510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K200509

B Applicant

Siemens Healthcare Diagnostics Inc.

C Proprietary and Established Names

ADVIA Centaur® Vitamin D Total (VitD)

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:

Modified device to reinstate plasma (sodium heparin, lithium heparin, K_2 EDTA, K_3 EDTA) as a sample matrix.

B Measurand:

25-hydroxyvitamin D

C Type of Test:

Quantitative chemiluminescent

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The ADVIA Centaur® Vitamin D Total (VitD) assay is for in vitro diagnostic use in the quantitative determination of total 25(OH)vitamin D in human serum and plasma (EDTA, lithium heparin, sodium heparin) using ADVIA Centaur® analyzers. The ADVIA Centaur® VitD assay is intended as an aid in the determination of vitamin D sufficiency.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

ADVIA Centaur® XP analyzer

IV Device/System Characteristics:

A Device Description:

The ADVIA Centaur® Vitamin D reagent kit comes in two configurations (100 or 500 test kit) and each kit contains the following:

- ReadyPack® primary reagent pack containing ADVIA Centaur Vit D Lite Reagent, Solid Phase Reagent, and Ancillary Well Reagent
- ReadyPack ® ancillary pack containing ADVIA Centaur Vit D Ancillary Reagent
- ADVIA Centaur® Vit D Low Calibrator
- ADVIA Centaur® Vit D High Calibrator
- ADVIA Centaur® systems Vit D Master Curve card
- ADVIA Centaur® systems Vit D Calibrator Assigned Value Card

The Vit D Reagents consists of the following:

<u>Lite Reagent 5.0 mL/reagent pack</u>:
The reagent contains anti-Vit D (monoclonal mouse) antibody labeled with acridinium ester (~0.8 µg/mL) in buffer with bovine serum albumin, mouse IgG, and sodium azide (< 0.1%)

<u>Solid Phase Reagent 10.0 mL/reagent pack</u>:
The Solid Phase Reagent contains anti-fluorescein (monoclonal mouse)-coated paramagnetic particles (PMP) (~0.60 mg/mL) in buffer with bovine serum albumin, surfactant, and sodium azide (< 0.1%)

Ancillary Well Reagent 5.0 mL/reagent pack:
The Ancillary Well Reagent contains vitamin D-analog conjugated to fluorescein (~0.2 µg/mL) and 1-anilinonaphthalene-8-sulfonic acid in buffer with bovine serum albumin and sodium azide (< 0.1%)

Ancillary Reagent Pack 25.0 mL/reagent pack:
The Ancillary Reagent Pack contains releasing agent in buffered saline with sodium azide (<0.1%) and stabilizers

Material Required but Not Provided

- ADVIA Centaur® Wash 1

Optional Materials

- ADVIA Centaur® Vit D Quality Control Material
- ADVIA Centaur® Vit D Diluent
- ADVIA Centaur® Vit D Master Curve Material (MCM)

B Principle of Operation:

The ADVIA Centaur® Vit D assay is an 18-minute antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH)vitamin D monoclonal mouse antibody labeled with acridinium ester (AE), and a vitamin D analog labeled with fluorescein.

An inverse relationship exists between the total amount of 25(OH) vitamin D present in the patient sample and the amount of relative light units (RLU) detected by the system.

V Substantial Equivalence Information:

A Predicate Device Name(s):

ADVIA Centaur® Vitamin D Total (VitD) Assay

B Predicate 510(k) Number(s):

K133156

C Comparison with Predicate(s):

Device & Predicate Device(s):	K200509	K133156
Device Trade Name	ADVIA Centaur® Vitamin D Total (Vit D) assay	Same
General Device Characteristic Similarities		

Device & Predicate Device(s):	K200509	K133156
Intended Use/Indications For Use	For the quantitative determination of total 25(OH)vitamin D. The assay is intended as an aid in the determination of vitamin D sufficiency.	Same
Measurement	Quantitative	Same
Assay Principle	Competitive immunoassay	Same
Technology	Chemiluminescence	Same
Detection Antibody	Monoclonal mouse antibody labeled with acridium ester (AE)	Same
Capture Antibody	Anti-fluorescein labeled (FITC) monoclonal mouse antibody covalently bound to paramagnetic particles (PMP).	Same
Measuring Range	4.2 to 150 ng/mL	Same
General Device Characteristic Differences		
Ancillary Pack Reagent	Buffering agent (high molarity) and enhanced releasing agent (low molecular weight)	Buffering agent (low molarity) and releasing agent (high molecular weight)

