# 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
# DECISION SUMMARY
ASSAY ONLY

## I Background Information:

### A 510(k) Number

K243979

### B Applicant

Inova Diagnostics, inc.

### C Proprietary and Established Names

Aptiva APS IgA Reagent

### D Regulatory Information

| Product Code(s) | Classification | Regulation Section | Panel |
|---|---|---|---|
| MID<br>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:

Anti-cardiolipin IgA autoantibodies (aCL IgA)
Anti-beta 2 glycoprotein 1 IgA autoantibodies (aβ2GPI IgA)

### 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:

The Aptiva APS IgA 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) IgA autoantibodies in human serum as an aid in the diagnosis of primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.

The Aptiva APS IgA 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:

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

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

<u>Materials Required but Not Provided</u>

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

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

**B Principle of Operation:**

The Aptiva APS IgA reagent utilize particle based multi-analyte technology (PMAT) in a cartridge format and contains two different populations of particles; one particle population coated with cardiolipin, and another particle population coated with human purified β2GPI. The two analyte microparticles, along with the control microparticle, are stored in the reagent cartridge for the Aptiva instrument.

The Aptiva System dilutes the sample 1:8, then combines an aliquot of diluted sample, and reagent into a cuvette. The mixture is incubated at 37°C. After a wash cycle, conjugated anti-human IgA antibodies are added to the particles and this mixture is incubated 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 (2) unique analyte particles, as well as determine the amount of conjugate on each particle. A third particle, coated with goat anti-human IgA antibodies, is present in the reagent as a control to flag low concentrations of IgA 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 IgA, which is proportional to the concentration of IgA 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 IgA Reagent is assigned a predefined lot specific master curve. The analyte specific master curve is stored on the reagent cartridge 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):**

QUANTA Flash aCL IgA
QUANTA Flash β2GP1 IgA

**B Predicate 510(k) Number(s):**

K120817

**C Comparison with Predicate(s):**

| Device & Predicate Device(s): | K243979 (Candidate Device) | K120817 (Predicate Device) |
|---|---|---|
| Device Trade Name | Aptiva APS IgA Reagent | QUANTA Flash aCL IgA, QUANTA Flash β2GP1 IgA |
| **General Device Characteristic Similarities** | | |
| Intended Use/ Indications For Use | The Aptiva APS IgA Reagent is an immunoassay utilizing particle-based multi-analyte technology for the semi-quantitative determination of anti-cardiolipin (aCL) and anti-beta 2 glycoprotein I (aβ2GPI) IgA autoantibodies in human serum as an aid in the diagnosis of primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.<br>The Aptiva APS IgA Reagent is intended for use with the Aptiva System. | Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-cardiolipin (aCL)/anti-ß2 glycoprotein-I (β2GPI) IgA 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. |
| Type of Test | Semi-quantitative | Same |
| Quality Control | Two Control Levels | Same |
| **General Device Characteristic Differences** | | |
| Instrumentation | Aptiva System | BIO-FLASH instrument |
| Technology | Fluorescent immunoassay | Chemiluminescent immunoassay |
| Clinical Cut-off | 5.00 FLU | 20.0 CU |
| Analytical Measuring Interval (AMI) | 0.41 – 68.00 FLU (aCL IgA)<br>0.66 – 80.00 FLU (aβ2GPI IgA) | 1.4 – 351.6 CU (aCL IgA)<br>4.0 – 512 CU (β2GP1 IgA) |
| Calibrator | Three Calibrator Levels | Two Calibrator Levels |
| Conjugate | Phycoerythrin conjugated polyclonal anti-human IgA antibody | Isoluminol conjugated monoclonal anti-human IgA antibody |
| Sample Type | Serum | Serum or Citrated Plasma |

## 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 EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline – Second Edition.
- CLSI EP25-Ed2: Evaluation of Stability of In Vitro Medical Laboratory Test Reagents – Second Edition.
- 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 IgA Reagent was evaluated in accordance with the CLSI guideline EP05-A3.

