DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

ROCHE DIAGNOSTICS
LINDA MCCAMMACK
REGULATORY PROGRAM MANAGER
9115 HAGUE ROAD
INDIANAPOLIS IN 46250

February 8, 2017

Re: K162840
Trade/Device Name: Elecsys Vitamin D total II, CalSet Vitamin D total II, CalCheck Vitamin D total II, PreciControl Vitamin D total II
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D test system
Regulatory Class: II
Product Code: MRG, JIT, JJX
Dated: December 22, 2016
Received: December 23, 2016

Dear Linda McCammack:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
k162840

Device Name
Elecsys Vitamin D total II CalCheckVitamin D total II
CalSet Vitamin D total II
PreciControl Vitamin D total II

Indications for Use *(Describe)*

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 the Elecsys Vitamin D total II assay on the 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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

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Roche Diagnostics
Indianapolis, IN 46250-0416

K162840

Elecsys Vitamin D total II
510(k) Summary

This summary of 510(k) safety and effectiveness information is being submitted in accordance with the requirements of 21 CFR 807.92.

In accordance with 21 CFR 807.87, Roche Diagnostics hereby submits official notification as required by Section 510(k) of the Federal Food, Drug and Cosmetics Act of our intention to market the device described in this Premarket Notification 510(k).

The purpose of this Traditional 510(k) Premarket Notification is to obtain FDA review and clearance for the Elecsys Vitamin D total II Test System.

Submitter Name	Roche Diagnostics
Address	9115 Hague Road P.O. Box 50416 Indianapolis, IN 46250-0457
Contact	Linda McCammack Phone: (317) 521-7144 FAX: (317) 521-2324 Email: linda.mccammack@roche.com
Date Prepared	September 19, 2016
Proprietary Name	1- Elecsys Vitamin D total II 2-CalSet Vitamin D total II 3- PreciControl Vitamin D total II 4- CalCheck Vitamin D
Common Name	1-Vitamin D total 2 -CalSet Vitamin D total II 3 - PreciControl Vitamin D total II 4 - CalCheck Vitamin D
Classification Name	1 - System, Test, Vitamin D, 2 - Calibrator, Secondary, 3 - Single (Specified) Analyte Controls (Assayed and Unassayed), 4 - Single (Specified) Analyte Controls (Assayed and Unassayed)
Product Codes, Regulation Numbers	1 -MRG, 862.1825 2- JIT, 862.1150 3- JJX, 862.1660 4- JJX, 862.1660
Predicate Devices	Elecsys Vitamin D Assay (k113546)
Establishment Registration	For the Elecsys Vitamin D total II, the establishment registration numbers: Roche Diagnostics GmbH in Mannheim, Germany is 9610126, Roche Diagnostics Penzberg, Germany is 9610529 Roche Diagnostics in the United States is 1823260

1. DEVICE DESCRIPTION

Elecsys Vitamin D total II is a second generation assay by Roche Diagnostics for the in vitro quantitative determination of 25-hydroxyvitamin D in human serum and plasma. It is intended for use on the **cobas e** 411 immunoassay analyzer. The **cobas e** family of analyzers employs the electrochemiluminescence "ECLIA" technology. The assay is a 27 minute assay utilizing a competition principle and a pretreatment step to release the bound 25-hydroxyvitamin D from the vitamin D binding protein.

Results are determined via a calibration curve which is instrument specifically generated by 2-point calibration against the master curve for that reagent lot.

1.1. Reagents

The **Elecsys Vitamin D total II** reagent working solutions include:

- Pretreatment rackpack (kit placed on instrument)
  - Pretreatment Reagent 1 (Dithiothreitol)
  - Pretreatment Reagent 2 (Sodium hydroxide)
- Rackpack (kit placed on instrument)
  - Streptavidin-coated microparticles
  - Reagent 1 (ruthenium labeled vitamin D binding protein)
  - Reagent 2 (biotinylated 25-hydroxyvitamin D)

1.2. Calibrator

The assay will be calibrated using the **CalSet Vitamin D total II**, which is a two concentration level set of lyophilized human serum matrix that 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. The CalSet includes:

- Cal 1 (approximately 2 ng/mL 25-hydroxyvitamin D in human serum matrix)
- Cal 2 (approximately 45 ng/mL 25-hydroxyvitamin D in human serum matrix)

1.3. Control

PreciControl Vitamin D total II is used for the quality control verification of the performance of the assay on the **cobas e** 411 analyzer. The control materials are lyophilized serum based on human serum in two concentration ranges.

