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

A. 510(k) Number:

k162840

B. Purpose for Submission:

New Device

C. Measurand:

Total 25-hydroxyvitamin D (25-OH vitamin D)

D. Type of Test:

Quantitative electrochemiluminescence immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Vitamin D total II

Vitamin D total II CalSet

PreciControl Vitamin D total II

Vitamin D CalCheck

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1825, Vitamin D Test System

   21 CFR 862.1150, Calibrator

   21 CFR 862.1660, Quality Control Material (assayed and unassayed)

2. Classification:

   Class II

   Class II

   Class I, reserved

3. Product code:

   MRG – Vitamin D Test System

   JIT – Calibrator, secondary

   JJX – Quality Control Material

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   Elecsys Vitamin D total II

   The Elecsys Vitamin D total II assay is intended for the quantitative determination of total 25-hydroxyvitamin D in human serum and plasma. This assay is to be used as an aid in the assessment of vitamin D sufficiency in adults. The electrochemiluminescence binding assay is intended for use on the cobas e 411 immunoassay analyzer.

   CalSet Vitamin D total II

   CalSet Vitamin D total II is used for calibrating the quantitative Elecsys Vitamin D total II assay on the cobas e 411 immunoassay analyzer.

   PreciControl Vitamin D total II

   PreciControl Vitamin D total II is used for quality control of Elecsys Vitamin D total II assay on cobas e 411 immunoassay analyzer.

CalCheck Vitamin D total II

This CalCheck set is an assayed control for use in calibration verification and for use in the verification of the assay range established by the Elecsys Vitamin D total II reagent on the cobas e 411 immunoassay analyzer.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Roche Cobas e 411 analyzer

I. Device Description:

1. The Elecsys Vitamin D total II reagent working solutions include:

- PT1 Pretreatment reagent 1 (white cap), 1 bottle, 4 mL: Dithiothreitol 1 g/L, pH 5.5.
- PT2 Pretreatment reagent 2 (gray cap), 1 bottle, 4 mL: Sodium hydroxide 28 g/L.
- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Vitamin D binding protein‑Ru/(bpy) (gray cap), 1 bottle, 6.5 mL: Ruthenium labeled vitamin D binding protein 100 µg/L; bis‑tris propane buffer 100 mmol/L; albumin (human) 40 g/L; pH 6.4; preservative.
- R2 25‑hydroxyvitamin D~biotin (black cap), 1 bottle, 6.5 mL: Biotinylated 25‑hydroxyvitamin D 140 µg/L; bis‑tris propane buffer 100 mmol/L; pH 8.6; preservative.

2. Calibrator:

CalSet Vitamin D total II is a two concentration level set of lyophilized human serum . The CalSet includes Cal 1 (approximately 2 ng/mL 25-hydroxyvitamin D_3) and Cal 2 (approximately 45 ng/mL 25-hydroxyvitamin D_3).

3. Control:

PreciControl Vitamin D total II materials are lyophilized human sera in two concentration ranges. Controls 1 and 2 (CTL 1 & CTL 2) are available in 1.0 mL bottles with two defined concentrations of total 25-hydroxy vitamin D The target concentrations are approximately 13 ng/mL and 30 ng/mL.

4. Calibration Verification Materials:

Each set of CalCheck Vitamin D total II contains 5 lyophilized serum levels (1.0 mL).
- Check 1: Approximate target range of ≤ 2 ng/mL

- Check 2: Approximate target range of 17.5 – 22.4 ng/mL
- Check 3: Approximate target range of 46.5 – 54.4 ng/mL
- Check 4: Approximate target range of 75.5 – 84.4 ng/mL
- Check 5: Approximate target range of 94.5 – > 100 ng/mL

The labeling for above materials that contain human blood or serum matrices include the following statement:

All human material should be considered potentially infectious. All products derived from human blood are prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Elecsys Vitamin D Assay