*Within-Laboratory Precision:*

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

| **aCL IgA** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Sample** | **N** | **Mean (FLU)** | **Repeatability** | | **Between-Run** | | **Between-Day** | | **Within-Laboratory** | |
| | | | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** | **SD** | **%CV** |
| 1 | 80 | 1.49 | 0.07 | 5.0 | 0.05 | 3.3 | 0.08 | 5.6 | 0.12 | 8.2 |
| 2 | 80 | 3.56 | 0.19 | 5.5 | 0.05 | 1.4 | 0.23 | 6.5 | 0.31 | 8.6 |
| 3 | 80 | 5.50 | 0.24 | 4.3 | 0.12 | 2.2 | 0.32 | 5.8 | 0.42 | 7.6 |
| 4 | 80 | 11.64 | 0.30 | 2.6 | 0.27 | 2.3 | 0.51 | 4.4 | 0.66 | 5.6 |
| 5 | 80 | 24.94 | 0.93 | 3.7 | 1.26 | 5.1 | 1.15 | 4.6 | 1.94 | 7.8 |
| 6 | 80 | 45.39 | 1.32 | 2.9 | 1.50 | 3.3 | 2.16 | 4.8 | 2.94 | 6.5 |

| aβ2GPI IgA | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | N | Mean (FLU) | Repeatability | | Between-Run | | Between-Day | | Within-Laboratory | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 80 | 2.05 | 0.14 | 6.7 | 0.14 | 6.9 | 0.12 | 5.7 | 0.23 | 11.2 |
| 2 | 80 | 4.82 | 0.30 | 6.2 | 0.13 | 2.7 | 0.45 | 9.3 | 0.55 | 11.5 |
| 3 | 80 | 7.43 | 0.35 | 4.7 | 0.22 | 2.9 | 0.68 | 9.2 | 0.80 | 10.7 |
| 4 | 80 | 15.58 | 0.49 | 3.2 | 0.42 | 2.7 | 0.83 | 5.3 | 1.05 | 6.7 |
| 5 | 80 | 28.44 | 1.08 | 3.8 | 1.23 | 4.3 | 1.87 | 6.6 | 2.49 | 8.7 |
| 6 | 80 | 56.85 | 1.49 | 2.6 | 2.06 | 3.6 | 3.12 | 5.5 | 4.03 | 7.1 |

*Lot-to-Lot Imprecision:*

The lot-to-lot imprecision of the Aptiva APS IgA Reagent was evaluated by testing five serum samples for aCL IgA and aβ2GPI IgA with three lots of the Aptiva APS IgA 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.

| aCL IgA | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | N | Mean (FLU) | Within-Day | | Between-Day | | Between-Lot | | Total | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 75 | 1.97 | 0.17 | 8.4 | 0.09 | 4.6 | 0.02 | 1.0 | 0.19 | 9.6 |
| 2 | 75 | 5.01 | 0.33 | 6.6 | 0.22 | 4.4 | 0.00 | 0.0 | 0.40 | 7.9 |
| 3 | 75 | 5.09 | 0.22 | 4.4 | 0.15 | 2.8 | 0.05 | 0.9 | 0.27 | 5.3 |
| 4 | 75 | 17.42 | 0.90 | 5.2 | 0.39 | 2.2 | 0.42 | 2.4 | 1.07 | 6.1 |
| 5 | 75 | 50.12 | 1.67 | 3.3 | 0.85 | 1.7 | 4.01 | 8.0 | 4.43 | 8.8 |

