DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

March 25, 2016

Phadia US Inc.
Martin Mann
Sr. Regulatory Affairs Manager
4169 Commercial Avenue
Portage, MI 49002

Re: K151799
Trade/Device Name: EliA™ anti-TG Immunoassay
EliA™ anti-TPO Immunoassay
EliA™ Thyroid Positive Control 250
EliA™ Thyroid Positive Control 2500/5000
Regulation Number: 21 CFR §866.5870
Regulation Name: Thyroid autoantibody immunological test system
Regulatory Class: II
Product Code: JZO, JJY
Dated: February 24, 2016
Received: February 25, 2016

Dear Mr. Mann:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements

as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Leonthena R. Carrington -S

Leonthena R. Carrington, MS, MBA, MT(ASCP)
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostics and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
k151799

Device Name
EliA™ anti-TG Immunoassay, EliA™ anti-TPO Immunoassay, EliA™ Thyroid Positive Control 250, and EliA™ Thyroid Positive Control 2500/5000.

Indications for Use *(Describe)*
EliA anti-TG Immunoassay 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 instrument Phadia 250.

EliA anti-TG Immunoassay 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 instrument Phadia 2500/5000.

EliA anti-TPO Immunoassay 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 instrument Phadia 250.

EliA anti-TPO Immunoassay 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 instrument Phadia 2500/5000.

EliA Thyroid Positive Control 250 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 using the EliA IgG method.

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 2500/5000 using the EliA IgG method.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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F.1 510k Decision Summary Input

510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
K151799

B. Purpose for Submission:
New devices

C. Measurand:
IgG Antibodies to TG and TPO

D. Type of Test:
Quantitative immunofluorescence assays

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 (Assays)
   Class I (Controls)

3. Product code:
   JZO System, Test, Thyroid Autoantibody
   JJY Multi-Analyte Controls, All Kinds (assayed)

4. Panel:
   Immunology

H. Intended Use:

1. Intended use(s):

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.

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.

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.

2. Indication(s) for use:
Same as intended use

3. Special conditions for use statement(s):
For prescription use only

4. Special instrument requirements:
Phadia® 250
or Phadia® 2500/5000

I. Device Description:

The method specific reagents on Phadia® 250 and Phadia® 2500/5000 are identical; they are only filled in different containers. Each device consists of:

- EliA anti-TG wells are coated with a human thyroglobulin antigen – 4 carriers (16 wells each), ready to use; or
  EliA anti-TPO wells are coated with a human recombinant thyroid peroxidase antigen – 4 carriers (16 wells each), ready to use;
- EliA Sample Diluent: PBS containing BSA, detergent and 0.095% sodium azide – 6 bottles, 48 mL each, ready to use; or 6 bottles, 400 mL each, ready to use;
- EliA IgG Conjugate 50 or 200: β-Galactosidase labeled anti-IgG (mouse monoclonal antibodies) in PBS containing BSA and 0.06% sodium azide – 6 wedge shaped bottles, 5 mL each, ready to use; or 6 wedge shaped bottles, 19 mL each, ready to use
- EliA 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 – 6 single use vials, 0.3 mL each, ready to use;
- EliA Negative Control 250 or 2500/5000: Human sera from healthy donors in PBS containing BSA, detergent and 0.095% sodium azide – 6 single-use vials, 0.3 mL each, ready to use;
- EliA IgG Calibrator Strips: Human IgG (0, 4, 10, 20, 100, 600 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide – 5 strips, 6 single-use vials per strip, 0.3 mL each, ready to use;
- EliA IgG Curve Control Strips: Human IgG (20 µg/L) in PBS containing BSA, detergent and 0.095% sodium azide – 5 strips, 6 single-use vials per strip, 0.3 mL each, ready to use;
- EliA IgG Calibrator Well: Coated with mouse monoclonal antibodies – 4 carriers (12 wells each), ready to use.