VI Standards/Guidance Documents Referenced:

• CLSI. EP17-A2, 2012 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

• CLSI EP06-A, 2003 Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline – First Edition

• CLSI EP05-A3, 2014; Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

• CLSI EP34, 2018; Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking – First Edition

• CLSI EP28-A3c – formerly C28-A3c, 2010; Defining, Establishing and Verifying

Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition

• CLSI EP25-A, 2009; Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline– First Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Within run and total imprecision were evaluated by analyzing six serum samples at the concentrations below. Each sample was assayed in 2 replicates per run, 2 runs per day for 20 days for a total of 80 replicates per sample. Testing was performed with one reagent lot on one ADVIA Centaur® XP analyzer. Results are summarized below:

Sample	Mean (ng/mL)	Repeatability		Within-Lab	
		SD	% CV	SD	% CV
Serum 1	21.29	1.36	6.4	2.04	9.6
Serum 2	26.10	1.56	6.0	2.37	9.1
Serum 3	32.16	1.71	5.3	2.38	7.4
Serum 4	65.47	2.52	3.8	3.60	5.5
Serum 5	84.12	1.90	2.3	3.34	4.0
Serum 6	132.32	3.13	2.4	4.76	3.6

2. Linearity:

This study was conducted following the guidelines of CLSI EP06-A: Evaluation of the Linearity of Quantitative Measurement Procedures.

A high (>150 ng/mL) Vitamin D sample was made by spiking 25(OH) Vitamin D into a human serum sample. Vitamin D stripped human serum was used as the low sample. A dilution series was then made by mixing the high and low samples to encompass the measuring interval of the Vitamin D assay. Each resulting sample was run in triplicate on one reagent lot on one ADVIA Centaur® XP analyzer.

The sponsor concluded that the modified assay is linear from 2.78 ng/mL to 151.00 ng/mL on the ADVIA Centaur® XP analyzer. A measuring range from 4.2 – 150 ng/mL is claimed in the labeling.

Dilution Recovery:

To validate the labeling instructions that patient samples with Vitamin D levels above the claimed measuring range can be manually or automatically diluted 1:2, 5 unique serum samples were spiked with 25(OH) Vitamin D to produce concentrations ranging from 186 - 211 ng/mL. The samples were then diluted 1:2 with ADVIA Centaur® VitD diluent and assayed for recovery. The recoveries ranged from 97% to 109%.

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery %
1	1:2	96.0	97.5	98
2	1:2	96.3	93.0	103
3	1:2	102.6	103.5	99
4	1:2	102.5	105.5	97
5	1:2	102.1	93.5	109

3. Analytical Specificity/Interference:

   Assay specificity was established during the review of k133156.

4. Assay Reportable Range:

   The ADVIA Centaur® VitD assay measures 25(OH) vitamin D from concentrations of 4.2 to 150 ng/mL (10.5 to 375 nmol/L). The claimed measuring range is defined by the limit of quantitation (LoQ), linearity, and method comparison studies.

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

   The ADVIA Centaur® VitD calibrators are traceable to the NIST SRM972a 25-OH Vitamin D reference material.

6. Detection Limit:

   A limit of blank (LoB) study was conducted following the guidelines of CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures. Four blank samples with no Vitamin D were run n=6 for 6 days, 2 runs/day on 2 reagent lots for a total n=72 reps per sample per lot, on one ADVIA Centaur® XP analyzer. The LoB was calculated as 1.52 ng/mL.

   A limit of detection (LoD) study was conducted following the guidelines of CLSI EP17-A2. Four (4) low level samples were made by diluting individual patient samples with Vitamin D stripped serum to concentrations around the expected LoD. Samples were run n=6 for 5 days, 2 runs per day on 2 reagent lots on one ADVIA Centaur® XP analyzer for a total n=60 replicates per sample, and lot. The LoD was calculated as 3.11 ng/mL.

   A limit of quantitation (LoQ) study was conducted following the guidelines of CLSI EP17-A2. Thirteen (13) unique native serum specimens were diluted with Vitamin D stripped serum to produce concentrations ranging from 1.24 to 25.6 ng/mL, Samples were run n=6 for 5 days, 2 runs per day on two reagent lots, on one ADVIA Centaur® XP analyzer for a total n=60 replicates per sample, lot, platform, across 5 days. Data was analyzed 2 point using a stored calibration. LoQ was calculated by precision profile and was defined as the concentration at which the within-lab coefficient of variation was 20%. The LoQ was calculated as 4.24 ng/mL.

