510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K213403

B Applicant

Inova Diagnostics, Inc.

C Proprietary and Established Names

Aptiva CTD Essential Reagent

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LJM, LKO, LKP, LLL, LSW, MQA, OBE	Class II	21 CFR 866.5100 - Antinuclear antibody immunological test system	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Human anti-dsDNA, anti-RNP, anti-Sm, anti-Ro60, anti-Ro52, anti-SS-B, anti-Scl-70, anti-Jo-1, anti-Centromere, and anti-Ribo-P IgG autoantibodies.

For ease of reference, the aforementioned autoantibody measurands are referred to in this decision summary by their cognate autoantigens.

Analyte	Description	Abbreviated Term	Other Names
Anti-dsDNA	Anti-double-stranded deoxyribonucleic acid IgG autoantibodies	dsDNA	
Anti-RNP	Anti-ribonucleoprotein IgG autoantibodies	RNP	Anti-U1RNP
Anti-Sm	Anti-Smith antigen IgG autoantibodies	Sm	Anti-Smith
Anti-Ro60	Anti-Ro 60kDa subunit autoantibodies	Ro60	Anti-SS-A2 Anti-SSA (Anti-Sjögren's Syndrome A)
Anti-Ro52	Anti-Ro 52kDa subunit autoantibodies	Ro52	Anti-SSA (Anti-Sjögren's Syndrome A)
Anti-SS-B	Anti-SS-B (Anti-Sjögren's Syndrome B) autoantibodies	SS-B	Anti-La
Anti-Scl-70	Anti-Scl 70 Antigen autoantibodies	Scl-70	Anti-DNA Topoisomerase 1
Anti-Jo-1	Anti-Jo 1 Antigen autoantibodies	Jo-1	Anti-Histidyl-tRNA Synthetase
Anti-Centromere	Anti-Centromere autoantibodies	Centromere	
Anti-Ribo-P	Anti-ribosomal P body IgG autoantibodies	Ribo-P	

C Type of Test:

Quantitative, 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 CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum:

- The presence of dsDNA antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus.

- The presence of RNP antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of mixed connective tissue disease and systemic lupus erythematosus.
- The presence of Sm antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus.
- The presence of Ro52 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, and idiopathic inflammatory myositis.
- The presence of Ro60 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus and Sjögren's syndrome.
- The presence of SS-B antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus and Sjögren's syndrome.
- The presence of Scl-70 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic sclerosis.
- The presence of Jo-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myositis.
- The presence of centromere antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic sclerosis.
- The presence of Ribo-P antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus.

The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

Inova Diagnostics Aptiva System

IV Device/System Characteristics:

A Device Description:

The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays that utilize particle-based multi-analyte technology (PMAT) in a cartridge format. Each Aptiva CTD Essential reagent kit contains the following reagents for 250 determinations:

- dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo-1, Centromere, Ribo-P and Control paramagnetic particles, preserved.
- Assay Buffer – colored pink, containing protein stabilizers and preservatives.
- PE Tracer IgG – PE labeled anti-human IgG antibody, containing buffer, protein stabilizers and preservative.
- Rehydration Buffer - containing protein stabilizers and preservatives.

The Aptiva CTD Essential Calibrators and Aptiva CTD Essential Controls are sold separately.

- The Aptiva CTD Essential Calibrators includes six calibrators. The calibrators contain human antibodies to dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo-1, Centromere, and Ribo-P in stabilizers and preservatives.
- The Aptiva CTD Essential Controls includes two controls. The controls contain human antibodies to dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo-1, Centromere, and Ribo-P in stabilizers and preservatives.

B Principle of Operation:

The Aptiva CTD Essential reagent utilizes particle based multi-analyte technology (PMAT). Ten unique populations of microparticles coated with dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo- 1, Centromere or Ribo-P autoantigens, along with one for the control microparticle, are stored in the reagent cartridge under conditions that preserve the autoantigens in their reactive states. When the assay cartridge is ready to be used, the reagent tube seals are pierced, and the reagent cartridge is then loaded onto the Aptiva Multi-Analyte Instrument.

The Aptiva System dilutes the patient sample 1:44.4 fold with Aptiva system rinse by the instrument in a disposable cuvette and a small amount of the diluted sample is combined with reagent into a second cuvette. The mixture is incubated at 37°C. After a series of wash cycles, phycoerythrin-conjugated polyclonal anti-human IgG (known as PE Tracer IgG) is 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 Aptiva system fluid.

Following the wash steps, the microparticles are transferred to the optical module of the instrument, where a charge coupled device (CCD) camera takes multiple images to identify and count the microparticle regions and determine the amount of conjugate on the microparticles. A control microparticle coated with goat anti-human IgG is present in the reagent as a control to flag low concentrations of IgG in the patient serum sample as an assay verification step. The median fluorescent intensity (MFI) is proportional to the amount of PE tracer that is bound to the human IgG, which is proportional to the amount of IgG antibodies bound to the corresponding microparticle regions.

For quantitation, each of the 10 assays in the Aptiva CTD Essential Reagent utilizes a predefined lot specific master curve that is uploaded onto the instrument through the RFID tag on the reagent cartridge. The first time a reagent cartridge of a new lot of Aptiva CTD Essential is placed in the instrument, it must be calibrated. The calibration process utilizes the six calibrators that are included in the calibrators kit to adjust the predefined lot specific dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo-1, Centromere and Ribo-P master curves into instrument specific working curves. These working curves are used to calculate Fluorescent Light Units (FLU) or IU/mL for dsDNA values from the measured MFI. The working curves are lot and instrument specific and stored in the system for use with any reagent cartridge from that lot.

Based on the defined cut-off value for each analyte, the test results are reported for each sample as “positive” or “negative” with a test value in FLU for the semi-quantitative assays or in IU/mL for quantitative dsDNA assay.

V Substantial Equivalence Information:

A Predicate Device Names and 510k Numbers

Predicate Name	510(k) Number
QUANTA Flash dsDNA	K152013
QUANTA Flash RNP	K123593
Orgentec Sm ELISA	K954830
QUANTA Flash Ro52	K141655
QUANTA Flash Ro60	K141328
QUANTA Flash SS-B	K141210
QUANTA Flash Scl-70	K152635
QUANTA Flash Jo-1	K151429
QUANTA Flash Centromere	K123880
QUANTA Lite Ribo-P	K981237

B Comparison with Predicate(s):

Device & Predicate Device(s):	K213403 (Device)	K152013 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (dsDNA)	QUANTA Flash dsDNA
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of dsDNA antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	QUANTA Flash dsDNA is a chemiluminescent immunoassay for the quantitative determination of IgG anti-double stranded deoxyribonucleic acid (dsDNA) antibodies in human serum. The presence of anti-dsDNA antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus.
Assay Methodology	Solid phase immunoassay	Same

Device & Predicate Device(s):	K213403 (Device)	K152013 (Predicate)
Antigen	Synthetic DNA	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Units	International Units (IU/mL)	Same
Cut-off	27.00 IU/mL – 35.00 IU/mL	Same
Controls	Two dsDNA controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Analytical Measuring Range	2.30 IU/mL – 814.10 IU/mL	9.8 IU/mL – 666.9 IU/mL
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K123593 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (RNP)	QUANTA Flash RNP
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of RNP antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of mixed connective tissue disease and systemic lupus erythematosus. … The individual assays included in the Aptiva CTD Essential Reagent	The QUANTA Flash RNP is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-ribonucleoprotein (RNP) antibodies in human serum. The presence of anti-RNP antibodies, in conjunction with clinical findings and other laboratory tests, can aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and Mixed Connective Tissue Disease (MCTD).

Device & Predicate Device(s):	K213403 (Device)	K123593 (Predicate)
	are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Native RNP	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two RNP controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.50 FLU – 181.99 FLU	3.5 CU – 643.8 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K954830 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Sm)	Orgentec anti-Sm
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of Sm antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus. … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova	Anti-Sm is an ELISA test system for the quantitative measurement of IgG class autoantibodies against Sm in human serum or plasma. This product is intended for professional in vitro diagnostic use only. The detection of autoantibodies against Sm proteins is a component of the multi-parametric ACR criteria for the diagnosis of systemic lupus erythematosus (SLE). The detection of Sm antibodies serves as a prognostic marker for SLE, there is a relationship between the appearance of Sm antibodies and severe organ manifestations of the disease. Evaluation of a test result should always take into account all clinical and

Device & Predicate Device(s):	K213403 (Device)	K954830 (Predicate)
	Diagnostics Aptiva System.	laboratory diagnostic findings.
Assay Methodology	Solid phase immunoassay	Same
Antigen	Synthetic Sm peptide	Same
Controls	Two Sm controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chromogenic immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	HRP conjugated anti-human IgG antibody
Sample Matrix	Human serum	Human Serum or Plasma
Solid Phase	Paramagnetic microparticles	ELISA
Units	Fluorescent light units (FLU)	Units (U/mL)
Cut-off	5.00 FLU	25.00 U/mL
Analytical Measuring Range	0.25 FLU – 256.00 FLU	1 U/mL – 200.0 U/mL
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Calibration curve using 6 calibrators (included)

Device & Predicate Device(s):	K213403 (Device)	K141655 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Ro52)	QUANTA Flash Ro52
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of Ro52 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus, Sjögren's syndrome, systemic sclerosis, and idiopathic inflammatory myositis. … The individual assays included in the Aptiva CTD Essential Reagent	QUANTA Flash Ro52 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro52 autoantibodies in human serum. The presence of anti-Ro52 autoantibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of Systemic Lupus Erythematosus, Sjögren's Syndrome, Systemic Sclerosis, Idiopathic Inflammatory Myopathies.

