510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K221890

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys Tg II

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MSW	Class II	21 CFR 866.6010 – Tumor Associated Antigen Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Thyroglobulin

C Type of Test:

Quantitative, Electrochemiluminescence immunoassay (ECLIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Immunoassay for the in vitro quantitative determination of thyroglobulin in human serum and plasma. Determination of Tg is used as an aid in monitoring for the presence of persistent or recurrent/metastatic disease in patients who have differentiated thyroid cancer (DTC) and have had thyroid surgery (with or without ablative therapy).

The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** immunoassay analyzers.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
In Vitro Diagnostic Use Only

The Instruction for Use of the Elecsys Tg II contains the following blackbox statement:

Thyroglobulin (Tg) determinations can be affected by the presence of Tg autoantibodies (anti-Tg) in some patient samples. These autoantibodies may interfere with the assay used to measure Tg, causing false high or false low Tg values.

The measured Tg value of a patient's sample can also vary depending on the assay used.The laboratory finding must therefore always contain a statement on the Tg assay method used. Tg values determined on patient samples by different assays cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the Tg assay procedure used while patient monitoring, the Tg values obtained upon changing to the new procedure must be confirmed by parallel measurements with both methods.

D Special Instrument Requirements:

cobas e 411 analyzer (cleared under K062279)

IV Device/System Characteristics:

A Device Description:

Materials included in the Elecsys TgII assay

Elecsys Tg II consists of the reagent rack-pack. Each reagent rack-pack is for 100 tests and contains:

- M (6.5 mL): Streptavidin-coated microparticles (transparent cap) 0.72 mg/mL and preservative.

- R1 (9 mL): Biotinylated monoclonal anti Tg antibody (mouse) (gray cap) 1 mg/L in Bis Tris buffer 50 mmol/L, pH 6.3 and preservative.
- R2 (9 mL): Monoclonal anti Tg antibodies (mouse) labeled with ruthenium complex (black cap) 3.1 mg/L in Bis Tris buffer 50 mmol/L, pH 6.3 and preservative.

Materials required (but not supplied)

- Tg II CalSet (4 x 1.0 mL): 2 vials each of Tg at 0.15 ng/mL, and 180 ng/mL
- PreciControl Universal (4 x 3.0 mL): 2 vials each of Tg at 25 ng/mL, and 100 ng/mL
- PreciControl TS (4 x 2.0 mL): 4 vials of 1 ng/mL
- Diluent MultiAssay (2 x 16 mL): sample diluent
- Anti-Tg assay: to verify the presence of antibodies to Tg in patient samples
- Distilled or deionized water

B Principle of Operation:

The Elecsys Tg II assay is a quantitative, two-step, double antigen sandwich assay. In the first incubation, thyroglobulin (Tg) in the patient sample reacts with a biotinylated monoclonal Tg-specific antibody and monoclonal Tg-specific antibodies labeled with a ruthenium complex to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex bounds to the solid phase via interaction of biotin and streptavidin in second incubation. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results for the Tg concentration of samples are determined via a calibration curve, which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Access Thyroglobulin Reagents On The Access Immunoassay Systems

B Predicate 510(k) Number(s):

K002905

C Comparison with Predicate(s):

Device & Predicate Device(s):	K221890 Candidate Device	K002905 Predicate
Device Trade Name	Elecsys Tg II	Access Thyroglobulin
General Device Characteristic Similarities		
Intended Use / Indications For Use	Immunoassay for the in vitro quantitative determination of thyroglobulin in human serum and plasma. Determination of Tg is used as an aid in monitoring for the presence of persistent or recurrent/metastatic disease in patients who have differentiated thyroid cancer (DTC) and have had thyroid surgery (with or without ablative therapy). The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.	The Access Thyroglobulin assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of thyroglobulin levels in human serum and plasma, using Access Immunoassay Systems. This device is intended to aid in the monitoring for the presence of local and metastatic thyroid tissue in patients who have had thyroid gland ablation (using thyroid surgery with or without radioactivity) and who lack serum thyroglobulin antibodies.
Analyte	Thyroglobulin	Same
Test format	Quantitative	Same
Traceability / Standardization	Standardized against CRM (Certified Reference Material) 457 of the BCR (Community Bureau of Reference) of the European Union.	Calibrators are traceable to the European Community Bureau of Reference (BCR) CRM 457 thyroglobulin standard
Measuring Range	0.1 ng/mL – 500 ng/mL	Same
General Device Characteristic Differences		
Sample Type / Matrix	Serum and Plasma (Li-Heparin, K2-EDTA, and K3-EDTA)	Serum and Plasma (heparinized)
Calibrator	Tg II CalSet (0.15, and 180 ng/mL)	Access Thyroglobulin Calibrators (0, 1.0, 10, 100, 250, and 500 ng/mL)
Calibration Interval	• 28 days when using the same reagent lot • 7 days (when using the same reagent kit on the analyzer) • as required: e.g., quality controls outside the defined limits	Calibration is required every 56 days or whenever new lot numbers of reagents are placed into use
Controls	• PreciControl Universal • PreciControl TS • Other suitable control material	Commercial Control Materials
Reagent Stability	• Up to the stated expiration date (unopened at 2–8°C) • 84 days (12 weeks) after opening, stored at 2–8°C	• Up to stated expiration date (unopened at 2–10°C) • 28 days (4 weeks) after opening, stored as 2–10°C

