently-disease free samples for this study are provided below:

ACA IgA		ACA IgG		ACA IgM		ACA IgA/IgG/IgM	
n	% pos	n	% pos	n	% pos	n	% pos
167	1.2%	164	3.0%	154	2.6%	158	1.9%

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.