510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:
k132828

B. Purpose for Submission:
New Device

C. Measurand:
Calcitonin

D. Type of Test:
Quantitative**,** Electrochemiluminescence Immunoassay (ECLIA)

E. Applicant:
Roche Diagnostics

F. Proprietary and Established Names:
Elecsys Calcitonin Immunoassay
Elecsys Calcitonin CalSet
Elecsys Calcitonin CalCheck5
Elecsys PreciControl Varia

G. Regulatory Information:

1. Regulation section:
   21 CFR 862.1140, Radioimmunoassay, Calcitonin
   21 CFR 862.1150, Calibrator, Secondary
   21 CFR 862.1660, Quality control material

2. Classification:
   Class II
   Class II
   Class I, reserved

3. Product code:
   JKR
   JIT
   JJY

4. Panel:
   Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See Indication(s) for use

2. Indication(s) for use:
   Elecsys Calcitonin Immunoassay:
   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 the indicated Elecsys and cobas e immunoassay analyzers.

   Elecsys Calcitonin CalSet:
   Calcitonin CalSet is used for calibrating the quantitative Elecsys Calcitonin assay on the Elecsys and **cobas e** immunoassay analyzers.

   Elecsys Calcitonin CalCheck 5:
   The Elecsys CalCheck 5 is an assayed control for use in calibration verification and for use in the verification of the calcitonin assay range established by the Elecsys and cobas e immunoassay analyzers.

   Elecsys PreciControl Varia:
   The Elecsys PreciControl Varia is used for quality control of the Elecsys immunoassays on Elecsys and cobas e immunoassay analyzers.

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

4. Special instrument requirements:

   cobas e 411 Immunoassay Analyzer

I. Device Description:

The Elecsys Calcitonin Reagent kit consists of 3working solutions:

M- Streptavidin-coated microparticles (0.72 mg/mL) and preservative.
R1- Anti-hCT-Ab~biotin: Biotinylated monoclononal anti-hCT antibody (mouse), phosphate buffer and preservative.

R2- Anti-hCT-Ab~Ru(bpy): monoclonal anti-hCT antibody (mouse) labeled with ruthenium complex; phosphate buffer, and preservative.

The Elecsys Calcitonin CalSet consists of 2 levels, hCT Cal 1 and hCT Cal 2. The Calcitonin calibrator set contains lyophilized calcitonin (synthetic) in two concentration ranges (approximately 2.0 pg/mL and 500 pg/mL) in an equine serum matrix.

The Elecsys Calcitonin CalCheck 5: Each set contains 5 lyophilized levels of human calcitonin (synthetic) in buffered equine serum. The approximate target range for each level is listed below:

Check 1 ≤1.00 pg/mL
Check 2 34.5-65.5 pg/mL
Check 3 345-655 pg/mL
Check 4 690-1310 pg/mL
Check 5 1380->2000 pg/mL

PreciControl Varia is a multi-composite lyophilized 3 level control set based on human source material. The PreciControl V1 and V2 level contains lyophilized calcitonin (synthetic) in two concentration ranges (approximately 10 pg/mL and 100 pg/mL). The PreciControl V0 does not contain any calcitonin.

All products derived from human blood are prepared exclusively from the blood of donors tested individually by FDA approved methods and shown to be free from HbsAg and antibodies to HCV and HIV.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   Immulite 1000 Calcitonin
   Elecsys Vitamin D CalSet
   Elecsys T4 CalCheck 5
   Elecsys PreciControl Varia

2. Predicate 510(k) number(s):
   k023304
   k113546
   k112528
   k111506

3. Comparison with predicate:

Assay Reagents

Similarities and Differences		
Item	Device Elecsys Calcitonin Immunoassay	Predicate Immulite 1000 Calcitonin (k023304)
Intended Use	Immunoassay for the in vitro quantitative determination of human calcitonin (thyrocalcitonin) in serum and plasma.	Same
Traceability	Standardized against the WHO 2nd IRP 89/620	Same
	Electrochemiluminescent Immunoassay	Chemiluminescent Immunometric assay
Reaction time	18 minutes	60 minutes
Sample type	Human serum and plasma treated with K2-EDTA, K3-EDTA, lithium heparin and lithium heparin plasma gel separation tubes.	Human serum and heparinized plasma
Sample volume	50 µL	75 µL
Calibration interval	2 months when using the same reagent lot. 7 days when using the same reagent kit on the analyzer.	4 weeks
Reagent Stability	Unopened: 2-8°C - Up to the stated expiration date Opened 2-8°C - 12 weeks On Analyzers – 4 weeks	Stable at 2-8ºC until expiration date
Analytical Sensitivity	1 pg/mL	2 pg/mL

