510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K223093

B Applicant

INOVA Diagnostics, Inc.

C Proprietary and Established Names

Aptiva APS IgG Reagent
Aptiva APS IgM Reagent

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MID MSV	Class II	21 CFR 866.5660 – Multiple Autoantibodies Immunological Test System	IM – Immunology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Aptiva APS IgG Reagent
Anti-cardiolipin IgG autoantibodies (aCL IgG)
Anti-beta 2 glycoprotein 1 IgG autoantibodies (aβ2GPI IgG)

Aptiva APS IgM Reagent
Anti-cardiolipin IgM autoantibodies (aCL IgM)
Anti-beta 2 glycoprotein 1 IgM autoantibodies (aβ2GPI IgM)

C Type of Test:

Semi quantitative, Particle-based multi-analyte assay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Aptiva APS IgG Reagent

The Aptiva APS IgG Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-cardiolipin (aCL) and anti-beta 2 glycoprotein 1 (aβ2GPI) IgG autoantibodies in human serum as an aid in the diagnosis of primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other clinical and laboratory findings.

The Aptiva APS IgG Reagent is intended for use with the Aptiva System.

Aptiva APS IgM Reagent

The Aptiva APS IgM Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-cardiolipin (aCL) and anti-beta 2 glycoprotein 1 (aβ2GPI) IgM autoantibodies in human serum as an aid in the diagnosis of primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other clinical and laboratory findings.

The Aptiva APS IgM Reagent is intended for use with the Aptiva System.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Inova Diagnostics Aptiva System (K193604)

IV Device/System Characteristics:

A Device Description:

Aptiva APS IgG Reagent and Aptiva APS IgM Reagent utilize particle-based multi-analyte technology (PMAT) in a cartridge format. Both assays are run on Inova Diagnostics Aptiva System.

The **Aptiva APS IgG Reagent** contains one cartridge with the following reagents for 200 determinations:

- APS IgG Beads (1×0.5 mL): Paramagnetic beads coated with native Cardiolipin (CL) plus β2GPI antigens, native β2GPI antigen, AffiniPure goat polyclonal anti-human IgG, and stabilizer
- Assay Buffer (1×17 mL): Containing protein stabilizers and preservatives
- PE Tracer IgG (1×17 mL): Phycoerythrin (PE) labeled goat anti-human IgG antibody with stabilizer and preservative
- Rehydration Buffer (1×6.5 mL): Containing protein stabilizer and preservative

Materials Required but Not Provided

- Aptiva APS IgG Calibrators: 3 Calibrators (2×0.3mL/each) of human antibodies, each contains human aCL IgG and aβ2GPI IgG in stabilizers and preservatives;
- Aptiva APS IgG Controls: 2 Controls (2×0.5 mL/each) of human antibodies, each contain human aCL IgG and aβ2GPI IgG in stabilizers and preservatives

The **Aptiva APS IgM Reagent** kit contains one cartridge with the following reagents for 200 determinations:

- APS IgM Beads (1×0.5 mL): Paramagnetic beads coated with native Cardiolipin (CL) plus β2GPI antigens, native β2GPI antigen, AffiniPure goat polyclonal anti-human IgM, and stabilizer
- Assay Buffer (1×17 mL): Containing protein stabilizers and preservatives
- PE Tracer IgM (1×17 mL): PE labeled goat anti-human IgM antibody with stabilizer and preservative
- Rehydration Buffer (1×6.5 mL): Containing protein stabilizer and preservative

Materials Required but Not Provided

- Aptiva APS IgM Calibrators: 3 Calibrators (2×0.3mL/each) of human antibodies, each contains human aCL IgM and aβ2GPI IgM in stabilizers and preservatives
- Aptiva APS IgM Controls: 2 Controls (2×0.5 mL/each) of human antibodies, each contains human aCL IgM and aβ2GPI IgM in stabilizers and preservatives

B Principle of Operation:

Both the Aptiva APS IgG Reagent and Aptiva APS IgM Reagent utilize particle based multi-analyte technology (PMAT) in a cartridge format. Both the Aptiva APS IgG Reagent and the

Aptiva APS IgM Reagent contain two different populations of particles: one particle population coated with cardiolipin along with human purified β2GPI and another particle population coated with only human purified β2GPI antigen. The two analyte microparticles, along with the control microparticle, are stored in the reagent cartridge.

The Aptiva System dilutes the sample 1:8, then combines an aliquot of diluted sample, and reagent into a cuvette. After incubation at 37°C and followed with a wash cycle, conjugated anti-human IgG or IgM antibodies are added to the particles. Following the incubation of this mixture at 37°C, excess conjugate is removed in another wash cycle, and the particles are re-suspended in system fluid. Multiple images are generated by the system to identify and count the two unique analyte particles, as well as determine the amount of conjugate on each particle. A third particle, coated with goat anti-human IgG or IgM antibodies, is present in the reagent as a control to flag low concentrations of IgG or IgM in the sample as an assay verification step. The median fluorescent intensity (MFI) for each analyte is proportional to the concentration of conjugate bound to human IgG or IgM, which is proportional to the concentration of IgG or IgM antibodies bound to the corresponding particle population. The system uses the MFI from at least 50 particles of each population. The identity of the particles is determined by the unique signature of the particles.

