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

A. 510(k) Number:

k142362

B. Purpose for Submission:

New device

C. Measurand:

25-hydroxyvitamin D

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Beckman Coulter Inc.

F. Proprietary and Established Names:

Access 25(OH) Vitamin D Total Assay for use on the UniCel DxI Immunoassay System
Access 25(OH) Vitamin D Total Calibrator for use on the UniCel DxI Immunoassay System

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1825, Vitamin D Test System
   21 CFR 862.1150, Calibrator

2. Classification:

   Class II

3. Product code:

   MRG
   JIT

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See Indications for Use below.

2. Indication(s) for use:

   The Access 25(OH) Vitamin D Total assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total 25-hydroxyvitamin D [25(OH) vitamin D] levels in human serum and plasma using the UniCel DxI Immunoassay Systems. Results are to be used as an aid in the assessment of vitamin D sufficiency.

   The Access 25(OH) Vitamin D Total Calibrators are intended to calibrate the Access 25(OH) Vitamin D Total assay for the quantitative determination of total 25-hydroxyvitamin D [25(OH) vitamin D] levels in human serum and plasma using the UniCel DxI Immunoassay Systems.

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   The UniCel DxI 800 System

I. Device Description:

The Access 25(OH) Vitamin D Total assay consists of the reagent pack and calibrators. Other items needed to run the assay include substrate and wash buffers. The Access 25(OH) Vitamin D Total assay reagent pack, Access 25(OH) Vitamin D Total assay calibrators, along with the Access wash buffer and substrate are designed for use with the UniCel DxI Immunoassay Analyzers in a clinical laboratory setting.

Reagent kit:
The Access 25(OH) Vitamin D Total reagent kit consists of two reagent packs. Each reagent pack contains four reagents:

- R1a: Paramagnetic particles coated with sheep monoclonal anti-25(OH) vitamin D antibody suspended in TRIS buffered saline, goat IgG, bovine serum albumin (BSA),< 0.1% sodium azide, and 0.1% ProClin 300.
- R1b: Formic Acid, Poly (vinyl alcohol) and 0.1% ProClin 300.
- R1c: Formic Acid, Poly (vinyl alcohol) and 0.1% ProClin 300.

- R1d: Vitamin D analog-alkaline phosphatase conjugate, ACES, <0.1% sodium azide, and 0.1% ProClin 300.

Each reagent pack contains enough materials for 50 tests. Reagent packs are stored at 2-10°C

Calibrator kit:

The calibrator set provides calibrators at six levels – zero and approximately 6, 17, 37, 87 and 210 ng/mL (0, 15, 43, 93, 218 and 525 nmol/L). The calibrators are prepared gravimetrically from Human Serum with 25(OH) vitamin D. A description of the calibrators is provided below.

- S0: Human Serum, <0.1% sodium azide, and 0.1% ProClin 300
- S1-S5: Human Serum with 25(OH) vitamin D levels of approximately 6, 17, 37, 87 and 210 ng/mL (15, 43, 93, 218 and 525 nmol/L), <0.1% sodium azide, and 0.1% ProClin 300.

The calibration card, included with each kit, contains the bar code that provides the instrument with the individual concentrations for each calibrator level and which Assay Protocol File to run for this particular assay.

Human source material used in the preparation of the reagent has been tested and found negative or non-reactive for Hepatitis B, Hepatitis C (HCV) and Human Immunodeficiency Virus (HIV-1 and HIV-2).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   DiaSorin LIAISON 25 OH Vitamin D Total Assay
   DiaSorin LIAISON 25 OH Vitamin D Total Calibrators

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

   k112725

3. Comparison with predicate:

   Vitamin D assay:

Similarities		
Item	Predicate (k112725) The LIAISON® 25 OH Vitamin D TOTAL Assay	The Access 25(OH) Vitamin D Total Assay
Intended Use	For the quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites in human serum.	Same

Similarities		
Item	Predicate (k112725) The LIAISON® 25 OH Vitamin D TOTAL Assay	The Access 25(OH) Vitamin D Total Assay
Format	Chemiluminescent immunoassay	Same
Method	Automated	Same
Product Type	Reagent	Same
Assay Components	Reagent pack, calibrators	Same
Calibration	Utilizes a stored calibration curve	Same
Reportable Units	ng/ml	Same