The Phadia EliA Immunodiagnostic System is an automated system for immunodiagnostic testing. 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.

J. Substantial Equivalence Information:

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

VarelisA TG Antibodies, Phadia GmbH	K003414
VarelisA TPO Antibodies, Phadia GmbH	K993585
EliA™ IgG/IgM/IgA Negative Control 250	K091845
EliA IgG Calibrator Strips	K061165

2. Comparison with predicate device:

EliA anti-TG Immunoassay – Similarities to predicate device

Feature	**Predicate Device** VarelisA TG	**New Device** EliA anti-TG
Intended Use	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	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.
Internal Controls	Positive and Negative Control included in the kit	Positive and negative Control provided with the EliA Thyroid Positive Control 250 / 2500/5000 and EliA IgG/IgM/IgA Negative Control 250 / 2500/5000, respectively
Assay Type	ELISA	
Type of test	quantitative	
Reported Unit	IU/ml	
Solid Phase	Microwells	
Antigen	human thyroglobulin antigen	

EliA anti-TG – Differences to predicate device

Feature	**Predicate Device** VarelisA TG	**New Device** EliA anti-TG
Instrumentation	ELISA-Reader needed	Phadia 250 and 2500/5000 are fully automated immunoassay analyzers
Reaction temperature	Room temperature	37°C controlled
Incubation times	Positive and negative Controls, diluted patient samples: 30 min. Conjugate: 30 min. Substrate: 10 min (in dark)	Diluted patient samples: 30 min. Conjugate: 28 min. Development Solution: 39 min.
Detection antibody (conjugate)	IgG conjugate: anti-human IgG horse radish peroxidase (goat)	IgG conjugate: anti-human IgG ß-Galactosidase (mouse monoclonal antibodies)
Signal	Optical density (at 450nm)	Fluorescence
Calibration	6-point Calibration	6-point total IgG Calibration
Calibrators	6 vials of TG-specific IgG at concentrations of 0 – 30 – 100 – 300 – 1000 – 3000 IU/ml	6 vials of human IgG at concentrations of 0 – 4 – 10 – 20 – 100 – 600 µg/l
Calibration curve	n.a.	Option to store curve for up to 28 days and run curve controls in each assay for calibration
Concept	All reagents in a single kit	Modular reagents concept (test-method specific and general reagents)
Sample Dilution	1:101	1:100
Cut-off	< 60 IU/ml negative 60-100 IU/ml equivocal > 100 IU/ml positive	< 40 IU/ml negative 40-60 IU/ml equivocal > 60 IU/ml positive
Substrate	TMB Chromogen	Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside & <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] (3:1))

EliA anti-TPO Immunoassay – Similarities to predicate device

Feature	**Predicate Device** VarelisA TPO	**New Device** EliA anti-TPO
Intended Use	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.	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.
Internal Controls	Positive and Negative Control included in the kit	Positive and negative Control provided with the EliA Thyroid Positive Control 250 / 2500/5000 and EliA IgG/IgM/IgA Negative Control 250 / 2500/5000, respectively
Assay Type	ELISA	
Type of test	quantitative	
Reported Unit	IU/ml	
Solid Phase	Microwells	
Antigen	human recombinant TPO antigen	

