510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K210902

B Applicant

Phadia AB

C Proprietary and Established Names

EliA Ro52
EliA Ro60

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LKJ	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:

IgG autoantibodies specific to SS-A/Ro proteins (52 kDa and 60 kDa)

C Type of Test:

Automated semi-quantitative solid phase fluoroenzymeimmunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

EliA Ro52 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Ro52 in human serum as an aid in the diagnosis of Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) in conjunction with other laboratory and clinical findings. EliA Ro52 uses the EliA IgG method.

EliA Ro60 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Ro60 in human serum as an aid in the diagnosis of Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA Ro60 uses the EliA IgG method.

B Indication(s) for Use:

Same as intended use.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

For use on the Phadia 250 instrument and the Phadia 2500 and Phadia 5000 instrument series (E-modules).

IV Device/System Characteristics:

A Device Description:

The EliA Ro52 and the EliA Ro60 are composed of assay-specific reagents, EliA method-specific reagents, and general reagents.

Assay-Specific Reagents include:

- For EliA Ro52
  EliA Ro52 Wells: coated with human recombinant SS-A/Ro (52 kDa) protein – 2 carriers (12 wells each), ready to use;
- For EliA Ro60
  EliA Ro60 Wells: coated with human recombinant SS-A/Ro (60 kDa) protein – 4 carriers (12 wells each), ready to use;
- EliA ANA Positive Control 250 or 2500/5000: Human serum in PBS containing IgG antibodies to dsDNA, RNP, Sm, Ro, La, Scl-70, CENP and Jo-1 – 6 single use vials, 0.3 mL each, ready to use;

- EliA ANA 3 Positive Control 250 or 2500/5000: Human monoclonal antibodies in Tris buffer containing IgG antibodies to Ro52, Rib-P and RNA Pol III – 6 single use vials, 0.3 mL each, ready to use;
- EliA IgG/IgM/IgA Negative Control 250 or 2500/5000: Human blood preparation from healthy donors in PBS containing BSA, detergent and 0.095% sodium azide – 6 single-use vials, 0.3 mL each, ready to use.

EliA Method-Specific Reagents include:

- EliA Sample Diluent: PBS containing BSA, detergent and 0.095% sodium azide – 6 bottles, 48 mL each, ready to use; or 6 bottles, 400 mL each, ready to use.
- EliA IgG Conjugate 50 or 200: β-Galactosidase labeled anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and 0.06% sodium azide – 6 wedge shaped bottles, 5 mL each, ready to use; or 6 wedge shaped bottles, 19 mL each, ready to use.
- EliA IgG Calibrator Strips: Human IgG (0, 4, 10, 20, 100, 600 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide – 5 strips, 6 single-use vials per strip, 0.3 mL each, ready to use.
- EliA IgG Curve Control Strips: Human IgG (20 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide – 5 strips, 6 single-use vials per strip, 0.3 mL each, ready to use.
- EliA IgG Calibrator Well: coated with mouse monoclonal antibodies – 4 carriers (12 wells each), ready to use.

General Reagents include:

- Development Solution: 0.01% 4-Methylumbelliferyl-β-D-galactoside, <0.0010% preservative
- Stop Solution: 4% Sodium Carbonate
- Washing Solution Additive: detergent, preservative <0.13%
- Washing Solution Concentrate: phosphate buffer

B Principle of Operation:

The EliA Ro52 and the EliA Ro60 are semi-quantitative solid-phase fluoroimmunoassay for the determination of autoantibodies against SS-A/Ro 52 kDa and 60 kDa proteins. Both assays are fully integrated and automated system which comprises of assay-specific reagents, EliA method-specific reagents, and general reagents.

The specific antigen (SS-A/Ro 52kDa protein or SS-A/Ro 60 kDa protein) is coated to the EliA solid phase component (EliA Well). The EliA wells are molded cups comparable to excised wells from a microtiter plate. If present in the patient's specimen, antibodies to the SS-A/Ro 52 kDa protein or SS-A/Ro 60 kDa protein bind to these specific antigens coated on the EliA well. After washing away non-bound antibodies, enzyme-labeled antibodies against human IgG antibodies (EliA IgG Conjugate) are added to form an antibody-conjugate complex. After incubation, non-bound conjugate is washed away, and the bound complex is incubated with a Development Solution. Following a reaction period, the reaction is stopped and the fluorescence of the reaction mixture is measured. As the extent of the fluorescent signal corresponds to the amount of antibody of interest that is bound to the antigen coated EliA well, the higher the value of fluorescent signal detected by the instrument, the higher the amount of antibody bound and

detected in the sample tested. To evaluate the test results, the fluorescence from patient samples is directly compared to a calibrator control curve.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Quanta Flash Ro52, Quanta Flash Ro52 Calibrators, Quanta Flash Ro52 Controls
Quanta Flash Ro60, Quanta Flash Ro60 Calibrators, Quanta Flash Ro60 Controls

B Predicate 510(k) Number(s):

K141655
K141328

C Comparison with Predicate(s):

EliA Ro52

Device & Predicate Device(s):	K210902	K141655
Device Trade Name	EliA Ro52	QUANTA Flash Ro52
General Device Characteristic Similarities		
Intended Use/ Indications For Use	EliA Ro52 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Ro52 in human serum as an aid in the diagnosis of Sjögren's syndrome (SS), systemic lupus erythematosus (SLE), systemic sclerosis (SSc) and idiopathic inflammatory myopathies (IIM) in conjunction with other laboratory and clinical findings. EliA Ro52 uses the EliA IgG method.	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 (SLE), Sjögren's Syndrome, Systemic Sclerosis, Idiopathic Inflammatory Myopathies.
Classification	Class II	Same
Regulation Number	21 CFR 866.5100	Same
Assay Technique	ELISA	Same
Type of Test	Semi-quantitative	Same
Reaction Temperature	37°C controlled	Same
General Device Characteristic Differences		
Antigen	Human recombinant SSA/Ro 52 (kDa) protein	Purified recombinant Ro52 antigen

