510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K162788

B. Purpose for Submission:

New device

C. Measurand:

IgA autoantibodies specific for β_2-GP1
IgG autoantibodies specific for β_2-GP1
IgM autoantibodies specific for β_2-GP1
IgA/IgG/IgM autoantibodies specific for β_2-GP1

D. Type of Test:

Immunoassay, semi-quantitative or qualitative

E. Applicant:

IMMCO Diagnostics, Inc.

F. Proprietary and Established Names:

ImmuLisa Enhanced™ B2GP1 IgA Antibody ELISA
ImmuLisa Enhanced™ B2GP1 IgG Antibody ELISA
ImmuLisa Enhanced™ B2GP1 IgM Antibody ELISA
ImmuLisa Enhanced™ B2GP1 IgA/IgG/IgM Antibody ELISA

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5660, Multiple Autoantibodies Immunological Test System

2. Classification:

   Class II

3. Product code:

   MSV, System, Test, Antibodies, β2-Glycoprotein I (β2-GPI)

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended uses:

   ImmuLisa Enhanced™ B2GP1 IgA Antibody ELISA: Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgA antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

   ImmuLisa Enhanced™ B2GP1 IgG Antibody ELISA: Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgG antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

   ImmuLisa Enhanced™ B2GP1 IgM Antibody ELISA: Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgM antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

   ImmuLisa Enhanced™ B2GP1 IgA/IgG/IGM Antibody ELISA: Enzyme-linked immunosorbent assay (ELISA) for the qualitative detection of β2-GPI IgA, IgG and IgM antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.

2. Indication for use:

   Same as intended use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   An ELISA 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 450/630

nm. An automatic microplate washer capable of accurately dispensing 200 µL of fluid is also required.

I. Device Description:

For ImmuLisa Enhanced™ B2GP1 IgA, or ImmuLisa Enhanced™ B2GP1 IgG, or ImmuLisa Enhanced™ B2GP1 IgM Antibody ELISA:

Each kit consists of 12 x 8 β2-GPI antigen coated microplate with individual break-away microwells; one negative control; three positive controls (one positive for β2-GPI IgA antibodies, one positive for β2-GPI IgG antibodies, and one positive for β2-GPI IgM antibodies); one set of five-level calibrators; three Horse radish peroxidase (HRP) conjugates (HRP goat anti-human IgA conjugate, HRP goat anti-human IgG conjugate and HRP goat anti-human IgM conjugate); TMB enzyme substrate; stop solution; and wash buffer. The results are read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL). Semi-quantitative results are reported as: Negative (< 20 EU/mL), Borderline/ Indeterminate (20–25 EU/mL) and Positive (>25 EU/mL).

ImmuLisa Enhanced™ B2GP1 IgA/IgG/IgM Antibody ELISA:

Each kit consists of 12 x 8 β2-GPI antigen coated microplate with individual break-away microwells; negative control; positive control for β2-GPI antibodies; one-level calibrator; Horse radish peroxidase (HRP) goat anti-human IgA/IgG/IgM conjugate; TMB enzyme substrate; stop solution; wash buffer; and diluent. The results are read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL). Qualitative results are reported as Negative (< 20 EU/mL) and Positive (≥20 EU/mL).

J. Substantial Equivalence Information:

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

   Inova Quanta Lite® β_2GP1 IgA ELISA, K973006
   Inova Quanta Lite® β_2GP1 IgG ELISA, K970551
   Inova Quanta Lite® β_2GP1 IgM ELISA, K973014

2. Comparison with predicate:

Similarities		
Item	**New Device** ImmuLisa Enhanced™ B2GP1 IgA Antibody ELISA	**Predicate** Quanta Lite® β_2 GP1 IgA ELISA
Intended Use	Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgA antibodies in human serum to aid in diagnosis of autoimmune thrombotic	Enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of β2-GPI IgA antibodies in human serum. The presence of β2-GPI IgA antibodies

	disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.	can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases.
Assay Type	Manual ELISA	Same
Solid Phase	Polystyrene microplate wells	Same
Capture Antigen	Purified human β2-GPI	Same
Sample Type	Serum	Same
Kit component set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate well	Same
Labeled Detection Antibody (Conjugate)	Goat anti-human IgA conjugated to horseradish peroxidase (HRP)	Same
Substrate/ Chromogen	Tetramethylbenzidene (TMB)	Same
Stop Solution	Sulfuric Acid	Same
Sample Dilution	1:101	Same
Incubation Times	Positive and negative controls, diluted patient samples: 30 minutes Conjugate: 30 minutes Substrate: 30 minutes (in the dark)	Same
Reaction Temperature	Room temperature (20–25°C)	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Positive Control	β2-GPI IgA antibodies	Same
Signal	Optical density	Same
Instrumentation	Microwell plate reader (450 nm)	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced™ B2GP1 IgA Antibody ELISA	**Predicate** Quanta Lite® β2 GP1 IgA ELISA
Assay Format	Semi-quantitative and qualitative	Semi-quantitative
Reported Unit	EU/mL	Standard IgA anti-β2-GPI unit (SAU)
Calibrators	Set of 5: Values in EU/mL: 160, 80 40, 20, 1	Set of 5: Values in SAU: 150, 75, 37.5 18.75 and 9.375
Wash Buffer	Powdered or optional liquid concentrate	Liquid concentrate

Linear Range	3.6–160 EU/mL	Not specified
Limit of Detection	3.6 EU/mL	Not specified
Results Interpretation	Negative: < 20 EU/mL Borderline: 20–25 EU/mL Positive: > 25 UE/mL	Negative: ≤ 20 SAU Positive: > 20 SAU