2. Predicate 510(k) number(s):

   k113546

3. Comparison with predicate:

Similarities and Differences for Assay		
Item	Candidate Device Elecsys Vitamin D total II k162840	Predicate device Elecsys Vitamin D Assay k113546
Intended Use	This assay is intended for the quantitative determination of total 25-hydroxyvitamin D in human serum and plasma. This assay is to be used as an aid in the assessment of vitamin D sufficiency in adults.	Same
Assay Method	Competition principle binding protein	Same
Detection Method	Electrochemiluminescence	Same
Applications/Test Time	27 minutes	Same
Calibration Method	2-point calibration based on master curve for specific reagent lot	Same
LoB, LoD, LoQ	2 ng/mL, 3 ng/mL, 5 ng/mL	Same
Instrument Platform	cobas e 411	Elecsys 2010, MODULAR

Similarities and Differences for Assay		
Item	Candidate Device Elecsys Vitamin D total II k162840	Predicate device Elecsys Vitamin D Assay k113546
		ANALYTICS E170, cobas e 411, cobas e 601, and cobas e 602
Sample Type	Serum and Li-heparin, K2-EDTA, K3-EDTA, Plasma Gel Seperation tubes (Li-Heparin)	Serum and Li-heparin, K2-EDTA, K3-EDTA, Plasma Gel Seperation tubes (Li-Heparin)
Traceability	ID-LC-MS/MS traceable to NIST RMP 2972	LC-MS/MS
Measuring Range	5 – 100.0 ng/mL	5 – 60.0 ng/mL

Similarities and Differences for Calibrator		
Item	Candidate Device CalSet Vitamin D total II k162840	Predicate device Vitamin D CalSet k113546
Intended Use/	CalSet Vitamin D total II is used for calibrating the quantitative Elecsys Vitamin D total II	Same
Analyte	25-hydroxyvitamin D3	25-hydroxyvitamin D
Matrix	Human serum matrix with added 25-hydroxyvitamin D3	Human serum matrix with added 25-hydroxyvitamin D
Target Ranges	Cal 1: approximately 2 ng/mL Cal 2: approximately 45 ng/mL	Cal 1: approximately 2 ng/mL Cal 2: approximately 37 ng/mL
Storage and Stability	Reconstituted calibrators: · At 2-8°C – 72 hours · At -20°C – 12 weeks (freeze only once) · On the cobas e 411 analyzer – up to 6 hours	Reconstituted calibrators: · At 2-8°C – 120 hours · At -20°C – 90 days (freeze only once) · On the cobas e 411 analyzer – up to 5 hours
Calibration Interval	Calibration must be performed once per reagent lot using fresh reagent (< 24 hours since registered). Renewed calibration: · After 3 months (12 weeks) using the same reagent lot · After 7 days (when using	Calibration must be performed once per reagent lot using fresh reagent (< 24 hours since registered). Renewed calibration: · After 1 month (28 days) using the same reagent lot · After 7 days (when using

Similarities and Differences for Calibrator		
Item	Candidate Device CalSet Vitamin D total II k162840	Predicate device Vitamin D CalSet k113546
	the same reagent kit on the analyzer) · As required e.g. quality control findings outside the defined limits	the same reagent kit on the analyzer) · As required e.g. quality control findings outside the defined limits
Format	Lyophilized	Same

Similarities and Differences for Control		
Item	Candidate Device PreciControl Vitamin D total II k162840	Predicate device PreciControl Varia k113546
Intended Use	PreciControl Vitamin D total II is used for quality control of the Elecsys Vitamin D total II assay	Same
Analyte	25-hydroxyvitamin D	Vitamin B12, Ferritin, Folate β-CTx, Osteocalcin, Parathyroid hormone 25-hydroxyvitamin D, Calcitonin
Matrix	Human serum matrix	Same
25-hydroxy Vitamin D Target Value	Target range: Level 1: Approx. 13 ng/mL Level 2: Approx. 30 ng/mL	Target range: Level 0: Approx. 12.8 ng/mL Level 1: Approx. 17 ng/mL Level 2: Approx. 32 ng/mL
Storage and Stability	Reconstituted control serum: · At -20°C – 31 days (freeze only once) · At 2-8°C – 72 hours · At 20-25°C - up to 5 hours	Same
Format	Lyophilized	Same