Device & Predicate Device(s):	K210902	K141655
Product Code	LKJ	OBE
Sample Dilution	1:100	1:23
Reporting of Results	EliA U/mL (arbitrary)	Chemiluminescent units (CU)
Instrumentation	Phadia 250 and the E-Modules of the Phadia 2500 and Phadia 5000 series	BIO-FLASH instrument.
Detection antibody	ß-Galactosidase conjugated mouse anti-human IgG (monoclonal antibodies)	Isoluminol conjugated anti-human IgG
Signal	Fluorescence	Relative Light Units
Calibration	6-point total IgG Calibration. 6 vials of human IgG at concentrations of 0, 4, 10, 20, 100, 600 µg/L	Lot specific Master Curve and two Calibrators
Interpretation of results	Negative: < 7 EliA U/mL Equivocal: 7-10 EliA U/mL Positive: > 10 EliA U/mL	Negative: < 20 CU Positive: > 20 CU
Substrate	0.01% 4-Methylumbelliferyl-β-D-galactoside with preservative	"Trigger" reagents

EliA Ro60

Device & Predicate Device(s):	K210902	K141328
Device Trade Name	EliA Ro60	QUANTA Flash Ro60
General Device Characteristic Similarities		
Intended Use/ Indications For Use	EliA Ro60 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Ro60 in human serum as an aid in the diagnosis of Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA Ro60 uses the EliA IgG method.	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, is an aid in the diagnosis of Systemic Lupus Erythematosus and Sjögren's Syndrome.
Classification	Class II	Same
Regulation Number	21 CFR 866.5100	Same
Assay Technique	ELISA	Same
Type of Test	Semi-quantitative	Same

Device & Predicate Device(s):	K210902	K141328
Reaction Temperature	37°C controlled	Same
General Device Characteristic Differences		
Antigen	Human recombinant SS-A/Ro 60 (kDa) protein	Purified recombinant Ro60 antigen
Product Code	LKJ	LLL
Sample Dilution	1:100	1:23
Reporting of Results	EliA U/mL (arbitrary)	Chemiluminescent units (CU)
Instrumentation	EliA Ro60 uses the EliA IgG method on the instruments Phadia 250 and the E-Modules of the Phadia 2500 and Phadia 5000 series.	Quanta Flash Ro60 is run on the BIO-FLASH instrument.
Detection Antibody	ß-Galactosidase conjugated anti-human IgG (mouse monoclonal antibodies)	Isoluminol conjugated anti-human IgG
Signal	Fluorescence	Relative Light Units (RLU)
Calibration	6-point total IgG Calibration 6 vials of human IgG at concentrations of 0, 4, 10, 20, 100, 600 µg/L	Lot specific Master Curve and two Calibrators
Interpretation of results	Negative < 7 EliA U/mL Equivocal 7-10 EliA U/mL Positive > 10 EliA U/mL	Negative: < 20 CU Positive: > 20 CU
Substrate	0.01% 4-Methylumbelliferyl-β-D-galactoside with preservative	"Trigger" reagents

VI Standards/Guidance Documents Referenced:

- 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
- CLSI EP07, 3rd Edition, Interference Testing in Clinical Chemistry
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- 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
- CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents, Approved Guideline
- CLSI EP37, 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility.

Within-lab Imprecision:

Within-lab imprecision of the EliA Ro52 and the EliA Ro60 was assessed by testing four serum samples using one lot of reagent on one Phadia 250 instrument. Samples were tested in duplicate per run, two runs per day for 20 days, for a total of 80 observations per sample. The results are presented in the tables below.

Within-Lab Precision of the EliA Ro52										
Sample	**N**	**Mean (EliA U/mL)**	**Within-Run**		**Between-Run**		**Between-Day**		**Within-Lab**	
			SD	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	80	3.0	0.1	2.9	0.1	3.6	0.0	1.0	0.1	4.6
2	80	7.9	0.2	2.9	0.2	2.6	0.2	2.0	0.3	3.9
3	80	10.5	0.3	2.5	0.5	4.7	0.3	3.2	0.6	5.3
4	80	69.1	1.9	2.7	4.6	6.6	3.6	5.2	4.9	7.1

Within-Lab Precision of the EliA Ro60										
Sample	**N**	**Mean (EliA U/mL)**	**Within-Run**		**Between-Run**		**Between-Day**		**Within-Lab**	
			SD	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	80	2.6	0.1	5.4	0.2	7.1	0.1	2.2	0.2	8.9
2	80	6.3	0.2	2.6	0.6	9.3	0.3	5.4	0.6	9.7
3	80	10.0	0.2	2.0	0.7	6.6	0.4	3.8	0.7	6.9
4	80	33.3	1.3	3.8	2.2	6.8	0.0	0.0	2.6	7.7

Reproducibility

The reproducibility of the EliA Ro52 and the EliA Ro60 was determined by testing five samples on three Phadia 250 instruments. Each sample was assessed in quadruplicate per run, one run per day for seven days using three reagent lots to generate 84 observations per sample on each instrument, or a total of 252 observations per sample. The results are presented in the tables below.

