510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

k140225

B. Purpose for Submission:

New Device

C. Measurand:

IgG antibodies specific for human PR3 protein, human MPO protein and human alpha3 chain of collagen IV

D. Type of Test:

Fluoroenzyme immunoassay, Semi-quantitative

E. Applicant:

Phadia US Inc.

F. Proprietary and Established Names:

EliA™ PR3^{S} Immunoassay
EliA™ MPO^{S} Immunoassay
EliA™ GBM Immunoassay
EliA™ ANCA/GBM Positive Control 100
EliA™ ANCA/GBM Positive Control 250

G. Regulatory Information:

1. Regulation section:

   21 CFR§866.5660 – Multiple autoantibodies immunological test system
   21 CFR§866.1660 – Quality Control Material (Assayed and Unassayed)

2. Classification:

   Class II (assay)
   Class I (control)

3. Product code:

   MOB, Test system, anti-neutrophil cytoplasmic antibodies (ANCA)
   MVJ, Devices, measure, antibodies to glomerular basement membrane (GBM)
   JJY, Multi-analyte controls, all kinds (assayed)

4. Panel:

   Immunology (82)
   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   EliA PR3^{S} is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to proteinase 3 (PR3) in human serum and plasma (heparin, EDTA, citrate) to aid in the clinical diagnosis of Glomerulonephritis with Polyangiitis (GPA; formerly known as Wegener's granulomatosis) in conjunction with other laboratory and clinical findings. EliA PR3^{S} uses the EliA IgG method on the instrument Phadia 100 and Phadia 250.

   EliA MPO^{S} is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to myeloperoxidase (MPO) in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of microscopic polyangiitis (MPA) in conjunction with other laboratory and clinical findings. EliA MPO^{S} uses the EliA IgG method on the instrument Phadia 100 and Phadia 250.

   EliA GBM is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to alpha3 chain of collagen IV in human serum and plasma (heparin, EDTA, citrate) as an aid in the clinical diagnosis of Goodpasture syndrome in conjunction with other laboratory and clinical findings. EliA GBM uses the EliA IgG method on the instrument Phadia 100 and Phadia 250.

   EliA ANCA/GBM Positive Control 100 is intended for laboratory use in monitoring the performance of in vitro measurement of ANCA/GBM antibodies with Phadia 100 using the EliA IgG method.

   EliA ANCA/GBM Positive Control 250 is intended for laboratory use in monitoring the performance of in vitro measurement of ANCA/GBM antibodies with Phadia 250 using the EliA IgG method.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   For use on the Phadia 100 and Phadia 250 instruments

I. Device Description:

EliA™ uses a modular reagent system. The test specific, method specific and general reagents are packaged and purchased as separate units. The reagents on Phadia 100 and Phadia 250 are identical; they are only filled in different containers.

Test Specific Reagents consist of:

- EliA™ well, coated with antigen (human PR3 protein, human MPO protein, or human recombinant α3 chain of collagen IV)
- EliA™ ANCA/GBM positive control, a multiparameter control containing IgG antibodies to PR3, MPO and GBM
- EliA™ IgG/IgM/IgA Negative Control, a multiparameter control containing normal human serum from healthy donors.

EliA™ Method-Specific Reagents:

- EliA™ method-specific sample diluent (PBS containing BSA, detergent and 0.095% sodium azide);
- EliA™ IgG conjugate (β-galactosidase labeled mouse monoclonal anti-IgG antibodies);
- EliA™ IgG calibrators (human IgG in PBS at measured concentrations of 0, 4, 10, 20, 100, 600 µg/L);
- EliA™ IgG Curve Control;
- EliA™ IgG Calibrator Well

General Reagents:

- Development Solution, Stop Solution, and Washing Solution

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   QUANTA Lite™ PR-3 ELISA (k981328)
   QUANTA Lite™ MPO IgG ELISA (k981330)
   Wielisa GBM (k974169)

2. Comparison with predicate:

EliA PR3^{S}

Similarities		
Item	Device EliA PR3^{S}	Predicate QUANTA Lite PR-3
Intended Use / Indication for Use	For the in vitro semi-quantitative measurement of IgG antibodies directed to proteinase 3 (PR3) in human serum and plasma (heparin, EDTA, citrate) to aid in the diagnosis of Granulomatosis with Polyangiitis (GPA, formally called Wegener's Granulomatosis) in conjunction with other laboratory and clinical findings.	For the semi-quantitative detection of IgG autoantibodies to serine protease 3 (PR-3) in human serum. This test is to be used in conjunction with other clinical findings to aid in assessment of certain autoimmune vasculitides such as Wegener's granulomatosis.
Type of Test	Semi-quantitative	Same
Antigen Used	native purified human PR3 protein	Purified human PR-3
Solid Phase	Microwells	Same
Internal Controls	Positive and negative controls provided with the assay	Low and high positives, and negative controls included in the kit

Differences		
Item	Device EliA PR3^{S}	Predicate QUANTA Lite PR-3
Sample Matrix	Serum and plasma (Li-heparin, EDTA, citrate)	Serum
Assay type	Automated immunoassay	Manual ELISA
Instrumentation	Phadia 100 and 250 automated immunoassay analyzers	ELISA-Reader needed
Reaction Temperature	37°C controlled	20-26°C
Detection Antibody (conjugate)	β-galactosidase labeled anti-IgG (mouse monoclonal antibodies)	Horseradish peroxidase labeled anti-human IgG (goat)
Substrate	4-methylumbelliferyl-β-D-galactoside (MUG)	Tetramethylbenzidene (TMB)
Signal	Fluorescence	Optical density
Calibrators	6 levels	N/A
Calibration	Total IgG Calibration	1-point Calibration