Each analyte in the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent is assigned a predefined lot specific master curve. The analyte specific master curve is stored on the reagent cartridge radio frequency identification (RFID) label. Based on results obtained by running calibrators (supplied separately), the system creates individual working curves. Working curves are used by the software to calculate Fluorescent Light Units (FLU) for each analyte from the MFI values obtained for each sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

For Aptiva APS IgG Reagent:
HemosIL AcuStar Cardiolipin IgG
QUANTA Lite β2GPI IgG ELISA

For Aptiva APS IgM Reagent:
HemosIL AcuStar Cardiolipin IgM
HemoIL AcuStar anti-β2 Glycoprotein-I IgM

B Predicate 510(k) Number(s):

K092181 - HemosIL AcuStar Cardiolipin IgG; HemosIL AcuStar Cardiolipin IgM
K970551 - QUANTA Lite β2GPI IgG ELISA
K091556 - HemosIL AcuStar anti-β2 Glycoprotein-I IgM

C Comparison with Predicate(s):

Aptiva APS IgG Reagent

Device & Predicate Device(s):	**K223093** New Device	**K092181** Predicate	**K970551** Predicate
Device Trade Name	Aptiva APS IgG Reagent	HemosIL AcuStar Cardiolipin IgG	QUANTA Lite β2GPI IgG ELISA
General Device Characteristic Similarities			
Intended Use/ Indications for Use	The Aptiva APS IgG Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-cardiolipin (aCL) and anti-beta 2 glycoprotein 1 (aβ2GPI) IgG autoantibodies in human serum as an aid in the diagnosis of primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory findings. The Aptiva APS IgG Reagent is intended for use with the Aptiva System.	Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-cardiolipin (aCL) IgG antibodies in human citrate plasma and serum on the BIO-FLASH instrument as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.	A semi-quantitative enzyme linked immunoassay for detecting IgG class autoantibody to β2 glycoprotein I (β2 GPI) for use as an 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.
Antigen	Bovine cardiolipin antigen	Same	N/A
	Purified human β2GPI	N/A	Same
Quality Control	2 controls (sold separately)	Same (sold separately)	Same (included in the kit)
General Device Characteristic Differences			
Solid Phase	Paramagnetic microparticles	Same	96-well polystyrene plate
Assay Format	Fluorescent immunoassay	Chemiluminescent immunoassay	Enzyme-linked immunosorbent assay
Instrumentation	INOVA Aptiva System	BIO-FLASH Instrument	Manual, ELISA reader
Sample Type	Human Serum	Human serum and citrated plasma	Human serum
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody	Anti-human IgG horseradish peroxidase (HRP)
Units	Fluorescent light units (FLU)	Chemiluminescent Units	Standard β2GPI IgG Units (SGU)

Result Interpretation	Negative: <5.00 FLU Positive: ≥5.00 FLU	Negative: <20 U/mL Positive: ≥20 U/mL	20.0 SGU as cut-off
Analytical Measuring Interval	0.29–328.94 FLU (aCL IgG)	2.6 – 2024.0 U/mL	
	0.21–256.70 FLU (aβ2GPI IgG)		9.4 – 150.0 SGU
Calibration	Lot specific Master Curve + 3 calibrators (sold separately)	2 calibrator levels (included in test kit)	1 calibrator diluted to a 5-point standard curve (included in test kit)

Aptiva APS IgM Reagent

Device & Predicate Device(s):	**K223095** New Device	**K092181** Predicate	**K091556** Predicate
Device Trade Name	Aptiva APS IgM Reagent	HemosIL AcuStar Cardiolipin IgM	HemosIL AcuStar anti-β2 Glycoprotein-I IgM
General Device Characteristic Similarities			
Intended Use/ Indications for Use	The Aptiva APS IgM Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-cardiolipin (aCL) and anti-beta 2 glycoprotein 1 (aβ2GPI) IgM autoantibodies in human serum as an aid in the diagnosis of primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory findings. The Aptiva APS IgM Reagent is intended for use with the Aptiva System.	Fully automated chemiluminescent assay for the semi-quantitative measurement of anti-cardiolipin (aCL) IgM antibodies in human citrated plasma and serum on the BIO-FLASH instrument, as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.	Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-β2 glycoprotein-I (anti-β2GPI) IgM antibodies in human citrated plasma and serum on the BIO-FLASH instrument as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.
Solid phase	Paramagnetic microparticles	Same	Same
Antigen	Bovine cardiolipin antigen	Same	N/A
	Purified human β2GPI	N/A	Same
Quality Control	2 control levels (sold separately)	Same	Same

General Device Characteristic Differences			
Sample Type	Human serum	Human serum and citrated plasma	
Assay Format	Fluorescent immunoassay	Chemiluminescent immunoassay	
Instrumentation	INOVA Aptiva System	BIO-FLASH instrument	
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgM antibody	Isoluminol conjugated monoclonal anti-human IgM antibody	
Units	Fluorescent light units	Chemiluminescent units	
Result Interpretation	Negative: <5.00 FLU Positive: ≥5.00 FLU	Negative: <20 U/mL Positive: ≥20 U/mL	
Analytical Measuring Interval	0.1–114.68 FLU (aCL IgM)	1.0 – 774.0 U/mL	
	0.1– 95.86 FLU (aβ2GPI IgM)		1.1– 841 U/mL
Calibration	Lot specific Master Curve + 3 calibrators (sold separately)	Two calibrator levels (included in test kit)	

