

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM**

A. 510(k) Number:

K151799

B. Purpose for Submission:

New device

C. Measurand:

IgG Antibodies to TG (Thyroglobulin) and TPO (Thyroid peroxidase)

D. Type of Test:

Fully automated, quantitative, Immunofluorescence

E. Applicant:

Phadia AB, Sweden

F. Proprietary and Established Names:

EliA™ anti-TG Immunoassay

EliA™ anti-TPO Immunoassay

EliA™ Thyroid Positive Control 250

EliA™ Thyroid Positive Control 2500/5000

G. Regulatory Information:

1. Regulation section:

21 CFR § 866.5870: Thyroid autoantibody immunological test system

21 CFR § 862.1660: Quality control material (assayed and unassayed)

2. Classification:

Class II (Assay)

Class I (Controls)

3. Product code:

JZO: System, Test, Thyroid Autoantibody

JJY: Multi-Analyte Controls, All Kinds (assayed)

4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):

a. *EliA™ anti-TG Immunoassay*

EliA™ anti-TG is intended for the in vitro quantitative measurement of IgG antibodies directed to thyroglobulin (TG) in human serum and plasma (Li-heparin, EDTA) as an aid in the clinical diagnosis of autoimmune thyroiditis and Graves' disease in conjunction with other laboratory and clinical findings. EliA anti-TG uses the EliA IgG method on the instruments Phadia 250 and Phadia 2500/5000.

b. *EliA™ anti-TPO Immunoassay*

EliA™ anti-TPO is intended for the in vitro quantitative measurement of IgG antibodies directed to thyroid peroxidase (TPO) in human serum and plasma (Li-heparin, EDTA,) as an aid in the clinical diagnosis of autoimmune thyroiditis and Graves' disease in conjunction with other laboratory and clinical findings. EliA anti-TPO uses the EliA IgG method on the instruments Phadia 250 and Phadia 2500/5000.

c. *EliA™ Thyroid Positive Control 250 and 2500/5000*

EliA™ Thyroid Positive Control 250 and EliA Thyroid Positive Control 2500/5000 is intended for laboratory use in monitoring the performance of in vitro measurement of antibodies against thyroid peroxidase (TPO) and thyroglobulin (TG) with Phadia 250 and Phadia 2500/5000 using the EliA IgG method.

d. *EliA™ Thyroid Negative Control 250 and 2500/5000*

EliA™ IgG/IgM/IgA Negative Control 250 and EliA™ IgG/IgM/IgA Negative Control 2500/5000 is intended for laboratory use in monitoring the performance of in vitro measurement of autoantibodies with Phadia 250 and Phadia 2500/5000 using the EliA™ IgG or IgM or IgA method.

2. Indication(s) for use:

Same as above

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

For use on the Phadia® 250 or Phadia® 2500/5000 instruments

I. Device Description:

The new devices belong to a fully integrated and automated system for immunodiagnostic testing. It comprises a Fluorescence-Immunoassay test system using EliA single wells as the solid phase and is intended to be performed on the Phadia 250 and Phadia 2500/5000 instruments.

The EliA™ reagents are available as modular packages, each purchased separately. All packages except the positive and negative controls are required to carry out an EliA™ anti-TG or anti-TPO test.

1. Antibody-Coated Wells

- a. EliA™ anti-TG wells are coated with a human thyroglobulin antigen – four carriers (16 wells each), ready to use.
- b. EliA™ anti-TPO wells are coated with a human recombinant thyroid peroxidase antigen – four carriers (16 wells each), ready to use.

2. EliA™ IgG Conjugate 50 or 200:

β-Galactosidase labeled anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and 0.06% sodium azide – six wedge shaped bottles, 5 mL each, ready to use; or six wedge-shaped bottles, 19 mL each, ready to use.

3. EliA™ Sample Diluent

PBS containing BSA, detergent and 0.095% sodium azide – six bottles, 48 mL each, ready to use; or six bottles, 400 mL each, ready to use.

4. EliA™ Thyroid Positive Control 250 or 2500/5000

Human serum containing IgG antibodies to TG and TPO in PBS containing BSA, detergent and 0.095% sodium azide – six single use vials, 0.3 mL each, ready to use.

5. EliA™ Negative Control 250 or 2500/5000:

Human sera from healthy donors in PBS containing BSA, detergent and 0.095% sodium azide – six single-use vials, 0.3 mL each, ready to use.

6. EliA™ IgG Calibrator Strips:

Human IgG (0, 4, 10, 20, 100, 600 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide – five strips, six single-use vials per strip, 0.3 mL each, ready to use.

7. EliA™ IgG Curve Control Strips:

Human IgG (20 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide – five strips, six single-use vials per strip, 0.3 mL each, ready to use.

8. EliA™ IgG Calibrator Well:

Coated with mouse monoclonal antibodies – four carriers (12 wells each), ready to use.

J. Substantial Equivalence Information:

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

VarelisA TG Antibodies (K003414)
 VarelisA TPO Antibodies (K993585)
 Negative controls (K091845)
 Calibrators (K061165)

2. Comparison with predicate:

Similarities: EliA™ anti-TG Immunoassay		
Item	New Device	VarelisA TG Antibodies K003414
Intended Use	EliA anti-TG is intended for the in vitro quantitative measurement of IgG antibodies directed to thyroglobulin (TG) in human serum and plasma (heparin, EDTA) as an aid in the clinical diagnosis of autoimmune thyroiditis and Graves' disease. EliA anti-TG uses the EliA IgG method on the instruments Phadia 250 and Phadia 2500/5000.	The VarelisA TG (Thyroglobulin) Antibodies EIA kit is designed for the quantitative and qualitative determination of thyroglobulin antibodies in serum or plasma to aid in the diagnosis of thyroid diseases such as autoimmune thyroiditis and Graves' disease.
Assay Type	ELISA	Same
Type of test	Quantitative	Same
Antigen	Human Thyroglobulin	Same