Differences		
Item	Device EliA PR3^{S}	Predicate QUANTA Lite PR-3
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	N/A
Sample Dilution	1:100	1:101
Assay Cut-off /Interpretation	<2.0 U/mL, Negative 2.0 – 3.0 U/mL, Equivocal >3.0 U/mL, Positive	≤ 20 U/mL, Negative 21 – 30 U/mL, Weak positive >30 U/mL, Moderate to strong positive

EliA MPO^{S}

Similarities		
Item	Device EliA MPO^{S}	Predicate QUANTA Lite MPO IgG
Intended Use / Indication for Use	For the in vitro semi-quantitative measurement of IgG antibodies directed to myeloperoxidase (MPO) in human serum and plasma (heparin, EDTA, citrate) to aid in the diagnosis of microscopic polyangiitis (MPA) in conjunction with other laboratory and clinical findings.	For the semi- quantitative detection of IgG autoantibodies to myeloperoxidase (MPO) in human serum. This test is to be used in conjunction with other clinical findings to aid in assessment of certain autoimmune vasculitides such as microscopic polyarteritis, and crescentic glomerulonephritis.
Type of Test	Semi-quantitative	Same
Antigen Used	native human MPO protein	Same
Solid Phase	Microwells	Same
Internal Controls	Positive and negative controls provided with the assay	Low and high positives, and negative controls included in the kit

Differences		
Item	Device EliA MPO^{S}	Predicate QUANTA Lite MPO IgG
Sample Matrix	Serum and plasma (Li-heparin, EDTA, citrate)	Serum
Assay type	Automated immunoassay	Manual ELISA
Instrumentation	Phadia 100 and 250 automated immunoassay analyzers	ELISA-Reader needed

Differences		
Item	Device EliA MPO^S	Predicate QUANTA Lite MPO IgG
Reaction Temperature	37°C controlled	20-26°C, Room temperature
Detection Antibody (conjugate)	β-galactosidase labeled anti-IgG (mouse monoclonal antibodies)	Horse-Radish Peroxidase labeled anti-human IgG (goat)
Substrate	MUG	TMB
Signal	Fluorescence	Optical density
Calibrators	6 levels	N/A
Calibration	Total IgG Calibration	1-point Calibration
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	N/A
Sample Dilution	1:50	1:101
Assay Cut-off /Interpretation	<3.5 U/mL, Negative 3.5 – 5.0 U/mL, Equivocal >5.0 U/mL, Positive	≤ 20 U/mL, Negative 21 – 30 U/mL, Weak positive > 30 U/mL, Moderate to strong positive

EliA GBM

Similarities		
Item	Device EliA GBM	Predicate Wielisa GBM
Intended Use /Indication for Use	For the in vitro semi-quantitative measurement of IgG antibodies to α3 chain of collagen IV in human serum and plasma (heparin, EDTA, citrate) to aid in the diagnosis of Goodpasture syndrome in conjunction with other laboratory and clinical findings.	For detection and semi-quantitation of IgG antibodies to glomerular basement membrane (GBM) in human sera. The assay is used to detect antibodies in a single serum specimen. The results of the assay are to be used as an aid to the diagnosis of Goodpasture syndrome. The analysis should be performed by trained laboratory professionals.
Type of Test	Semi-quantitative	Same
Antigen Used	Human recombinant α3 chain of collagen IV	GBM antigen (α3 chain)
Solid Phase	Microwells	Same
Internal Controls	Positive and negative controls provided with the assay	Low and high positives, and negative controls included in the kit

Differences		
Item	Device EliA GBM	Predicate Wielisa GBM
Sample Matrix	Serum and plasma (Li-heparin, EDTA, citrate)	Serum
Assay type	Automated immunoassay	Manual ELISA
Instrumentation	Phadia 100 and 250 automated immunoassay analyzers	Microplate-Reader (405 nm)
Reaction Temperature	37°C controlled	Room temperature, 20-26°C
Detection Antibody (conjugate)	β-galactosidase labeled anti-IgG (mouse monoclonal antibodies)	Alkaline phosphatase labeled anti-human IgG
Substrate	MUG	p-Nitrophenylphosphate (pNPP)
Signal	Fluorescence	Optical density
Calibrators	6 levels 0, 4, 10, 20, 100, 600 µg/l	5 levels 10, 40, 80, 160, 320 U/ml
Calibration	Total IgG calibration	Analyte-specific calibration
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	5-point standard curve
Sample Dilution	1:100	1:80
Assay Cut-off /Interpretation	<7 U/mL, Negative 7 – 10 U/mL, Equivocal >10 U/mL, Positive	≤ 10 U/mL, Negative 10 – 20 U/mL, Weak positive >20 U/mL, Moderate to strong positive

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

The EliA wells are molded cups comparable to excised wells from a microtiter plate. They are made of polystyrene and are coated with the respective antigen. The wells are at the same time a holder of the coupled antigen for convenient automation and a reaction chamber with reaction/washing solution handling based on pipetting to add and aspiration to remove liquids.