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3: Evaluation of Precision of Quantitative Measurement Procedures, Approved Guideline – Third Edition.
- CLSI EP06-Ed2: Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A3: Interference Testing in Clinical Chemistry – Third Edition.
- CLSI EP12-A2: User Protocol for Evaluation of Qualitative Test Performance – Second Edition.
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline – Second Edition.
- CLSI EP25-A: Evaluation of Stability of *In Vitro* Diagnostic Reagents, Approved Guideline
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guideline – Third Edition.
- CLSI EP37: Supplemental Tables for Interference Testing in Clinical Chemistry
- CLSI EP39: A Hierarchical Approach to Selecting Surrogate Samples for the Evaluation of *In Vitro* Medical Laboratory Tests

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision and reproducibility of the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent were evaluated in accordance with the CLSI guideline EP05-A3.

Within-Laboratory Precision:

Within-lab precision of the Aptiva APS IgG reagent was performed with by testing seven serum samples containing various concentrations of aCL IgG, aβ2GPI IgG, aCL IgM and aβ2GPI IgM autoantibodies. All samples were run in duplicate, twice a day, for 20 days using single lot of reagents on single instrument. Data was analyzed for repeatability (within-run), between-run, between-day and within-laboratory precision. Results are summarized below.

Aptiva APS IgG Reagent

aCL IgG										
Sample	**N**	**Mean (FLU)**	**Repeatability**		**Between-Run**		**Between-Day**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	80	2.33	0.13	5.5	0.08	3.3	0.10	4.3	0.18	7.8
2	80	5.64	0.29	5.1	0.10	1.8	0.28	5.0	0.42	7.4
3	80	8.77	0.37	4.2	0.32	3.7	0.00	0.0	0.49	5.6
4	80	26.15	1.41	5.4	0.00	0.0	1.42	5.4	2.00	7.6
5	80	66.29	2.53	3.8	2.22	3.4	2.71	4.1	4.32	6.5
6	80	201.45	7.94	3.9	5.58	2.8	10.65	5.3	14.40	7.2
7	80	265.15	11.06	4.2	10.94	4.1	19.96	7.5	25.30	9.5

aβ2GPI IgG										
Sample	**N**	**Mean (FLU)**	**Repeatability**		**Between-Run**		**Between-Day**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	80	2.04	0.15	7.3	0.04	1.9	0.18	9.0	0.24	11.7
2	80	5.12	0.29	5.7	0.22	4.4	0.00	0.0	0.37	7.1
3	80	9.39	0.50	5.3	0.29	3.1	0.89	9.5	1.06	11.3
4	80	24.55	1.22	5.0	0.22	0.9	1.69	6.9	2.10	8.5
5	80	53.51	2.11	3.9	2.08	3.9	2.22	4.1	3.70	6.9
6	80	170.02	6.40	3.8	5.05	3.0	7.83	4.6	11.30	6.6
7	80	212.10	8.19	3.9	6.60	3.1	14.44	6.8	17.86	8.4

Aptiva APS IgM Reagent

aCL IgM										
Sample	**N**	**Mean (FLU)**	**Repeatability**		**Between-Run**		**Between-Day**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	80	2.93	0.12	4.1	0.06	2.1	0.25	8.5	0.28	9.7
2	80	5.54	0.15	2.8	0.10	1.9	0.20	3.6	0.27	4.9
3	80	10.67	0.34	3.2	0.14	1.3	0.50	4.7	0.62	5.8
4	80	21.94	0.59	2.7	0.55	2.5	0.51	2.3	0.95	4.3
5	80	56.16	1.30	2.3	0.47	0.8	3.07	5.5	3.36	6.0
6	80	73.51	2.25	3.1	1.23	1.7	3.06	4.2	3.99	5.4
7	80	116.09	2.62	2.3	2.55	2.2	5.90	5.1	6.94	6.0

aβ2GPI IgM										
Sample	**N**	**Mean (FLU)**	**Repeatability**		**Between-Run**		**Between-Day**		**Within-Laboratory**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	80	2.47	0.10	3.9	0.07	2.9	0.22	9.0	0.25	10.2
2	80	5.39	0.13	2.5	0.27	5.0	0.22	4.1	0.38	7.0
3	80	8.75	0.28	3.2	0.19	2.1	0.38	4.4	0.51	5.8
4	80	27.26	1.23	4.5	0.51	1.9	1.37	5.0	1.91	7.0
5	80	46.00	1.22	2.7	0.74	1.6	2.29	5.0	2.70	5.9
6	80	63.00	2.10	3.3	0.63	1.0	2.70	4.3	3.48	5.5
7	80	93.18	2.97	3.2	2.03	2.2	4.59	4.9	5.83	6.3

Lot-to-Lot Imprecision:

The lot-to-lot imprecision of the Aptiva APS IgG Reagent and Aptiva APS IgM Reagent was evaluated by testing seven serum samples for aCL IgG and aβ2GPI IgG with three lots of the Aptiva APS IgG Reagent and six serum samples for aCL IgM and aβ2GPI IgM with three lots of the Aptiva APS IgM Reagent. Samples were tested in replicates of five, once a day, for five days using one instrument to generate a total of 75 data points for each sample. The results are summarized in the tables below.

