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

A. 510(k) Number:

k133156

B. Purpose for Submission:

Modification of traceability for standardization, and addition of pediatric reference range

C. Measurand:

25-hydroxyvitamin D

D. Type of Test:

Quantitative chemiluminescent

E. Applicant:

Siemens Healthcare Diagnostics

F. Proprietary and Established Names:

ADVIA Centaur Vitamin D Total (VitD) Assay

ADVIA Centaur Vitamin D Total (VitD) Calibrators

ADVIA Centaur Vitamin D Total (VitD) Quality Control (QC)

ADVIA Centaur Vitamin D Total Master (VitD) Curve Material

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1825, Vitamin D Test System

   21 CFR 862.1150, Calibrator

   21 CFR 862.1660, Quality Control Material (Assayed and Unassayed)

2. Classification:

Class II

Class II

Class I, reserved

3. Product code:

   MRG, Vitamin D Test System

   JJX, Single (specified) Analyte Controls

   JIT, Calibrator, secondary

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for use below

2. 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 the ADVIA Centaur XP system. The ADVIA Centaur VitD assay is intended as an aid in the determination of vitamin D sufficiency.

   The ADVIA Centaur Vitamin D Total (VitD) Calibrators are for in vitro diagnostic use in calibrating ADVIA Centaur systems Vitamin D Total (VitD) assay.

   The ADVIA Centaur Vitamin D Total (VitD) QC is for in vitro diagnostic use to monitor the precision and accuracy of the ADVIA Centaur VitD assay on the ADVIA Centaur systems.

   The ADVIA Centaur Vitamin D Total (VitD) Master Curve Material is for in vitro diagnostic use in the verification of calibration and reportable range of the ADVIA Centaur VitD assay.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   For use on the ADVIA Centaur XP instrument

I. Device Description:

The ADVIA Centaur VitD reagent kit comes in two configurations (100 or 500 test kit) and each kit consists of the following:

1. ReadyPack primary reagent:

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

   Solid Phase: 10.0 mL/reagent pack: 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: vitamin D-analog conjugated to fluorescein (~0.2 µg/mL) and 1-anilinoaphthatlene-8-sulfonic acid in buffer with bovine serum albumin and sodium azide (<0.1%).

2. The ADVIA Centaur Vitamin D Total calibrators are lyophilized human plasma with 2 levels of 25 (OH) vitamin D concentrations. After reconstitution, calibrators contain low or high levels of 25 (OH) vitamin D in buffered, defibrinated human plasma with bovine serum albumin, cholesterol, preservatives, and sodium azide (< 0.1%).

   The ADVIA Centaur Vitamin D Total QC and The ADVIA Centaur Master Curve Materials (MCM) are sold separately and are described below.

3. The ADVIA Centaur Vitamin D Total QC (sold separately) are lyophilized human plasma with 2 levels of 25 (OH) vitamin D concentrations. After reconstitution, calibrators contain low or high levels of 25 (OH) vitamin D in buffered, defibrinated human plasma with bovine serum albumin, cholesterol, preservatives, and sodium azide (< 0.1%).

4. The ADVIA Centaur Master Curve Materials (MCM) (sold separately) are lyophilized bovine serum albumin with 5 levels of 25 (OH) vitamin D concentrations. After reconstitution, materials contain different levels of 25 (OH) vitamin D in buffered, defibrinated human plasma with bovine serum albumin, cholesterol, preservatives, and sodium azide (< 0.1%).

   The ADVIA Centaur Vitamin D Total (VitD) calibrators, controls and MCMs were prepared from human blood components tested using FDA approved methods and shown to be negative for hepatitis B surface antigen, anti-hepatitis C and anti-HIV 1 and 2 antibodies.

   The ADVIA Centaur VitD calibrators, controls, and master curve verifier have been

previously cleared in k110586. There are no reagent or material changes for the calibrators, controls, and master curve verifier, just a change in the traceability. The calibrators are no longer sold in separate package but sold together with the reagent kit.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ADVIA Centaur Vitamin D Total (VitD) Assay, including calibrators, controls, and master curve verifiers.