Differences: EliA™ anti-TG Immunoassay		
Item	New Device	VarelisA TG Antibodies K003414
Instrument	Phadia 250 and 2500/5000, fully automated immunoassay analyzers	ELISA-Reader
Instrumentation	Phadia 250 and 2500/5000 are fully Automated immunoassay analyzers	ELISA-Reader
Reaction Temperature	37°C (controlled)	Room temperature
Assay Range	12–4794 IU/mL	3–3000 IU/mL
Incubation times	Diluted patient samples: 30 minutes Conjugate: 28 minutes Development Solution: 39 minutes	Positive and negative controls, diluted patient samples: 30 minutes. Conjugate: 30 minutes Substrate: 10 minutes (in dark)
Detection antibody (conjugate)	IgG conjugate: anti-human IgG β-Galactosidase (mouse monoclonal)	IgG conjugate: anti-human IgG horse radish peroxidase (goat)

Differences: EliA™ anti-TG Immunoassay		
Item	New Device	VarelisA TG Antibodies K003414
	antibodies)	
Signal	Fluorescence	Optical density (at 450nm)
Calibrators	Six vials of human IgG at concentrations of 0, 4, 10, 20, 100 and 600 µg/L	Six vials of TG-specific IgG at concentrations of 0, 30, 100, 300, 1000 and 3000 IU/mL
Cut-off	< 40 IU/mL negative 40–60 IU/mL equivocal > 60 IU/mL positive	< 60 IU/mL negative 60–100 IU/mL equivocal > 100 IU/mL positive
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	N/A
Substrate	Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside and < 0.0010% preservative (mixture of 5-chloro- 2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-Methyl-2H-isothiazol-3-one [EC no. 220-239-6] at a 3:1 ratio.	TMB Chromogen
Sample Dilution	1:100	1:101
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit

Similarities: EliA™ anti-TPO Immunoassay		
Item	New Device	VarelisA TG Antibodies K993585
Intended Use	EliA anti-TPO is intended for the in vitro quantitative measurement of IgG antibodies directed to thyroid peroxidase (TPO) in human serum and plasma (heparin, EDTA) as an aid in the clinical diagnosis of autoimmune thyroiditis and Graves' disease. EliA anti-TPO uses the EliA IgG method on the instruments Phadia 250 and Phadia 2500/5000.	The VarelisA TPO Antibodies EIA kit is designed for the quantitative and qualitative determination of TPO (thyroid peroxidase) antibodies in serum or plasma to aid in the diagnosis of thyroid diseases such as autoimmune thyroiditis and Graves' disease.
Assay Type	ELISA	Same
Type of test	Quantitative	Same
Antigen	Human Recombinant TPO	Same

Differences: ELiA™ anti-TPO Immunoassay		
Item	New Device	VareliSA TG Antibodies K993585
Instrumentation	Phadia 250 and 2500/5000, fully automated immunoassay analyzers	ELISA-Reader
Reaction Temperature	37°C (controlled)	Room temperature
Assay Range	4–1542 IU/mL	1–3000 IU/mL
Incubation times	Diluted patient samples: 30 minutes Conjugate: 28 minutes Development Solution: 39 minutes	Positive and negative controls: diluted patient samples: 30 minutes Conjugate: 30 minutes Substrate: 10 minutes (in dark)
Detection antibody (conjugate)	IgG conjugate: anti-human IgG β-Galactosidase (mouse monoclonal antibodies)	IgG conjugate: anti-human IgG horse radish peroxidase (goat)
Signal	Fluorescence	Optical density (at 450nm)
Calibrators	Six vials of human IgG at concentrations of 0, 4, 10, 20, 100 and 600 µg/L	Six vials of TG-specific IgG at concentrations of 0, 30, 100, 300, 1000 and 3000 IU/mL
Cut-off	< 25 IU/mL negative 25–35 IU/mL equivocal > 35 IU/mL positive	< 60 IU/mL negative 60–100 IU/mL equivocal > 100 IU/mL positive
Calibration curve	Option to store curve for up to 28 days and run curve controls in each assay for calibration	N/A
Substrate	Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside and <0.0010% preservative (mixture of 5-chloro- 2-methyl-2H-isothiazol-3-one [EC no. 247-500-7] and 2-Methyl-2H-isothiazol-3-one [EC no. 220-239-6] at a 3:1 ratio	TMB Chromogen
Sample Dilution	1:100	1:101
Concept	Modular reagents concept (test-method specific and general reagents)	All reagents in a single kit

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

CLSI EP6-A - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP17-A2 - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline Second Edition.

CLSI H18-A3: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline

L. Test Principle:

The EliA™ test wells are coated with a human thyroglobulin protein, or a human recombinant thyroid peroxidase protein. If present in the patient's specimen, antibodies to TG or TPO bind to their specific antigen. After washing away unbound 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 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Note: All results below met the manufacturer's pre-specified acceptance criteria.

a. Precision/Reproducibility:

The precision studies were performed using eight patient serum samples selected from different parts of the claimed measuring range of the assay on both Phadia 250 and Phadia 2500/5000 instruments to establish intra- and inter-assay precision. A total of 21 runs (three instruments/seven runs each) over seven days were performed on samples with 252 replicate determinations per sample with a calibration curve in each run.

Two lots of EliA™ anti-TG well were included in the study. On each instrument the same eight samples were tested. All samples were neat specimens from different patients. The study was performed on three Phadia 250 and three Phadia 2500/5000 instruments.