1.4. Calibration Verification Materials

CalCheck Vitamin D total II is used for the calibration verification and the assessment of the measuring range as needed by the laboratory certification agencies such as College of American Pathologists or CLIA certification. The CalChecks are a customer convenience product and not required to assess the performance.

2. INTENDED USE

Following are the Intended Uses for the Elecsys Vitamin D total II Test System.

2.1. 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.

2.2. 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.

2.3. PreciControl Vitamin D total II

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

2.4. CalCheck Vitamin D total II

The 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. TECHNOLOGICAL CHARACTERISTICS

Elecsys Vitamin D total II utilizes electrochemiluminescence "ECLIA" technology for the quantitative determination of 25-hydroxyvitamin D for the assessment of vitamin D sufficiency on the **cobas e** 411 immunoassay analyzer.

The following tables compare the Elecsys Vitamin D total II with its predicate device, Elecsys Vitamin D Assay (k113546).

Table 1: Assay Comparison

Feature	Predicate Device: Elecsys Vitamin D Assay (k113546)	Candidate Device: Elecsys Vitamin D total II
Intended Use/ Indications for Use	This Vitamin D assay in 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 Elecsys and **cobas e** immunoassay analyzers.	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. The electrochemiluminescence binding assay is intended for use on the **cobas e** 411 immunoassay analyzer.
Assay Method	Competition principle binding protein	Same
Detection Method	Electrochemiluminescence	Same
Applications/Test Time	27 minutes	Same
Instrument Platform	Elecsys 2010, MODULAR *ANALYTICS* E170, **cobas e** 411, **cobas e** 601, and **cobas e** 602	**cobas e** 411
Sample/Reagent Ratio	15 µL / 185 µL	20 µL / 180 µL
Sample Type/Matrix	Human serum, plasma	Same
Sample Anticoagulants	Li-heparin, K_2-EDTA, K_3-EDTA, Li-heparin PST.	Li-heparin, K_2-EDTA, K_3-EDTA, PST tubes for corresponding plasma types.
Calibrator	Vitamin D CalSet	CalSet Vitamin D total II
Calibration Method	2-point calibration based on master curve for specific reagent lot	Same
Calibration Interval	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 the same reagent kit on the analyzer) • As required e.g. quality control findings outside the defined limits	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 the same reagent kit on the analyzer) • As required e.g. quality control findings outside the defined limits
Controls	PreciControl Varia	PreciControl Vitamin D total II
Traceability/ Standardization	LC-MS/MS traceable to NIST SRM 2972	ID-LC-MS/MS RMP traceable to NIST 2972.
Reagent Stability	• Unopened at 2-8°C up to the stated expiration date • After opening at 2-8°C 56 days (8 weeks) • On the analyzer 21 days	• Unopened at 2-8°C up to the stated expiration date • After opening at 2-8°C 56 days (8 weeks) • On the analyzer 28 days (4 weeks)
Measuring Range	5 – 60 ng/mL	5 – 100 ng/mL