CalSet (calibrator)

Similarities and Differences		
Item	Device Elecsys Calcitonin CalSet	Predicate Elecsys Vitamin D CalSet (k133546)
Intended Use	For calibrating the assay on the Elecsys and cobas e immunoassay analyzers.	Same
Levels	Two	Same
Format	Lyophilized	Same

Similarities and Differences		
Item	Device Elecsys Calcitonin CalSet	Predicate Elecsys Vitamin D CalSet (k133546)
Matrix	Buffered (50 mmol/L HEPES) equine serum	Human serum
Stability	Unopened: up to the stated expiration date After reconstituting: At 2-8°C – 72 hours At -20°C – 84 days (freeze only once) At 20-25°C: up to 5 hours	Unopened: up to the stated expiration date After reconstituting: At 2-8C – 120 hours At -20C – 90 days (freeze only once) On Elecsys 2010/**cobas e** 411 at 20-25°C: up to 5 hours

CalCheck (**Calibration Verifiers)**

Similarities and Differences		
Item	Device Elecsys Calcitonin CalCheck 5	Predicate Elecsys T4 CalCheck5 (k112528)
Intended Use	It is an assayed control for use in calibration verification and for use in the verification of the assay range.	Same
Levels	Five	Same
Format	Lyophilized	Same
Matrix	Buffered (50 mmol/L HEPES) equine serum	Level 1: BSA Level 2-5:Human serum
Stability	Unopened: store at 2-8°C up to the stated expiration date After reconstituting: At 20-25°C: up to 4 hours	Unopened: store at 2-8°C up to the stated expiration date After reconstituting: At 15-25°C : up to 5 hours

Controls (PreciControl Varia)

Similarities and Differences		
Item	Device Elecsys PreciControl Varia	Predicate Elecsys PreciControl Varia (k111506)
Intended Use	It is used for quality control of Elecsys immunoassays on the Elecsys and cobas e immunoassay analyzers	Same
Volume (reconstituted)	3.0 mL	Same
Stability	Unopened: store at 2-8°C up to the stated expiration date After reconstituting: At -20°C: 31 days At 2-8°C: 72 hours At 20-25°C: on board the analyzer: up to 5 hours	Same
Calcitonin Concentration	Level 1: 10 pg/mL Level 2: 100 pg/mL	No calcitonin concentration present

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

CLSI EP5-A2 Evaluation of Precision of Clinical Chemistry Devices; Approved Guideline 2nd Edition
CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline, 1st Edition
CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline, 1st Edition
CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline 2nd Edition
CLSI EP09-A2-IR Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, 2nd Edition

L. Test Principle:

The Roche Calcitonin Immunoassay is an Electrochemiluminescence Immunoassay (ECLIA) which is based on the sandwich principle. During the first 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.
During the second incubation, streptavidin-coated microparticles are added and form a complex which 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision studies were performed on one cobas e 411 Immunoassay analyzer with one reagent lot. The samples consisted of PreciControl Varia and human sera pools. Two replicates of each control and human sera were run per day for 21 days for a total of 84 replicates. Unaltered serum sample pools were used in addition to spiked and diluted pools in order to obtain samples to cover the measuring range. CLSI EP5-A2 guidelines were followed. The precision results are summarized in the table below:

			Within-run		**Total precision**	
Sample	n	Mean (pg/mL)	SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)
Human serum 1	84	1.21	0.034	2.8	0.043	3.6
Human serum 2	84	11.5	0.345	3.0	0.413	3.6
Human serum 3	84	48.5	1.24	2.5	1.71	3.5
Human serum 4	84	482	13.8	2.9	19.2	4.0
Human serum 5	84	1910	42.6	2.2	65.0	3.4
PC Varia 1	84	8.88	0.191	2.1	0.261	2.9
PC Varia 2	84	97.7	1.44	1.5	2.51	2.6

Reproducibility of the Elecsys Calcitonin assay was evaluated over three sites using three different cobas e 411 Immunoassay analyzers based on CLSI EP5-A2 guidelines. Sources of variability included testing for 21 days, 2 runs per day, with 2 replicates for each panel member per run at three sites. One reagent lot was evaluated. Two controls (PCV = PreciControl Varia, Level 1 & 2) and five human serum pools (HSP) were tested. The human serum pools used were native as well as spiked and diluted.

The following precision study result summary is from one site and is representative of the results obtained from all 3 sites.