Differences		
Item	Predicate (k112725) The LIAISON® 25 OH Vitamin D TOTAL Assay	The Access 25(OH) Vitamin D Total Assay (candidate device)
Sample Type	Serum	Serum and Li Hep Plasma
Instrument	DiaSorin LIAISON	UniCel DxI Immunoassay System
Reagent closed vial stability	2 to 8°C until the expiration date	2 to 10°C until the expiration date
Reagent open vial stability	2 to 10°C for up to 4 weeks after opening	2 to 10°C for up to 28 days after opening
Antibody	Goat polyclonal anti-25(OH) vitamin D	Sheep monoclonal anti-25(OH) vitamin D
Measuring Range (ng/mL)	4-150 ng/mL	7.0-120 ng/mL
Standardization	Standard prep: UV ε	NIST-Ghent ID-LCMS/ MS

Vitamin D Calibrators Similarities and Differences		
Item	Predicate (k112725) The LIAISON® 25 OH Vitamin D TOTAL Calibrators	Access 25(OH) Vitamin D Total Calibrators
Intended Use	Calibrators are intended to calibrate the 25(OH) Vitamin D assay.	Same
Calibrators Formulation	25-OH-D Horse serum, phosphate, surfactants, NaN3	Human Serum with 25(OH) vitamin D
Levels	2 levels Low and High	6 levels 0 and approximately 6, 17, 37, 87 and 210 ng/mL

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

- CLSI EP5-A2: *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline –Second Edition*
- CLSI EP7-A2: *Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition*
- CLSI EP17-A2: *Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline -Second Edition*
- CLSI EP9-A2: *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Second Edition (Interim Revision)*
- CLSI EP25-A: *Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline*
- CLSI EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline*

L. Test Principle:

The Access 25(OH) Vitamin D Total assay is a two-step competitive binding immunoenzymatic assay. In the initial incubation, sample is added to a reaction vessel with a vitamin D binding protein (DBP) releasing agent and paramagnetic particles coated with sheep monoclonal anti-25(OH) vitamin D antibody. 25(OH) vitamin D is released from DBP and binds to the immobilized monoclonal anti-25(OH) vitamin D on the solid phase. Subsequently, a 25(OH) vitamin D analogue-alkaline phosphatase conjugate is added which competes for binding to the immobilized monoclonal anti-25(OH) vitamin D. After a second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of 25(OH) vitamin D in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

M. Performance Characteristics (if/when applicable):

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

   a. Precision/Reproducibility:

   Precision was evaluated according to the CLSI Document EP5-A2, *Evaluation of Precision Performance of Quantitative Measurement Methods*. Four patient samples with different concentrations were run in duplicates over 20 days (2 runs per day) for a total of 40 runs and 80 replicates per sample. The study was run at an internal site on three Unicel DxI 800 instruments, using three reagent pack lots, one calibrator lot and one lot of reagent pack per instrument. The study results of representative reagent lot are presented in the table below:

Instrument 1 and Reagent lot 1

25 (OH) vitamin D (ng/mL)			Within-run		Between-run		Total	
Sample	N	Mean	SD (ng/ml)	%CV	SD (ng/ml)	%CV	SD (ng/ml)	%CV
Sample 1	80	15.6	0.7	4.6%	1.3	8.1%	1.5	9.3%
Sample 2	80	26.0	1.2	4.7%	1.5	5.7%	1.9	7.4%
Sample 3	80	53.0	1.6	3.0%	3.5	6.5%	3.8	7.2%
Sample 4	80	110.9	3.3	3.0%	6.5	5.9%	7.3	6.6%

b. Linearity/assay reportable range:

The linearity study was carried out according to the CLSI EP6-A, *Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.*
A linearity study was performed across the assay measuring range using a high and a low sample. Additionally, seven evenly spaced dilutions were created by mixing the high and low samples to cover the range of the assay of 7.0 ng/mL - 120 ng/mL. The study was carried out on three UniCel DxI 800 instruments, with three reagent pack lots and one calibrator lot. The low and high samples were run in replicates of eight while the rest of the dilutions were run in replicates of four. The linearity study results using one representative reagent lot with the polynomial regression equations are summarized and presented in the table below.