Reproducibility of EliA Ro52												
Sample	**N**	**Mean (EliA U/mL)**	**Within-Run**		**Between-Run/Day**		**Between-Instrument**		**Between-Lot**		**Total**	
			SD	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	252	4.4	0.2	3.7	0.3	5.8	0.1	1.3	0.0	0.0	0.3	6.9
2	252	8.3	0.3	3.0	0.3	3.2	0.0	0.0	0.2	2.2	0.4	5.0
3	252	10.6	0.3	2.5	0.3	2.9	0.1	0.7	0.2	1.6	0.5	4.1
4	252	32.8	0.8	2.4	1.2	3.6	0.6	1.7	1.3	3.9	2.0	5.9
5	252	215.4	8.3	3.9	7.9	3.7	8.2	3.8	4.2	2.0	14.7	5.7

Reproducibility of EliA Ro60												
Sample	**N**	**Mean (EliA U/mL)**	**Within-Run**		**Between-Run/Day**		**Between-Instrument**		**Between-Lot**		**Total**	
			SD	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	252	3.5	0.1	3.6	0.3	7.2	0.1	1.5	0.1	1.7	0.3	8.2
2	252	6.1	0.2	3.0	0.3	4.5	0.0	0.0	0.0	0.4	0.3	5.4
3	252	10.2	0.2	2.3	0.3	3.2	0.1	0.7	0.2	2.1	0.5	4.4
4	252	32.2	1.1	3.3	1.3	3.9	0.0	0.0	0.9	2.9	1.9	5.8
5	252	201.0	9.6	4.8	11.6	5.7	2.9	1.4	10.6	5.3	18.6	9.1

Reproducibility on the Phadia 2500E and Phadia 5000E instrument series:

The reproducibility of the EliA Ro52 and the EliA Ro60 was determined by testing five samples on three Phadia 2500E instruments. Each sample was assessed in quadruplicate per run, one run per day for seven days using one reagent lot to generate 84 observations per sample. The results are presented in the tables below.

Reproducibility of EliA Ro52 on Phadia 2500E										
Sample	**N**	**Mean (EliA U/mL)**	**Within-Run**		**Between-Run/Day**		**Between-Instrument**		**Total**	
			SD	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	84	4.7	0.2	4.0	0.1	3.0	0.1	2.2	0.3	5.0
2	84	8.6	0.3	3.5	0.2	2.3	0.1	0.7	0.4	4.2
3	84	11.0	0.3	2.5	0.5	4.7	0.2	1.9	0.6	5.4
4	84	36.3	1.5	4.0	1.0	2.8	0.0	0.0	1.8	4.9
5	84	212.9	10.3	4.8	9.1	4.3	0.0	0.0	13.7	6.4

Reproducibility of EliA Ro60 on Phadia 2500E										
Sample	**N**	**Mean (EliA U/mL)**	**Within-Run**		**Between-Run/Day**		**Between-Instrument**		**Total**	
			SD	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
1	84	3.6	0.2	5.3	0.3	6.9	0.1	1.4	0.3	8.7
2	84	6.6	0.2	2.9	0.3	4.3	0.2	2.9	0.4	5.2
3	84	10.5	0.3	2.9	0.4	3.4	0.1	0.8	0.5	4.5
4	84	35.8	1.2	3.3	1.3	3.7	0.0	0.0	1.8	4.9
5	84	219.9	11.7	5.3	10.3	4.7	10.7	4.9	18.9	7.1

2. Linearity:

The linearity of the EliA Ro52 and the EliA Ro60 was evaluated based on the recommendations of CLSI EP06-Ed2.

Three patient serum samples were serially diluted using a normal blood donor serum sample for a total of 9 dilutions for each dilution series across. The following patient serum sample concentrations were used: 266.1 EliA U/mL, 89.5 EliA U/mL, and 15.9 EliA U/mL were used for EliA Ro52 testing on the Phadia 250 instrument; 275.5 EliA U/mL, 97.7 EliA U/mL, 16.2 EliA U/mL were used for EliA Ro52 testing on the Phadia 2500E instrument; 249.4 EliA U/mL, 88.5 EliA U/mL, and 15.4 EliA U/mL were used for EliA Ro60 testing on the Phadia 250 instrument; and 263.6 EliA U/mL, 92.3 EliA U/mL, and 12.3 EliA U/mL were used for EliA Ro60 testing on the Phadia 2500E instrument. Each sample was then tested in quadruplicate using one lot of EliA Ro52 Well and EliA Ro60 Well, and a single Phadia 250 instrument or Phadia 2500E instrument, respectively. The results were analyzed using by fitting a regression model, assuming proportionality between the observed concentration and the expected concentration, using the weighted least squares method.

EliA Ro52 on Phadia 250:

Sample Number	Range [EliA U/mL]	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	8.1 - 266.1	0.987 (0.961 - 1.012)	1.226 (0.865 – 1.587)	0.999	99.7 – 116.7
2	3.2 – 89.5	0.994 (0.977 - 1.011)	0.435 (0.320 – 0.551)	0.999	97.8 – 117.4
3	0.7 - 15.9	0.948 (0.884 - 1.013)	0.091 (-0.053 – 0.235)	0.990	92.4 – 127.5
Combined	0.7 – 266.1	1.014 (0.983 – 1.045)	0.025 (-0.08 – 0.130)	0.993	92.3 – 118.5

EliA Ro52 on Phadia 2500E:

Sample Number	Range [EliA U/mL]	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	7.1 - 275.5	0.862 (0.811 – 0.913)	0.776 (-0.291 – 1.842)	0.994	83.9 – 100
2	2.4 – 97.7	0.911 (0.884 – 0.938)	0.490 (0.265 – 0.715)	0.998	92.8 – 116.7
3	0.7 - 16.2	0.904 (0.831 – 0.976)	-0.045 (-0.163 – 0.07)	0.987	82.6 – 104.4
Combined	0.7 – 275.5	0.928 (0.902 – 0.953)	-0.01 (-0.114 – 0.094)	0.994	80.0 – 114.3

For the EliA Ro52 assay, the results support the linearity of the claimed analytical measuring range of 0.7 – 240 EliA U/mL.