Device & Predicate Device(s):	K213403 (Device)	K141655 (Predicate)
	are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Recombinant Ro52	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two Ro52 controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.25 FLU – 196.27 FLU	2.3 CU – 1685.3 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K141328 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Ro60)	QUANTA Flash Ro60
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of Ro60 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus and Sjögren's syndrome. ….	QUANTA Flash Ro60 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Ro60 autoantibodies in human serum. The presence of anti-Ro60 autoantibodies, in conjunction with clinical findings and other laboratory tests, aids in the diagnosis of Systemic Lupus Erythematosus and Sjögren's Syndrome.

Device & Predicate Device(s):	K213403 (Device)	K141328 (Predicate)
	The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Recombinant Ro60	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two Ro60 controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.5 FLU – 583.72 FLU	4.9 CU – 1374.8 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K141210 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (SS-B)	QUANTA Flash SS-B
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, Centromere, and Ribo-P in human serum: … • The presence of SS-B antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic lupus erythematosus and Sjögren's	QUANTA Flash SS-B is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-SS-B autoantibodies in human serum. The presence of anti-SS-B autoantibodies, in conjunction with clinical findings and other laboratory tests is an aid in the diagnosis of Sjögren's Syndrome and Systemic Lupus Erythematosus.

Device & Predicate Device(s):	K213403 (Device)	K141210 (Predicate)
	syndrome. … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Recombinant SS-B	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two SS-B controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.40 FLU – 195.84 FLU	3.3 CU – 1550.0 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K152635 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Scl-70)	QUANTA Flash Scl-70
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of Scl-70 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of systemic	QUANTA Flash Scl-70 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Scl-70 autoantibodies in human serum. The presence of anti-Scl-70 autoantibodies, in conjunction with clinical findings and other laboratory tests, aids in the diagnosis of systemic sclerosis.

Device & Predicate Device(s):	K213403 (Device)	K152635 (Predicate)
	sclerosis. … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Recombinant Scl-70	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two Scl-70 controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.50 FLU – 371.24 FLU	1.2 CU – 786.3 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K151429 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Jo-1)	QUANTA Flash Jo-1
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of Jo-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic	QUANTA Flash Jo-1 is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-Jo-1 antibodies in human serum. The presence of anti-Jo-1 antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the diagnosis of idiopathic inflammatory myopathy.

Device & Predicate Device(s):	K213403 (Device)	K151429 (Predicate)
	inflammatory myositis. … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Recombinant Jo-1	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two Jo-1 controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.25 FLU – 153.60 FLU	2.2 CU – 1147.2 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K123880 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Centromere)	QUANTA Flash Centromere
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of centromere antibodies, in conjunction with clinical findings and other laboratory tests, is an aid in the	QUANTA Flash Centromere is a chemiluminescent immunoassay for the semi-quantitative determination of IgG anti-centromere protein B autoantibodies in human serum. The presence of anti-centromere protein B autoantibodies is used as an aid in the diagnosis of systemic sclerosis, in conjunction with clinical finding and other laboratory tests.

Device & Predicate Device(s):	K213403 (Device)	K123880 (Predicate)
	diagnosis of systemic sclerosis. … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Recombinant centromere	Same
Sample Matrix	Human serum	Same
Solid Phase	Paramagnetic microparticles	Same
Controls	Two Centromere controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Detection Principle	Fluorescent immunoassay	Chemiluminescent immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	Isoluminol conjugated monoclonal anti-human IgG antibody
Units	Fluorescent light units (FLU)	Chemiluminescent units (CU)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.50 FLU – 187.69 FLU	3.4 CU – 708.9 CU
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Lot specific Master Curve + 2 calibrators (sold separately)

Device & Predicate Device(s):	K213403 (Device)	K981237 (Predicate)
Device Trade Name	Aptiva CTD Essential Reagent (Ribo-P)	QUANTA Lite Ribosomal P
General Device Characteristic Similarities		
Intended Use/ Indications For Use	The Aptiva CTD Essential Reagent consists of 10 multiplexed immunoassays utilizing particle-based multi-analyte technology for the quantitative determination of IgG autoantibodies against dsDNA, and semi-quantitative determination of IgG autoantibodies against RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, centromere, and Ribo-P in human serum: … • The presence of Ribo-P antibodies, in conjunction with clinical findings and other laboratory tests, is an aid	QUANTA Lite Ribosome P is an enzyme-linked immunosorbent assay (ELISA) for the semi-quantitative detection of Ribosome P antibodies in human serum. The presence of Ribosome P antibodies can be used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of Systemic Lupus Erythematosus (SLE) and other related connective tissue diseases.

Device & Predicate Device(s):	K213403 (Device)	K981237 (Predicate)
	in the diagnosis of systemic lupus erythematosus. … The individual assays included in the Aptiva CTD Essential Reagent are intended for use with the Inova Diagnostics Aptiva System.	
Assay Methodology	Solid phase immunoassay	Same
Antigen	Synthetic Ribosomal P peptide	Same
Sample Matrix	Human serum	Same
Controls	Two Ribo-P controls with lot specific values assigned.	Same
General Device Characteristic Differences		
Solid Phase	Paramagnetic microparticles	ELISA
Detection Principle	Fluorescent immunoassay	Chromogenic immunoassay
Conjugate	Phycoerythrin conjugated polyclonal anti-human IgG antibody	HRP conjugated anti-human IgG antibody
Units	Fluorescent light units (FLU)	Units (U)
Cut-off	5.00 FLU	20.00 CU
Analytical Measuring Range	0.25 FLU – 86.86 FLU	N/A
Calibration	Lot specific Master Curve + 6 calibrators (sold separately)	Single point calibration

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. Reaffirmed September 2019.
- CLSI EP06-Ed2: Evaluation of the Linearity of Quantitative Measurement Procedures – 2nd Edition.
- CLSI EP07-A2: Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition.
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, Approved Guideline – Second Edition.
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory, Approved Guideline – Third Edition.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Precision and reproducibility of the Aptiva CTD Essential Reagent were evaluated in accordance with the CLSI guideline EP05-A3.

Within-Laboratory Precision:

To evaluate within-laboratory precision of the Aptiva CTD Essential Reagent, a variable number of samples containing various concentrations of antibodies were assayed in duplicate, twice a day, for 20 days, for a total of 80 measurements per sample, using one reagent lot at one laboratory site, by one operator.

The resulting data was analyzed using Analyze-it for Excel software by analysis of variance (ANOVA) methods and the within-run (repeatability), between-run, between-day, and within-laboratory precision were determined. As the number of samples and the values of measurand assayed varies by analyte, the study results are summarized on a per-analyte basis below:

dsDNA Within-Laboratory Precision

		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**Mean (IU/mL)**	**SD (IU/mL)**	**%CV**	**SD (IU/mL)**	**%CV**	**SD (IU/mL)**	**%CV**	**SD (IU/mL)**	**%CV**
1	16.19	1.13	7.0	0.64	4.0	1.39	8.6	1.90	11.8
2	25.81	1.97	7.6	1.65	6.4	1.30	5.0	2.88	11.1
3	33.39	2.08	6.2	1.99	6.0	1.03	3.1	3.06	9.2
4	47.86	3.03	6.3	2.07	4.3	3.34	7.0	4.96	10.4
5	67.19	4.53	6.7	2.86	4.3	3.28	4.9	6.28	9.3
6	98.34	5.16	5.2	0.7	0.7	5.7	5.8	7.72	7.9
7	208.89	13.01	6.2	0.00	0.0	9.46	4.5	16.09	7.7
8	425.21	31.59	7.4	28.14	6.6	14.65	3.4	44.77	10.5
9	600.13	35.66	5.9	53.43	8.9	0.00	0.0	64.24	10.7

RNP Within-Laboratory Precision

		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	**Mean (FLU)**	**SD (FLU)**	**%CV**	**SD (FLU)**	**%CV**	**SD (FLU)**	**%CV**	**SD (FLU)**	**%CV**
1	1.96	0.09	4.8	0.09	4.8	0.15	7.5	0.20	10.1
2	2.93	0.14	4.7	0.13	4.3	0.28	9.7	0.34	11.6
3	5.26	0.31	5.9	0.24	4.5	0.42	8.0	0.57	10.9
4	14.59	0.53	3.6	0.57	3.9	0.82	5.6	1.13	7.8
5	45.76	2.71	5.9	1.47	3.2	2.85	6.2	4.20	9.2
6	131.34	8.27	6.3	2.20	1.7	9.41	7.2	12.72	9.7

Sm Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	3.98	0.18	4.5	0.17	4.2	0.39	9.8	0.46	11.6
2	5.17	0.22	4.3	0.18	3.5	0.40	7.8	0.50	9.6
3	9.53	0.29	3.0	0.38	4.0	0.43	4.5	0.64	6.7
4	51.21	2.62	5.1	0.00	0.0	3.19	6.2	4.13	8.1
5	122.68	5.04	4.1	6.59	5.4	8.95	7.3	12.20	9.9
6	156.91	9.72	6.2	10.53	6.7	9.42	6.0	17.15	10.9
7	188.62	13.87	7.4	12.55	6.7	6.05	3.2	19.66	10.4

