510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K210901

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys Vitamin D total III

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 - Vitamin D Test System	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

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

C Type of Test:

Quantitative immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Binding assay for the in vitro 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 cobas e immunoassay analyzers.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

cobas e 601 analyzer

IV Device/System Characteristics:

A Device Description:

The Elecsys Vitamin D total III assay is comprised of a kit for multiple use with three ready to use liquid assay reagents and two pretreatment reagents:

Pretreatment reagent 1: Dithiothreitol 1 g/L.

Pretreatment reagent 2: Sodium hydroxide 57.5 g/L.

Ruthenium labeled vitamin D binding protein 150 µg/L; bis-tris propane buffer 200 mmol/L; albumin 25 g/L; preservative.

Biotinylated 25-hydroxyvitamin D 20 µg/L; bis-tris propane buffer 200 mmol/L; preservative.

Streptavidin-coated microparticles: Streptavidin-coated microparticles 0.72 mg/mL and preservative.

B Principle of Operation:

Elecsys Vitamin D total III assay is a back titration immunoassay, comprising several discrete steps: pretreatment step to release 25-OH vitamin D bound to vitamin D binding protein; incubation of pretreated sample with ruthenium tris-bipyridine labeled vitamin D binding protein; and second incubation of sample with streptavidin-coated microparticles and biotinylated 25-OH vitamin D labeled to bind any unbound ruthenium tris-bipyridine labeled vitamin D binding protein. The sample is drawn into the analyzer's electrochemiluminescence (ECL) detection module, and the ECL signal generated is inversely proportional to amount of 25-OH vitamin D in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys Vitamin D total II

B Predicate 510(k) Number(s):

k162840

C Comparison with Predicate(s):

Device & Predicate Device(s):	k210901	k162840
Device Trade Name	Elecsys Vitamin D total III	Elecsys Vitamin D total II
General Device Characteristic Similarities		
Intended Use/Indications For Use	In vitro quantitative determination of total 25-hydroxy vitamin D in human serum and plasma.	Same
General Device Characteristic Differences		
Measuring Range	6.0 - 120 ng/mL	5.0 - 100 ng/mL
Biotin interference levels	>600 ng/mL	>30 ng/mL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; approved guideline- third edition.

CLSI EP06-A Evaluation of the Linearity of Quantitative Measurement Procedures.

CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition.

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:
   The precision performance of the Elecsys Vitamin D total III assay on the cobas e 601 analyzer was established by two studies to evaluate within-run (repeatability) precision and within-laboratory precision following the recommendations in the CLSI guideline EP05-A3.

Study #1 for 21 days

In the study, 10 human serum samples and two controls were tested with one lot and one instrument in duplicate per run, with two runs per day for 21 days with a total of 84 measurements per sample. The result were analyzed for within-run and within-laboratory precision:

Sample	Mean (ng/mL)	Within-run		Within-laboratory	
		SD (ng/mL)	CV%	SD (ng/mL)	CV%
HS 1	12.3	0.905	7.4	1.20	9.8
HS 2	15.3	0.809	5.3	1.10	7.2
HS 3	28.7	1.28	4.4	1.74	6.0
HS 4	33.0	1.39	4.2	1.85	5.6
HS 5	52.3	1.68	3.2	2.09	4.0
HS 6	61.0	1.39	2.3	2.23	3.7
HS 7	96.5	2.6	2.8	2.92	3.0
HS 8	99.6	2.45	2.5	2.76	2.8
HS 9	111	3.00	2.7	3.63	3.3
HS 10	112	3.37	3.0	3.65	3.3
Control 1	23.0	1.27	5.5	1.49	6.5
Control 2	41.9	1.52	3.6	1.87	4.5

Study #2 for five days

In the study, human serum samples and controls were tested with each of three lots and one instrument in replicates of five per run for five days with a total of 25 measurements per sample. Ten serum samples were tested with two of the lots and 9 serum samples with a third lot. For each lot, the result were analyzed for within-run and within-laboratory precision. The results from one representative lot are shown in the following table:

Sample	Mean (ng/mL)	Within-run		Within-lab	
		SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
HS 1	11.6	1.11	9.6	1.16	10.0
HS 2	15.0	1.36	9.1	1.36	9.1
HS 3	24.4	1.85	7.6	1.85	7.6
HS 4	32.8	1.92	5.9	1.92	5.9
HS 5	56.8	3.13	5.5	3.13	5.5
HS 6	55.0	2.60	4.7	2.93	5.3
HS 7	69.0	2.55	3.7	2.97	4.3
HS 8	102	3.37	3.3	3.37	3.3
HS 9	107	3.45	3.2	3.59	3.4
HS 10	119	3.41	2.9	3.78	3.2
Control 1	24.0	1.48	6.2	1.48	6.2
Control 2	45.1	2.47	5.5	2.47	5.5