Similarities		
Item	**New Device** ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	**Predicate** Quanta Lite® β2 GPI IgG ELISA
Intended Use	Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgG antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.	Enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of β2 GPI IgG antibodies in human serum. The presence of β2-GPI IgG antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases.
Assay Type	Manual ELISA	Same
Solid Phase	Polystyrene microplate wells	Same
Capture Antigen	Purified human β2-GPI	Same
Sample Type	Serum	Same
Kit Component Set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate well.	Same
Labeled Detection Antibody (Conjugate)	Goat anti-human IgG conjugated to HRP	Same
Substrate/Chromogen	TMB	Same
Stop Solution	Sulfuric Acid	Same
Sample Dilution	1:101	Same
Incubation Times	Positive and negative controls, diluted patient samples: 30 minutes Conjugate: 30 minutes Substrate: 30 minutes (in the dark)	Same
Reaction Temperature	Room temperature (20–25°C)	Same
Traceability	International Reference Preparation is not available. Results are traceable to	Same

	in-house standards.	
Positive Control	β2-GPI IgG antibodies	Same
Instrumentation	Microwell plate reader (450 nm)	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	**Predicate** Quanta Lite® β2 GPI IgG ELISA
Assay Format	Semi-quantitative and qualitative	Same
Reported Unit	EU/mL	Standard IgG anti-β2-GPI unit (SGU)
Calibrators	Set of 5: Values in EU/mL: 160, 80 40, 20, 1	Set of 5: Values in SGU: 150, 75, 37.5 18.75 and 9.375
Wash Buffer	Powdered or optional liquid concentrate	Liquid concentrate
Linear Range	3.1–160 EU/mL	Not specified
Limit of Detection	3.1 EU/mL	Not specified
Results Interpretation	Negative: < 20 EU/mL Borderline: 20–25 EU/mL Positive: > 25 EU/mL	Negative: ≤ 20 SGU Positive: > 20 SGU

Similarities		
Item	**New Device** ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	**Predicate** Quanta Lite® β2 GPI IgM ELISA
Intended Use	Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgM antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.	Enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of β2-GPI IgM antibodies in human serum. The presence of β2-GPI IgM antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases.
Assay Type	Manual ELISA	Same
Solid Phase	Polystyrene microplate wells	Same
Capture Antigen	Purified human β2-GPI	Same
Sample Type	Serum	Same

Kit Component Set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate well.	Same
Labeled Detection Antibody Conjugate	Goat anti-human IgM conjugated to HRP	Same
Substrate/ Chromogen	TMB	Same
Stop Solution	Sulfuric Acid	Same
Sample Dilution	1:101	Same
Incubation Times	Positive and negative controls diluted patient samples: 30 min. Conjugate: 30 minutes. Substrate: 30 minutes (in the dark).	Same
Reaction Temperature	Room temperature (20–25°C)	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Positive Control	β2-GPI IgM antibodies	Same
Instrumentation	Microwell plate reader (450 nm)	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	**Predicate** Quanta Lite® β2 GPI IgM ELISA
Assay Format	Semi-quantitative and qualitative	Semi-quantitative
Reported Unit	EU/mL	Standard IgM anti-β2-GPI unit (SMU)
Calibrators	Set of 5: Values in EU/mL: 160, 80 40, 20, 1	Set of 5: Values in SMU: 150, 75, 37.5 18.75 and 9.375
Wash Buffer	Powdered or optional liquid concentrate	Liquid concentrate
Linear Range	2.3–160 EU/mL	Not specified
Limit of Detection	2.3 EU/mL	Not specified
Results Interpretation	Negative: < 20 EU/mL Borderline: 20–25 EU/mL Positive: > 25 EU/mL	Negative: ≤ 20 SMU Positive: > 20 SMU

Similarities		
Item	**New Device** ImmuLisa Enhanced™ B2GPI IgA/IgG/IgM Antibody ELISA	**Predicates** Quanta Lite® β2 GPI IgA ELISA Quanta Lite® β2 GPI IgG ELISA Quanta Lite® β2 GPI IgM ELISA

Intended Use	Enzyme-linked immunosorbent assay (ELISA) for the qualitative or semi-quantitative detection of β2-GPI IgA/IgG/IgM antibodies in human serum to aid in diagnosis of autoimmune thrombotic disorders associated with antiphospholipid syndrome (APS) and APS with systemic lupus erythematosus (SLE) in conjunction with other laboratory tests and clinical findings.	Enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of β2-GPI IgA, IgG or IgM antibodies in human serum. The presence of β2-GPI IgA, IgG or IgM antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases.
Assay Type	Manual ELISA	Same
Solid Phase	Polystyrene microplate wells	Same
Capture Antigen	Purified human β2-GPI	Same
Sample Type	Serum	Same
Kit Component Set	Includes positive control, negative control, calibrators, conjugate, substrate, diluent, wash buffer, stop solution, microplate well.	Same
Labeled Detection Antibody(Conjugate)	Goat anti-human IgA conjugated to HRP, Goat anti-human IgG conjugated to HRP and Goat anti-human IgM conjugated to HRP	Same
Substrate/Chromogen	TMB	Same
Stop Solution	Sulfuric Acid	Same
Sample Dilution	1:101	Same
Incubation Times	Positive and negative controls diluted patient samples: 30 minutes Conjugate: 30 minutes Substrate: 30 minutes (in the dark).	Same
Reaction Temperature	Room temperature (20–25°C)	Same
Traceability	International Reference Preparation is not available. Results are traceable to in-house standards.	Same
Positive Control	β2-GPI IgA/IgG/IgM antibodies	Same
Instrumentation	Microwell plate reader (450 nm)	Same
Storage	2–8°C	Same

Differences		
Item	**New Device** ImmuLisa Enhanced™ B2GPI IgA/IgG/IgM Antibody ELISA	**Predicate** QuantaLite® β2GPI IgA ELISA QuantaLite® β2GPI IgG ELISA QuantaLite® β2GPI IgM ELISA
Assay Format	Qualitative	Semi-quantitative
Reported Unit	EU/mL	SAU, SGU and SMU
Calibrators	30 EU/mL (one-point calibration)	Set of 5: Values in SAU, SGU or SMU: 150, 75, 37.5 18.75 and 9.375
Wash Buffer	Powdered or optional liquid concentrate	Liquid Concentrate
Limit of Detection	2.7 EU/mL	Not specified
Results Interpretation	Negative: < 20 EU/ mL Positive: ≥ 20 EU/ mL	Negative: ≤ 20 SAU,SGU or SMU Positive: > 20 SAU,SGU or SMU

K. Standard/Guidance Document Referenced:

CLSI guideline EP05-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline."

CLSI guideline EP06-A, "Evaluation of the Linearity of Quantitative Analytical Measurement Procedures: A Statistical Approach."

CLSI guideline EP07-A2, "Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition."

CLSI guideline EP09-A2, "Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline."

CLSI guideline EP12-A2, "User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline – Second Edition."

CLSI guideline EP17-A, "Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline."

L. Test Principle:

The test is performed as a solid phase immunoassay. Controls, calibrators and patient sera are incubated in the β2-GPI antigen coated wells to allow specific antibodies present in the serum to bind to the antigen. Unbound antibodies and other serum proteins are removed by washing the microplate wells. Bound antibodies are detected by adding an enzyme-labeled anti-human IgA, IgG or IgM conjugate or combined anti-human IgA/IgG/IgM conjugate to the microplate wells. Unbound conjugate is removed by washing. Enzyme substrate (TMB) is then added to the wells and the presence of antibodies is

detected by a color change produced by the conversion of TMB 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. Results are expressed in ELISA units per milliliter (EU/mL) and reported as positive or negative.