| aβ2GPI IgA | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | N | Mean (FLU) | Within-Day | | Between-Day | | Between-Lot | | Total | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 75 | 2.12 | 0.19 | 9.0 | 0.12 | 5.5 | 0.11 | 5.1 | 0.25 | 11.7 |
| 2 | 75 | 5.53 | 0.29 | 5.3 | 0.22 | 4.0 | 0.34 | 6.1 | 0.50 | 9.0 |
| 3 | 75 | 5.63 | 0.28 | 4.9 | 0.10 | 1.8 | 0.25 | 4.5 | 0.39 | 6.9 |
| 4 | 75 | 26.53 | 1.21 | 4.6 | 0.61 | 2.3 | 1.15 | 4.3 | 1.78 | 6.7 |
| 5 | 75 | 60.69 | 1.95 | 3.2 | 3.63 | 6.0 | 2.99 | 4.9 | 5.09 | 8.4 |

*Site-to-Site Precision (Reproducibility):*

The reproducibility of the Aptiva APS IgA Reagent was conducted at three different sites/instruments using seven serum samples for aCL IgA and aβ2GPI IgA. 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.

| aCL IgA | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | N | Mean (FLU) | Within-Day | | Between-Day | | Between-Site | | Total | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 75 | 2.21 | 0.11 | 5.1 | 0.04 | 1.7 | 0.13 | 5.8 | 0.18 | 7.9 |
| 2 | 75 | 4.85 | 0.19 | 3.8 | 0.15 | 3.2 | 0.24 | 5.0 | 0.34 | 7.1 |
| 3 | 75 | 5.45 | 0.23 | 4.3 | 0.19 | 3.5 | 0.28 | 5.1 | 0.41 | 7.6 |
| 4 | 75 | 7.84 | 0.35 | 4.4 | 0.24 | 3.1 | 0.50 | 6.3 | 0.65 | 8.3 |
| 5 | 75 | 16.51 | 0.56 | 3.4 | 0.60 | 3.6 | 1.39 | 8.4 | 1.61 | 9.8 |
| 6 | 75 | 37.16 | 1.27 | 3.4 | 0.97 | 2.6 | 3.91 | 10.5 | 4.23 | 11.4 |
| 7 | 75 | 54.80 | 1.95 | 3.6 | 0.91 | 1.7 | 2.06 | 3.8 | 2.98 | 5.4 |

| aβ2GPI IgA | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | N | Mean (FLU) | Within-Day | | Between-Day | | Between-Site | | Total | |
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1 | 75 | 2.50 | 0.13 | 5.1 | 0.08 | 3.2 | 0.16 | 6.5 | 0.22 | 8.8 |
| 2 | 75 | 5.10 | 0.20 | 3.9 | 0.17 | 3.4 | 0.30 | 5.9 | 0.40 | 7.8 |
| 3 | 75 | 6.41 | 0.24 | 3.7 | 0.22 | 3.4 | 0.41 | 6.4 | 0.52 | 8.2 |
| 4 | 75 | 9.36 | 0.42 | 4.5 | 0.17 | 1.8 | 0.65 | 7.0 | 0.80 | 8.5 |
| 5 | 75 | 19.77 | 0.56 | 2.8 | 0.65 | 3.3 | 1.63 | 8.3 | 1.84 | 9.3 |
| 6 | 75 | 35.86 | 1.06 | 2.9 | 1.06 | 2.9 | 3.13 | 8.7 | 3.47 | 9.7 |
| 7 | 75 | 64.91 | 1.95 | 3.0 | 2.98 | 4.6 | 1.91 | 2.9 | 4.04 | 6.2 |

2. Linearity:

The linearity of the Aptiva APS IgA Reagent according to CLSI EP06-Ed2.