Ro52 Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.16	0.06	5.3	0.05	3.9	0.06	5.0	0.10	8.3
2	2.57	0.16	6.4	0.14	5.3	0.17	6.8	0.28	10.8
3	5.59	0.32	5.7	0.17	3.1	0.40	7.1	0.54	9.6
4	8.14	0.28	3.4	0.23	2.8	0.46	5.6	0.58	7.1
5	44.31	1.33	3.0	1.48	3.3	3.17	7.2	3.75	8.5
6	73.45	1.80	2.4	2.45	3.3	3.56	4.9	4.68	6.4
7	118.51	6.22	5.2	4.90	4.1	5.49	4.6	9.63	8.1
8	150.71	7.42	4.9	2.14	1.4	7.04	4.7	10.45	6.9
9	186.21	9.86	5.3	6.60	3.5	8.88	4.8	14.82	8.0

Ro60 Within-Laboratory Precision									
Sample	Mean (FLU)	Repeatability		Between-Run		Between-Day		Within-Laboratory	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	2.96	0.19	6.5	0.25	8.5	0.07	2.2	0.32	10.9
2	5.53	0.32	5.7	0.26	4.7	0.38	6.9	0.56	10.1
3	8.48	0.55	6.5	0.39	4.6	0.62	7.3	0.91	10.8
4	19.73	0.81	4.1	0.41	2.1	1.01	5.1	1.36	6.9
5	58.82	2.59	4.4	1.92	3.3	3.47	5.9	4.74	8.1
6	80.96	3.98	4.9	4.99	6.2	4.51	5.6	7.82	9.7
7	226.76	14.37	6.3	16.15	7.1	9.72	4.3	23.70	10.5
8	400.89	24.72	6.2	27.87	7.0	20.96	5.2	42.75	10.7

SS-B Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.33	0.05	4.0	0.06	4.2	0.12	9.0	0.14	10.7
2	4.69	0.16	3.3	0.18	3.8	0.49	10.5	0.55	11.7
3	64.34	1.87	2.9	1.60	2.5	3.74	5.8	4.48	7.0
4	138.52	5.76	4.2	2.62	1.9	7.95	5.7	10.16	7.3
5	157.86	6.60	4.2	5.43	3.4	10.42	6.6	13.48	8.5

Scl-70 Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	3.21	0.11	3.3	0.18	5.5	0.28	8.6	0.34	10.7
2	5.38	0.25	4.7	0.23	4.3	0.51	9.5	0.62	11.5
3	11.49	0.33	2.8	0.32	2.8	0.62	5.4	0.77	6.7
4	62.11	2.48	4.0	0.59	0.9	3.89	6.3	4.65	7.5
5	109.42	3.49	3.2	1.97	1.8	6.83	6.2	7.92	7.2
6	307.35	18.93	6.2	25.47	8.3	9.60	3.1	33.16	10.8

Jo-1 Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.07	0.10	4.8	0.07	3.4	0.14	6.8	0.19	9.0
2	5.15	0.27	5.2	0.22	4.3	0.24	4.6	0.42	8.1
3	18.54	0.75	4.0	0.93	5.0	1.41	7.6	1.85	10.0
4	81.42	4.76	5.8	5.37	6.6	4.28	5.3	8.36	10.3
5	107.75	7.29	6.8	2.98	2.8	6.19	5.7	10.01	9.3

Centromere Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	4.05	0.16	4.0	0.18	4.4	0.38	9.4	0.45	11.1
2	5.00	0.19	3.7	0.23	4.6	0.38	7.6	0.48	9.6
3	7.41	0.26	3.5	0.23	3.1	0.43	5.7	0.55	7.4
4	30.72	1.11	3.6	1.71	5.6	2.23	7.3	3.02	9.8
5	134.91	9.40	7.0	8.44	6.3	5.78	4.3	13.89	10.3

Ribo-P Within-Laboratory Precision									
		Repeatability		Between-Run		Between-Day		Within-Laboratory	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.42	0.09	6.5	0.00	0.0	0.10	7.1	0.14	9.6
2	3.39	0.16	4.8	0.12	3.4	0.21	6.1	0.29	8.4
3	4.85	0.27	5.5	0.25	5.2	0.39	8.1	0.54	11.1
4	21.77	1.23	5.6	1.07	4.9	0.71	3.3	1.77	8.1
5	38.98	2.76	7.1	0.00	0.0	2.63	6.7	3.81	9.8
6	66.90	4.89	7.3	2.54	3.8	2.95	4.4	6.25	9.3

Reproducibility:

The reproducibility of the Aptiva CTD Essential Reagent was conducted at three sites using a variable number of samples containing various concentrations of antibodies. Samples were assayed in quintuplicate, once a day, for five days to generate 25 data points per sample per site using one reagent lot and a total of 75 replicates per sample. Instrument and operator variables were nested within the multiple site component – i.e., a different operator and instrument was used at each of the three sites.

The resulting data was analyzed using Analyze-it for Excel software by ANOVA methods and precision between sites was determined. As the number of samples assayed varies by analyte, the results are summarized on a per analyte basis below.

dsDNA Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (IU/mL)	SD (IU/mL)	%CV	SD (IU/mL)	%CV	SD (IU/mL)	%CV	SD (IU/mL)	%CV
1	24.31	1.48	6.1	1.15	4.7	2	8.2	2.74	11.3
2	36.29	2.32	6.4	1.64	4.5	2.9	8.0	4.06	11.2
3	120.5	7.07	5.9	2.57	2.1	6.74	5.6	10.1	8.4
4	248.98	11.90	4.8	8.06	3.2	4.79	1.9	15.15	6.1
5	554.77	34.39	6.2	22.84	4.1	62.44	11.3	74.85	13.5

RNP Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.09	0.09	4.5	0.13	6.2	0.23	11.2	0.28	13.6
2	5.64	0.30	5.3	0.24	4.3	0.61	10.9	0.72	12.8
3	14.59	0.54	3.7	0.37	2.6	1.32	9.1	1.48	10.1
4	45.09	1.93	4.3	2.20	4.9	3.98	8.8	4.94	11.0
5	130.87	5.91	4.5	3.15	2.4	8.29	6.3	10.66	8.1

Sm Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.93	0.11	3.8	0.17	5.6	0.29	9.9	0.35	12.0
2	5.01	0.20	4.0	0.19	3.8	0.46	9.2	0.54	10.7
3	7.13	0.30	4.2	0.38	5.3	0.69	9.7	0.84	11.8
4	50.85	2.35	4.6	2.47	4.9	4.19	8.2	5.40	10.6
5	84.84	3.19	3.8	1.72	2.0	7.73	9.1	8.54	10.1
6	142.01	6.59	4.6	6.89	4.9	16.28	11.5	18.87	13.3

Ro52 Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.80	0.06	3.3	0.05	2.9	0.18	9.8	0.19	10.7
2	4.62	0.16	3.5	0.15	3.2	0.37	8.1	0.43	9.4
3	7.90	0.28	3.5	0.14	1.8	0.65	8.2	0.72	9.1
4	43.21	0.93	2.1	0.96	2.2	2.88	6.7	3.17	7.3
5	77.74	2.86	3.7	2.50	3.2	9.01	11.6	9.78	12.6
6	130.60	5.80	4.4	3.76	2.9	15.07	11.5	16.58	12.7

Ro60 Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.69	0.17	6.5	0.08	2.9	0.19	7.1	0.27	10.0
2	5.99	0.34	5.6	0.21	3.6	0.70	11.6	0.80	13.4
3	29.52	1.29	4.4	1.10	3.7	2.07	7.0	2.68	9.1
4	79.82	3.90	4.9	1.02	1.3	5.12	6.4	6.52	8.2
5	212.42	10.48	4.9	7.77	3.7	7.80	3.7	15.20	7.2
6	325.49	25.27	7.8	19.39	6.0	25.95	8.0	41.09	12.6

SS-B Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.27	0.06	4.5	0.05	4.3	0.14	10.7	0.16	12.4
2	4.17	0.16	3.8	0.15	3.6	0.29	6.9	0.36	8.6
3	22.80	0.88	3.9	1.20	5.2	1.71	7.5	2.27	10.0

SS-B Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
4	61.69	1.80	2.9	2.21	3.6	3.31	5.4	4.37	7.1
5	136.07	5.76	4.2	3.91	2.9	10.08	7.4	12.25	9.0
6	149.63	6.64	4.4	4.44	3.0	10.67	7.1	13.33	8.9

Scl-70 Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	3.39	0.20	6.0	0.23	6.8	0.26	7.7	0.40	11.9
2	5.93	0.26	4.4	0.17	2.9	0.47	8.0	0.57	9.6
3	12.51	0.41	3.2	0.37	2.9	0.60	4.8	0.81	6.5
4	70.19	2.23	3.2	3.19	4.5	2.37	3.4	4.55	6.5
5	123.54	3.76	3.0	5.14	4.2	3.50	2.8	7.27	5.9
6	220.48	8.57	3.9	7.79	3.5	26.09	11.8	28.54	12.9

Jo-1 Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	3.23	0.17	5.1	0.12	3.6	0.24	7.3	0.31	9.7
2	5.41	0.22	4.1	0.20	3.7	0.43	7.9	0.52	9.6
3	17.49	0.60	3.5	0.55	3.2	1.03	5.9	1.32	7.5
4	79.52	3.72	4.7	4.09	5.1	9.30	11.7	10.82	13.6
5	108.64	7.01	6.5	2.60	2.4	13.06	12.0	15.05	13.9

Centromere Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.28	0.13	5.6	0.15	6.8	0.15	6.4	0.25	10.9
2	4.34	0.14	3.2	0.16	3.7	0.37	8.6	0.43	9.9
3	6.79	0.22	3.2	0.21	3.1	0.66	9.7	0.73	10.7
4	28.61	1.04	3.6	1.20	4.2	1.32	4.6	2.07	7.2
5	43.74	1.75	4.0	1.88	4.3	5.01	11.5	5.63	12.9
6	112.03	6.74	6.0	6.21	5.5	8.78	7.8	12.69	11.3

Ribo-P Multi-Site Reproducibility									
		Repeatability		Between-Run		Between-Site/Instrument		Reproducibility	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.56	0.07	4.4	0.04	2.5	0.18	11.7	0.20	12.8
2	4.78	0.25	5.2	0.27	5.7	0.31	6.6	0.48	10.1
3	21.96	1.21	5.5	0.26	1.2	2.17	9.9	2.50	11.4
4	38.20	1.76	4.6	1.40	3.7	3.17	8.3	3.89	10.2
5	61.32	3.38	5.5	1.15	1.9	5.46	8.9	6.53	10.6

Lot-to-lot imprecision:

To evaluate the between-lot imprecision of the Aptiva CTD Essential Reagent, a variable number of samples containing various concentrations of antibodies were assayed in quintuplicate, once a day, for 5 days, using three reagent lots, using one instrument, for a total of 75 replicates per sample.