These studies support the following labeling claims:

Limit of blank	1.7 ng/mL
Limit of detection	3.2 ng/mL
Limit of quantitation	4.2 ng/mL

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

To compare results from the modified device to the predicate, one hundred twenty-six (126) serum samples (including 118 native and 8 contrived) were run in singlicate over 2 days using two lots of modified reagent and compared to the predicate assay on one ADVIA Centaur® XP analyzer. Contrived samples were made by spiking 25(OH) Vitamin D into individual samples to obtain Vitamin D levels at the high end of the Vitamin D assay measuring range. Results using Deming regression are summarized below:

Reagent Lot (y)	Reagent Lot 1	Reagent Lot 2
N	126	126
Range (ng/mL)	5.93 - 130.85	5.93 - 130.85
Intercept Slope	0.85 1.03	1.74 1.04
R Value	0.99	0.99

2. Matrix Comparison:

To validate use of the ADVIA Centaur® VitD assay with different claimed sample types, the sponsor evaluated 66 native and 8 contrived matched sample sets (serum, serum separator tube, lithium heparin, sodium heparin, K2 EDTA, K3 EDTA) ranging in concentration from 13.0 to 142.9 ng/mL. Samples were run along with calibrators and control on one ADVIA Centaur® XP analyzer with one lot of reagent. All samples were tested in replicates of 3 and the first replicate was used to perform a Weighted-Deming fit regression analysis comparing all tube types to serum collected in a plain tube without a serum separator. Pearson correlation coefficient (R) was calculated and the results are summarized below:

Tube Type	Slope	Intercept	R
Serum Separator	0.97	0.87	0.99
Sodium Heparin	1.02	-1.13	0.99
Lithium Heparin	0.99	-0.18	0.99
K_2 EDTA	0.97	0.64	0.99
K_3 EDTA	0.96	0.68	0.99

The results of this study support the use of the ADVIA Centaur® Vitamin D Total (VitD) assay with venous blood collected in serum tubes with or without a separator as well as plasma from venous blood collected in tubes with the following anticoagulants: sodium heparin, lithium heparin, K_2 EDTA, and K_3 EDTA.

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:

The sponsor performed a study referencing CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory to verify that the existing Reference Interval could be transferred to the modified Vitamin D assay. Twenty (20) samples from apparently healthy individuals were analyzed on one ADVIA Centaur® XP analyzer using one lot of the modified reagent. Results of the verification study and the performance studies support that the reference interval could be transferred to the modified reagent.

	Adults	**Pediatric (12 months up to 21 years)**
Median	22.5 ng/mL	23.8 ng/mL
Observed Range 2.5^{th} to 97.5^{th} Percentile	7.4 – 44.0 ng/mL	11.4 – 45.8 ng/mL

In addition, the labeling contains the following information on Vitamin D levels, and which is drawn from literature references:

Vitamin D Status	**Range, Adult ng/mL (nmol/L)**	**Range, Pediatric ng/mL (nmol/L)**
Deficiency	< 20 ng/mL (50 nmol/L)	< 15 ng/mL (37.5 nmol/L)
Insufficiency	20– < 30 ng/mL (50– < 75 nmol/L)	15 – < 20 ng/mL (37.5– < 50 nmol/L)

Vitamin D Status	Range, Adult ng/mL (nmol/L)	Range, Pediatric ng/mL (nmol/L)
Sufficiency	30–100 ng/mL (75–250 nmol/L)	20–100 ng/mL (50–250 nmol/L)

Holick MF. Vitamin D Deficiency. N Engl J Med. 2007;357:266–81.

Holick MF. MrOs is D-ficient. J Clin Endocrinol Metab. 2009;94(4):1092–3.

Rollins G. Vitamin D Testing–What's the Right Answer? Labs Grapple with Confusing Analytics, Evidence. Clinical Laboratory News. July 2009;35(7): 1,6.

Freeman R. Vitamin D: The sunshine hormone. How and when to treat deficiencies. Menopausal Medicine. May 2009; S8–11.

Misra M, Pacaud D, Petryk A, Collett-Solberg PF, Kappy M. Vitamin D Deficiency in Children and Its Management: Review of Current Knowledge and Recommendations. Pediatrics. 2008;122:398–417

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.