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

**A. 510(k) Number:**

k110586

**B. Purpose for Submission:**

New device

**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 (Vit D) Assay
ADVIA Centaur Vitamin D Total (Vit D) Calibrators
ADVIA Centaur Vitamin D Total (Vit D) QC
ADVIA Centaur Vitamin D Total (Vit D) Master Curve Material

**G. Regulatory Information:**

| Product Code | Classification | Regulation Section | Panel |
|---|---|---|---|
| MRG | Class II | 21 CFR 862.1825<br>Vitamin D Test System | Clinical Chemistry (75) |
| JIS | Class II | 21 CFR 862.1150<br>Calibrator | Clinical Chemistry (75) |
| JJX | Class I, reserved | 21 CFR 862.1660<br>Quality Control Material | 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 is for *in vitr*o 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:

   The ADVIA Centaur XP instrument

## I. Device Description:

The ADVIA Centaur VitD ReadyPack primary reagent pack consists of the following reagents:

1. **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 and sodium azide (< 0.1%).
2. **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%).
3. **Ancillary Well Reagent:** 5.0 mL/reagent pack: vitamin D-analog conjugated to fluorescein (~0.2 µg/mL) in buffer with bovine serum albumin and sodium azide (< 0.1%).
4. **VitD Ancillary Pack Reagent:** 25.0 mL/reagent pack releasing agent in buffered saline with sodium azide (< 0.1%).

The ADVIA Centaur Vitamin D Total calibrators are lyophilized human plasma with 2 levels of 25OHD 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 are lyophilized human plasma with 2 levels of 25OHD 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 Master Curve Materials (MCM) are lyophilized bovine serum

albumin with 5 levels of 25OHD 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%) .

These 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.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

   IDS OCTEIA 25-Hydroxy Vitamin D kit - method, calibrator and controls, VALIDATE Thyroid Calibration Verification Test Set

2. Predicate 510(k) number(s):
   k021163
   k062501

3. Comparison with predicate:

| **Similarities** | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay | Predicate (k021163) IDS 25-OH Vitamin D EIA assay |
| Intended use/Indications for use | 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). It is intended as an aid in the determination of vitamin D sufficiency. | same |
| Sample type | Serum and plasma | same |
| Reagent storage temperature | 2 – 8 $^{o}$C | same |
| Interpretation of results | Standard curve | same |
| Expected values | Ranges established for healthy adults:<br><br>10.6 – 43.4 ng/mL | Ranges established for healthy adults<br><br>19.1 ng/mL to 57.6 ng/mL |

| Similarities | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay | Predicate (k021163) IDS 25-OH Vitamin D EIA assay |
| | n = 542 | n = 36 |

| Differences | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay | Predicate (k021163) IDS 25-OH Vitamin D EIA assay |
| Platform | ADVIA Centaur XP | ELISA plate read on a plate reader |
| Assay principle | Chemiluminescence | ELISA |
| Assay procedure | Automated | Manual |
| Approximate Assay time | 18 minute | 3.5 hours |
| Traceability | Traceable to LC-MS/MS | Standardized using UV quantification of 25-(OH) vitamin |

| Similarities | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay calibrators | Predicate (k021163) IDS 25-OH Vitamin D EIA assay calibrators |
| Intended use | For *in vitr*o diagnostic use in calibrating Vitamin D Total (VitD) assay. | same |
| Antigen used in calibrators | 25-(OH) vitamin $D_3$ | same |
| Storage temperature | 2-8°C | same |

| Differences | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay calibrators | Predicate (k021163) IDS 25-OH Vitamin D EIA assay calibrators |
| Number of calibrators | 2 | 6 |
| Matrix | Lyophilized human plasma | Lyophilized human serum |

| Similarities | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay controls | IDS 25-OH Vitamin D EIA assay controls (Predicate Device) k021163 |
| Intended Use | For *in vitro* diagnostic use to monitor the precision and accuracy of the VitD assay. | same |
| Antigen used in controls | 25-(OH) vitamin $D_3$ | same |
| Number of vials | 2 | same |
| Storage temperature | 2-8°C | same |

| Differences | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay controls | IDS 25-OH Vitamin D EIA assay controls (Predicate Device) k021163 |
| Matrix | Lyophilized human plasma | Lyophilized human serum |

| Similarities and Differences | | |
|---|---|---|
| Item | ADVIA Centaur Vitamin D Total assay Master Curve Materials (MCM) | VALIDATE Thyroid Calibration Verification Test Set (k062501) |
| Intended use | It is for in vitro diagnostic use in the verification of calibration and reportable range of the assay. | same |
| Number of levels | 5 | same |
| Measurands | Total vitamin D test | Thyroid tests |

