510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number
K193313

B Applicant
Roche Diagnostics

C Proprietary and Established Names
Elecsys Anti-TSHR

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 to the predicate

B Measurand:
IgG autoantibodies specific for the thyroid stimulating hormone receptor (TSHR)

C Type of Test:
Quantitative electrochemiluminescence 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 autoantibodies to thyroid stimulating hormone (TSH) receptor in human serum using a human thyroid stimulating monoclonal antibody. The anti-TSH receptor determination is used in the assessment of patients suspected of Graves' disease (autoimmune hyperthyroidism).

The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e 601 immunoassay analyzers.

C Special Conditions for Use Statement(s):
For Prescription Use Only

D Special Instrument Requirements:
Roche cobas e 601

IV Device/System Characteristics:

A Device Description:
The Elecsys Anti-TSHR reagent kit consists of a Reagent Pack (R1, R2, and Microparticles), lyophilized calibrators 1 and 2, and a Pretreatment Pack (PT1, PT2, PTR, PTB).

The Reagent Pack's components are:

- M: Streptavidin-coated microparticles (transparent cap), Streptavidin-coated microparticles (0.72 mg/mL) and preservative in a 6.5 mL-bottle.
- R1: Buffer solution (gray cap), containing 20 mmol/L phosphate buffer (pH 7.4); stabilizers; and preservative in a 7 mL-bottle.
- R2: Anti TSHR~Ru(bpy) (black cap), Monoclonal anti-TSHR antibody M22 (human) labeled with ruthenium complex (approximately 0.3 mg/L) in 20 mmol/L phosphate buffer (pH 7.4); stabilizers, and preservative in a 7 mL-bottle.

The Pretreatment Pack's components are:

- PT1: Pretreatment buffer solution (black cap), 20 mmol/L phosphate buffer (pH 7.4); stabilizers, and preservative in a 4 mL-bottle.
- PT2: Empty bottle (white cap) for pretreatment reagent (PTR) reconstituted with pretreatment buffer (PTB).
- PTR: Pretreatment reagent (white cap): lyophilized porcine TSHR-anti-pTSHR-Ab~biotin complex; 40 mmol/L phosphate buffer (pH 7.2); and stabilizers in a 4 mL-bottle.
- PTB: Pretreatment buffer (white cap), reconstitution medium for PTR containing phosphate buffer 10 mmol/L (pH 7.2) and stabilizer in 5 mL-bottle.

The reformulated reagents are designed to block the interference of free biotin with the anti-TSHR assay and to improve the binding between the conjugate and the streptavidin-coated magnetic microparticles. A biotin-binding scavenger antibody was added to the assay to bind free biotin in the sample. The antibodies are specific only to free biotin and do not bind to, or interact with, the biotin-linker conjugates. The biotinylated antibody conjugate was modified by changing the chemical spacer between the biotin molecule and the detection antibody. Lengthening the distance between the biotin and the immunological component is designed to improve binding of the conjugate to the streptavidin-coated magnetic bead. No change was made to the capture and detection antibodies which still recognize the same epitope.

B Principle of Operation:

The Elecsys Anti-TSHR immunoassay is a three-step competitive immunoassay with streptavidin-coated microparticles and electrochemiluminescence detection. Patient serum sample is incubated with pretreatment reagent PT1 and the reconstituted PT2 containing a pre-formed immunocomplex of solubilized porcine TSH receptor (pTSHR) and biotinylated anti-pTSHR mouse monoclonal antibody. Anti-TSHR antibodies (TRAb) in patient's sera interact with the TSHR immunocomplex. After addition of streptavidin-coated microparticles and a human thyroid stimulating monoclonal autoantibody (M22) labeled with a ruthenium complex, bound TRAb are detected by their ability to inhibit the binding of labeled M22. The entire complex 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/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Results are determined using a calibration curve that is generated specifically on each instrument by a two-point calibration and master curve provided with the reagent bar code. Test values of ≤1.75 IU/L are negative while test values >1.75 IU/L are positive.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys Anti-TSHR Immunoassay