Aptiva APS IgG Reagent

aCL IgG										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Lot**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	1.61	0.08	5.2	0.07	4.4	0.12	7.2	0.16	9.9
2	75	4.16	0.21	5.0	0.18	4.3	0.19	4.6	0.34	8.1
3	75	19.71	0.98	5.0	0.99	5.0	2.17	11.0	2.58	13.1
4	75	51.11	2.67	5.2	1.63	3.2	6.06	11.9	6.82	13.3
5	75	124.49	5.93	4.8	5.92	4.8	0.00	0.0	8.37	6.7
6	75	168.77	7.11	4.2	8.61	5.1	0.00	0.0	11.17	6.6
7	75	285.27	10.73	3.8	5.73	2.0	11.38	4.0	16.66	5.8

aβ2GPI IgG										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Lot**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	1.97	0.10	5.0	0.13	6.7	0.03	1.7	0.17	8.5
2	75	4.94	0.26	5.3	0.35	7.2	0.40	8.2	0.60	12.1
3	75	23.39	1.08	4.6	1.24	5.3	1.10	4.7	1.98	8.5
4	75	58.42	3.18	5.5	2.47	4.2	3.87	6.6	5.59	9.6
5	75	100.56	5.55	5.5	6.40	6.4	5.21	5.2	9.94	9.9
6	75	144.20	6.89	4.8	9.57	6.6	10.69	7.4	15.92	11.0
7	75	213.10	8.78	4.1	5.46	2.6	21.18	9.9	23.57	11.1

Aptiva APS IgM Reagent

aCL IgM										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Lot**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	2.07	0.07	3.3	0.10	4.9	0.03	1.7	0.13	6.1
2	75	4.39	0.12	2.8	0.19	4.3	0.20	4.6	0.30	6.9
3	75	5.25	0.15	2.9	0.24	4.5	0.24	4.5	0.36	6.9
4	75	31.61	0.83	2.6	0.85	2.7	2.16	6.8	2.46	7.8
5	75	50.07	1.54	3.1	1.89	3.8	5.18	10.3	5.72	11.4
6	75	88.20	3.24	3.7	3.80	4.3	8.10	9.2	9.51	10.8

aβ2GPI IgM										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Lot**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	1.57	0.05	3.3	0.09	5.6	0.06	4.0	0.12	7.6
2	75	4.62	0.15	3.2	0.25	5.4	0.15	3.3	0.33	7.0
3	75	5.73	0.20	3.5	0.28	4.8	0.00	0.0	0.34	6.0
4	75	24.76	0.61	2.5	0.68	2.7	1.86	7.5	2.07	8.4
5	75	54.40	1.63	3.0	2.45	4.5	4.54	8.3	5.41	9.9
6	75	80.53	2.52	3.1	3.27	4.1	7.35	9.1	8.43	10.5

Site-to-Site Precision (Reproducibility):

The reproducibility of the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent was conducted at three different sites using seven serum samples for aCL IgG and aβ2GPI IgG and six serum samples for aCL IgM and aβ2GPI IgM. Samples were tested in replicates of five, once a day, for five days using one reagent lot to generate a total of 75 replicates per sample. The results are summarized below.

Aptiva APS IgG Reagent

aCL IgG										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Site**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	1.97	0.10	5.2	0.04	2.3	0.12	6.0	0.16	8.3
2	75	5.60	0.33	5.9	0.20	3.6	0.13	2.3	0.41	7.2
3	75	25.24	1.35	5.3	1.06	4.2	0.49	1.9	1.78	7.1
4	75	53.83	2.46	4.6	1.48	2.7	1.40	2.6	3.19	5.9
5	75	123.94	6.00	4.8	2.43	1.0	0.00	0.0	6.48	5.2
6	75	164.49	9.10	5.5	5.26	3.2	2.78	1.7	10.87	6.6
7	75	279.39	14.03	5.0	5.79	2.1	20.98	7.5	25.89	9.3

aβ2GPI IgG										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Site**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	1.99	0.11	5.7	0.08	4.0	0.14	7.1	0.20	10.0
2	75	5.26	0.24	4.6	0.20	3.8	0.19	3.6	0.36	6.9
3	75	23.58	1.08	4.6	1.18	5.0	0.96	4.1	1.87	7.9
4	75	52.83	2.49	4.7	1.76	3.3	1.47	2.8	3.38	6.4
5	75	138.12	8.47	6.1	4.03	2.9	7.36	5.3	11.92	8.6
6	75	161.78	6.16	3.8	4.09	2.5	10.78	6.7	13.07	8.1
7	75	217.27	9.54	4.4	4.86	2.2	12.35	5.7	16.35	7.5