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

CLSI EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*

CLSI EP6-A2, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach*

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

CLSI EP17-A, *Protocols for Determination of Limits of Detection and Limits of Quantitation*
CLSI C28-A2, *How to Define and Determine Reference Interval in the Clinical Laboratory*

## L. Test Principle:

The ADVIA Centaur Vitamin D Total assay is a one-pass, 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 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:*

Precision was evaluated according to the CLSI Document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*. 2 patient samples (~12.0 and 18.0 ng/mL) and 4 medical decision pools (~32.0, 50.0, 55.8 and 132.0 ng/mL) were assayed twice a day in replicates of 4 using 3 lots of reagents on one system for 20 days. These studies were performed by 2 operators. Results are summarized below:

| | Data Points | Mean | Within run | | Between run | | Between day | | Total precision | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| Serum sample 1 | 160 | 11.66 | 0.81 | 7.0 | 0.68 | 5.8 | 0.75 | 6.4 | 1.30 | 11.1 |
| Serum sample 2 | 160 | 18.04 | 1.20 | 6.6 | 0.98 | 5.4 | 0.79 | 4.4 | 1.74 | 9.6 |
| Serum pool 1 | 160 | 32.40 | 1.87 | 5.8 | 1.68 | 5.2 | 1.93 | 6.0 | 3.17 | 9.8 |
| Serum pool 2 | 160 | 49.89 | 2.22 | 4.5 | 1.95 | 3.9 | 2.80 | 5.6 | 4.07 | 8.2 |
| Serum pool 3 | 160 | 55.75 | 2.66 | 4.8 | 1.73 | 3.1 | 3.02 | 5.4 | 4.38 | 7.8 |
| Serum pool 4 | 160 | 132.05 | 3.53 | 2.7 | 3.21 | 2.4 | 4.16 | 3.2 | 6.33 | 4.8 |

*b. Linearity/assay reportable range:*

A linearity study was performed across the assay measuring range using a high and a low sample. The samples were prepared using a patient serum pool

prescreened for Vitamin D levels >200ng/mL and a low concentration sample containing BSA and cholesterol. The high serum pool sample and the low sample were combined in different ratios to produce 11 dilutions covering the assay range. 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 | Observed concentration | % Recovery |
|---|---|---|---|
| 1 | 3.8 | 3.8 | 100.0 |
| 2 | 8.5 | 8.8 | 103.3 |
| 3 | 13.1 | 13.8 | 105.7 |
| 4 | 22.3 | 23.4 | 104.7 |
| 5 | 40.8 | 40.7 | 99.8 |
| 6 | 59.3 | 58.8 | 99.2 |
| 7 | 77.8 | 73.6 | 94.6 |
| 8 | 96.3 | 92.9 | 96.4 |
| 9 | 114.8 | 112.1 | 97.6 |
| 10 | 133.3 | 134.5 | 100.9 |
| 11 | 151.8 | 151.8 | 100.0 |

The measured vs. expected linear regression analysis for all samples generated a linear regression as follows:

$y = 1.026x – 3.586$, $r^2 = 0.997$

The linear study data supports the sponsor's claim that the measuring range of the Vitamin D assay is 4.2 to 150 ng/mL.