2. Predicate 510(k) number(s):
   k110586

3. Comparison with predicate:

Assay:

Similarities		
Item	Candidate Device ADVIA Centaur VitD Assay (modified)	Predicate Device ADVIA Centaur VitD Assay (k110586)
Intended Use	For the 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 the ADVIA Centaur XP system.	Same
Indications for Use	This assay is intended to be used as an aid in the determination of vitamin D sufficiency.	Same
Sample type	Serum and Plasma	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
Assay Range	4.2-150 mg/mL	Same
Calibration	2 point	Same

Similarities		
Item	Candidate Device ADVIA Centaur VitD Assay (modified)	Predicate Device ADVIA Centaur VitD Assay (k110586)
Calibrators	ADVIA Centaur VitD Calibrators	Same

Differences		
Item	Candidate Device ADVIA Centaur VitD Assay (modified)	Predicate Device ADVIA Centaur VitD Assay (k110586)
Standardization/Traceability	Traceable to the ID-LC/MS/MS 25 (OH) vitamin D Reference Measurement Procedure (University of Ghent) via patient sample correlation	Traceable to an internal LC-MS/MS via patient sample correlation
Calibrators packaging	Provided with reagent kit	Provided separately
Expected Values (pediatric)	Pediatric:11.4 to 45.8 ng/mL	Pediatric: not provided
Expected Values (adult)	Adult: 7.4 to 44.0 ng/mL	Adult: 10.6-43.4 ng/mL

Controls:

Similarities		
Item	Candidate Device ADVIA Centaur VitD Controls (modified)	Predicate Device ADVIA Centaur VitD Controls (k110586)
Intended Use	For the in vitro diagnostic use to monitor the precision and accuracy of the ADVIA Centaur VitD assay on the ADVIA Centaur systems.	Same
Antigen	25 (OH) vitamin D_3	Same
Number of levels	2	Same
Matrix	lyophilized human plasma	Same
Packaging	Provided separately	Same
Storage temperature	2-8°C	Same

Differences		
Item	Candidate Device ADVIA Centaur VitD Controls (modified)	Predicate Device ADVIA Centaur VitD Controls (k110586)
Standardization/Traceability	Traceable to the ID-LC/MS/MS 25 (OH) vitamin D Reference Measurement Procedure (University of Ghent) via patient sample correlation	Traceable to an internal LC-MS/MS via patient sample correlation

Master curve materials (MCM):

Similarities		
Item	Candidate Device ADVIA Centaur VitD MCM (modified)	Predicate Device ADVIA Centaur VitD MCM (k110586)
Intended Use	For in vitro diagnostic use in the verification of calibration and reportable range of the ADVIA Centaur VitD assay	Same
Antigen	25 (OH) vitamin D	Same
Number of levels	5	Same
Matrix	Lyophilized human plasma	Same
Packaging	Provided separately	Same
Storage temperature	2-8°C	Same

Differences		
Item	Candidate Device ADVIA Centaur VitD MCM (modified)	Predicate device ADVIA Centaur VitD MCM (k110586)
Standardization/Traceability	Traceable to the ID-LC/MS/MS 25 (OH) vitamin D Reference Measurement Procedure (University of Ghent) via patient sample correlation	Traceable to an internal LC-MS/MS via patient sample correlation

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

CLSI Guideline EP5-A2: Evaluation of Precision Performance of Qualitative Measurement Methods

CLSI Guideline EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation.

CLSI Guideline EP6-A: Evaluation of the Linearity of Qualitative Measurement Methods

CLSI Guideline C28-A2: How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline

CLSI Guideline EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline

L. Test Principle:

The ADVIA Centaur Vitamin D Total assay is a one-pass 18 minute competitive immunoassay that uses an anti-fluorescein labeled (FITC) monoclonal antibody covalently bound to paramagnetic particles (PMP), one monoclonal antibody labeled with acridium ester (AE), and a vitamin D analog labeled with fluorescein. An inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLUs) detected by the system.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Within run and total imprecision were evaluated by testing two natural patient serum samples and four Medical Decision Pools (MDP) which consisted of 25 (OH) vitamin D spiked into human serum sample pools. Each sample was assayed in 2 replicates per run, 2 runs per day for 20 days for a total of 80 replicates. Testing was performed with two reagent lots on two analyzers. The precision results from a representative reagent lot are summarized below:

			Within Run		Total	
Sample	N	Mean (ng/mL)	SD	%CV	SD	%CV
Patient 1	80	13.6	0.64	4.7	1.61	11.9
Patient 2	80	17.2	0.91	5.3	1.70	9.9
MDP pool 1	80	28.2	1.45	5.2	2.02	7.2
MDP pool 2	80	46.1	1.79	3.9	2.79	6.1
MDP pool 3	80	73.2	2.71	3.7	4.36	6.0
MDP pool 4	80	114.1	3.44	3.0	4.71	4.2

b. Linearity/assay reportable range:

Dilutions were prepared using an individual patient serum sample with a 25 (OH)

vitamin D value of 22 ng/mL spiked with 25 (OH) vitamin D_3 to obtain a 25 (OH) vitamin D total value of approximately 180 ng/mL, and a low sample which was prepared by acid treating human serum followed by the addition of charcoal and centrifugation then spiked with low level 25(OH) vitamin D3 near the LoD of the assay. The high serum sample and the low sample were combined in different ratios to produce 9 dilutions covering the assay range. The dilutions were run in replicates of 3 on a single analyzer using 1 lot of reagent. Linearity was evaluated by calculating a linear regression comparing observed values versus expected values. Results of the percent recovery of all the samples are summarized below:

Sample	Expected concentration (ng/mL)	Observed concentration (ng/mL)	% Recovery
P1	2.92	2.92	100
P2	24.32	26.2	108
P3	45.72	42.83	94
P4	67.12	65.92	98
P5	88.52	87.06	98
P6	109.91	112.88	103
P7	131.31	124.19	95
P8	152.71	149.58	98
P9	174.11	174.11	100

A weighted least squares linear regression analysis resulted in the following equation:

y=0.99x + 0.04 r=0.999

The sponsor claims a measuring range of 4.2-150 ng/mL for the ADVIA Centaur Vitamin D assay.

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

Standardization and Traceability

The Siemens ADVIA Centaur Vitamin D Total was originally cleared under 510(k) k110586. Siemens has modified the assay by standardizing the cleared vitamin D assay in accordance with the Vitamin D Standardization Program (VDSP).The VDSP is an international collaborative effort to standardize the laboratory measurement of serum 25-OH vitamin D This collaboration involves the coordinated efforts of the National Institutes of Health, Office of Dietary Supplements (ODS), the Centers for Disease Control and Prevention (CDC), the National Institutes for Standards and Technology (NIST), Ghent University, and other institutions. Please refer to http://ods.od.nih.gov/Research/VitaminD.aspx for more information on the VDSP program.

To achieve standardization against the VDSP recognized Reference Measurement Procedure (RMP), one hundred and seventy unique serum samples were obtained with 25 (OH) vitamin D values assigned using the University of Ghent's ID-LC/MS/MS 25 (OH) vitamin D Reference Measurement Procedure (RMP). The values ranged from 5 to 100 ng/mL. Using these samples, a set of 10 standards have been manufactured whose values have been assigned by method correlation to the RMP. This value assignment has aligned the candidate device to the RMP. The RMP is traceable to the National Institute of Standards and Technology Standard Reference Material 2972.

The relationship between the ADVIA Centaur VitD Assay and ID-LC/MS/MS 25-OH vitamin D RMP is described using Deming regression:

$y=0.99\ x + 0.53$ ng/mL, $r=0.96$

The ADVIA Centaur Vitamin D Total assay (candidate device) has passed the certification process for the CDC VDSP and a certificate has been provided by the CDC. Please see the certification process at http://www.cdc.gov/labstandards/hs.html.

Value assignment:

The ADVIA Centaur VitD calibrators, controls, and master curve verifier have been previously cleared in k110586. There are no reagent or material changes for the calibrators, controls, and master curve verifier, just a change in the traceability.

Calibrators, 2-level controls (Level 1: 20 ng/mL and Level 2: 100 ng/ml) and MCMs are traceable to internal standards, which are traceable to the Ghent University ID-LC-MS/MS. The internal standards were value assigned via method correlation using clinical samples with the Ghent University ID-LC/MS/MS assigned values.

Production lots of calibrators, controls and master curve materials (MCM) are value assigned against the internal standards using two reagent lots, 2 runs on two different instruments for a total of 24 replicates. The average dose for each calibrator, control and MCM assigns their value.