Feature	Predicate Device: Elecsys Vitamin D Assay (k113546)				Candidate Device: Elecsys Vitamin D total II			
Precision	Elecsys 2010 and **cobas e** 411 analyzers:							
	Repeatability				Repeatability			
		mean [ng/mL]	SD [ng/mL]	CV [%]		mean [ng/mL]	SD [ng/mL]	CV [%]
	HS1	6.20	0.445	7.2	HS1	11.1	0.725	6.6
	HS2	11.2	1.42	5.1	HS2	20.8	0.849	4.1
	HS3	23.2	0.666	2.9	HS3	25.6	0.774	3.0
	HS4	43.5	1.04	2.4	HS4	47.5	0.749	1.6
	HS5	52.6	0.858	1.6	HS5	92.6	1.76	1.9
	Intermediate				Intermediate			
	HS1	6.20	0.640	10.3	HS1	11.1	0.965	8.7
	HS2	11.2	0.849	7.6	HS2	20.8	1.09	5.2
	HS3	23.2	1.24	5.3	HS3	25.6	1.43	5.6
	HS4	43.5	1.46	3.4	HS4	47.5	1.77	3.7
	HS5	52.6	1.50	2.9	HS5	92.6	2.40	2.6
LoB	2 ng/mL				Same			
LoD	3 ng/mL				Same			
LoQ	5 ng/mL				Same			
Analytical Specificity	25-hydroxyvitamin D_3			100%	25-hydroxyvitamin D_3			100%
	25-hydroxyvitamin D_2			92%	25-hydroxyvitamin D_2			93.7%
	24, 25-dihydroxyvitamin D_3			149%	24, 25-dihydroxyvitamin D_3			13.7%
	C3-epimer of 25-hydroxyvitamin D_3			91%	3-epi-25-hydroxyvitamin D_3			112.8%
					3-epi-25-hydroxyvitamin D_2			91.4%
Method Comparison Candidate Assay against ID-LC-MS/MS RMP	Deming Regression y = 0.954x – 0.707 r = 0.982 Passing Bablok y = 0.937x – 0.360 $_T$ = 0.902 Using 111 samples with sample concentrations between approximately 5.6 ng/mL and 93 ng/mL							
Method Comparison Candidate Assay against Predicate Device Elecsys Vitamin D Assay	Deming Regression y = 0.849x + 0.983 r = 0.955 Passing Bablok y = 0.867x + 0.724 $_T$ = 0.840 Using 105 samples with sample concentrations between approximately 6.1 ng/mL and 56 ng/mL							
Limitations	Hemolysis		≤ 2 g/L		Hemolysis		≤ 600 mg/dL	
	Bilirubin		≤ 66 mg/dL		Bilirubin		≤ 66 mg/dL	
	Lipemia (Intralipid)		≤ 400 mg/dL		Intralipid		≤ 300 mg/dL	
	Biotin		≤ 70 ng/mL		Biotin		≤ 30 ng/mL	

Feature	Predicate Device: Elecsys Vitamin D Assay (k113546)		Candidate Device: Elecsys Vitamin D total II	
	Serum albumin	≤ 7 g/dL	Serum albumin	≤ 7 g/dL
	Cholesterol	≤ 380 mg/dL	Cholesterol	≤ 300mg/dL
			Triglyceride	≤ 300 mg/dL
			Rheumatoid Factor	≤ 1200 IU/mL
			Total Protein	≤ 9 g/dL
			IgG	≤ 7 g/dL

Table 2: Calibrator Comparison

Feature	Predicate Device	Candidate Device
Intended Use/ Indications for Use	Vitamin D CalSet is used for calibrating the quantitative Elecsys Vitamin D assay on the Elecsys and **cobas e** immunoassay analyzers.	CalSet Vitamin D total II is used for calibrating the quantitative Elecsys Vitamin D total II on the **cobas e** 411 immunoassay analyzer.
Analyte	25-hydroxyvitamin D	25-hydroxyvitamin D_3
Matrix	Human serum matrix with added 25-hydroxyvitamin D	Human serum matrix with added 25-hydroxyvitamin D_3
Levels	Two	Same
Target Ranges	Cal 1: approximately 2 ng/mL Cal 2: approximately 37 ng/mL	Cal 1: approximately 2 ng/mL Cal 2: approximately 45 ng/mL
Format	Lyophilized	Same
Handling	Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding the foam formation.	Same
Storage and Stability	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	Reconstituted calibrators: • At 2-8°C – 72 hours • At -20°C – 12 weeks (freeze only once) • On the **cobas e** 411 analyzer – up to 5 hours

Table 3: Control Comparison

Feature	Predicate Device	Candidate Device
Intended Use/ Indications for Use	PreciControl Varia is used for the quality control of Elecsys immunoassays on the Elecsys and **cobas e** immunoassay analyzers.	PreciControl Vitamin D total II is used for quality control of the Elecsys Vitamin D total II assay on the **cobas e** 411 immunoassay analyzer.

