

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

A. 510(k) Number:

k113546

B. Purpose for Submission:

New assay

C. Measurand:

25-OH Vitamin D

D. Type of Test:

Quantitative, automated electrochemiluminescence immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys Vitamin D assay, Elecsys Vitamin D CalSet, Elecsys PreciControl Varia 3, and Elecsys Vitamin D Calcheck 5.

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

2. Classification:

Class II, (controls – Class I, reserved)

3. Product code:

MRG, JIT, JJY, JJX

4. Panel:

H. Intended Use:

1. Intended use(s):

See indications for use.

2. Indication(s) for use:

The Elecsys Vitamin D assay is intended for the quantitative determination of total 25-hydroxy vitamin D in human serum and plasma. The Elecsys Vitamin D 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 Elecsys and **Cobas e** immunoassay analyzers.

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

The Elecsys Vitamin D CalCheck 5 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 reagent on the indicated Elecsys and **cobas e** immunoassay analyzers.

Elecsys PreciControl Varia 3 is used for quality control of specified Elecsys immunoassays on Elecsys and **cobas e** immunoassay analyzers.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Performance studies were conducted on the cobas e411 Analyzer

I. Device Description:

The devices cleared under this 510(k) are:

- (1) Elecsys Vitamin D Assay
- (2) Elecsys Vitamin D CalSet
- (3) Elecsys PreciControl Varia 3
- (4) Elecsys Vitamin D CalCheck 5

The assay consists of

Pre-treatment reagent 1 – dithiothreitol;

Pretreatment reagent 2 – NaOH;

Solution M containing streptavidin-coated microparticles with preservative;

R1 - Ruthenium labeled vitamin D binding protein in buffer with human albumin and preservative;

R2- Biotinylated vitamin D (25-OH) in buffer with preservative

The Vitamin D Calset contains two bottles of Vitamin D 25-OH in two concentration ranges (approximately 2 and 37 ng/mL) in a human serum matrix with preservative.

The Vitamin D Calcheck 5 contains 5 levels of lyophilized synthetic Vitamin D (25-OH) from human serum with preservative.

The Precicontrol Varia 3 contains three levels of lyophilized serum-based control. Analytes include Vitamin D, as well as analytes previously cleared under k111506 (Vitamin B12, Ferritin, Folate III, β -Crosslaps/Serum (β -CTx), Osteocalcin, Parathyroid Hormone (PTH)).

Package inserts for the 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. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A. However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen.

J. Substantial Equivalence Information:

1. Predicate device name(s):

ARCHITECT 25-OH Vitamin D assay (k110619)
Elecsys hGH CalSet (k103221)
Predicate Device: Elecsys PreciControl Multimarker (k102157)
Predicate Device: Elecsys T4 CalCheck 5 (k112528)

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

k110619
k103221
k102157
k112528

3. Comparison with predicate:

Feature	Elecsys Vitamin D Assay	Predicate Device: Abbott Architect 25-OH Vitamin D (K110619)
General Assay Features		
Intended Use/ Indications for Use	The Vitamin D 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 Technology	Quantitative protein binding assay	Quantitative immunoassay
Detection Protocol	Electrochemiluminescence	Chemiluminescence
Sample Type	Human serum and plasma treated with K ₂ -EDTA, K ₃ -EDTA or lithium heparin.	Human serum, plasma treated with lithium heparin, sodium heparin, potassium EDTA, or sodium citrate.
Reagents	The Elecsys Vitamin D assay is a competitive binding assay which includes vitamin D (25-OH) labeled with biotin, and a ruthenium labeled vitamin D binding protein, and streptavidin coated microparticles.	The Abbott Architect 25-OH Vitamin D assay is a competitive one-step delayed immunometric assay which includes anti-vitamin D coated microparticles, an assay diluent, and biotinylated vitamin D anti-biotin acridinium labeled conjugate.
Calibration	Elecsys Vitamin D CalSet, 2 levels	Architect 25-OH Vitamin D Calibrators, 6 levels
Traceability / Standardization	The Elecsys Vitamin D Assay has been standardized against LC-MS/MS which in turn has been standardized to the NIST standard.	Architect 25-OH Vitamin D is traceable to a manufacturer's internal standard (Primary Calibrator), which is anchored against Absorbance at 264 nm.
Measuring Range	5-60 ng/mL	13-96 ng/mL