Detection Limit	LoB: 0.02 ng/mL LoD: 0.04 ng/mL LoQ: 0.1 ng/mL	LoB: 0.03 ng/mL LoD: Not available LoQ: 0.1 ng/mL

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures
- CLSI EP06 2nd Edition, Evaluation of Linearity of Quantitative Measurement Procedures
- CLSI EP07 3rd Edition, Interference Testing in Clinical Chemistry
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP12 3rd Edition, Evaluation of Qualitative, Binary Output Examination Performance
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP37 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the Elecsys Tg II assay was evaluated according the CLSI EP05-A3 guideline.

Within-Laboratory Precision:

A panel of six native serum samples (Sample #1–6) and one pooled serum sample (Sample #7) and three controls (PreciControl Universal 1 and 2 (PCU1 and PCU2), and PreciControl TS (PC TS)) were tested on one **cobas e** 411 analyzer using one reagent lot. Each sample was tested with two replicates per run, two runs per day for 21 days, yielding a total of 84 measurements.

The data were analyzed for repeatability (within-run), between-run, between-day, and within-laboratory precision. The mean (ng/mL), standard deviation (SD) (ng/mL) and percent coefficient of variation (%CV) are summarized in the table below:

Sample	N	Mean (ng/mL)	Repeatability (Within Run)		Between-Run		Between-Day		Within-Laboratory	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	84	0.13	0.01	7.56	0.00	0.00	0.01	5.49	0.01	9.34
2	84	0.15	0.01	5.86	0.00	0.00	0.01	6.50	0.01	8.75
3	84	0.23	0.01	4.00	0.00	1.81	0.01	3.59	0.01	5.67
4	84	1.67	0.05	2.67	0.02	0.91	0.03	1.50	0.05	3.20
5	84	38.70	0.50	1.30	0.00	0.00	0.46	1.19	0.68	1.76
6	84	235	4.52	1.92	0.00	0.00	2.90	1.23	5.37	2.28
7	84	459	7.76	1.69	5.00	1.09	4.39	0.96	10.20	2.23
PCU 1	84	24.10	0.27	1.11	0.04	0.16	0.31	1.28	0.41	1.70
PCU 2	84	84.40	0.87	1.02	0.51	0.60	1.03	1.22	1.44	1.71
PC TS	84	1.05	0.02	1.55	0.00	0.00	0.02	1.79	0.03	2.37

Lot-to-lot imprecision and reproducibility:

Testing was performed on three **cobas e** 411 analyzers (at three external sites) with three reagent lots. Two of three lots were distributed to each of the three sites as below:

Testing Site	Lot 1	Lot 2	Lot 3
Site 1		x	x
Site 2	x		x
Site 3	x	x	

Seven serum samples (Sample 1–5: native serum samples, Sample 6–7: pooled native sera) and three controls (PCU1, PCU2, and PC TS) were tested in five replicates per run, one run per day, for five days, yielding N=25 datapoints per sample per lot and N=50 datapoints per sample at each site. The results are summarized in the following table:

Sample	N*	Mean (ng/mL)	Within-Run		Between-Day		Between-Lot		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	148	0.22	0.02	7.56	0.01	6.66	0.01	6.49	0.01	6.45	0.03	13.61
2	148	0.32	0.02	6.62	0.02	7.14	0.01	3.77	0.02	6.88	0.04	12.50
3	146	2.00	0.08	3.79	0.09	4.52	0.01	0.62	0.07	3.34	0.14	6.81
4	148	39.70	1.26	3.17	2.02	5.08	0.54	1.36	1.53	3.86	2.88	7.26
5	149	261	10.97	4.20	12.10	4.64	8.02	3.07	13.43	5.15	22.62	8.67
6	148	407	13.95	3.43	17.79	4.37	10.62	2.61	20.39	5.01	32.24	7.92
7	149	464	17.55	3.79	19.27	4.16	0.00	0.00	18.47	3.98	31.95	6.89
PCU 1	149	24.20	1.16	4.80	1.79	7.43	0.89	3.70	1.26	5.2	2.63	10.91
PCU 2	148	92.99	4.31	4.63	4.62	4.97	3.32	3.57	3.10	3.34	7.78	8.36
PC TS	149	1.08	0.03	3.15	0.05	4.23	0.03	3.08	0.02	1.57	0.07	6.30

* Reason for N being less than 150: Failed quality control, handling errors, or flag from instrument; Outliers were removed for analysis as per CLSI EP05-A3 guideline.

2. Linearity:

The linearity of the Elecys Tg II assay was assessed based on CLSI document EP06 2nd Edition. Linearity series were prepared by mixing High and low native serum samples to create 13 levels that span across the analytical measuring interval (AMI). Each level was measured with four replicates using three lots of reagents on the **cobas e** 411 analyzer. For each level, the mean value of the measured values, predicted value and the deviation from linearity were calculated. Predicted values were calculated using a best fitted straight line by a weighted least squares linear regression analysis. Percent deviations from linearity were calculated as differences between the observed values (mean values) and the predicted values divided by the predicted values. The table below summarizes the linearity data analysis:

Lot (Sample)	Range (ng/mL)	Slope	Intercept	%Deviation*	Deviation**
1 (1)	0.029 − 568	0.926	-0.009	-4.4% − 8.0%	-0.0004 − 0.017
1 (2)	0.006 − 506	0.917	0.007	-9.7% − 9.5%	-0.013 − 0.0002
2 (1)	0.022 − 578	0.924	-0.004	-6.6% − 8.2%	0.003 − 0.005
2 (2)	0.005 − 516	0.925	0.006	-7.7% − 8.2%	-0.015 − 0.0001
3 (1)	0.035 − 567	0.932	0.005	-6.1% − 7.3%	-0.003 − 0.0005
3 (2)	0.089 − 521	0.936	-0.015	-8.8% − 6.8%	0.002 − 0.026

* % Deviation from Linearity for concentrations ≥0.3 ng/mL

** Deviation (ng/mL) from Linearity for concentrations <0.3 ng/mL

The data support the linearity interval from 0.005 to 578 ng/mL with deviations from linearity within ±10% for values ≥ 0.3 ng/mL and ±0.03 ng/mL for values < 0.3 ng/mL. The study results support the linearity of the claimed analytical measuring interval (AMI): 0.1 – 500 ng/mL.

High Dose Hook Effect:

The high-dose hook effect of the Elecsys Tg II assay was assessed using three samples spiked with purified human Thyroglobulin up to concentrations at 226,021, 229,820, and 244,927 ng/mL. Each sample was diluted with Diluent MultiAssay to produce a set of 11 samples with concentrations spanning between 55.1 and 226,021, 56.1 and 229,820, and 59.8 and 244,927 ng/mL. All samples were tested in singleton using the Elecsys Tg II on one **cobas e** 411 analyzer. The hook concentration reported corresponds to the highest analyte concentration that generates a signal ≥ 10% above the upper limit of the measuring range. No hook effect was observed for the Elecsys Tg II up to 120,000 ng/mL.

3. Analytical Specificity/Interference:

The studies were performed to evaluate potential interference and cross reactivity of the Elecsys Tg II assay following the CLSI EP07 (3rd Edition) and EP37 (1st Edition) guidelines.

Endogenous Interfering Substances:

Potential endogenous interfering substances were tested for their ability to interfere with the Elecsys Tg II assay using three serum samples with thyroglobulin concentration around 0.2

ng/mL, 2 ng/mL, and 40 ng/mL. For each interfering substance, test samples were spiked with the test substance and results were compared to control samples spiked with an equal volume of solvent. All samples were measured in five replicates using one Elecsys Tg II assay on one **cobas e** 411 analyzer. The interference was calculated by comparing the mean measurements of the test and control samples. No-significant interference (≤ ±10% difference of test from control) were observed for the Elecsys Tg II up to the following concentrations for each endogenous substance tested:

Endogenous Substance	Concentration
Bilirubin*	66 mg/dL
Hemoglobin	600 mg/dL
Intralipid	2,000 mg/dL
Biotin	1,200 ng/mL
IgG	2 g/dL
Albumin	7 mg/dL