The resulting data was analyzed using Analyze-it for Excel software by ANOVA methods and precision between lots was determined. As the number of samples assayed varies by analyte, the results are summarized on a per analyte basis below:

dsDNA Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (IU/mL)	SD (IU/mL)	%CV	SD (IU/mL)	%CV	SD (IU/mL)	%CV	SD (IU/mL)	%CV
1	14.26	1.04	7.3	0.59	4.2	0.97	6.8	1.54	10.8
2	26.41	2.30	8.7	1.92	7.3	1.75	6.6	3.47	13.1
3	87.69	6.04	6.9	4.73	5.4	3.64	4.2	8.49	9.7
4	191.71	10.49	5.5	9.92	5.2	1.98	1.0	14.57	7.6
5	402.15	32.20	8.0	5.28	1.3	33.19	8.3	46.54	11.6
6	557.44	30.79	5.5	18.13	3.3	61.67	11.1	71.27	12.8

RNP Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.72	0.16	9.4	0.10	5.9	0.04	2.3	0.19	11.3
2	4.36	0.30	6.9	0.19	4.4	0.25	5.8	0.44	10.0
3	21.30	1.48	7.0	0.65	3.0	1.33	6.2	2.09	9.8
4	114.31	7.08	6.2	2.24	2.0	4.21	3.7	8.54	7.5

Sm Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.90	0.13	4.6	0.24	8.3	0.35	12.0	0.44	15.3

Sm Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
2	3.90	0.26	6.8	0.23	6.0	0.34	8.8	0.49	12.6
3	47.73	1.71	3.6	3.35	7.0	3.25	6.8	4.97	10.4
4	115.48	4.70	4.1	9.17	7.9	5.14	4.5	11.51	10.0
5	163.02	6.28	3.9	10.02	6.1	15.23	9.3	19.28	11.8

Ro52 Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.77	0.07	4.2	0.10	5.8	0.17	9.4	0.21	11.9
2	4.11	0.16	3.9	0.23	5.6	0.11	2.7	0.30	7.3
3	7.50	0.33	4.4	0.45	6.0	0.52	7.0	0.77	10.2
4	39.74	2.11	5.3	2.79	7.0	1.38	3.5	3.76	9.5
5	71.05	2.15	3.0	6.03	8.5	6.23	8.8	8.93	12.6
6	118.01	7.98	6.8	5.99	5.1	10.71	9.1	14.64	12.4

Ro60 Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	3.06	0.44	14.5	0.20	6.6	0.14	4.7	0.51	16.6
2	4.72	0.37	7.9	0.20	4.2	0.09	1.9	0.43	9.1
3	72.00	4.61	6.4	2.08	2.9	6.00	8.3	7.84	10.9
4	251.18	12.67	5.0	11.98	4.8	19.10	7.6	25.86	10.3
5	359.05	19.11	5.3	9.87	2.7	22.33	6.2	31.00	8.6

SS-B Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.31	0.07	5.2	0.07	5.7	0.12	8.9	0.15	11.8
2	4.66	0.19	4.0	0.29	6.2	0.50	10.8	0.61	13.1
3	65.82	1.81	2.7	3.54	5.4	5.86	8.9	7.08	10.8
4	138.38	5.93	4.3	7.58	5.5	5.30	3.8	10.99	7.9

Scl-70 Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	2.23	0.16	7.3	0.10	4.6	0.25	11.1	0.31	14.1
2	4.58	0.20	4.5	0.26	5.6	0.49	10.8	0.59	12.9
3	85.97	3.19	3.7	7.35	8.6	3.27	3.8	8.66	10.1
4	287.33	13.38	4.7	17.36	6.0	19.27	6.7	29.19	10.2

Jo-1 Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	3.05	0.13	4.4	0.20	6.6	0.07	2.2	0.25	8.2
2	4.96	0.30	6.1	0.27	5.5	0.10	2.0	0.42	8.4
3	16.14	0.89	5.5	0.82	5.1	1.01	6.2	1.58	9.8
4	69.67	5.20	7.5	2.21	3.2	6.10	8.8	8.31	11.9
5	97.37	7.35	7.6	2.02	2.1	9.19	9.4	11.94	12.3

Centromere Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.69	0.25	14.9	0.17	9.9	0.09	5.1	0.31	18.6
2	4.36	0.18	4.2	0.35	8.1	0.16	3.6	0.43	9.8
3	28.59	1.16	4.1	2.66	9.3	1.58	5.5	3.30	11.5
4	128.28	6.57	5.1	6.06	4.7	13.72	10.7	16.38	12.8

Ribo-P Multi-Lot Imprecision									
		Repeatability		Between-Day		Between-Lot		Total	
Sample	Mean (FLU)	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV	SD (FLU)	%CV
1	1.33	0.08	5.8	0.09	6.7	0.00	0.0	0.12	8.9
2	4.81	0.32	6.7	0.16	3.3	0.34	7.0	0.49	10.2
3	23.81	0.99	4.1	0.87	3.6	0.91	3.8	1.60	6.7
4	62.64	2.54	4.0	2.89	4.6	3.49	5.6	5.19	8.3

2. Linearity:

The linearity of the analytical measuring range was calculated separately for each of the Aptiva CTD Essential Reagent analytes (dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo-1, Ribo-P and Centromere).

The linearity for all analytes was evaluated by a study following CLSI EP06-Ed2. Various samples were prepared as overlapping dilution series by mixing human serum samples with high antibody concentrations and samples with low antibody concentrations to cover portions of the analytical measuring interval (AMI) of each analyte. Each sample was tested in duplicate and the results from each sample were analyzed separately using a weighted least squares regression analysis.

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. For values on the low end of the measuring interval, absolute deviations were used in lieu of the percentage. The results are summarized on a per analyte basis below.

dsDNA Linearity				
Sample	**Test Range (IU/mL)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	120.85 – 1208.48	1.02 (0.98 – 1.05)	0.99	-9.2% – 6.4%
2	13.09 – 130.89	0.98 (0.96 – 0.99)	1.00	-4.5% – 4.1%
3	1.99 – 19.89	0.98 (0.94 – 1.02)	0.99	-5.0% – 5.3% and -1.12 – 0.08 IU/mL

RNP Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	20.36 – 203.59	0.96 (0.93 – 1.00)	0.99	-9.6% – 5.8%
2	4.55 – 45.46	1.00 (0.97 – 1.03)	0.99	-11.6% – 6.9%
3	0.71 – 7.09	0.93 (0.88 – 0.99)	0.98	-13.9% – 7.0% and -0.11 FLU
4	0.14 – 1.43	0.99 (0.94 – 1.04)	0.99	-0.07 – -0.08 FLU

Sm Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	33.02 – 330.25	0.92 (0.89 – 0.96)	0.98	-12.8% – 8.3%
2	5.87 – 58.72	1.02 (1.00 – 1.04)	1.00	-2.3% – 10.9%
3	0.73 – 7.31	0.96 (0.93 – 0.99)	0.99	-9.0% – 4.2% and -0.30 – -0.21 FLU
4	0.13 – 1.33	1.01 (0.97 – 1.04)	0.99	-0.06 – 0.04 FLU

Ro52 Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	20.07 – 200.73	0.98 (0.95 – 1.01)	0.99	-8.8% – 4.5%
2	4.81 – 48.10	1.02 (1.00 – 1.03)	1.00	-9.8% – 3.5%
3	0.96 – 9.58	1.01 (0.96 – 1.06)	0.98	-8.8% – 7.0% and -0.60 – 0.27 FLU
4	0.12 – 1.25	0.97 (0.94 – 0.99)	0.99	-0.04 – 0.04 FLU

Ro60 Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	71.74 – 717.36	0.93 (0.89 to 0.98)	0.98	-8.1% to 7.1%
2	17.17 – 85.85	1.04 (1.01 to 1.07)	0.99	-3.6% to 5.4%
3	5.94 – 59.41	1.02 (0.98 to 1.06)	0.99	-7.0% to 14.4%
4	1.48 – 7.41	1.02 (0.98 to 1.05)	0.99	-5.9% to 11.3%
5	0.30 – 2.96	0.91 (0.84 to 0.97)	0.95	-0.38 to 0.28 FLU