Semi-quantitative results are determined from a series of five calibrators (160 EU/mL, 80 EU/mL, 40 EU/mL, 20 EU/mL, and 1 EU/mL). Values less than 20 EU/mL are considered negative results while values greater than 25 EU/mL are considered positive; results between 20 EU/mL and 25 EU/mL are considered 'indeterminate'. The sponsor states the following recommendations in the Package Insert: "Indeterminate results should be retested and evaluated along with other laboratory methods; and when a negative result occurs in the presence of clinical indications or when indeterminate results are obtained, lupus anti-coagulant and anti-cardiolipin antibody testing is recommended as additional testing."

Qualitative results are determined using a ratio of the absorbance of the sample to the absorbance of the cut-off calibrator (20 EU/mL). The ratio is multiplied by the concentration of the cut-off calibrator to give a numerical value. Values greater than or equal to 20 EU/mL are considered positive and <20 EU/mL values are considered negative.

M. Performance Characteristics:

1. Analytical performance:

   The results of all studies met the manufacturer's pre-determined acceptance criteria.

   a. Precision/Reproducibility:

   Semi-Quantitative Precision:

   Precision performance was evaluated in accordance with CLSI guideline EP05-A2. Native sera from the Intended Use population that cover the analytical measuring range were tested and included samples in the negative range, ~20% below cut-off, around the cut-off, ~20% above cut-off and in the moderate positive range of the assays. All specimens were assayed 12 times on one day to determine intra-assay repeatability. The same five specimens were also assayed across 13 runs with six repetitions in each over a period of four weeks to assess reproducibility at three sites using two sets of equipment. An additional 12 replicates were run on the first set of equipment (total replicates = 90). Assays were performed by two operators using one reagent lot and different equipment sets. The first operator performed the assay manually with a multi-channel pipettor, microplate washer and microplate reader. The second operator used an automatic system with a liquid handling system including a microplate reader. One assay lot was used for the study. Tabulated below are the precision and reproducibility results:

   B2GPI IgA Semi-Quantitative Precision:

Specimen	**Mean** (EU/mL)	**Repeatability**		**Between Days**		**Total Imprecision**	
		SD	CV%	SD	CV%	SD	CV%

1	9.1	0.8	8.1	1.0	10.8	1.2	13.6
2	15.7	1.4	8.6	1.4	8.7	1.9	12.2
3	20.9	1.8	8.7	2.1	10.2	2.8	13.5
4	24.2	1.4	6.2	1.4	5.9	2.0	8.3
5	54.0	1.4	2.7	2.4	4.4	2.7	5.1
6	106.7	4.2	4.2	4.6	4.3	6.3	5.9
7	153.1	9.2	6.5	9.0	5.8	12.9	8.4

Specimen	**Mean** (EU/mL)	**Total Imprecision Manual/ Operator #1**		**Total Imprecision Automated/ Operator # 2**	
		SD	CV%	SD	%CV
1	9.1	0.85	9.3	1.01	11.1
2	15.7	1.32	8.4	1.39	8.8
3	20.9	1.88	9.0	2.17	10.4
4	24.2	1.66	6.9	1.26	5.2
5	54.0	2.19	4.1	2.27	4.2
6	106.7	4.15	3.9	5.52	5.2
7	153.1	8.34	5.4	10.1	6.6

B2GPI IgG Semi-Quantitative Precision:

Specimen	**Mean** (EU/mL)	**Repeatability**		**Between Days**		**Total Imprecision**	
		SD	CV%	SD	CV%	SD	CV%
1	10.1	0.9	8.9	1.2	11.7	1.5	14.6
2	15.6	0.7	4.5	0.8	5.3	0.8	5.4
3	21.2	1.2	5.7	1.2	5.7	1.2	5.7
4	25.0	1.0	4.1	1.2	4.6	1.2	4.8
5	56.0	1.7	3.2	3.6	6.4	3.5	6.2
6	109.8	4.6	4.2	6.0	5.5	5.8	5.3
7	154.0	11.1	7.2	8.1	5.3	8.5	5.5

Specimen	**Mean** (EU/mL)	**Total Imprecision Manual/ Operator #1**		**Total Imprecision Automated/ Operator # 2**	
		SD	CV%	SD	CV%
1	10.1	1.01	10.0	1.22	12.1
2	15.6	0.89	5.7	0.70	4.5
3	21.2	1.10	5.2	1.23	5.8
4	25.0	1.24	5.0	1.09	4.4
5	56.0	2.69	4.8	3.41	6.1

6	109.8	6.04	5.5	5.63	5.1
7	154.0	9.06	5.9	7.47	4.9

B2GPI IgM Semi-Quantitative Precision:

Specimen	**Mean** (EU/mL)	**Repeatability**		**Between Days**		**Total Imprecision**	
		SD	CV%	SD	CV%	SD	CV%
1	11.1	1.1	8.9	1.2	11.2	1.7	15.0
2	17.3	1.2	6.9	1.6	9.3	2.0	11.5
3	19.7	0.7	3.8	2.1	10.8	2.1	10.6
4	26.1	1.4	5.7	1.6	5.9	1.6	6.1
5	83.7	3.5	4.2	4.1	4.8	4.0	4.7
6	114.8	7.6	6.8	9.3	8.1	9.2	8.0
7	159.4	9.1	6.1	8.5	5.3	9.4	5.9

Specimen	**Mean** (EU/mL)	**Total Imprecision Manual/ Operator #1**		**Total Imprecision Automated/ Operator # 2**	
		SD	CV%	SD	CV%
1	11.1	0.98	8.8	1.35	12.2
2	17.3	1.93	11.2	1.36	7.9
3	19.7	1.88	9.6	2.18	11.1
4	26.1	1.20	4.6	1.60	6.1
5	83.7	3.74	4.5	4.07	4.9
6	114.8	8.51	7.4	9.51	8.3
7	159.4	6.42	4.0	9.00	5.6

Qualitative Reproducibility:

Studies were performed under the guidance of CLSI EP12-A2. Ninety replicates each of native sera in the negative range, ~20% below cut-off, at ~cut-off, ~20% above cut-off and in the moderate positive range of the assays were tested for qualitative reproducibility. These samples were tested in multiple runs same as in semi-quantitative precision study. The results of these studies are presented in the tables below.