B Predicate 510(k) Number(s):

K080092

C Comparison with Predicate(s):

Device & Predicate Device(s):	Device K193313	Predicate K080092
Device Trade Name	Elecsys Anti-TSHR	Elecsys Anti-TSHR Immunoassay
General Device Characteristic Similarities		
Intended Use/ Indications For Use	Immunoassay for the in vitro quantitative determination of autoantibodies to thyroid stimulating hormone (TSH) receptor in human serum using a human thyroid stimulating monoclonal antibody. The anti-TSH receptor determination is used in the assessment of patients	Elecsys Anti-TSHR Immunoassay is an immunoassay for the in vitro quantitative determination of autoantibodies to TSH receptor in human serum using a human thyroid stimulating monoclonal antibody. The anti-TSH receptor determination is used in the assessment of patients with

Device & Predicate Device(s):	Device K193313	Predicate K080092
Device Trade Name	Elecsys Anti-TSHR	Elecsys Anti-TSHR Immunoassay
	suspected of Graves' disease (autoimmune hyperthyroidism). The electrochemiluminescence immunoassay "ECLIA" is intended for use on cobas e 601 immunoassay analyzers.	suspect Graves' disease (autoimmune hyperthyroidism). The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.
Assay Method	Electrochemiluminescence (ECLIA)	Same
Assay Type	Competitive	Same
Assay Protocol	Sample+PT1+PT2, incubation, +R1, incubation, +beads+R2, incubation	Same
Sample Type	Serum	Same
Traceability/ Standardization	NIBSC 1st IS 90/672 Standard	Same
Cut-off	1.75 IU/L	Same
Measuring Range	0.8–40.0 IU/L	Same
Calibrators	2 calibrators against the master curve for that reagent lot	Same
General Device Characteristic Differences	Device K193313	Predicate K080092
Instrument Platform	cobas e 601 analyzer	Roche automated clinical chemistry analyzers
Biotin Tolerance	Up to 600 ng/mL	Up to 10 ng/mL
Biotinylated antibody	MAK<TSHR>M-4E31-IgG-Bi (PEG** mono)	MAK<TSHR>M-4E31-IgG-Bi (DDS* mono)
Buffer 2	Phosphate buffer 20 mmol/L	Same
	Anti-Biotin Antibody; specific for free, unconjugated biotin ("scavenger antibody")	Not applicable

VI Standards/Guidance Documents Referenced:

EP05-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Edition

EP6-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline, Second Edition

EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition

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

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision of the Elecsys Anti-TSHR assay was evaluated on one cobas e 601 immunoassay analyzer with one reagent lot. Human sera and two PreciControl ThyroAB levels were tested with two replicates per run, two runs per day for 21 days (n = 84 per sample). The samples were run in randomized order on the analyzer. The human serum samples used were native human serum pools (human serum 1–4) and spiked human serum (human serum 5). Calculations were performed according to EP05-A3.

		Repeatability		Between Run		Between Day		Within Laboratory	
Sample	**Mean (IU/L)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Sera 1	1.41	0.10	7.5	0	0	0.08	5.3	0.13	9.1
Sera 2	1.87	0.14	7.5	0	0	0.08	4.2	0.16	8.6
Sera 3	1.99	0.11	5.7	0	0	0.09	4.3	0.14	7.2
Sera 4	22.70	0.25	1.1	0.18	0.8	0.16	0.7	0.35	1.5
Sera 5	37.50	0.30	0.8	0.16	0.4	0.38	1.0	0.50	1.3
Control 1	4.42	0.14	3.3	0.04	0.9	0.09	2.1	0.18	4.0
Control 2	18.10	0.34	1.9	0.18	1.0	0.10	0.5	0.40	2.2

The reproducibility of the Elecsys Anti-TSHR assay was evaluated on one cobas e 601 immunoassay analyzer with three reagent lots and the five human serum samples described above. The protocol consisted of testing two replicates of each sample per run, two runs per day, for seven days (n=28 replicates per lot, n=84 replicates per sample). Calculations were performed according to EP05-A3.