Characteristic	CalSet for Elecsys Vitamin D Assay	Predicate Device: Elecsys hGH CalSet (K103221)
Intended Use	Elecsys Vitamin D CalSet is used for calibrating the quantitative Elecsys Vitamin D assay on the Elecsys and cobas e immunoassay analyzers.	Elecsys hGH CalSet is used for calibrating the quantitative Elecsys hGH assay on the Elecsys and cobas e immunoassay analyzers.
Levels	Two	Same
Matrix	Human serum	Same
Format	Lyophilized	Same

Characteristic	Elecsys PreciControl Varia 3	Predicate Device: Elecsys PreciControl Multimarker (K102157)
Intended Use	Elecsys PreciControl Varia 3 is used for quality control of Elecsys immunoassays on the Elecsys and cobas e immunoassay analyzers.	Elecsys PreciControl Multimarker is used for quality control of specified Elecsys immunoassays on the Elecsys and cobas e immunoassay analyzers.
Levels	Three	Two
Format	Lyophilized	Same
Matrix	Human serum	Equine serum

Characteristic	Elecsys Vitamin D CalCheck 5	Predicate Device: Elecsys T4 CalCheck 5 (K112528)
Intended Use	The Elecsys Vitamin D CalCheck 5 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 reagent on the indicated Elecsys and cobas e immunoassay analyzers.	The Elecsys T4 CalCheck 5 is an assayed control for use in calibration verification and for use in the verification of the assay range established by the Elecsys T4 reagent on the indicated Elecsys and cobas e immunoassay analyzers.
Analyte	(25-OH) Vitamin D	Thyroxine (T4)
Levels	Five	Same
Matrix	Human serum	Check 1: BSA/Buffer matrix Check 2-5: human serum
Format	Lyophilized	Same

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

CLSI EP6, Evaluation of Linearity of Quantitative Measurement Procedures: A statistical Approach.

CLSI EP5, Evaluation of Precision Performance of Clinical Chemistry Devices.

L. Test Principle:

The Elecsys Vitamin D Assay is a competitive protein binding assay which uses Vitamin D Binding Protein for detection of 25-Hydroxyvitamin 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 vitamin D (25-OH) and the ruthenylated vitamin D binding protein. In the 3rd and final incubation, streptavidin-coated microparticles are added along with vitamin D (25-OH) 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):

Evaluations described below were performed on the cobas e 411

1. Analytical performance:

a. Precision/Reproducibility:

Precision of the Elecsys® Vitamin D assay was evaluated according to CLSI EP5-A2 guideline. Two reagent lots were evaluated. The protocol consisted of testing 2 replicates of each control (PC Varia = PreciControl Varia 3) and human sera (HS) per run, 2 runs per day for 21 days. The human sera were all native, single donors. Repeatability and Intermediate precision were calculated. Results are tabulated below:

Lot 1		Repeatability		Intermediate (within lab) precision		
Sample	Mean (ng/mL)	SD (ng/mL)	CV (%) (UCL* 95%)	SD (ng/mL)	CV (%) (UCL* 95%)	n
Human serum 1	52.6	0.858	1.6 (2.0)	1.5	2.9 (3.6)	84
Human serum 2	6.2	0.445	7.2 (8.8)	0.64	10.3 (12.3)	84
Human serum 3	13	0.618	4.7 (5.8)	1.09	8.4 (10.4)	84
Human serum 4	23.2	0.666	2.9 (3.5)	1.24	5.3 (6.6)	84
Human serum 5	43.5	1.04	2.4 (2.9)	1.46	3.4 (4.0)	84