Feature	Predicate Device	Candidate Device
Analyte	Vitamin B12 Ferritin Folate β-CTx Osteocalcin Parathyroid hormone 25-hydroxyvitamin D Calcitonin	25-hydroxyvitamin D
Matrix	Human serum matrix	Same
Levels	Three	Two
Target Ranges	Target range for the 25-hydroxyvitamin D: • Level 0: Approximately 12.8 ng/mL • Level 1: Approximately 17 ng/mL • Level 2: Approximately 32 ng/mL	Target range: • Level 1: Approximately 13 ng/mL • Level 2: Approximately 30 ng/mL
Format	Lyophilized	Same
Handling	Carefully dissolve the contents of one bottle by adding exactly 3.0 mL of distilled or deionized water and allow to stand closed for 30 minutes to reconstitute. Mix carefully, avoiding the foam formation.	Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding the foam formation.
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

Table 4: CalCheck Comparison

Feature	Predicate Device	Candidate Device
Intended Use/Indications for 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 Elecsys and **cobas e** immunoassay analyzers.	CalCheck Vitamin D total II 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.
Analyte	25-hyroxyvitamin D_3	Same
Matrix	Human serum	Same
Levels	Five	Same

Feature	Predicate Device	Candidate Device
Target Ranges	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	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
Format	Lyophilized	Same
Handling	Reconstitute the contents of Check 1, Check 2, Check 3, Check 4 and Check 5 with exactly 1.0 mL distilled or deionized water. Allow the bottles to stand closed for 15 minutes. Mix gently by inversion to ensure homogeneity.	Same
Storage and Stability	Reconstituted control serum: • At 20-25°C – 5 hours	Same

4. NON-CLINICAL PERFORMANCE EVALUATION

Non-clinical performance evaluation for Elecsys Vitamin D total II executed with the study briefly summarized.

4.1. Precision

4.1.1. Repeatability and Intermediate Precision

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 each of two levels of control, PreciControl Vitamin D total II and five human sera per run, 2 runs per day for 21 days. 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).

4.2. Analytical Sensitivity

4.2.1. Limit of Blank (LoB)

Experimental Design included three reagent lots evaluated on one **cobas e** 411 analyzer, six runs over three or more days with one blank sample with ten replicates per run. The zero-level (blank) sample used was a Vitamin D depleted human serum sample pool. In total, 60 determinations for analyte free samples have been obtained. The LoB was calculated according to CLSI EP17-A2.

4.2.2. Limit of Detection (LoD)

Experimental Design included three reagent lots evaluated on one **cobas e** 411 analyzer, six runs over three or more days with five samples with two replicates/sample/run. The five samples were low-level human serum sample pools (diluted). A pooled estimate of the precision (SD total) for the 5 low-level samples was calculated. The LoD was calculated according to CLSI EP17-A2.

4.2.3. Limit of Quantitation (LoQ)

Experimental Design included three reagent lots evaluated on one **cobas e** 411 analyzer, one run per day over five days. Five replicates per each sample per run with at least four low level samples of serum, Li -heparin, K_2-EDTA and K_3-EDTA plasma and 25 replicates/sample/reagent lot.

The mean value and the intermediate precision (CV) and standard deviation (SD) for each LoQ sample were calculated.

LoQ samples were sorted according to the concentration of their measured mean value. LoQ is defined as the mean value of that sample which is the first that fulfills the specification for the intermediate precision and for which no sample with higher concentration exists that exceeds this specification.

4.3. Linearity/Assay Reportable Range

The linearity study was conducted on the Elecsys Vitamin D total II assay to demonstrate that measurements across the claimed measuring range for each parameter are linear. The study was performed according to CLSI guideline EP6-A using serum and plasma samples on the **cobas e** 411 immunoassay analyzer.

A high analyte serum and K_3-EDTA plasma sample (single donors, spiked) was diluted with a depleted low analyte human serum. At least 9 concentrations (dilutions) throughout the measuring range were prepared. Samples were assayed in 3-fold determination within a single run.