Qualitative Reproducibility:

B2GPI IgA Qualitative Precision:

Specimen	**Mean** (EU/mL)	**% Negative**	**% Positive**
Low Negative	8.3	100	0
Cut-off -20%	15.4	100	0

Cut-off	20.9	23.3	76.7
Cut-off +20%	24.2	0	100
Moderate Positive	64.8	0	100
Moderate to High Pos	128.6	0	100

B2GPI IgG Qualitative Precision:

Specimen	**Mean** (EU/mL)	**% Negative**	**% Positive**
Low Negative	9.8	100	0
Cut-off -20%	14.8	100	0
Cut-off	20.8	23.3	76.7
Cut-off +20%	24.5	0	100
Moderate Positive	59.4	0	100
Moderate to High Pos	96.7	0	100

B2GPI IgM Qualitative Precision:

Specimen	**Mean** (EU/mL)	**% Negative**	**% Positive**
Low Negative	10.3	100	0
Cut-off -20%	14.5	100	0
Cut-off	19.7	61.1	38.9
Cut-off +20%	26.1	0	100
Moderate Positive	100.5	0	100
Moderate to High Pos	151.4	0	100

B2GPI IgA/IgG/IgM Qualitative Precision:

Specimen	**Mean** (EU/mL)	**% Negative**	**% Positive**
Low Negative	8.0	100	0
Cut-off -20%	17.4	97.8	2.2
Cut-off	19.8	50	50
Cut-off +20%	24.8	0	100
Moderate Positive	62.7	0	100
Moderate to High Pos	135.9	0	100

Lot-to-Lot Reproducibility:

Lot-to-lot reproducibility was tested by using samples spanning the assay range, similar to the samples used in the precision study, above. Three assay lots were used in the study. Assays were performed by two operators using different equipment sets. The first operator performed the assay manually with a multichannel pipettor, microplate washer and microplate reader. The second

operator used an automatic system with a liquid handling system including a microplate reader. Each of the seven samples below was tested in three runs with three replicates over three days on each of the three assay lots (81 replicates/sample).

B2GPI IgA Lot-to-Lot Reproducibility:

Specimen	Mean (EU/mL)	Lot 1 CV%	Lot 2 CV%	Lot 3 CV%	Inter-Lot CV%
1	10.2	9.6	8.6	10.3	12.6
2	16.0	7.0	6.3	6.6	9.5
3	20.8	6.3	4.6	4.6	7.9
4	25.7	6.1	7.1	5.9	8.7
5	56.5	3.6	4.0	3.0	6.4
6	114.9	2.9	2.6	4.0	5.6
7	168.1	3.4	4.5	5.0	8.3

B2GPI IgG Lot-to-Lot Reproducibility:

Specimen	Mean (EU/mL)	Lot 1 CV%	Lot 2 CV%	Lot 3 CV%	Inter-Lot CV%
1	12.6	7.5	7.0	6.3	9.0
2	15.7	5.8	5.7	5.3	6.6
3	21.3	6.4	6.0	5.1	8.4
4	26.3	7.2	7.4	5.9	8.4
5	52.8	5.1	4.7	5.4	5.3
6	110.7	7.1	6.1	6.9	6.6
7	158.3	5.4	3.7	5.4	5.1

B2GPI IgM Lot-to-Lot Reproducibility:

B

Specimen	Mean (EU/mL)	Lot 1 CV%	Lot 2 CV%	Lot 3 CV%	Inter-Lot CV%
1	10.5	6.6	13.0	13.8	13.7
2	15.3	6.1	8.8	9.5	10.1
3	20.7	4.8	7.3	7.1	6.8
4	27.2	6.8	4.4	4.5	6.3
5	85.3	4.9	4.0	3.9	6.0
6	112.7	8.5	8.2	7.8	9.2
7	160.6	7.0	4.0	4.2	6.3

B2GPI IgA/IgG/IgM Lot-to-Lot Reproducibility:

Specimen	Mean (EU/mL)	Lot 1 CV%	Lot 2 CV%	Lot 3 CV%	Inter-Lot CV%
1	8.2	9.7	12.1	11.3	12.4
2	16.7	7.7	5.8	8.1	7.5
3	18.7	6.6	5.4	7.8	7.7
4	24.7	8.2	4.6	4.2	7.2
5	63.4	6.4	8.0	9.1	8.0
6	132.8	6.4	5.2	5.3	6.7

b. Linearity/assay reportable range*:*

Linearity and recovery were tested by diluting positive specimens with equidistant dilutions across the measuring range and comparing actual vs. expected results. The linear range of the IgA assay was determined to be 3.6–160 EU/ml. The linear range of the IgG assay was determined to be 3.1–160 EU/ml. The linear range of the IgM assay was determined to be 2.3–160 EU/ml. Results of the linear regression analysis are summarized below:

B2GPI IgA:

Specimen	Dilution Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	2.3 to 17.8	1.06 (0.89 to 1.21)	-0.59 (-2.4 to 1.2)	0.9767	88% to 112%
2	11.0 to 63.9	1.05 (0.97 to 1.14)	-0.77 (-4.2 to 2.6)	0.9934	93% to 105%
3	59.0 to 168.3	1.08 (0.94 to 1.22)	-0.68 (-16.7 to 15.4)	0.9915	90% to 100%

B2GPI IgG:

Specimen	Dilution Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	2.1 to 41.4	1.04 (0.89 to1.19)	0.2 (-1.8 to 4.9)	0.9807	82% to 100%
2	4.1 to 140.9	1.03 (0.93 to 1.13)	4.13 (-3.9 to 12.1)	0.9905	86% to 100%
3	3.3 to 198.1	0.99 (0.91 to 1.07)	7.25 (-1.8 to 16.3)	0.9936	82% to 100%

B2GPI IgM:

B2GPI IgM:

Specimen	Dilution Range (EU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R^2	% Recovery
1	1.7 to 63.4	1.00 (0.95 to 1.05)	1.8 (-0.1 to 3.6)	0.9974	81% to 100%
2	5.4 to 87.5	1.02 (0.93 to 1.10)	2.3 (-1.9 to 6.4)	0.9936	89% to 100%
3	54.7 to 173.5	0.96 (0.82 to 1.09)	3.1 (-13.1 to 19.4)	0.9963	86% to 100%

High dose hook effect: High antibody concentrations specimens above 160 EU/mL were tested. No hook effect was demonstrated in dilution sample levels up to 228.6 EU/mL for IgA; 597.6 EU/mL for IgG; and 605.5 EU/mL for IgM.

