510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K181556

B. Purpose for Submission:

Adding a previously cleared assay on a new instrument platform (Phadia 2500/5000)

C. Measurand:

IgG autoantibodies specific to native pyruvate dehydrogenase complex from mitochondria and recombinant M2-antigen (M2)

D. Type of Test:

Automated semi-quantitative solid-phase fluoroimmunoassay

E. Applicant:

Phadia US, Inc.

F. Proprietary and Established Name:

EliA M2 Immunoassay

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5090, Antimitochondrial antibody immunological test system

2. Classification:

   Class II

3. Product code:

   DBM, Antimitochondrial Antibody, Indirect Immunofluorescent, Antigen, Control

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   EliA M2 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to M2 in human serum and plasma (Li-heparin, EDTA) to aid in the clinical diagnosis of primary biliary cirrhosis in conjunction with other laboratory and clinical findings. EliA M2 uses the EliA IgG method on the instrument Phadia 2500/5000.

2. Indication for use:

   Same as intended use

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   For use on the Phadia 2500 and Phadia 5000 instruments

I. Device Description:

EliA uses a modular reagent system. The assay-specific, method-specific and general reagents are packaged and purchased as separate units. The reagents on Phadia 2500 and Phadia 5000 are identical; they are only filled in different containers.

EliA Assay-specific reagent:

- EliA M2 Wells are coated with native pyruvate dehydrogenase complex from mitochondria and recombinant M2-antigen– 4 carriers (12 wells each), ready to use

EliA Method-specific reagents:

- EliA IgG Calibrator Strips: Human IgG (0, 4, 10, 20, 100, 600 µg/L) in PBS containing BSA, detergent and 0.095% (w/v) 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% (w/v) sodium azide – 5 strips, 6 single-use vials per strip, 0.3 mL each, ready to use
- EliA Sample Diluent: PBS containing BSA, detergent and 0.095% (w/v) 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% (w/v) 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 Well: Coated with mouse monoclonal antibodies – 4 carriers (12 wells each), ready to use

EliA General reagents:

- Development Solution (0.01 %4-Methylumbelliferyl-β-D-galacto-side, <0.0010% preservative), ready for use
- Stop Solution (4% Sodium Carbonate), ready for use
- Dilution Wells (high density polyethylene wells), 50 carriers, ready to use
- Pipette Tips in Racks (polyethylene tips), 24 racks × 160 tips, ready for use
- Washing Solution (information in separate Washing Solution package insert)

Phadia 2500 and Phadia 5000 are identical instruments except for sample throughput. The Phadia 2500 consists of one process module (two process lines), whereas Phadia 5000 consists of two process modules (2 x 2 process lines). Instrument operation is handled by onboard Instrument Software (ISW). Data output is administered by Information Data Manager (IDM). All steps of an assay are performed within a single process line. Thus, study protocols used for Phadia 2500 are also valid for Phadia 5000.

J. Substantial Equivalence Information:

1. Predicate device name:

   EliA M2 Immunoassay on Phadia 250

2. Predicate 510(k) number:

   K141375

3. Comparison with predicate:

Similarities		
Item	**Device** EliA M2 on Phadia 2500/5000	**Predicate** EliA M2 on Phadia 250
Intended Use/ Indications for Use	EliA M2 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to M2 in human serum and plasma (Li-heparin, EDTA) to aid in the clinical diagnosis of primary biliary cirrhosis in conjunction with other laboratory and clinical findings. EliA M2 uses the EliA IgG method on the instrument Phadia 2500/5000.	EliA M2 is intended for the in vitro semi-quantitative measurement of IgG antibodies directed to M2 in human serum and plasma (heparin, EDTA) to aid in the clinical diagnosis of primary biliary cirrhosis in conjunction with other laboratory and clinical findings. EliA M2 uses the EliA IgG method on the instrument Phadia 250.
Assay Type	Automated fluoroenzyme immunoassay	Same
Assay Format	Semi-quantitative	Same
Assay Set-up	Random access	Same
Solid Phase	Polystyrene microwells	Same