Human serum 6	11.2	0.566	5.1 (6.2)	0.849	7.6 (9.0)	84
Lot 2		Repeatability		Intermediate (within lab) precision		
Sample	Mean (ng/mL)	SD (ng/mL)	CV (%) (UCL* 95%)	SD (ng/mL)	CV (%) (UCL* 95%)	n
Human serum 1	55.5	1.07	1.9 (2.4)	1.38	2.5 (2.9)	84
Human serum 2	6.08	0.467	7.7 (9.4)	0.583	9.6 (11.3)	84
Human serum 3	13.8	0.557	4.0 (4.9)	0.803	5.8 (6.8)	84
Human serum 4	24.6	1.12	4.6 (5.6)	1.25	5.1 (5.9)	84
Human serum 5	44.7	0.871	1.9 (2.4)	1.28	2.9 (3.4)	84
Human serum 6	8.44	0.695	8.2 (10.1)	0.918	10.9 (13.0)	84
Human serum 7	11.4	0.649	5.7 (7.0)	0.843	7.4 (8.7)	84

		Repeatability		Intermediate (within lab) precision		
Sample	Mean (ng/mL)	SD (ng/mL)	CV (%) (UCL* 95%)	SD (ng/mL)	CV (%) (UCL* 95%)	n
PC Varia 0	11.6	0.529	4.6 (5.6)	0.705	6.1 (7.1)	84
PC Varia 1	17.0	0.745	4.4 (5.4)	0.964	5.7 (6.8)	84
PC Varia 2	31.1	0.802	2.6 (3.1)	1.08	3.5 (4.1)	84

b. Linearity/assay reportable range:

Linearity of the Elecsys Vitamin D assay was evaluated according to CLSI EP6-A.

A high analyte serum sample was diluted with a low analyte serum sample (depleted) to prepare concentrations throughout the measuring range and samples were assayed in 3-fold determination within a single run.

The linear regression equation yielded slope of 1.07 and intercept of -1.13 ($r^2 = 0.996$) for the measured sample ranged from 0 to 62 (0, 3, 7, 12, 18, 22, 28, 32, 38, 44, 48, 52, 56, 60, 62) ng/mL.

The results of the study support the sponsor's claim that the assay is linear across the measuring range of 5 – 60 ng/mL.

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

Calibrator

Calset Components:

The Elecsys Vitamin D Calset contains 25-hydroxyvitamin D in a lyophilized human serum matrix. Target values are 2.0 [target range: 1.0 to 3.0] and 37.0 ng/mL (target range: 33.0-41.0).

Traceability/value assignment:

The values are standardized against an LCMS/MS assay which is in turn standardized against the NIST standard. The Elecsys Vitamin D CalSet assigned values are determined with the Elecsys Vitamin D assay. A master calibrator set is available and traceable to a reference method (LC-MS/MS). The assigned values for Elecsys Vitamin D CalSet are read from the master calibration curve.

Stability:

Calset stability is evaluated under the manufacturer's recommended storage conditions including: after reconstitution, after freeze-thaw conditions, and unopened.

The sponsor claims that the CalSet is stable at up to hundred twenty (120) hours at 2 to 8°C; up to ninety (90) days at -15 to -25°C and for one freeze/thaw cycle (Table 14). Stability protocols and acceptance criteria were reviewed and found to be adequate.

Control materials:

Components:

Lyophilized Synthetic 25-OH Vitamin D in a human serum matrix, at target levels of 13, 17, 32 ng/mL. This control material also contains additional analytes previously cleared under k060755, k971833, k082340, k993706, k051297 and k070709.

Value assignment:

Values are assigned for each lot of Elecsys® PreciControl Varia 3 in combination with each assay reagent lot available. The controls are run in duplicate on a minimum of 3 analyzers (minimum n=6). The assigned value of each control level is defined as the median value obtained.

Stability:

Studies to support the claimed storage for unopened at 2-8°C up to the stated expiration date, for reconstituted at 2 - 8°C: 72 hours, and at -20°C: 31 days(freeze only once), on - analyzers at 20-25°C: up to 5 hours were reviewed. Stability protocols and acceptance criteria were reviewed and found to be adequate.

Calcheck

Components:

Five levels of lyophilized synthetic 25-OH Vitamin D in a human serum matrix.

Level	Target Value [ng/mL]	Target Range [ng/mL]
Check 1	≤ 1	–
Check 2	15	13 - 17
Check 3	30	27 - 33
Check 4	48	44 - 52
Check 5	60	56 - 64

Value assignment:

For each Elecsys Vitamin D CalCheck 5 lot manufactured, the CalChecks are run in duplicate on at least three E170/cobas e 601/cobas e 602 analyzer measuring cells. The assigned value of each CalCheck is defined as the median value obtained over at least 6 determinations (duplicate runs on at least 3 analyzers) of the respective CalCheck.