Dilution study:

Additional studies were performed to demonstrate that the samples with concentrations greater than 150mg/dL using a 1:2 dilution can be accurately measured by the ADVIA Centaur Vitamin D Total assay. These studies were performed with eight serum samples that were diluted 1:2 with ADVIA Centaur Vitamin D Total assay diluent. All samples were run in triplicate. Theoretical doses were calculated by dividing the measured dose for the undiluted sample by the dilution factor. The percent recoveries were determined by dividing the observed dose by the theoretical dose. The percent recovery was from 90% to 110%. Results are summarized below:

| Sample | Concentration | Dilution | Observed concentration (ng/mL) | Expected concentration (ng/mL) | % Recovery |
|---|---|---|---|---|---|
| 1 | 154.4 | 1:2 | 76.9 | 77.2 | 100 |
| 2 | 139.0 | 1:2 | 67.6 | 69.5 | 97 |
| 3 | 191.2 | 1:2 | 93.0 | 95.6 | 97 |
| 4 | 178.0 | 1:2 | 83.5 | 89.0 | 94 |
| 5 | 237.1 | 1:2 | 124.8 | 118.6 | 105 |
| 6 | 232.0 | 1:2 | 126.2 | 116.0 | 109 |
| 7 | 186.1 | 1:2 | 96.3 | 93.1 | 103 |
| 8 | 171.1 | 1:2 | 84.2 | 85.6 | 98 |

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

Traceability and value assignment:
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 LC-MS/MS. The internal standards were value assigned via method correlation using clinical samples with LC/MS/MS values. The relationship between the ADVIA Centaur Vitamin D assay internal standards and liquid chromatography coupled with tandem mass spectroscopy is described using linear regression as:
ADVIA Centaur VitD=1.01(LC/MS/MS) + 8.9, r=0.99

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.

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, MCM, and the calibrators have been reviewed and found to be acceptable. Calibrators, MCM, and controls 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 the Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ**)** for the ADVIA Centaur Vitamin D Total assay was

determined in accordance with the CLSI guideline EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*.

LoB was performed using 3 blank samples in replicates of 4 were assayed using 2 reagent lots and 2 instruments, 2 runs per day, over ten operating days generating 320 measurements. The LoB was determined to be 1.60 ng/mL, by calculating the 95$^{th}$ percentile of the distribution of the test values.

LoD was performed using seven samples prepared by diluting a patient sample with charcoal stripped human serum to concentrations ranging from 1.6 to 12.1 ng/mL. 7 samples in replicates of 4 were assayed using 2 reagent lots and 2 instruments, 2 runs per day, over ten operating days generating 320 measurements. The LoD was estimated to be 3.3 ng/mL.

LoQ was performed using four low samples. 4 samples were analyzed using 2 reagent lots and 2 instruments, 2 runs per over 10 days generating 320 measurements. LoQ was 4.2 ng/mL when %CV was 18.3 (inter assay precision n=320).

The measuring range of the ADVIA Centaur Vitamin D Total assay is 4.2 - 150ng/mL.

e. *Analytical specificity:*
Interference:
The sponsor performed studies to evaluate the effects of endogenous compounds (such as hemoglobin, cholesterol, uric acid, human IgG, triglycerides, unconjugated and conjugated bilirubin) on the performance of the ADVIA Centaur Vitamin D Total assay, following CLSI EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline.* Testing was done in the presence of 20 – 106 ng/mL 25(OH) vitamin D and different concentrations of the listed compounds. All samples were run in triplicate. Percent recovery was calculated relative to control samples containing vitamin D without spiked endogenous compounds. The table below lists all substances tested at concentrations with non-significant (<10%) interference defined by the sponsor when compared to the control samples:

| Compound | Concentration tested |
|---|---|
| Bilirubin (conjugated) | 40 mg/dL |
| Bilirubin (unconjugated) | 40 mg/dL |
| Cholesterol | 350 mg/dL |
| Hemoglobin | 155 mg/dL |
| Human immunoglobulin | 12 g/dL |
| Triglycerides | 540 mg/dL |
| Uric acid | 20 mg/dL |

Based on the study data, the sponsor stated in their labeling that hemolyzed sample should not be tested with the vitamin D assay.