Aptiva APS IgM Reagent

aCL IgM										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Site**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	2.16	0.10	4.6	0.09	4.3	0.05	2.2	0.14	6.7
2	75	4.56	0.16	3.4	0.19	4.1	0.04	0.8	0.24	5.4
3	75	6.68	0.28	4.1	0.19	2.8	0.33	5.0	0.47	7.1
4	75	40.83	1.47	3.6	1.33	3.3	3.56	8.7	4.07	10.0
5	75	57.09	2.30	4.0	1.61	2.8	4.83	8.5	5.59	9.8
6	75	92.71	4.27	4.6	2.36	2.5	8.24	8.9	9.58	10.3

aβ2GPI IgM										
Sample	**N**	**Mean (FLU)**	**Within-Day**		**Between-Day**		**Between-Site**		**Total**	
			SD	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
1	75	1.56	0.06	3.7	0.06	3.9	0.04	2.3	0.09	5.9
2	75	4.63	0.17	3.7	0.14	3.0	0.18	3.9	0.28	6.2
3	75	6.11	0.24	3.9	0.20	3.3	0.36	5.9	0.47	7.8
4	75	35.89	1.25	3.5	1.46	4.1	3.26	9.1	3.78	10.5
5	75	55.66	2.28	4.1	1.92	3.5	4.78	8.6	5.64	10.1
6	75	82.78	3.71	4.5	2.20	2.7	7.25	8.8	8.44	10.2

2. Linearity:

The linearity of the analytical measuring interval (AMI) was evaluated for the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent according to CLSI EP06-Ed2.

For the Aptiva APS IgG Reagent, five overlapping dilution series to cover the AMI of each of the aCL IgG and aβ2GPI IgG assays were prepared by mixing human serum samples with high antibody concentrations and samples with low antibody concentrations. Each sample was tested in duplicate using one lot of the Aptiva APS IgG Reagent. The overall testing range for linearity study is from 0.11–382.20 FLU for aCL IgG assay, and 0.11–301.21 FLU for a aβ2GPI IgG. The percent deviation from the weighted least squares regression analysis was used to assess the fit of the regression for each sample and each analyte. The data support the linearity of AMI of 0.29–328.94 FLU for the aCL IgG assay and the AMI of 0.21–256.70 FLU for the aβ2GPI IgG assay, as part of the Aptiva APS IgG Reagent.

For Aptiva APS IgM Reagent, four overlapping dilution series to cover portions of the AMI of each of the aCL IgM and aβ2GPI IgM assays were prepared by mixing human serum samples with high antibody concentrations and samples with low antibody concentrations. Each sample was tested in duplicate using one lot of the Aptiva APS IgM Reagent. The overall testing range for linearity study is from 0.04–118.12 FLU for aCL IgM assay, and 0.04–102.10 FLU for a aβ2GPI IgM. The percent deviation from the weighted least squares regression analysis was used to assess the fit of the regression for each sample and each analyte. The data support the linearity of AMI of 0.10–114.68 FLU for the aCL IgM assay and the AMI of 0.10–95.86 FLU for the aβ2GPI IgM assay, as part of the Aptiva APS IgM Reagent.

3. Hook effect

The hook effect for Aptiva APS IgG Reagent and Aptiva APS IgM Reagent on the Aptiva System was evaluated by testing high positive serum samples for each device: two high aCL IgG samples and two high aβ2GPI IgG samples for the Aptiva APS IgG Reagent; two high aCL IgM samples and two high aβ2GPI IgM samples for the Aptiva APS IgM Reagent.

For the Aptiva APS IgG Reagent, no antigen excess hook effect was observed up to 2645.36 FLU for aCL IgG and 1790.48 FLU for aβ2GPI IgG.

For the Aptiva APS IgM Reagent, no hook effect was observed up to 167.25 FLU for aCL IgM and 126.13 FLU for the aβ2GPI IgM.

4. Analytical Specificity/Interference:

Interference

An interference study was performed according to CLSI EP07-A2 for the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent. A set of three human serum specimens— one positive, one around the cut-off and one negative sample for each assay were used to prepare interferent spiked samples or corresponding control samples without the interfering

substance. All samples were tested in five replicates using the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent. The percent recovery for each sample spiked with the potential interfering substance was calculated by comparing its result to that of the corresponding control sample without the interfering substance. Based on the results, the following tables display the highest concentration at which no interference was observed for the Aptiva APS IgG Reagent and IgM Reagent assays.