Sample	Mean (pg/mL)	Within-run		Total precision		n
		SD (pg/mL)	CV (%)	SD (pg/mL)	CV (%)	
HSP01	1.11	0.0377	3.4	0.0596	5.4	84
HSP02	12.7	0.387	3.0	0.464	3.7	84
HSP03	52.6	1.32	2.5	1.87	3.6	84
HSP04	514	17.0	3.3	18.6	3.6	84
HSP05	1992	50.6	2.5	66.7	3.3	84
PCV L1	9.76	0.204	2.1	0.307	3.1	84
PCV L2	106	1.82	1.7	2.74	2.6	84

Summary of Reproducibility study over 3 sites

Sample	Repeatability		Between-run		Between-day		Between-lab		Total	
Mean pg/mL	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
HSP1 1.06	0.03	3.1	0.03	2.6	0.03	3.3	0.04	3.8	0.07	6.5
HSP2 12.4	0.27	2.2	0.14	1.1	0.22	1.8	0.26	2.1	0.46	3.7
HSP3 51.2	0.99	1.9	0.85	1.7	0.74	1.4	1.19	2.3	1.92	3.7
HSP4 500	12.0	2.4	4.89	1.0	8.2	1.6	11.8	2.4	19.4	3.9
HSP5 1924	36.8	1.9	31.2	1.6	24.7	1.3	58.7	3.1	79.9	4.2
PCV Ll 9.32	0.15	1.6	0.12	1.3	0.19	2.0	0.39	4.1	0.47	5.1
PCV L2 101	1.48	1.5	1.38	1.4	1.6	1.6	4.21	4.2	4.94	4.9

b. Linearity/assay reportable range:

Linearity of the Elecsys Calcitonin assay was assessed on the cobas e 411 Immunoassay analyzer according to CLSI EP6-A. A high analyte serum sample pool (spiked) was diluted with a low analyte serum sample pool. Twenty-one concentrations throughout the measuring range were prepared. Samples were assayed in 3-fold determination within a single run. Samples tested range between 0.28 to 2186.5 pg/mL.

The calcitonin linearity study resulted in a polynomial regression in which the 2nd order polynomial regression was considered significant and yielded a best fit square linearity equation of y=0.9707x+ 0.0617.

The linearity study supports the sponsor's claimed measuring range: 1.0 – 2000 pg/mL.

Hook Effect: To evaluate the hook effect, two human serum samples were spiked with analyte concentration up to 1,200,000 pg/mL. The samples were assessed on the cobas e 411. It was determined that here is no high-dose hook effect at calcitonin concentrations up to 1,000,000 pg/mL.

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

Traceability:

The Elecsys Calcitonin CalSet, Elecsys Calcitonin CalCheck 5, and the calcitonin component of the PreciControl Varia are traceable to the WHO 2nd IRP 89/620 Reference Standard.

Elecsys Calcitonin CalSet Stability: Real-time and accelerated stability studies were performed to determine the Elecsys Calcitonin CalSet stability. The study protocol and acceptance criteria were reviewed and found to be acceptable. The Elecsys Calcitonin CalSet stability studies support the following stability claims:
Unopened: up to 12 months when stored at 2-8°C.
After reconstituting: At 2-8°C, 72 hours; at -20°C, 84 days, at 20-25°C: up to 5 hours.

Elecsys Calcitonin CalCheck5 Stability: Real-time and accelerated stability studies were performed to determine Elecsys Calcitonin CalCheck 5 stability. The study protocol and acceptance criteria were reviewed and found to be acceptable. The Elecsys Calcitonin CalCheck 5 stability studies support the following stability claims:
Unopened: 12 months stored at 2-8°C.
Reconstituted: 20-25°C: up to 4 hours

Elecsys PreciControl Varia Stability: Real-time and accelerated stability studies were performed to determine Elecsys Calcitonin CalCheck 5 stability. The study protocol and acceptance criteria were reviewed and found to be acceptable. The Elecsys PreciControl Varia stability studies support the following stability claims: Unopened at 2 – 8 ºC ,up to 15 months; reconstituted/thawed serum at -20 ºC, for 31 days (freeze only once); at 2 – 8 ºC, for 72 hours; and at 20 – 25 ºC on-board the analyzers, up to 5 hours.