SS-B Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	20.55 – 205.54	0.98 (0.94 – 1.01)	0.99	-13.2% – 7.1%
2	8.89 – 88.85	0.94 (0.92 – 1.07)	0.99	-13.5% – 5.9%
3	1.15 – 11.51	0.88 (0.82 – 0.94)	0.98	-10.3% – 13.7% and -0.46 – -0.27 FLU
4	0.16 – 1.55	0.99 (0.96 – 1.02)	0.99	-0.09 – 0.06 FLU

Scl-70 Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	41.46 – 414.62	1.01 (0.99 – 1.03)	0.99	-4.7% – 6.9%
2	5.30 – 53.05	0.98 (0.96 – 0.99)	1.00	-12.9% – 2.5%
3	0.81 – 8.13	0.97 (0.92 – 1.01)	0.99	-14.5% – 4.9% and -0.43 – -0.25 FLU
4	0.16 – 1.57	0.94 (0.89 – 0.98)	0.98	-0.09 – 0.10 FLU

Jo-1 Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	20.60 – 206.02	0.95 (0.92 – 0.98)	0.99	-12.7% – 5.3%
2	5.78 – 57.76	0.96 (0.93 – 0.99)	0.99	-4.3% – 8.6%
3	0.76 – 7.63	1.07 (1.02 – 1.11)	0.98	-10.7% – 11.4%
4	0.14 – 1.38	0.96 (0.94 – 0.98)	0.99	-0.03 – 0.05 FLU

Centromere Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	40.48 – 202.38	1.01 (0.98 – 1.04)	0.99	-12.7% – 5.9%
2	5.96 – 59.56	1.00 (0.99 – 1.01)	1.00	-5.8% – 2.1%
3	0.91 – 9.08	1.01 (0.99 – 1.02)	1.00	-9.9% – 1.9%
4	0.19 – 1.86	0.96 (0.89 – 1.03)	0.97	-0.19 – 0.10 FLU

Ribo-P Linearity				
Sample	**Test Range (FLU)**	**Slope (95% CI)**	**R^2**	**Range of Linearity Deviations**
1	13.91 – 139.12	0.97 (0.95 – 1.00)	0.99	-3.7% – 6.2%
2	2.78 – 27.83	1.00 (0.95 – 1.04)	0.98	-11.9% – 7.2%
3	0.58 – 5.76	0.99 (0.93 – 1.05)	0.99	-7.8% – 6.4% and -0.48 – -0.27 FLU
4	0.09 – 0.95	1.05 (0.97 – 1.12)	0.95	-0.10 – 0.11 FLU

Auto-rerun and reportable results

The Aptiva software has an auto-rerun option available. If the option is selected, the instrument will automatically rerun any sample that has a result above the upper limit of the

analytical measuring range by performing a 10-fold dilution to bring the measured value to within the AMI, followed by a software calculation to account for the additional 10-fold dilution. To validate the auto-rerun function with 1:10 dilutions, two positive samples for each analyte, with concentrations well above the assay measuring range, were run with the auto-rerun function enabled on the Aptiva Instrument. The same set of samples were manually diluted 1:10, tested, and used as reference values. A percent recovery comparison of the values obtained by the auto-rerun and the manual dilution was conducted to determine the highest Auto-rerun values.

The highest value that is ≤15% of the manual concentration for each measurand is listed in the table below:

Assay	Auto rerun highest value
dsDNA	8141.00 IU/mL
RNP	1819.90 FLU
Sm	2560.00 FLU
Ro52	1962.70 FLU
Ro60	5837.20 FLU
SS-B	1958.40 FLU
Scl-70	3712.40 FLU
Jo-1	1536.00 FLU
Centromere	1876.90 FLU
Ribo-P	868.60 FLU

High concentration hook effect:

To assess the Aptiva CTD Essential Reagent for hook effects, two samples for dsDNA, RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, Centromere, and Ribo-P were tested at three increasing two-fold serial dilutions from the standard 1:44.4 dilution used by the Aptiva CTD Essential Reagent.

All samples showed an increase in FLU values as the dilution factor became more concentrated, demonstrating that high positive specimens above the AMR do not demonstrate a hook effect up to the following concentrations:

Assay	Highest concentrations tested
dsDNA	4314.85 IU/mL
RNP	25878.44 FLU
Sm	1864.64 FLU
Ro52	2962.51 FLU
Ro60	3653.43 FLU
SS-B	4562.22 FLU
Scl-70	10562.70 FLU
Jo-1	1932.57 FLU
Centromere	1523.35 FLU
Ribo-P	1144.80 FLU

3. Analytical Specificity/Interference:

Interference:

An interference study was performed based on the recommendations contained in CLSI EP07-A2. A set of three human serum specimens − one positive specimen, one specimen near the cutoff, and one negative specimen, were assessed as vehicle-control or interferent-spiked contrived specimens in triplicate using the Aptiva CTD Essential Reagent assays (dsDNA, RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Jo-1, Centromere and Ribo-P). The percent recovery for each sample that was spiked with the potential interfering substance was calculated by comparing its result to that of the corresponding control sample without the interfering substance.

Endogenous:
The following endogenous interferents were tested: bilirubin, hemoglobin, triglycerides, cholesterol, rheumatoid factor IgM and human IgG. The Aptiva CTD Essential Reagent showed <±15% recovery with bilirubin up to 1 mg/mL, hemoglobin up to 2 mg/mL, triglyceride up to 1000 mg/dL, cholesterol up to 332.5 mg/dL, RF IgM up to 153.4 IU/mL and human IgG up to 35 mg/mL.

Exogenous:
The following exogenous interferents were tested: ibuprofen, acetaminophen, prednisone, warfarin, diltiazem, azathioprine, sildenafil, cyclophosphamide, mycophenolate mofetil and heparin. The Aptiva CTD Essential Reagent showed <±15% recovery up to 21.9 mg/dL ibuprofen, 15.6 mg/dL acetaminophen, 0.0099 mg/dL prednisone, 7.5 mg/dL warfarin, 0.09 mg/dL diltiazem, 0.258 mg/dL azathioprine, 0.271 mg/dL sildenafil, 54.9 mg/dL cyclophosphamide, 1.125 mg/mL mycophenolate mofetil and 330 units/dL heparin. Rituximab was not evaluated for interference with the Aptiva CTD Essential Reagent.

Analytical Specificity:

CDC (Center for Disease Controls and Prevention) ANA Reference Panel samples 1–12 were tested using one lot of Aptiva CTD Essential Reagent to demonstrate the analytical specificity of the assays. The results have been outlined below.

CDC ID	Sample ID	CDC Description	Aptiva Result
IS2072	CDC ANA 01	ANA Homogeneous Positive/Anti-native DNA	dsDNA Positive
IS2073	CDC ANA 02	ANA Speckled Positive/Anti-SS-B Positive	SS-B, Ro52, Ro60 Positive
IS2074	CDC ANA 03	ANA Speckled Positive	RNP, Ro60, SS-B Positive
IS2075	CDC ANA 04	ANA-U1 RNP Positive	RNP Positive
IS2076	CDC ANA 05	Anti-Sm Positive	RNP and Sm Positive
IS2105	CDC ANA 07	Anti-SS-A Positive	Ro52 and Ro60 Positive
IS2134	CDC ANA 08	ANA Centromere Positive	Centromere Positive
IS2135	CDC ANA 09	Anti-Scl-70 Positive	Scl-70 Positive
IS2187	CDC ANA 10	Anti-Jo-1 Positive	Jo-1 and Ro52 Positive
IS2706	CDC ANA 12	Anti-Ribosomal P Positive	Ribo-P Positive

4. Assay Reportable Range:

The analytical measuring range (AMR) of the dsDNA, RNP, Sm, Ro60, Ro52, SS-B, Scl-70, Jo-1, Centromere and Ribo-P assays are outlined in the table below:

Assay	Analytical Measuring Interval (AMI)
dsDNA	2.3 – 814.10 IU/mL
RNP	0.50 – 181.99 FLU
Sm	0.25 – 256.00 FLU
Ro52	0.25 – 196.27 FLU
Ro60	0.50 – 583.72 FLU
SS-B	0.40 – 195.84 FLU
Scl-70	0.50 – 371.24 FLU
Jo-1	0.25 – 153.60 FLU
Centromere	0.50 – 187.69 FLU
Ribo-P	0.25 – 86.86 FLU

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

Traceability

For Aptiva dsDNA, the Master Curve Standards are traceable to the First International Standard Preparation (WHO code: Wo/80). Based upon this standardization, results are reported in International Units (IU)/mL. There are no international reference standards for the other analytes that allow the standardization of the assays. Calibrator and control values are directly traceable to in-house reference materials that are used to create the master curves for the Aptiva CTD Essential Reagent.

No international reference serum panel for anti-ANA antibodies is available that allows for the standardization of anti-ANA antibody assays.

Sample Stability

To determine the stability of patient samples, three samples were tested for each analyte in the Aptiva CTD Essential Reagent assay. The samples used in the study were contrived by combining native high and low measurand (auto-antibody) serum samples to yield a desired reactivity. The samples were assayed in duplicate for up to 21 days while stored at 2-8°C, up to 48 hours while stored at room temperature (e.g., 20–25°C), and after repeated freeze/thaw cycles up to 5 cycles. The results were compared to the result obtained from control samples and conditions (i.e., timepoint zero / zero cycles).

Based on these results, serum samples may be stored up to 48 hours at room temperature, up to 14 days at 2–8°C, and may be frozen/thawed for up to 5 cycles when stored at or below -20°C.