*90% unconjugated + 10% conjugated Bilirubin

Exogenous Interfering Substances:

Potential exogenous interfering substances (drugs) were tested for their ability to interfere with the Elecsys Tg II assay using three serum samples with thyroglobulin concentrations around 0.2 ng/mL, 2 ng/mL, and 50 ng/mL. For each interfering substance, test samples were spiked with the test substance and results were compared to control samples spiked with an equal volume of solvent (blank). All samples were measured in five replicates using one Elecsys Tg II assay on one **cobas e** 411 analyzer. The interference was calculated by comparing measurements of the test and control samples. No significant interference (≤ ±10% difference of test from control) for the Elecsys Tg II was observed up to the following concentrations of the potential exogenous interfering substances tested:

Exogenous Substance	Concentration	Exogenous Substance	Concentration
Acetaminophen	156 mg/L	Lenvatinib Mesylate	0.15 mg/L
Acetylcysteine	150 mg/L	Levodopa	7.5 mg/L
Acetylsalicylic Acid	30 mg/L	Methyldopa +1.5	22.5 mg/L
Amiodarone	200 mg/L	Metronidazole	123 mg/L
Ampicillin-Na	75 mg/L	Octreotide	0.3 mg/dL
Ascorbic acid	52.5 mg/L	Perchlorate	2,000 mg/L
Cabozantinib-S-	4.14 mg/L	Phenylbutazone	107* mg/L
Carbimazole	30 mg/L	Prednisolone	100 mg/L
Cefoxitin	750 mg/L	Propranolol	240 mg/L
Cyclosporine	1.8 mg/L	Propylthiouracil	300 mg/L
Doxycycline	6* mg/L	Rifampicin	16* mg/L
Fluocortolone	100 mg/L	Theophylline	60 mg/L
Heparin	3,300 IU/L	Thiamazole	80 mg/L
Hydrocortisone	200 mg/L	D-T3	0.5 mg/L
Ibuprofen	219 mg/L	L-T3	0.5 mg/L
Iodide	0.2 mg/L	D-T4	5 mg/L
Itraconazole	10* mg/L	L-T4	5 mg/L

*Corresponds to one-time maximum daily dose

Cross-reactivity:

Each potential cross-reacting substance was tested with serum samples at two thyroglobulin concentrations: 5 ng/mL and 50 ng/mL. Test samples were prepared by spiking with the potential cross-reacting substances. Results were compared to control samples which were spiked with an equal volume of solvent (blank) where appropriate. All samples were measured in five replicates using one Elecsys Tg II assay on one **cobas e** 411 analyzer. The cross-reactivity was calculated by comparing measurements of the test and control samples. No significant cross reactivity (≤ ±10% difference of test from control) for the Elecsys Tg II was observed up to the following concentrations of the potential cross-reacting substances tested:

Cross-reacting Substance	Concentration
Thyroxine Binding Globulin (TBG)	200,000 ng/mL
Thyroid Stimulating Hormone (TSH)	1,000 mIU/mL)

Blocking Effect of HAMA/Heterophilic Ab:

The effect of the presence of human anti-mouse antibodies (HAMA) on the Elecsys Tg II assay was assessed on the **cobas e** 411 analyzer. A serum pool with thyroglobulin concentration of 7 ng/mL was used to spike in HAMA. The spiked sample and the corresponding serum pool without HAMA were tested in four replicates. The recovery of the serum pool containing HAMA compared to the serum pool without HAMA was calculated. No interference was observed up to 805 µg/L of HAMA.

4. Assay Reportable Range:

The analytical measuring interval (AMI) for the Elecsys TgII is 0.1 – 500 ng/mL.

The instrument has a 1:10 auto-dilution feature for the device. The claimed extended measuring range is from 500 ng/mL to 5,000 ng/mL. Samples with concentrations above 5,000 ng/mL are reported as > 5,000 ng/mL.

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

Traceability:

The Elecsys Tg II was standardized against the Certified Reference Material (CRM) 457 of the Community Bureau of Reference of the European Union.

Stability:

Shelf-life stability:
The shelf-life stability of the Elecsys Tg II was conducted. The reagent packs were stored at 2–8ºC and the real-time stability was evaluated at baseline and testing timepoints up to 17 months. The data support a shelf-life of the Elecsys Tg II up to 15 months at 2–8ºC.