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

i. *Traceability*:

There are currently no recognized international standards for the measurement of β2-GP1 IgA, β2-GP1 IgG and β2-GP1 IgM antibodies. Calibrator and Control values are directly traceable to in-house standards.

ii. *Value Assignment:*
Calibrators and Positive Controls are dilutions of pooled β2-GP1 IgA, β2-GP1 IgG or β2-GP1 IgM antibody positive sera. IMMCO formulates new calibrator and control lots from an array of antibody positive sera obtained from various commercial plasma centers. The calibrators and controls are taken from different pooled sera. All source sera have been tested and found negative for infectious disease as stated in the product insert. 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.

iii. *Stability:*

Shelf life stability:
Accelerated and open kit stability studies for each device were performed on three lots of components/reagents. Accelerated studies were conducted with materials incubated at 37°C. In these conditions, one day is considered equivalent to one month stored at 2°–8°C. Materials are removed from the incubator for testing at three-day intervals for a minimum of 21 days.

Data from accelerated stability study support an18-month shelf life stability claim. Real-time stability testing is on-going.

Open Kit Stability:

For open kit stability studies, materials are opened and stored in a dark environment as required for bench-top usage, then assayed at 15, 45 and 90 day intervals. Open vial stability studies demonstrate opened reagents are stable at 45 days, but the sponsor chose a more restrictive one month open kit stability claim.

d. Detection limit:

The Limit of Blank (LoB) and the Limit of Detection (LoD) were determined by following CLSI EP17-A. Sixty samples of diluent were run as blank samples for LoB and six different normal human sera for LoD were each assayed 10 times. The LoB was determined to be 2.6 EU/ml for β2-GP1 IgA, 2.3 EU/mL for β2-GP1 IgG, 1.4 EU/mL for β2-GP1 IgM and 1.9 EU/mL β2-GP1 IgA/IgG/IgM. The LoD was determined to be 3.6 EU/mL for β2-GP1 IgA, 3.1 EU/mL for β2-GP1 IgG, 2.3 EU/mL for β2-GP1 IgM and 2.7 for β2-GP1 IgA/IgG/IgM.

e. Analytical specificity:

Endogenous Interference:

The studies were performed according to CLSI EP07-A2. Interference was studied by mixing sera with known β2-GP1 antibody levels with potentially interfering serum samples and studying deviation from expected results. Known levels of antibodies included negative, around the cut-off and low positive (i.e. 2–5 times the cut-off). No significant interference was demonstrated in for β2-GP1 IgA, β2-GP1 IgG, β2-GP1 IgM or β2-GP1 IgA/IgG/IgM with the following substances at the levels indicated: Hemoglobin (2 g/L), Bilirubin (342 µmol/L), Rheumatoid Factor (100 IU/mL), Triglycerides (37 mmol/L), Cholesterol (13 mmol/L), Heparin (3000 U/L), Acetylsalicylic Acid (3.62 mmol/L), Prednisone (0.84 µmol/L), Warfarin (32.5 µmol/L), Hydroxychloroquine (148 µmol/L), Rituximab (75 µg/mL) and Atorvastatin (600 µg EQ/L).

Cross-reactivity:

Numerous potentially cross-reactive autoimmune and infectious disease sera were tested for B2GP1 antibodies for β2-GP1 IgA, β2-GP1 IgG, β2-GP1 IgM or β2-GP1 IgA/IgG/IgM. Results for each assay are provided in the following table:

	ImmuLisa™ B2GP1 Antibody ELISAs							
Condition	**B2GP1 IgA**		**B2GP1 IgG**		**B2GP1 IgM**		**B2GP1 IgA/IgG/IgM**	
	n	% Pos	n	% Pos	n	% Pos	n	% Pos
Syphilis	7/40	17.5	3/40	7.5	5/40	12.5	8/37	21.6
HCV	0/32	0.0	2/32	6.3	1/32	3.1	2/32	6.3
Celiac	0/16	0.0	0	0.0	0	0.0	1/22	4.5
Polymyositis	3/17	17.6	0/17	0.0	1/17	5.9	2/17	11.8
Dermatomyositis	2/12	16.7	0/12	0.0	1/12	8.3	0/12	0.0

Rheumatoid Arthritis	6/46	13.0	4/60	6.7	0/60	0.0	3/45	6.7
Systemic Sclerosis	3/31	9.7	3/23	13.0	1/23	4.3	2/23	8.7
Sjögren's	2/37	5.4	5/37	13.5	2/37	5.4	2/37	5.4
Stroke	1/15	6.7	0/15	0.0	1/15	6.7	1/15	6.7
Epilepsy	1/14	7.1	0/14	0.0	1/14	7.1	1/14	7.1
Arterial Thrombosis	1/15	6.7	2/15	13.3	1/15	6.7	3/15	20.0
Venous Thrombosis	0/15	0.0	0/15	0.0	0/15	0.0	1/15	6.7
Myocardial Infarction	3/20	15.0	0/20	0.0	1/20	5.0	4/20	20.0
Acute Coronary Syndrome	1/10	10.0	0/10	0.0	0/10	0.0	1/10	10.0
Pre-eclampsia	0/20	0.0	3/20	15.0	0/20	0.0	0/20	0.0
SNHL	0/0	0.0	0/10	0.0	0/0	0.0	0/7	0.0
Hashimoto's	0/7	0.0	0/20	0.0	0/20	0.0	0/7	0.0
Graves	3/25	12.0	1/18	5.6	0/18	0.0	1/20	5.0
Wegener's	4/45	8.9	0/34	0.0	1/34	2.9	1/42	2.4
Churg-Strauss	3/23	13.0	1/23	4.3	0/23	0.0	2/23	8.7
Crohn's	0/20	0.0	1/20	5.0	0/20	0.0	1/20	5.0
Ulcerative Colitis	1/20	5.0	2/20	10.0	2/20	10.0	2/20	10.0
Toxoplasma	0/10	0.0	1/10	10.0	1/10	10.0	1/10	10.0
CMV	0/10	0.0	1/10	10.0	1/10	10.0	2/10	20.0
Rubella	0/10	0.0	0/10	0.0	0/10	0.0	0/10	0.0
Total	**41/623**	**6.5**	**29/534**	**5.4**	**20/515**	**3.8**	**41/581**	**7.0**

The '*Limitations of Procedure'* section of the Package Insert states: "*A diagnosis cannot be made on the basis of anti-B2GP1 results alone. The results of other laboratory tests supporting diagnosis of APS, SLE and associated thrombotic conditions in at-risk patients, particularly anticardiolipin antibody and lupus anticoagulant tests, must be considered along with clinical findings."*

f. Assay cut-off:

The cutoff was established by testing a population of 62 normal healthy subjects (NHS) for β2-GP1IgA, β2-GP1IgG, β2-GP1IgM and 60 NHS for β2-GP1IgA/IgG/IgM) and calculating the mean of the normal subjects plus 2.5 SD for IgA, mean plus 3 SD for IgG, mean plus 2.5 for IgM and mean plus 2 for IgA/IgG/IgM as supported by receiver operatory curve (ROC) analysis. The cutoff was assigned an arbitrary value of 20 EU/mL.