Reagent Stability

Shelf life:

A Real Time stability study was conducted at 3-month intervals using Aptiva CTD Essential Reagent stored at 2–8°C. At each timepoint, a low-negative sample, a mid-range positive

sample, and a high-range positive sample were tested at time 0, 6, 9, and 12-months with additional testing scheduled to be performed up to 24 months. The currently available results support a 12-month stability claim.

An accelerated stability study was performed for 5 weeks at 37°C ± 3°C to simulate 30-months at 5 ± 3°C. The recovery of the measured values relative to the control stored at 5 ± 3°C was determined and a linear regression analysis was performed between recovery values and the number of days for each bead component of the Aptiva CTD Essential Reagent. At each timepoint, a minimum of six samples were tested, including low-negative sample, mid-range positive sample, and high-range positive samples. The accelerated stability testing results support that the Aptiva CTD Essential Reagent may be stable for up to 24-months.

In use (onboard) stability:
To establish the in-use stability of the Aptiva CTD Essential reagent cartridge onboard the Aptiva instrument, one lot of reagent cartridge was tested using human serum samples for all analytes. The specimens were tested periodically for 45 days. At day 21, the reagent cartridge was recalibrated, and a new cartridge specific working curve was generated. Percent recoveries were calculated compared to the day zero average values, and a linear regression analysis was performed by plotting percent recovery against time.

The test results support an in-use (onboard) stability for the Aptiva CTD Essential Reagent as 36 days with an 18-day recalibration.

6. Detection Limit:

Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) were assessed for each analyte in the Aptiva CTD Essential Reagent and were calculated separately based on the recommendations contained in CLSI EP17-A2.

LoB:
To determine the Limit of Blank, four blank samples were prepared using three lots of Aptiva system rinse, and were run in quintuplicate, 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 using Analyse-it for Excel, at the 95^{th} 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 contrived for each analyte by mixing human serum samples with high and low levels of antibodies, and were assayed in quintuplicate on two reagent lots, twice per day, for three days, for a total of 120 data points for each assay on each reagent lot. The LoD was determined 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 were contrived by mixing human serum samples with high and low levels of antibodies, which were run in quintuplicate, using two reagent lots, twice per day for three days, 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 to be the lowest concentration level that meets the within laboratory imprecision of <20% for each lot. The LoQ for each assay was determined as the greatest LoQ across the two lots and set as the lower limit of the AMR.

The LoB, LoD, and LoQ for each measurand is summarized as follows:

Analyte	LoB	LoD	LoQ
dsDNA	0.11 IU/mL	1.64 IU/mL	2.30 IU/mL
RNP	0.03 FLU	0.12 FLU	0.15 FLU
Sm	0.01 FLU	0.13 FLU	0.18 FLU
Ro52	0.00 FLU	0.10 FLU	0.23 FLU
Ro60	0.01 FLU	0.06 FLU	0.14 FLU
SS-B	0.00 FLU	0.14 FLU	0.33 FLU
Scl-70	0.02 FLU	0.12 FLU	0.16 FLU
Jo-1	0.01 FLU	0.03 FLU	0.07 FLU
Centromere	0.00 FLU	0.06 FLU	0.21 FLU
Ribo-P	0.02 FLU	0.16 FLU	0.16 FLU

7. Assay Cut-Off:

A cut-off study was performed using clinical samples. Please refer to the Clinical Cut-off (section §VII.D) below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Samples for the method comparison analysis included the samples from the clinical validation study (see VII.C below). Samples were tested on both the Aptiva CTD Essential Reagent and their respective predicate devices. Positive percent agreement (PPA) and negative percent agreement (NPA), with 95% confidence intervals were calculated for each analyte comparison, excluding values that were outside of the measuring ranges of either assay. The following tables summarize these method comparison results:

Method comparison of the Aptiva dsDNA with the predicate device						
			Predicate			
			Positive	Indeterminate	Negative	Total
Aptiva dsDNA	Positive	>35.0	97	8	17	122
	Indeterminate	27.0−35.0	5	3	13	21
	Negative	<27.0	21	16	248	285
	Total		123	27	278	428

Indeterminate as positive results	PPA	75.3%	(67.9−81.5%)
	NPA	89.2%	(85.0−92.3%)
Indeterminate as negative results	PPA	78.9%	(70.8−85.1%)
	NPA	91.8%	(88.2−94.4%)

Method comparison of the Aptiva RNP with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva RNP	Positive	≥5.0	93	36	129
	Negative	<5.0	2	349	351
	Total		95	385	480

PPA	97.9%	(92.6−99.4%)
NPA	90.6%	(87.3−93.2%)

Method comparison of the Aptiva Sm with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Sm	Positive	≥5.0	30	7	37
	Negative	<5.0	5	376	381
	Total		35	383	418

PPA	85.7%	(70.6−93.7%)
NPA	98.2%	(96.3−99.1%)

Method comparison of the Aptiva Ro52 with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Ro52	Positive	≥5.0	203	18	221
	Negative	<5.0	5	802	807
	Total		208	820	1028

PPA	97.6%	(94.5−99.0%)
NPA	97.8%	(96.6−98.6%)

Method comparison of the Aptiva Ro60 with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Ro60	Positive	≥5.0	193	29	222
	Negative	<5.0	3	326	329
	Total		196	355	551

PPA	98.5%	(95.6−99.5%)
NPA	91.7%	(88.5−94.3%)

Method comparison of the Aptiva SS-B with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva SS-B	Positive	≥5.0	88	17	105
	Negative	<5.0	3	442	445
	Total		91	459	550

PPA	96.7%	(90.8−98.9%)
NPA	96.3%	(94.1−97.7%)

Method comparison of the Aptiva Scl-70 with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Scl-70	Positive	≥5.0	64	9	73
	Negative	<5.0	3	359	362
	Total		67	368	435

PPA	95.5%	(87.6−98.5%)
NPA	97.6%	(95.4−98.7%)

Method comparison of the Aptiva Jo-1 with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Jo-1	Positive	≥5.0	24	1	25
	Negative	<5.0	1	390	391
	Total		25	391	416

PPA	96.0%	(80.5−99.3%)
NPA	99.7%	(98.6−100%)

Method comparison of the Aptiva Centromere with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Centromere	Positive	≥5.0	101	6	107
	Negative	<5.0	4	338	342
	Total		105	344	449

PPA	96.2%	(90.6−98.5%)
NPA	98.3%	(96.2−99.2%)

Method comparison of the Aptiva Ribo-P with the predicate device					
			Predicate		
			Positive	Negative	Total
Aptiva Ribo-P	Positive	≥5.0	23	1	24
	Negative	<5.0	0	363	363
	Total		23	364	387

PPA	100%	(85.7−100%)
NPA	99.7%	(98.5−100%)

2. Matrix Comparison:

The Aptiva CTD Essential Reagent is intended to use only human serum specimens.

C Clinical Studies:

A cohort of characterized samples were used to validate the clinical performance of the Aptiva CTD Essential Reagent. The clinical validation study included 1269 total samples including samples from patients with Sjögren's syndrome (SjS, *N*=141), systemic lupus erythematosus (SLE, *N* =230), systemic sclerosis (SSc, *N* =217), mixed connective tissue disease (MCTD, *N* =91), idiopathic inflammatory myopathy (IIM, *N* =200) and control samples (*N* =390) from patients with various types of autoimmune and infectious diseases.

The performance summarized below is organized by autoantigen:

dsDNA:

Clinical sensitivity and specificity for the Aptiva dsDNA					
			Diagnosis		
			SLE	Controls	Totals
Aptiva dsDNA	Positive	>35.0	106	74	180
	Indeterminate	27.0−35.0	10	29	39
	Negative	<27.0	114	936	1050
	Total		230	1039	1269

Indeterminate treated as positive	Sensitivity	50.4%	(44.0−56.8%)
	Specificity	90.1%	(88.1−91.8%)
Indeterminate treated as negative	Sensitivity	46.1%	(39.8−52.5%)
	Specificity	92.9%	(91.2−94.3%)

dsDNA: Distribution of target and differential disease samples and antibody positivity rates:					
Diagnostic Group	***N***	**dsDNA Positive** (indeterminate as positive)		**dsDNA Positive** (indeterminate as negative)	
		n	**(%)**	***n***	**(%)**
Target diagnosis					
Systemic lupus erythematosus (SLE)	230	116	50.4%	106	46.1%
Differential diagnosis controls					
Autoimmune hepatitis type 1 (AIH-1)	10	3	30.0%	3	30.0%
Autoimmune hepatitis type 2 (AIH-2)	40	5	12.5%	4	10.0%
Antiphospholipid syndrome (APS)	12	2	16.7%	2	16.7%
Atopic dermatitis	16	0	0.0%	0	0.0%
Celiac disease (CD)	43	8	18.6%	6	14.0%
Crohn's disease (CrD)	20	5	25.0%	4	20.0%
Dermatitis herpetiformis (DH)	7	2	28.6%	0	0.0%
Drug-induced liver injury	9	1	11.1%	0	0.0%
Fibromyalgia	8	2	25.0%	2	25.0%
Gout	6	0	0.0%	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	2	10.5%	2	10.5%
Graves' Disease	16	4	25.0%	4	25.0%
Hashimoto's thyroiditis (HT)	20	1	5.0%	1	5.0%
Idiopathic inflammatory myopathy (IIM)	200	12	6.0%	10	5.0%
Infectious Disease	20	1	5.0%	0	0.0%
Mixed connective tissue disease (MCTD)	91	9	9.9%	9	9.9%
Nodal Osteoarthritis	19	0	0.0%	0	0.0%
Primary biliary cholangitis (PBC)	15	1	6.7%	0	0.0%
Polymyalgia Rheumatica	13	1	7.7%	1	7.7%
Prostate Cancer	15	1	6.7%	1	6.7%
Psoriasis	7	0	0.0%	0	0.0%
Psoriatic Arthritis	9	1	11.1%	1	11.1%
Rheumatoid arthritis (RA)	35	5	14.3%	2	5.7%
Sarcoidosis	15	2	13.3%	2	13.3%
Sjögren's Syndrome (SjS)	141	11	7.8%	6	4.3%
Spondyloarthritis	16	4	25.0%	0	0.0%
Systemic sclerosis (SSc)	217	20	9.2%	12	5.5%
Total Controls	**1039**	**103**	**9.9%**	**72**	**6.9%**