Value assignment for ElecsysCalcitonin CalSet, Elecsys Calcitonin CalCheck 5, and PreciControl Varia:

Value assignment is determined by performing multiple runs using multiple analyzers and the mean results are calculated based on the multiple replicate results. The target values for the ElecsysCalcitonin CalSet, Elecsys Calcitonin CalCheck 5, and PreciControl Varia are listed in the tables below:

Target Values for Elecsys Calcitonin CalSet

Level	Calcitonin Target Value (pg/mL)	Calcitonin Target Range (pg/mL)
Calibrator 1	2.0	1.5 – 2.5
Calibrator 2	500	400 – 600

Target Values for the Elecsys Calcitonin CalCheck5

Level	Calcitonin Target Value (pg/mL)	Calcitonin Target Range (pg/mL)
Check 1	<1	-
Check 2	50	45-55
Check 3	500	450-550
Check 4	1000	900-1100
Check 5	2000	1800-2200

Target Values for Elecsys PreciControl Varia

Control Level	Calcitonin Target Value (pg/mL)	Target Range (pg/mL)
Level 1	10.0	8.9-12.1
Level 2	100	89-121

d. Detection limit

Limit of Blank (LoB) was determined using three reagent lots on two cobas e 411 analyzers, measuring five zero-level human serum samples during three days, two runs per day for a total of 60 replicates per sample per reagent lot. The LoB was determined as the 95th percentile of measurement of blank samples.

Limit of Detection (LoD) was determined using three reagent lots on two cobas e 411 analyzers, measuring five low-level human serum samples during three days, two runs per day for a total of 60 replicates per sample per reagent lot. The LoD was determined as the lowest amount of analyte in a sample that can be detected with 95% probability.

Limit of Quantitation (LoQ) was determined using three reagent lots on two cobas e 411 analyzers, measuring nine low-level human serum samples during three days, two runs per day for a total of 60 replicates per sample per reagent lot. The LoQ was derived from a plot of the allowable error versus the expected concentration of Calcitonin at a total error of 20%.

The detection limits are summarized in the table below:

Elecsys Calcitonin Immunoassay

Limit of Blank (LoB)	Limit of Detection (LoD)	Limit of Quantitation (LoQ)
0.3 pg/mL	0.5 pg/mL	1.0 pg/mL

e. Analytical specificity:

Cross Reactivity: The specificity of the Elecsys Calcitonin assay was determined using two human serum sample pools spiked with potential cross-reactant compounds. The sample analyte concentrations were approximately 10 and 500 pg/mL calcitonin. The spiked and non-spiked samples were tested in duplicate on the cobas e 411 Immunoassay analyzer. The cross-reactivity results are summarized in the table below:

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Cross reactant	Max. concentration tested (ng/mL)	Highest cross-reactivity observed (%)
Salmon Calcitonin	200	0.017
Porcine Calcitonin	1000	0.007
Chicken Calcitonin	1000	0.005
ACTH (1-39) human	200	0.037
C-Peptide	80000	0.000
Calcitonin Gene Related Peptide	2000	0.002
PTH (1-84)	300	0.013
TSH	2000µIU/mL	0.009
Insulin	67000	0.000
Prolactin	2000	0.001
Gastrin I	4000	0.001
Elcatonin	200000	0.000
Katacalcin	80000	0.000

Endogenous Interference: The effect on quantitation of analyte in the presence of endogenous interfering substances using the Elecsys Calcitonin was determined on the cobas e 411 immunoassay analyzer for the following 8 interfering substances: Hemoglobin, Biotin, Lipemia, Bilirubin, Rheumatoid Factor, IgG, IgA, and IgM. For each interfering substance, three spiked human serum samples (one low, one medium, and one high concentrations of Calcitonin) were used to prepare dilution series of 11 dilutions that were tested in duplicate. The sponsor defined significant interference as greater than ± 10% of the unspiked (reference) value. The endogenous interference effects on analyte quantitation are summarized in the table below:

Substance	Highest Concentration at which no significant interference was observed.
Hemoglobin	200 mg/dL
Biotin	40 ng/dL
Intralipid (lipemia)	2000 mg/dL
Bilirubin	66 mg/dL
Rheumatoid Factor	1200 IU/mL
Human IgG	4 g/dL
Human IgM	0.7 g/dL
Human IgA	1.6 g/dL

The sponsor states the following limitation in the labeling:

"Samples showing visible signs of hemolysis may cause interference. Avoid analyzing samples with hemogloblin concentrations >0.2 g/L."

"Samples should not be taken from patients receiving therapy with high biotin doses (i.e. >5 mg/day) until at least 8 hours following the last biotin administration."