Open-pack reagent stability:
Reagent stability at 2−8ºC after first opening for the Elecsys Tg II assay was evaluated on one **cobas e** 411 analyzer. A fresh reagent rack pack was placed on the analyzer and calibrated. Five native single donor serum samples covering the AMI of the assay were tested at baseline and other time points up to 101 days. The data of each sample at each testing time point were compared to the data of initial value tested at baseline. The data support the reagent stability of 84 days at 2–8ºC after first opening.

On-board reagent stability:
On-board reagent stability for the Elecsys Tg II was evaluated on one **cobas e** 411 analyzer. Five native serum samples covering the AMI of the assay were tested at baseline and other time points up to 44 days. The data of each sample at each testing time point were compared to the data of initial value tested at baseline. The data support the claim of 28 days stability for the Elecsys Tg II reagents stored on-board on the **cobas e** 411 analyzer.

Calibration stability:
The Elecsys Tg II was calibrated with a fresh reagent kit on Day 0 using one **cobas e** 411 analyzer. Five native serum samples covering the AMI of the assay were tested up to 29 days. On each testing day, a new reagent kit of the same lot stored at 2–8ºC was used, and recovery of samples was determined using the calibration curve of day 0. The data support the claim of 28 days calibration stability on the **cobas e** 411.

On-board calibration stability:
On-board calibration stability for the Elecsys Tg II assay was evaluated on one **cobas e** 411 immunoassay analyzer. A fresh Elecsys Tg II reagent rack pack was placed on the analyzer. Five serum samples covering the AMI of the assay were tested at baseline and other time points up to 22 days. After specific time point, the same samples were measured using a separate Elecsys Tg II reagent kit kept under on-board condition (20 ± 3°C) without re-calibration of the reagent kit. The data support the claim of 7 days for the on-board calibration stability on the **cobas e** 411.

Sample stability:
The sample stability of serum, Li-Heparin plasma, K2-EDTA and K3-EDTA plasma was evaluated using samples stored at 2–8°C, 15–25°C, and -20°C (±5°C) (-25 to -15°C) for each sample matrix. Seven samples were prepared covering the AMI of the assay and tested using the Elecsys Tg II on the **cobas e** 411 analyzer at Day 0 and at various testing timepoints during storage: 2–8°C and 15–25°C up to 15 days, and -20°C (±5°C) up to 41 months. At each testing point, measurements were performed in three replicates and recovery was calculated as percent or absolute deviation compared to the values of Day 0. The data support the stability for all sample types for 14 days at 2−8°C, 14 days at 15−25°C, and 24 months at -20 (± 5)°C.

6. Detection Limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for Elecsys Tg II reagent pack were determined based on the CLSI EP17-A2 guidelines.

LoB:
The LoB was determined by testing five analyte-free serum samples with at least two Elecsys Tg II reagent lots on one **cobas e** 411 analyzer. Samples were tested in two replicates per run, two runs per day for two days, and one run per day for another two days, for a toal of 60 measurements per reaget lot. LoB was defined as the value corresponding to the 95th percentile of the rank position of the distribution of values. The highest LoB value observed across tested reagent lots was 0.017 ng/mL. The claimed LoD for the Elecsys Tg II is 0.02 ng/mL.

LoD:
The LoD was determined by testing five serum samples containing low levels of thyroglobulin with three reagent lots on one **cobas e** 411 analyzer. Samples were run in two replicates per run, two runs per day for two days, and one run per day for another two days, yielding a total of 60 measurements per lot. The LoD was calculated LoD was calculated based on LoD = LoB + 1.653 x SD (total of low analyte samples). The highest LoD value observed across three lots was 0.04 ng/mL. The claimed LoD for Elecsys Tg II test is 0.04 ng/mL.

LoQ:
The LoQ was determined by testing a set of seven low level of serum samples using three reagent lots on one **cobas e** 411 analyzer. The samples were analyzed in five replicates on one **cobas e** 411 analyzer, one run per day over five days, for a total of N=25 determinations per sample per reagent lot. The LoQ was determined to be the lowest analyte concentration that measured with a %CV of within-laboratory precision ≤ 20% and %bias within ±15%. The highest LoQ value observed across lots was 0.057 ng/mL. The claimed LoQ for Elecsys Tg II test is 0.1 ng/mL.

