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

A. 510(k) Number:

k162298

B. Purpose for Submission:

New Device

C. Measurand:

25-hydroxyvitamin D

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Siemens Healthcare Diagnostics

F. Proprietary and Established Names:

LOCI Vitamin D Total Assay

LOCI VITD CAL

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825	Clinical Chemistry (75)
JIT	Class II	21 CFR 862.1150	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indication for use below.

2. Indication(s) for use:

   The LOCI Vitamin D Total Assay is an *in vitro* diagnostic test for the quantitative measurement of total 25-hydroxyvitamin D (25-OH-D) in human serum and plasma on the Dimension® EXL™integrated chemistry system with LOCI® Module. Measurements of vitamin D are used in the assessment of vitamin D sufficiency.

   The LOCI VITD CAL is an *in vitro* diagnostic product for the calibration of the Vitamin D (VITD) Total assay on the Dimension® EXL™ integrated chemistry system with LOCI® module.

3. Special conditions for use statement(s):

   For Prescription use only.

4. Special instrument requirements:

   Dimension® EXL™ integrated chemistry system with LOCI® Module

I. Device Description:

The reagent cartridge of the LOCI VITD Total Assay consists of wells numbered consecutively from the wide end of the cartridge and includes the following:

Wells	Matrix	Ingredients	Volume	Concentration	Source
1-2 (Reagent 4)	Liquid	Releasing Reagent	25 tests per well		
3-4 (Reagent 1)	Liquid	Biotinylated antibody	25 tests per well	0.75µg/mL	Sheep Monoclonal
5-6 (Reagent 2)	Liquid	Chemibeads	25 tests per well	200 µg/mL	Sheep Monoclonal
7-8 (Reagent 3)	Liquid	Sensibeads	25 tests per well	500 µg/mL	

LOCI VITD CAL is a five level, single analyte frozen liquid human serum based product. The calibrator level 1 is a zero level, while levels 2, 3, 4, and 5 contain approximately 12, 30, 75, and 165 ng/mL respectively.

Each donor unit used in the preparation of this product was tested by FDA- approved methods for the presence of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), as well as for Hepatitis B surface Antigen and antibody to Hepatitis C (HCV), and found to be negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   ADVIA Centaur Vitamin D Total (VitD) Assay
   ADVIA Centaur Vitamin D Total (VitD) Calibrators

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

   k110586

3. Comparison with predicate:

Similarities/Differences		
Item	Candidate Device LOCI Vitamin D Total Assay (k162298)	Predicate Device ADVIA Centaur Vitamin D Total Assay (k110586)
Intended Use	For the quantitative measurement of total 25-hydroxyvitamin D in human serum and plasma. Measurements of Vitamin D are used in the assessment of Vitamin D sufficiency.	Same
Assay format	Competitive immunoassay	Same
Detection	Direct chemiluminescent technology	Same
Antibody	Sheep monoclonal	Mouse monoclonal
Sample size	8µL	20µL
Analyzers	Dimension EXL with LM, Dimension EXL with LM-PMT and Dimension EXL 200 systems	ADVIA Centaur, ADVIA Centaur XP, and ADVIA Centaur XPT systems
Sample type	Serum, K2 EDTA plasma, and lithium heparin plasma	Serum plasma (EDTA, lithium heparin and sodium heparin)
Measuring Range	5.0 - 150.0 ng/mL	4.2 to 150 ng/mL

Similarities/Differences		
Item	Candidate device LOCI VITD CAL (k162298)	Predicate device ADVIA Centaur VitaminD Total Calibrators (k110586)
Intended Use	For the calibration of the total Vitamin D total (VitD) assay.	Same
Calibrator base	Human serum	Human plasma
Calibrator form	Liquid	Lyophilized

Similarities/Differences		
Item	Candidate device LOCI VITD CAL (k162298)	Predicate device ADVIA Centaur VitaminD Total Calibrators (k110586)
Traceable to:	Internal standards which are traceable to the ID-LC-MS/MS 25(OH)vitamin D Reference Method Procedure (RMP)	Internal standards which are traceable to the LC-MS/MS 25(OH)vitamin D.
Number of levels	Five	Two
Packaging Content	2 vials: Level 1 (1.5 mL), Level 2 (2.0 mL), Level 3, 4, 5, (1.5 mL) per vial	2 vials Low - 2 mL per vial; 2 vials High -2 mL per vial
Storage	-25 to -15°C	2° to 8°C

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

- Clinical and Laboratory Standards Institute (CLSI) Guideline EP09-A2: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Second Edition.
- CLSI Guideline EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition.
- CLSI Guideline EP-6A: Evaluation of Linearity of Quantitative Measurement Procedures: a Statistical approach; Approved Guideline.
- CLSI Guideline EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition.
- CLSI Guideline EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition.
- CLSI Guideline EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition.
- CLSI Guideline EP25: An Evaluation of Stability of InVitro Diagnostic Reagents; Approved Guideline.