EliA anti-TPO – Differences to predicate device

Feature	**Predicate Device** VarelisA TPO	**New Device** EliA anti-TPO
Instrumentation	ELISA-Reader needed	Phadia 250 and 2500/5000 are fully automated immunoassay analyzers
Reaction temperature	Room temperature	37°C controlled
Incubation times	Positive and negative Controls, diluted patient samples: 30 min. Conjugate: 30 min. Substrate: 10 min (in dark)	Diluted patient samples: 30 min. Conjugate: 28 min. Development Solution: 39 min.
Detection antibody (conjugate)	IgG conjugate: anti-human IgG horse radish peroxidase (goat)	IgG conjugate: anti-human IgG ß-Galactosidase (mouse monoclonal antibodies)
Signal	Optical density (at 450nm)	Fluorescence
Calibration	6-point antigen-specific Calibration	6-point total IgG Calibration
Calibrators	6 vials of TPO-specific IgG at concentrations of 0 – 30 – 100 – 300 – 1000 – 3000 IU/ml	6 vials of human IgG at concentrations of 0 – 4 – 10 – 20 – 100 – 600 µg/l
Calibration curve	n.a.	Option to store curve for up to 28 days and run curve controls in each assay for calibration
Concept	All reagents in a single kit	Modular reagents concept (test-method specific and general reagents)
Sample Dilution	1:101	1:200
Cut-off	< 60 IU/ml negative 60-100 IU/ml equivocal > 100 IU/ml positive	< 25 IU/ml negative 25-35 IU/ml equivocal > 35 IU/ml positive
Substrate	TMB Chromogen	Development Solution 0.01 % 4-Methylumbelliferyl-β-D-galactoside & <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] (3:1))

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

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantification, 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 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 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:

a. Precision/Reproducibility:

To determine the precision of the assays on Phadia 250 and Phadia 2500/5000 instrument, the variability was assessed on 8 samples.

Two batches were used to determine the precision of the assays on Phadia 250 and on Phadia 2500/5000. The results are summarized in the tables below:

EliA anti-TG Immunoassay:

To determine the precision of the assay on the Phadia 250 instrument, the variability was assessed in a study with a total of 21 runs (3 instruments x 7 runs each) over 7 days performed on samples with 252 replicate determinations per sample with a calibration curve in each run.

EliA anti-TG on Phadia 250 (n = 252)

Mean value IU/mL	Intra Run CV%	Inter Run CV%	Total Imprecision CV%
30.6	8.7	4.9	11.9
37.3	6.2	4.7	11.4
74.9	4.3	0.9	6.7
124.9	3.6	0.7	4.6
184.8	2.8	0.6	3.1
771.5	2.7	1.1	3.2
2065.1	3.3	1.4	4.3
4147.2	4.0	1.3	4.7

To determine the precision of the assay on the Phadia 2500/5000 instrument, the variability was assessed in a study with a total of 21 runs (3 instruments x 7 runs each) over 7 days performed on samples with 252 replicate determinations per sample with a calibration curve in each run.

EliA anti-TG on Phadia 2500/5000 (n = 252)

Mean value IU/mL	Intra-Run CV%	Inter-Run CV%	Total Imprecision CV%
31.4	13.5	3.2	17.8
38.9	11.9	2.3	16.0
76.7	5.2	1.9	9.2
134.5	5.3	2.0	8.3
193.0	4.3	2.0	5.6
754.2	5.2	1.2	5.4
1869.6	5.4	0.0	6.9
3797.5	8.9	1.3	10.2

EliA anti-TPO Immunoassay:

To determine the precision of the assay on the Phadia 250 instrument, the variability was assessed in a study with a total of 21 runs (3 instruments x 7 runs each) over 7 days performed on samples with 252 replicate determinations per sample with a calibration curve in each run.

EliA anti-TPO on Phadia 250 (n = 252)

Mean value IU/mL	Intra Run CV%	Inter Run CV%	Total Imprecision CV%
15.7	5.7	2.3	8.7
27.4	4.6	1.5	5.7
39.4	3.8	0.5	6.2
66.7	3.9	1.0	4.5
218.0	4.6	1.3	4.9
480.1	4.7	1.0	5.2
814.9	3.9	0.4	4.6
1212.6	4.0	0.0	6.2

To determine the precision of the assay on the Phadia 2500/5000 instrument, the variability was assessed in a study with a total of 21 runs (3 instruments x 7 runs each) over 7 days performed on samples with 252 replicate determinations per sample with a calibration curve in each run.