EliA Ro60 on Phadia 250:

Sample Number	Range [EliA U/mL]	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	2.7 – 88.5	1.016 (0.967 – 1.064)	0.674 (0.397 – 0.951)	0.996	100 – 119.3
2	11.0 – 249.4	1.047 (1.030 – 1.064)	1.282 (0.515 – 2.048)	0.999	100 – 116.1
3	0.5 – 15.4	0.988 (0.971 – 1.005)	0.053 (0.20 – 0.086)	0.999	95.1 – 117.1
Combined	0.5 – 249.4	1.063 (1.044 – 1.081)	-0.004 (-0.076 – 0.07)	0.998	94.0 – 117.4

EliA Ro60 on Phadia 2500E:

Sample Number	Range [EliA U/mL]	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	2.6 – 92.3	1.013 (0.968 – 1.058)	0.136 (-0.125 – 0.396)	0.997	95.9 – 108.1
2	4.8 – 263.6	1.033 (0.980 – 1.086)	0.910 (0.171 – 1.649)	0.995	96.6 – 114.3
3	0.6 - 12.3	0.963 (0.942 – 0.984)	0.040 (0.009 – 0.071)	0.999	95.2 – 104.4
Combined	0.6 – 263.6	1.069 (1.045 – 1.093)	-0.039 (-0.131– 0.053)	0.996	90.9 – 114.5

For the EliA Ro60 assay, the results support the linearity of the claimed analytical measuring range of 0.5 – 240 EliA U/mL.

Hook Effect/Over the Range Results:

Not applicable. Results above the upper limit of the measuring range are reported as ">240" for both assays. No recommendations are made for dilution of samples outside of the analytical measuring range in the Directions for Use of each assay.

3. Analytical Specificity/Interference:

Comparison to Reference Sera:

CDC (Center for Disease Controls and Prevention) ANA human reference sera panel samples #1– #12 were tested using one lot of the Ro52 and one lot of the EliA Ro60 EliA Rib-P assay reagents.

For the EliA Ro52, CDC ANA #2 (speckled pattern in FANA), CDC ANA #7 (SS-A/Ro) and CDC ANA #10 (Jo-1) showed positive results with 13.9 EliA U/mL, 19.5 EliA U/mL and 247.4 EliA U/mL, respectively. CDC ANA #3 (RNP-Sm, SS-A/Ro, SS-B/La) showed an equivocal result with 7.4 EliA U/mL. All other CDC ANA samples remained negative.

For the EliA Ro60, CDC ANA #2 (speckled pattern in FANA), CDC ANA #3 (RNP-Sm, SS-A/Ro, SS-B/La), and CDC ANA #7 (SS-A/Ro) showed positive results with 143.0 EliA U/mL, 146.2 EliA U/mL and 207.2 EliA U/mL, respectively. All other CDC ANA samples remained negative.

Endogenous and Exogenous Interference:

Three serum samples: one negative sample, one sample with a concentration within the equivocal range, and one positive sample, were spiked with the different interfering substances or blank solution. For the EliA Ro52, the concentration of the samples were 1.0 EliA U/mL, 10.4 EliA U/mL, and 116.5 EliA U/mL. For the EliA Ro60, the concentration of the samples were 0.5 EliA U/mL, 8.0 EliA U/mL, and 102.5 EliA U/mL. All samples were tested in triplicate in two runs using one lot of EliA antigen specific wells and one lot of system reagents on the Phadia 250 instrument. The quotient between serum sample spiked with interference substance and serum sample spiked with blank was calculated for each sample spiked with the interfering substance. No interference, the quotient between samples

spiked with interference substance or blank within 0.90 – 1.10, was observed up to the concentrations listed in the table below:

Potential Interfering Compound	Concentration with No Interference
Endogenous substance	
Bilirubin F	40 mg/dL
Bilirubin C	40 mg/dL
Hemoglobin	1000 mg/dL
Lipemic factor	2000 mg/dL
Rheumatoid factor	500 IU/mL
Human IgG	3500 mg/dL
Exogenous substance	
Ibuprofen	21.9 mg/dL
Losartan	1.14 mg/dL
Hydroxychloroquine	0.23 mg/dL
Azathioprine	0.26 mg/dL
Prednisone	0.01 mg/dL
Rituximab	109 mg/dL
Infliximab	26.4 mg/dL
Omeprazole	0.84 mg/dL
Diltiazem	0.09 mg/dL

4. Assay Reportable Range:

   The reportable range for the EliA Ro52 is 0.7 – 240.0 EliA U/mL.

   The reportable range for the EliA Ro60 is 0.5 – 240.0 EliA U/mL.

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

   Traceability:

   The EliA IgG calibrators are traceable (via unbroken chain of calibrations) to the International Reference Preparation (IRP) 67/86 of Human Serum Immunoglobulins A, G and M from WHO. New batches of IgG calibrators are compared to a secondary standard (standardized with the IRP) or the IRP directly and adjusted accordingly to meet the correct concentration.

   The instrument measures specific IgG concentrations in µg/L. By using a conversion factor given by the lot-specific code of the EliA test well, the results are automatically converted to EliA U/mL.

   Stability:

   Stability (open and closed vial, and on-board) of EliA IgG method-specific reagents and general EliA reagents were previously established through the clearance of other EliA tests,

e.g., K141375, K061165. The stability for the EliA Ro52 and the EliA Ro60 assay-specific reagents (i.e., EliA Ro52 Wells and EliA Ro60 Wells) were determined as follows:

Shelf-life:
The stability of the EliA Ro52 Wells and the EliA Ro60 Wells was evaluated with a real-time study on the Phadia 250 instrument. Three lots EliA Ro52 Wells were stored under standard conditions in sealed foil bags with desiccant bags inside, at 2–8°C, and tested 0, 7, 13, 19, and 25 months. Each lot of wells was initially tested in three identical initial runs and in two identical test runs at the later time points. The wells were tested with four positive samples and with nine negative samples in single determination with a full calibration curve.