The EliA wells are coated with human PR3 protein, or human MPO protein, or human recombinant α3 chain of collagen IV. If present in the patient's specimen, antibodies to these proteins bind to the 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 higher the response value, the more specific IgG is present in the specimen. To evaluate test results, the response for patient samples is compared directly to the response for calibrators.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

EliA PR3^S Assay:

EliA PR3^S on Phadia 100: The precision of the assay on the Phadia 100 instrument was evaluated on samples containing various concentrations of antibodies. Each sample was run on three instruments, four replicates per run, one run a day, for seven days with one reagent lot (total of 84 observations per sample). The results are summarized in the table below:

EliA PR3^S on Phadia 100									
Sample	**Mean (U/mL)**	**Within-Run**		**Between-Run**		**Between-Instrument**		**Total**	
		SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	2.3	0.2	8.7	0.1	5.7	0.2	8.1	0.3	13.2
2	17.7	1.1	6.4	0.8	4.6	0.5	2.5	1.5	8.3
3	130.9	10.4	7.9	8.9	6.8	12.5	9.6	18.5	14.2
4	1.3	0.1	5.8	0.1	5.1	0.0	0.0	0.1	7.7
6	3.6	0.2	4.7	0.2	4.7	0.0	0.0	0.2	6.6
5	43.5	2.6	6.0	2.0	4.6	0.4	0.9	3.3	7.7

EliA PR3^S on Phadia 250: The precision of the assay on the Phadia 250 instrument was evaluated on samples containing various concentrations of antibodies. Each sample was run on three instruments, four replicates per run, one run a day, for seven days with three reagent lots (total of 252 replicates per sample). Additional samples were tested with one reagent lots with a total of 84 observations per sample. The results are summarized in the table below:

EliA PR3^S on Phadia 250											
Sample	**Mean (U/mL)**	**Within-Run**		**Between-Run**		**Between-Instrument**		**Between-Lot***		**Total**	
		SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
n=252											
1	2.2	0.1	4.2	0.2	7.7	0.0	2.0	0.2	9.1	0.3	12.8
2	17.5	0.6	3.5	1.2	6.8	0.4	2.2	1.1	6.1	1.8	10.0
3	120.7	5.0	4.2	10.1	8.3	7.6	6.3	11.6	9.6	17.8	14.8
n=84											
4	1.4	0.1	6.9	0.1	4.2	0.0	0.0			0.1	8.1
5	3.5	0.1	3.9	0.1	3.7	0.1	2.9			0.2	6.1
6	43.3	2.1	4.9	1.2	2.8	1.6	3.6			2.9	6.7

EliA MPO^S Assay:

EliA MPO^S on Phadia 100: The precision of the assay on the Phadia 100 instrument was evaluated on three samples containing various concentrations of antibodies. Each sample was run on three instruments, four replicates per run, one run a day, for six days with one reagent lots (total of 72 replicates per sample). Three additional samples were tested with the same protocol for seven days (total of 84 observations per sample). The results are summarized in the table below:

EliA MPO^S on Phadia 100									
Sample	**Mean (U/mL)**	**Within-Run**		**Between-Run**		**Between-Instrument**		**Total**	
		SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
n=72									
1	4.1	0.1	1.9	0.2	4.0	0.3	6.9	0.2	4.4
2	11.1	0.2	2.1	0.5	4.8	0.6	5.4	0.6	5.3
3*	124.7	6.1	4.9	7.6	6.1	5.4	4.3	9.8	7.8
n=84									
4	2.2	0.1	3.7	0.1	5.6	0.1	3.7	0.2	7.6
5	5.5	0.1	2.1	0.3	4.6	0.1	2.5	0.3	5.6
6**	46.2	2.2	4.7	2.3	5.0	0.0	0.0	3.2	6.9

* n=71 (missing one observation due to instrument error)

**n=83 (missing one observation due to pipetting error)

EliA MPO^S on Phadia 250: The precision of the assay on the Phadia 250 instrument was evaluated first on four samples containing various concentrations of antibodies. Each sample was run on three instruments, four replicates per run, one run a day, for six days with three reagent lots (total of 216 replicates per sample). Three additional samples were run with the same protocol for seven days but using one reagent lot for a total of 84 observations per sample. The combined the results are summarized in the table below:

EliA MPO ^{S} on Phadia 250											
Sample	**Mean (U/mL)**	**Within-Run**		**Between-Run**		**Between-Instrument**		**Between-Lot**		**Total**	
		SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
n=216											
1	4.5	0.1	2.8	0.1	2.5	0.1	2.2	0.1	1.7	0.2	3.8
2	12.1	0.4	3.2	0.3	2.6	0.2	2.0	0.2	1.9	0.5	4.1
3	126.0	5.1	4.0	6.3	5.0	2.6	2.1	5.2	4.1	8.1	6.4
4	3.0	0.1	2.8	0.1	2.4	0.0	0.7	0.1	3.5	0.1	3.7
n=84											
5	2.2	0.1	4.4	0.1	2.2	0.0	0.0			0.1	4.9
6	5.8	0.2	3.8	0.1	2.3	0.0	0.0			0.3	4.5
7	47.3	2.2	4.7	1.2	2.6	1.2	2.6			2.8	6.0

EliA GBM Assay**:**

The precision of the EliA GBM assay on the Phadia 100 and Phadia 250 was evaluated on six samples containing various concentrations of antibodies. Each sample was run on three instruments, four replicates per run, one run a day, for seven days with one reagent lot (total of 84 observations per sample). The results are summarized in the table below:

EliA GBM on Phadia 100									
Sample	**Mean (U/mL)**	**Within-Run**		**Between-Run**		**Between-Instrument**		**Total**	
		SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	9.1	0.7	7.5	0.5	5.0	0.4	4.3	0.9	10.0
2	102.4	5.3	5.1	3.3	3.2	4.8	4.7	7.8	7.6
3	571.6	22.9	4.0	25.2	4.4	50.3	8.8	60.5	10.6
4	5.5	0.2	3.0	0.2	3.7	0.2	4.3	0.4	6.5
5	10.6	0.2	1.8	0.3	2.8	0.2	1.9	0.4	3.8
6	287.9	13.8	4.8	10.8	3.7	0.0	0.0	17.5	6.1