For the Aptiva APS IgA Reagent, multiple dilution series were prepared to cover the AMI of each of the aCL IgA and aβ2GPI IgA assays by diluting human serum samples with various antibody concentrations in human negative serum. Four unique specimens were used for aCL IgA and three for aβ2GPI IgA, with dilutions made in 10% increments that overlapped other samples' dilution series. Each sample was tested in duplicate using two lots of the Aptiva APS IgA Reagent on the Aptiva Multi-Analyte Instrument. The overall testing range for the linearity study was from 0.41–301.63 FLU for the aCL IgA assay, and 0.57–81.66 FLU for the aβ2GPI IgA assay. The percent deviation from the weighted least squares regression analysis was used to assess the fit of the regression for each sample and analyte.

| aCL IgA | | | | | |
|---|---|---|---|---|---|
| Sample | Test Range (FLU) | Slope (95% CI) | $R^2$ | % Deviation from Linearity | FLU of Deviation from Linearity# |
| 1 | 30.16–301.63 | 1.07 (1.03–1.12) | 0.99 | -6.8% to 10.4%* | N/A |
| 2 | 5.51–55.11 | 1.00 (0.97–1.03) | 1.00 | -7.9% to 5.0% | N/A |
| 3 | 2.72–27.20 | 1.02 (1.00–1.03) | 1.00 | -1.8% to 6.6% | 0.48 FLU |
| 4 | 0.41–4.05 | 1.00 (0.95–1.05) | 1.00 | -14.1%** to 6.4% | -0.19 to -0.15 FLU |
| Combined | 301.63 – 0.41 | 0.93 (0.91 – 0.95) | 1.00 | -14.1% to 10.4% | -0.19 to 0.48 FLU |

# For sample below 5 FLU.

* % Deviation from linearity of 10.4% for sample level with aCL IgA concentration of 60.33 FLU

** % Deviation from linearity of -14.1% for sample level with aCL IgA concentration of 1.62 FLU

| aβ2GPI IgA | | | | | |
|---|---|---|---|---|---|
| **Sample** | **Test Range (FLU)** | **Slope (95% CI)** | **$R^2$** | **% Deviation from Linearity** | **FLU of Deviation from Linearity#** |
| 1 | 1.26–12.57 | 0.94 (0.90–0.98) | 0.99 | -11.7%* to 6.7% | -0.48 FLU |
| 2 | 8.17–81.66 | 1.00 (0.97–1.03) | 1.00 | -13.9%** to 5.1% | N/A |
| 3 | 0.57–5.71 | 0.99 (0.94–1.04) | 0.99 | -4.2% to 6.1% | -0.44 to -0.18 FLU |
| Combined | 81.66 – 0.57 | 0.99 (0.97 – 1.00) | 1.00 | -13.9% to 6.7% | -0.48 to -0.18 FLU |

# For sample below 5 FLU.

* % Deviation from linearity of -11.7% for sample level with aβ2GPI IgA concentration of 3.77 FLU

** % Deviation from linearity of -13.9% for sample level with aβ2GPI IgA concentration of 16.33 FLU

The data summarized in the tables below support the linearity of AMI of 0.41–68.00 FLU for the aCL IgA assay and the AMI of 0.66–80.00 FLU for the aβ2GPI IgA assay, as part of the Aptiva APS IgA Reagent.

3. Hook effect

The hook effect for Aptiva APS IgA Reagent on the Aptiva System was evaluated by testing high positive serum samples for each device: three high aCL IgA samples and three high aβ2GPI IgA samples for the Aptiva APS IgA Reagent. No antigen excess hook effect was observed up to 98.19 FLU for aCL IgA and 133.48 FLU for aβ2GPI IgA.

4. Analytical Specificity/Interference:

An interference study was performed according to CLSI EP07-A3 and CLSI EP37-A1 for the Aptiva APS IgA Reagent. A set of human serum specimens—one positive, one around the cut-off (±25%), 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 IgA Reagent for both aCL IgA and aβ2GPI IgA analytes. 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 table displays the highest concentration at which no interference was observed for both analytes in the Aptiva APS IgA Reagent assay.

