510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K222610

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys Anti-Tg

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JZO	Class II	21 CFR 866.5870 - Thyroid Autoantibody Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Modification of a previously cleared assay to decrease interference to biotin.

B Measurand:

Anti-Thyroglobulin (anti-Tg) autoantibody

C Type of Test:

Quantitative, electrochemiluminescent immunoassay

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 antibodies to thyroglobulin in human serum and plasma. The anti-Tg autoantibodies determination is used as an aid in the detection of autoimmune thyroid diseases in conjunction with other laboratory and clinical findings.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on **cobas e** 411 immunoassay analyzer.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For in vitro diagnostic use.

D Special Instrument Requirements:

cobas e 411 immunoassay analyzer

IV Device/System Characteristics:

A Device Description:

The Elecsys reagent rackpack (M, R1, R2) consists of:

- M: Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL:
  Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1: Tg~biotin (gray cap), 1 bottle, 10 mL:
  Biotinylated Tg (human) 0.200 mg/L; TRIS buffer 100 mmol/L, pH 7.0; preservative.
- R2: Anti Tg-Ab~Ru(bpy) (black cap), 1 bottle, 10 mL:
  Monoclonal anti-Tg antibodies (human) labeled with ruthenium complex 0.620 mg/L; TRIS buffer 100 mmol/L, pH 7.0; preservative.

The Elecsys Anti-Tg has been modified from the previously cleared format (K053426) to decrease potential biotin interference in the assay. Briefly, free biotin in serum and plasma is neutralized by the addition of a biotin-neutralizing antibody in reagents. The antibodies are specific for free biotin and do not bind to, or interact with, the biotin-linker conjugates.

B Principle of Operation:

The Elecsys Anti-Tg assay is a two-step sandwich immunoassay that makes use of a competitive test principle and uses biotinylated Tg and a monoclonal Anti-Tg specific antibody labeled with ruthenium complex.

The Elecsys Anti-Tg assay has an assay time of 18 minutes. During the first incubation, 10 µL of sample is combined with a biotinylated-Tg and antibodies from the patient sample bind the Tg antigen. During the second incubation, after the addition of anti-Tg antibodies labeled with ruthenium complex and streptavidin-coated microparticles, the immunocomplex produced becomes bound to the solid phase via interaction of biotin and streptavidin. 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. The application of voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The results are determined via a calibration curve which is instrument-specifically generated by a 2-point calibration and a master curve provided via the reagent barcode or 2-barcode.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Roche Elecsys Anti-Tg

B Predicate 510(k) Number(s):

K053426

C Comparison with Predicate(s):

Device & Predicate Device(s):	**K222610 (Device)**	**K053426 (Predicate)**
Device Trade Name	Elecsys Anti-Tg	Elecsys Anti-Tg
General Device Characteristic Similarities		
Intended Use/ Indications For Use	Immunoassay for the in vitro quantitative determination of antibodies to thyroglobulin in human serum and plasma. The anti-Tg autoantibodies determination is used as an aid in the detection of autoimmune thyroid diseases in conjunction with other laboratory and clinical findings. The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e 411 immunoassay analyzer.	Immunoassay for the in vitro quantitative determination of antibodies to thyroglobulin in human serum and plasma. The anti-Tg determination is used as an aid in the detection of autoimmune thyroid diseases. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.
Detection method	Electrochemiluminescence "ECLIA"	Same

Test format	Quantitative competitive	Same
Assay Reaction Time	18 Minutes	Same
Traceability	Standardized against the NIBSC (National Institute for Biological Standards and Control) 65/93 Standard	Same
General Device Characteristic Differences		
Sample Matrices	Human serum and plasma (K2-EDTA, K3-EDTA)	Human serum and plasma (sodium heparin, K2/K3-EDTA)
Calibrators	Anti-Tg CalSet (sold separately)	Anti-Tg calibrator 1 and 2 (included in test kit)
Controls	PreciControl ThyroAB (sold separately)	PreciControl Anti-Tg 1 and 2 (included in test kit)
Biotin Tolerance	No interference up to 1200 ng/mL	No interference up to 60 ng/mL
Measuring Range	15 – 4000 IU/mL	10 – 4000 IU/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; Approved Guideline – Third Edition
- CLSI EP06-Ed2, Evaluation of Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-Ed3, Interference Testing in Clinical Chemistry.
- CLSI EP09c 3rd Edition, Measurement Procedure Comparison and Bias Estimation Using Patient Samples
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition
- CLSI EP37-Ed1, Supplemental Tables for Interference Testing in Clinical Chemistry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within-laboratory precision of the modified Elecsys Anti-Tg was conducted following the CLSI EP05-A3. Aliquots of six human serum samples including one native sample and five