EliA GBM on Phadia 250									
Sample	**Mean (U/mL)**	**Within-Run**		**Between-Run**		**Between-Instrument**		**Total**	
		SD	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**	**SD**	**CV (%)**
1	9.1	0.7	7.7	0.3	0.8	0.0	0.0	0.8	8.4
2	109.4	9.3	8.5	3.6	3.3	0.0	0.0	10.0	9.1
3	623.4	42.8	6.9	30.3	4.9	34.3	5.5	62.8	10.1
4	5.2	0.4	6.6	0.2	2.8	0.1	1.8	0.4	7.4
5	10.4	0.6	5.3	0.2	2.1	0.2	1.4	0.6	5.9
6	270.5	9.5	3.5	6.9	2.6	0.0	0.0	11.8	4.4

Lot-to-lot reproducibility for EliA GBM assay was tested on Phadia 100 with four samples with mean concentration of 0.2, 13.1, 23.9, and 48.1 U/mL. Each sample was run with duplicate per run, one run a day on three instruments for six days with three lots of reagents for a total of 108 observations per sample. The results show the CV% between 3.6% and 8.4% for the lot-to-lot variation.

b. Linearity/assay reportable range:

Linearity: For each of EliA PR3^S, EliA MPO^S, and EliA GBM assays, at least five positive patient serum samples for each assay were serially diluted and tested for each assay. Each dilution was tested in triplicate on Phadia 100 and Phadia 250. The results of the dilutions were compared with their expected value. The linear regression analysis for each assay gives the following equation:

Linearity of EliA PR3^S				
Sample	**Range (U/mL)**	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**
Phadia 100				
1	0.4 – 25.5	1.03 (0.98 – 1.07)	-0.28 (-0.76 – 0.20)	1.00
2	1.2 – 101.9	1.02 (0.99 – 1.05)	-0.57 (-1.59 – 0.45)	1.00
3	1.4 – 124.3	1.02 (0.98 – 1.05)	-0.30 (-1.18 – 1.78)	1.00
4	1.1 – 112.6	1.00 (0.98 – 1.02)	0.98 (0.05 – 1.91)	1.00
5	4.7 – 226.6	0.98 (0.96 – 1.00)	1.77 (-0.21 – 3.75)	1.00
Phadia 250				
1	0.4 – 25.2	1.02 (0.98 – 1.06)	-0.21(-0.62 – 0.20)	1.00
2	1.2 – 96.7	1.01 (0.99 – 1.02)	-0.55 (-1.16 – 0.05)	1.00
3	1.3 – 104.1	0.99 (0.97 – 1.01)	-0.86 (-1.82 – 0.09)	1.00
4	1.3 – 113.1	1.02 (0.98 – 1.06)	1.02 (-0.75 – 2.79)	1.00
5	2.0 – 222.3	0.96 (0.93 – 1.00)	2.28 (-1.05 – 5.61)	1.00

The technical measuring range (detection limit, upper limit) for EliA is PR3^S from 0.7 to ≥ 177 EliA U/mL. The upper limit of the reported results in EliA U/mL can vary due to a lot-specific conversion from µg/L to EliA U/mL. Results above the upper limit are reported as "above". Linearity was shown for samples in the range from 0.4–226.6 EliA U/mL. The labeling states that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the technical measuring range.

Linearity of EliA MPO^S				
Sample	**Range (U/mL)**	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**
Phadia 100				
1	1.2 – 104.2	1.03 (0.97 – 1.08)	1.60 (-0.40 – 3.60)	0.99
2	0.5 – 30.9	1.00 (0.98 – 1.02)	-0.10 (-0.30 – 0.09)	1.00
3	1.3 – 98.8	1.00 (0.99 – 1.01)	-0.45 (-0.82 – -0.07)	1.00
4	0.9 – 88.2	0.99 (0.97 – 1.01)	-0.19 (-0.77 – 0.39)	1.00

Linearity of EliA MPO^S				
Sample	**Range (U/mL)**	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**
5	1.5 – 153.5	1.00 (0.98 – 1.02)	0.99 (-0.52 – 2.49)	1.00
6	0.3 – 15.9	1.01 (0.98 – 1.05)	0.09 (-0.12 – 0.30)	1.00
Phadia 250				
1	1.1 – 93.7	1.02 (0.99 – 1.05)	0.07 (-0.96 – 1.10)	1.00
2	0.6 – 36.3	1.02 (0.99 – 1.04)	-0.02(-0.40 – 0.36)	1.00
3	1.4 – 104.4	0.99 (0.96 – 1.02)	-1.13(-2.27 – 0.01)	1.00
4	1.0 – 91.7	0.98 (0.96 – 1.01)	-0.14 (-1.07 – 0.79)	1.00
5	1.6 – 146.4	0.98 (0.97 – 1.01)	-0.46 (-1.60 – 0.67)	1.00
6	0.4 – 17.0	1.01 (0.99 – 1.03)	0.04 (-0.10 – 0.19)	1.00

The technical measuring range (detection limit, upper limit) for EliA MPO^S is from 0.3 to ≥ 134 EliA U/mL. The upper limit of the reported results in EliA U/mL can vary due to a lot-specific conversion from µg/L to EliA U/mL. Results above the upper limit are reported as "above". Linearity was shown for samples in the range from 0.3–153.5 EliA U/mL. The labeling states that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the technical measuring range.