2. Linearity:

The linearity performance of the Elecsys Vitamin D total III assay on the cobas e 601 analyzer was established in a study conducted following the recommendations of CLSI guideline EP06-A. In the study, 11 samples were prepared by intermixing known volumes of

a high analyte serum sample of known concentration with a depleted low analyte serum sample, and covering a range of 1.67 to 133 ng/mL. Each sample was assayed in triplicate using one lot and one instrument. The mean of the observed results were compared by regression analysis to the expected results based on the dilution volumes. Regression analysis identified that the coefficients for polynomial fit were statistically significant, and therefore the polynomial better than the linear model. Further analysis found that the deviation from linearity at each concentration (i.e. difference between polynomial and linear fit) to be insignificant as defined by the sponsor with absolute bias less than ±2 ng/mL and percent bias less then ±10%. Based on these results, the sponsor concluded that the test system demonstrated a proportional response over the claimed measuring range of 6 – 120 ng/mL.

Sample Dilution study

A sample dilution study was conducted to support the claim in the product labeling that 25-OH vitamin D samples with results above the measuring range may be manually diluted with the sponsor's Diluent Universal or serum with a low analyte concentration. In the study, four samples were spiked with 25-OH vitamin D to prepare concentrations with expected values ranging from 125 - 183 ng/mL. Each of the four samples were diluted 1:2 with Universal Diluent, and diluted 1:2 with analyte depleted serum. Each diluted sample was assayed in triplicate, and analyzed for recovery after correcting for dilution. The recoveries ranged from 95% to 109%. The dilution study results support the sponsor's labeling claims that samples with 25-OH vitamin D concentrations above 120 ng/mL may be manually diluted 1:2 to obtain results up to 240 ng/mL.

3. Analytical Specificity/Interference:

The analytical specificity performance of the Elecsys Vitamin D total III assay on the cobas e 601 analyzer was established by conducting a cross-reactivity study and interference testing for endogenous and exogenous substances.

Endogenous

Interference from certain endogenous substances was assessed using three serum samples with 25-OH Vitamin D concentrations of low, mid, and high. 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). For each of the three original serum samples, the control and test samples were further prepared into a series of 11 samples of varying substance concentration by inter-mixing the control and test samples with known volumes. Each sample of the dilution series was assayed in five replicates using one lot and one instrument. From measurement of the dilution series for the three original serum samples of low, mid, and high 25-OH Vitamin D concentration, the highest substances concentration within the allowable error was found, and identified as the highest concentration at which no significant interference is observed. The sponsor defined the allowable error as:

LoQ to 20.0 ng/mL: ≤ 2.5 ng/mL
>20.0 ng/mL to 50 ng/mL: ≤ 10%
>50.0 ng/mL: ≤ 15%

The results of the study are summarized as follows:

Substance	Highest concentration tested at which no significant interference is observed
Albumin	7 g/dL
Bilirubin	66 mg/dL
Hemoglobin	600 mg/dL
Intralipid	300 mg/dL
IgG	7 g/dL
IgA	1.3 g/dL
IgM	1 g/dL
Rheumatoid Factor	1200 IU/mL
Triglyceride	300 mg/dL

Biotin Interference
Biotin interference was tested with three serum samples containing 25-OH Vitamin D concentrations of 10, 28, and 77 ng/mL. Each of the three samples were spiked at two levels of biotin at 1550 and 3600 ng/mL, along with controls samples with no added biotin. For each of the serum samples, the control and test samples were further prepared into a series of 11 samples of varying substance concentration by inter-mixing the control and test samples with known volumes. Each sample of the dilution series was assayed in five replicates using one lot and one instrument. From measurement of the dilution series for the serum samples of low, mid, and high 25-OH Vitamin D concentration, the highest biotin concentration within the allowable error of ±10% was found. The results of the study support the labeling claim that specimens containing biotin up to a concentration of 600 ng/mL demonstrated ≤ 10% change in 25-hydroxyvitamin D assay results.