<u>Reagent Lot 1 Calibrator Lot 1 Regression</u>
Linear fit:
Observed= -0.446295 + 1.0992361*Expected
R^2 = 0.996641
Polynomial fit degree 2:
Observed= -0.758483 + 1.159853*Expected- 0.0008362*(Expected-6.76296)^2
R^2 =0.997396
Polynomial fit degree 3:
Observed= -0.073718 + 1.0080187*Expected + 0.0051549*(Expected-6.76296)^2- 4.2283e-5*(Expected-6. 76296)^3
R^2 =0.999426

Reagent Lot 1, Calibrator Lot 1					
Observed (ng/mL)	Expected (ng/mL)	Linear fit (ng/mL)	Cubic fit (ng/mL)	Cubic Absolute Difference	Cubic Relative % Difference
4.42	4.42	4.41	4.41	0.0	0.0%
20.96	20.46	22.05	21.41	-0.6	-2.9%
40.38	36.51	39.68	40.17	0.5	1.2%
62.00	52.55	57.32	59.65	2.3	4.1%
77.31	68.6	74.96	78.79	3.8	5.1%
97.49	84.64	92.59	96.54	4.0	4.3%
108.36	100.69	110.23	111.86	1.6	1.5%
123.85	116.73	127.87	123.71	-4.2	-3.3%
132.78	132.78	145.5	131.02	-14.5	-10.0%

The linear study data supports the sponsor's claim that the measuring range of the Vitamin D assay is 7 to 120 ng/mL.

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

Traceability:
The calibrators are traceable to a Joint Committee for Traceability in Laboratory Medicine (JCTLM) - approved isotope dilution mass spectrometry (ID-LC-MS/MS) reference method procedure (RMP) developed at Ghent University. This RMP is further traceable to the NIST SRM 2972.

The traceability of Access 25(OH) Vitamin D Total Calibrators for the UniCel DxI was verified using a panel of forty (40) serum samples from the Vitamin D Standardization-Certification Program that were assigned by the ID-LC-MS/MS RMP for Vitamin D from Ghent University (Termed VDSCP samples). The traceability verification study was run on two UniCel Dxl 800 instruments using two reagent pack lots and three calibrator lots. Each sample was tested in singleton on two instruments. The correlation between the two methods was analyzed by fitting the observed Access 25 (OH) Vitamin D into a linear regression model using a weighted Deming method. The results of weighted Deming regression are presented below:

N	Range (ng/mL)	Slope	Intercept	R
40	9.0-79.2	0.97	-1.88	0.96

Calibrator value assignment:
Primary reference calibrators are prepared from 25(0H) Vitamin D and human serum. A stock is prepared by volumetrically mixing the 25(0H) Vitamin D to a known concentration based on the ID-LC-MS/MS reference method. The stock is diluted to designated (assigned) concentrations using serum based matrix. Primary working calibrators are prepared at six calibrator levels; zero, and approximately 6, 17, 37, 87 and 210 ng/mL.

Product (commercial) calibrators are value assigned using the Primary reference calibrators on the Access 2 Immunoassay System through an internal procedure. Verification of these assigned values is performed in a statistical comparison of calibration curves generated by both the Primary reference and Product calibrators at concentrations spanning the measuring interval. The product calibrators are prepared at six levels; zero, and approximately 6, 17, 37, 87 and 210 ng/mL.

Calibrator stability:
The sponsor claims that the calibrators are stable until the expiration date when stored unopened at -15 - 30°C. Once opened, the sponsor claims that the calibrators are stable for 56 days when stored at 2 to 8°C.

The sponsor recommends use of commercially available controls to assess validity of the 25(OH) Vitamin D assay in the labeling.

d. Detection limit:

LoB:
LoB was tested using a protocol based on CLSI EP17-A2, *Protocols for Determination of Limits of Detection and Limit of Quantitation; Approved Guideline*. A total of 156 replicates of a zero analyte sample (Access 25(OH) Vitamin D Total Calibrator S0) were measured in 12 runs on three UniCel DxI 800 instruments, using three reagent pack lots and two calibrator lots. This study determined the LoB for the Access 25(OH) Vitamin D assay to be 0.98 ng/mL.

LoD:
LoD was tested using a protocol based on CLSI EP17-A2, *Protocols for Determination of Limits of Detection and Limit of Quantitation; Approved Guideline*. Three replicates from five low-level diluted patient samples were measured in 12 runs on three UniCel DxI 800 instruments, using three reagent pack lots and two calibrator lots. A total of 180 replicates were generated over 4 days. This study determined the LoD for the Access 25(OH) Vitamin D assay to be 1.47 ng/mL.