| Aptiva APS IgA Reagent (aCL IgA / aβ2GPI IgA) | |
|---|---|
| **Endogenous Interfering Substances** | **Concentration without Interference** |
| Bilirubin, Conjugated | 100.0 mg/dL |
| Hemoglobin | 10.0 g/L |
| Triglyceride | 1000.0 mg/dL |
| Cholesterol | 332.5 mg/dL |
| RF IgM | 196.5 IU/mL |
| Human IgG | 2000.0 mg/dL |

| Aptiva APS IgA Reagent (aCL IgA / aβ2GPI IgA) | |
|---|---|
| **Exogenous Interfering Substances** | **Concentration without Interference** |
| Ibuprofen | 21.9 mg/dL |
| Warfarin | 7.5 mg/dL |
| Prednisone | 0.0099 mg/dL |
| Acetaminophen | 15.6 mg/dL |
| Aspirin | 3.0 mg/dL |
| Hydroxychloroquine | 0.465 mg/dL |
| Omeprazole | 0.84 mg/dL |
| Simvastatin | 0.168 mg/dL |
| Heparin | 330.0 units/dL |

5. Assay Reportable Range:

The reportable range for the Aptiva APS IgA Reagent is the same as the analytical measuring interval (AMI) for both measurands. The AMI for each measurand is shown in the table below:

| Aptiva APG IgA Reagent | Analytical Measuring Interval |
|---|---|
| aCL IgA | 0.41 – 68.00 FLU |
| aβ2GPI IgA | 0.66 – 80.00 FLU |

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

*Traceability:*

There are no international reference materials 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 analyte of the Aptiva APS IgA Reagent.

*Stability:*

*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 IgA Reagent. The accelerated study was performed by testing three reagent lots with incubation at 37°C and 47°C. Five serum samples spanning the analytical measuring range for both aCL IgA and aβ2GPI IgA analytes were tested at multiple timepoints. The collected data showed the initial shelf-life of 11 months for the Aptiva APS IgA Reagent when stored at 2–8°C.

*Reagent in-use (on-board) stability:* An in-use stability study for the Aptiva APS IgA Reagent was conducted by testing multiple samples with different assay levels at different time points using one lot of reagent stored on board. The in-use (on-board) stability of the Aptiva APS IgA Reagent was set at 28 days, with a 14-day recalibration.

*Reagent shipping stability:* A transport simulation study was performed to assess the shipping stability of the Aptiva APS IgA Reagent kits during transport. The study was performed under simulated conditions, including high-temperature exposure and thermal

cycling, that the products may potentially be exposed to during transport. The collected data support that shipping conditions at 2–8°C mitigate the potential impact of prolonged or repeated temperature stressing during transport.

*Sample stability:* Serum samples with different antibody levels were stored at various storage temperatures and tested with the Aptiva APS IgA 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 four freeze/thaw cycles when stored at -20°C or lower.

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 IgA 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 analyte, on each reagent lot separately, at the 95th percentile using the non-parametric method for all analyses.

LoD: To determine the LoD for each analyte, five low level samples were assayed in five replicates, twice per day for three days using two reagent lots, to generate a total of 150 data points for each analyte on each reagent lot. The LoD was calculated using the equation $LoD = LoB + SD_L \times c_p$, where $SD_L$ is the pooled standard deviation of the low-level samples and $c_p$ is a multiplier for the 95th percentile.

LoQ: To determine the LoQ for each analyte, five low level samples were run in five replicates, twice per day for three days using two reagent lots, to generate 150 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 claimed LoB, LoD, and LoQ for the Aptiva APS IgA Reagent are summarized in the following table:

| | **LoB (FLU)** | **LoD (FLU)** | **LoQ (FLU)** |
|---|---|---|---|
| **Aptiva APS IgA Reagent** | | | |
| aCL IgA | 0.06 | 0.11 | 0.41 |
| aβ2GPI IgA | 0.38 | 0.48 | 0.66 |