Linearity of EliA GBM				
Sample	**Range (U/mL)**	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**
Phadia 100				
1	16.8 – 521.1	0.97 (0.93 – 1.01)	2.79 (-6.76 – 12.35)	1.00
2	1.6 – 49.1	0.98 (0.95 – 1.02)	1.03 (0.35 – 1.91)	1.00
3	16.4 – 510.0	0.96 (0.90 – 1.02)	1.03 (-12.6 – 14.7)	0.99
4	5.3 – 570.4	0.97 (0.93 – 1.00)	0.82 (-6.97 – 8.61)	1.00
5	20.5 – 932.1	0.99 (0.93 – 1.04)	-5.26 (-27.14 – 16.61)	0.99
6	1.8 – 80.3	0.98 (0.93 – 1.04)	-1.31 (-3.14 – 0.53)	1.00
Phadia 250				
1	6.1 – 425.9	0.95 (0.91 – 1.01)	0.09 (-8.92 – 9.10)	0.99
2	5.0 – 522.1	0.95 (0.90 – 1.01)	-2.96 (-14.19 – 8.27)	0.99
3	8.8 – 422.8	0.97 (0.93 – 1.01)	0.76 (-6.50 – 8.02)	1.00
4	8.4 – 390.5	0.96 (0.91 – 1.01)	2.12 (-6.45 – 10.70)	1.00
5	18.3 – 875.1	0.97 (0.92 – 1.02)	-6.74 (-25.88 – 12.40)	1.00
6	1.2 – 109.7	0.99 (0.97 – 1.02)	-1.34 (-2.42 – -0.26)	1.00

The technical measuring range (detection limit, upper limit) for EliA GBM is from 1.9 to ≥ 680 EliA U/mL. The upper limit of the reported results in EliA U/ml can vary due to a lot-specific conversion from µg/L to EliA U/ml. Results above the upper limit are reported as "above". Linearity was shown for samples in the range from 1.2–932.1 EliA U/mL. The labeling states that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the technical measuring range.

Hook effect: For each of three assays, hook effect was assessed by analyzing a high positive serum sample with antibody concentration above the upper limit of the measuring range of each assay. The results are summarized as follows:

EliA PR3^{S}	No hook effect was observed up to 20,154 U/mL
EliA MPO^{S}	No hook effect was observed up to 23,823 U/mL
EliA GBM	No hook effect was observed up to 18,540 U/mL

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability: There is no international reference standard for IgG antibodies that specifically recognize PR3, MPO or the human α3 chain of collagen IV. 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 U/mL for EliA PR3^{S}, EliA MPO^{S} and EliA GBM.

Calibrators: The EliA IgG calibration is a total IgG calibration. The 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 standardization of the assay is based on a set of six WHO-standardized IgG Calibrators derived from human serum with the assigned values at 0, 4, 10, 20, 100, 600 µg/mL.

The calibrators are required to perform an initial calibration curve, which can be stored in the Phadia instrument and may be used for up to 28 days on this and additional IgG assays. Each additional assay outside of a calibration run includes curve controls that have to fall within defined ranges to verify that the stored calibration curve is still valid.

Controls: Both EliA ANCA/GBM Positive Control 100/250 and EliA IgG/IgM/IgA Negative Control 100/250 are for single use only. The EliA™ IgG/IgM/IgA Negative Control were cleared under k131821. The EliA ANCA/GBM Positive Control is prepared from selected pooled human sera and contains IgG antibodies specific to PR3, MPO and GBM. The mean values for every lot are determined with four consecutive control assays, each in six replicates. Ranges are calculated as respective mean ± 3SD. The target ranges for the EliA ANCA/GBM positive controls and EliA IgG/IgM/IgA negative control for each of EliA PR3^{S}, EliA MPO^{S}, and EliA GBM assays are shown below:

EliA ANCA/GBM Positive Control		
Assay	**Instrument**	**Range**
EliA PR3^S	Phadia 100	9.3 – 21.8 U/mL
	Phadia 250	9.0 – 21.0 U/mL
EliA MPO^S	Phadia 100	25.1 – 58.6 U/mL
	Phadia 250	27.0 – 63.0 U/mL
EliA GBM	Phadia 100	22.6 – 52.7 U/mL
	Phadia 250	22.6 – 52.7 U/mL

EliA IgG/IgM/IgA Negative Control		
Assay	**Instrument**	**Range**
EliA PR3^S	Phadia 100	< 2.0 U/mL
	Phadia 250	
EliA MPO^S	Phadia 100	< 3.5 U/mL
	Phadia 250	
EliA GBM	Phadia 100	< 7.0 U/mL
	Phadia 250	

Stability:

Shelf life: : The stability of EliA PR3^S wells, EliA MPO^S wells and EliA GBM wells was evaluated with both the real time and accelerated/stress study. The results support stability of the kits under the recommended storage of 2 – 8°C for up to 18 months.

The stability of EliA ANCA/GBM positive control was evaluated with real time stability study. The results support the stability of the EliA ANCA/GBM Positive Control up to 9 months at 2-8°C.

On-board stability: The on-board stability EliA PR3^S, EliA MPO^S and EliA GBM carriers (containing the antigen coated wells) was tested over four weeks using three positive and two negative samples only on the Phadia 250 instrument since Phadia 100 reagents are stored off the instrument and are only loaded as needed for an assay. The on-board stability for the Phadia 250 instrument was determined to be 28 days at 2-8°C.

Open vial stability: Stability of the foilbag containing the EliA PR3^S wells, EliA MPO^S wells and EliA GBM wells after first opening was tested and determined to be 9 months at 2-8°C.

d. Detection limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) study was modeled after CLSI EP17 by testing six blood donor samples from apparently healthy subjects on three Phadia 100 and two Phadia 250 instruments. On

each instrument system, each sample was tested in 12 replicates, one run a day for six days. The sample with the lowest signal was used to estimate the LoB while the other five samples with antibody concentration between the blank and up to 2.5X blank were used to determine the LoD. The results are summarized in the tables below:

EliA PR3^S	**LoB (U/mL)**	**LoD (U/mL)**	**LoQ(U/mL)**
Phadia 100	0.38	0.61	0.7
Phadia 250	0.24	0.32	

EliA MPO^S	**LoB (U/mL)**	**LoD (U/mL)**	**LoQ (U/mL)**
Phadia 100	0.13	0.26	0.3
Phadia 250	0.09	0.14	