Similarities and Differences for CalCheck		
Item	Candidate Device CalCheck Vitamin D total II k162840	Predicate device Elecsys Vitamin D CalCheck 5 k113546
Intended Use	CalCheck Vitamin D total II is an assayed control for use in calibration verification and for use in the verification of the assay range	Same
Target Ranges	Approximate target concentrations: · ≤ 5 ng/mL · 17.5 – 22.4 ng/mL · 46.5 – 54.4 ng/mL · 75.5 – 84.4 ng/mL · 94.5 – >100 ng/mL	Approximate target concentrations: · ≤ 5.00 ng/mL · 10.5 – 19.5 ng/mL · 21.0 – 39.0 ng/mL · 33.6 – > 60.0 ng/mL · 42.0 – > 60.0 ng/mL
Analyte	25-hydroxyvitamin D3	Same
Matrix	Human serum	Same
Storage and Stability	Reconstituted control serum: 5 hours at 20-25°C	Same
Format	Lyophilized	Same

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

CLSI EP5-A3: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Third Edition

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

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

CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition

L. Test Principle:

The Elecsys Vitamin D total II assay employs a competitive protein binding assay which uses Vitamin D Binding Protein for detection of 25-OH Vitamin D. The sample is treated with pretreatment reagent in the first incubation period. This releases vitamin D from the endogenous vitamin D binding protein present in the patient's sample. In the next incubation, vitamin D binding protein labeled with ruthenium is added and a complex is formed between

the 25-OH Vitamin D and the ruthenylated vitamin D binding protein. In the 3rd and final incubation, streptavidin-coated microparticles are added along with 25-OH Vitamin D labeled with biotin. Any unbound ruthenium labeled vitamin D binding proteins become occupied with biotin-labeled vitamin D (25-OH). The complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D (25-OH) becomes bound to the solid phase via interaction of the biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the electrochemiluminescence emission is detected. Results are determined using a calibration curve that is generated on each instrument by a 2 point calibration provided with the reagent bar code.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated on a single cobas e 411 analyzer according to CLSI guideline EP5-A3 using one reagent lot for evaluation. The protocol consisted of testing 2 replicates of two levels of control, PreciControl Vitamin D total II, and five human serum samples per run, 2 runs per day for 21 days (n=84). The samples were run in randomized order on the cobas e 411 analyzer. Serum samples used were all single donors (native as well as spiked).

Precision Results Summary:

			Within Run Precision		Total Precision	
	n	Mean (ng/mL)	SD (ng/mL)	CV%	SD (ng/mL)	CV%
Human Serum 1	84	11.1	0.725	6.6	0.965	8.7
Human Serum 2	84	20.8	0.849	4.1	1.09	5.2
Human Serum 3	84	25.6	0.774	3.0	1.43	5.6
Human Serum 4	84	47.5	0.749	1.6	1.77	3.7
Human Serum 5	84	92.6	1.76	1.9	2.40	2.6
PC Control 1	82	15.4	0.748	4.8	1.30	8.4
PC Control 2	84	29.1	1.04	3.6	1.56	5.4

b. Linearity/assay reportable range:

The linearity study on the Elecsys Vitamin D total II was performed according to CLSI guideline EP06-A. A linearity sample set was prepared by diluting a high 25-OH vitamin D concentration serum and K3-EDTA plasma sample (single donors, spiked) with a depleted serum/plasma sample with a low 25-OH vitamin D concentration to obtain 11 concentrations throughout the measuring range. Samples were assayed in triplicate within a single run and analyzed by linear regression.

