510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K252431

B Applicant

Roche Diagnostics GmbH

C Proprietary and Established Names

Elecsys Calcitonin

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
JKR	Class II	21 CFR 862.1140 - Calcitonin Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

Modified device

B Measurand:

Calcitonin

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:
The Calcitonin immunoassay is intended for the in vitro quantitative determination of human calcitonin (thyrocalcitonin) in serum and plasma. The calcitonin determination is intended to be used as an aid in the diagnosis and treatment of diseases involving the thyroid and parathyroid glands, including carcinoma and hyperparathyroidism in conjunction with other clinical and laboratory findings.

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

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

D Special Instrument Requirements:

cobas e 411 analyzer

IV Device/System Characteristics:

A Device Description:

The Elecsys Calcitonin consists of the following:

The Elecsys Calcitonin reagents "M", "R1" and "R2" are combined in the reagent rackpack, which is a bundle of the three reagent bottles, which is placed on the instrument as a single unit. The reagent rackpack is labeled as hCT.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-hCT-Ab~biotin (gray cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-hCT antibody (mouse) 1.50 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

R2 Anti-hCT-Ab~$Ru(bpy)^{2+}_3$ (black cap), 1 bottle, 8 mL: Monoclonal anti-hCT antibody (mouse) labeled with ruthenium complex 1.0 mg/L; phosphate buffer 100 mmol/L, pH 7.2; preservative.

B Principle of Operation:
Elecsys Calcitonin is a sandwich principle assay with a total duration of 18 minutes including the following steps:

1st Incubation: 50 µL of sample, a biotinylated monoclonal hCT-specific antibody and a monoclonal hCT-specific antibody labeled with a ruthenium complex react to form a sandwich complex. The presence of a scavenger antibody depletes free, unconjugated biotin present in the sample.

2nd Incubation: After addition of streptavidin-coated microparticles, the 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 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):
Elecsys Calcitonin Immunoassay

B Predicate 510(k) Number(s):
K132828

C Comparison with Predicate(s):

Device & Predicate Device(s):	K252431	K132828
Device Trade Name	Elecsys Calcitonin	Elecsys Calcitonin Immunoassay
General Device Characteristic Similarities		
Intended Use/Indications For Use	Immunoassay for the in vitro quantitative determination of human calcitonin (thyrocalcitonin) in serum and plasma.	Same
Traceability	Traceable to WHO 2nd IRP 89/620	Same
General Device Characteristic Differences		
Biotin tolerance	≤ 1200 ng/mL	≤ 40 ng/mL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition

CLSI EP06-Ed2, Evaluation of Linearity of Quantitative Measurement Procedures, 2nd Edition

CLSI EP07-Ed3, Interference Testing in Clinical Chemistry

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-2nd Edition

CLSI EP09c-Ed3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
Precision was evaluated on one cobas e 411 analyzer with one reagent lot. The protocol consisted of testing two replicates of five human serum samples and each of the levels of control, PreciControl Varia. The sample types tested were native sample pools (S1 - S2) and spiked sample pools (S3 - S5). The protocol consisted of testing two replicates per run for each control and sample, two runs per day over 21 days using one reagent lot. Repeatability and intermediate precision were calculated according to CLSI EP05-A3.

Sample	n	Mean (pg/mL)	**Repeatability**		**Intermediate Precision**	
			SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
Sample 1	84	1.02	0.080	7.9	0.120	11.8
Sample 2	84	10.2	0.261	2.5	0.466	4.6
Sample 3	84	62.1	1.91	3.1	2.95	4.7
Sample 4	84	905	21.8	2.4	41.3	4.6
Sample 5	84	1882	83.0	4.4	107	5.7
Control 1	84	9.35	0.197	2.1	0.391	4.2
Control 2	84	84.5	1.93	2.3	3.23	3.8

A reproducibility study was performed using repeated measurements of five human serum pools (HSP) and two control levels of PreciControl Varia. Data were collected at two external sites, each using one reagent lot, and one internal site using three reagent lots. 25-repeated measurements were performed for each sample appearing in a combination of sample (concentration level, site and lot) with five repetitions per day for five days on a cobas e 411 analyzer. Testing was completed at all three sites for a total N = 125 per sample. The data were analyzed for reproducibility and are summarized below. Data calculation was performed according to CLSI EP05-A3.