		Repeatability		Between Run		Between Day		Between Lot		Total	
Sample	**Mean (IU/L)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Sera 1	1.38	0.01	6.9	0.03	2.3	0.9	6.5	0.07	4.7	0.15	10.9
Sera 2	1.90	0.12	6.3	0.03	1.8	0.05	2.8	0.07	3.4	0.15	7.9
Sera 3	1.99	0.10	5.0	0.02	1.0	0.10	4.9	0.06	3.0	0.15	7.7
Sera 4	23.0	0.28	1.2	0.22	0.9	0.05	0.2	0.21	0.9	0.41	1.8
Sera 5	37.8	0.29	0.8	0.06	0.2	0.26	0.7	0.33	0.9	0.51	1.4

2. Linearity:

Linearity of the Elecsys anti-TSHR assay was assessed on the cobas e 601 analyzer according to CLSI EP06-A. Three high analyte human serum sample pools were diluted with anti-TSHR free serum and concentrations covering the measuring range were measured. Sample dilutions were assayed in triplicate within a single run.

Sample	Dilution Range (IU/L)	Regression Equation	Slope (95% CI)	Intercept (95% CI)	R^2
1	0.79–43.4	y=1.000x-0.005	0.958–1.042	-0.019–0.176	0.983
2	0.77–42.8	y=0.997x+0.051	0.957–1.037	-0.117–0.220	0.984
3	0.71–44.9	y=0.993x-0.126	0.947–1.039	0.073–0.179	0.979

3. Analytical Specificity/Interference:

Endogenous Interference:

The interference study was performed according to CLSI EP07-A2. Three specimens were tested (around the cut-off, a moderate positive, and a high positive) for each compound tested. One aliquot of each serum sample was spiked with the interfering substance, another aliquot was spiked with the same volume of isotonic NaCl solution to create a dilution pool. The interferent pool was then diluted into the dilution pool in 10 % increments. The recovery for each sample was calculated by comparison to the reference (unspiked) sample. No interference was observed at these concentrations: hemoglobin at ≤400 mg/dL: bilirubin at ≤ 25 mg/dL; Intralipid at ≤1500 mg/dL; and Rheumatoid Factor at ≤600 IU/mL.

Exogenous Interference:

The effect on quantitation of TSH in the presence of drugs was determined by comparing values obtained from samples spiked with 17 commonly and 13 specialty pharmaceutical compounds with the reference sample (unspiked). The drug concentrations tested correspond at least to the three times maximum daily doses (or the one-time maximum daily dose, respectively). Drug interferences are measured based on recommendations given in CLSI guidelines EP7-ED3 and EP37-ED1 and other published literature.

Two human serum samples (native serum pools) with analyte concentration approximately 3 and 30 IU/L were divided into aliquots and spiked with the potential interferences. The reference sample, without interferent, was spiked with the respective amount of solvent only and tested on the cobas e 601.

Each of the 17 commonly used drugs was found to be non-interfering at the listed concentration: Acetaminophen at 156 mg/L; N-Acetylcysteine at 553 mg/L; Acetylsalicylic Acid at 1000 mg/L; Ampicillin-Na at 1000 mg/L; Ascorbic acid at 300 mg/L; Cefoxitin at 2500 mg/L; Cyclosporine at 1.8 mg/L; Doxycycline at 50 mg/L; Sodium Heparin at 50 IU/L; Ibuprofen at 500 mg/L; Itraconazole at 30 mg/L; Levodopa at 20 mg/L; Methyldopa +1,5 at 22.5 mg/L; Metronidazole at 200 mg/L; Phenylbutazone at 321 mg/L; Rifampicin at 60 mg/L; and Theophylline at 60 mg/L.