L. Test Principle:

The LOCI Vitamin D Total assay is a homogeneous competitive chemiluminescent immunoassay based on LOCI technology. The assay measures total 25 (OH) vitamin D concentration comprising of both 25(OH) vitamin D2 and 25(OH) vitamin D3 in both serum and plasma. LOCI Vitamin D Total Assay reagents include the releasing reagent, biotinylated monoclonal antibody, and two synthetic bead reagents. The patient sample is incubated with the releasing reagent to release 25(OH) vitamin D molecules from vitamin D-binding proteins. The reaction mixture is then incubated with biotinylated antibody to form a 25(OH) vitamin

D/biotinylated antibody complex. Chemibeads coated with a 25(OH) vitamin D3 analog and chemiluminescent dye are added to remove the excess free biotinylated antibody. Streptavidin-coated sensibeads containing a photosensitive dye are added to bind the biotinylated antibody. Aggregates of the Chemibead analog/biotinylated antibody/streptavidin sensibeads are formed as a result. Illumination of the reaction mixture by light at 680 nm generates singlet oxygen from the sensibeads, which diffuses into the chemibeads and triggers a chemiluminescent reaction. The resulting chemiluminescent signal is measured at 612 nm and is inversely proportional to the concentration of total 25(OH) vitamin D in the sample.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

a. Precision/Reproducibility:

Precision testing was performed in accordance with CLSI EP05-A2. The samples used consisted of three serum based commercial quality controls with mean vitamin D concentration values of 18.9, 38.7, 89.6 ng/mL, respectively. A total of four patient samples were used, including three serum samples with mean concentration values of 8.2, 29.4 ng/mL, and 76.54 and one K2 EDTA plasma sample with a mean concentration value of 25.2 ng/mL. Testing was performed over twenty days, for two runs per day for a total of 80 replicates for each sample. Single tests from two independent cups were analyzed for each test material using one lot of the reagent.

Results obtained are summarized below:

Samples	N	Mean	Repeatability		Within-Lab Precision	
		ng/mL	SD	%CV	SD	%CV
QC (Low)	80	18.9	0.58	3.1	1.01	5.4
QC (Level 1)	80	38.7	1.02	2.6	2.02	5.2
QC (Level 2)	80	89.6	1.72	1.9	3.67	4.1
Serum 1	80	8.2	0.46	5.6	0.71	8.7
Serum 2	80	29.4	0.76	2.6	1.46	5.0
Serum 3	80	76.5	1.63	2.1	3.11	4.1
Plasma	80	25.2	0.44	1.8	0.78	3.1

b. Linearity/assay reportable range:

A linearity study was performed according to CLSI EP 06-A to assess the linearity of the LOCI VIT D Total Assay across the assay measuring ranges (5 to 150 ng/mL). A serum sample with a high concentration of vitamin D was serially diluted with a low concentration serum sample to generate nine samples with vitamin D concentration values of 4.4, 24.7, 44.9, 65.1, 85.4, 105.6, 125.8, 146.1 and 163.3 ng/mL respectively. Each dilution was assayed in replicates of five and a linear regression

analysis of the data was performed. The results of the linear regression analyses are summarized below:

$y = 1.0222x + 1.3862$, $r^2 = 0.998$

The linearity study data support the claimed measuring range of 5.0 to 150 ng/mL.

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

LOCI VITD CAL Calibrator Traceability:

The assay is standardized through 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), the LOCI VITD CAL master calibration parameters were aligned to the CDC VDSP by using 163 human serum samples from the CDC VSDP program which were value assigned by the RMP, Ghent University ID-LC-MS/MS and traceable to NIST SRM2972.

Value Assignment for calibrator:

Target values are assigned to each lot of calibrator from the master pool using the Dimension EXL with Loci ®Module system. Subsequent LOCI VITD CAL calibrators will be generated based on previous master pool values obtained by ID-LC-MS/MS and adjusted if the mean recovery percent bias exceeds the manufacturer's acceptance criteria. The commercial lot of LOCI VITD CAL are made from the master pool to have the following target values.
Level 1 = 0 ng/mL
Level 2 = 12 mg/mL
Level 3 = 30 ng/mL
Level 4 = 75 ng/mL
Level 5 = 165 ng/mL

Stability of Calibrator:

Protocols and acceptance criteria for the calibrator stability study were reviewed and found to be acceptable to support the manufacturer's following stability claims for the LOCI VITD CAL.