Stability:
Shelf life stability studies: Real-time stability studies were performed for the control materials, MCM and the calibrators. The stability study protocol and the acceptance criteria have been reviewed and found to be acceptable. The real time ongoing stability study supports a stability of 26 weeks (6 months) when materials are stored at 2-8^{o} C.

Open vial stability: The stability study protocol and the acceptance criteria to determine open-vial stability of the control materials, calibrators, and the MCMs have been reviewed and found to be acceptable. Controls, calibrators, and MCMs are stable for up to 120 days when stored at -20^{o} C, 28 days when stored at 2-8^{o} C, and up to 7 days stability when stored at 25^{o} C.

d. Detection limit:

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined following CLSI Document EP17-A2 guidelines. The LoD is defined as the lowest concentration of 25 (OH) vitamin D that can be detected with 95% probability. LoB was performed using 5 treated human serum samples and LoD was performed using 6 low serum samples. All samples were assayed with 4 replicates per run, 2 runs per day, for over 5 days, over 2 lots of reagent and on 2 analyzers. LoQ was determined using 6 low human samples (25(OH) vitamin D total values 1.4 to 13 ng/mL), over 4 days, 2 runs per day, 4 replicates, using 2 lots of reagent on 1 analyzer for a total of 40 replicates per sample. LoQ was defined as the lowest concentration with an inter-assay precision of ≤20% CV.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
1.7 ng/mL	3.2 ng/mL	4.2 ng/mL

The ADVIA vitamin D assay has a measuring range of 4.2 to 150 ng/mL.

e. Analytical specificity:

See k110586

An additional interferent, Fluorescein, was detected since k110586 was cleared.

An interference study was conducted by the sponsor to characterize the interference with sodium fluorescein in two steps, which includes an initial screening step and a dose response confirmation step. At the first step, sodium fluorescein was spiked into a serum sample with a 25(OH) vitamin D concentration of approximately 22 ng/mL at the following levels: 250 mcg/mL, 2.5 mcg/mL, 0.025 mcg/mL and 0.00025 mcg/mL. The samples were tested in triplicate on the ADVIA Centaur XP. The spiked samples were compared to the unspiked samples. Fluorescein interference was seen at fluorescein levels of 250 mcg/mL and 2.5mcg/mL.

To more accurately determine the level of fluorescein that would no longer interfere with the assay, a dose response testing was performed. Sodium fluorescein was spiked into 2 serum pools with a 25(OH) vitamin D concentration of approximately 25 ng/mL and 49 ng/mL at serially diluted levels: 3.13, 1.56, 0.78, 0.39, 0.20, and 0.10 mcg/mL. These samples were tested along with control samples which did not contain fluorescein. The sponsor defined non-significant interference as the level of fluorescein that will result in ≤ 10% bias. The results of the study are summarized in the table below:

Fluorescein mcg/mL	25 (OH) Vit D Concentration ng/mL	Percent Interference
0	25.2	NA
3.13	129.3	414%
1.56	67.6	169%
0.78	42.8	70%
0.39	32.2	28%
0.20	28.4	13%
0.10	27.2	8%
0	49.1	NA
3.13	>150	NA
1.56	118.7	142%
0.78	78.9	61%
0.39	60.4	23%
0.20	55.7	14%
0.10	52.7	7%

The sponsor concluded that the study demonstrated that at a sodium fluorescein level of ≥ 0.10 mcg/mL will cause significant interference.

The following limitation statement has been added to the labeling:

"Do not use samples which contain fluorescein. Fluorescein levels > 0.10 µg/mL can produce falsely elevated results in this assay. Interference testing with varying levels of sodium fluorescein as high as 3.13 ug/mL at clinically relevant concentrations of 25(OH) vitamin D (~ 25 – 50 ng/mL) can result in 25(OH) vitamin D concentrations to be greater than 150 ng/mL. Evidence suggests that patients undergoing retinal fluorescein angiography can retain amounts of fluorescein in the body for up to 48 to 72 hours post-treatment. In the cases of patients with renal insufficiency, including many diabetics, retention could be much longer. Such samples can produce falsely elevated values when tested with this assay, and should not be tested. *"