Sodium Heparin was found to interfere at concentrations >50 IU/L. A limitation stating: "*Do not use samples from patients under sodium heparin treatment. Sodium heparin showed no interference up to a concentration of 50 IU/L.*" was added to the package insert.

Each of the 13 specialty drugs was found to be non-interfering at the listed concentration: Amiodarone at 200 mg/L; Carbimazole at 30 mg/L; Fluocortolone at 20 mg/L; Hydrocortisone at 200 mg/L; Iodide at 0.040 mg/L; Levothyroxine at 0.250 mg/L; Liothyronine at 0.015 mg/L; Thiamazole at 16 mg/L; Octreotide at 0.300 mg/L; Perchlorate at 400 mg/L; Prednisolone at 20 mg/L; Propranolol at 240 mg/L; and Propylthiouracil at 60 mg/L.

Biotin Interference:
Three human serum samples were spiked with a range of biotin concentrations and tested with the Elecsys anti-TSHR assay on the cobas e 601 analyzer.

% Bias for samples containing various concentrations of Biotin					
Unspiked Sample Conc (IU/L)	**Biotin concentrations (ng/mL)**				
	150	**225**	**300**	**375**	**450**
1.68	-1.6	-0.2	-2.9	6.7	-4.5
11.2	1.0	-2.8	-0.6	-1.8	0.1
32.2	2.0	2.0	2.1	3.2	4.0

% Bias for samples containing various concentrations of Biotin				
Unspiked Sample Conc (IU/L)	**Biotin concentrations (ng/mL)**			
	525	**600**	**675**	**1200**
1.68	-3.1	5.4	12.4	71.9
11.2	2.1	3.3	5.2	24.2
32.2	5.7	5.6	5.1	15.8

Based on this study, the sponsor claims that biotin does not affect the anti-TSHR assay ≤600 ng/mL.

Cross-reacting Substances:
The effect on quantitation of analyte in the presence of potential cross-reacting compounds using the Elecsys anti-TSHR was determined on the cobas e 601 analytical unit using a native human serum sample pool. For each potential cross-reacting compound a human serum sample with a low concentration level and an elevated level of anti-TSHR was tested. Results from these spiked serum samples were matched against the unspiked references and the % cross-reactivity was calculated. Less than 1% cross reactivity was observed for these potential cross-reactants and the concentration tested: anti-thyroglobulin (TG) antibodies at 4,000,000 IU/L; anti-thyroid peroxidase (TPO) at 600,000 IU/L; luteinizing hormone (LH) at 10,000 IU/L; and follicle stimulating hormone (FSH) at 10,000 IU/L.

4. Assay Reportable Range:
See K080092. The assay's reportable range is 0.8–40 IU/L (defined by the Limit of Detection and the maximum of the master curve). Because the %CV of low concentration samples is high, values below the LoQ should be reported with caution. The package insert states: "*Please note: When reporting values <1.1 IU/L, the client report should be annotated with the following information. "Values <1.1 IU/L are not reliable as the intermediate precision CV is > 20%.""*

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
This assay is standardized against NIBSC 1st IS 90/672 Standard.

Stability:
Shelf life stability: Accelerated stability testing comparing the current and the updated (biotin) assay reagent supports the transfer of the shelf-life claim of 18 months to the updated anti-TSHR assay. Real-time stability testing is ongoing for the updated Elecsys Anti-TSHR, supporting a shelf-life of 15 months at this time for unopened reagents and pretreatment kit components stored at 2–8°C.

On-board Reagent Stability: An isochronous study compared unstressed (refrigerated at 2–8°C) to stressed (20–25°C) reagents and pretreatment components. The study supported the claim that the reagents can be stored on board for 3 weeks. The study also supported the claim that: the pretreatment components can be stored for 72 hours if continuously stored onboard; or for 3 weeks total for up to 7 uses for 8 hours total on the analyzer (20–25°C) and stored in the refrigerator (2–8°C) when not in use. The sponsor recommends that the pretreatment bottles (PT2 containing the reconstituted PTR) be stored in the refrigerator when not in use (at 2–8°C).