EliA anti-TPO on Phadia 2500/5000 (n = 252)

Mean value IU/mL	Intra-Run CV%	Inter-Run CV%	Total Imprecision CV%
17.7	8.7	2.2	13.1
26.6	6.4	3.1	8.0
38.5	5.9	3.4	7.3
65.3	6.1	2.6	7.0
202.9	6.9	2.7	7.6
466.6	7.2	2.2	7.8
802.8	7.4	1.7	7.8
1340.2	9.0	2.1	9.5

b. Linearity/assay reportable range:

6-7 patient serum samples were diluted in sample diluent and tested with one batch of EliA anti-TG Immunoassay and EliA anti-TPO Immunoassay and one set of system reagents on Phadia 250 or Phadia 2500/5000. The ratios of observed/expected values were calculated. Depending on the lot specific EliA well factor, the upper limit of the measuring range may vary between different solid phase batches. The results are summarized below:

EliA anti-TG on Phadia 250

Dilution range (IU/mL)	Slope	Intercept	R^2
2655.8 – 31.1	1.002	-1.46	0.9990
2935.0 – 33.5	1.008	13.21	0.9983
4827.9 – 62.5	1.025	58.32	0.9967
375.3 – 10.1*	0.998	1.01	0.9999
375.3 – 15.4	0.998	0.93	0.9999
468.8 - 17.5	0.995	1.34	0.9995
394.2 - 14.2	1.005	-0.07	0.9998
326.9 – 12.5	1.001	1.57	0.9994

*Evaluation including 1st dilution step below LoD

The claimed linear range for anti-TG is 12 – 4794 IU/mL

EliA anti-TG on Phadia 2500/5000

Dilution range (IU/mL)	Slope	Intercept	R^2
2626.4 – 31.1	0.995	23.48	0.9993
2052.8 – 36.2	1.008	17.61	0.9991
5127.8 – 63.2	1.006	60.01	0.9981
375.9 – 11.1*	0.977	8.21	0.9963
375.9 – 17.6	0.971	9.70	0.9963
470.7 – 12.1	1.011	2.09	0.9992
412.0 – 15.4	0.987	-1.38	0.9990
327.9 – 13.6	1.016	4.09	0.9973

*Evaluation including 1st dilution step below LoD

The claimed linear range for anti-TG is 12 – 4794 IU/mL

EliA anti-TPO on Phadia 250

Dilution range (IU/mL)	Slope	Intercept	R^2
1536.1 – 16.5	1.004	-1.78	0.9999
2201.2 – 22.1	1.048	-1.48	0.9946
479.8 – 7.0	1.008	-0.50	0.9999
310.9 – 3.2*	1.005	0.42	0.9998
310.9 – 4.9	1.005	0.55	0.9998
349.2 – 6.3	0.993	0.90	0.9998
458.0 – 5.6	0.998	1.33	0.9998

*Evaluation including 1st dilution step below LoD

The claimed linear range for anti-TPO is 4 – 1542 IU/mL

EliA anti-TPO on Phadia 2500/5000

Dilution range (IU/mL)	Slope	Intercept	R^2
1400.8 – 15.1	0.987	-0.70	0.9993
1955.9 – 22.1	1.008	2.43	0.9997
471.6 – 4.6	0.988	1.55	0.9993
205.7 – 3.6*	1.071	0.40	0.9996
205.7 – 5.8	1.015	2.37	0.9979
349.4 – 5.8	0.997	0.90	0.9999
464.3 – 5.3	0.987	1.61	0.9994

*Evaluation including 1st dilution step below LoD

The claimed linear range for anti-TPO is 4 – 1542 IU/mL

Hook Effect/Over the Range Results:

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 (4794 IU/ml for EliA anti-TG and 1542 IU/ml for TPO EliA anti-TPO).

EliA anti-TG Immunoassay:

No hook effect was observed when analyzing five high positive samples that had concentrations up to 27.7 times above the upper limit of the technical measuring range.

