510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K202540

B Applicant

Phadia AB

C Proprietary and Established Names

EliA Rib-P

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MQA	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 Rib-P proteins

C Type of Test:

Automated semi-quantitative solid phase fluoroimmunoassay

Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002
www.fda.gov

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

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

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:

EliA uses a modular reagent system. The assay-specific, method-specific and general reagents are packaged and sold separately.

The EliA Rib-P Assay-Specific Reagents include:

- EliA Rib-P Wells: coated with human recombinant ribosomal P-proteins P0, P1 and P2 – two carriers (12 wells 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, 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, ready to use.

EliA Method-Specific Reagents include:

- EliA Sample Diluent: PBS containing BSA, detergent and 0.095% sodium azide, 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, 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, ready to use.
- EliA IgG Curve Control Strips: Human IgG (20 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide, ready to use.
- EliA IgG Calibrator Well: coated with mouse monoclonal antibodies, ready to use.

General Reagents are not included but required:

- 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 Rib-P is a semi-quantitative solid-phase fluoroimmunoassay for the determination of autoantibodies against Rib-P. The EliA Rib-P test is fully integrated and automated system which comprises of assay-specific reagents, EliA method-specific reagents, and general reagents.

The antigen (human recombinant ribosomal P-proteins P0, P1, and P2) is immobilized on 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 ribosomal P-proteins P0, P1, and P2 bind to their specific antigen. 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. After stopping the reaction, the fluorescence in the reaction mixture is measured. The assay directly measures the amount of antibody of interest bound to the antigen coating the EliA well, therefore 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 test results, the response for patient samples is compared directly to the response for calibrators.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Quanta Lite Ribosome P Elisa

B Predicate 510(k) Number(s):

K981237

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K202540**	**K981237**
Device Trade Name	EliA Rib-P	QUANTA Lite Ribosome P ELISA
General Device Characteristic Similarities		

Intended Use/ Indications for Use	EliA Rib-P is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to Rib-P in human serum as an aid in the diagnosis of systemic lupus erythematosus (SLE) in conjunction with other laboratory and clinical findings. EliA Rib-P uses the EliA IgG method.	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.
Methodology	ELISA	Same
Dilution	1:100	1:101
Analyte	Autoantibodies to Ribosome P	Same
Sample matrix	Serum	Same
General Device Characteristic Differences		
Type of test	Automated semi-quantitative	Manual semi-quantitative
Antigen	Human recombinant P-proteins P0, P1 and P2	Synthetic Ribosome P antigen
Detection	Fluorescence	Optical density (OD)
Conjugate	ß-Galactosidase conjugated anti-human IgG (mouse monoclonal antibodies)	Horseradish peroxidase conjugated anti-human IgG, (goat)
Controls	EliA ANA 3 Positive Control and EliA IgG/IgM/IgA Negative Control	The Ribosome P ELISA Low Positive, the Ribosome P ELISA High Positive and the ELISA Negative Control
Calibration	6-point total IgG Calibration at concentrations of 0 – 4 – 10 – 20 – 100 – 600 µg/L	One-point calibration
Instrumentation	Phadia 250 and the E-modules of the Phadia 2500 and Phadia 5000 series	Microwell plate reader measuring OD at 450nm and 620nm
Cut-offs	Negative: < 7 EliA U/mL Equivocal: 7–10 EliA U/mL Positive: > 10 EliA U/mL	Negative <20 Units Weak positive 20 – 39 Units Moderate positive 40 – 80 Units Strong positive >80 Units

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: All results presented below were within the manufacturer's pre-determined acceptance criteria for each study.

1. Precision/Reproducibility:

Reproducibility:
The reproducibility of the EliA Rib-P was assessed by testing five serum samples using three lots of assay reagents on three Phadia 250 instruments. Each sample was tested in four replicates per run, one run per day for seven days using three lots of reagent to generate 84 replicates per sample on each instrument, or a total of 252 measurements. The results are summarized in the table below:

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	2.8	0.2	5.9	0.2	6.6	0.2	8.5	0.1	5.0	0.4	13.3
2	252	7.8	0.3	3.2	0.3	4.1	0.3	4.2	0.0	0.0	0.5	6.7
3	252	9.5	0.4	3.8	0.3	2.9	0.5	5.3	0.4	3.9	0.8	8.1
4	252	56.8	1.8	3.2	2.9	5.1	1.0	1.8	0.6	1.0	3.6	6.4
5	252	316.4	9.3	2.9	7.5	2.4	2.6	0.8	3.0	1.0	12.5	4.0

Within-laboratory precision:
Within-laboratory imprecision of EliA Rib-P assay was evaluated by testing four serum samples using one lot of EliA Rib-P Well on one Phadia 250 instrument. Samples were tested in two replicates per run, two runs per day for 20 days, for a total of 80 replicates per sample. The results are shown in the following table:

Sample	N	Mean (EliA U/mL)	Within-Run		Between-Run		Between-Day		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	80	3.0	0.2	7.2	0.4	11.8	0.1	3.6	0.4	13.8
2	80	7.6	0.3	3.9	0.4	5.9	0.0	0.0	0.5	7.0
3	80	11.0	0.2	2.2	0.5	4.2	0.3	2.3	0.5	4.7
4	80	87.6	2.0	2.2	2.3	2.7	1.6	1.8	3.0	3.5

2. Linearity:

Three patient serum samples with following concentrations: 452.6 EliA U/mL, 357.6 EliA U/mL, 122.9 EliA U/mL, and 32.2 EliA U/mL were used for testing on the Phadia 250. Four patient serum samples with following concentrations: 427.6 EliA U/mL, 34.3 EliA U/mL, and 22.6 EliA U/mL were used for testing on the Phadia 2500E. All samples were serially diluted with EliA Sample Diluent. Each dilution was tested in triplicate on one lot of the EliA Rib-P assay reagents and one set of system reagents on Phadia 250 and Phadia 2500E instruments. The ratios of observed/expected values were calculated. The observed values were graphed against the calculated values and a linear regression analysis was performed. The results are summarized in the tables below.

Results on Phadia 250:

Sample Number	Range [EliA U/mL]	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	55.7–357.6	0.97 (0.94–1.00)	12.25 (6.24–18.25)	1.00	99.0–117.0
2	2.7–122.9	1.00 (0.98–1.03)	1.28 (0.12–2.45)	1.00	103.0–120.0
3	0.5–32.2	1.00 (0.97–1.03)	0.24 (-0.12–0.61)	1.00	81.0–112.0
4	45.1–452.6	0.95 (0.92–0.99)	9.01 (1.59–16.43)	1.00	95.0–116.0

Results on Phadia 2500E:

Sample Number	Range [EliA U/mL]	Slope (95% CI)	Intercept (95% CI)	R^2	% Recovery
1	8.5–427.6	1.03 (0.98–1.09)	2.96 (-6.70–12.61)	0.99	100.0–117.0
2	0.5–34.3	1.00 (0.97–1.03)	0.44 (-0.01–0.89)	1.00	89.0–116.0
3	0.7–22.6	1.02 (0.96–1.09)	0.30 (-0.38–0.97)	0.99	84.0–116.0

The results support the linearity of the claimed analytical measuring range (1.9–403 EliA U/mL) for EliA Rib-P assay.

Hook Effect/Over the Range Results:

Results above the upper limit of the measuring range are reported as ">403". No recommendations are made for dilution of samples outside measuring range in the Package Insert.

3. Analytical Specificity/Interference:

Comparison to Reference Sera:

CDC (Center for Disease Controls and Prevention) ANA Reference Panel samples 1–12 were tested using one lot of EliA Rib-P assay reagents. Among the 12 sera in CDC Panel, the only sera (#12) that was known to be positive for Rib-P was found to be reactive in the EliA Rib-P assay as expected.

Endogenous and Exogenous Interference:

Three serum samples with concentration at 2.3 EliA U/mL (negative), around 9 EliA U/mL (within the equivocal range), and 126.4 EliA U/mL (positive) were spiked with the different interfering substances or the corresponding substance-specific blank solution. For lipemic factor (ClinOleic), distilled water was used as the blank solution, and for the exogenous substances EliA Sample Diluent was used as the blank solution. Additionally, three samples with following concentrations: 87.7 EliA U/mL, 9 EliA U/mL, and 7.8 EliA U/mL were tested for Rheumatoid factor interference. The samples were tested in triplicate in two runs using one lot of EliA Rib-P Wells on a Phadia 250. 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. The quotient between samples spiked with interference substance or blank were within the range of 0.90 – 1.10 The results are listed in the table below:

Compound	**No Interference up to Concentration**
Endogenous substance	
Bilirubin F	40 mg/dL
Bilirubin C	40 mg/dL
Hemoglobin	1000 mg/dL
Lipemic factor	2000 mg/dL
Rheumatoid factor	550 IU/mL
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

4. Assay Reportable Range:

The reportable range for the EliA Rib-P is 1.9–403.0 EliA U/mL.

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

Traceability
There is no international reference standard that recognizes Rib-P. EliA IgG calibrators and EliA IgG Curve Controls are derived from a purchased immunoglobulin preparation (GAMMANORM, Biovitrum AB, Stockholm, Sweden). The EliA IgG calibrators are traceable via an 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 EliA IgG Calibrators are compared to a secondary standard (standardized with the IRP) or the IRP directly and adjusted accordingly to meet the correct concentration.