6. Detection Limit:
The limit of blank and limit of detection were determined in accordance with CLSI EP17-A2.

The Limit of Blank (LoB) is the 95th percentile value from 60 measurements of analyte-free samples over several independent series. The limit of blank corresponds to the concentration below which analyte-free samples are found with a probability of 95%. The LoB of each assay was determined by assaying five analyte-free samples in duplicate per sample over six days for each lot. A total of 60 data points per sample per lot; three lots were tested. The LoB for each lot was calculated separately. The LoB was determined as 0.5 IU/mL.

The Limit of Detection (LoD) is determined based on the limit of blank and the standard deviation of low concentration samples. The limit of detection corresponds to the lowest analyte concentration which can be detected (value above the limit of blank with a probability of 95%). The LoD of each assay was determined by assaying five low analyte samples in duplicate per sample over six days for each lot. A total of 60 data points per lot were generated and three lots were tested. The LoD for each lot was calculated separately. The LoD was determined as 0.8 IU/mL.

The Limit of Quantitation (LoQ) was defined as the lowest analyte concentration which can be quantified with a total error of ≤ 20%. The LoQ was determined by assaying nine low concentration anti-TSHR native samples tested in replicates of five, one run per day, for five days (25 replicates per sample) on three reagent lots. The LoQ was calculated by determining the lowest analyte concentration that resulted in a total within laboratory CV <20%. The LoQ was 1.1 IU/mL.

Because the %CV of low concentration samples is high, values below the LoQ should be reported with caution. The package insert states: *"Please note: When reporting values <1.1 IU/L, the client report should be annotated with the following information. "Values <1.1 IU/L are not reliable as the intermediate precision CV is >20%.""*

7. Assay Cut-Off:
See K080092. Test values of ≤1.75 IU/L are negative while test values >1.75 IU/L are positive.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   The sponsor conducted a method comparison of the Elecsys Anti-TSHR updated assay (candidate device) and the current Elecsys Anti-TSHR immunoassay (predicate device K080092). A total of 260 clinical samples from the intended use population were measured with both assays in singleton on the cobas e 601 analyzer covering the entire measuring range. The tested group was 198 samples from patients diagnosed with Graves' disease and 62 samples from patients diagnosed with other thyroid issues (e.g., Hashimoto's disease, non-toxic goiter, thyroid carcinoma, etc.). Separate quantitative and qualitative analyses of the data set were performed:

   Because the assay is quantitative, a Passing-Bablok regression analysis of 120 samples within the measuring range was performed. The samples were tested once with both assays.

Parameter	Result
Range (IU/L)	0.800–35.7 IU/L (Predicate Assay, X) 0.917–40.0 IU/L (Candidate Assay, Y)
Slope (95th CI)	1.047 (1.029–1.064)
Intercept (95th CI)	-0.068 (-0.188–0.032)
Correlation Coefficient	0.998
Absolute Bias at 1.75 IU/L (95th CI)	0.014 (-0.085–0.095)
Percent Bias at 1.75 IU/L	0.80

   The agreement between the assays was calculated using the results of all samples tested in the study (n=260):

		Predicate Assay		
		Positive	Negative	Total
Candidate Assay	Positive	148	5	153
	Negative	4	103	107
	Total	152	108	260

   Positive percent agreement: 97.37% (148/152) 95% CI: 93.43%–98.97%
   Negative percent agreement: 95.37% (103/108) 95% CI: 89.62%–98.01%

2. Matrix Comparison:
   A matrix comparison is not needed because serum is the only matrix indicated.

C Clinical Studies:

1. Clinical Sensitivity:
   See K080092

2. Clinical Specificity:
   See K080092

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

D Clinical Cut-Off:
See K080092

E Expected Values/Reference Range:
See K080092

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.