8. Assay Cut-Off:

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

Positive: ≥5.00 FLU
Negative: <5.00 FLU

## B Comparison Studies:

1. Method Comparison with Predicate Device:

The samples for the Aptiva APS IgA method comparison analysis were serum samples from the clinical validation study (see VII.C) and additional samples known to be around the cut-off that were within the analytical measuring interval of the assay and the respective predicate devices (QUANTA Flash aCL IgA and QUANTA Flash β2GP1 IgA). 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:

| **aCL IgA** | | | | |
|---|---|---|---|---|
| | | **QUANTA Flash aCL IgA** | | |
| | | Positive | Negative | Total |
| **Aptiva APS IgA Reagent (aCL IgA)** | Positive | 31 | 6 | 37 |
| | Negative | 4 | 205 | 209 |
| | Total | 35 | 211 | 246 |
| PPA: 88.6% (31/35) (95% CI: 74.0−95.5%)<br>NPA: 97.2% (205/211) (95% CI: 93.9−98.7%)<br>TPA: 95.9% (236/246) (95% CI: 92.7−97.8%) | | | | |

| **aB2GPI IgA** | | | | |
|---|---|---|---|---|
| | | **QUANTA Flash aCL IgA** | | |
| | | Positive | Negative | Total |
| **Aptiva APS IgA Reagent (aB2GPI IgA)** | Positive | 37 | 9 | 46 |
| | Negative | 3 | 51 | 54 |
| | Total | 40 | 60 | 100 |
| PPA: 92.5% (37/40) (95% CI: 80.1−97.4%)<br>NPA: 85.0% (51/60) (95% CI: 73.0−91.9%)<br>TPA: 88.0% (88/100) (95% CI: 80.2−93.0%) | | | | |

2. Matrix Comparison:

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

## C Clinical Studies:

1. Clinical Sensitivity and Specificity:

The clinical validation study for the Aptiva APS IgA Reagent involved 615 characterized serum samples. This cohort included 70 samples from patients with primary antiphospholipid syndrome (pAPS), 83 samples from patients with secondary antiphospholipid syndrome (sAPS), and 462 samples from patients with various autoimmune and infectious diseases that could be considered in the differential diagnosis of APS. All samples were tested using the Aptiva APS IgA Reagent and the clinical performance of the Aptiva APS IgA Reagent, as an aid in the diagnosis of APS, is summarized in the following tables.

| aCL IgA | | | | |
|---|---|---|---|---|
| | | Clinical Diagnosis | | |
| | | APS | Non-APS | Total |
| **Aptiva APS IgA Reagent (aCL IgA)** | Positive (≥5.0 FLU) | 48 | 10 | 58 |
| | Negative (<5.0 FLU) | 105 | 452 | 557 |
| | Total | 153 | 462 | 615 |
| Clinical Sensitivity: 31.4% (48/153) (95% CI: 24.6−39.1%)<br>Clinical Specificity: 97.8% (452/462) (95% CI: 96.1−98.8%) | | | | |

| aβ2GPI IgA | | | | |
|---|---|---|---|---|
| | | Clinical Diagnosis | | |
| | | APS | Non-APS | Total |
| **Aptiva APS IgA Reagent (aβ2GPI IgA)** | Positive (≥5.0 FLU) | 49 | 7 | 56 |
| | Negative (<5.0 FLU) | 104 | 455 | 559 |
| | Total | 153 | 462 | 615 |
| Clinical Sensitivity: 32.0% (49/153) (95% CI: 25.2−39.8%)<br>Clinical Specificity: 98.5% (455/462) (95% CI: 96.9−99.3%) | | | | |