RNP:

Clinical sensitivity and specificity for the Aptiva RNP					
		Diagnosis			
		SLE	MCTD	Controls	Totals
Aptiva RNP	Positive ≥5.0	86	62	49	197
	Negative <5.0	144	29	899	1072
	Total	230	91	948	1269

SLE	Sensitivity	37.4%	(31.4−43.8%)
MCTD	Sensitivity	68.1%	(58.0−76.8%)
	Specificity	94.8%	(93.2−96.1%)

RNP: Distribution of target and differential disease samples and antibody positivity rates:			
Diagnostic Group	***N***	**RNP Positive**	
		n	**(%)**
Target conditions			
Systemic lupus erythematosus (SLE)	230	86	37.4%
Mixed connective tissue disease (MCTD)	91	62	68.1%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	0	0.0%
Autoimmune hepatitis type 2 (AIH-2)	40	0	0.0%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	0	0.0%
Crohn's disease (CrD)	20	2	10.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	0	0.0%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	1	6.3%
Hashimoto's thyroiditis (HT)	20	1	5.0%
Idiopathic inflammatory myopathy (IIM)	200	10	5.0%
Infectious Disease	20	0	0.0%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	0	0.0%
Polymyalgia Rheumatica	13	1	7.7%
Prostate Cancer	15	1	6.7%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	0	0.0%
Rheumatoid arthritis (RA)	35	0	0.0%
Sarcoidosis	15	0	0.0%
Sjögren's Syndrome (SjS)	141	18	12.8%
Spondyloarthritis	16	0	0.0%
Systemic sclerosis (SSc)	217	15	6.9%
Total Controls	**948**	**49**	**5.2%**

Sm:

Clinical sensitivity and specificity for the Aptiva Sm				
		Diagnosis		
		SLE	Controls	Totals
Aptiva Sm	Positive ≥5.0	24	4	28
	Negative <5.0	206	1035	1241
	Total	230	1039	1269

Sensitivity	10.4%	(7.1–15.1%)
Specificity	99.6%	(99.0–99.9%)

Sm: Distribution of target and differential disease samples and antibody positivity rates:			
Diagnostic Group	***N***	**Sm Positive**	
		n	**(%)**
Target condition			
Systemic lupus erythematosus (SLE)	230	24	10.4%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	0	0.0%
Autoimmune hepatitis type 2 (AIH-2)	40	0	0.0%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	0	0.0%
Crohn's disease (CrD)	20	0	0.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	0	0.0%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	0	0.0%
Hashimoto's thyroiditis (HT)	20	0	0.0%
Idiopathic inflammatory myopathy (IIM)	200	0	0.0%
Infectious Disease	20	0	0.0%
Mixed connective tissue disease (MCTD)	91	1	1.1%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	0	0.0%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	0	0.0%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	0	0.0%
Rheumatoid arthritis (RA)	35	0	0.0%
Sarcoidosis	15	0	0.0%
Sjögren's Syndrome (SjS)	141	1	0.7%
Spondyloarthritis	16	0	0.0%
Systemic sclerosis (SSc)	217	1	0.5%
Total Controls	**1039**	**3**	**0.3%**

Ro52:

Clinical sensitivity and specificity for the Aptiva Ro52								
			Diagnosis					
			SLE	SjS	SSc	IIM	Controls	Total
Aptiva Ro52	Positive	≥5.0	56	85	33	38	31	243
	Negative	<5.0	174	56	184	162	450	1026
	Total		230	141	217	200	481	1269

SLE	Sensitivity	24.3%	(19.3−30.3%)
SjS	Sensitivity	60.3%	(52.0−68.0%)
SSc	Sensitivity	15.2%	(11.0−20.6%)
IIM	Sensitivity	19.0%	(14.2−25.0%)
	Specificity	93.6%	(91.0−95.4%)

Ro52: Distribution of target and differential disease samples and antibody positivity rates:			
Diagnostic Group	***N***	**Ro52 Positive**	
		n	**(%)**
Target conditions			
Systemic lupus erythematosus (SLE)	230	56	24.3%
Sjögren's Syndrome (SjS)	141	85	60.3%
Systemic sclerosis (SSc)	217	33	15.2%
Idiopathic inflammatory myopathy (IIM)	200	38	19.0%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	1	10.0%
Autoimmune hepatitis type 2 (AIH-2)	40	0	0.0%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	1	2.3%
Crohn's disease (CrD)	20	0	0.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	1	11.1%
Fibromyalgia	8	1	12.5%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	1	5.3%
Graves' Disease	16	1	6.3%
Hashimoto's thyroiditis (HT)	20	0	0.0%
Infectious Disease	20	0	0.0%
Mixed connective tissue disease (MCTD)*	91	18	19.8%
Nodal Osteoarthritis	19	1	5.3%
Primary biliary cholangitis (PBC)	15	1	6.7%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	1	6.7%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	1	11.1%
Rheumatoid arthritis (RA)	35	2	5.7%
Sarcoidosis	15	1	6.7%
Spondyloarthritis	16	0	0.0%
Total Controls	**481**	**31**	**6.4%**

*Note: Some MCTD samples may test positive for Ro52 antibodies due to associations with this disease.

Ro60:

Clinical sensitivity and specificity for the Aptiva Ro60					
		Diagnosis			
		SLE	SjS	Controls	Totals
Aptiva Ro60	Positive ≥5.0	120	94	101	315
	Negative <5.0	110	47	797	954
	Total	230	141	898	1269

SLE	Sensitivity	52.2%	(45.7−58.5%)
SjS	Sensitivity	66.7%	(58.5−73.9%)
	Specificity	88.8%	(86.5−90.7%)

Ro60: Distribution of target and differential disease samples and antibody positivity rates:			
Diagnostic Group	***N***	**Ro60 Positive**	
		n	**(%)**
Target condition			
Systemic lupus erythematosus (SLE)	230	120	52.2%
Sjögren's Syndrome (SjS)	141	94	66.7%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	0	0.0%
Autoimmune hepatitis type 2 (AIH-2)	40	1	2.5%
Antiphospholipid syndrome (APS)	12	3	25.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	2	4.7%
Crohn's disease (CrD)	20	2	10.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	1	11.1%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	2	12.5%
Hashimoto's thyroiditis (HT)	20	0	0.0%
Idiopathic inflammatory myopathy (IIM)	200	26	13.0%
Infectious Disease	20	2	10.0%
Mixed connective tissue disease (MCTD)	91	19	20.9%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	1	6.7%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	1	6.7%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	1	11.1%
Rheumatoid arthritis (RA)	35	2	5.7%
Sarcoidosis	15	1	6.7%
Spondyloarthritis	16	1	6.3%
Systemic sclerosis (SSc)	217	36	16.6%
Total Controls	**898**	**101**	**11.2%**

SS-B:

Clinical sensitivity and specificity for the Aptiva SS-B					
		Diagnosis			
		SLE	SjS	Controls	Totals
Aptiva SS-B	Positive ≥5.0	36	66	31	
	Negative <5.0	194	75	867	
	Total	230	141	898	1269

SLE	Sensitivity	15.7%	(11.5−20.9%)
SjS	Sensitivity	46.8%	(38.8−55.0%)
	Specificity	96.5%	(95.1−97.6%)

SS-B: Distribution of target and differential disease samples and antibody positivity rates:			
Diagnostic Group	***N***	**SS-B Positive**	
		n	**(%)**
Target condition			
Systemic lupus erythematosus (SLE)	230	36	15.7%
Sjögren's Syndrome (SjS)	141	66	46.8%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	0	0.0%
Autoimmune hepatitis type 2 (AIH-2)	40	0	0.0%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	1	2.3%
Crohn's disease (CrD)	20	0	0.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	1	11.1%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	2	12.5%
Hashimoto's thyroiditis (HT)	20	0	0.0%
Idiopathic inflammatory myopathy (IIM)	200	9	4.5%
Infectious Disease	20	1	5.0%
Mixed connective tissue disease (MCTD)	91	11	12.1%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	0	0.0%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	0	0.0%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	1	11.1%
Rheumatoid arthritis (RA)	35	0	0.0%
Sarcoidosis	15	0	0.0%
Spondyloarthritis	16	0	0.0%
Systemic sclerosis (SSc)	217	5	2.3%
Total Controls	**898**	**31**	**3.5%**

Scl-70:

Clinical sensitivity and specificity for the Aptiva Scl-70				
		Diagnosis		
		SSc	Controls	Totals
Aptiva Scl-70	Positive ≥5.0	66	60	126
	Negative <5.0	151	992	1143
	Total	217	1052	1269

Sensitivity	30.4%	(24.7−36.8%)
Specificity	94.3%	(92.7−95.5%)