The shelf-life was determined to be up to 24 months at 2–8°C for the EliA Ro52 and up to 18 months at 2–8°C for EliA Ro60.

On-board stability:
The on-board stability the EliA Ro52 Wells and the EliA Ro60 Wells was tested over 8 weeks using three positive and two negative samples on the Phadia 250 instrument. For this study, the carriers were stored at 10°C ±20% and 80% ±20% humidity in the Phadia 250 carrier storage tray in a temperature- and humidity-test cabinet for up to 8 weeks. The following conditions were tested: 8 weeks at 10°C and 80% humidity, 8 weeks at 2–8°C, 4 weeks at 2–8°C and 4 weeks at 10°C and 80% humidity, and 2 weeks at 2–8°C and 6 weeks at 10°C with 80% humidity. At 12 different days during the study, the carriers were removed from the holder, shaken three times to simulate dispensing, and were returned to the carrier storage tray. The wells were measured together after 8 weeks, using three positive and two negative samples, which were assayed in duplicate.

The on-board stability for the Phadia 250 was determined to be 28 days at 2–8°C.

Open (in-use) Stability:
Stability after first opening of the foil bag containing the EliA Ro52 and EliA Ro60 wells was tested with a real-time study. In this study, EliA Ro52 and EliA Ro60 wells in the carrier (stored at 2–8°C) were removed from the foil bag, stored 5 minutes at room temperature, closed again by the zipper, and stored at +10°C and 80% humidity in a climate chamber until use. This process of opening and sealing the wells in the carrier was repeated six times within 2 months. The wells were stored at +10°C and 80% humidity in a climate chamber for up to 10 months. At 0, 4, 6, and 10 months, the wells were compared to EliA Ro52 and EliA Ro 60 wells stored under standard conditions at +2 to +8°C. Each positive sample, three for EliA Ro52 and two for EliA Ro60, were tested in triplicate. Each negative sample, two for EliA Ro52 and one for EliA Ro60, were tested in single determinations.

The in-use stability was determined to be 6 months at 2–8°C for the EliA Ro52 and EliA Ro60 wells.

6. Detection Limit:

Limit of blank (LoB), Limit of detection (LoD) and limit of quantitation (LoQ) of the EliA Ro52 assay were determined on both Phadia 250 and Phadia 2500E.

LoB was determined from the measurement of four blank samples (analyte-free, i.e. IgG depleted sera) tested in five replicates per sample in one run on a Phadia 250 and Phadia 2500E instrument, respectively, over three consecutive days using two different lots of test reagents. The LoB was calculated as the 95th percentile for each lot (60 observations per lot). The LoB was determined as the higher value of these two lots for each type of instrument. The claimed LoB for EliA Ro52 and EliA Ro60 is the highest 95th percentile LoB value across the two lots and instrument types.

LoD was determined by testing four low-level samples in five replicates per sample in one run on a Phadia 250 and Phadia 2500E instrument, respectively, over three consecutive days using two different lots of test reagents. The LoD for each lot was calculated based on 240 determinations with 120 blank and 120 low level replicates per instrument type, following the recommendations of CLSI document EP17-A and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%. The claimed LoD for EliA Ro52 and EliA Ro60 is the greatest LoD across the two lots and both instrument types.

LoQ was determined by testing four low-levels samples in five replicates per sample over three consecutive days, in one run on a Phadia 250 and Phadia 2500E instrument, and two reagent lots. The LoQ was defined as the lowest concentration of measurand that meets the predefined imprecision goal of <20% for each lot. The greatest LoQ across the two lots and both instrument types was set as the claimed LoQ for the EliA Ro52 and EliA Ro60 assays.

EliA Ro52			
Instrument	**LoB EliA U/mL**	**LoD EliA U/mL**	**LoQ EliA U/mL**
Phadia 250	0.0	0.2	0.6
E-module of the Phadia 2500E	0.1	0.3	0.7
The claim for both instrument types	**0.1**	**0.3**	**0.7**

EliA Ro60			
Instrument	**LoB EliA U/mL**	**LoD EliA U/mL**	**LoQ EliA U/mL**
Phadia 250	0.1	0.2	0.4
E-module of the Phadia 2500E	0.1	0.2	0.5
The claim for both instrument types	**0.1**	**0.4**	**0.5**

7. Assay Cut-Off:

The assay cut-off and the equivocal range for the EliA Ro52 were established by testing a cohort consisting of 69 apparently healthy blood donors, 19 samples from SLE patients and 9 samples from SS patients on a Phadia 250 instrument. The established cut-off was further verified for the additional intended use groups of IIM and SSc patients by testing an additional 10 IIM and 14 SSc patient sera.

The assay cut-off and the equivocal range of the EliA Ro60 was established by testing a cohort consisting of 70 samples from apparently healthy blood donors, 22 samples from SLE patients and 6 samples from SS patients on a Phadia 250 instrument.

The cut-off was set as follows for the EliA Ro52 and the EliA Ro60:

Test Result	Interpretation
<7 EliA U/mL	Negative
7–10 EliA U/mL	Equivocal
>10 EliA U/mL	Positive

In case of equivocal results, it is recommended to retest the patient after 8–12 weeks.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A total of 208 patient serum samples with antibody concentrations covering the measuring range were tested with the EliA Ro52, the EliA Ro60 and their predicates, QUANTA Flash Ro52 and QUANTA Flash Ro60 assays. The tests were performed in single determination according to the Directions for Use for each assay. Only samples that were found within the measuring intervals of the EliA and/or QUANTA Flash tests were used for the method comparison analysis. Positive percent agreement (PPA), negative percent agreement (NPA), and total agreement were calculated with equivocal results considered as negative or as positive, respectively. The results are summarized in the tables below:

EliA Ro52:

EliA Ro52: equivocal results considered negative		**QUANTA Flash Ro52**		
		Positive: ≥ 20 Units	**Negative: < 20 Units**	**Total**
EliA Ro52	**Positive: > 10 EliA U/mL**	42	2	44
	Negative: < 10 EliA U/mL	10	127	137
	Total	52	129	181

Equivocal Results Considered Negative	Calculation	Agreement (%)	95% CI
PPA	42 / 52	80.8	67.5 – 90.4
NPA	127 / 129	98.4	94.5 – 99.8
Total	(42+127) / 181	93.4	88.8 – 96.5

EliA Ro52: equivocal results considered positive		**QUANTA Flash Ro52**		
		Positive: ≥ 20 Units	**Negative: < 20 Units**	**Total**
EliA Ro52	**Positive: > 7 EliA U/mL**	48	12	60
	Negative: < 7 EliA U/mL	4	117	121
	Total	52	129	181

Equivocal Results Considered Negative	Calculation	Agreement (%)	95% CI
PPA	48 / 52	92.3	81.5 – 97.9
NPA	117 / 129	90.7	84.3 – 95.1
Total	(48+117) / 181	91.2	86.1 – 94.9

EliA Ro60

EliA Ro60: equivocal results considered negative		**QUANTA Flash Ro60**		
		Positive: ≥ 20 Units	**Negative: < 20 Units**	**Total**
EliA Ro60	**Positive: > 10 EliA U/mL**	62	3	65
	Negative: < 10 EliA U/mL	4	35	39
	Total	66	38	104

Equivocal Results Considered Negative	Calculation	Agreement (%)	95% CI
PPA	62 / 66	93.9	85.2 – 98.3
NPA	35 / 38	92.1	78.6 – 98.3
Total	(62+35) / 104	93.3	86.6 – 97.3

EliA Ro60: equivocal results considered positive		**QUANTA Flash Ro60**		
		Positive: ≥ 20 Units	**Negative: < 20 Units**	**Total**
EliA Ro60	**Positive: > 7 EliA U/mL**	64	7	71
	Negative: < 7 EliA U/mL	2	31	33
	Total	66	38	104

Equivocal Results Considered Negative	Calculation	Agreement (%)	95% CI
PPA	64 / 66	97.0	89.5 – 99.6
NPA	31 / 38	81.6	65.7 – 92.3
Total	(64+31) / 104	91.3	84.2 – 96.0

2. Matrix Comparison:

Not applicable, serum is the claimed sample type for the EliA Ro52 and the EliA Ro60.

3. Instrument Comparison:

EliA Ro52:
Performance of EliA Ro52 was evaluated on the Phadia 250 and Phadia 2500E instrument using 57 positive, 6 equivocal and 30 negative samples. The samples were analyzed in single determination on one Phadia 250 and one Phadia 2500E instrument each. A Passing-Bablok regression analysis was performed and demonstrated a slope of 0.94 (0.93 – 0.97 95% CI) and an intercept of 0.91 (0.46 – 1.14 95% CI). The PPA, NPA, and total agreement were calculated with equivocal results considered as negative or as positive, respectively. The results are summarized in the table below:

Percent Ro52 Agreement	Agreement (%), (95% CI)	Agreement (%), (95% CI)
	Equivocal = Negative	Equivocal = Positive
PPA	80.8% (67.5 – 90.4%)	80.8% (67.5 – 90.4%)
NPA	98.4% (94.5 – 99.8%)	98.4% (94.5 – 99.8%)
Total	93.4% (88.8 – 96.5%)	93.4% (88.8 – 96.5%)

EliA Ro60:
Performance of EliA Ro60 was evaluated on the Phadia 250 and Phadia 2500E instrument using 42 positive, 9 equivocal and 39 negative samples. The samples were analyzed in single determination on one Phadia 250 and one Phadia 2500E instrument each. A Passing-Bablok regression analysis was performed and demonstrated a slope of 1.01 (0.97 – 1.04 95% CI) and an intercept of 0.24 (0.15 – 0.48 95% CI). The PPA, NPA, and total agreement were calculated with equivocal results considered as negative or as positive, respectively. The results are summarized in the table below:

Percent Ro60 Agreement	Agreement (%), (95% CI)	Agreement (%), (95% CI)
	Equivocal = Negative	Equivocal = Positive
PPA	93.9% (85.2 – 98.3%)	97.0% (89.5 – 99.6%)
NPA	92.1% (78.6 – 98.3%)	81.6% (65.7 – 92.3%)
Total	93.3% (86.6 – 97.3%)	91.3% (84.2 – 96.0%)

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

EliA Ro52:

A total of 755 clinically and ethnically defined serum samples were used to determine the clinical sensitivity and specificity of the EliA Ro52 assay. Samples with a diagnosis of

systemic lupus erythematosus (SLE) (n=120), Sjögren's syndrome (SS) (n=60), idiopathic inflammatory myopathies (IIM) (n=94) and systemic sclerosis (SSc) (n=91) represent the "Target Disease". Samples with a diagnosis of various other autoimmune and infectious diseases are represented as the "Control Disease" group (n=390). The distribution of the diseases and the EliA Ro52 result for each clinical subgroup is summarized in the following table.