			Reproducibility	
Sample	N	Mean (pg/mL)	SD (pg/mL)	CV (%)
Sample 1	125	1.48	0.185	12.5
Sample 2	125	10.2	0.367	3.59
Sample 3	125	54.0	2.21	4.10
Sample 4	125	1035	40.8	3.94

			Reproducibility	
Sample	N	Mean (pg/mL)	SD (pg/mL)	CV (%)
Sample 5	125	1856	61.4	3.31
Control 1	125	9.35	0.309	3.31
Control 2	125	88.7	2.82	3.18

2. Linearity:
A linearity study was conducted according to CLSI EP06-2nd edition. A total of 9 levels of serum samples ranging from 0.312 to 2015 pg/mL were prepared by mixing different proportions of a native serum sample containing a high concentration of calcitonin with a native serum sample containing a low concentration of calcitonin. Samples were run in 4 replicates in a single run. Samples were tested on one cobas e411 analyzer using 3 reagent lots. The data were analyzed using a weighted linear regression model. The deviation from linearity did not exceed 4.2% for concentrations within the measuring interval. The results support the claimed measuring interval of 1 – 2,000 pg/mL.

Dilution:
The sponsor conducted a study to demonstrate that samples with calcitonin concentrations above the measuring range and up to 200,000 pg/mL can be diluted with Diluent MultiAssay; the recommended dilution is 1:100.

3. Analytical Specificity/Interference:

Endogenous interference
Potential interference from endogenous substances was evaluated per CLSI EP07 Ed 03. For each potential interferent, samples with calcitonin concentration at around 8, 1000 and 1500 pg/mL were measured in the presence of interferent at 11 interferent concentrations and with control samples (with no interferent added). Percent recovery at each concentration was calculated as the mean value of 5 replicates at that concentration compared to the control sample. The endogenous interference effects on analyte quantitation are summarized in the table below:

Endogenous Substances

Substance	**Highest concentration tested that did not cause significant interference**
Bilirubin	66 mg/dL
Hemoglobin	200 mg/dL
Intralipid	2,000 mg/dL
Biotin	1,200 ng/mL
Rheumatoid factor	1,200 IU/mL
IgG	4 g/dL
IgA	0.7 g/dL
IgM	0.4 g/dL

Exogenous interference
Potential interference from exogenous substances was evaluated per CLSI EP07 Ed 03. To assess drug interference on the measurement of calcitonin, potentially interfering drugs were spiked into two human serum sample pools and tested with the Elecsys Calcitonin assay on

the cobas e 411 analyzer. The analyte concentration of the sample pools were approximately 10 and 500 pg/mL Calcitonin (spiked). Each sample was measured in 5-fold determination. The mean % Recovery and absolute deviation were calculated when comparing the drug spiked portions to control samples (with no interferent added). At the following concentrations, the percent difference between the results of the spiked samples compared to the results of the control samples was less than ± 10.0%. The results are summarized in the table below:

Drug	Highest concentration tested that did not cause significant interference
Acetaminophen	156 mg/L
Acetylcysteine	150 mg/L
Acetylsalicylic Acid	30 mg/L
Ampicillin-Na	75 mg/L
Ascorbic acid	52.5 mg/L
Cyclosporine	1.8 mg/L
Cefoxitin	750 mg/L
Doxycycline	18 mg/L
Heparin	3300 IU/L
Ibuprofen	219 mg/L
Itraconazol	20 mg/L
Levodopa	7.5 mg/L
Methyldopa +1.5	22.5 mg/L
Metronidazole	123 mg/L
Phenylbutazone	321 mg/L
Rifampicin	48 mg/L
Theophylline	60 mg/L