The linearity data were analyzed with regards to linear, quadratic and cubic polynomials according to CLSI EP6-A.

4.4. 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 in two-fold determination. One human serum sample was spiked with analyte to achieve a high 25-OH Vitamin D concentration of approx.10,000 ng/mL. A dilution series was performed using a low level analyte (Vitamin D depleted) serum. The hook concentration reported corresponds to the highest analyte concentration that generates a signal within the primary measuring range of the assay.

4.5. 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.

The specified HAMA-serum (HAMA L2) and the related basis serum (without interferent) was measured in two-fold determination each. Recovery of the HAMA-serum compared to the basis serum was calculated.

4.6. Endogenous Interferences

4.6.1. Hemolysis/Bilirubin/Lipemia/Biotin

The purpose of this study was to evaluate endogenous substances for potential interference with the parameters measured with the Elecsys Vitamin D total II on the **cobas e** 411 immunoassay analyzer using human serum samples (single donors, native as well as spiked).

For each interfering substance three human serum samples containing low, mid and high concentrations of 25-OH Vitamin D were tested in accordance with CLSI EP07-A2.

4.6.2. Triglycerides Interference

One aliquot of each serum sample was spiked with the interfering substance (Triglyceride concentrate), another aliquot was spiked with the same volume of isotonic NaCl solution (dilution pool). The interfering pool was then diluted into the dilution pool in 10% increments. The recovery for each sample was calculated by comparison to the reference sample.

4.6.3. Rheumatoid Factors Interference

One aliquot of each serum sample was spiked with the interfering substance, and another aliquot with the same volume of the solvent (buffer matrix) of the interfering substance (dilution pool). The interfering pool was then diluted into the dilution pool in 10% increments. The recovery for each sample was calculated by comparison to the reference sample.

4.6.4. Total Protein/Albumin/Immunoglobulin (IgG)/Cholesterol Interference

One aliquot of each serum sample was spiked with the interfering substances. Another aliquot without any additives (since the interfering substance is a lyophilisate) is used as dilution pool. The interfering pool was then diluted into the dilution pool in 10% increments.

4.7. Analytical Specificity/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 that occur in the human body during anabolism and catabolism. 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 Elecsys Vitamin D total II on the **cobas e** 411analyzer. The concentrations of the cross-reactants where chosen i) to ensure results within the measuring range depending on their expected cross-reactivity and ii) to reflect at least a >10 times higher concentration as typically found in native serum. Results from these spiked serum samples were matched against the unspiked references and the % cross-reactivity (normalized and non-normalized) was calculated.

The analysis was done in nmol/L to take into account i) the different molecular weights of the vitamin D metabolites and ii) the 1:1 equimolar binding of metabolite and vitamin D binding protein.

4.8. Exogenous Interferences – Drugs

The effect on quantitation of analyte in the presence of drugs was determined by comparing values obtained from samples spiked with 16 commonly and 3 specially used pharmaceutical compounds with the reference sample (unspiked). Two human serum samples (single donors,

native as well as spiked) were used and tested on the **cobas e** 411 immunoassay analyzer. The analyte concentration of the samples were approximately 30 and 70 ng/mL.

The drug concentrations tested are according to the recommendation (if available) given in the CLSI guideline EP7-A2. When concentrations are not given in the guideline, concentrations of at least 3-times of the maximum recommended daily dose were tested.

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 Elecsys Vitamin D total II concentration of the spiked aliquots was determined in 10-fold determination and compared to the Vitamin D total II result determined for the reference aliquot (also in 10-fold determination) on one **cobas e** 411 immunoassay analyzer.

4.9. Sample Matrix Comparison

The effect on quantitation of analyte in the presence of anticoagulants with the

Elecsys Vitamin D total II Immunoassay was determined by comparing values obtained from samples (single donors - native, diluted as well as spiked) drawn into serum, Li-Heparin, K_2-EDTA- , K_3-EDTA-plasma primary tubes and Plasma Gel Separation Tubes (Li-Heparin).