native sample pools, and two PreciControl ThyroAB controls were measured in duplicate per run, two runs per day, for 21 days using one reagent lot on one **cobas e** 411 analyzer, resulting a total of 84 measurements per sample. The within-laboratory precision is shown in the table below:

Sample	N	Mean (IU/mL)	Repeatability		Between-Run		Between-Day		Within-Lab Precision	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	84	15.7	1.3	8.4	1.0	6.4	1.6	10.0	2.3	14.5
2	84	110.0	7.8	7.1	2.9	2.6	0.4	0.4	8.3	7.6
3	84	1953.0	64.0	3.3	31.7	1.6	29.9	1.5	77.4	4.0
4	84	2495.0	106.0	4.2	0.0	0.0	67.5	2.7	125.0	5.0
5	84	3816.0	171.0	4.5	0.0	0.0	0.0	0.0	171.0	4.5
Control 1	84	79.4	5.4	6.8	2.4	3.0	3.1	3.9	6.7	8.4
Control 2	84	177.0	14.0	7.9	9.7	5.5	2.2	1.2	17.2	9.7

Lot-to-lot precision was evaluated on one **cobas e** 411 analyzer by testing five serum samples, including one native donor sample and four native donor pools, and two PreciControl ThyroAB controls, in five replicates per run, one run per day for five days using three reagent lots, yielding 25 measurements per lot for each sample. The results are summarized in the table below.

Sample	N	Mean (IU/mL)	Repeatability		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	17.9	1.9	10.8	0.0	0.0	0.5	3.0	2.0	11.3
2	75	112.0	6.0	5.4	0.0	0.0	6.2	5.6	8.7	7.8
3	75	1868.0	76.4	4.1	21.2	1.1	78.0	4.2	111.0	6.0
4	75	3708.0	238.0	6.4	0.0	0.0	297.0	8.0	381.0	10.3
5	75	3903.0	131.0	3.4	0.0	0.0	346.0	8.9	370.0	9.5
Control 1	75	86.4	3.8	4.3	2.8	3.2	0.0*	0.0	4.7	5.4
Control 2	75	212.0	9.8	4.6	3.6	1.7	4.9	2.3	11.6	5.5

* Negative variance estimates were set to 0.

2. Linearity:

Linearity of the Elecsys anti-Tg assay was assessed according to CLSI EP06-Ed2. Native human serum samples and normal serum sample pools were used to create six dilution series for a total of 26 concentration levels across the measuring range. The samples were assayed in triplicate within a single run on the **cobas e** 411 analyzer. For each level, the mean 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 results of the study were summarized in the following table:

Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R2	Deviation from Linearity (%)
14.18 – 4071	1.052 (1.024 – 1.081)	-2.181 (-4.673 – 0.311)	0.996	-15.05% – 11.28%*

* One mid-range sample gave a higher deviation from linearity of -15.05% and one sample on the low end of the range gave a deviation from linearity of 11.28% which corresponded to a deviation of 1.4 IU/mL. The deviation from linearity for the remaining samples were between -7.6% to 10.7%.

The results support the linearity of the claimed measuring interval (AMI): 15 – 4000 IU/mL.

3. Analytical Specificity/Interference:

Endogenous Interference:

Potential interference from some endogenous substances was assessed using three native serum pools with anti-Tg concentrations of low (~25 IU/mL), mid (~120 IU/mL), and high-range (~3000 IU/mL). For difference substance interferent levels, each of the three samples was further divided into two aliquots for a control sample (with no added interferent) and test sample (with added interferent). The anti-Tg level in the test and control samples were assayed in five replicates using one modified Elecsys anti-Tg reagent lot using one instrument. For each interferent concentration level, the recovery was based on the mean of the replicates of test sample value comparing to the control sample.