β2-GP1	Interpretation
<20 EU/mL	Negative
20 – 25 EU/mL	Indeterminate (Borderline)*
>25 EU/mL	Positive

*The B2GP1 IgA/IgG/IgM ELISA is qualitative and values greater than or equal to 20 EU/mL are reported as positive.

2. Comparison studies:

a. *Method comparison with predicate devices:* ImmuLisa™ B2GP1 Antibody ELISAs were tested in comparison with analogous Quanta Lite kits using sera from patients with autoimmune diseases associated with β2-GP1 antibodies (disease-associated population: APS, SLE, APS with SLE), patients with a suspected diagnosis of APS and SLE submitted for reference laboratory testing and patients with infectious diseases and other autoimmune conditions (disease control population). The number of specimens tested for each ImmuLisa™ B2GP1 Antibody ELISA varies and is indicated in the respective tables.

Only specimens in the linear range of the assays were included in the method comparison. Greater than 15% of the test cases were the cutoff of the assay. Normal human sera were excluded from the method comparison.

The samples used in the method comparison study for the β2-GP1 IgA, β2-GP1 IgG, β2-GP1 IgM and β2-GP1 IgA/IgG/IgM are shown in the table below:

ImmuLisa™ B2GP1 Antibody ELISAs	B2GP1 IgA	B2GP1 IgG	B2GP1 IgM	B2GP1 IgA/IgG/IgM
Number and type of clinical samples tested on each ImmuLisa™ B2GP1 Antibody ELISA				
Total Specimens	**n=328**	**n=338**	**n=355**	**n=331**
Disease-associated population	**n= 153**	**n=154**	**n=169**	**n=176**
Antiphospholipid syndrome (APS)	91	82	96	87
Systemic lupus erythematosus (SLE)	26	26	27	21
APS with SLE	36	46	46	68
Suspected diagnosis	**n=50**	**n=52**	**n=52**	**n=48**
Suspected diagnosis of APS	31	32	32	28
Suspected diagnosis of SLE	19	20	20	20
Disease controls	**n=125**	**n=132**	**n=134**	**n=107**
Autoimmune conditions	n=70	n=75	n=81	n=56
Celiac	5	0	0	0
Rheumatoid Arthritis	24	23	25	19
Systemic Sclerosis	7	6	7	6
Sjögren's	14	14	13	14
Wegener’s granulomatosus	7	0	0	3
Churg-Strauss	0	8	8	1
Graves’ Disease	13	13	12	13
Hashimoto’s Disease	0	5	16	0

ImmuLisa™ B2GP1 Antibody ELISAs	B2GP1 IgA	B2GP1 IgG	B2GP1 IgM	B2GP1 IgA/IgG/IgM
Sensory Neuronal Hearing Loss	0	6	0	0
Infectious diseases	**n=31**	**n=32**	**n=28**	**n=26**
Syphilis	19	20	16	16
Hepatitis C virus (HCV)	12	12	12	10
Other conditions	**n=24**	**n=25**	**n=25**	**n=25**
Thrombocytopenia	9	10	10	10
Pre-eclampsia	15	15	15	15

The method comparison results between the new devices and the predicates are shown below:

B2GPI IgA:

Indeterminate B2GPI IgA Samples Called Positive

N=328 (Disease-associated: 153, Suspected diagnosis: 50, Disease control: 125)		**QuantaLite® β2GP1 IgA ELISA** (SAU)		**Total**
		Positive: >20	Negative: ≤20	
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA (EU/mL)	Positive: ≥20	86	2	88
	Negative: <20	29	211	240
	Total	115	213	328

Positive percent agreement: 74.8% (86/115) 95% CI: 65.7% – 82.2%
Negative percent agreement: 99.1% (211/213) 95% CI: 96.3% – 99.8%
Total percent agreement: 90.5% (297/328) 95% CI: 87.7% – 93.4%

Indeterminate B2GPI IgA Samples Called Negative

N=328 (Disease-associated: 153, Suspected diagnosis: 50, Disease control: 125)		**QuantaLite® β2GP1 IgA ELISA** (SAU)		**Total**
		Positive: >20	Negative: ≤20	
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA (EU/mL)	Positive: ≥25	84	0	84
	Negative: <25	31	213	244
	Total	115	213	328

Positive percent agreement: 73.0% (84/115) 95% CI: 63.8% – 80.7%
Negative percent agreement: 100.0% (213/213) 95% CI: 97.8% – 100.0%

Total percent agreement: 90.5% (297/328) 95% CI : 86.7% – 93.4%

B2GPI IgG:

Indeterminate B2GPI IgG Samples Called Positive

N=338 (Disease-associated: 154, Suspected diagnosis: 52 , Disease control: 132)		**QuantaLite® β2GP1 IgG ELISA** (SGU)		**Total**
		Positive: >20	Negative: ≤20	
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA (EU/mL)	Positive: ≥20	64	21	85
	Negative: <20	13	240	253
	Total	77	261	338

Positive percent agreement: 83.1% (64/77) 95% CI: 72.5% – 90.4%
Negative percent agreement: 92.0% (240/261) 95% CI: 87.8% – 94.8%
Total percent agreement: 89.9% (304/338) 95% CI: 86.2% – 92.8%

Indeterminate B2GPI IgG Samples Called Negative

N=338 (Disease-associated: 154, Suspected diagnosis: 52, Disease control: 132)		**QuantaLite® β2GP1 IgG ELISA** (SGU)		**Total**
		Positive: >20	Negative: ≤20	
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA (EU/mL)	Positive: ≥25	63	15	78
	Negative: <25	14	246	260
	Total	77	261	338

Positive percent agreement: 81.8% (63/77) 95% CI: 71.0% – 89.4%
Negative percent agreement: 94.3% (246/261) 95% CI: 90.5% – 96.6%
Total percent agreement: 91.4% (309/338) 95% CI : 87.8% – 94.0%

B2GPI IgM:

Indeterminate B2GPI IgM Samples Called Positive

N=355 (Disease-associated: 169, Suspected diagnosis: 52 , Disease control: 134)		**QuantaLite® β2GP1 IgM ELISA** (SMU)		**Total**
		Positive: >20	Negative: ≤20	
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA (EU/mL)	Positive: ≥20	110	2	112
	Negative: <20	10	233	243
	Total	120	235	355

Positive percent agreement: 91.7% (110/120) 95% CI: 84.8% – 95.7%

Negative percent agreement: 99.1% (233/235) 95% CI: 96.7% – 99.9%
Total percent agreement: 96.6% (343/355) 95% CI: 94.3% – 98.4%

Indeterminate B2GPI IgM Samples Called Negative

N=355 (Disease-associated: 169, Suspected diagnosis: 52, Disease control: 134)		**QuantaLite® β2GP1 IgM ELISA** (SMU)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA (EU/mL)	Positive: ≥25	109	2	111
	Negative: <25	11	233	244
	Total	120	235	355

Positive percent agreement: 90.8% (109/120) 95% CI: 83.8% – 95.1%
Negative percent agreement: 99.1% (233/235) 95% CI: 96.7% – 99.9%
Total percent agreement: 96.3% (342/355) 95% CI : 94.0% – 98.2%

B2GPI IgA/IgG/IgM results

N=331 (Disease-associated: 176, Suspected diagnosis: 48 , Disease control: 107)		**QuantaLite® β2GP1 IgA/IgG/IgM** (SMU)		
		Positive: >20	Negative: ≤20	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA (EU/mL)	Positive: ≥20	142	5	147
	Negative: <20	35	149	184
	Total	177	154	331

Positive percent agreement: 80.2% (142/177) 95% CI: 73.4% – 85.7%
Negative percent agreement: 96.8% (149/154) 95% CI: 92.2% – 98.8%
Total percent agreement: 87.9% (291/331) 95% CI: 83.8% – 91.1%

b. Matrix comparison:

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

3. Clinical studies:

a. Clinical sensitivity and specificity:

Serum samples from cohorts of patients with the target condition(s) or other autoimmune and infectious conditions expected to be found in the intended use population were tested and each assay performance was assessed against the clinical diagnosis.

The samples used in the clinical study for the β2-GP1 IgA, β2-GP1 IgG, β2-GP1 IgM and β2-GP1 IgA/IgG/IgM are shown in the table below:

ImmuLisa™ B2GP1 Antibody ELISAs	B2GP1 IgA	B2GP1 IgG	B2GP1 IgM	B2GP1 IgA/IgG/IgM
Number and type of clinical samples tested on each ImmuLisa™ B2GP1 Antibody ELISA				
Disease-associated population	**n= 294**	**n= 318**	**n= 345**	**n= 266**
Antiphospholipid syndrome (APS)	128	97	145	105
Systemic lupus erythematosus (SLE)	64	98	101	61
APS with SLE	103	123	99	100
Suspected diagnosis	**n= 259**	**n= 259**	**n= 259**	**n= 259**
Suspected diagnosis of APS	88	88	88	85
Suspected diagnosis of SLE	171	171	171	171
Disease controls	**n= 438**	**n= 442**	**n= 432**	**n= 432**
Autoimmune conditions	n= 301	n= 305	n= 295	n= 298
Celiac	16	0	0	22
Polymyositis	17	17	17	17
Dermatomyositis	12	12	12	12
Rheumatoid Arthritis	46	60	60	45
Systemic Sclerosis	31	23	23	23
Sjögren's	37	37	37	37
Wegener’s granulomatosus	45	45	45	45
Churg-Strauss	23	23	23	23
Ulcerative Colitis	20	20	20	20
Crohn’s	20	20	20	20
Graves’ Disease	27	18	18	20
Hashimoto’s Disease	7	20	20	7
Sensory Neuronal Hearing Loss	0	10	0	7
Infectious diseases	n= 102	n=102	n= 102	n= 99
Syphilis	40	40	40	37
Hepatitis C virus (HCV)	12	12	12	12
HCV	20	20	20	20
Toxoplasma	10	10	10	10
Cytomegalovirus (CMV)	10	10	10	10
Rubella	10	10	10	10
Other conditions	n= 124	n= 124	n= 124	n= 124
Thrombocytopenia	15	15	15	15
Pre-eclampsia	20	20	20	20
Stroke	15	15	15	15
Epilepsy	14	14	14	14

Arterial thrombosis	15	15	15	15
Venous thrombosis	15	15	15	15
Myocardial infarction	20	20	20	20
Acute coronary syndrome	10	10	10	10

The clinical study results are tabulated below:

B2GP1 IgA:

Results Where Indeterminate Samples Called Positive

N=653 (Disease-associated: 128 , Disease control: 525)		**APS Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA	Positive	49	42	91
	Negative	79	483	562
	Total	128	525	653

Clinical sensitivity: 38.3% (49/128) 95% CI: 30.0% – 47.3%
Clinical specificity: 92.0% (483/525) 95% CI: 89.3% – 94.1%

N=628 (Disease-associated: 103, Disease control: 525)		**APS with SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA	Positive	20	42	62
	Negative	83	483	566
	Total	103	525	628

Clinical sensitivity: 19.4% (20/103) 95% CI: 12.5% – 28.6%
Clinical specificity: 92.0% (483/525) 95% CI: 89.3% – 94.1%

N=589 (Disease-associated: 64, Disease control: 525)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA	Positive	7	42	49
	Negative	57	483	540
	Total	64	525	589

Clinical sensitivity: 10.9% (7/64) 95% CI: 4.9% – 21.8%
Clinical specificity: 92.0% (483/525) 95% CI: 89.3% – 94.1%

Results Where Indeterminate Samples Called Negative

N=653 (Disease-associated: 128 , Disease control: 525)	**APS Diagnosis**		
	Positive	Negative	**Total**

ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA	Positive	47	30	77
	Negative	81	495	576
	Total	128	525	653

Clinical sensitivity: 36.7% (49/128) 95% CI: 28.5% – 47.5%
Clinical specificity: 94.3% (583/525) 95% CI: 91.9% – 96.0%

N=628 (Disease-associated: 103, Disease control: 525)		**APS with SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA	Positive	17	30	47
	Negative	86	495	581
	Total	103	525	628

Clinical sensitivity: 16.5% (17/103) 95% CI: 10.2% – 25.4%
Clinical specificity: 94.3% (583/525) 95% CI: 91.9% – 96.0%

N=589 (Disease-associated: 64, Disease control: 525)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA Antibody ELISA	Positive	6	30	36
	Negative	58	495	553
	Total	64	525	589