EliA™ anti-TG on Phadia 250 (n = 252)									
	Mean (IU/mL)	Inter-Run		Intra-Run		Inter-Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	30.6	1.5	4.9	2.7	8.7	2.0	6.4	3.6	11.9
Sample 2	37.3	1.8	4.7	2.3	6.2	3.1	8.4	4.3	11.4
Sample 3	74.9	0.7	0.9	3.2	4.3	3.7	5.0	5.0	6.7
Sample 4	124.9	0.9	0.7	4.5	3.6	3.5	2.8	5.8	4.6
Sample 5	184.8	1.1	0.6	5.2	2.8	0.8	0.5	5.7	3.1
Sample 6	771.5	8.8	1.1	21.2	2.7	0.8	0.1	24.8	3.2
Sample 7	2065.1	29.7	1.4	67.9	3.3	38.6	1.9	88.6	4.3
Sample 8	4147.2	54.5	1.3	164.6	4.0	85.6	2.1	194.6	4.7

EliA™ anti-TG on Phadia 2500/5000 (n = 252)									
	Mean (IU/mL)	Inter-Run		Intra-Run		Inter- Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	31.4	1.0	3.2	4.3	13.5	3.4	10.9	5.6	17.8
Sample 2	38.9	0.9	2.3	4.6	11.9	4.1	10.6	6.2	16.0
Sample 3	76.7	1.5	1.9	4.0	5.2	5.4	7.0	7.1	9.2
Sample 4	134.5	2.7	2.0	7.1	5.3	8.0	5.9	11.1	8.3
Sample 5	193.0	4.0	2.0	8.4	4.3	5.3	2.8	10.8	5.6
Sample 6	754.2	9.1	1.2	39.1	5.2	7.5	1.0	40.8	5.4
Sample 7	1869.6	0.0*	0.0*	100.5	5.4	79.5	4.3	128.4	6.9
Sample 8	3797.5	50.9	1.3	338.9	8.9	118.1	3.1	387.5	10.2

* Inter-Run standard deviation of 0.0 and %CV of 0.0 indicates that this part of the total variation could not be determined as the Intra-Run variation completely explained the total variation.

EliA™ anti-TPO on Phadia 250 (n = 252)									
	Mean (IU/mL)	Inter-Run		Intra-Run		Inter- Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	15.7	0.4	2.3	0.9	5.7	0.9	5.9	1.4	8.7
Sample 2	27.4	0.4	1.5	1.3	4.6	0.8	2.9	1.6	5.7
Sample 3	39.4	0.2	0.5	1.5	3.8	1.8	4.5	2.4	6.2
Sample 4	66.7	0.7	1.0	2.6	3.9	1.2	1.7	3.0	4.5
Sample 5	218.0	2.8	1.3	10.0	4.6	1.4	0.7	10.6	4.9
Sample 6	480.1	4.7	1.0	22.7	4.7	6.5	1.4	24.7	5.2
Sample 7	814.9	3.2	0.4	31.9	3.9	19.6	2.4	37.8	4.6
Sample 8	1212.6	0.0*	0.0*	48.0	4.0	57.6	4.8	75.0	6.2

* Inter-Run standard deviation of 0.0 and %CV of 0.0% indicates that this part of the total variation could not be determined as the Intra-Run variation completely explained the total variation.

EliA™ anti-TPO on Phadia 2500/5000 (n = 252)									
	Mean (IU/mL)	Inter-Run		Intra-Run		Inter-Instrument		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Sample 1	17.7	0.4	2.2	1.5	8.7	1.6	8.9	2.3	13.1
Sample 2	26.6	0.8	3.1	1.7	6.4	0.4	1.5	2.1	8.0
Sample 3	38.5	1.3	3.4	2.3	5.9	0.9	2.3	2.8	7.3
Sample 4	65.3	1.7	2.6	4.0	6.1	1.1	1.7	4.5	7.0
Sample 5	202.9	5.4	2.7	14.0	6.9	3.4	1.7	15.4	7.6
Sample 6	466.6	10.2	2.2	33.7	7.2	7.2	1.5	36.5	7.8
Sample 7	802.8	13.8	1.7	59.1	7.4	16.3	2.0	62.8	7.8
Sample 8	1340.2	28.0	2.1	120.2	9.0	30.8	2.3	127.5	9.5

b. Linearity/assay reportable range:

For EliA™ anti-TG, seven patient serum samples and for EliA™ anti-TPO, six patient samples were serially diluted using EliA Sample Diluent and tested in three replicates with one batch of EliA™ anti-TG immunoassay and EliA™ anti-TPO immunoassay and one set of system reagents on the Phadia® 250 or Phadia® 2500/5000 instruments. The observed values were graphed against the calculated values and a linear regression was performed. Results are summarized below:

EliA™ anti-TG on Phadia 250				
Sample	Dilution Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²
1	2655.8–31.1	1.002 (0.979–1.024)	-1.46 (-24.59–21.66)	0.9990
2	2935.0–33.5	1.008 (0.979–1.037)	13.21 (-19.92–46.35)	0.9983
3	4827.9–62.5	1.025 (0.983–1.006)	58.32 (-19.33–135.98)	0.9967
4	375.3–10.1	0.998 (0.991–1.005)	1.01 (-0.07–2.09)	0.9999
5	468.8–17.5	0.995 (0.976–1.015)	1.34 (-2.73–5.42)	0.9995

6	394.2–14.2	1.005 (0.992–1.018)	-0.07 (-2.39–2.25)	0.9998
7	326.9–12.5	1.001 (0.979–1.023)	1.57 (-1.65–4.80)	0.9994