Stability:

The package insert claim for stability of reconstituted Elecsys Vitamin D CalCheck 5 is up to 5 hours at 20-25°C. The CalCheck products are not stored on-board the analyzer, therefore no on-board stability claims are made. Stability protocols and acceptance criteria were reviewed and found to be adequate.

d. Detection limit:

The LoB and LoD were determined according to protocols in CLSI EP-17 to support the LoB claim of 2 ng/mL and LoD claim of 3 ng/mL.

LoQ: The precision at low concentrations was determined to support the lower assay limit. Eight native human serum sample pools with concentrations ranging from 2.25 to 20.5 ng/mL were tested once per run, 2 runs per day over 5 days. The mean, standard deviation and coefficient of variation for each sample were calculated. LoQ is calculated to be 5.00 ng/mL. The CV at this concentration is less than ≤ 20%.

e. Analytical specificity:

Cross reactivity:

Cross-reactivity from compounds listed below were evaluated. Results are tabulated:

Cross-reactant	cross reactivity (%)
Vitamin D3 (25-OH)	100
Vitamin D2 (25-OH)	92

24,25-OH Vitamin D3	149
1,25-OH Vitamin D3	not detectable
1,25-OH Vitamin D2	not detectable
Vitamin D3	not detectable
Vitamin D2	not detectable
C3-epimers 25-OH D3	91

Exogenous interferences:

Two samples with approx. 10 and 26 ng/mL (25-OH) Vitamin D were divided into aliquots and spiked with potential interferences. Concentrations tested were equal to or greater than concentrations listed in the CLSI EP7 guideline. Recovery was evaluated relative to a control samples without interference. Compounds tested are shown in the table below. Test samples containing these compounds all recovered within +/- 10% of the control sample.

Drug	Conc. of spiked drug [ng/mL – unless noted otherwise]
Acetylcysteine	1663
Ampicillin-Na	1000
Ascorbic acid	300
Cyclosporine	105
Cefoxitin	660
Heparin	5000 U
Intralipid	< 400 mg/dL
Levodopa	140
Methyldopa +1.5	15
Metronidazole	200
Phenylbutazone	400
Doxycycline	30
Acetylsalicylic Acid	1000

Rifampicin	64
Acetaminophen	200
Ibuprofen	500
Theophylline	100

In addition, the presence of any of the following: bondronate (50 mg/L), alpha-calcidol (0.003 mg/L), alendronate (350 mg/L), pamidronate (90 mg/L), zolendronate (4 mg/L) and PTH 1-34 (0.02 mg/L) showed no significant interference (defined by the sponsor as recovery within 10%).

Endogenous interferents:

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 (50 ng/mL) concentrations of Vitamin D were tested. No significant interference is defined by the sponsor as <10% difference between the spiked and control samples. The highest concentrations at which no significant interference was observed are shown below:

Hemoglobin: < 200 mg/dL (The sponsor notes in the package insert that samples showing visible signs of hemolysis may cause interference and hemoglobin >2g/dL may lead to elevated results)

Biotin: < 70 ng/mL.

Lipemia: < 400 mg/dL.

Bilirubin: No interference up to <66

Cholesterol: Cholesterol at varying levels was spiked into human serum samples containing Vitamin D concentration near 10 ng/mL, Recoveries for samples containing < 380 mg/dL cholesterol were within the manufacturers acceptance criteria of +/- 90%.

Serum proteins: Albumin and IgG were spiked into human serum samples at concentrations ranging from near 4 g/dL to > 7 g/dL. Vitamin D in the samples ranged from near 20 ng/mL to near 45 ng/mL. Percent recovery observed up to 7 g/dL albumin was within +/- 90%.

Percent recovery for samples containing up to 3.5 g /dL IgG was within +/-10%. Higher concentrations of IgG interfered with the assay.

f. Assay cut-off:

Not applicable – this is a quantitative assay.