	Aptiva APS IgG Reagent (aCL IgG / aβ2GPI IgG)	**Aptiva APS IgM Reagent (aCL IgM / aβ2GPI IgM)**
Interferent	**Concentration without Interference**	
Endogenous		
Bilirubin, Conjugated	1.0 mg/mL	1.0 mg/mL
Hemoglobin	10.00 g/L	10.00 g/L
Triglyceride	1000.0 mg/dL	1000.0 mg/dL
Cholesterol	332.5 mg/dL	332.5 mg/dL
RF IgM	153.4 IU/mL	153.4 IU/mL
Human IgG	20.0 mg/mL	20.0 mg/mL
Exogenous		
Ibuprofen	21.9 mg/dL	21.9 mg/dL
Warfarin	7.5 mg/dL	7.5 mg/dL
Prednisone	0.0099 mg/dL	0.0099 mg/dL
Acetaminophen	15.6 mg/dL	15.6 mg/dL
Aspirin	3.00 mg/dL	3.00 mg/dL
Hydroxychloroquine	0.465 mg/dL	0.465 mg/dL
Omeprazole	0.840 mg/dL	0.840 mg/dL
Simvastatin	0.168 mg/dL	0.168 mg/dL
Heparin	330 units/dL	330 units/dL

5. Assay Reportable Range:

The reportable range for the Aptiva APS IgG Reagent and Aptiva APS IgM Reagent is the same as the analytical measuring interval (AMI) for both devices. The AMI for each assay is shown in the table below:

	Analytical Measuring Interval
Aptiva APG IgG Reagent	
aCL IgG	0.29 – 328.94 FLU
aβ2GPI IgG	0.21 – 256.70 FLU
Aptiva APG IgM Reagent	
aCL IgM	0.10 – 114.68 FLU
aβ2GPI IgM	0.10 – 95.86 FLU

6. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability:

There is no international reference material for anti-cardiolipin antibodies and anti-β2-GPI antibodies. Calibrator and control values are directly traceable to in-house reference materials that are used to create the master curves for each assay of the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent.

Stability:

a. Reagent shelf-life stability: An on-going real-time stability and an accelerated stability study were conducted to establish shelf-life stability for the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent. The study required testing at least three serum samples at different levels for each assay using three lots of reagents at different timepoints. The collected data showed the initial shelf-life of 9 months for the Aptiva APS IgG Reagent and 7 months for the Aptiva IgM Reagent when stored at 2–8°C.

b. Reagent in-use (on-board) stability: An initial in-use stability for the Aptiva APS IgG Reagent and Aptiva APS IgM Reagent was conducted by testing multiple samples with different assay levels at different time points using the reagent stored on board. The in-use (on-board) stability of the Aptiva APS IgG and Aptiva APS IgM Reagents was set at 28 days, with a 14-day recalibration.

c. Reagent shipping stability: A transport simulation study was performed to assess the shipping stability of the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent kits during transport. The study was performed under simulated conditions that the products may potentially be exposed to during transport. The collected data support the reagent packaging mitigates the potential impact of temperature stressing during transport.

d. Sample stability: Five serum samples with different antibody levels were stored at various storage temperatures and tested with the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent at various timepoints. The collected data showed that the sample can be stored up to 14 days at 2–8°C, up to 48 hours at room temperature (20–26°C), and up to five freeze/thaw cycles when stored at -20°C.

7. Detection Limit:

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were assessed for each analyte in the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent following CLSI EP17-A2.

LoB:
To determine the LoB, four blank samples were run in five replicates, once a day for three days using two reagent lots, for a total of 60 data points per lot. The LoB was determined for each assay, on each reagent lot separately, at the 95th percentile using the non-parametric method for all analyses. The higher LoB result between the two lots was selected for the final LoB value.

LoD:
To determine the LoD for each analyte, four low level samples were assayed in five replicates, twice per day for three days using two reagent lots, to generate a total of 120 data points for each assay on each reagent lot. The LoD was calculated as the LoB + 1.65 x SD of the replicates for the samples separately for each assay on each reagent lot and the highest LoD result was selected for the final LoD value.

LoQ:
To determine the LoQ for each analyte, four low level samples (different from LoD study) were run in five replicates, twice per day for three days using two reagent lots, to generate 120 data points for each assay on each reagent lot. The LoQ was determined separately for each assay on each reagent lot by calculating the total imprecision of each sample. The LoQ was defined as the lowest concentration level that meets the within-laboratory imprecision of <20% for each lot. The greatest LoQ across the two lots was set as the LoQ value and as the lower limit of the AMI for each assay.

The LoB, LoD, and LoQ for the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent are summarized in the following table:

	LoB (FLU)	**LoD (FLU)**	**LoQ (FLU)**
Aptiva APS IgG Reagent			
aCL IgG	0.00	0.07	0.29
aβ2GPI IgG	0.02	0.09	0.21
Aptiva APS IgM Reagent			
aCL IgM	0.01	0.04	0.10
aβ2GPI IgM	0.03	0.06	0.10

8. Assay Cut-Off:

The following cut-off is used for the aCL IgG, aβ2GPI IgG assays in the Aptiva APS IgG Reagent and aCL IgM and aβ2GPI IgM assays in the Aptiva APS IgM Reagent:

Positive: ≥5.00 FLU
Negative: <5.00 FLU

The cut-off was established based on greater than the 99th percentile of the results obtained on the reference healthy population.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Samples for the Aptiva APS IgG and Aptiva APS IgM method comparison analysis included serum samples from the clinical validation study (**see VII.C**) that display results within the analytical measuring range of the assay and the respective predicate device(s). Positive percent agreement (PPA), negative percent agreement (NPA), and total percent agreement