EliA™ anti-TG on Phadia 2500/5000				
Sample	Dilution Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²
1	2626.4–31.1	0.995 (0.977–1.04)	23.48 (4.45–42.51)	0.9993
2	2052.8–36.2	1.008 (0.986–1.031)	17.61 (0.89–36.12)	0.9991
3	5127.8–63.2	1.006 (0.974–1.037)	60.01 (-1.87–121.88)	0.9981
4	375.9–11.1	0.977 (0.928–1.026)	8.21 (0.42–16.01)	0.9962
5	470.7–12.1	1.01 (0.987–1.034)	2.09 (-2.60–6.78)	0.9992
6	412.0–15.4	0.987 (0.959–1.015)	-1.38 (-6.53–3.78)	0.9990
7	327.9–13.6	1.016 (0.969–1.063)	4.09 (-2.78–10.96)	0.9973

The technical measuring range (detection limit, upper limit of the calibration curve) for EliA™ anti-TG is from 12 to 4797 IU/mL. The upper limit of the reported results in EliA IU/ml can vary due to a lot-specific conversion from µg/L to EliA IU/mL. The transfer of the upper limit of the measuring range to IU/ml is defined by the formula: $600 \mu\text{g/l} \times 9.98$ (for anti-TG), where 600 µg/L is the highest calibration point. Results above the upper limit are generally reported as “above”. Linearity was shown for the range between 10.1 – 4827.9 IU/mL on Phadia 250, and between 11.1–5127.8 on Phadia 2500/5000. The claimed linear range of EliA™ anti-TG for instruments is 12–4797 IU/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.

EliA™ anti-TPO on Phadia 250				
Sample	Dilution Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²
1	1536.1–16.5	1.004 (0.996–1.013)	-1.78 (-6.68–3.12)	0.9999
2	2201.2–22.1	1.048 (0.993–1.102)	-1.48 (-47.76–44.80)	0.9946
3	479.8–7.0	1.008 (0.999–1.017)	-0.50 (-2.28–1.28)	0.9999
4	310.9–3.2	1.005 (0.996–1.015)	0.42 (-0.71–1.55)	0.9998
5	349.2–6.3	0.993 (0.983–1.003)	0.90 (-0.47–2.27)	0.9998
6	458.0–5.6	0.998 (0.988–1.007)	1.33 (-0.34–3.01)	0.9998

EliA™ anti-TPO on Phadia 2500/5000				
Sample	Dilution Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²
1	1400.8–15.1	0.987 (0.968–1.005)	-0.70 (-10.60–9.20)	0.9993
2	1955.9–22.1	1.008 (0.996–1.020)	2.43 (-6.55–11.42)	0.9997
3	471.6–4.6	0.988 (0.970–1.007)	1.55 (-1.76–4.85)	0.9993
4	205.7–3.6	1.071 (1.054–1.087)	0.40 (-0.56–1.36)	0.9996
5	349.4–5.8	0.997 (0.968–1.005)	0.90 (-0.32–2.12)	0.9999

6	464.3–5.3	0.987 (0.970–1.004)	1.61 (-1.46–4.68)	0.9994
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The technical measuring range (LoD/LoQ, upper limit of the calibration curve) for EliA™ anti-TPO is from 4 to 1542 IU/mL. The upper limit of the reported results in EliA IU/mL can vary due to a lot-specific conversion from µg/L to EliA IU/mL. The conversion of the upper limit of the measuring range to IU/mL is calculated by the formula: 600 µg/L x 3.21 (for anti-TG), where 600 µg/L is the highest calibration point. Results above the upper limit are generally reported as “above”. Linearity was shown for the range between 3.2–2201.2 U/mL on Phadia 250, and 3.6–1955.9 on Phadia 2500/5000. The claimed linear range of EliA™ anti-TPO for instruments is 4–1542 IU/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.

High dose Hook effect:

Hook effect was investigated by using five serum samples above the upper limit of the measuring range. Five high positive samples were diluted and the dilutions were measured in two replicates and compared to the upper limit of the technical measuring range.

EliA™ anti-TG Immunoassay: No hook effect was observed when analyzing a high positive serum sample up to 135,582 IU/mL.

EliA™ anti-TPO Immunoassay: No hook effect was observed when analyzing a high positive serum sample up to 20,725 IU/mL.

Results above the upper limit of the measuring range are reported as “above”. Package insert recommends not making dilution of samples outside measuring range.

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

i) *Traceability*

Calibrators – 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 the World Health Organization (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.

EliA™ anti-TG is calibrated against the 1st International Reference Preparation for human anti-TG serum code 65/93 and EliA™ anti-TPO is calibrated against the NIBSC research standard for anti-thyroid microsome serum code 66/387. 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 IU/mL for EliA™ anti-TG and EliA™ anti-TPO.

The calibrators are a set of six WHO-standardized IgG calibrators derived from human serum with assigned values from 0–600 µg/mL. The calibrator curve is acquired by fitting the values of the six calibrators and can be stored for up to 28 days by the instrument to be used on additional assays. Each additional assay includes calibrator (curve) controls that have to recover in defined ranges to ensure that the stored calibration curve is still valid.

Controls – EliA Thyroid Positive Control is prepared from selected pooled human sera and contains IgG antibodies to TG and TPO. 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. The target ranges of the EliA™ Controls tested with the EliA™ TG and TPO on the two Phadia® instruments are summarized below:

Instrument	EliA™ Positive Control-TG	EliA™ Positive Control-TPO
Phadia® 250	467.9–1091.7 IU/mL	104.1–243.0 IU/mL
Phadia® 2500/5000	505.3–1179.1 IU/mL	100.0–233.3 IU/mL
Instrument	EliA™ IgG/IgM/IgA Negative Control	EliA™ IgG/IgM/IgA Negative Control
Phadia® 250	< 40 IU/mL	< 25 IU/mL
Phadia® 2500/5000	< 40 IU/mL	< 25 IU/mL

ii) Kit Stability:

Data for open and closed real time stability and on-board stability of EliA™ IgG reagents and general EliA reagents on Phadia 250 and Phadia 2500/5000 instrument were submitted and cleared with several other EliA tests, e.g., K141375 for EliA M2, for Phadia 250 instrument and with K061165 for EliA CCP for the Phadia 2500/5000.