EliA GBM	**LoB (U/mL)**	**LoD (U/mL)**	**LoQ (U/mL)**
Phadia 100	1.16	1.88	1.9
Phadia 250	0.52	0.97	

e. Analytical specificity:

Endogenous Interference: For each assay, interferences were assessed by testing four serum samples: one negative, two positives with concentration levels around the cut-off, and one high positive sample. Each sample was spiked with the interference substances or substance-specific blank, and analyzed in two runs, three replicates per run (n=6) using one lot of EliA antigen wells and one lot of system reagents. The ratio of spiked and blank samples was calculated. The data demonstrated that the three analytes were not adversely affected by high levels of the following substances tested up to the concentrations listed in the table below:

EliA PR3^{S}Assay	
Potential Interfering Substances	**Concentration Tested**
Bilirubin F	191 mg/dl
Bilirubin C	216 mg/dl
Hemoglobin	5190 mg/dl
Lipemic factor	1%
Rheumatoid factor	500 IU/ml

EliA MPO^{S}Assay	
Potential Interfering Substances	**Concentration Tested**
Bilirubin F	191 mg/dl
Bilirubin C	216 mg/dl
Hemoglobin	4940 mg/dl
Lipemic factor	1%
Rheumatoid factor	500 IU/ml

EliA GBM Assay		
Potential Interfering Substances	**Concentration Tested (for low positive)**	**Concentration Tested (for high positive)**
Bilirubin F	191 mg/dl	193 mg/dl
Bilirubin C	216 mg/dl	210 mg/dl
Hemoglobin	5190 mg/dl	4700 mg/dl
Lipemic factor	1%	n.a.
Chyle	n.a.	19000 FTU
Rheumatoid factor	500 IU/ml	550 IU/ml

f. Assay cut-off:

Based on the results of the expected values/reference range study described below in Section M.5, the cut-offs were setup so that: 1) the 99th percentile of the results from 400 apparently healthy blood donor samples must be below the upper limit of the equivocal range for EliA PR3^{S}s and EliA MPO^S; and the 95th percentile below the lower limit of the equivocal range for EliA GBM; 2) the lower limit of the equivocal range is equal or higher than the lowest positive calibrator; 3) the upper limit of the equivocal range is at least 1.4 fold above the lower limit of the equivocal range. The assay cut-offs were set as follows:

EliA PR3^S	
IgG Concentration	**Interpretation**
< 2.0 U/mL	Negative
2.0 – 3.0 U/mL	Equivocal
> 3.0 U/mL	Positive

EliA MPO^S	
IgG Concentration	**Interpretation**
< 3.5 U/mL	Negative
3.5 – 5.0 U/mL	Equivocal
> 5.0 U/mL	Positive

EliA GBM	
IgG Concentration	**Interpretation**
< 7 U/mL	Negative
7 – 10 U/mL	Equivocal
> 10 U/mL	Positive

Where samples yield equivocal results, the sponsor recommends retesting the patient again after 8-12 weeks.

2. Comparison studies:

a. Method comparison with predicate device:

EliA PR3^{S} Assay**:**

A total of 455 samples used in the clinical validation study were tested with EliA for EliA PR3^{S} assay and predicate device QUANTA Lite PR-3. Among these samples, the 227 samples within the assay measuring range of both assays were included in the method comparison analysis. The results are summarized below:

		QUANTA Lite PR-3		
		Positive	Negative	Total
EliA PR3^{S}	Positive	72	13	85
	Equivocal	4	7	11
	Negative	2	129	131
	Total	78	149	227

Equivocal considered as positive:
Positive agreement: 97.4% (95% CI: 91.0 – 99.7%)
Negative agreement: 86.6% (95% CI: 80.0 – 91.6%)
Overall agreement: 90.3% (95% CI: 85.7 – 93.8%)

Equivocal considered as negative:
Positive agreement: 92.3% (95% CI: 84.0 – 97.1%)
Negative agreement: 91.3% (95% CI: 85.5 – 95.3%)
Overall agreement: 91.6% (95% CI: 87.2 – 94.9%)

EliA MPO^{S} Assay:

A total of 425 samples from the samples used in the clinical validation study were tested with EliA MPO^{S} assay and predicate device QUANTA Lite MPO IgG. Among these samples, the 284 samples within the assay measuring range of both assays were included in the method comparison analysis. The results are summarized below:

		QUANTA Lite MPO		
		Positive	Negative	Total
EliA MPO^S	Positive	29	33	62
	Equivocal	0	5	5
	Negative	1	216	217
	Total	30	254	284

Equivocal considered as positive:
Positive agreement: 96.7% (95% CI: 82.8 – 99.9%)
Negative agreement: 85.0% (95% CI: 80.0 – 89.2%)
Overall agreement: 86.3% (95% CI: 81.7 – 90.0%)

Equivocal considered as negative:
Positive agreement: 96.7% (95% CI: 82.8 – 99.9%)
Negative agreement: 87.0% (95% CI: 82.2 – 90.9%)
Overall agreement: 88.0% (95% CI: 83.7 – 91.6%)

EliA GBM Assay**:**

A total of 527 samples, including 460 samples from the samples used in the clinical validation study, were tested with EliA GBM assay and predicate Wielisa anti-GBM assay. Among these samples, the 295 samples within the assay measuring range of both assays were included in the method comparison analysis. The results are summarized below:

		Wielisa Anti-GBM			
		Positive	Equivocal	Negative	Total
EliA GBM	Positive	44	9	6	59
	Equivocal	0	0	3	3
	Negative	0	0	233	233
	Total	44	9	242	295