The results are summarized in the following table.

Endogenous Interferent	Highest concentration tested without interference	Concentration of interference claimed in labeling
Biotin	2400 ng/mL	1200 ng/mL
Bilirubin	66 mg/dL	66 mg/dL
Hemoglobin	420 mg/dL	420 mg/dL
Intralipid	2000 mg/dL	2000 mg/dL
Rheumatoid Factor	300 IU/mL	300 IU/mL
Tg	200 ng/mL	100 ng/mL

Exogenous Interference:

Interference from 17 common and 14 pharmaceutical drugs that might be used in the clinical context of the assay's intended use were assessed using two serum samples, one with a low concentration of anti-Tg (~25 IU/mL) and one with a high concentration of anti-Tg (1500 IU/mL). The two samples were divided into aliquots and spiked with the potential interferents or with solvent only. No significant interference (a recovery of 100% ± 10%) is observed for the interferents at the concentrations tested in the table below.

Common and Pharmaceutical Drugs	Concentration Tested (mg/L)	Common and Pharmaceutical Drugs	Concentration Tested (mg/L)
Acetaminophen	156	Levodopa	7.5
Acetylcysteine	150	Levothyroxine	0.25
Acetylsalicycic Acid	30	Liothyronine	0.045
Amiodarone	40.0	Methimazole	16.0
Ampicillin-Na	75	Methyldopa	22.5
Ascorbic acid	52.5	Metronidazole	123
Carbimazole	30.0	Nivolumab	96.0
Cyclosporine	1.8	Octreotide	0.300
Cefoxitin	750	Perchlorate	2000
Doxycycline	18	Phenylbutazone	321
Flucortolone	100	Prednisolone	100
Heparin	3300 IU/L	Propranolol	48.0
Hydrocortisone	200	Propylthiouracil	180
Ibuprofen	219	Rifampin	48
Iodine	50.0	Theophylline	60
Itraconazole	10		

Cross reactivity:

Potential cross-reactivity between anti-Tg and anti-TPO was assessed in a cross-reactivity study. Two native human serum pools with anti-Tg concentrations of 26 IU/mL and 96 IU/mL were spiked with anti-TPO antibodies to a level of 1500 IU/mL and assessed using one reagent lot of the modified Elecsys Anti-Tg and a **cobas e** 411 immunoassay analyzer. The average results from three replicates of the paired spiked human serum pools were compared with the un-spiked human serum pool to determine the percent of cross reactivity. Significant Cross Reactivity was defined as ≥10% Cross Reactivity between spiked and un-spiked native human serum pools. The results demonstrate that there is no notable cross reactivity between anti-Tg and anti-TPO (up to 1500 IU/ml) in the Elecsys Anti-Tg assay.

4. Assay Reportable Range:

The reportable range for the Elecsys Anti-Tg assay is 15 – 4000 IU/mL.

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

Traceability:

The Elecsys Anti-Tg assay is standardized against the NIBSC (National Institute for Biological Standards and Control) 65/93 Standard.

Stability:

An on-board stability study of the modified assay reagents was performed using five samples, including two native single serum samples and three native serum pools, across the

measuring range and tested with one lot of the modified Elecsys Anti-Tg stored on the analyzer. The data support the package insert claim of 6 weeks on-board stability.

A stability study to determine the stability of opened reagents when stored at 2–8°C was performed using five samples, including two native single serum samples and three native serum pools, across the measuring range and tested at various time points with one lot of the modified Elecsys Anti-Tg stored at 2–8°C for up to 50 days. The data support the package insert claim of 6 weeks.