(TPA) with 95% confidence intervals (95%CI) were calculated for each analyte comparison, excluding values that were outside of the measuring ranges of either assay. The results are summarized in the following tables:

Aptiva APS IgG Reagent

Method comparison (aCL IgG vs. the predicate device)				
		HemosIL AcuStar Cardiolipin IgG (Predicate)		
		Positive	Negative	Total
Aptiva APS IgG Reagent (aCL IgG)	Positive (≥5.0 FLU)	31	7	38
	Negative (<5.0 FLU)	7	157	164
	Total	38	164	202
PPA: 81.6% (31/38) (95% CI: 66.6−90.8%) NPA: 95.7% (157/164) (95% CI: 91.5−97.9%) TPA: 93.1% (188/202) (95% CI: 88.7−95.8%)				

Method comparison (aβ2GPI IgG vs. the predicate device)				
		QUANTA Lite aβ2GPI IgG ELISA (Predicate)		
		Positive	Negative	Total
Aptiva APS IgG Reagent (aβ2GPI IgG)	Positive (≥5.0 FLU)	44	6	50
	Negative (<5.0 FLU)	6	52	58
	Total	50	58	108
PPA: 88.0% (44/50) (95% CI: 76.2−94.4%) NPA: 89.7% (52/58) (95% CI: 79.2−95.2%) TPA: 88.9% (96/108) (95% CI: 81.6−93.5%)				

Aptiva APS IgM Reagent

Method comparison (aCL IgM vs. the predicate device)				
		HemosIL AcuStar Cardiolipin IgM (Predicate)		
		Positive	Negative	Total
Aptiva APS IgM Reagent (aCL IgM)	Positive (≥5.0 FLU)	40	37	77
	Negative (<5.0 FLU)	6	339	345
	Total	46	376	422
PPA: 87.0% (40/46) (95% CI: 74.3−93.9%) NPA: 90.2% (339/376) (95% CI: 86.7−92.8%) TPA: 89.8% (379/422) (95% CI: 86.6−92.3%)				

Method comparison (aβ2GPI IgM vs. the predicate device)				
		HemosIL AcuStar anti-β2 Glycoprotein-I IgM (Predicate)		
		Positive	Negative	Total
Aptiva APS IgM Reagent (aβ2GPI IgM)	Positive (≥5.0 FLU)	24	34	58
	Negative (<5.0 FLU)	3	183	186
	Total	27	217	244
PPA: 88.9% (24/27) (95% CI: 71.9−96.1%) NPA: 84.3% (183/217) (95% CI: 78.9−88.6%) TPA: 84.8% (207/244) (95% CI: 79.9−88.8%)				

2. Matrix Comparison:

Not applicable. Only human serum specimens are intended use sample type for the Aptiva APS IgG Reagent and Aptiva APS IgM Reagent.

C Clinical Studies:

Aptiva APS IgG Reagent

The clinical validation study for the Aptiva APS IgG Reagent was performed using a cohort of samples including a total of 526 characterized serum samples. This study cohort included 60 samples from patients with primary antiphospholipid syndrome (pAPS), 62 samples from patients with secondary antiphospholipid syndrome (sAPS), and 404 samples from patients with various types of autoimmune and infectious diseases. All samples were tested with the Aptiva APS IgG Reagent according to the instruction for use. The clinical performance of the Aptiva APS IgG Reagent as an aid in the diagnosis of APS are summarized in the following tables:

Clinical performance (aCL IgG)				
		Clinical Diagnosis		
		APS	Non-APS	Total
Aptiva APS IgG Reagent (aCL IgG)	Positive (≥5.0 FLU)	66	2	68
	Negative (<5.0 FLU)	56	402	458
	Total	122	404	526
Clinical Sensitivity: 54.1% (66/122) (95% CI: 45.3−62.7%) Clinical Specificity: 99.5% (402/404) (95% CI: 98.2−99.9%)				

Clinical performance (aβ2GPI IgG)				
		Clinical Diagnosis		
		APS	Non-APS	Total
Aptiva APS IgG Reagent (aβ2GPI IgG)	Positive (≥5.0 FLU)	65	4	69
	Negative (<5.0 FLU)	57	400	457
	Total	122	404	526
Clinical Sensitivity: 53.3% (65/122) (95% CI: 44.5−61.94%) Clinical Specificity: 99.0% (400/404) (95% CI: 97.5−99.6%)				

Distribution of target and differential disease samples and antibody positive rates are shown in the table below:

Aptiva APS IgG Reagent					
Diagnostic Group	***N*=526**	**aCL IgG**		**aβ2GPI IgG**	
		n	**(%)**	***n***	**(%)**
Target diagnosis	**122**	**66**	**54.1%**	**65**	**53.3%**
pAPS	60	33	55.0%	32	53.3%
sAPS	62	33	53.2%	33	53.2%
Differential diagnostic controls	**404**	**2**	**0.5%**	**4**	**1.0%**
Infectious Disease	58	0	0.0%	0	0.0%
PREPI	37	1	2.7%	1	2.7%
SLE no APS	27	0	0.0%	0	0.0%
Systemic sclerosis	12	1	8.3%	1	8.3%
Crohn's Disease	29	0	0.0%	0	0.0%
Ulcerative Colitis (UC)	27	0	0.0%	0	0.0%
Rheumatoid Arthritis	21	0	0.0%	1	4.8%
Fetal Loss no APS	15	0	0.0%	0	0.0%
Thrombosis no APS	4	0	0.0%	0	0.0%
ANCA-associated vasculitis (AAV)	15	0	0.0%	0	0.0%
Autoimmune Thyroid	30	0	0.0%	0	0.0%
Celiac Disease (CD)	30	0	0.0%	0	0.0%
COVID-19 related thrombosis	20	0	0.0%	1	5.0%
Hematologic malignancies	16	0	0.0%	0	0.0%
Idiopathic thrombocytopenic purpura (ITP)	17	0	0.0%	0	0.0%
Solid tumor malignancies	16	0	0.0%	0	0.0%
Deep vein thrombosis	30	0	0.0%	0	0.0%

Aptiva APS IgM Reagent

The clinical validation study for the Aptiva APS IgM Reagent was performed using a cohort of samples including a total of 689 characterized serum samples. This study cohort included 219 samples from patients with primary antiphospholipid syndrome (pAPS), 72 samples from patients with secondary antiphospholipid syndrome (sAPS), and 398 samples from patients with various types of autoimmune and infectious diseases. All samples were tested with the Aptiva APS IgM Reagent according to the instruction for use. The clinical performance of the Aptiva APS IgM as an aid in the diagnosis of APS are summarized in the following tables:

Clinical performance (aCL IgM)				
		Clinical Diagnosis		
		APS	Non-APS	Total
Aptiva APS IgM Reagent (aCL IgM)	Positive (≥5.0 FLU)	80	10	90
	Negative (<5.0 FLU)	211	388	599
	Total	291	398	689
Clinical Sensitivity: 27.5% (80/291) (95% CI: 22.7−32.9%) Clinical Specificity: 97.5% (388/398) (95% CI: 95.4−98.6%)				

Clinical performance (aβ2GPI IgM)				
		Clinical Diagnosis		
		APS	Non-APS	Total
Aptiva APS IgM Reagent (aβ2GPI IgM)	Positive (≥5.0 FLU)	72	6	78
	Negative (<5.0 FLU)	219	392	611
	Total	291	398	689
Clinical Sensitivity: 24.7% (72/291) (95% CI: 20.1−30.0%) Clinical Specificity: 98.5% (392/398) (95% CI: 96.8−99.3%)				

Distribution of target and differential disease samples and antibody positive rates are shown in the table below:

Aptiva APS IgM Reagent					
Diagnostic Group	***N*=689**	**aCL IgM**		**aβ2GPI IgM**	
		n	**(%)**	***n***	**(%)**
Target diagnosis	**291**	**80**	**27.5%**	**72**	**24.7%**
pAPS	219	60	27.4%	52	23.7%
sAPS	72	20	27.8%	20	27.8%
Differential diagnosis controls	**398**	**10**	**2.5%**	**6**	**1.5%**
Infectious Disease	69	0	0.0%	0	0.0%
Myositis	20	1	5.0%	0	0.0%
Autoimmune Thyroiditis	35	3	8.6%	2	5.7%
Celiac Disease (CD)	50	0	0.0%	0	0.0%
Ulcerative Colitis (UC)	19	1	5.3%	1	5.3%
Rheumatoid Arthritis	13	1	7.7%	1	7.7%
Atopic Dermatitis	11	0	0.0%	0	0.0%
Fetal Loss no APS	27	0	0.0%	0	0.0%
Pre-Eclampsia	17	0	0.0%	0	0.0%
Thrombosis no APS	7	1	14.3%	1	14.3%
ANCA-associated vasculitis (AAV)	15	0	0.0%	0	0.0%
COVID-19 related thrombosis	20	1	5.0%	0	0.0%
Hematologic malignancies	16	0	0.0%	0	0.0%
Solid tumor malignancies	16	1	6.3%	1	6.3%
Idiopathic thrombocytopenic purpura (ITP)	17	0	0.0%	0	0.0%
Other disease controls	16	0	0.0%	0	0.0%
Deep vein thrombosis	30	1	3.3%	0	0.0%

D Clinical Cut-Off:

Refer to assay cut-off.

E Expected Values/Reference Range:

To determine the reference range of the Aptiva APS IgG and Aptiva APS IgM, a panel of 200 apparently healthy blood donors (106 females and 94 males, ages 18–70 years, and median age of 37 years) were tested following CLSI EP28-A3c. With the cut-off of 5.0 FLU, no samples tested positive for both the Aptiva APS IgG Reagent and the Aptiva APS IgM Reagent. The results are summarized in the following table:

Measurand	N	Min (FLU)	Max (FLU)	Medium (FLU)	97.5th Percentile (FLU)
Aptiva APS IgG Reagent					
aCL IgG	200	0.29	0.42	0.29	0.29
aβ2GPI IgG	200	0.21	0.47	0.21	0.21
Aptiva APS IgM Reagent					
aCL IgM	200	0.10	2.54	0.10	0.51
aβ2GPI IgM	200	0.10	3.11	0.10	0.47

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.