The linearity test results are summarized below:

Serum linearity results:

Mean observed concentration (ng/mL)	Expected concentration(ng/mL)	Absolute Difference	% Difference
0.022	0.022	0.003	14.5
6.13	6.16	-0.671	-10.0
11.6	12.3	-0.954	-7.2
26.6	24.5	-0.622	-2.3
40.3	36.6	0.454	1.1
55.2	48.8	1.77	3.3
68.5	61.0	2.80	4.2
82.8	73.2	3.01	3.8
94.2	85.4	1.89	2.0
104	97.6	-1.10	-1.0
113	110	-6.58	-5.5

The linearity study has the following linear regression for serum samples:

$y = 1.085x - 0.005$, $R^2 = 0.9880$

K3 EDTA plasma linearity results:

Mean observed concentration (ng/mL)	Expected concentration(ng/mL	Absolute Difference	% Difference
0.814	0.814	0.007	0.9
6.09	6.27	-0.433	-6.5
11.1	11.7	-0.571	-4.6
24.5	22.7	-0.142	-0.6
37.6	33.6	0.884	2.4
50.7	44.5	2.10	4.4
63	55.4	3.11	5.2
74.6	66.3	3.51	4.9
85.5	77.2	2.89	3.5
95.1	88.2	0.829	0.9
103	99.1	-6.304	-2.9
110	110	-9.14	-7.7

Parameter of the linear regression for plasma samples:

$y = 1.078x - 0.011$, $R^2 = 0.9971$

The linearity study results support the claimed measuring range of 5.0 -100 ng/mL.

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

Standardization and Traceability

This method is in the process of being standardized in accordance with the Vitamin D Standardization Program (VDSP). The VDSP is an international collaborative effort to standardize the laboratory measurement of serum 25-OH vitamin D. This collaboration involves the coordinated efforts of the National Institutes of Health, Office of Dietary Supplements (ODS), the Centers for Disease Control and Prevention (CDC), the National Institutes for Standards and Technology (NIST), Ghent University, and other institutions. Please refer to http://ods.od.nih.gov/Research/VitaminD.aspx for more information on the VDSP program.

CalSet value assignment:

The CalSet Vitamin D total II assigned values are determined with the Elecsys Vitamin D total II assay. The CalSet Vitamin D total II is traceable to the ID-LC-MS/MS 25- hydroxyvitamin D Reference Measurement Procedure. The ID-LC-MS/MS is traceable to the National Institute of Standards and Technology Standard Reference Material 2972. A native human serum sample panel (single donors) with RMP-assigned target values (RMP = Reference Measurement Procedure: ID-LC-MS/MS of the University of Ghent) is used for Reference Standardization to assign values to the Master Calibrators (sample curve consisting of human serum samples from single donors covering the entire measuring range).

For each CalSet Vitamin D total II manufactured, the calibrators are run in duplicate on at least three (3) cobas e 411 analyzers and at least two (2) MODULAR ANALYTICS E170/cobas e 601/cobas e 602 analyzers with all Vitamin D total II reagent lots available. The assigned value of each calibrator is defined as the median value obtained over at least six (6) runs on at least three (3) analyzers of the respective calibrator.The assigned values for CalSet Vitamin D total II are read from the master calibration curve. The values must fall within specified acceptable ranges for each lot.

Control value assignment:

The PreciControl Vitamin D total II assigned values are determined with the Elecsys Vitamin D total II assay. The assigned values for PreciControl Vitamin D total II are read from the master calibration curve. Values are assigned for each lot of PreciControl Vitamin D total II in combination with each assay reagent lot available. The controls are run in duplicate on the MODULAR ANALYTICS E170 /cobas e 601/cobas e602 Analyzers. The assigned values obtained on the additional analyzer (cobas e 411) are compared to those obtained on the master analyzer platform (cobas e 601). The assigned value of each control level is defined as the median value obtained over at least six (6) determinations of the respective control level.

CalCheck value assignment:

For each CalCheck Vitamin D total II lot manufactured, the CalChecks are run in duplicate on at least two cobas analyzers with at least two runs. The assigned value of each CalCheck is defined as the median value obtained over at least six (6) determinations (runs) of the respective CalCheck and are read from the master calibration curve.