Shelf life stability: 12 months stored at -15^{0}C to 25^{0}C.
Opened bottle stability: 30 days at 2-8^{0}C.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) studies were performed according to the CLSI EP-17-A2 guideline. LoB study was performed with five blank samples composed of serum-based calibrator 1 at 0 ng/mL and tested over three days using three reagent lots on one instrument for a total of 60 measurements per lot. The LoB was determined to be 0.8 ng/mL.

The LoD study was performed with five low samples prepared using 3 native serum samples diluted with different lots of calibrator Level 1 and tested over three days using 3 reagent lots on one instrument for a total of 60 measurements per lot. The LoD was determined to be 1.3 ng/mL.

The LoQ study was performed with five low serum samples between 4-5 ng/mL of vitamin D concentrations. The samples were tested for 3 days, one run per day, in 3 replicates per run, with three reagent flex lots on one instrument. These generated 27 data points.

LoQ was determined to be 5.0 ng/mL based on total precision (≤20%) using all measurements observed on the low serum samples.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
0.8 ng/mL	1.3 ng/mL	5.0 ng/mL

The measuring range for the assay is 5.0-150 ng/mL.

e. Analytical specificity:

Interference testing was performed according to CLSI EP07-A2 to determine the effect of various endogenous and exogenous substances on the LOCI Vitamin D Total assay. For all interferents, the percent bias was determined by testing a control sample without the interferent and comparing it to the value obtained from a test sample to which the potential interferent had been added. Interferents were tested at three levels of vitamin D concentrations: 13.9-16.9 ng/mL, 28.4-32.0 ng/mL, and 70.2-77.3 ng/mL. The spiked and control samples were assayed with 5 replicates each.

The table below lists all substances tested at concentrations with non-significant (<10%) interference as defined by the sponsor, when compared to the control sample.

Substances	Highest Concentration tested that did not demonstrate significant interference
Acetaminophen	20 mg/dL
Ascorbic Acid	3 mg/dL
Biotin	200 ng/mL
Cholesterol	300 mg/dL
Dextran 40	6000 mg/dL
Heparin	3U/mL
Ibuprofen	30 mg/dL
Lipemia (intralipid)	300 mg/dL
Protein(Albumin)	5 g/dL
Protein (Total)	15.9 g/dL
Rheumatoid Factor	500 IU/mL
Salicyclic Acid	65 mg/dL
Triglycerides	686 mg/dL
Uric Acid	20 mg/dL
Bilirubin (conjugated)	20 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Hemoglobin(hemolysate)	250 mg/dL

Based on the study data, the sponsor states the following in their labeling:

- Total Protein at 19 g/dL causes a decrease in the vitamin D concentration by 6% at 14.3 ng/mL, 11% at 30.9 ng/mL, and 12% at 77.3 ng/mL.
- Hemoglobin at 500 mg/dL causes an increase in the vitamin D concentration by 11% at 15 ng/mL, 19% at 31.7 ng/mL, and 13% at 75.1 ng/mL.
- Unconjugated bilirubin at 80 mg/dL causes an increase in the vitamin D concentration by 13% at 13.5 ng/mL, 12% at 27.3 ng/mL, and 6% at 66.7 ng/mL.
- Conjugated bilirubin at 40 mg/dL causes an increase in vitamin D concentration by 17% at 14.9 ng/mL, 12% at 31.7 ng/mL, and 11% at 75.3 ng/mL.
- Lipemia (Intralipid) at 500 mg/dL causes a decrease in vitamin D concentration by 10% at 14.9 ng/mL, 11% at 32.3 ng/mL and 80% at 75.1 ng/mL.
- Cholesterol at 350 mg/dL causes a decrease in vitamin D concentration by 10% at 25.2 ng/mL and 12% at 64.0 mg/dL.
- Paricalcitol (Zemplar) at 24 ng/mL has shown a 93.8% cross-reactivity at 28.8 ng/mL of vitamin D and 70.8% cross-reactivity at 54.7 ng/mL of vitamin D.

To test for interference from Human Anti-Mouse Antibodies (HAMA), 20 patient samples containing vitamin D (at 5.0 ng/mL to 87.5 ng/mL) and varying

concentrations of HAMA (ranging from 8 ng/mL to 161,000 ng/mL), were spiked at a 1:10 ratio with control or neutralizing test solution made of several non-specific binding (NSB) inhibitory agents. Samples were tested in 5 replicates, and the average vitamin D values were obtained. The bias and % bias of each sample were calculated using the equations:

Bias = Test – control
%Bias = 100 x (Test – control)/control

The study results support the claim that HAMA up to 106 ng/mL does not interfere with LOCI Vitamin D Total Assay.