Stability:
The stability for the EliA Rib-P assay-specific reagent (EliA Rib-P Wells) were determined as follows:

Kit stability (shelf-life): The real-time shelf-life of the EliA Rib-P Wells was performed using Phadia 250 instrument. Three lots of the wells were stored at 2–8°C and tested at seven timepoints (0, 7, 13, 19, 25, 31, and 37 months) in triplicate. The wells were tested with five positive samples in triplicate determination and with nine negative samples in single determination with a full calibration curve. The EliA IgG/IgM/IgA Negative Control was additionally tested in triplicate. The shelf-life of the EliA Rib-P assay was determined to be 36 months stored at 2–8°C.

Open-vial (in-use) stability: The real-time stability of the EliA Rib-P Wells stored at 2–8°C after first opening of the foil bag was performed. The wells were tested at 4, 7 and 10 months in two identical test runs with three positive samples in triple determination and two negative samples in single determination on the Phadia 250. Each test run contained a full calibration curve. The in-use stability for the EliA Rib-P assay was determined to be 9 months at 2–8°C.

On-board stability: The on-board stability of EliA Rib-P Wells was tested over four weeks using five samples (three positive and two negative) on the Phadia 250 instrument. The on-board stability EliA Rib-P assay was determined to be 28 days at 2–8°C.

6. Detection Limit:

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

The LoB was determined from the measurement of four analyte-free (i.e., IgG-depleted sera) samples tested in five replicates per sample over three consecutive days using two lots of EliA Rib-P reagents on two instruments. The LoB was calculated as the 95^{th} percentile for each lot (60 determinations per lot). LoB was determined as the higher value of these two lots for each type of instrument. The claimed LoB for EliA Rib-P assay is the greatest LoB across the two lots and both instrument types.

The LoD was determined by testing four low-level samples in five replicates per run per sample over three consecutive days using two lots of EliA Rib-P reagents on two instruments. The LoD for each lot was calculated based on 240 determinations with 120 blank and 120 low level replicates following 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 Rib-P assay is the greatest LoD across the two lots and both instrument types.

The LoQ was determined by testing four low-level samples in five replicates per sample over three days using two lots of EliA Rib-P reagents on two instruments. The LoQ was defined to be the lowest concentration level that meets the within-laboratory imprecision of <20% for each lot. The greatest LoQ across the two lots and both instrument types were set as the claimed LoQ for EliA Rib-P assay.

The results are summarized in the table below:

Instrument	LoB EliA U/mL	LoD EliA U/mL	LoQ EliA U/mL
Phadia 250	0.0	0.5	1.9
E-module of the Phadia 2500E	0.1	0.4	1.1
The claim for both instrument types	**0.1**	**0.5**	**1.9**

7. Assay Cut-Off:

The assay cut-off and the equivocal range were established by testing a cohort consisting of 70 apparently healthy blood donors and 30 samples from SLE patients on a Phadia 250 instrument. The assay cut-offs were set as follows:

Decision Point	Interpretation
< 7 EliA U/mL	Negative
7–10 EliA U/mL	Equivocal
> 10 EliA U/mL	Positive

In case of equivocal results, the manufacturer recommends retesting the patient after 8–12 weeks.

B Comparison Studies:

1. Method Comparison with Predicate Device:

Three hundred twenty-three (323) samples with antibody concentrations covering the analytical measuring range were analyzed with EliA Rib-P and the predicate Quanta Lite Ribosome P ELISA assay. All samples were found within the measuring ranges of both tests and 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 Rib-P positive: Equivocal results considered negative		**QUANTA Lite Ribosome P ELISA**		
		Positive: ≥ 20 Units	**Negative: < 20 Units**	**Total**
EliA Rib-P positive	**Positive: > 10 EliA U/mL**	36	4	40
	Negative: < 10 EliA U/mL	2	281	283
	Total	38	285	323

Equivocal results considered negative	Agreement (%)	95% CI
PPA	94.7	82.3–99.4
NPA	98.6	96.4–99.6
Total	98.1	96.0–99.3

EliA Rib-P positive: Equivocal results considered positive		QUANTA Lite Ribosome P ELISA		
		Positive: ≥ 20 Units	Negative: < 20 Units	Total
EliA Rib-P positive	Positive: > 7 EliA U/mL	38	34	72
	Negative: < 7 EliA U/mL	0	251	251
	Total	38	285	323

Equivocal results considered positive	Agreement (%)	95% CI
PPA	100.0	90.7–100
NPA	88.1	83.7–91.6
Total	85.5	85.6–92.6

2. Matrix Comparison:

   Not applicable, serum is the claimed sample type for this assay.