* Inloes R, Clark, D and Drobnies A: Interference of fluorescein, used in retinal angiography with certain clinical laboratory tests. Clin Chem 1987, 33:2126-2127.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the modified (standardized) ADVIA Centaur Vitamin D assay (candidate device) to the non-standardized ADVIA Centaur Vitamin D assay (predicate device), one hundred and seventy-seven native human serum samples with 25 (OH) vitamin D values ranging from 5.0 ng/ml to 140.0 ng/mL were assayed in singlicate using one reagent lot of the candidate device and predicate device. Deming regression was used for the regression analysis and the results are summarized below:

y= 0.78x + 3.34, r=0.99

The slope showed an approximate bias of -22% between the candidate device and the predicate device; however, this shift is expected because the purpose of the device modification was to adjust the calibration to better align with the VDSP reference sample concentration target levels. Therefore, test results from the candidate device do not, and are not expected to, directly correlate with test results from the predicate device. Laboratories that use the ADVIA Centaur Vitamin D assay should be aware of the significantly different performance of the modified assay.

An additional method comparison study was conducted to evaluate the accuracy between the candidate device and the RMP, University of Ghent's ID-LC/MS/MS method. The method comparison against the RMP was the basis of the substantial equivalence determination.

A method comparison study was performed to compare the 25-OH vitamin D concentrations of serum samples using the candidate device and the University of Ghent ID-LC/MS/MS 25-OH vitamin D Reference Measurement Procedure (RMP). One hundred and twenty-two independent serum samples, composed of 116 native and 6 spiked samples, with RMP assigned concentrations ranging from 7.8 ng/mL to 148.1 ng/mL were assayed in a singlicate using one lot of the candidate device. Deming regression was used for the regression analysis and results are summarized below:

y= 0.93x + 2.89, r=0.99

Results correlate well around medical decision range of 10 to 30 ng/mL; however results above 50 ng/mL starts to show some scatter. However, the scatter in that part of the assay range is clinically insignificant.

b. Matrix comparison:

See k110586.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

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

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Expected Values for Adults:
A reference range study was conducted using adult subjects aged between 21 to 93 years. Samples from 291 apparently healthy adults from the North and South of the U.S. were tested. These samples were collected during different seasons (summer and winter). The samples numbers were split evenly between the North and South, summer and winter, and with and without supplements containing vitamin D. Samples were only included in this study if the samples had normal PTH, TSH, and calcium values. Based on the 95% confidence interval, the following values were established following CLSI guideline C28-A2.

Median 25(OH) vitamin D	Observed Range (2.5^{th} to 97.5^{th} percentile)
22.5 ng/L	7.4 to 44.0 ng/mL

Expected Values for Pediatric patients:

A reference range study was conducted using samples collected from 237 pediatric patients aged between 1 year and up to 21 years. These samples were collected from apparently healthy pediatric subjects from the North and South of the U.S. as well as from different seasons (summer and winter). Serum samples from pediatric patients meeting the following criteria were included:

1. Subjects aged 1 year up to 21-year-old.

2. No active chronic diseases, with the exception of allergic diseases for which no allergy medication has been taken within the last 7 days preceding sample collection.

3. No active acute disease.

4. No prescribed medications in the 7 days preceding sample collection.

5. No contraceptive medication in the 90 days preceding sample collection.

6. No over the counter medications in the 7 days preceding sample collection.

7. No suspicion of premature of delayed puberty, based on physical examination.

8. No pregnant female subjects.

9. Ages 1 yr to 2yr: Length, weight, and weight for length all within the 10^{th} and 90^{th} percentiles, based on WHO growth charts.

10. < 2 yr: Weight, height, and BMI all within the 10^{th} and 90^{th} percentiles based on CDC growth charts.

11. Serum iPTH, TSH levels within published normal ranges

12. Only subjects taking < 1000 IU/Day of Vitamin D supplement

13. Only subjects with no personal or family history of thyroid disease.

14. Serum, iPTH and TSH, levels with published normal ranges.

15. Specimens with serum hemoglobin ≤ 155 mg/dL.

Based on the 95% confidence interval, the following expected values for pediatric patients were established following CLSI guideline C28-A2.

Median 25(OH) vitamin D	23.4 ng/mL
Observed Range 2.5^{th} to 97.5^{th} percentile	11.4 ng/mL to 46.0 ng/mL

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.