Similarities		
Item	**Device** EliA M2 on Phadia 2500/5000	**Predicate** EliA M2 on Phadia 250
Measurand	Autoantibodies to M2	Same
Coating Antigens	Native pyruvate dehydrogenase complex from mitochondria and recombinant M2-antigen	Same
Sample Volume	90 µL (20 µL of undiluted (sample)	Same
Incubation Temperature	37°C (controlled)	Same
Incubation Times	Diluted patient samples: 30 min. Conjugate: 28 min. Development Solution: 39 min.	
Time to 1st result	~2 h	Same
Detection Antibody (Conjugate) Volume	90 µL	Same
Substrate/Chromogen (Development) Volume	90 µL	Same
Stop Solution Volume	200 µL	
Calibration Strip	Human IgG (0, 4, 10, 20, 100, 600 µg/L)	Same
Calibration Frequency	28 days	Same
Assay Cut-off/ Results Interpretation	< 4 U/mL: Negative 4 – 6 U/mL: Equivocal > 6 U/mL: Positive	Same
Result Calculation Software	Phadia Information Data Manager (IDM)	Same

Differences		
Item	**Device** EliA M2 on Phadia 2500/5000	**Predicate** EliA M2 on Phadia 250
Instrument Platform	Phadia 2500/5000	Phadia 250
Measuring Range	0.5 – 276 U/mL	0.8 – 220 U/mL
Barcode Reader	On the Phadia 2500/5000, the reagents are on a moving belt which conveys them past the barcode reader. The lot-specific information will be read automatically by the instrument during loading.	The Phadia 250 instrument has a built-in barcode reader at the front of the instrument, but the operator needs to scan the barcodes manually by showing the reagents to the barcode reader. Alternatively, the operator can also enter the characters below the barcode manually.
Loading of EliA Carriers	The Phadia 2500/5000 instruments do not have a Loading Tray. The EliA carriers are loaded into racks which are directly transferred to the	The EliA carriers are loaded manually on the Loading Tray from where they can be processed directly or transferred to the cooled

Differences		
Item	**Device** EliA M2 on Phadia 2500/5000	**Predicate** EliA M2 on Phadia 250
	cooled storage compartment.	storage compartment.
Process Time/Time to Patient Result	Phadia 2500/5000 instruments process two Wells in parallel in 48 seconds. Phadia 2500/5000 provides the results at a 24 seconds interval.	Phadia 250 needs 1 minute to process one Well. Phadia 250 provides the results at a one minute interval.
Daily Sample Throughput	Phadia 2500: ~2500 tests/day Phadia 5000: ~5000 tests/day	~250 tests/day
Sample Dilution	Use disposable Pipette Tips in Racks for pipetting samples in Dilution Well	Use steel pipette to dilute the samples in Dilution Plates
Carry-over due to Reagent Re-use	No carry-over from samples to conjugate because Phadia 2500/5000 instruments use disposable tips for pipetting samples and a separate pipette for the conjugate.	Conjugate should not be reused due to risk of carryover contamination.

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

CLSI EP05-A3; Evaluation of Precision Performance of Quantitative Measurement Methods; September 2014

CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; April 2003

CSLI EP17-A2: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

EliA tests are fluorescence immunoassays for the detection and measurement of human antibodies based on EliA solid-phase components, which contain specific antigens for the antibodies to be measured.

EliA M2: Polystyrene EliA wells are coated with native pyruvate dehydrogenase complex from mitochondria and recombinant M2-antigen. If present in the patient's specimen, antibodies to M2 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 higher the value of fluorescent signal, 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.

Phadia 2500/5000: The Phadia 2500 and Phadia 5000 instruments are automated platforms for EliA test procedures from sample and reagent handling up to processing of results.