7. Assay Cut-Off:

   See Clinical Cut-Off below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   Method comparison study were conducted by testing 141 native serum samples in singleton at one clinical site using one reagent lot of Elecsys Tg II (candidate device) on **cobas e** 411 analyzer, and one reagent lot of Access Thyroglobulin assay (predicate device) on Beckman Access DxI 800. Among 141 samples, 126 samples had values within the measuring ranges of both candidate and predicate devices and analyzed with Passing-Bablok regression. The results showed a slope of 1.394 with an intercept of -0.102. Such results indicated a systematic difference between the candidate and predicate devices. Therefore, in addition to the method comparison study, the recovery study was conducted as follows:

   The recovery study used the master calibrator material traceable to the Certified Reference Material (CRM) for spiking into the Diluent MultiAssay (sample diluent used for the Elecsys Tg II). A dilution series including 17 samples were prepared by diluting the sample with value of 557 ng/mL with Diluent MultiAssay. Samples with spiked values from 1 ng/mL to

557 ng/mL were measured in four replicates and samples with spiked values from 0.0557 ng/mL to 0.195 ng/mL were measured in 10 replicates. All measurements were performed using one Elecsys Tg II reagent lot on one **cobas e** 411 analyzer. The mean-value of replicates for each sample level was compared to its expected concentration level, and the percent recovery (% recovery) was calculated. The results showed that the % recovery for 17 samples is from 91.7% to 110.5%.

2. Matrix Comparison:

A study was performed to demonstrate that lithium heparin, K2-EDTA, and K3-EDTA plasma matrices yield comparable values as serum with the Elecsys Tg II. The study included 65 matched native single and pooled samples with thyroglobulin concentration covering the measuring range of the assay. Samples were tested in singleton using one reagent lot on **cobas e** 401. Passing-Bablok regression analyses were performed by comparing the results of samples from different plasma samples (y) to the results of corresponding serum samples (x) for two different ranges of the serum sample concentrations (0.197 to 19.9 ng/mL and 20 to 490 ng/mL). The results are summarized in the tables below:

Plasma/Serum	N	Range (ng/mL)	Slope (95% CI)	Intercept (95% CI)	R
Li-heparin plasma vs serum	49	0.197 –19.9	0.9752 (0.9553; 0.9940)	-0.0001 (-0.0900; 0.09745	0.997
	16	20 – 490	1.0133 (0.9962; 1.0452)	-0.9379 (-3.0174; -0.1074)	1.000
K2-EDTA plasma vs serum	49	0.197 – 19.9	0.9904 (0.9774; 1.0059)	0.0005 (-0.1142; 0.0620)	0.996
	16	20 – 490	1.0070 (0.9546; 1.0196)	-0.5143 (-1.1575; 0.7477)	1.000
K3-EDTA plasma vs serum	49	0.197 – 19.9	0.9535 (0.9350; 0.9731)	0.0291 (-0.0367; 0.1401)	0.998
	16	20 – 490	0.9919 (0.9219; 1.0043)	-1.0145 (-1.9147; 1.1720)	0.999

	Li-Heparin vs Serum	K2-EDTA vs Serum	K3-EDTA vs Serum
Bias at 0.2 ng/mL	-2.5%	-0.7%	9.9%
Bias at 1 ng/mL	-2.5%	-0.9%	-1.7%
Bias at 2 ng/mL	-2.5%	-0.9%	-3.2%
Bias at 10 ng/mL	-2.5%	-1.0%	-4.4%
Bias at 100 ng/mL	0.4%	0.2%	-1.8%
Bias at 200 ng/mL	0.9%	0.4%	-1.3%
Bias at 400 ng/mL	1.1%	0.6%	-1.1%

C Clinical Studies:

The effectiveness of the Elecsys Tg II assay as an aid in monitoring of disease status in patients who have differentiated thyroid cancer (DTC) and have had thyroid surgery (with or without ablative therapy) was determined from a prospective, multi-center study. The clinical performance of the Elecsys Tg II at a cut-off of 0.2 ng/mL was evaluated by comparing the test results with the clinical status, the presence of structural disease (SD) over a 24-month period after surgery in subjects with a diagnosis of DTC based on an estimated real-world structural disease prevalence. The clinical study consists of two sets of patients: longitudinal cohort and cross-sectional cohort.