Clinical sensitivity: 9.4% (6/64) 95% CI: 3.9% – 19.9%
Clinical specificity: 94.3% (583/525) 95% CI: 91.9% – 96.0%

<u>B2GP1 IgG:</u>

Indeterminate B2GPI IgG Samples Called Positive

N=617 (Disease-associated: 97, Disease control: 520)		**APS Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	Positive	57	32	89
	Negative	40	488	528
	Total	97	520	617

Clinical sensitivity: 58.8% (57/97) 95% CI: 48.3 – 68.5%
Clinical specificity: 93.8% (488/520) 95% CI: 91.3 – 95.7%

N=643 (Disease-associated: 123, Disease control: 520)	**APS with SLE Diagnosis**	**Total**

		Positive	Negative	
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	Positive	71	32	103
	Negative	52	488	540
	Total	123	520	643

Clinical sensitivity: 57.7% (71/123) 95% CI: 48.5% – 66.5%
Clinical specificity: 93.8% (488/520) 95% CI: 92% – 96%

N=618 (Disease-associated: 98, Disease control: 520)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	Positive	26	32	58
	Negative	72	488	560
	Total	98	520	618

Clinical sensitivity: 26.5% (26/98) 95% CI: 18.4% – 36.6%
Clinical specificity: 93.8% (488/520) 95% CI: 91.3% – 95.7%

Indeterminate B2GPI IgG Samples Called Negative

N=617 (Disease-associated: 97, Disease control: 520)		**APS Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	Positive	53	25	78
	Negative	44	495	539
	Total	97	520	617

Clinical sensitivity: 54.6% (53/97) 95% CI: 44.2 – 64.7%
Clinical specificity: 95.2% (495/520) 95% CI: 92.9 – 96.8%

N=643 (Disease-associated: 123, Disease control: 520)		**APS with SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgG Antibody ELISA	Positive	64	25	89
	Negative	59	495	554
	Total	123	520	643

Clinical sensitivity: 52.0% (64/123) 95% CI: 42.9% – 61.1%
Clinical specificity: 95.2% (495/520) 95% CI: 92.9 – 96.8%

N=618 (Disease-associated: 98, Disease control: 520)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™	Positive	21	25	46

B2GPI IgG Antibody ELISA	Negative	77	495	572
	Total	98	520	618

Clinical sensitivity: 21.4% (21/98) 95% CI: 14.0% – 31.1%
Clinical specificity: 95.2% (495/520) 95% CI: 92.9 – 96.8%

B2GP1 IgM:

Indeterminate B2GPI IgM Samples Called Positive

N=655 (Disease-associated: 145 , Disease control: 510)		**APS Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	Positive	93	20	113
	Negative	52	490	542
	Total	145	510	655

Clinical sensitivity: 64.1% (93/145) 95% CI: 55.7– 71.8%
Clinical specificity: 96.1% (490/510) 95% CI: 93.9 – 97.5%

N=609 (Disease-associated: 99, Disease control: 510)		**APS with SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	Positive	46	20	66
	Negative	53	490	543
	Total	99	510	609

Clinical sensitivity: 46.5% (46/99) 95% CI: 36.5 – 56.7%
Clinical specificity: 96.1% (490/510) 95% CI: 93.9 – 97.5%

N=673 (Disease-associated: 101, Disease control: 510)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	Positive	19	20	39
	Negative	82	490	572
	Total	101	510	611

Clinical sensitivity: 18.8% (19/101) 95% CI: 12.0 – 28.1%
Clinical specificity: 96.1% (490/510) 95% CI: 93.9 – 97.5%

Indeterminate B2GPI IgM Samples Called Negative

N=655 (Disease-associated: 145 , Disease control: 510)		**APS Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	Positive	89	13	102
	Negative	56	497	553
	Total	145	510	655

Clinical sensitivity: 61.4% (89/145) 95% CI: 52.9– 69.2%
Clinical specificity: 97.5% (497/510) 95% CI: 95.6 – 98.6%

N=609 (Disease-associated: 99, Disease control: 510)		**APS with SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	Positive	42	13	55
	Negative	57	497	554
	Total	99	510	609

Clinical sensitivity: 42.4% (42/99) 95% CI: 32.7 – 52.8%
Clinical specificity: 97.5% (497/510) 95% CI: 95.6 – 98.6%

N=673 (Disease-associated: 101, Disease control: 510)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgM Antibody ELISA	Positive	16	13	29
	Negative	85	497	582
	Total	101	510	611

Clinical sensitivity: 15.8% (16/101) 95% CI: 9.6 – 24.8%
Clinical specificity: 97.5% (497/510) 95% CI: 95.6 – 98.6%

B2GP1 IgA/IgG/IgM:

N=623 (Disease-associated: 105, Disease control: 518)		**APS Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA/IgG/IgM Antibody ELISA	Positive	89	42	131
	Negative	16	476	492
	Total	105	518	623

Clinical sensitivity: 84.8% (89/105) 95% CI: 76.1 – 90.8%
Clinical specificity: 91.9% (476/518) 95% CI: 89.1 – 94.0%

N=618 (Disease-associated: 100 , Disease control: 518)	**APS with SLE Diagnosis**		
	Positive	Negative	**Total**

ImmuLisa Enhanced™ B2GPI IgA/IgG/IgM Antibody ELISA	Positive	66	42	108
	Negative	34	476	510
	Total	100	518	618

Clinical sensitivity: 66.0% (66/100) 95% CI: 55.8 – 75.0%
Clinical specificity: 91.9% (476/518) 95% CI: 89.1 – 94.0%

N=579 (Disease-associated: 61 , Disease control: 518)		**SLE Diagnosis**		
		Positive	Negative	**Total**
ImmuLisa Enhanced™ B2GPI IgA/IgG/IgM Antibody ELISA	Positive	15	42	57
	Negative	46	476	522
	Total	61	518	579

Clinical sensitivity: 24.6% (15/61) 95% CI: 14.8 – 37.6%
Clinical specificity: 91.9% (476/518) 95% CI: 89.1 – 94.0%

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   Test results in a normal population are expected to be negative. A study of 174 normal, apparently disease-free samples tested with the B2GP1 IgA assay yielded seven positive results (4.0%). A study of 151 normal, apparently disease-free samples tested with the B2GP1 IgG yielded five positive results (3.3%). A study of 151 normal, apparently disease-free samples tested with the B2GP1 IgM assay yielded three positive results (2.0%). A study of 206 normal, apparently disease-free samples tested with the B2GP1 IgA/IgG/IgM assay yielded seven positive results (3.4%).

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.