Stability:

The calibrator, CalCheck, and control shelf-life and open-vial stability testing protocols and acceptance criteria were reviewed and found to be acceptable as summary tables below:

CalSet Vitamin D total II	
Shelf life (lyophilized) at 2-8°C	15 months
Open vial (reconstituted) at -20°C	12 weeks (freeze only once)
Open vial (reconstituted) 2-8°C	72 hours
On board stability on cobas e 411 analyzer at 20-25°C	Up to 5 hours
On board stability on E170, cobas e 601, cobas e 602 analyzer at 20-25°C	Use only once

PreciControl Vitamin D total II	
Shelf life (lyophilized) at 2-8°C	15 months
Open vial (reconstituted) at -20°C	31 days (freeze only once)
Open vial (reconstituted) at 2-8°C	72 hours
On the analyzers at 20-25°C	Up to 5 hours

CalCheck Vitamin D total II	
Shelf life (lyophilized) at 2-8°C	18 months
Open vial (econstituted) at 20-25°C	4 hours

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) studies were performed according to CLSI EP-17A2.

Limit of Blank

To establish the Limit of Blank (LoB), one 25-hydroxyvitamin D depleted human serum sample pool was assayed in 10 replicates per run, 2 runs per day over 3 or more days with three reagent lots on one cobas e 411 analyzer for 60 total measurements. The Limit of Blank was determined as the 95th percentile of measurements of blank samples.

Based on this study, the sponsor defined the Limit of Blank as 2 ng/mL.

Limit of Detection

To determine the Limit of Detection (LoD), 5 low level diluted serum pools were measured using 3 reagents lots over 3 days, 2 runs per day, on one cobas e 411 analyzer with two replicates per sample per run for total measurements of 36. LoD was calculated according to as the following equation: LoD = LoB + 1.653 x SD total (of low analyte samples).

Based on this study, the sponsor defined the Limit of Detection as 3 ng/mL.

Limit of Quantitation

To determine the Limit of Quantitation (LoQ), intermediate precision was used to calculate the LoQ for serum as well as Li-Heparin, K2-EDTA, K3-EDTA plasma samples. Below are the studies for Serum and Plasma samples:

Serum: A minimum of 6 low level samples were prepared (native human serum samples as well as diluted) with concentrations around the expected LoQ. Each sample was evaluated in five replicates per run, three reagent lots, one run per day over five days (n=25 per sample).

Li-Heparin: 5 samples were evaluated in five replicates per run, one lot, one run per day over five days (n=25 per sample).

K2 and K3-EDTA: 7 samples were evaluated in five replicates per run, one lot, one run per day over five days (n=35 per sample).

The LoQ was defined as the lowest concentration with an inter-assay precision of ≤ 20% CV. Based on this study, the sponsor defined the Limit of Quantitation as 5 ng/mL.

Detection limits results:

Limit of Blank	Limit of Detection	Limit of Quantitation
2 ng/mL	3 ng/mL	5 ng/mL

e. *Analytical specificity:*

Endogenous Interferences:

Potential interference from endogenous substances was evaluated. For each interfering substance, 3 serum samples containing low (10 ng/mL), mid (30 ng/mL), and high (70 ng/mL) concentrations of 25-hydroxyvitamin D were tested. An aliquot of each serum sample was spiked with the interfering substances. Another aliquot without any additives was used as the dilution pool. The interfering pool was diluted

into the dilution pool in 10% increments. For bilirubin, 90% bilirubin and 10% ditaurobilirubin was used to mimic the natural ratio of both forms in human blood. The recovery for each sample was calculated by comparison to the reference sample. The sponsor considered significant interference as:

≤ 20 ng/mL 25-hydroxyvitamin D: > 2.0 ng/mL deviation from the unspiked reference value

> 20 ng/mL 25-hydroxyvitamin D: > 10% deviation from the unspiked reference value

The highest concentrations at which no significant interference was observed are shown below:

Substance	Highest concentration of substance tested that demonstrated no significant interference
Bilirubin	66 mg/dL
Hemoglobin	600 mg/dL
Intralipid	300 mg/dL
Triglyceride	300 mg/dL
Cholestrol	300 mg/dL
Biotin	30 ng/mL
Total Protein	9 g/dL
Rheumatoid facror(RF)	1200 IU/mL
Immunoglobulin (IgG)	7g/dL
Serum Albumin	7g/dL

The labeling contains the following limitation:

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. Patients taking > 20 mg biotin per day may need to discontinue biotin intake for longer than 8 hours prior to testing with this assay.