Scl-70: Distribution of target and differential disease samples and antibody positivity rate:			
Diagnostic Group	***N***	**Scl-70 Positive**	
		n	**(%)**
Target condition			
Systemic sclerosis (SSc)	217	66	30.4%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	3	30.0%
Autoimmune hepatitis type 2 (AIH-2)	40	0	0.0%
Antiphospholipid syndrome (APS)	12	2	16.7%
Atopic dermatitis	16	1	6.3%
Celiac disease (CD)	43	2	4.7%
Crohn's disease (CrD)	20	1	5.0%
Dermatitis herpetiformis (DH)	7	1	14.3%
Drug-induced liver injury	9	1	11.1%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	2	12.5%
Hashimoto's thyroiditis (HT)	20	2	10.0%
Idiopathic inflammatory myopathy (IIM)	200	4	2.0%
Infectious Disease	20	0	0.0%
Mixed connective tissue disease (MCTD)	91	8	8.8%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	0	0.0%
Polymyalgia Rheumatica	13	1	7.7%
Prostate Cancer	15	2	13.3%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	0	0.0%
Rheumatoid arthritis (RA)	35	1	2.9%
Sarcoidosis	15	0	0.0%
Sjögren's Syndrome (SjS)	141	13	9.2%
Spondyloarthritis	16	0	0.0%
Systemic lupus erythematosus (SLE)	230	16	7.0%
Total Controls	**1052**	**60**	**5.7%**

Jo-1:

Clinical sensitivity and specificity for the Aptiva Jo-1				
		Diagnosis		
		IIM	Controls	Totals
Aptiva Jo-1	Positive ≥5.0	23	7	30
	Negative <5.0	177	1062	1239
	Total	200	1069	1269

Sensitivity	11.5%	(7.8−16.7%)
Specificity	99.3%	(98.7−99.7%)

Jo-1: Distribution of target and differential disease samples and antibody positivity rate:			
Diagnostic Group	***N***	**Jo-1 Positive**	
		n	**(%)**
Target condition			
Idiopathic inflammatory myopathy (IIM)	217	23	11.5%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	0	0.0%
Autoimmune hepatitis type 2 (AIH-2)	40	0	0.0%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	0	0.0%
Crohn's disease (CrD)	20	0	0.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	0	0.0%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	0	0.0%
Hashimoto's thyroiditis (HT)	20	0	0.0%
Infectious Disease	20	0	0.0%
Systemic sclerosis (SSc)	217	1	0.5%
Mixed connective tissue disease (MCTD)	91	0	0.0%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	0	0.0%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	0	0.0%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	0	0.0%
Rheumatoid arthritis (RA)	35	0	0.0%
Sarcoidosis	15	0	0.0%
Syphilis	3	0	0.0%
Sjögren's Syndrome (SjS)	141	5	3.5%
Spondyloarthritis	16	0	0.0%
Systemic lupus erythematosus (SLE)	230	1	0.4%
Total Controls	**1069**	**7**	**0.7%**

Centromere:

Clinical sensitivity and specificity for the Aptiva Centromere				
		Diagnosis		
		SSc	Controls	Totals
Aptiva Centromere	Positive ≥5.0	102	32	134
	Negative <5.0	115	1020	1135
	Total	217	1052	1269

Sensitivity	47.0%	(40.5−53.6%)
Specificity	97.0%	(95.7−97.8%)

Centromere: Distribution of target and differential disease samples and antibody positivity rate:			
Diagnostic Group	***N***	**Centromere Positive**	
		n	**(%)**
Target condition			
Systemic sclerosis (SSc)	217	102	47.0%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	1	10.0%
Autoimmune hepatitis type 2 (AIH-2)	40	3	7.5%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	4	9.3%
Crohn's disease (CrD)	20	1	5.0%
Dermatitis herpetiformis (DH)	7	1	14.3%
Drug-induced liver injury	9	0	0.0%
Fibromyalgia	8	1	12.5%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	2	12.5%
Hashimoto's thyroiditis (HT)	20	1	5.0%
Idiopathic inflammatory myopathy (IIM)	200	4	2.0%
Infectious Disease	20	0	0.0%
Mixed connective tissue disease (MCTD)	91	1	1.1%
Nodal Osteoarthritis	19	1	5.3%
Primary biliary cholangitis (PBC)	15	2	13.3%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	1	6.7%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	0	0.0%
Rheumatoid arthritis (RA)	35	1	2.9%
Sarcoidosis	15	0	0.0%
Sjögren's Syndrome (SjS)	141	3	2.1%
Spondyloarthritis	16	0	0.0%
Systemic lupus erythematosus (SLE)	230	5	2.2%
Total Controls	**1052**	**32**	**3.0%**

Ribo-P:

Clinical sensitivity and specificity for the Aptiva Ribo-P				
		Diagnosis		
		SLE	Controls	Totals
Aptiva Ribo-P	Positive ≥5.0	27	3	30
	Negative <5.0	203	1036	1239
	Total	230	1039	1269

Sensitivity	11.7%	(8.2−16.5%)
Specificity	99.7%	(99.2−99.9%)

Ribo-P: Distribution of target and differential disease samples and antibody positivity rate:			
Diagnostic Group	***N***	**Ribo-P Positive**	
		n	**(%)**
Target condition			
Systemic lupus erythematosus (SLE)	230	27	11.7%
Differential diagnosis controls			
Autoimmune hepatitis type 1 (AIH-1)	10	0	0.0%
Autoimmune hepatitis type 2 (AIH-2)	40	1	2.5%
Antiphospholipid syndrome (APS)	12	0	0.0%
Atopic dermatitis	16	0	0.0%
Celiac disease (CD)	43	0	0.0%
Crohn's disease (CrD)	20	0	0.0%
Dermatitis herpetiformis (DH)	7	0	0.0%
Drug-induced liver injury	9	0	0.0%
Fibromyalgia	8	0	0.0%
Gout	6	0	0.0%
Granulomatosis with polyangiitis (GPA)	19	0	0.0%
Graves' Disease	16	0	0.0%
Hashimoto's thyroiditis (HT)	20	0	0.0%
Idiopathic inflammatory myopathy (IIM)	200	0	0.0%
Infectious Disease	20	0	0.0%
Mixed connective tissue disease (MCTD)	91	1	1.1%
Nodal Osteoarthritis	19	0	0.0%
Primary biliary cholangitis (PBC)	15	0	0.0%
Polymyalgia Rheumatica	13	0	0.0%
Prostate Cancer	15	0	0.0%
Psoriasis	7	0	0.0%
Psoriatic Arthritis	9	0	0.0%
Rheumatoid arthritis (RA)	35	0	0.0%
Sarcoidosis	15	0	0.0%
Sjögren's Syndrome (SjS)	141	1	0.7%
Spondyloarthritis	16	0	0.0%
Systemic sclerosis (SSc)	217	0	0.0%
Total Controls	**1039**	**3**	**0.3%**

D Clinical Cut-Off:

The cut-off for the dsDNA, SS-B, Ro60, Ro52, Sm, RNP, Scl-70, Jo-1, Centromere and Ribo-P assay was determined using a cohort of 120 native serum samples from subjects consisting of 16 patients with celiac disease, 18 patients with Hashimoto's thyroiditis, 25 patients with infectious diseases, 7 patients with primary biliary cholangitis (PBC), 2 patients with PBC/autoimmune hepatitis (AIH), 8 patients with primary sclerosing cholangitis (PSC), 1 patient with PSC/AIH, 30 patients with rheumatoid arthritis and 13 patients with Lyme disease. This cohort was used to determine a preliminary cut-off value. The preliminary cut-off was verified using samples from the intended use population to ensure optimal differentiation for the Aptiva CTD Essential Reagent analytes.

The following cutoff is used for the RNP, Sm, Ro52, Ro60, SS-B, Scl-70, Centromere and Ribo-P assays in the Aptiva CTD Essential Reagent:

Negative	<5.00 FLU
Positive	≥5.00 FLU

For the dsDNA assay, the following cutoff is used in the Aptiva CTD Essential Reagent:

Negative	<27.00 IU/mL
Indeterminate	27.00 – 35.00 IU/mL
Positive	≥35.00 IU/mL

E Expected Values/Reference Range:

A negative Aptiva CTD Essential Reagent test result is expected for apparently healthy individuals. To determine the expected values for the Aptiva CTD Essential Reagent, a panel of 115 apparently healthy blood donors (71 females/44 males, ages 18 to 59 years, with an average and median age of 33 years) were tested on the Aptiva CTD Essential Reagent. The results are as follows:

Assay	Number of samples positive	Mean concentration	Range
dsDNA	0 (0.0%)*	5.84 IU/mL	2.30 – 27.71 IU/mL
RNP	2 (1.7%)	0.97 FLU	0.50 - 31.32 FLU
Sm	0 (0.0%)	0.26 FLU	0.25 – 1.12 FLU
Ro52	1 (0.9%)	0.40 FLU	0.25 – 5.64 FLU
Ro60	2 (1.7%)	2.84 FLU	0.50 – 214.80 FLU
SS-B	0 (0.0%)	0.67 FLU	0.40 – 4.84 FLU
Scl-70	1 (0.9%)	1.14 FLU	0.50 – 6.58 FLU
Jo-1	0 (0.0%)	0.27 FLU	0.25 – 0.77 FLU
Centromere	1 (0.9%)	0.88 FLU	0.50 – 13.83 FLU
Ribo-P	0 (0.0%)	0.29 FLU	0.25 – 1.30 FLU

* – One sample (0.9%) was determined to be indeterminate for the dsDNA analyte.

VIII Proposed Labeling:

The labeling supports a substantial equivalence finding for this device.

IX Conclusion:

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