A minimum of 40 serum/plasma pairs per sample material were tested in singleton with one reagent lot on one **cobas e** 411 immunoassay analyzer. Potential effects are assessed by Passing/Bablok regression analysis.

4.10. Method Comparison to Predicate

A method comparison was performed using the Elecsys Vitamin D total II assay (candidate device, Y) and the Elecsys Vitamin D Assay (predicate device, X) to assess the bias between the two assays (for information only).

A total of 105 serum samples (single donors, native) were measured in singleton on the **cobas e** 411 analyzer in one run covering the entire measuring range. Vitamin D values ranged between 6.1 and 55.9 ng/mL for the Reference Method (X) according to the recommendations of CLSI EP9-A2.

4.11. Method Comparison to Reference Method

A total of 111 native single donor patient serum samples (reference sample set 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 in one run covering the entire measuring range. 25-hydroxyvitamin D values ranged between 5.64 and 92.8 ng/mL for the Reference Method.

4.12. Reagent Stability

To test reagent stability, three studies were executed with two studies completed including:

- Study 1: Reagent stability after first opening at 2-8°C (56 days)
- Study 2: On board reagent stability (28 days)
- Study 3: A real-time stability study is ongoing to support shelf-life stability claim.

4.12.1. Reagent Stability After First Opening

Reagent stability after first opening for the Elecsys Vitamin D total II assay was tested on one **cobas e** 411 immunoassay analyzer. A fresh reagent rackpack was placed on the analyzer and calibrated. Reference values for the samples tested were determined. After initial measurement the kit was removed from the analyzer and kept at 2-8 °C up to 64 days. After 64 days the kit was placed on the analyzer again, calibrated and the test samples were determined. Samples tested in duplicate include five human serum (HS) samples and two controls (PreciControl Vitamin D total II) for run qualification. The human serum samples used were all single donors (native as well as spiked).

4.12.2. On-board Reagent Stability

Reagent On-board Stability for the Elecsys Vitamin D total II assay was tested on one **cobas e** 411 immunoassay analyzer. A fresh Reagent Rack-Pack was placed on the analyzer and calibrated. All samples were measured on day 0. On day 8, 15, 22, 29 and day 36, the same samples were measured with the same reagent kit kept at 20°C ± 3°C (on-board condition) using the calibration curves established on day 0, 8, 15, 22 and 29, respectively. Samples tested include

five human serum (HS) samples and two controls (PreciControl Vitamin D total II) for run qualification. Each sample was tested in two-fold determination. The human serum samples used were all single donors (native as well as spiked).

4.12.3. Real-time Stability

In the ongoing real-time stability study, the Elecsys Vitamin D total II reagent is stored at 2-8°C. The stored assay reagents are tested at time point T=0 (after manufacturing) and at specified intervals over the shelf life of the device up to the planned shelf life plus one month. Testing will be performed using three human serum samples (stored at -80°C). The average on-test recovery value will be calculated as percent recovery compared to the reference value measured at T=0 for the human serum samples.

4.13. Calibrator Stability

Five studies were performed in order to verify the stability claims for the CalSet Vitamin D total II.

- Study 1: Stability at -20°C after reconstitution
- Study 2: Stability at 2-8°C after reconstitution
- Study 3: On-board stability at 20-25°C after reconstitution
- Study 4: Accelerated stability at 35°C
- Study 4: Real-time stability

4.13.1. Stability at -20°C after reconstitution

The on-test and reference materials were tested in duplicate on the **cobas e** 411 immunoassay analyzer. The on-test material was reconstituted and stored in closed vials for 14 weeks at -20°C. The on-test signal recovery was calculated as percent of the reference value.

4.13.2. Stability at 2-8°C after reconstitution

The on-test and reference materials were tested in duplicate on the **cobas e** 411 immunoassay analyzer. The on-test material was reconstituted and stored in closed vials for 73 hours at 2-8°C. The on-test signal recovery was calculated as percent of the reference value.