Subject inclusion and exclusion criteria are as follows:

Inclusion criteria:

- Subject ≥ 22 years of age at the time of enrollment
- Subject able and willing to provide informed consent
- Histologically confirmed and documented papillary or follicular thyroid carcinoma, including Hurthle and follicular variants of papillary carcinoma

For Longitudinal Cohort

- Subject diagnosed with DTC and has undergone total/near- total thyroidectomy within 4 – 12 weeks prior to enrollment
- Subjects who have a total/near total thyroidectomy as a result of a completion thyroidectomy following lobectomy and who meet all other entry requirements may be enrolled once at least 4 weeks have elapsed following the completion surgery
- Subject willing and able to tolerate removal of up to 20 mL of blood per visit

For Cross-sectional cohort

- Subject diagnosed with DTC and has undergone total/near total thyroidectomy > 12 weeks prior to enrollment with current evidence of structural disease
- Subject willing and able to tolerate removal of up to 20 mL of blood or in the case of residual samples, approximately 1 mL of serum is available**.**

Exclusion criteria:

- Subjects with medullary or anaplastic histology (including tumors that have any component of poorly differentiated histology if not classified as anaplastic cancer)
- Subjects with TSH-secreting pituitary adenomas
- Presence of Tg antibodies (Elecsys anti-Tg ≥ 22 IU/mL or local anti-Tg test positive)
- Known pregnancy or lactating
- Participation in an investigational medicinal product known to interfere with Tg synthesis or secretion or any other substance known to interfere directly or indirectly with Tg synthesis or secretion within the last 3 months (90 days) before enrollment, if applicable.

From nine different sites in U.S., a total of 772 samples from 219 subjects were collected for longitudinal cohort (Group 1) and a total of 72 samples from 72 subjects were collected from cross-sectional cohort (Group 2). Among them, 242 samples were excluded (due to subject

discontinuation or other reasons) and therefore, a total of 530 samples including 461 samples in Group 1 and 69 samples in Group 2 were used in the study analysis.

For the samples of 530 evaluated in the study, the distributions of demographic and clinical variables are described in the table below:

N=530			
Category	**Demographic or Clinical Variable**	**N**	**% Total**
Race	White, Caucasian	430	81.1
	African American	30	5.7
	Asian or Asian American	13	2.5
	Native Hawaiian or other Pacific Islander	4	0.8
	Other	23	4.3
	Unknown/ Not reported	30	5.7
Ethnicity	Hispanic or Latino	58	10.9
	Non-Hispanic or Latino	470	88.7
	Unknown	2	0.4
Sex	Female	363	68.5
	Male	167	31.5
Age	Minimum	23 years	
	Median	53 years	
	Maximum	97 years	
RAI*-Treated	Yes	108	20.4
	No	422	79.6
ATA Risk Classification	Low	237	44.7
	Intermediate	172	32.5
	High	121	22.8

*RAI: Radioactive iodine

For the Group 1 longitudinal cohort, serum samples were collected from subjects within 4 – 12 weeks following total or near total thyroidectomy but before radioiodine ablation (if planned). Thyroglobulin levels were measured at four additional time points (approximately 6 months, 12 months, 18 months, and 24 months post-surgery/radioiodine ablation) resulting in five planned visits per patient. For the Group 2 cross-sectional cohort, samples from subjects had only one single visit. All samples were tested with the Elecsys Tg II on **cobas e** 411 analyzer.

For each sample at each visit, structural disease (SD+) was defined as evidence of disease on ultrasound, cross-sectional or functional imaging, or biopsy proven disease as determined by the investigator.

Results:

In this study, samples from the cross-sectional cohort (Group 2) were used to increase the number of observations from the patients with structural disease due to the low prevalence of structural disease in the longitudinal cohort. This increased number of observations from patients

with structural disease provides estimations of sensitivity and negative predictive value (NPV) with less uncertainty. Because the longitudinal and the cross-sectional cohorts were combined, the prevalence of structural disease was larger than the real-life prevalence, the estimates of NPV and positive predictive value (PPV) were adjusted using the real-world prevalence of structural disease observed in the longitudinal cohort (4.99 % = 23/461).

The following tables represent the combined data from two groups and the clinical performance:

		Disease Status		Total
		SD+	SD-	
Elecsys Tg II	≥0.2 ng/mL	91	204	295
	<0.2 ng/mL	1	234	235
	Total	92	438	530

Clinical Performance Measures	Estimate	95% CI
Sensitivity	98.91% (91/92)	(94.10%; 99.81%)
Specificity	53.42% (234/438)	(48.74%; 58.05%)
Prevalence	4.99% (23/461)	(3.35%; 7.37%)
NPV	99.89%	(99.42%; 99.98%)
PPV	10.03%	(9.16%; 11.03%)

The pre-defined suppressed Tg concentrations according to the ATA Response Classification categories is shown in the table below:

Excellent Response	Tg < 0.2 ng/mL
Indeterminate Response	Tg ≥ 0.2 ng/mL AND Tg < 1.0 ng/mL
Biochemical Incomplete Response	Tg ≥ 1.0 ng/mL

Likelihood ratios for the above three different Tg value ranges were calculated for the RAI (radioactive iodine)- treated patients and Not-RAI treated patients separately. Results of the calculations were presented in the tables below:

Combined Cohorts- RAI-Treated

		Disease Status		Total	LR* (95%CI)
		SD+	SD-		
Elecsys Tg II (ng/mL)	Tg < 0.2	1	27	28	0.03 (0.00; 0.14)
	Tg ≥0.2 and Tg<1.0	0	11	11	0.00 (0.00; 0.23)
	Tg ≥1.0	63	6	69	7.22 (3.67; 15.44)
Total		64	44	108	

*The confidence intervals for the LR were calculated as confidence intervals for the ratios of two independent binomial proportions by the asymptotic method.