EliA anti-TPO Immunoassay:

No hook effect was observed when analyzing five high positive samples that had concentrations up to 13.4 times above the upper limit of the technical measuring range.

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

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

Traceability:

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 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.

Stability:

The EliA IgG system reagents and the EliA IgG/IgM/IgA Negative Control are already FDA cleared under k063775 and k072393, respectively.

Shelf life:

An accelerated study was done to determine the shelf life for EliA anti-TG wells and EliA anti-TPO wells and it was determined to be 18 months. The real time stability test confirmed the determined shelf life.

On-board stability:

The on-board stability EliA anti-TG and EliA anti-TPO carriers (containing the antigen coated wells) was tested over 4 weeks using 3 positive and 2 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.

Open Stability:

Stability of the foilbag 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.

d. Detection limit:

The limit of blank (LoB) and limit of detection (LoD) studies were done on both Phadia 250 and Phadia 2500/5000. Four different analyte-free samples and four blood donor samples with low antibody concentration were measured in six replicates in each of six runs at six different days (6 replicates x 6 runs = 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 3 runs on instrument 2) with two lots of wells and reagents. The results are summarized in the tables below:

EliA anti-TG (IU/mL)	LoB	LoD
Phadia 250	5.0	10.1
Phadia 2500/5000	4.9	8.5

It was decided to use a single LoD of 12.0 IU/mL for both instruments.

EliA anti-TPO (IU/mL)	LoB	LoD
Phadia 250	1.7	2.9
Phadia 2500/5000	1.5	2.8

It was decided to use a single LoD of 4.0 IU/mL for both instruments.

e. Analytical specificity:

Endogenous Interference: A study was run to investigate whether high concentrations of potentially interfering substances in serum, like bilirubin, hemoglobin, lipemic factor, rheumatoid factor, thyroxine and iodide adversely affect the results of the new device. Three serum samples were prediluted in EliA Sample Diluent and spiked with the different interfering substances or their respective blank solutions, and analyzed in triplicates. A calibration curve was run in duplicate. The runs were repeated twice. One batch of EliA antigen wells and one batch of system reagents were used throughout the studies.

One negative sample, one sample with concentration around the cut-off and one high positive sample were tested. The ratio of blank/spiked for equivocal and positive samples was 0.94 – 1.09 for EliA anti-TG and 0.93 – 1.05 for EliA anti-TPO. No interference was observed up to the concentrations listed in the table below:

EliA anti-TG 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

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

Reference sera: Externally defined sera should be measured according to their target values as mentioned by the institution. For CAP, the target is set based on the complete peer group, if >80% consensus of all participants could be reached. For UK-NEQAS the target is set by the provider based on all responses; an equivocal target means that positive and negative responses are acceptable. The targets of the reference samples were hit.

Evaluation of QAS sera on EliA anti-TG

Sample No.	Sample ID supplier	Institution	Target	EliA anti-TG (IU/ml)	
				Neg <40 Equ 40-60 **Pos >60**	
1	S2-03	CAP	**Pos**	**4511.7**	**OK**
2	S2-11	CAP	**Pos**	**5979.9**	**OK**
3	S2-19	CAP	Neg	0.7	**OK**
4	S2-11	CAP	**Pos**	**4650.7**	**OK**
5	S2-19	CAP	**Pos**	**6083.5**	**OK**
6	S2-02	CAP	**Pos**	**5333.6**	**OK**
7	S2-10	CAP	Neg	0.8	**OK**
8	S2-18	CAP	**Pos**	**3612.1**	**OK**