Special Thyroid Drugs	Highest concentration tested that did not cause significant interference
Iodide	100 mg/L
Levothyroxine	1.0 mg/L
Carbimazole	30 mg/L
Thiamazole	80 mg/L
Propylthiouracil	60 mg/L
Perchlorate	2000 mg/L
Propranolol	240 mg/L
Amiodarone	200 mg/L
Prednisolone	100 mg/L
Hydrocortisone	200 mg/L
Fluocortolone	100 mg/L

Special Thyroid Drugs	Highest concentration tested that did not cause significant interference
Octreotide	0.3 mg/L

Cross-Reactivity
A cross-reactivity study was conducted to evaluate the potential cross-reactivity of the assay. The potential cross-reactants were added at defined concentrations to a single native human serum pool sample with an approximate Calcitonin concentration of 8 pg/mL and analyzed with the Elecsys Calcitonin on the cobas e 411 analyzer. Samples were measured in the presence and absence of the potential cross-reactants and cross-reactivity (%) was calculated using the following equation: *% Cross Reactivity = 100 x (Average "spike" concentration – Average "blank concentration) / Spike concentration of cross reactant*

Results are summarized below.

Cross-Reactant	Concentration Cross-Reactant [ng/mL]	Cross reactivity [%]
Salmon Calcitonin	200	0.000
Porcine Calcitonin	1000	0.000
Chicken Calcitonin	1000	0.000
ACTH (1-39) human	200	0.000
C-Peptide	80000	0.000
Calcitonin Gene Related Peptide	2000	0.000
PTH (1-84) human	300	0.000
TSH	2000 µIU/mL	0.000
Insulin	67000	0.000
Prolactin	2000	0.000
Gastrin I	4000	0.000
Elcatonin	200000	0.000
Katacalcin	80000	0.000

HAMA
The effect of the presence of human anti-mouse antibodies on the Elecsys Calcitonin was assessed on the cobas e 411 analyzer. Human serum samples were tested in two-fold determinations. Each sample was divided, with one part used as the control sample and the other part was spiked with HAMA. Testing with HAMA levels up to a concentration of 81 ng/mL showed < ±10 % bias. Testing with HAMA levels of 805 ng/mL, at calcitonin concentrations above 18 pg/mL, showed negative biases up to 20%.

The labeling includes the following limitations:
For assays using antibodies, the possibility exists for interference by heterophile antibodies in the patient's sample. Patients who have been regularly exposed to animals or have received murine-derived immunotherapy or diagnostic procedures using murine immunoglobulin or

immunoglobulin fragments may produce antibodies (e.g. human anti-mouse antibodies, or HAMA) that can interfere with immunoassays. The Elecsys Calcitonin assay is affected by HAMA interference at high HAMA levels. Internal Study data show, for HAMA levels of 805 ng/mL, at calcitonin concentrations above 18 pg/mL, negative biases up to 20 % have been observed. Less than ±10 % bias was observed with HAMA levels of 81 ng/mL. In rare cases when samples contain high levels of HAMA, a negative bias may be observed. The interference is most critical in patients with distant metastasis where HAMA interference may impact clinical decision-making regarding additional patient management / treatment. In cases where interference is suspected, carefully evaluate the results and laboratories should follow their validated process for identifying HAMA interference.

High Dose Hook Effect
High-dose hook effect of the Elecsys Calcitonin was assessed on the cobas e 411 analyzer. Three high concentration spiked serum samples were used to prepare a dilution series. The hook-samples were diluted with Diluent MultiAssay to create an 11 member sample panel with concentrations ranging from 0.1 pg/mL to >1,000,000 pg/mL. No hook effect was observed at concentrations up to 1,000,000 pg/mL (1 µg/mL).