25-OH Vitamin D conc.		**Biotin concentration (ng/mL)**						
		155	**310**	**620**	**930**	**1240**	**1395**	**1550**
9.8 ng/mL	Absolute difference	0.45	0.48	2.05	2.63	3.63	4.18	4.67
28.3 ng/mL	% difference	3%	4%	8%	11%	12%	15%	16%
77.0 ng/mL		-1%	-2 %	-1%	-2%	-2%	-1%	-2%

25-OH Vitamin D conc.		**Biotin concentration (ng/mL)**						
		360	**620**	**1240**	**1860**	**2480**	**2790**	**3600**
10.5 ng/mL	Absolute difference	0.16	0.58	3.68	4.41	8.55	11.4	13.2
36.3 ng/mL	% difference	-1%	1%	15%	18%	36%	47%	55%
76.7 ng/mL		0%	5%	7%	14%	27%	31%	36%

Exogenous
Interference from 20 drugs was assessed using two serum samples with 25-OH Vitamin D concentrations of low and high. Each of the samples was further divided into two aliquots for a control sample with no added interferent and test sample with added interferent. For each of the substances, the test sample and control sample were assayed in 10 replicates using one lot and one instrument. The results were analyzed as percent recovery between the test sample and control sample. The substance under test was considered to have no significant

interference if the recovery was 100 ± 10%. The substances and concentrations tested which did not caused significant interference are listed below.

Substance	Concentration tested at which no significant interference was observed
Acetaminophen	15.6 mg/dL
Acetylcysteine	15 mg/dL
Acetylsalicylic acid	3.0 mg/dL
Alphacalcidol	0.00018 mg/dL
Ampicillin-Na	7.5 mg/dL
Ascorbic acid	5.25 mg/dL
Cefoxitin	75 mg/dL
Cyclosporine	0.18 mg/dL
Doxycycline	1.8 mg/dL
Heparin	3300 IU/L
Ibuprofen	21.9 mg/dL
Itraconazole	3.0 mg/dL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Phenylbutazone	32.1 mg/dL
Rifampicin	4.8 mg/dL
Rocaltrol (Calcitriol)	0.000102 mg/dL
Theophylline	6.0 mg/dL
Zemplar (Paricalcitol)	0.00012 mg/dL

Cross-reactivity

A cross-reactivity study was conducted to quantify the level of cross-reactivity from certain vitamin D metabolites with the assay. In the study, three test samples were prepared by spiking individually various vitamin D metabolites into native human serum samples with approximate 25-OH vitamin D concentrations of 20, 40, and 60 ng/mL. The concentrations of the cross-reactants were chosen to ensure results within the measuring range depending on their expected cross-reactivity and to reflect at least >10 times higher concentration as typically found in native serum. These samples were paired with three reference samples of the same serum that were not spiked and with the same 25-OH vitamin D concentrations. Each sample was assayed in replicates of five using one lot and one instrument. The results from each of the three test samples and reference samples were averaged. The difference in results between test and reference samples was calculated for each of the three 25-OH vitamin D concentrations, and each analyzed for %cross-reactivity using the below equation. The table summarizes the cross-reactivities from the study as the mean of the three results. The product labeling lists %cross-reactivity and %cross-reactivity normalized to the 25-hydroxyvitamin D3 %cross-reactivity.

% Cross-Reactivity = 25-OH vitamin D result from test sample – 25-OH vitamin D result from reference sample x 100% / Concentration of cross-reactive substance

Cross-Reactant	Concentration of Cross-Reactant, ng/mL	%Cross Reactivity	%Cross Reactivity normalized to 25-hydroxyvitamin D3
25-hydroxyvitamin D3	50	72.2%	100.0%
25-hydroxyvitamin D2	50	75.7%	103.3%
24,25-dihydroxyvitamin D3	100	5.8%	8.1%
3-epi-25-hydroxyvitamin D3	50	88.2%	121.6%
3-epi-25-hydroxyvitamin D2	50	74.7%	102.7%
1,25-dihydroxyvitamin D3	100	None detected	-
1,25-dihydroxyvitamin D2	100	0.3%	0.9%
Vitamin D3 (Cholecalciferol)	1000	0.6%	0.9%
Vitamin D2 (Ergocalciferol)	1000	0.5%	0.7%

Human Anti-Mouse Antibodies (HAMA)
The effect of human anti-mouse antibodies with the Elecsys Vitamin D total III assay on the cobas e 601 analyzer was assessed. In the study, serum containing 805 ng/mL HAMA and control serum without HAMA were each measured in duplicate. The results demonstrated that there was no significant interference from HAMA with the assay when using the sponsor's criteria of 100 ± 10% recovery.

4. Assay Reportable Range:

   See Linearity A.2

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

   Metrological traceability:
   The device has been standardized using internal standards which are 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.