M. Performance Characteristics:

1. Analytical performance:

All results presented below met the manufacturer's pre-determined acceptance criteria for all analytical performance studies.

a. Precision/Reproducibility:

The precision of the assay was assessed in a study with a total of 21 runs, one run/day over a period of seven days. Each sample was tested in four replicates/run giving a total of 84 replicates per sample (3 instruments × 7 runs x 4 replicates = 84). The data was calculated against the calibration curve from Day 1. Five native patient serum samples were tested: one negative, two in the equivocal range, and two positive specimens. All samples were tested on all three Phadia 2500/5000 instruments.

One lot of EliA M2 reagents was used in the study because inter-lot-variation was determined in the predicate device K141375. The results for all three instruments are summarized in the table below:

Mean (U/mL)	**Within-Run**		**Between-Run**		**Between-Instrument**		**Total Imprecision**	
	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1.7	0.1	7.3	0.1	4.8	0.2	13.1	0.3	15.8
4.0	0.2	4.3	0.1	2.9	0.3	6.6	0.3	8.4
5.9	0.2	3.7	0.1	2.5	0.3	4.2	0.4	6.2
74.8	2.3	3.1	1.7	2.2	4.0	5.3	4.9	6.5
175.9	8.0	4.5	6.8	3.9	12.1	6.9	16.0	9.1

b. Linearity/assay reportable range:

Four patient serum samples were diluted in EliA Sample Diluent and tested in triplicates with one lot of EliA M2 reagents and one set of system reagents on Phadia 2500/5000. The results of the dilutions were compared with their expected values. The ratio observed/expected (O/E) was calculated. Mean observed value was used in the calculation. The results of the linear regression analysis are summarized below:

Sample	**Dilution range (U/mL)**	**Slope (95% CI)**	**Intercept (95% CI)**	**R^2**	**%CV Range**
1	0.7 – 48.3	0.99 (0.97 to 1.01)	-0.32 (-0.78 to 0.14)	1.00	1.2 – 5.4

Sample	Dilution range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2	%CV Range
2	2.1 – 211.3	1.02 (0.99 to 1.05)	1.90 (-1.01 to 4.81)	1.00	0.5 – 3.5
3	5.7 – 253.2	1.03 (0.97 to 1.08)	2.36 (-3.78 to 8.5)	1.00	2.1 – 6.6
4	0.5 – 16.6	1.02 (0.98 to 1.07)	0.13 (-0.18 to 0.44)	1.00	1.0 – 4.1

The reportable range (Limit of Detection to upper limit) is from 0.5 to 220 U/mL. The measuring range (Limit of Quantitation to upper limit) is from 0.8 to 220 U/mL.

The following statements are included in the package insert: "*Please note that concentration values between LoD and LoQ may show a higher uncertainty"* and "*Please note that due to differing binding characteristics of the antibodies in patient samples, not all sera can be diluted linearly within the measuring range."*

High dose hook effect: The hook effect was previously reviewed in K141375.

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

i) *Traceability:*

The EliA IgG calibration method and traceability was previously reviewed in K141375.

Controls:

Controls are sold separately from the assay. The EliA M2 Positive is prepared from selected pooled human sera and contains IgG antibodies to M2. The EliA IgG/IgM/IgA Negative Control is prepared from selected pooled sera from normal, healthy donors. The controls are pre-diluted and ready for use. Each EliA Control package contains a Control Certificate listing predefined acceptance criteria for the EliA products the Controls can be used with.

ii) *Kit Stability:*

Shelf-life stability, open-stability and on-board stability were previously reviewed in K141375.

d. *Detection limit:*

The limit of blank (LoB) is based on 72 blank replicates (1 blank sample tested in 12 replicates × one run/day × six days). The limit of detection (LoD) is based on 72 replicates (12 replicates × one run/day × six days) for each of the five low-level serum samples tested. The limit of quantitation (LoQ) is based on the concentration of one of the five low-level serum samples tested with a %CV closest but below the precision target level of 20% CV.