	N	EliA Ro52		
		Positive n	Equivocal n	Negative n
Target Disease				
Systemic lupus erythematosus (SLE)	**120**	**57**	**4**	**59**
Primary Sjögren's syndrome	**60**	**30**	**3**	**27**
Idiopathic inflammatory myopathies (IIM)	**94**	**34**	**1**	**59**
IIM PM	40	7	0	33
IIM DM	13	3	1	9
IIM Myositis/CTD-overlap	18	3	0	15
IIM Overlap / MCTD	9	9	0	0
IIM Jo-1 Positive	10	9	0	1
IIM Sporadic inclusion body myositis	4	3	0	1
Systemic sclerosis (SSc)	**91**	**19**	**5**	**67**
SSc, diffuse	56	11	3	42
SSc, limited	27	8	2	17
SSc, various*	8	0	0	8
Target Disease (Total)	**365**	**140**	**13**	**212**
Control Disease				
Celiac disease	13	0	0	13
Crohn's disease	12	0	0	12
Ulcerative colitis	35	0	1	34
CTD overlap Non-MCTD	10	0	1	9
Graves' disease	12	0	0	12
Primary antiphospholipid syndrome	12	1	0	11
Primary biliary cholangitis	25	1	2	22
Primary sclerosing cholangitis	24	0	0	24
Type 1 Diabetes	12	0	0	12
Lymphoma	21	0	0	21
Leukemia	20	0	0	20
Varied Cancer	9	0	0	9
Mixed connective tissue disease	10	2	0	8
Rheumatoid arthritis	35	2	0	33
Bacterial infections [total]	17	1	0	16
Bacterial infections (Mycoplasma)	3	0	0	3
Bacterial infections (Borrelia)	7	0	0	7

	N	EliA Ro52		
		Positive n	Equivocal n	Negative n
Bacterial infections (var. Staphylococcus)	3	1	0	2
Bacterial infections (various)	4	0	0	4
Viral infections [total]	80	4	1	75
Viral infections (Dengue)	4	0	0	4
Viral infections (EBV)	3	0	0	3
Viral infections (HBV)	11	1	0	10
Viral infections (HCV)	31	2	1	28
Viral infections (HIV)	24	1	0	23
Viral infections (various)	7	0	0	7
Hashimoto's disease	10	1	0	9
Vasculitis [total]	33	0	0	33
Granulomatosis with Polyangiitis	10	0	0	10
Eosinophilic granulomatosis with polyangiitis	4	0	0	4
Microscopic polyangiitis	13	0	0	13
Polyarteritis nodosa	3	0	0	3
Giant cell arteritis	3	0	0	3
Control Disease (Total)	**390**	**12**	**5**	**373**
TOTAL	**755**	**152**	**18**	**585**

* The "SSc various" group is composed of the patients having SSc, classified according to the ACR/EULAR van den Hoogen 2013 criterion. The patients were not further subgrouped.

Clinical sensitivity and specificity for the EliA Ro52 as an aid in the diagnosis of SLE were shown in the following tables with excluded SS, SSc and IIM samples from the Target Disease group:

Equivocal = negative

EliA Ro52	Diagnosis		
	SLE	Controls	Total
Positive	57	12	69
Negative	63	378	441
Total	120	390	510

	Value	95% CI
Sensitivity	47.5%	38.3 – 56.8
Specificity	96.9%	94.7 – 98.4

Equivocal = positive

EliA Ro52	Diagnosis		
	SLE	Controls	Total
Positive	61	17	78
Negative	59	373	432
Total	120	390	510

	Value	95% CI
Sensitivity	50.8%	41.6 – 60.1
Specificity	95.6%	93.1 – 97.4

Clinical sensitivity and specificity for the EliA Ro52 as an aid in the diagnosis of SS were shown in the following tables with excluded SLE, SSc and IIM samples from the Target Disease group:

Equivocal = negative

EliA Ro52	Diagnosis		
	SS	Controls	Total
Positive	30	12	42
Negative	30	378	408
Total	60	390	450

	Value	95% CI
Sensitivity	50.0%	36.8 – 63.2
Specificity	96.9%	94.7 – 98.4

Equivocal = positive

EliA Ro52	Diagnosis		
	SS	Controls	Total
Positive	33	17	50
Negative	27	373	400
Total	60	390	450

	Value	95% CI
Sensitivity	55.0%	41.6 – 67.9
Specificity	95.6%	93.1 – 97.4

Clinical sensitivity and specificity for the EliA Ro52 as an aid in the diagnosis of IIM were shown in the following tables with excluded SLE, SS, and SSc samples from the Target Disease group:

Equivocal = negative

EliA Ro52	Diagnosis		
	IIM	Controls	Total
Positive	34	12	46
Negative	60	378	438
Total	94	390	484

	Value	95% CI
Sensitivity	36.2%	26.5 – 46.7
Specificity	96.9%	94.7 – 98.4

Equivocal = positive

EliA Ro52	Diagnosis		
	IIM	Controls	Total
Positive	35	17	52
Negative	59	373	432
Total	94	390	484

	Value	95% CI
Sensitivity	37.2%	27.5 – 47.8
Specificity	95.6%	93.1 – 97.4

Clinical sensitivity and specificity for the EliA Ro52 as an aid in the diagnosis of SSc were shown in the following tables with SLE, SS, and IIM samples excluded from the Target Disease group:

Equivocal = negative

EliA Ro52	Diagnosis		
	SSc	Controls	Total
Positive	19	12	31
Negative	72	378	450
Total	91	390	481

	Value	95% CI
Sensitivity	20.9%	13.1 – 30.7
Specificity	96.9%	94.7 – 98.4

Equivocal = positive

EliA Ro52	Diagnosis		
	SSc	Controls	Total
Positive	24	17	41
Negative	67	373	440
Total	91	390	481

	Value	95% CI
Sensitivity	26.4%	17.7 – 36.7
Specificity	95.6%	93.1 – 97.4

EliA Ro60:

A total of 713 clinically and ethnically defined serum samples were used to determine the clinical sensitivity and specificity of the EliA Ro52 assay. Samples with a diagnosis of systemic lupus erythematosus (SLE) (n=120) and Sjögren's syndrome (SS) (n=60) represent the "Target Disease". Samples with a diagnosis of various other autoimmune and infectious diseases are represented as the "Control Disease" group (n=390). The distribution of the diseases and the EliA Ro52 result for each clinical subgroup is summarized in the following table.