Cross Reactivity:

A cross reactivity study was performed according to CLSI EP07-A2 to determine the effect of various cross reactive substances on the LOCI Vitamin D Total assay. Cross reactivity was examined as a percentage ratio of observed analyte concentration versus a cross-reactant concentration. Testing was performed using two serum samples containing Vitamin D at medical decision levels of 25-30 ng/mL and 50-60 ng/mL respectively. Cross-reactant compounds were spiked into separate serum samples at a defined test concentration. Control samples were prepared by adding the same fixed volume of diluent to the base samples. Samples were processed and % Cross-reactivity was determined as:
% Cross Reactivity = [(mean test result – mean control result)/concentration of cross-reactant compound] x100.

The results of the study are summarized in table below:

Cross-Reactant	Cross-reactant Concentration (ng/mL)	% Cross-reactivity	
		Vitamin D conc. 25-30 ng/mL	Vitamin D conc. 50-60 ng/mL
Vitamin D_2 (ergocalciferol)	1000	0.1	-0.1
Vitamin D_3(cholecalciferol)	1000	- 0.1	0.1
1,25-dihydroxyvitamin D_2	0.5	151.9	1.3
1,25-dihydroxyvitamin D_3	0.5	1.7	-224.5
3-epi 25-hydroxyvitamin D_3	100	3.9	2.5
1αOHVitaminD_3 (alfacalcidol)	3000	0.1	0
24, 25$(OH)_2$ vitamin D_3	60	2.5	1.2
Paricalcitol	24	93.8	70.8
25(OH) vitamin D_2	30	95.1	94.1
25 (OH) vitamin D_3	30	88.6	90.1

f. Assay cut-off:

Not applicable.

2. Comparison studies*:*

a. Method Comparison with predicate *device:*

A method comparison study was performed in accordance to CLSI EP09-A3 to evaluate the accuracy between LOCI Vitamin D Total Assay on the Dimension EXL with LOCI® Module system against the reference method procedure (RMP), University of Ghent's ID-LC-MS/MS. On hundred and sixty three human serum samples (154 native and 9 contrived) with value assigned concentration of 5.2 to 150 ng/mL by ID-LC-MS/MS RMP (Ghent University) were tested with the candidate device. The results were analyzed by standard Passing Bablok regression yielding the following results:

n	Sample Range (ng/mL)	Slope (95%CI)	Intercept (95%CI)	r-Value
163	5.2 - 126.1	1.06 (1.01 to 1.12)	0.4 (-0.54 to1.42)	0.977

b. Matrix comparison:

Studies were performed to compare the performance of the LOCI Vitamin D Total assay with different sample matrices. Matched lithium heparin plasma, K2 EDTA plasma, serum separator tube (SST) and red-top serum samples were collected from 70 patients. Out of the 70 matched serum/plasma sets, 62 were native samples and eight were spiked to cover the upper range of the assay. The results were analyzed by standard Passing Bablok regression and Least Squares (Standard Linear Regression). A summary of the results is presented below.

Tube Type	Slope	Intercept ng/mL	Correlation Coefficient	n	Test range ng/mL
Lithium heparin plasma vs serum (red-top)	0.991	0.13	0.992	70	11.5 – 146.5
K2EDTA plasma vs serum (red top)	0.982	1.37	0.997	70	11.6 – 142.5
Serum SST vs serum (red top)	0.989	0.48	0.997	70	11.4 – 148.6

The study supports that Lithium heparin, Serum, K2EDTA plasma are acceptable sample types to use with this assay.

3. <u>Clinical studies:</u>

a. Clinical Sensitivity:

Not applicable.

b. Clinical specificity:

Not applicable.

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

Not applicable.

4. Clinical cut-off:

Not applicable.

5. Expected Values/Reference range:

To determine a reference range for LOCI Vitamin D Total assay, blood was drawn and tested from two hundred and fifty-two healthy adults ranging from 21 to 93 years of age. This study was performed in accordance with CLSI EP28-A3c. The samples were collected from apparently healthy subject from North, Central and South of the U.S as well as from different seasons (spring, summer and winter).These individuals were not taking Vitamin D supplements of more than 2000 IU. Specimens with abnormal PTH, TSH, Calcium, Magnesium and Phosphorus were excluded from the data. The central 95th percentile encompassed results from the 2.5th -97.5th ranks. The following values were obtained:

Expected ranges for adults (n=252)

Observed values	
Median 25(OH) vitamin D	28.3 ng/mL
Observed Range 2.5th to 97.5th percentile	13.9 - 61.0 ng/mL

The sponsor includes the following in the package insert labeling: "To assure proper representation of specific population, each laboratory should establish its own reference intervals."

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.