6. Detection Limit:
   Detection capability studies of the Elecsys Vitamin D total III assay on the cobas e 601 analyzer were conducted for limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) following the recommendations in CLSI EP17-A2.

   LoB
   The LoB of the assay was evaluated using Vitamin D depleted human serum sample pool, and three reagent kit lots and one instrument. The sample was measured in replicates of 10 per run, across six runs over four days for a total of 60 replicates per lot. The LoB was analyzed as the 95th percentile of measurements by the non-parametric method of the guidance by ranking ordering of the results. The highest LoB of the three lots was found to be 1.6 ng/mL; supporting the claimed LoB in the product labeling of 2.0 ng/mL.

LoD
The LoD was evaluated using five low-level samples prepared from diluted serum pools using three reagent lots and one instrument. The samples were tested in replicates of two per run, with six runs over four days for a total of 60 measurements per lot. For each lot, the LoD was analyzed using a parametric data analysis which was the lowest concentration at which the analyte can be detected with 95% probability. The LoD of the assay was selected from the highest value obtained across the three lots, which was 2.8 ng/mL, supporting the claimed LoD in the product labeling of 3.0 ng/mL.

LoQ
The LoQ was evaluated using 8 low-level diluted serum samples using three reagent lots and one instrument. Each of the samples was tested in replicates of five across each lot on five different days for a total of 25 measurements per sample per lot. The LoQ was defined as the lowest concentration of analyte which has imprecision was less than or equal to 20% CV. The LoQ of the assay was selected from the highest value obtained across the three lots, which was 5.1 ng/mL, supporting the claimed LoQ in the product labeling of 6.0 ng/mL.

7. Assay Cut-Off:
Not applicable.

B Comparison Studies:

1. Method Comparison:
The accuracy performance of the Elecsys Vitamin D total III assay on the cobas e 601 analyzer was established by a method comparison against a reference method for vitamin D. In the study, 157 native, single donor patient serum samples were tested in singlicate on the candidate device and the reference method. The samples were CDC Verification Samples provided by the Vitamin D Standardization and Certification Program with assigned values by the candidate Reference Method Procedure: ID-LCMS/MS at the CDC Vitamin D Reference Laboratory. The results of the study were analyzed graphically for slope and intercept by Deming regression analysis, and summarized as follows:

Concentration range, comparator device	Regression equation	r
5.6 to 118.4 ng/mL	y = 0.981x + 0.795	0.982

The predicted bias at medical decision point (30 ng/mL) was calculated as the percent difference between the Deming regression line and the unity line at the medical decision point and was found to be 0.8%.

2. Matrix Comparison:
A matrix equivalency study to serum was conducted to support use of the Elecsys Vitamin D total III assay on the cobas e 601 analyzer with additional specimen matrix types claimed in the product labeling: Serum Separation Tubes, and Li-Heparin plasma, K2-EDTA plasma, and K3-EDTA plasma. In the study, 46 donor matched venous specimens of the aforementioned were collected. Each specimen was tested in singlicate using one lot of the reagent kit and one instrument, and the results compared to the mean of singlicate serum measurements. The results were analyzed by Passing-Bablok linear regression with the

concentration from the first replicate of each donor's evaluation tube (y-axis) versus the mean concentration of the serum results (x-axis). The slope and intercept of the regression line were calculated, and summarized as follows:

Specimen	Range	r	Intercept	Slope
Li-Heparin plasma	8.28 to 103 ng/mL	0.985	-0.62	1.03
K2-EDTA plasma	9.27 to 99.8 ng/mL	0.957	0.072	0.973
K3-EDTA plasma	9.32 to 100 ng/mL	0.990	0.14	0.92
Serum Separation Tube	8.88 to 107 ng/mL	0.987	-0.26	1.02

The study results support the sponsor's claim that human serum and plasma (lithium heparin, K2-EDTA and K3 EDTA) are acceptable sample types to be used with this assay.

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:

A reference range study with Elecsys Vitamin D total III assay on the cobas e 601 analyzer was conducted testing serum samples collected from apparently healthy donors from southern, middle, and northern sites of the United States during both summer and winter. There were approximately equal numbers of males and females, and approximately 30% of the donors had dark skin complexion. The age range was 22 to 79 years. The results of the study are summarized as follows:

		Seasonal	
	All (n = 463)	Summer (n = 245)	Winter (n = 218)
Mean, ng/mL	26.6	29.2	23.6
Median	25.7	27.7	22.8
2.5th percentile	10.2	12.5	9.4
97.5th percentile	49.4	52.4	44.1

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.