The results are summarized in the table below:

	LoB	LoD	LoQ
EliA M2 (U/mL)	0.3	0.5	0.8

e. Analytical specificity:

i) Endogenous Interference:

Interference by endogenous substances was previously reviewed in K141375.

ii) Cross-reactivity:

Cross-reactivity was previously reviewed in K141375.

iii) Carry-over:

The Phadia 2500/5000 instruments use disposable tips for pipetting samples and a separate pipette for the conjugate, therefore carry-over from samples to conjugate was not evaluated.

f. Assay cut-off:

The assay cut-offs are the same as in the predicate device (refer to K141375) and are summarized in the table below:

Assay cut-off	Interpretation
<4 U/mL	Negative
4 – 6 U/mL	Equivocal
>6 U/mL	Positive

2. Comparison studies:

a. Method comparison with predicate device:

Instrument comparison was performed with the predicate device, see 2c. below.

b. Matrix comparison:

Matrix comparison between serum and plasma (Li-Heparin, and EDTA) was reviewed in K141375.

c. *Instrument comparison:*

The purpose of this study was to evaluate conformance and show comparability of EliA M2 on the Phadia 250 instrument versus the Phadia 2500/5000 instrument. A

total of 103 samples (84 positive, 12 negative and 7 equivocal) were run in singlicate on one Phadia 250 instrument and one Phadia 2500/5000 instrument for comparison. Only samples inside the measuring range were included in the calculations. Results were analyzed by Passing-Bablok regression. The results are summarized below:

Range tested (U/mL)	**Slope (95% CI)**	**Intercept (95% CI)**	***R**
0.9 – 211.6	1.04 (1.02 to 1.06)	-0.14 (-0.46 to 0.03)	0.995
*Pearson correlation coefficient			

No sample switched from negative to positive or vice versa. One sample in the equivocal range (5.7 U/mL) tested positive (6.3 U/mL), and one negative sample (3.8 U/mL) yielded test result in the equivocal range (4.4 U/mL) on the Phadia 2500/5000 instrument. The results are summarized below:

		EliA M2 on Phadia 250			**Total**
		Positive >6	Equivocal:4 6	Negative ≤ 4	
EliA M2 on Phadia 2500/5000	Positive: >6	84	1	0	85
	Equivocal: 4 6	0	6	1	7
	Negative: <4	0	0	11	11
Total		84	7	12	103

Positive percent agreement, negative percent agreement, and total percent agreement were evaluated with equivocal results considered positive in the table below:

Equivocal EliA M2 results considered as positive				
		EliA M2 on Phadia 250		**Total**
		Positive: ≥ 4	Negative: < 4	
EliA M2 on Phadia 2500/5000	Positive: ≥ 4	91	1	92
	Negative: < 4	0	11	11
Total		91	12	103
Positive percent agreement: 100% (91/91) 95% CI: 96.0 – 100%				
Negative percent agreement: 91.7% (11/12) 95% CI: 61.5 – 99.8%				
Total percent agreement: 99.0% (103/103) 95% CI: 94.7 – 100%				

Results when equivocal results are considered negative are presented in the table below:

Equivocal EliA M2 results considered as negative				
		EliA M2 on Phadia 250		
		Positive: >6	Negative: ≤6	**Total**
EliA M2 on Phadia 2500/5000	Positive: > 6	84	1	85
	Negative: ≤ 6	0	18	18
Total		84	19	103
Positive percent agreement: 100% (84/84) 95% CI: 95.7 – 100%				
Negative percent agreement: 95.5% (18/19) 95% CI: 77.2 – 99.9%				
Total percent agreement: 99.0% (102/103) 95% CI: 94.9 – 100%				

3. Clinical studies:

   Clinical performance was previously reviewed in K141375.

   a. Clinical sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

4. Clinical cut-off**:**

   See assay cut-off

5. Expected values/Reference range:

   A total of 400 apparently healthy blood donor samples from a Caucasian population matched by age and gender were tested on the Phadia 2500/5000 instrument to evaluate expected values in the normal population. Seven samples were found in the equivocal range and two were found with positive results. There was no significant difference between male and female. Expected values may vary depending on the population tested. The results are summarized below:

	U/mL
Mean	1.1
Median	0.9
Rang7e	0.2 33.8
95th percentile	1.9
99th percentile	5.2

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.