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

| Aptiva APS IgA Reagent | | | | | |
|---|---|---|---|---|---|
| **Diagnostic Group** | ***N*=615** | **aCL IgA** | | **aβ2GPI IgA** | |
| | | ***n*** | **(%)** | ***n*** | **(%)** |
| **Target diagnosis** | **153** | **48** | **31.4%** | **49** | **32.0%** |
| pAPS | 70 | 14 | 20.0% | 17 | 24.3% |
| sAPS | 83 | 34 | 41.0% | 32 | 38.6% |
| **Differential diagnostic controls** | **462** | **10** | **2.2%** | **7** | **1.5%** |
| Viral Infectious Disease | 53 | 2 | 3.8% | 0 | 0.0% |
| Bacterial Infectious Disease | 45 | 2 | 4.4% | 0 | 0.0% |
| Myositis | 24 | 0 | 0.0% | 0 | 0.0% |
| Preeclampsia or Placental Insufficiency (PREPI) | 36 | 0 | 0.0% | 0 | 0.0% |
| Systemic Lupus Erythematosus (without APS) | 34 | 1 | 2.9% | 1 | 2.9% |
| Inflammatory Bowel Disease (IBD) | 19 | 0 | 0.0% | 0 | 0.0% |
| Rheumatoid Arthritis (RA) | 20 | 0 | 0.0% | 0 | 0.0% |
| Fetal loss (without APS) | 13 | 0 | 0.0% | 0 | 0.0% |
| Thrombosis (without APS) | 1 | 0 | 0.0% | 0 | 0.0% |
| ANCA-Associated Vasculitis | 18 | 0 | 0.0% | 0 | 0.0% |
| Autoimmune Thyroiditis | 49 | 1 | 2.0% | 2 | 4.1% |
| Celiac Disease (CeD) | 44 | 1 | 2.3% | 0 | 0.0% |
| COVID-19 Related Thrombosis | 22 | 0 | 0.0% | 0 | 0.0% |
| Hematologic Malignancies | 20 | 1 | 5.0% | 1 | 5.0% |
| Idiopathic thrombocytopenic purpura (ITP) | 14 | 1 | 7.1% | 1 | 7.1% |
| Solid Tumor Malignancies | 20 | 0 | 0.0% | 1 | 5.0% |
| Deep Vein Thrombosis | 18 | 1 | 5.6% | 1 | 5.6% |
| Myocardial Infarction (MI) | 12 | 0 | 0.0% | 0 | 0.0% |

2. Comparison of the Aptiva APS IgA and Predicates

Clinical Performance of the Aptiva APS IgA was compared with the predicates using the same samples for aCL IgA (n=332) and aβ2GPI IgA (n=362). The clinical sensitivity and specificity are summarized in the following table.

| Assay | n= | Sensitivity | Specificity |
|---|---|---|---|
| **aCL IgA** | | | |
| Aptiva APS IgA | 332 | 25.4% (18.3 - 34.1%) | 97.2% (94.1 - 98.7%) |
| QUANTA Flash aCL IgA | | 26.3% (19.1 - 35.1%) | 98.6% (96.0 - 99.5%) |
| **aβ2GPI IgA** | | | |
| Aptiva APS IgA | 362 | 33.8% (26.3 - 42.2% | 97.4% (94.4 - 98.8%) |
| QUANTA Flash β2GP1 IgA | | 30.8% (23.6 - 39.1%) | 98.3% (95.6 - 99.3%) |

**D Clinical Cut-Off:**

Refer to Assay Cut-off

**E Expected Values/Reference Range:**

To determine the reference range of the Aptiva APS IgA, a panel of 126 apparently healthy blood donors (ages 18–70 years, with a mean age of 38 years and median age of 35 years) were tested and analyzed following CLSI C28-A3c. For Aptiva APS IgA aCL IgA, with a cut-off of 5.00 FLU, two samples (1.6%) were positive. For aβ2GPI IgA, with a cut-off of 5.00 FLU, no samples were positive. The results are summarized in the following table:

| | N | Min (FLU) | Max (FLU) | Median (FLU) | 97.5$^{th}$ Percentile (FLU) |
|---|---|---|---|---|---|
| aCL IgA | 126 | 0.41 | 9.13 | 0.41 | 2.83 |
| aβ2GPI IgA | 126 | 0.66 | 1.52 | 0.66 | 0.77 |

## 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.