Evaluation of QAS sera on EliA anti-TPO

Sample No.	Sample ID supplier	Institution	Target	EliA anti-TPO (IU/ml)	
				Neg <25 Equ 25-35 **Pos >35**	
1	S2-03	CAP	**Pos**	**150.1**	**OK**
2	S2-11	CAP	**Pos**	**171.3**	**OK**
3	S2-19	CAP	Neg	0.0	**OK**
4	S2-11	CAP	**Pos**	**97.0**	**OK**
5	S2-19	CAP	**Pos**	**164.4**	**OK**
6	S2-02	CAP	**Pos**	**155.6**	**OK**
7	S2-10	CAP	Neg	0.0	**OK**
8	S2-18	CAP	**Pos**	**89.6**	**OK**
9	1012	NEQAS	Equ	**41.4**	**OK**
10	1032	NEQAS	**Pos**	**102.1**	**OK**
11	1042	NEQAS	**Pos**	**136.5**	**OK**
12	1052	NEQAS	**Pos**	**98.3**	**OK**
13	1062	NEQAS	**Pos**	**87.5**	**OK**
14	111-2	NEQAS	**Pos**	**132.3**	**OK**
15	112-2	NEQAS	Equ	21.8	**OK**
16	114-2	NEQAS	**Pos**	**174.5**	**OK**
17	116-2	NEQAS	**Pos**	**90.4**	**OK**
18	122-2	NEQAS	**Pos**	**84.9**	**OK**
19	123-2	NEQAS	Equ	1.6	**OK**
20	124-2	NEQAS	**Pos**	**107.6**	**OK**

Carry-over: A study was carried out on a Phadia 250 instrument using the test EliA Ro, cleared under k082759. A serum sample was diluted 1:2 and 1:20 using instrument dilution and manual dilution. A lower dilution factor than the default one (1:100) was chosen to challenge the system. Only a few RUs difference compared to the reference pipetting could be seen, which is too low to be expressed in U/mL. The observed carry over effect is therefore negligible without any influence to assay results.

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 impossible.

f. Assay cut-off:

A 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 in order to evaluate expected values for each of the antigens in the submission in the normal population and to confirm the defined cut-off. The samples were measured on the Phadia 250 instrument for EliA anti-TG and EliA anti-TPO. 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 following values were selected for the cut-off:

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 serum samples were collected with a diagnosis from patients with Graves' disease (n = 100), autoimmune thyroiditis (n = 100), non-AI thyroid disease (n = 48), connective tissue disease (n = 15), Crohn`s disease (n = 20), ulcerative colitis (n = 20), primary biliary cirrhosis (n = 10), HIV infection (n = 20), HCV infection (n = 20), HBV infection (n = 20), other infection (n = 20), cancer (n = 56), rheumatoid arthritis (n = 12), hypergamma-globulinemia (n = 15),

systemic lupus erythematosus (n = 24), Sjögren's syndrome (n = 14), celiac disease (n = 15), type 1 diabetes mellitus (n = 15), type II diabetes mellitus (n = 15), pregnant women of all trimesters (n = 90), pre-eclampsia (n = 5), miscarriage (n=9), thyroid cancer (n = 21), myasthenia gravis (n = 7), pernicious anemia (n = 8), chronic lymphocytic thyroiditis (n = 10), sub-acute thyroiditis (n = 5), multi-nodular goiter (n = 4).

Samples were analyzed with the EliA anti-TG and VarelisA TG assays. The test was run in single determination and values outside the overlapping measuring range for both tests (12-3000 IU/ml) were excluded from statistical analyses (n=280). The results are summarized in the tables below:

EliA anti-TG – Equivocal results evaluated as negative:

		VarelisA TG (IU/mL)		
		Positive >100	Negative <100	Total
EliA anti-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%** (268/302) (95% CI: 84.6% – 92.1%)
Total percent agreement: **89.0%** [(122+268)/438] (95% CI: 85.7% – 91.8%)

EliA anti-TG – Equivocal results evaluated as positive:

		VarelisA TG (IU/mL)		
		Positive >60	Negative <60	Total
EliA anti-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%** [(170+220)/438] (95% CI: 85.7% – 91.8%)