4.13.3. On-board stability at 20-25°C after reconstitution

The on-test and reference materials were tested in duplicate on the **cobas e** 411 immunoassay analyzer. The on-test material was reconstituted and stored in open vials for 7 hours at 20-25°C. The on-test signal recovery was calculated as percent of the reference value.

4.13.4. Accelerated stability at 35°C

The on-test and reference materials were tested in duplicate on the **cobas e** 411 immunoassay analyzer. The on-test material was stored lyophilized (as supplied to the user) at 35°C for 3 weeks. The reference material was a set of CalSet Vitamin D total II (stored at 2 - 8°C). After 3 weeks, the on-test and reference materials were tested in duplicate. The on-test signal recovery was calculated as a percent of the reference value.

4.13.5. Real-time stability

In the on-going real-time stability study, the CalSet Vitamin D total II test material is stored at 2-8°C. The CalSets are tested in duplicate at specified intervals over the shelf life of the device up to the planned shelf life plus one month (16 months).

Real-time stability is calculated based on the recovery of signal of stressed calibrator (stored at 2-8°C) vs. unstressed calibrator (stored at -80°C). At the specified intervals over the shelf life, the mean value of the stressed calibrator was calculated as percent recovery of the unstressed value (each tested in duplicates at the same time point).

4.14. PreciControl Vitamin D total II

4.14.1. Value Assignment

The PreciControl Vitamin D total II assigned values are determined with the Elecsys Vitamin D total II assay. 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). These Master Calibrators are for routine use in subsequent standardizations. 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 at least two (2) modules of the master analyzer platform. 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.

4.14.2. Stability

Three studies were performed in order to verify the stability claims for the PreciControl Vitamin D total II.

- Study 1: Stability after Reconstitution
- Study 2: Accelerated Stability
- Study 3: Real-time Stability

4.14.2.1. Stability after reconstitution

The on-test and reference materials were tested in duplicate. The on-test material was reconstituted and stored for 32 days at -20°C, 73 hours at 2 - 8ºC and for 6 hours at 20 - 25ºC. The reference material was freshly reconstituted PreciControl Vitamin D total II stored at 2 – 8ºC. The deviation of the on-test material compared to the reference value was calculated. The PreciControl Vitamin D total II was evaluated in duplicate on the **cobas e** 411 immunoassay analyzer.

4.14.2.2. Accelerated Stability

The on-test material was stored lyophilized (as supplied to the user) at 35°C for 3 weeks. The reference material was PreciControl Vitamin D total II stored at 2 – 8ºC. After 3 weeks, the on-test and reference material was tested in duplicate. The deviation of the on-test material compared to the reference value was calculated. The PreciControl Vitamin D total II was evaluated in duplicate on the **cobas e** 411 immunoassay analyzer.

4.14.2.3. Real-time Stability

In the on-going real-time stability study, the PreciControl Vitamin D total II on-test material is stored at 2 - 8°C. The controls are tested at specified intervals over the shelf life of the device up

to the planned shelf life plus one month. Data for the time-points after manufacturing, 7, 10, 13 and 16 months tested in duplicate will be available. The deviation of the on-test material compared to the reference value (stored at -80°C) will be calculated.

4.15. Calibration Stability

To test calibration stability, two studies were completed, including:

- Study 1: Lot calibration stability
- Study 2: On-board calibration stability

4.15.1. Lot Calibration Stability

Calibration of an Elecsys Vitamin D total II reagent lot is recommended every 12 weeks (3 months). During that time period fresh reagent kits of the same lot can be used without calibration using the calibration curve of the day 0 reagent kit. Elecsys Vitamin D total II was calibrated with a fresh reagent kit on day 0 using a **cobas e** 411 immunoassay analyzer. After 13 weeks a new reagent kit of the same lot was used and recovery of samples was determined using the calibration curve of day 0. Five human serum (HS) samples and two controls (PreciControl Vitamin D total II) for run qualification were tested; each sample was tested with two-fold determination. Pools of human serum samples were used (native as well as spiked).