Shelf-life stability – The real time stability test confirmed the shelf life for EliA™ anti-TG wells and EliA™ anti-TPO wells and it was determined to be 18 months.

The shelf life stability of the EliA™ Thyroid Positive Control was determined to be 15 months. The shelf life stability of the EliA IgG/IgM/IgA Negative Control was 24 months.

Open Stability – Stability of the foil bag containing the EliA anti-TG and EliA anti-TPO wells after first opening was tested and determined to be 9 months at 2–8°C.

Controls – EliA™ Thyroid Positive Control 250, EliA™ Thyroid Positive Control 2500/5000, EliA™ IgG/IgM/IgA Negative Control 250, and EliA™ IgG/IgM/IgA Negative Control 2500/5000 are for single use only. Therefore, stability after first

opening study is not required.

On-board stability – The on-board stability EliA™ anti-TG and EliA™ anti-TPO carriers (containing the antigen coated wells) were tested over four weeks using three positive and two negative samples only on the Phadia 250 instrument. As the storage conditions in the Phadia 2500/5000 instrument are similar, the results can also be used for stability claims for the Phadia 2500/5000. The on-board stability for the Phadia 250 instrument was determined to be 28 days at 2–8°C.

iii) Sample Storage

The package insert recommends following the guidelines in CLSI H18-A3: *Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline* for sample storage. Separated serum/plasma should remain at room temperature for no longer than 8 hours. If assays will not be completed within 8 hours, serum/plasma should be refrigerated (2– 8°C). If assays are not completed within 48 hours, or the separated serum/plasma will be stored beyond 48 hours, serum/plasma should be frozen at or below -20°C. Freezing and thawing should be avoided.

d. Detection limit:

The Limit of Blank (LoB) and Limit of Detection (LoD) were determined in accordance with CLSI EP17-A2. On each Phadia instrument (Phadia® 250 and Phadia® 2500/5000), four immunoglobulin depleted serum (for LoB) and four low antibody samples (for LoD) were assayed in six replicates in each of six runs at six different days (36 replicates per sample). For both instrument types (Phadia 250 and Phadia 2500/5000), each sample was run in a total of six runs on two instruments (3 runs on instrument #1 and three runs on instrument #2) with two lots of wells and reagents. The results are summarized in the tables below:

EliA™ anti-TG	LoB (IU/mL)	LoD (IU/mL)
Phadia 250	5.0	10.1
Phadia 2500/5000	4.9	8.5
A single LoD of 12.0 IU/mL for anti-TG was chosen.		
EliA™ anti-TPO	LoB (IU/mL)	LoD (IU/mL)
Phadia 250	1.7	2.9
Phadia 2500/5000	1.5	2.8
A single LoD of 4 IU/mL for anti-TPO was chosen.		

Limit of Quantitation (LoQ)

For LoQ determination for both assays, bias was set to 12% which leads to estimated CV_{tot} of 30%. The target value for the total error = $12+2*\%CV = 72\%$. Because estimated total error for the LoD in all cases was less than the target value, the LoQ was determined to equal the LoD for both assays.

e. Analytical specificity:

i) Endogenous Interference

Interferences were assessed by testing three samples (one negative, one around the cut-off, and a high positive sample). Each sample was spiked with the interfering substances or substance-specific blanks, and analyzed in two runs, each in duplicates ($n=4$), on one lot of EliA™ TG or EliA™ TPO Well and one lot of each system reagents. The data demonstrated that EliA™ TG or EliA™ TPO was not adversely affected (percent recoveries ranged from 94 to 109% for EliA™ TG and 93 to 105% for EliA™ TPO) by high levels of the following substances up to the concentrations listed in the tables below:

EliA™ anti-TG and EliA™ anti-TPO Immunoassay	
Potential Interfering Compound	Concentration in undiluted sample
Bilirubin F	192 mg/dL
Bilirubin C	201 mg/dL
Hemoglobin	4960 mg/dL
Lipemic factor	1%
Rheumatoid factor	500 IU/mL
Thyroxine	2.5 ng/dL
Iodide	3 mM

ii) Carry-over

In order to demonstrate the efficacy of the Phadia 250 system of washing the sample pipette between samples, carry-over study was previously evaluated in K082759, EliA Ro assay. The data demonstrate no carryover effect as the results of the Diluent sample results were not affected by the results of the previous sample.

Phadia 2500/5000 instruments use disposable tips for pipetting samples and a separate pipette for the conjugate, therefore carry-over from samples to conjugate is unlikely.

f. Assay cut-off:

The study was done on 604 apparently healthy blood donor samples from Caucasian, African American, Hispanic and Asian individuals almost equally distributed by sex and

age, the expected values for each of the antigens in the submission in the normal population and to confirm the defined cut-off. Based on the results of the expected values/reference range study, the 90th and 95th percentile of the 604 samples were calculated and the 90th percentile was taken into account for setting the cut-off. The assay cut-offs were set as follows:

EliA™ anti-TG	
< 40 IU/mL	Negative
40–60 IU/mL	Equivocal
> 60 IU/mL	Positive

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

EliA™ anti-TPO	
< 25 IU/mL	Negative
25–35 IU/mL	Equivocal
> 35 IU/mL	Positive

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

2. Comparison studies:

a. *Method comparison with predicate device:*

EliA™ anti-TG Immunoassay: A total of 718 clinically defined serum samples from patients with autoimmune thyroiditis or Graves' disease, non-autoimmune thyroid diseases, bacterial or viral infections, cancer or connective tissue diseases, type II diabetes mellitus, Sjögren's syndrome, rheumatoid arthritis, hypergammaglobulinemia, Systemic lupus erythematosus (SLE), ulcerative colitis, celiac disease, Crohn's disease, type I diabetes, chronic thyroiditis, sub-acute thyroiditis, multi-nodular goiter, pernicious anemia, myasthenia gravis, miscarriage, pre-eclampsia, thyroid carcinoma and pregnant women across all trimesters (see table below under clinical studies) were tested on EliA™ anti-TG (new device) and VarelisA TG Antibodies (predicate device) using Phadia® 250 instrument. The results are summarized below:

n=718	VarelisA TG Abs. positive: > 100 IU/mL	VarelisA TG Abs. equivocal: 60–100 IU/mL	VarelisA TG Abs. negative: < 60 IU/mL	Total
EliA™ anti-TG positive: > 60 IU/mL	142	25	9	176
EliA™ anti-TG Equiv.: 40–60 IU/mL	10	13	13	36
EliA™ anti-TG negative: < 40 IU/mL	7	28	471	506

Total	159	66	493	718
Positive percent agreement:	89.3% (142/159)	95% CI: 83.1–93.6%		
Equivocal percent agreement:	19.7% (13/66)	95% CI: 10.9–31.3%		
Negative percent agreement:	95.5% (471/493)	95% CI: 93.3–97.2%		
Total percent agreement:	87.2% (616/718)	95% CI: 84.5–89.5%		

For the calculation of agreement, from 718 samples, 280 samples (260 samples below and 20 samples above the measuring range limits) were excluded resulting in a total of 438 samples. Agreements were calculated by grouping EliA™ TG equivocal results with its test negative results, and then agreements were calculated again by grouping EliA™ TG equivocal results with the test positive results:

Equivocal EliA™ TG results considered as negative				
n = 438		VarelisA TG (IU/mL)		Total
		Positive: > 100	Negative: ≤ 100	
EliA™ TG (IU/mL)	Positive: > 60	122	34	156
	Negative: ≤ 60	14	268	282
Total		136	302	438
Positive percent agreement:		89.7% (122/136)	95% CI: 83.3–94.3%	
Negative percent agreement:		88.7% (269/302)	95% CI: 84.6–92.1%	
Total percent agreement:		89.0% (390/438)	95% CI: 85.7–91.8%	

Equivocal EliA™ TG results considered as positive				
n = 438		VarelisA TG (IU/mL)		Total
		Positive: > 100	Negative: ≤ 100	
EliA™ TG (IU/mL)	Positive: > 40	170	21	191
	Negative: ≤ 40	27	220	247
Total		197	241	438
Positive percent agreement:		86.3% (170/197)	95% CI: 80.7–90.8%	
Negative percent agreement:		91.3% (220/241)	95% CI: 87.0–94.5%	
Total percent agreement:		89.0% (390/438)	95% CI: 85.7–91.8%	

EliA™ anti-TPO Immunoassay: A total of 718 clinically defined serum samples with a diagnosis patients with Hashimoto's or Graves' disease, non-autoimmune thyroid diseases, bacterial or viral infections, cancer or connective tissue diseases, type II diabetes mellitus, Sjögren's syndrome, rheumatoid arthritis, hypergammaglobulinemia, SLE, ulcerative colitis, celiac disease, Crohn's disease, type I diabetes, chronic

thyroiditis, sub-acute thyroiditis, multi-nodular goiter, pernicious anemia, myasthenia gravis, miscarriage, pre-eclampsia, thyroid carcinoma and pregnant women across all trimesters (refer to the Table 3 in Clinical studies) were tested on EliATM anti-TPO (new device) and VarelisA TPO Antibodies (predicate device) using Phadia® 250 instrument. Additionally, 77 technical samples without clinical diagnosis were also included in the test on EliA anti-TPO (new device) and VarelisA TPO Antibodies (predicate device) to cover the equivocal range. The technical samples are blood samples without a clinical diagnosis. So, the total number of samples tested was 795 (718 + 77). The results are summarized below:

n=795	VarelisA TPO Abs. positive: > 100 (IU/mL)	VarelisA TPO Abs. equivocal: 60–100 (IU/mL)	VarelisA TPO Abs. negative: < 60 (IU/mL)	Total
EliA™ TPO positive: > 35 (IU/mL)	191	24	10	225
EliA™ TPO Equiv.: 25–35 (IU/mL)	0	22	65	87
EliA™ TPO negative: < 25 (IU/mL)	0	0	483	483
Total	191	46	558	795
Positive percent agreement: 100% (191/191) 95% CI: 98.1–100%				
Equivocal percent agreement: 47.8% (22/46) 95% CI: 32.9–63.1%				
Negative percent agreement: 86.6% (483/558) 95% CI: 83.4–89.3%				
Total percent agreement: 87.5% (696/795) 95% CI: 85.0–89.8%				

For the calculation of agreement, 297 samples (273 samples below and 24 samples above the measuring range limits) were excluded resulting in a total of 498 samples. Agreements were calculated by grouping EliA™ TPO equivocal results with the test negative results, and then agreements were calculated again by grouping EliA™ TPO equivocal results with the test positive results:

Equivocal EliA™ anti-TPO results considered as negative				
n = 498		VarelisA TPO (IU/mL)		Total
		Positive: > 100	Negative: ≤ 100	
EliA™ TPO (IU/mL)	Positive: > 35	167	34	201
	Negative: ≤ 35	0	297	297
Total		167	331	498
Positive percent agreement: 100% (167/167) 95% CI: 97.8–100%				
Negative percent agreement: 89.7.6% (297/331) 95% CI: 85.9–92.8%				
Total percent agreement: 93.2% (464/498) 95% CI: 90.6–95.2%				