Equivocal considered as positive:
Positive agreement: 100.0% (95% CI: 93.3 – 100%)
Negative agreement: 96.3% (95% CI: 93.1 – 98.3%)
Overall agreement: 97.5% (95% CI: 94.3 – 98.6%)

Equivocal considered as negative:
Positive agreement: 100.0% (95% CI: 92.0 – 100%)
Negative agreement: 94.0% (95% CI: 90.3 – 96.6%)
Overall agreement: 94.9% (95% CI: 91.8 – 97.1%)

b. Matrix comparison:

Potential differences between sample matrices (serum, plasma heparin, plasma

EDTA, and plasma citrate) were evaluated in each assay by testing 41 paired spiked samples containing different levels of analyte on Phadia 100. Sample concentrations spanned the assay ranges, but each set contained at least three negative samples and three equivocal samples. A Passing and Bablok analysis was performed and the results are presented in the following table:

EliA PR3^S Assay				
	Test Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
Serum vs. Heparin plasma	1.5 – 138.4	1.01 (0.96 – 1.05)	-0.27 (-0.60 – 0.18)	1.00
Serum vs. EDTA plasma	1.3 – 127.4	0.99 (0.96 – 1.02)	-0.10 (-0.46 – 0.09)	0.99
Serum vs. Citrate plasma	1.4 – 134	0.99 (0.95 – 1.02)	-0.04 (-0.27 – 0.24)	0.99

EliA MPO^S Assay				
	Test Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
Serum vs. Heparin plasma	0.5 – 93.4	0.99 (0.96 – 1.02)	0.20 (-0.22 – 0.90)	1.00
Serum vs. EDTA plasma	0.5 – 113.3	1.11 (1.08 – 1.15)	-0.05 (0.83 – 0.24)	1.00
Serum vs. Citrate plasma	0.6 – 98.2	1.01 (0.98 – 1.08)	0.19 (-0.29 – 0.85)	0.99

EliA GBM Assay				
	Test Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
Serum vs. Heparin plasma	4.6 – 605.2	1.01 (0.98 – 1.05)	-0.04 (-1.42 – 0.39)	0.99
Serum vs. EDTA plasma	4.2 – 613.4	1.04 (1.01 – 1.08)	-0.43 (-0.98 – 0.33)	0.99
Serum vs. Citrate plasma	4.2 – 598.5	1.03 (1.00 – 1.07)	-0.52 (-1.39 – -0.04)	0.99

c. Instrument comparison:

The performance of EliA PR3^S, EliA MPO^S and EliA GBM was evaluated on the Phadia 100 and Phadia 250 instruments using 32 positive and four negative samples. The samples were analyzed in six runs in single replicate on three Phadia 100 and three Phadia 250, with two runs on each instrument. The results of the Passing-Bablok regression analyses are summarized below:

	Slope (95% CI)	Intercept (95% CI)
EliA PR3^S	0.91 (0.89 – 0.94)	-0.03 (-0.11 – 0.18)
EliA MPO^S	1.04 (1.02 – 1.08)	0.20 (-0.08 – 0.41)
EliA GBM	1.09 (1.06 – 1.10)	-0.53 (-0.88 – -0.07)

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

EliA PR3^S Assay:

A total of 455 samples were included in the clinical validation for the EliA PR3^S assay. The validation set of samples includes 100 samples from patients diagnosed with glomerulonephritis with polyangiitis (GPA) and 355 samples from patients with other autoimmune diseases and infectious conditions. Clinical sensitivity and specificity to aid in diagnosis of GPA are summarized in the following table:

		Clinical Diagnosis of GPA		
		Positive	Negative	Total
EliA PR3^S	Positive	79	6	85
	Equivocal	4	9	13
	Negative	17	340	357
	Total	100	355	455

Equivocal considered as positive:
Sensitivity: 83.0% (95% CI: 74.2 - 89.8%)
Specificity: 95.8% (95% CI: 93.1 - 97.6%)

Equivocal considered as negative:
Sensitivity: 79.0% (95% CI: 69.7 – 86.5%)
Specificity: 98.3% (95% CI: 96.4 – 99.4%)

The table below shows the results for non-GPA samples tested with EliA PR3^S:

Performance of EliA PR3^S on Non-GPA Samples		
Non-GPA Diseases	**N**	**No (%) Positive***
Microscopic Polyangiitis (MPA)	77	3 (4%)
Churg Strauss Syndrome (CSS)	23	0 (0%)
Non-ANCA Associated Vasculitis (NANCA)	20	0 (0%)
Crohn's disease	30	2 (7%)
Ulcerative colitis (UC)	30	1 (3%)
Mixed Connective Tissue Disease (MCTD)	5	0 (0%)
Systemic Lupus Erythematosus (SLE)	32	0 (0%)
Sjögren's Syndrome (SS)	8	0 (0%)
Rheumatoid Arthritis (RA)	20	0 (0%)
Acute renal failure	15	0 (0%)
Severe pneumonia	15	0 (0%)

Performance of EliA PR3^S on Non-GPA Samples		
Non-GPA Diseases	**N**	**No (%) Positive***
Asthma	15	0 (0%)
Sinusitis	15	0 (0%)
Hepatitis C virus infection (HCV)	3	0 (0%)
Hepatitis B virus infection (HBV)	23	0 (0%)
Human Immunodeficiency virus infection (HIV)	1	0 (0%)
Borrelia	5	0 (0%)
Yersinia	15	0 (0%)
Helicobacter pylori	2	0 (0%)
Toxoplasmosis	1	0 (0%)
TOTAL	**355**	**6 (1.7%)**

**Equivocal considered as negative for this analysis*

EliA MPO^S Assay**:**

A total of 425 samples were included in the clinical validation for the EliA MPO^S assay. The validation set of samples includes 80 samples from patients diagnosed with Microscopic polyangiitis (MPA), and 345 samples from patients with other diseases including autoimmune and infectious disease. Clinical sensitivity and specificity to aid in diagnosis of MPA are summarized in the following tables:

		Clinical Diagnosis of MPA		
		Positive	Negative	Total
EliA MPO^S	Positive	44	25	69
	Equivocal	3	2	5
	Negative	33	318	351
	Total	80	345	425

Equivocal considered as positive:
Sensitivity: 58.8% (95% CI: 47.2– 69.6%)
Specificity: 92.2% (95% CI: 88.8 – 94.8%)

Equivocal considered as negative:
Sensitivity: 55.0% (95% CI: 43.5 – 66.2%)
Specificity: 92.8% (95% CI: 89.5 – 95.3%)

The table below shows the results for non-MPA samples tested with EliA MPO^S:

Performance of EliA MPO^S on Non-MPA Samples		
Diseases	**N**	**No (%) Positive***
Churg Strauss Syndrome (CSS)	60	11 (18%)
Necrotizing Crescentic Glomerulonephritis (NCGN)	12	8 (67%)
Glomerulonephritis with Polyangiitis (GPA)	18	4 (23.5%)
Non-ANCA Associated Vasculitis (NAAV)	22	0 (0%)

Performance of EliA MPO^S on Non-MPA Samples		
Diseases	**N**	**No (%) Positive***
Ulcerative colitis (UC)	30	0 (0%)
Crohn's disease	30	0 (0%)
Systemic Lupus Erythematosus (SLE)	40	0 (0%)
Progressive Systemic Scleroderma (PSS)	15	0 (0%)
Rheumatoid Arthritis (RA)	20	0 (0%)
Acute renal failure	15	0 (0%)
Severe pneumonia	15	1 (6.7%)
Asthma	15	0 (0%)
Sinusitis	15	0 (0%)
Helicobacter pylori	5	0 (0%)
Borrelia	5	0 (0%)
Parvovirus	10	0 (0%)
Human Immunodeficiency virus infection (HIV)	5	0 (0%)
Epstein-Barr virus (EBV)	10	1 (11%)
Yersinia	3	0 (0%)
TOTAL	**345**	**25 (7.2%)**

**Equivocal considered as negative for this analysis*

EliA GBM Assay**:**

A total of 460 samples were included in the clinical validation for the EliA GBM assay. The validation set of samples includes 69 samples from patients diagnosed with Goodpasture syndrome and 391 samples from patients with other autoimmune and infectious conditions. Clinical sensitivity and specificity to aid in diagnosis of Goodpasture Syndrome are summarized in the following tables:

		Clinical Diagnosis of Goodpasture Syndrome		
		Positive	Negative	Total
EliA GBM	Positive	65	2	67
	Equivocal	3	0	3
	Negative	1	389	322
	Total	69	391	460

Equivocal considered as positive:
Sensitivity: 98.6% (95% CI: 92.2 – 100%)
Specificity: 99.5% (95% CI: 98.2 – 99.9%)

Equivocal considered as negative:
Sensitivity: 94.2% (95% CI: 85.8 – 98.4%)
Specificity: 99.5% (95% CI: 98.2 – 99.9%)

The table below shows the results for non-Goodpasture syndrome samples tested with EliA GBM:

Performance of EliA GBM on Non-Goodpasture Syndrome Samples		
Diseases	**N**	**No (%) Positive**
Microscopic Polyangiitis (MPA)	52	2 (4%)
Glomerulonephritis with Polyangiitis (GPA)	50	0 (0%)
Systemic Lupus Erythematosus (SLE)	58	0 (0%)
Systemic sclerosis (SSc)	42	0 (0%)
Rheumatoid Arthritis (RA)	20	0 (0%)
Acute renal failure	15	0 (0%)
Diabetic nephropathy	20	0 (%)
Deep vein thrombosis	13	0 (0%)
Bacterial infection[1]	19	0 (0%)
Viral infection[2]	31	0 (0%)
Others[3] (Non-Goodpasture syndrome)	71	0 (0%)
TOTAL	**391**	**2 (0.5%)**

[1] *including 10 Yersinia, 2 Mycoplasma, 2 multibacterial infection, 1 Acinetobacter baumannii, 1 Staphylococcus aureus, 1 Pseudomonia aureus, 1 Enterococcus, and 1 Helicobacter pylori*

[2] *including 8 HBV, 11 HCV and 12 EBV*

[3] *patients suspected of Goodpasture Syndrome*

b. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off**:**

See assay cut-off

5. Expected values/Reference range:

Four hundred (400) samples from apparently healthy Caucasian individuals with equally distributed by sex and age were measured with each of EliA PR3***S***, EliA MPO***S*** and EliA GBM assays. The results are shown in the following tables:

EliA PR3***S***

	Phadia 100 U/mL	Phadia 250 U/mL
Mean	0.5	0.3
Median	0.4	0.3
Range	0.2 – 3.0	0.1 – 4.1
95th percentile	0.7	0.6
99th percentile	1.0	0.7

One sample was positive (>3 U/mL) on the Phadia 250 in this study, and one sample fell in the equivocal range on the Phadia 100. Expected values may vary depending on the population tested.

EliA MPO*s*

	Phadia 250 U/mL
Mean	0.6
Media	0.5
Range	0.1 – 2.4
95th percentile	0.9
99th percentile	1.5

No samples were positive (>5 U/mL) or equivocal (3.5 – 5 U/mL). Expected values may vary depending on the population tested.

EliA GBM

	Phadia 100 U/mL
Mean	0.3
Media	0.2
Range	0 – 11.4
95th percentile	0.9
99th percentile	2.0

One sample was positive (>10 U/mL) in this study, but no samples fell in the equivocal range. Expected values may vary depending on the population tested.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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