A real time stability study was performed to examine the long term stability of shelf-life of the modified Elecsys Anti-Tg reagents when stored at 2–8°C. At various testing points, two lots of PreciControl ThyroAB Level 1 and Level 2 controls were tested in triplicate on one **cobas e** 411 using three lots of the modified Elecsys Anti-Tg reagents stored at 2–8°C. The data support the package insert claim that the Elecsys Anti-Tg reagents are stable when stored at 2–8°C for up to 15 months.

6. Detection Limit:

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were determined in accordance with CLSI EP17-A2.

Limit of Blank (LoB):

For determination of the LoB, the LoB was determined as the 95th percentile of blank sample measurements. Briefly, an analyte-free native human serum sample was measured using three reagent lots of the modified Elecsys Anti-Tg, one **cobas e** 411 analyzer, over the course of six runs with 10-replicates per run, resulting in a total of 60 blank replicates per reagent lot. The LoB determined in all three lots were less than the claimed LoB in the labeling, 9.0 IU/mL.

Limit of Detection (LoD):

For the determination of the LoD, five low-level native human serum samples, including three single native donors and two native sample pools, were measured in duplicate in six runs, over six days, with three lots of the modified Elecsys Anti-Tg on one **cobas e** 411 analyzer. In total, 60 measured values of samples with low analyte concentrations were obtained per lot. The LoD corresponds to the concentration determined by LoD = LoB + 1.653 x SD total (of low analyte samples). The LoD determined in all three lots were less than the claimed LoD in the labeling, 10.0 IU/mL.

Limit of Quantitation (LoQ):

For the determination of LoQ, four low-level single native human serum samples were measured in quintuplicate per run, one run per day for five days using three different lots of the modified Elecsys Anti-Tg on one **cobas e** 411 analyzer, resulting in 25 replicates per sample per lot. The LoQ was set as the lowest concentration of analyte that can be reproducibly measured with a total error of ≤ 20% at ≤ 15 IU/mL. The LoQ determined in all three lots were less than the claimed LoQ in the labeling, 15 IU/mL.

7. Assay Cut-Off:

Refer to K053426.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed to show comparability between the performance of the current Elecsys anti-Tg assay (predicate) and the modified Elecsys anti-Tg assay (candidate). A total of 129 native human samples, including 90 native single donor samples and 39 native serum pools, spanning the predicate measuring range (23.1 – 3609 IU/mL) were tested on one **cobas e** 411 analyzer. The results of Passing-Bablok regression analysis are shown below:

N	Range (IU/mL)	Slope (95% CI)	Y-Intercept (95% CI)	R	Bias at 115 U/mL (95% CI)
129	23.1 – 3609	0.974 (0.942 – 1.018)	1.72 (-4.35 – 8.21)	0.930	-1.14 (-4.0 – 2.9)

2. Matrix Comparison:

A matrix equivalency study to serum was conducted to support use of the Elecsys anti-Tg assay on the **cobas e** 411 analyzer with the following sample matrices: Serum, dipotassium (K2) EDTA plasma, and tripotassium (K3) EDTA plasma. In the study, at least 44 serum/plasma donor matched venous specimens were collected and evaluated. Each specimen was tested in singlicate using one reagent lot of the modified Elecsys Anti-Tg. The results were analyzed by Passing-Bablok regression with serum results as the reference (x-axis). The results are summarized as follows:

	N	Serum Sample Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	Pearson r Value
K2-EDTA Plasma vs Serum	44	17.0 – 3678	1.018 (1.004 – 1.035)	-1.17 (-7.24 – 1.10)	0.997
K3-EDTA Plasma vs Serum	44	17.0 – 3678	1.021 (0.998 – 1.041)	-1.37 (-8.09 – 0.944)	0.996

C Clinical Studies:

1. Clinical Sensitivity:

Refer to K053426.

2. Clinical Specificity:

Refer to K053426.

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Refer to K053426.

D Clinical Cut-Off:

Refer to K053426.

E Expected Values/Reference Range:

The reference range of the modified Elecsys Anti-Tg was verified be the same as the reference range of the predicate which was established based on a total of 391 healthy subjects and confirmed the threshold value of 115 IU/mL (which corresponds to the 94th percentile).

The labeling of the Elecsys Anti-Tg includes the following statement: "*Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.*"

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.