Equivocal EliA™ anti-TPO results considered as positive				
n = 498		VarelisA TPO (IU/mL)		Total
		Positive: >60	Negative: ≤60	
EliA™ TPO (IU/mL)	Positive: > 25	213	75	288
	Negative: ≤ 25	0	210	210
Total		213	285	498
Positive percent agreement:		100% (213/213)	95% CI: 98.3–100%	
Negative percent agreement:		73.7% (210/285)	95% CI: 68.2–78.7%	
Total percent agreement:		84.9% (423/498)	95% CI: 81.5–88.0%	

b. Matrix comparison:

The purpose of this study was to demonstrate that the new device EliA™ anti-TG and EliA™ anti-TPO give the same results for serum, Li-heparin plasma and K3-EDTA plasma collected from the same patient. A total of 57 matrix-matched samples spread across the assay range were assayed in duplicate on the Phadia 250. Out of 57 samples, 25 samples were spiked with serum samples of high antibody titer to cover the measuring range as it was not feasible to obtain a relevant number of matched serum- and plasma-samples from thyroid disease patients. However, the seven samples within the equivocal range and 25 negative samples were neat samples. Negative samples did not switch to positive in any serum/plasma combination.

Weighted Deming regression plots were generated and the corresponding slopes of regression and coefficients were determined. The manufacturer’s acceptance criterion was that the quotient of plasma to serum concentration should be between 0.80–1.20 for all equivocal/positive sera. All of the samples met the acceptance criterion. The results are summarized in the tables below:

	EliA™ anti-TG Immunoassay			
	Range Tested (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R²
Serum vs K3-EDTA Plasma	25.5–4323.7	1.00 (0.97–1.03)	-3.16 (-6.79–0.47)	1.00
Serum vs Li-Heparin Plasma	24.3–5639.9	100 (0.97–1.03)	-0.43 (-3.96–3.11)	1.00

	EliA™ anti-TPO Immunoassay			
	Range Tested (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R ²
Serum vs K₃-EDTA Plasma	10.8–1445.2	1.00 (0.90–1.10)	-3.07 (-8.90–2.75)	0.99
Serum vs Li-Heparin Plasma	11.0–1389.9	0.99 (0.95–1.03)	-0.78 (-2.25–0.68)	0.99

c. Instrument comparison

A study was performed to demonstrate that the performance of EliA™ anti-TG or EliA™ anti-TPO was equivalent on the Phadia® 250 and Phadia® 2500/5000 instruments. A total of 50 samples (30 positive, 8 equivocal and 12 negative) spanning the assay range were analyzed in six runs in single replicates on three Phadia®250 and three Phadia® 2500/5000 instruments, with two runs on each instrument. The results of a Passing Bablock regression analysis are shown below:

	EliA™ anti-TG Immunoassay	
	Slope (95% CI)	Intercept (95% CI)
Phadia®250 vs Phadia®2500/5000	0.96 (0.93–0.97)	1.70 (0.70–3.80)

	EliA™ anti-TPO Immunoassay	
	Slope (95% CI)	Intercept (95% CI)
Phadia®250 vs Phadia®2500/5000	0.98 (0.97–0.99)	0.20 (-0.40–0.80)

3. Clinical studies:

a. *Clinical sensitivity and specificity:*

EliA™ anti-TG Immunoassay

The performance of EliA™ anti-TG was compared to a clinical diagnosis autoimmune Thyroiditis. The validation set consisted of clinically characterized sera from AI Thyroiditis and various disease controls for a total of 718 patient samples. The results of EliA™ anti-TG for each disease category are shown below:

Condition	Number of samples	(%) positive on EliA™ TG	(%) positive on predicate
Graves` Disease	100	48 (48%)	48 (48%)
Autoimmune thyroiditis	115	72 (62.6%)	69 (60%)
Non-AI Thyroid Disease	48	7 (14.6%)	9 (18.8%)
Connective Tissue Disease	15	2 (13.3%)	2 (13.3%)
Crohn`s Disease	20	1 (5%)	0 (0%)
Ulcerative Colitis	20	1 (5%)	0 (0%)
Primary Biliary Cirrhosis	10	0 (0%)	0 (0%)
HIV infection	20	0 (0%)	3 (15%)
HCV infection	20	1 (5%)	2 (10%)
HBV infection	20	1 (5%)	2 (10%)
Other infection	20	1 (5%)	1 (5%)
Cancer (Various)	56	13 (23.2%)	7 (12.5%)
Rheumatoid Arthritis	12	2 (16.7%)	1 (8.3%)
Hypergammaglobulinemia	15	1 (6.7%)	1 (6.7%)
Systemic Lupus Erythematosus	24	5 (20.8%)	3 (12.5%)
Sjögren`s Syndrome	14	2 (14.3%)	2 (14.3%)
Celiac Disease	15	3 (20%)	2 (13.3%)
Type I Diabetes Mellitus	15	2 (13.3%)	2 (13.3%)
Type II Diabetes Mellitus	15	1 (6.7%)	1 (6.7%)
Pregnant Women (all trimesters)	90	5 (5.6%)	2 (2.2%)
Pre-eclampsia	5	0 (0%)	0 (0%)
Miscarriage	9	6 (66.7%)	1 (11.1%)
Thyroid Cancer	21	0 (0%)	0 (0%)
Myasthenia gravis	7	0 (0%)	0 (0%)
Pernicious Anemia	8	2 (25%)	2 (25%)
Chronic Lymph. Thyroiditis	10	0 (0%)	0 (0%)
Sub-acute Thyroiditis and Chron. Lymph. Thyroiditis	5	5 (100%)	5 (100%)
Multi-nodular Goiter	4	0 (0%)	0 (0%)

Clinical performance with equivocal samples considered negative is summarized in the following table:

		Diagnosis		Total
		Positive	Negative	
EliA™ anti-TG (IU/mL)	Positive: > 60	120	56	176
	Negative: ≤ 60	95	447	542
Total		215	503	718
Clinical sensitivity:		55.8% (120/215)	95% CI: 48.9–62.6%	
Clinical specificity:		88.9% (447/503)	95% CI: 85.8–91.5%	

Clinical performance with equivocal samples considered positive is summarized in the following table:

		Diagnosis		Total
		Positive	Negative	
EliA™ anti-TG (IU/mL)	Positive: > 40	134	78	212
	Negative: ≤ 40	81	425	506
Total		215	503	718
Clinical sensitivity: 62.3% (134/215)		95% CI: 55.5–68.8%		
Clinical specificity: 84.5% (425/503)		95% CI: 81.0–87.5%		

EliA™ anti-TPO Immunoassay

The performance of EliA™ anti-TPO was compared to a clinical diagnosis autoimmune Thyroiditis. The validation set consisted of clinically characterized sera from AI Thyroiditis and various disease controls for a total of 718 patient samples. The results of EliA™ anti-TPO for each disease category are shown below:

Condition	Number of samples	(%) positive on EliA™ TPO	(%) positive on predicate
Graves` Disease	100	81 (81%)	68 (68%)
Autoimmune thyroiditis	115	96 (83.5%)	88 (76.5%)
Non-AI Thyroid Disease	48	5 (10.4%)	5 (10.4%)
Connective Tissue Disease	15	1 (6.7%)	1 (6.7%)
Crohn`s Disease	20	0 (0%)	0 (0%)
Ulcerative Colitis	20	0 (0%)	0 (0%)
Primary Biliary Cirrhosis	10	0 (0%)	0 (0%)
HIV infection	20	1 (5%)	0 (0%)
HCV infection	20	1 (5%)	1 (5%)
HBV infection	20	0 (0%)	0 (0%)
Other infection	20	0 (0%)	0 (0%)
Cancer	56	11 (20%)	5 (9%)
Rheumatoid Arthritis	12	1 (8%)	1 (8%)
Hypergammaglobulinemia	15	1 (6%)	0 (0%)
Systemic Lupus Erythematosus	24	1 (4%)	0 (0%)
Sjögren`s Syndrome	14	1 (7%)	1 (7%)
Celiac Disease	15	2 (13%)	2 (13%)
Type I Diabetes Mellitus	15	2 (13%)	2 (13%)
Type II Diabetes Mellitus	15	2 (13%)	2 (13%)
Pregnant Women (all trimesters)	90	10 (11%)	10 (11%)
Pre-eclampsia	5	4 (80%)	3 (60%)
Miscarriage	9	2 (22%)	1 (11%)
Thyroid Cancer	21	2 (9%)	1 (5%)

Myasthenia gravis	7	1 (14%)	0 (0%)
Pernicious Anemia	8	1 (12%)	0 (0%)
Chronic Lymph. Thyroiditis	10	1 (10%)	1 (10%)
Sub-acute Thyroiditis and Chron.	5	4 (80%)	4 (80%)
Multi-nodular Goiter	4	0 (0%)	0 (0%)

Clinical performance with equivocal samples considered negative is summarized in the following table:

		Diagnosis		Total
		Positive	Negative	
EliA™ anti-TPO (IU/mL)	Positive: > 35	177	48	225
	Negative: ≤ 35	38	455	493
Total		215	503	718
Clinical sensitivity: 82.3% (177/215)		95% CI: 76.6%–87.2%		
Clinical specificity: 90.5% (455/503)		95% CI: 87.5%–92.9%		

Clinical performance with equivocal samples considered positive is summarized in the following table:

		Diagnosis		Total
		Positive	Negative	
EliA™ anti-TPO (IU/mL)	Positive: > 25	182	53	235
	Negative: ≤ 25	33	450	483
Total		215	503	718
Clinical sensitivity: 84.7% (182/215)		95% CI: 79.1%–89.2%		
Clinical specificity: 89.5% (450/503)		95% CI: 86.4%–92.0%		

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

Not applicable

4. Clinical cut-off:

Same as assay cut-off

5. Expected values/Reference range:

A total of 604 apparently healthy, age- and gender-matched blood donor samples from a population to better reflect the ethnic composition of the US population (~60.5% White Americans, ~14% African Americans, ~17% Hispanics, ~6% Asians, ~2.5% others) were used. Note: The 90th percentile was chosen to determine the cut-off because according to literature >10% of the healthy subjects are reported to have anti-thyroglobulin / anti-thyroid

peroxidase antibodies. The results are summarized below:

	EliA™ TG IU/mL
Mean	61.2
Median	12.2
Range	12–5071.7
90 th percentile	35.6
95 th percentile	97.9
	EliA™ TPO
Mean	17.3
Median	4.4
Range	4 – 1004.3
90 th percentile	14.2
95 th percentile	59.9

EliA™ anti-TG Immunoassay:

For EliA™ anti-TG, the 90th percentile lies at 35.6 IU/mL. The lower limit of the equivocal range was chosen to be at least equal to lowest positive calibrator (40 IU/mL). The upper cut-off value was determined as 56 IU/mL (40 IU/ml x 1.4) which was rounded up to 60 IU/mL.

EliA™ anti-TPO Immunoassay:

For EliA™ anti-TPO, the 90th percentile lies at 14.2 IU/ml. The lower limit of the equivocal range was chosen to be at least equal to lowest positive calibrator (25 IU/mL). The upper cut-off value was calculated as 35 IU/mL (25 IU/ml x 1.4).

For both assays, the cut-off and the equivocal range were supported by the findings of the clinical study and the correlation to the predicate device assay.

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.