4. Assay Reportable Range:

   1-2,000 pg/mL

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

   Traceable to the WHO 2nd IRP 89/620 reference standard.

   The sponsor has provided information to support the following claims: The reagent kit can be stored on-board the analyzer for up to 28 days. A new calibration of the kit kept on-board is recommended every 7 days. Information was also provided to support all claimed calibration recommendations.

6. Detection Limit:
   The limit of blank (LoB), limit of detection (LoD), and limit of quantification (LoQ) were established following the recommendations in the CLSI EP17-A2 guideline.

   Limit of Blank (LoB)
   The LoB study was performed on 1 blank sample measured in 10 replicates per run, 1 run per day, 6 days using 3 reagent lots and 1 cobas e 411 analyzer. The LoB was determined as the 95th percentile of the measurements of blank samples. The LoB for the Elecsys Calcitonin assay was estimated to be 0.3 pg/mL.

   Limit of Detection (LoD)
   The LoD study was performed on 5 low-level native human serum samples measured in 2 replicates per run, 1 run per day, 6 days using 3 reagent lots and 1 cobas e 411 analyzer. The $LoD = LoB + 1.653 \times SD_{total}$. The LoD for the Elecsys Calcitonin assay was estimated to be 0.5 pg/mL.

Limit of Quantification (LoQ)
The LoQ study was performed on 10 low-level native human serum samples, each sample measured in 2 replicates, 1 run per day, 6 days of testing using 3 reagent lots. The LoQ is determined as the lowest concentration of analyte that can be reproducibly measured with a total error of ≤ 20%. The LoQ for the Elecsys Calcitonin assay was estimated to be 1.0 pg/mL.

7. Assay Cut-Off:

Not applicable

B Comparison Studies:

1. Method Comparison with Predicate Device:
A method comparison study of the candidate, Elecsys Calcitonin assay versus the predicate device, Elecsys Calcitonin (k132828), was performed. The study was conducted on one cobas e 411 analyzer according to CLSI Guideline EP09c, 3rd Edition. 120 serum samples were tested including native samples and native sample pools, and some samples (10%) spiked with recombinant hCT to cover the measuring interval. Calcitonin concentrations ranged from 1.07-1817 pg/mL. Passing-Bablok analysis was used to analyze the data.

Method Comparison Summary
Elecsys Calcitonin (candidate) vs Elecsys Calcitonin (predicate) in pg/mL:

N	Concentration Range	Intercept (95% CI)	Slope (95% CI)	r
120	1.07-1817	0.318 (0.096, 0.655)	0.983 (0.972, 1.013)	0.996

2. Matrix Comparison:
The effect of anticoagulants on the measurement of calcitonin with the candidate device was assessed by comparing values obtained from serum, lithium heparin plasma, k2-EDTA plasma, K3-EDTA plasma, and tubes with separating gel for both serum (SST) and plasma (Li-PST). At least 40 sample pairs were tested in singleton with one reagent lot on one cobas e 411 analyzer. Passing/Bablok regression analysis was performed. Results are summarized below.

Serum vs	n	Slope	Intercept	Range (pg/mL)	r
Lithium Heparin	45	1.027	0.12	1.18-1880	0.998
Li-Heparin PST	47	1.009	0.002	1.18-1897	0.999
K2-EDTA	47	0.957	0.019	1.18-1897	0.999
K3-EDTA	46	0.940	0.099	1.18-1897	0.999
Serum SST	49	0.980	-0.035	1.18-1897	1.000

The data support the package insert claim that serum, Li-Heparin, K2-EDTA and K3-EDTA plasma and tubes with separating gel for both serum (SST) and plasma (Li-PST) are acceptable sample types for use with the Elecsys Calcitonin immunoassay.

C Clinical Studies:

1. Clinical Sensitivity:

   Not applicable

2. Clinical Specificity:

   Not applicable

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

   Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

Previously established in K132828.

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.