Cross reactivity:
The sponsor performed studies to estimate if compounds similar to 25-hydroxy Vitamin D (25(OH) D2 and D3) cross react with the ADVIA Centaur Vitamin D Total assay. These studies were performed in accordance with CLSI EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline.* Specificity of the ADVIA Centaur Vitamin D Total assay was determined by adding the cross reactants to 2 medical decision serum pools containing varying amounts of 25(OH) vitamin D, approximately 35.0, 55ng/mL and 115 ng/mL. Each test sample was compared to a matched unspiked serum pool. The study was carried out using 2 reagent lots and each sample was run in triplicate. Based on the results summarized in the below table, the sponsor concluded that the assay did not significantly cross react with Vitamin D2, Vitamin D3, 1,25-OH Vitamin D2, 1,25-OH vitamin D3, 3-epi-25-OH vitamin D3 and Paricalcitol (Zemplar). The assay recovers both 25-OH Vitamin D3 and 25-OH vitamin D2.

| Cross-Reactant | Vitamin D2 | Vitamin D3 | 25-OH Vitamin D2 | 25-OH Vitamin D3 | 1,25-OH Vitamin D2 | 1,25-OH Vitamin D3 | 3-epi-25-OH vitamin D3 | Paricalcitol (Zemplar) |
|---|---|---|---|---|---|---|---|---|
| Spiked (ng/mL) | 1000 | 1000 | 30.0 | 30.0 | 100.0 | 100.0 | 100.0 | 24.0 |
| Cross Reactivity | 0.5% | 0.3% | 104.5% | 100.7% | 4.0% | 1.0% | 1.1% | 0.1% |

*f. Assay cut-off:*

Not applicable

2. Comparison studies:

*a. Method comparison with predicate device:*

The sponsor performed studies to compare the performance of the ADVIA Centaur Vitamin D Total assay with the performance of the predicate IDS assay (k021163). A total of 196 specimens in the range of 6.2 to 150 ng/mL, were used in this comparison. Results of Deming regression analysis are summarized below:

| x axis | y axis | n | r | Slope | Intercept |
|---|---|---|---|---|---|
| Predicate | Advia Centaur | 196 | 0.96 | 0.98 | 3.05 |

*b. Matrix comparison:*

The sponsor performed studies to compare the performance of the ADVIA Centaur Vitamin D Total assay with different sample matrices. Specifically, 200 commercially obtained matched sets of serum (red top serum tube), SST serum, EDTA, lithium heparin and sodium heparin in the range of 11.9 to 136.9 ng/mL were used in these studies. Correlation was performed on the 231 matched sets comparing the serum samples with the other tube types. Linear regression analysis is summarized below:

| Tube type | N | r | Slope | Intercept |
|---|---|---|---|---|
| Serum vs. SST | 231 | 0.994 | 1.01 | -0.33 |
| Serum vs. EDTA | 231 | 0.993 | 1.09 | -0.17 |
| Serum vs. Lithium Heparin | 231 | 0.992 | 1.04 | 0.18 |
| Serum vs. Sodium Heparin | 231 | 0.992 | 1.04 | 0.90 |

Based on the study data, the sponsor claims that EDTA, lithium heparin, and sodium heparin are acceptable anti-coagulants for the vitamin D assay.

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):

Sample Stability Study:
This study was performed to monitor the stability of serum and plasma samples for use with the ADVIA Centaur Vitamin D Total Assay. Specifically, samples ranging from 24.99 ng/mL to 124.10 ng/mL were examined after being frozen and thawed 3, 6, 9, and 12 times. Based on the results from this study, the sponsor concluded that the samples subjected to up to 12 freeze/thaw cycles can be safely used with the ADVIA Centaur Vitamin D Total Assay.

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:
   To determine a reference range for the ADVIA Centaur Vitamin D Total assay, samples obtained from 542 apparently healthy adults from the North and South of the U.S. were examined. These samples were collected during different weather 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. 258 adults took supplement containing vitamin D and 284 adults took supplement containing vitamin D. Approximately 100 samples per group were collected. Samples were only included in this study if the samples had normal PTH, TSH, calcium, magnesium and phosphorus values. Two ranges based on the 95% confidence intervals were determined following CLSI C28-A2. The samples were run in singleton on the ADVIA Centaur Vitamin D Total assay. The following results were obtained:

   Expected Ranges for adults: (n = 542)

| Observed values | |
|---|---|
| Median 25 (OH) vitamin D | 21.1 ng/mL (52.8 nmol/L) |
| Observed Range 2.5th to 97.5th percentile | 10.6 – 43.4 ng/mL (26.5 – 108.5 nmol/L) |

## 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.