3. Instrument Comparison:

   This study compared the performance of the Phadia 250 and Phadia 2500E instruments using the EliA Rib-P assay by evaluating 47 positive, 10 equivocal and 28 negative serum samples. The samples were analyzed in single determination on one Phadia 250 and one Phadia 2500E instrument each. The Passing-Bablok regression analysis showed the slope of 0.94 (95% CI: 0.93–0.98) and the intercept of -0.76 (95% CI: -1.20 – -0.48). The PPA, NPA, and total agreement were calculated with equivocal results considered as negative or as positive, respectively. The results are summarized in the tables below.

Positive/Negative Agreement if equivocal samples are considered positive.

	Agreement (%), (95% CI)
PPA	91.2 (80.7–97.1)
NPA	96.4 (81.7–99.9)
Total Agreement	92.9 (85.3–97.4)

Positive/Negative Agreement if equivocal samples are considered negative.

	Agreement (%), (95% CI)
PPA	93.6 (82.5–98.7)
NPA	100.0 (90.8–100.0)
Total Agreement	96.5 (90.0–99.3)

C Clinical Studies:

1. Clinical Sensitivity and Specificity:

A total of 560 clinically defined serum samples were included in the clinical evaluation for the EliA Rib-P. This validation cohort includes 146 samples from individuals diagnosed with systemic lupus erythematosus (SLE), and 414 samples from patients with non-SLE diseases/conditions. The distribution of the cohort and the EliA Rib-P positivity rate for each clinical subgroup was summarized in the following table.

Diagnostic groups	**n**	**No (%) Positive EliA Rib-P**
Systemic lupus erythematosus (SLE)	136	38 (28%)
SLE with secondary antiphospholipid syndrome	10	3 (30%)
Target disease (Total)	**146**	41 (28%)
Non-SLE Specimens		
Celiac disease	13	0
Crohn's disease	12	0
CTD* overlap non-MCTD**	10	0
Dermatomyositis	4	0
Polymyositis	6	0
Graves' disease	12	0
Primary antiphospholipid syndrome	12	0
Primary biliary cholangitis	21	0
Sjögren's syndrome	23	0
Type 1 diabetes	12	0
Ulcerative colitis	11	0
Varied cancer	10	0
MCTD**	10	0
Rheumatoid arthritis	30	0
Bacterial infections	36	0
Viral infections	56	0
Hashimoto's disease	10	0
Granulomatosis with Polyangiitis	4	0
Systemic sclerosis, diffuse	48	1 (2%)
Autoimmune hepatitis	16	0
Polymyalgia rheumatica	25	0
Systemic sclerosis, limited	33	0
Non-SLE (Total)	**414**	
Total	**560**	

*CTD: connective tissue disease

**MCTD: mixed connective tissue disease

Clinical sensitivity and specificity of the assay are summarized in the tables below:

EliA Rib-P – equivocal results evaluated as positive:

	Diagnosis		Total
	SLE	**Controls**	
Positive test ≥ 7 EliA U/mL	51	3	54
Negative test < 7 EliA U/mL	95	411	506
Total	146	414	560

Sensitivity (95% CI): 34.9% (27.2%–43.3%)
Specificity (95% CI): 99.3% (97.9%–99.9%)

EliA Rib-P – equivocal results evaluated as negative:

	Diagnosis		Total
	SLE	**Controls**	
Positive test ≥ 10 EliA U/mL	41	1	42
Negative test < 10 EliA U/mL	105	413	518
Total	146	414	560

Sensitivity (95% CI): 28.1% (21.0%–36.1%)
Specificity (95% CI): 99.8% (98.7%–100.0%)

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. A panel of 638 serum samples from apparently healthy blood donors were collected from the Biobank at Phadia GmbH (Germany). The set consisted following age groups: age ≤30 years (64 males, 64 females), 31–40 years (64 males, 64 females), 41–50 years (64 males, 64 females), 51–60 years (64 males, 64 females), >60 years (62 males, 64 females). The panel included 400 samples from Caucasian origin, 238 samples from multiple ethnicities (80 samples from African Americans, 80 samples from Hispanics, and 78 samples from Asian blood donors). One sample was found equivocal (7–10 EliA U/mL). The results are presented in the following table:

	Female					Male					Total
Age, years	≤30	31–40	41–50	51–60	>60	≤30	31–40	41–50	51–60	>60	
N	64	64	64	64	62	64	64	64	62	62	638
Median	2.1	1.8	1.7	1.5	1.4	1.4	1.6	1.5	1.6	1.8	1.6
95th Percentile	3.4	3.4	3.3	4.0	3.2	2.9	2.9	3.3	3.7	3.3	3.4
99th Percentile	4.3	5.2	4.1	5.6	5.7	3.5	3.2	5.9	5.5	3.9	5.0

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.