4.15.2. On-board Calibration Stability

Elecsys Vitamin D total II reagent kits can be stored on board of the analyzers for up to 7 days without a new calibration. Reagent On-board Calibration stability for the Elecsys Vitamin D total II assay was tested on one **cobas e** 411 immunoassay analyzer. A fresh Reagent Rack-Pack was placed on the analyzer and calibrated. All samples were measured on day 0. On day 8 the same samples were measured with the same reagent kit kept at 20°C ± 3°C (on-board condition) using the calibration curve established on day 0. Samples tested include five human serum (HS) samples and two controls (PreciControl Vitamin D total II) for run qualification. Each sample was tested in two-fold determination. Pools of human serum samples were used (native as well as spiked).

4.16. CalCheck Vitamin D total II

The CalCheck Vitamin D total II matrix, human serum, is identical to the Master Calibrators (human serum sample panel) used for the Elecsys Vitamin D total II assay.

4.16.1. Value Assignment

For each CalCheck Vitamin D total II lot manufactured, the CalChecks are run in duplicate on at least two (2) modules 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.

4.16.2. Stability

Two studies were performed in order to verify the stability claims for the CalCheck Vitamin D total II.

- Study 1: Open Vial Stability
- Study 2: Accelerated Stability
- Study 3: Real-time Stability

4.16.2.1. Open Vial Stability

The on-test and reference materials were tested in duplicate. The on-test material was reconstituted and stored for 6 hours at 25ºC (in an open vial). The reference material was a freshly reconstituted set of CalChecks. The on-test recovery was calculated as a percent of the reference value.

4.16.2.2. Accelerated Stability

The on-test material was stored lyophilized (as supplied to the user) at 35°C for 3 weeks. The reference material was a set of CalChecks (stored at 2-8°C). After 3 weeks, the test and reference materials were tested in duplicate. The on-test recovery was calculated as a percent of the reference value. One CalCheck Vitamin D total II lot was evaluated in duplicate on the **cobas e** 411 immunoassay analyzer.

4.16.2.3. Real-time Stability

In the on-going real-time stability study, the CalCheck Vitamin D total II test material is stored at 2-8°C. The CalChecks are tested at T=0 and at specified intervals over the shelf life of the device up to the planned shelf life plus one month. For the lot stability, data for the time-points at 0, 7, 13 and 19 months tested in duplicate will be available. The average on-test recovery value will be calculated as percent recovery compared to the unstressed reference value (stored at -20°C).

4.17. Sample Stability

No new data was collected using the Elecsys Vitamin D total II assay. Sample stability data generated with the Elecsys Vitamin D Assay (k113546) is applicable since the claims and sample materials did not change (sample stability is not dependent on assay reagent formulation).

5. EXTERNAL (CLINICAL) TESTING

Clinical samples were collected at three US sites in order to establish the reference range values for the Elecsys Vitamin D total II assay. Serum samples were prospectively collected from approximately 200 apparently healthy adults at each of the three locations (northern site, midwest site and southern site) during summer and winter seasons (totaling approximately 600 Study Subjects). Study subjects were pre-screened to exclude subjects taking supplements or various drugs, those who were over or under weight, those with a medical history that might suggest variations in bone health, and pregnant and lactating women. Conditions such as parathyroid or thyroid disorders, and kidney disease, as well as electrolyte composition were determined by testing the samples. Those samples with properties outside of the published reference range for the particular test were excluded from the calculation of the Elecsys Vitamin D total II reference range study. They were assayed with Elecsys Vitamin D total II 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 is 7.61 – 55.5 ng/mL. The established reference ranges for Elecsys Vitamin D total II are provided below in Table 5.

Table 5: Results of 2.5th and 97.5th Percentiles for Elecsys Vitamin D total II

N	Mean	Median	2.5th Percentile (90 % CI[a)])	97.5th Percentile (90 % CI)	Units
421	25.8	23.6	7.61 (6.11, 8.44)	55.5 (52.4, 64.5)	ng/mL

a) Confidence interval

6. CONCLUSIONS

The information provided in this 510(k) Premarket Notification will support a determination of substantial equivalence for Elecsys Vitamin D total II, CalSet Vitamin D total II, PreciControl Vitamin D total II and CalCheck Vitamin D total II. The data supports a safe, effective device which performs as well or better than the predicate devices.