HAMA Effect: The effect of the presence of human anti-mouse antibodies on the Elecsys Calcitonin assay was assessed on the **cobas e** 411 Immunoassay Analyzer. A high HAMA human serum pool 805 µg/L (0.805 µg/mL) was spiked with analyte to yield calcitonin concentrations of approx. 10 and 500 pg/mL. The high HAMA serum pool was diluted in 11 dilution series with a serum pool without HAMA. The expected analyte concentrations were calculated and compared with the observed concentrations for each diluted sample. The sponsor defined significant interference of HAMA as greater than ±10% of the expected concentrations. All results were within ±10% of the expected concentrations.

The sponsor states the following limitation in the labeling:

"Samples from patients routinely exposed to animal or animal serum products may contain heterophilic antibodies causing an atypical result. This assay has been formulated to mitigate the risk of this type of interference. However, potential interactions between rare sera and test components can occur."

Drug Interference: To assess drug interference on the measurement of calcitonin, 17 pharmaceutical compounds were spiked into two human serum sample pools and tested with the Elecsys Calcitonin assay on the **cobas e** 411 Immunoassay analyzer. The analyte concentration of the sample pools were approx. 10 and 500 pg/mL Calcitonin (spiked). The sponsor defines significant interference as greater than ± 10% of the reference value (unspiked sample). The drug interference results are summarized in the table below:

Drug	Highest concentration of drug tested which showed no significant interference (mg/L)	Drug	Highest concentration of drug tested which showed no significant interference (mg/L)
Acetylcystein	1700	Levothyroxine	1
Ampicillin-Na	1000	Carbimazol	30
Ascorbic acid	300	Thiamazol	80
Cyclosporine	5	Propylthiouracil	60
Cefoxitin	2500	Sodium Perchlorate	2000
Heparin	5000 U	Propranolol	240
Intralipid	10000	Amiodaron	200
Levodopa	20	Prednisolone	100
Methyldopa +1.5	20	Hydrocortisone	200
Metronidazole	200	Fluocortolon	100
Phenylbutazone	400	Octreotid	0.3
Doxycycline	50	Iodide	400
Acetylsalicylic acid	1000	Theophylline	100
Rifampicin	65	Ibuprofen	500
Acetaminophen	200		

f. Assay cut-off:
Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed comparing the Siemens Immulite 1000 Calcitonin assay run on the Immulite 1000 analyzer (predicate device) to the Elecsys Calcitonin assay (candidate device) run on the cobas e 411 analyzer. A total of 150 human serum samples were measured in singlicate over a minimum of 5 days. Of those samples, 19 (12.7%) were altered in order to cover the entire measuring range. Calcitonin values ranged between 1.61 and 1936 pg/mL for the samples tested. Results of the Deming Regression analysis are presented below:

Deming Regression: y= 0.998x – 3.05, r=0.991

95% CI of the slope: 0.967/1.03

95% CI of the intercept: -0.452/-0.159

b. Matrix comparison:

The effect on quantitation of calcitonin in the presence of anticoagulants with the Elecsys Calcitonin Immunoassay was determined by comparing values obtained from samples drawn into Li-Heparin tubes with and without serum separator gel, K_2 EDTA and K_3 EDTA plasma tubes. Fifty serum/plasma pairs per sample material were tested in singleton with one reagent lot on one cobas e 411 Immunoassay analyzer. The Passing/Bablok regression analysis results are summarized below:

Serum vs	n	Slope	Intercept	Range (pg/mL)
Lithium Heparin	53	0.951	0.282	1.65-1911
Li-Heparin Gel Separation	54	0.955	0.176	1.65-1911
K2-EDTA	52	0.956	0.129	1.65-1911
K3-EDTA	53	0.958	0.080	1.65-1911

The resulting data support the package insert claim that serum, Li-Heparin plasma from tubes with and without gel separation, K_2-EDTA plasma, and K_3- EDTA-plasma are acceptable sample types for use with the Elecsys Calcitonin Immunoassay.

3. Clinical studies:

a. Clinical Sensitivity:
Not applicable

b. Clinical specificity:
Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):
Not applicable

4. Clinical cut-off:
Not applicable

5. Expected values/Reference range:

Reference ranges for apparently health males (n=184) and apparently healthy females (n=180) were determined using the 97.5th percentiles as upper limit of normal. The evaluation was done at three different sites with two reagent lots using three cobas e 411 analyzers. The reference range study result summary is presented below:

Cohort	N	97.5th percentile	Lower limit of 95% CI	Upper limit of 95% CI
Apparently healthy females	180	7.63 pg/mL	6.10 pg/mL	12.7 pg/mL
Apparently healthy males	184	14.3 pg/mL	10.4pg/mL	18.0 pg/mL

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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