	N	EliA Ro60		
		Positive n	Equivocal n	Negative n
Target Disease				
Systemic lupus erythematosus (SLE)	**120**	**58**	**3**	**59**
Primary Sjögren's syndrome	**60**	**41**	**2**	**17**
Target Disease (Total)	**180**	**99**	**5**	**76**
Control Disease				
Idiopathic inflammatory myopathies (IIM)	10	0	0	10
Systemic sclerosis (SSc) [total]	91	21	0	70
SSc, diffuse	56	11	0	45
SSc, limited	27	10	0	17
SSc, various*	8	0	0	8
Celiac disease	13	0	0	13
Crohn's disease	12	0	0	12
Ulcerative colitis	35	0	1	34
CTD overlap Non-MCTD	10	0	1	9
Graves' disease	12	0	0	12
Primary antiphospholipid syndrome	12	1	0	11
Primary biliary cholangitis	25	1	2	22
Primary sclerosing cholangitis	24	0	0	24
Type 1 Diabetes	12	0	0	12
Lymphoma	21	0	0	21
Leukemia	20	0	0	20
Varied Cancer	9	0	0	9
Mixed connective tissue disease	10	2	0	8
Rheumatoid arthritis	35	2	0	33
Bacterial infections [total]	17	1	0	16
Bacterial infections (Mycoplasma)	3	0	0	3
Bacterial infections (Borrelia)	7	0	0	7
Bacterial infections (var. Staphylococcus)	3	1	0	2
Bacterial infections (various)	4	0	0	4

	N	EliA Ro60		
		Positive n	Equivocal n	Negative n
Viral infections [total]	80	4	1	75
Viral infections (Dengue)	4	0	0	4
Viral infections (EBV)	3	0	0	3
Viral infections (HBV)	11	1	0	10
Viral infections (HCV)	31	2	1	28
Viral infections (HIV)	24	1	0	23
Viral infections (various)	7	0	0	7
Hashimoto's disease	10	1	0	9
Vasculitis [total]	33	0	0	33
Granulomatosis with Polyangiitis	10	0	0	10
Eosinophilic granulomatosis with polyangiitis	4	0	0	4
Microscopic polyangiitis	13	0	0	13
Polyarteritis nodosa	3	0	0	3
Giant cell arteritis	3	0	0	3
Control Disease (Total)	**533**	**27**	**1**	**505**
TOTAL	**713**	**126**	**6**	**581**

* The "SSc various" group is composed of the patients having SSc, classified according to the ACR/EULAR van den Hoogen 2013 criterion. The patients were not further subgrouped.

Clinical sensitivity and specificity for the EliA Ro60 as an aid in the diagnosis of SLE were shown in the following tables with excluded SS, SSc and IIM samples from the Target Disease group:

Equivocal = negative

EliA Ro60	Diagnosis		
	SLE	Controls	Total
Positive	58	6	64
Negative	62	436	498
Total	120	442	562

	Value	95% CI
Sensitivity	48.3%	39.1 – 57.6
Specificity	98.6%	97.1 – 99.5

Equivocal = positive

EliA Ro60	Diagnosis		
	SLE	Controls	Total
Positive	61	7	68
Negative	59	435	494
Total	120	442	562

	Value	95% CI
Sensitivity	50.8%	41.6 – 60.1
Specificity	98.4%	96.8 – 99.4

Clinical sensitivity and specificity for the EliA Ro52 as an aid in the diagnosis of SS were shown in the following tables with excluded SLE, SSc and IIM samples from the Target Disease group:

Equivocal = negative

EliA Ro60	Diagnosis		
	SS	Controls	Total
Positive	41	6	47
Negative	19	436	455
Total	60	442	502

	Value	95% CI
Sensitivity	68.3%	55.0 – 79.7
Specificity	98.6%	97.1 – 99.5

Equivocal = positive

EliA Ro60	Diagnosis		
	SS	Controls	Total
Positive	43	7	50
Negative	17	435	452
Total	60	442	502

	Value	95% CI
Sensitivity	71.7%	58.6 – 82.5
Specificity	98.4%	96.8 – 99.4

2. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

   Not applicable

D Clinical Cut-Off:

Same as assay cut-off.

E Expected Values/Reference Range:

The expected value in the normal population is negative. Antibody prevalence in autoimmune patients varies widely depending on disease area. The proportion of sera from a normal population found positive for the antinuclear antibodies covered by the EliA Ro52 and EliA Ro60 test is below 1%. Expected values may vary depending on the population tested. A panel of 400 serum samples from apparently healthy Caucasian blood donors were collected from the Biobank at Phadia GmbH (Germany). A supplemental set of 240 serum samples was added to the study to reflect the ethnic composition of the U.S. The 240 supplemental serum samples consisted of 80 African American, 80 Hispanic, and 80 Asian healthy blood donor specimens.

The distribution of samples and the test results are presented in the following tables:

Study Demographics	Female (N = 320)					Male (N=320)				
	Age									
	≤30	31–40	41–50	51-60	>60	≤30	31–40	41–50	51-60	>60
Caucasian	40	40	40	40	40	40	40	40	40	40
African American	8	8	8	8	8	8	8	8	8	8
Hispanic	8	8	8	8	8	8	8	8	8	8
Asian	8	8	8	8	8	8	8	8	8	8
Total	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**	**64**

	Caucasian		African American		Hispanic		Asian	
	Female	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**
N	200	200	40	40	40	40	40	40
EliA Ro52 (U/mL)								
Median	1.15	1.10	1.31	1.1	0.8	0.8	0.8	0.7
95th Percentile	1.89	1.85	1.89	1.5	1.70	1.3	2	1.52
EliA Ro60 (U/mL)								
Median	0.47	0.52	0.549	0.51	0.40	0.46	0.40	0.42
95th Percentile	0.78	0.87	1.77	0.92	0.73	1.10	0.86	0.74

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.