2. Comparison studies:

a. *Method comparison with predicate device:*

A method comparison was performed with a predicate device (k110619). A total of 165 human serum samples from US blood donors were measured (in singlicate). Results of the Deming Regression analysis are presented in the following table.

N	165
Range (ng/mL)	7 – 58 ng/mL (13-63 ng/mL on the predicate device)
Slope (95% CI)	1.01 (0.94 – 1.08)
Intercept (ng/mL) (95% CI)	0.53(-1.11 – 2.18)
Correlation Coefficient (Pearson's r) (95% CI)	0.92 [0.90 to 0.94]
Sy/x (ng/mL)	4.6

b. *Matrix comparison:*

Anticoagulants:

The effect on quantitation of analyte in the presence of anticoagulants with the Elecsys® Vitamin D assay was determined by comparing values obtained from samples (single donors, native as well as spiked) drawn into Serum, Li-Heparin, K₂-EDTA- , K₃-EDTA-plasma primary tubes and Li-Heparin Plasma Gel Separation Tubes. Forty serum/plasma pairs per sample material were tested with one reagent lot on one Cobas e 411 Immunoassay Analyzer. Samples range tested ranged from 5.6 to 58 ng/mL.

Anticoagulant	Slope	Intercept	R
K2-EDTA plasma	0.981	-0.485	0.996
K3-EDTA plasma	0.983	-1.00	0.996
Li Heparin	0.996	-0.197	0.997
Li Heparin separation tubes	0.980	0.062	0.998

3. Clinical studies:

a. *Clinical Sensitivity:* Not applicable; Clinical sensitivity and specificity is

not typically provided in 510(k)s for this type of assay.

b. *Clinical specificity:* See a, above.

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

4. Clinical cut-off:

Not applicable; this is a quantitative assay.

5. Expected values/Reference range:

Serum samples were prospectively collected from apparently healthy adult subjects at three geographically distinct regions of the US and tested on the Elecsys® Vitamin D during late fall season. Serum samples were first tested for calcium, magnesium and phosphate levels. To confirm normal thyroid and parathyroid functions of the consented subjects, T4, TSH, and PTH levels were also tested. Apparently healthy was defined as patients with a normal medical history (based on physical knowledge) and normal nutritional status. The subject samples with missing results, results outside of the normal range of the above analytes or results that could be affected by the interference were excluded.

A total of 560 eligible subjects from all sites were used to compute the reference intervals. The intermediate 95% nonparametric reference interval was computed. The analysis was performed separately for each site. N, min, max, 2.5, 50, 97.5, median, coverage for the 95% reference interval, and 90% nonparametric confidence for the 2.5 and the 97.5 quantile were computed for each site.

Site	Population	N	Mean	Median	P2.5	P97.5
All sites	All	560	25.53	24.43	6.36	49.53
	No VitD Supplement Usage	380	24.54	23.28	7.26	47.04
Site 1	All	139	27.53	25.33	10.19	50.99
	No VitD Supplement Usage	122	26.83	24.97	10.19	50.99
Site 2	All	149	25.56	23.80	8.83	47.04
	No VitD Supplement Usage	106	24.48	23.05	8.09	46.49
Site 3	All	142	23.81	23.31	4.48	48.63
	No VitD Supplement Usage	49	20.87	19.04	4.62	44.45
Site 4	All	130	25.25	24.65	7.26	52.66

Site	Population	N	Mean	Median	P2.5	P97.5
	No VitD Supplement Usage	103	23.65	22.74	6.97	42.71

Percent of various ethnic backgrounds at the sites are shown below:

	African American / Black		American Indian/Alaska Native		Asian		Caucasian/ White		Other		Unknown		Total	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Site 1	47	33.8	0	0.0	0	0.0	91	65.5	0	0.0	1	0.7	139	100.0
Site 2	3	2.0	1	0.7	3	2.0	140	94.0	2	1.3	0	0.0	149	100.0
Site 3	16	11.3	3	2.1	2	1.4	113	79.6	6	4.2	2	1.4	142	100.0
Site 4	16	12.3	0	0.0	8	6.2	104	80.0	2	1.5	0	0.0	130	100.0
Grand Total	82	14.6	4	0.7	13	2.3	448	80.0	10	1.8	3	0.5	560	100.0

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.