LoQ:
LoQ was tested using a protocol based on CLSI EP17-A2, *Protocols for Determination of Limits of Detection and Limit of Quantitation; Approved Guideline*. Three replicates of 3 naïve and 7 diluted patient samples (sample range from 1.0 to 16 ng/mL) were measured on three UniCel DxI 800 instruments, using three reagent pack lots and one calibrator lot in 22 runs. The mean concentration and total percent coefficient of variation (%CV) were calculated for each sample on each day for multiple days. LoQ was determined as the lowest concentration with a total imprecision ≤ 20% CV obtained using 3 different lots of reagent. This study determined the LoQ for the Access 25(OH) Vitamin D assay to be 4.4 ng/mL with a ≤ 20% CV.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
0.98 ng/mL	1.47 ng/mL	4.4 ng/mL

The measuring range of the Beckman Access 25(OH) Vitamin D Total assay is 7 – 120 ng/mL. The sponsor's claimed LoQ of the assay is 7 ng/mL.

e. Analytical specificity:

Interference:

The sponsor performed studies to evaluate the effects of potential interferents 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, 40 and 150 ng/mL 25(OH) vitamin D and different concentrations of the listed compounds. Eight replicates were tested for each control and spiked sample preparation at ~20 ng/mL and five replicates were tested for each control and spiked sample preparation at ~40 and ~150 ng/mL. 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:

Substance	Highest Concentration Added
Acetaminophen	20 mg/dL
Bilirubin (conjugated and unconjugated)	40 mg/dL
Acetylsalicylic Acid	65 mg/dL
Hemoglobin	50 mg/dL
Cholesterol	500 mg/dL
Heparin (low molecular weight)	3 U/mL
Ibuprofen	30 mg/dL
Rheumatoid factor	200 IU/mL
Protein (Gamma Globulin)	6 g/dL
Triglycerides	3280 mg/dL
Uric Acid	24 mg/dL
L-Ascorbic Acid	3 mg/dL
D-Biotin	180 ng/mL

The sponsor has the following limitations in their labeling:

"Vitamin D Levels should not be tested in patients who have received Paricalcitol within 24 hours of obtaining the sample"

Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies (e.g. human anti-sheep antibodies) may be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

The limitations section states that heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays.

The specimen collection section states: "Do not assay hemolyzed samples. Hemoglobin concentrations greater than 50 mg/dL may lead to falsely elevated results."

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 Access 25(OH) Vitamin D Total assay. These studies were performed in accordance with CLSI EP7-A2, *Interference Testing in Clinical Chemistry; Approved Guideline.* Specificity of the Access 25(OH) Vitamin D Total assay was determined by adding the cross reactants to the vitamin D samples with an approximate concentration of 20, 40, and 100 ng/mL. Each test sample was compared to a matched unspiked sample. Five replicates of each spiked and unspiked sample were tested on one UniCel DxI 800 instrument, using one reagent pack lot and one calibrator lot. Values for percent cross-reactivity were calculated using the equation below.

$$\text{Cross-reactivity} = \frac{\text{mean value spiked (ng/mL)} - \text{mean value unspiked (ng/mL)}}{\text{Concentration of cross-reactant added (ng/mL)}} \times 100$$

Results from this study are summarized in the table below:

Substance	Concentration added	% Cross Reactivity		
	ng/mL	Concentration of 25(OH) vitamin D in sample:		
		20ng/mL	40 ng/mL	100 ng/mL
3-epi-25(OH) vitamin D_3**	100	43	64	47
1,25(OH)2 vitamin D_2*	9	974	1140	1278
1,25(OH)2 vitamin D_3*	25	306	329	186
24,25$(OH)_2$ vitamin D_3	104	6	2	-11
Vitamin D_3 (Cholecalciferol)	19,832	0	0	0
Vitamin D_2 (Ergocalciferol)	19,232	0	0	0
1αOH vitamin D_3 (alfacalcidol)	8,013	0	0	0
Paricalcitol (Zemplar)	24	218	209	195
25(OH) vitamin D_2	40	57	69	80

Due to the insufficient spike recovery in vitamin D immunoassays[1] the % Cross Reactivity results obtained above were normalized by dividing by the observed % Cross Reactivity of 25(OH) vitamin D_3 to obtain the final % Cross Reactivity values below:

Substance	Concentration added	% Cross Reactivity		
	ng/mL	Concentration of 25(OH) vitamin D in sample:		
		20 ng/mL	40 ng/mL	100 ng/mL
3-epi-25(OH) vitamin D_3**	100	55	100	71
1,25(OH)2 vitamin D_2*	9	1253	1797	1927
1,25(OH)2 vitamin D_3*	25	393	518	281
24,25(OH)2 vitamin D_3	104	7	3	-16
Vitamin D_3 (Cholecalciferol)	19,832	0	0	0
Vitamin D_2 (Ergocalciferol)	19,232	0	0	0
1αOH vitamin D_3 (alfacalcidol)	8,013	0	0	0
Paricalcitol (Zemplar)	24	483	389	293
25(OH) vitamin D_2	40	86	86	103
25(OH) vitamin D_3	20/40	100	100	100

* Concentrations tested were 125 - 375 times the endogenous levels typically found for 1,25 $(OH)_2$ vitamin D.[2]

** Concentrations tested were approximately 50-200 times the average endogenous levels reported for 3-epi-25(OH) vitamin D_3 in infant, pediatric and adult subjects;

in these populations, the maximum 3-epi-25(OH) vitamin D_3 concentration found was 4.9 ng/mL.[3]

References:

1 Carter, GD et al. The anomalous behaviour of exogenous 25-hydroxyvitamin D in competitive binding assays. J Steroid Biochem 2007; 103: 480-482.

2 Juttmann JT, et al. Seasonal fluctuations in serum concentrations of vitamin D metabolites in normal subjects. British Medical Journal 1981; 282: 1349-1352.

3 Keevil B. Does the presence of 3-epi-25OHD3 affect the routine measurement of vitamin D using liquid chromatography tandem mass spectrometry; Clin Chem Lab Med 2012; 50 (1): 181–183.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed according to CLSI EP9-A3 to compare the performance of the Access 25(0H) Vitamin D Total Assay on the UniCel Dxl Immunoassay System with that of the 25(0H) Vitamin DID LC-MS/MS Reference Measurement Procedure (RMP) from Ghent University (Ghent RMP). One hundred and ten native independent patient samples value assigned by Ghent RMP were tested in duplicates and the singlet set of results was used to compare against the candidate method. The linear regression was calculated using the Passing-Bablok method and the method comparison study results are summarized below:

N	Intercept (ng/mL) (95% CI)	Slope (ng/mL) (95% CI)	R	Sample range tested
109	-3.96 (-5.48 to -1.04)	1.03 (0.91 to 1.07)	0.94	7.1 to 120 ng/mL

b. Matrix comparison:

A sample type comparison study was performed on the Access 25(OH) Vitamin D Total assay using 45 matched sets of serum and plasma (lithium-heparin) samples. The samples (40 neat and 5 spiked) spanned the reportable range of the assay (12.0 to 102.4 ng/mL) were tested on one UniCel DxI 800 instrument using one reagent pack lot and one calibrator lot. One singlet set of data was used for the data analysis. Following CLSI recommendations in EP9-A2, *Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, 2nd Edition,* Passing Bablok analysis was used to evaluate the sample type comparison. The study linear regression analyses are summarized in the table below:

Sample Type	N	Intercept	Slope	R
Serum (no gel) vs. Serum (gel)	45	1.65	0.97	0.99
Serum (no gel) vs. Lithium Heparin Plasma	45	- 0.30	1.01	0.99

Based on the study data, the sponsor claims that serum, serum with gel and lithium heparin (plasma) tubes 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):

   Not applicable

4. Clinical cut-off:

   Not applicable

5. Expected values/Reference range:
   A reference range study was conducted using serum samples from 367 apparently healthy adults between 21-89 years of age. The study population included male and female subjects from various geographically diverse regions of the US and reflected the overall US population in terms of gender, race and ethnicity. The samples were collected during the cold and warm weather. All subjects self-reported their health status. Normal levels of serum calcium, magnesium, phosphorous, PTH and TSH were confirmed following enrollment. Twenty percent of the subjects that participate in this study took vitamin D supplements. The observed range of 25(OH) Vitamin D concentrations is summarized in the table below:

N	Median	95% Reference Interval	
		2.5^{th} Percentile	97.5^{th} Percentile
367	27.4 ng/mL	14.0 ng/mL	49.8 ng/mL

The sponsor stated in their labeling that it is important for each laboratory to establish its own reference range, representative of its typical population.

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.