EliA anti-TPO Immunoassay:
A total of 718 serum samples were collected with a diagnosis from patients with Graves' disease (n = 100), autoimmune thyroiditis (n = 100), non-AI thyroid disease (n = 48), connective tissue disease (n = 15), Crohn`s disease (n = 20), ulcerative colitis (n = 20), primary biliary cirrhosis (n = 10), HIV infection (n = 20), HCV infection (n = 20), HBV infection (n = 20), other infection (n = 20), cancer (n = 56), rheumatoid arthritis (n = 12), hypergamma-globulinemia (n = 15), systemic lupus erythematosus (n = 24), Sjögren's syndrome (n = 14), celiac disease (n = 15), type 1 diabetes mellitus (n = 15), type II diabetes mellitus (n = 15), pregnant women of all trimesters (n = 90), pre-eclampsia (n = 5), miscarriage (n=9), thyroid cancer (n = 21), myasthenia gravis (n = 7), pernicious anemia (n = 8), chronic thyroiditis (n = 10), sub-acute thyroiditis (n = 5), multi-nodular goiter (n = 4).

Samples were analyzed with the EliA anti-TPO and VarelisA TPO assays. The test was run in single determination and values outside the overlapping measuring range for both tests (4-1542 IU/ml) were excluded from statistical analyses (n=297). The results are summarized in the tables below:

EliA anti-TPO – Equivocal results evaluated as negative:

		VarelisA TPO (IU/mL)		
		Positive >100	Negative <100	Total
EliA anti-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% – 100.0%)
Negative percent agreement: **89.7%** (297/331) (95% CI: 85.9% – 92.8%)
Total percent agreement: **93.2%** [(167+297)/498] (95% CI: 90.6% – 95.2%)

EliA anti-TPO – Equivocal results evaluated as positive:

		VarelisA TPO (IU/mL)		
		Positive >100	Negative <100	Total
EliA anti-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% – 100.0%)
Negative percent agreement: **73.7%** (210/285) (95% CI: 68.2% – 78.7%)
Total percent agreement: **84.9%** [(213+210)/498] (95% CI: 81.5% – 88.8%)

b. Matrix comparison:

Serum, lithium heparin plasma and EDTA plasma were collected from the same patients (n = 57) to demonstrate that the plasma results do not deviate from the corresponding serum results and are within the pre-defined specifications. Most samples were spiked with serum samples of high antibody titer to cover the measuring range; samples within the equivocal range were neat samples. Samples were tested in duplicates. Weighted Deming regression analysis was performed using the first replicate only and by plotting the concentration observed from the control tube (serum) versus the concentration for each test collection tube. The corresponding slopes of regression and coefficient determination are summarized in the tables below:

EliA anti-TG Immunoassay:

	Range tested (IU/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
Serum vs EDTA	25.5 – 4323.7	1.00 (0.97 to 1.03)	-3.16 (-6.79 to -0.47)	1.00
Serum vs Li-heparin	24.3 – 4202.4	1.00 (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 EDTA	10.8 – 1445.2	1.00 (0.90 to 1.10)	-3.07 (-8.90 to 2.75)	0.99
Serum vs Li-heparin	11.0 – 1389.9	0.99 (0.95 – 1.03)	-0.78 (-2.25 to -0.68)	0.99

c. *Instrument comparison*

Performance of EliA™ anti-TG and EliA anti-TPO was evaluated on the Phadia 250 and Phadia 2500/5000 instruments using 30 positive, 8 equivocal and 12 negative samples. The samples were analyzed in six runs in single replicates on three Phadia 250 and three Phadia 2500/5000 instruments (2 runs on each instrument). The regression analysis results are summarized as follows:

EliA anti-TG Immunoassay:

	Intercept	Slope
Estimate	1.7	0.96
95% CI	0.7 – 3.8	0.93 – 0.97

EliA anti-TPO Immunoassay:

	Intercept	Slope
Estimate	0.2	0.98
95% CI	-0.4 – 0.8	0.97 – 0.99

3. Clinical studies:

a. Clinical sensitivity and specificity:

EliA anti-TG Immunoassay:

The clinically defined AI Thyroiditis samples that were used for the method comparison were also used to determine sensitivity and specificity of the assay. The results are summarized in the tables below.