Human Anti-Mouse Antibodies (HAMA):

The effect of the presence of human anti-mouse antibodies on the Elecsys Vitamin D total II assay was assessed on the cobas e 411 immunoassay analyzer. Serum containing 805 ng/mL human anti-mouse antibody (HAMA) and control serum without interferent were measured in duplicate. The sponsor considered recovery of the HAMA-containing serum compared to the control serum of 100 ± 10% to be significant interference.

The study results support the sponsor's claim that HAMA up to 805 ng/mL does not significantly interfere with the Elecsys Vitamin D total II assay.

Exogenous Interferences – Drugs

The effect on quantitation of 25-hydroxyvitamin D using the Elecsys Vitamin D total II assay in the presence of drugs was determined by comparing values obtained from samples spiked with 19 pharmaceutical compounds with the reference sample (unspiked). Two human serum samples (single donors, native as well as spiked) with 25-hydroxyvitamin D concentrations of approximately 30 and 70 ng/mL were used in the study. The two serum samples were divided into aliquots and spiked with the potential interferents. The reference sample without interferent was spiked with the respective amount of solvent only. The samples were measured on the cobas e 411 immunoassay analyzer. The sponsor defined significant interference as greater or equal to ± 10% of the reference value (unspiked sample).

Drug	Highest concentration of spiked drug that demonstrated no significant interference (mg/L)
Acetaminophen	200
Acetylcystein	1660
Ampicillin-Na	1000
Ascorbic acid	300
Cyclosporine	5
Cefoxitin	2500
Heparin	5000 U
Ibuprofen	500
Levodopa	4
Methyldopa +1.5	4
Metronidazole	120
Phenylbutazone	400
Doxycycline	30
Acetylsalicylic Acid	1000
Rifampicin	60
Theophyllin	10
EinsAlpha (alfacalcidol)	0.003
ZEMPLAR (paricalcitol)	0.002
Rocaltrol (calcitriol)	0.0017

Cross reactivity:

A cross-reactivity study was conducted according to CLSI-EP7-A2 to evaluate the potential cross-reactivity of the assay with other vitamin D metabolites. The potential cross-reactants were added at defined concentrations to native human sera with approximate 25-hydroxyvitamin D concentrations of 25, 40, and 60 ng/mL, and analyzed with the Elecsys Vitamin D total II assay on the cobas e 411analyzer. Results from the spiked serum samples were matched against the unspiked references and the % cross-reactivity was calculated using the equation below.

% Cross-Reactivity= mean value spiked –mean value unspiked X 100
Spiked Concentration

Cross-reactant	Concentration of spiked cross reactants (ng/mL)	Non – Normalized Mean Cross Reactivity %	Normalized Mean Cross Reactivity %
25-hydroxy Vitamin D2	50	84.0	93.7
25-hydroxy Vitamin D3	50	89.8	100
24,25-dihydroxy Vitamin D3	100	12.4	13.7
3-epi 25-hydroxy Vitamin D3	50	101.5	112.8
3-epi 25-hydroxy Vitamin D2	50	82.3	91.4
1,25-dihydroxy Vitamin D3	100	Not detected	Not detected
1,25-dihydroxy Vitamin D2	100	Not detected	Not detected
Vitamin D3(Cholecalciferol)	1000	0.6	0.7
Vitamin D2(Ergocalciferol)	1000	0.2	0.3

To reflect the endogenous situation and binding behavior of the metabolite without spiking effect and to avoid potentially underreporting the cross-reactivity to 25-hydroxy Vitamin D2, the observed % cross-reactivities shown above were normalized to 25-hydroxyvitamin D3 by dividing the respective observed % cross-reactivity by the % cross-reactivity of 25-hydroxyvitamin D3. The table above shows the mean % cross-reactivity for both normalized and non-normalized samples.