Combined Cohorts- Not RAI-Treated

		Disease Status		Total	LR* (95%CI)
		SD+	SD-		
Elecsys Tg II (ng/mL)	Tg < 0.2	0	207	207	0.00 (0.00; 0.23)
	Tg ≥0.2 and Tg<1.0	0	124	124	0.00 (0.00; 0.39)
	Tg ≥1.0	28	63	91	6.25 (5.02; 7.88)
Total		28	394	**422**	

*The confidence intervals for the LR were calculated as confidence intervals for the ratios of two independent binomial proportions by the asymptotic method.

In 491 samples in the longitudinal study, 49 samples were from RAI-treated patients and 412 samples were from Not-RAI-treated patients. The prevalence of SD+ based on this study cohort was determined as: 10.20% (5/49) for RAI-treated patients and 4.37% (18/412) for Not-RAI-treated patients. The probability of SD+ for three ranges of the Elecsys Tg II described above is presented in the table below for RAI-treated and Not RAI-treated patients separately and these probabilities were adjusted for the SD+ real-world prevalence of 10.20% for RAI-treated and 4.37% for Not RAI-treated patients separately [PV probability of SD+ then PV/(1-PV)=LR*prevalence/(1-prevalence)].

	Prevalence	Probability of SD+, (95% CI)		
		Tg <0.2 ng/mL	Tg ≥0.2 ng/mL and Tg <1.0 ng/mL	Tg ≥1.0 ng/mL
RAI-Treated	10.20%	0.29% (0.05%; 1.55%)	0.00% (0.00%; 2.56%)	45.07% (29.44%; 63.70%)
Not RAI-Treated	4.37%	0.00% (0.00%; 1.04%)	0.00% (0.00%; 1.73%)	22.22% (18.64%; 26.46%)

The results from the clinical study demonstrated that at Tg concentrations ≥ 1.0 ng/mL (corresponding to ATA "Biochemical Incomplete Response"), the probability of structural disease (i.e., 45% for RAI-treated patients and 22% for Not RAI-treated patients) was much higher than the probability of structural disease for Tg concentrations <0.2 ng/mL (corresponding to ATA "Excellent Response").

D Clinical Cut-Off:

The clinical cut-off for the Elecsys Tg II is 0.2 ng/mL. This was set based on 2015 ATA Guidelines which introduced an "Excellent response" with "no clinical, biochemical or structural evidence of disease" and for patients without TSH stimulation is defined by negative imaging and a suppressed Tg < 0.2 ng/mL in the absence of Tg antibodies. Tg ≥ 0.2 AND Tg < 1.0 was used corresponding to the ATA response to therapy classification to determine "Indeterminate Response." Tg > 1.0 ng/mL was used corresponding to the ATA response to therapy classification to determine "Biochemical Incomplete Response."

E Expected Values/Reference Range:

A reference range of the Elecsys Tg II was established by testing serum samples from a total of 463 apparently healthy individuals including 244 males and 219 females. The results are summarized in the following table:

	Male	Female	Total
N	244	219	463
Age (years)	22–79	22–77	22–79
Min – Max (ng/mL)	2.66–82.5	1.65–234	1.65–234
Median (ng/mL)	15.1	18.3	16.6
2.5th percentile (ng/mL)	3.3	3.9	3.6
97.5th percentile (ng/mL)	63.2	104	77

Addtionally, the value of the Elecsys Tg II was evaluated in a total of 127 subjects with differentiated thyroid cancer with no evidence of disease for 4 or more years following total/near-total thyroidectomy. The study included 100 female patients aged 26 to 92 years, and 27 male patients aged 34 to 81 years. The results showed that the thyroglobulin concentration ranged 0.1 to 11.6 ng/mL. About 80.3% (102/127) of subjects had thyroglobulin level below 0.1 ng/mL, and 95% of patients had thyroglobulin level ≤ 0.786 ng/mL.

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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