EliA anti-TG – diagnostic group AI Thyroiditis - equivocal results evaluated as negative:

	Diagnostic Group - AI Thyroiditis		
	+	-	total
Positive test >60.0 IU/mL	120	56	176
Negative test ≤60.0 IU/mL	95	447	542
Total	215	503	718

Sensitivity (95% CI): 55.8% (48.9% - 62.6%)
Specificity (95% CI): 88.9% (85.8% - 91.5%)

The table below shows the results for each clinical subgroup:

Condition	Number of samples	No (%) pos. on EliA anti-TG	No (%) pos. on Predicate VarelisA TG
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	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%)
Multi-nodular goiter	4	0 (0%)	0 (0%)

EliA anti-TPO Immunoassay:
The clinically defined AI Thyroiditis samples that were used for the method comparison were also used to determine sensitivity and specificity of the assay. The results are summarized in the tables below.

EliA anti-TPO – diagnostic group AI Thyroiditis - equivocal results evaluated as negative:

	Diagnostic Group - AI Thyroiditis		
	+	-	total
Positive test >35.0 IU/mL	177	48	225
Negative test ≤35.0 IU/mL	38	455	493
Total	215	503	718

Sensitivity (95% CI): 82.3% (76.6% – 87.2%)
Specificity (95% CI): 90.5% (87.5% – 92.9%)

The table below shows the results for each clinical subgroup:

Condition	Number of samples	No (%) pos. on EliA anti-TPO	No (%) pos. on Predicate VarelisA TPO
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 (19.6%)	5 (8.9%)
Rheumatoid arthritis	12	1 (8.3%)	1 (8.3%)
Hypergammaglobulinemia	15	0 (0%)	0 (0%)
Systemic lupus erythematosus	24	1 (4.2%)	0 (0%)
Sjögren`s syndrome	14	1 (7.1%)	1 (7.1%)
Celiac disease	15	2 (13.3%)	2 (13.3%)
Type I diabetes mellitus	15	2 (13.3%)	2 (13.3%)
Type II diabetes mellitus	15	2 (13.3%)	2 (13.3%)
Pregnant women (all trimesters)	90	10 (11.1%)	10 (11.1%)
Pre-eclampsia	5	4 (80%)	3 (60%)
Miscarriage	9	2 (22.2%)	1 (11.1%)
Thyroid cancer	21	2 (9.5%)	1 (4.8%)
Myasthenia gravis	7	1 (14.3%)	0 (0%)
Pernicious anemia	8	1 (12.5%)	0 (0%)
Multi-nodular goiter	4	0 (0%)	0 (0%)

b. Other clinical supportive data: Not applicable

4. Clinical cut-off:

Same as assay cut-off

5. Expected values/Reference range:

EliA anti-TG Immunoassay:
Antibody prevalence in autoimmune patients varies widely depending on disease area. A significant percentage (>10%) of healthy subjects was reported to have anti-thyroglobulin antibodies. Expected values may vary depending on the population tested.

The 5th percentile of the tested normal samples was calculated as 5.4 IU/ml, the 95th percentile as 97.9 IU/ml.

EliA anti-TPO Immunoassay:
Antibody prevalence in autoimmune patients varies widely depending on disease area. A significant percentage (>10%) of healthy subjects was reported to have anti-thyroid peroxidase antibodies. Expected values may vary depending on the population tested.
The 5th percentile of the tested normal samples was calculated as 1.7 IU/ml, the 95th percentile as 59.9 IU/ml.