High Dose Hook Effect:

The high-dose hook effect of the Elecsys Vitamin D total II assay was assessed on the cobas e 411 analyzer. One human serum sample was spiked with analyte to achieve a high 25-OH Vitamin D concentration of approximatly10,000 ng/mL. A dilution series was performed using a low level analyte (Vitamin D depleted) serum. The study results show that no hook effect was observed up to 10,000 ng/mL for Elecsys Vitamin D total II.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method Comparison to Reference Method:

A method comparison study against the ID-LC-MS/MS 25(OH)D Reference Measurement Procedure (RMP) was the basis of the substantial equivalence determination for this submission. A method comparison study to compare the candidate device Elcsys Vitamin D total II with Reference method ID-LC-MS/MS method using a total of 111 native single donor patient serum samples (provided by the Vitamin D Standardization and Certification Program with assigned values by the RMP at CDC, independent from the samples used for standardization) were measured in singleton on the cobas e 411 analyzer. The 25-hydroxyvitamin D values ranged between 5.64 and 92.8 ng/mL as measured by the Reference Method, LC-MS/MS. The results are summarized in the table below:

	Deming regression results
n	111
Slope	0.954
Intercept	-0.707
Correlation Coefficient	0.982
Range(ng/mL)	5.64-92.8

b. Method comparison with predicate device:

A method comparison was performed using the candidate Elecsys Vitamin D total II assay and the predicate Elecsys Vitamin D Assay to assess the bias between the two assays. A total of 105 single native donors serum samples were measured in singleton on the cobas e 411 analyzer in one run covering the entire measuring range of the predicate device. The results are summarized in the table below:

	Deming regression results
n	105
Slope	0.849
Intercept	0.983
Correlation Coefficient	0.955
Range(ng/mL)	6.1-56

c. Matrix comparison:

The effect on quantitation of 25-OH Vitamin D with the Elecsys Vitamin D total II assay in the presence of different anticoagulants was determined by comparing values obtained from samples (single donors - native, diluted as well as spiked) drawn into serum, Li-Heparin, K2-EDTA, K3-EDTA plasma primary tubes, and Plasma Gel Separation Tubes (Li-Heparin). A minimum of 45 serum/plasma pairs per sample material were tested in singleton with one reagent lot on one cobas e 411 immunoassay analyzer. The linear regression equations from the comparisons are

given below:

Serum/Li-Heparin Plasma Comparison:

y = 0.984x - 0.0.10, r = 0.997

Serum/K2- EDTA Plasma Comparison:

y = 0.981x - 0.341, r = 0.998

Serum/K3- EDTA Plasma Comparison:

y =0.992x -1.25, r = 0.998

Serum/Li-Heparin Plasma Gel Separation Tubes Comparison:

y = 0.974x + 0.735, r = 0.999

The data support the package insert claim that serum, Li-Heparin, K2-EDTA and K3-EDTA-plasma as well as Li-Heparin Plasma Gel Separation Tubes are acceptable sample types for use with Elecsys Vitamin D total II.

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:

   The reference range study was conducted with reference to the CLSI C28-A3 guideline. Total of 421 serum samples from 50% males and 50% females, with 30% dark skin tone were collected from apparently healthy adults, from 21 to 88 years old. The samples were collected from three regionally diverse U.S regions during the summer and winter

seasons (approximately 50% summer and 50% winter time) to represent a broad spectrum of UV light exposure in the intended use population. The samples were assayed on the cobas e 411 immunoassay analyzer.

The Reference Interval for the total population of the study for the Elecsys Vitamin D total II assay was calculated using the 95% Reference Interval (2.5^{th} to 97.5^{th} percentile).

Normal Adults: 7.61 – 55.5 ng/mL (n= 421).

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.