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

A. 510(k) Number:

k142373

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 for use on the Access 2 Immunoassay System
Access 25 (OH) Vitamin D Total Calibrators for use on the Access 2 Immunoassay System.

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 Indications for Use below.

2. Indication(s) for use:

   The Access 25(OH) Vitamin D Total assay is a paramagnetic chemiluminescent immunoassay for the quantitative determination of total 25-hydroxyvitamin D [25(OH) vitamin D] levels in human serum and plasma using the Access 2 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 assay for the quantitative determination of total-hydroxyvitamin D [25(OH) vitamin D] levels in human serum and plasma using the Access 2 Immunoassay Systems.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   For use with the Access 2 Immunoassay Systems

I. Device Description:

The Access 25(OH) Vitamin D Total assay reagent is provided in 2 packs, 50 tests/pack for a total of 100 determinations. The reagent pack contains the following reagents:

R1a:	Dynabeads® 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

The Access 25(OH) Vitamin D Total Calibrators (for use on Access 2 Immunoassay Systems) is provided ready to use and contains a calibration card for exact concentrations. The calibrators are provided in 6 vials (S0, S1, S2, S3, S4 and S5) which contain 1.4 ml of calibrator/vial. Each vial of calibrator contains the following:

S0:	Human Serum, <0.1% sodium azide, and 0.1% ProClin 300
S1,S2,S3, S4,S5	Human Serum with 25(OH) vitamin D levels of approximately 7, 18, 35, 74 and 167 ng/mL (18, 45, 88, 185 and 418 nmol/L), <0.1% sodium azide and 0.1% ProClin 300
Calibration Card:	1

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 LIASON 25 OH Vitamin D Total Assay

2. Predicate 510(k) number(s):
   k112725

3. Comparison with predicate:

Assay:

Similarities and Differences		
Item	Diasorin 25 OH Vitamin D Total (k112725) Predicate Device	Access 25 (OH) Vitamin D Total Candidate Device
Intended Use	For the quantitative determination of 25-hydroxyvitamin and other hydroxylated vitamin D metabolites in human serum	Same
Standardization	Standard prep:UV	NIST-Ghent ID-LC-MS/MS
Technology	Competitive Immunoassay	Same
Format	Chemiluminescent	Same
Method	Automated	Same
Sample Type	Serum	Serum/Li Heparin Plasma
Measuring Range	4-150 ng/mL	7.0-120 ng/mL
Antibody	Goat polyclonal anti-25(OH) vitamin D	Sheep monoclonal anti-25(OH) 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 7, 18, 35, 74 and 167 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 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. Analytical performance:

a. Precision/Reproducibility:

A twenty day reproducibility/precision study based on CLSI EP5-A2 was performed at an internal site using three Access 2 analyzers and 3 reagent lots. Four patient serum 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 Access 2 instruments, using three reagent pack lots, one calibrator lot and one lot of reagent pack per instrument. The study results of one representative reagent lot are provided in the table below:

Sample	Mean (n=80) ng/mL	Within-run		Between-run		Total	
		SD	%CV	SD	%CV	SD	%CV
Sample 1	13.3	0.5	3.8	0.9	6.7	1.0	7.7
Sample 2	24.6	0.5	2.2	1.8	7.2	1.8	7.5
Sample 3	49.8	1.1	2.1	3.5	7.0	3.6	7.3
Sample 4	110.5	1.6	1.5	7.3	6.6	7.5	6.8

b. Linearity/assay reportable range:

A linearity study was conducted based on CLSI EP6-A. A high human serum based sample and a low human serum based sample were analyzed in addition to 7 evenly spaced dilutions which were created by mixing the high and low sample to cover the range of the assay (7.0-120 ng/mL). Each diluted sample was analyzed in replicates of 4 and the low and high samples were analyzed in replicates of eight using three Access 2 analyzers, three reagent pack lots and one calibrator lot. The linearity study results using one representative reagent lot with the polynomial regression equations are summarized and presented in the table below:

Reagent 3, Calibrator 1					
Observed (ng/mL)	**Expected (ng/mL)**	**Linear fit (ng/mL)**	**Quadratic fit (ng/mL)**	**Quad Absolute Difference**	**Quad Relative % Difference**
4.75	4.75	4.89	4.75	-0.1	-2.9%
21.61	18.95	20.09	21.66	1.6	7.8%
36.70	33.14	35.29	37.68	2.4	6.8%
54.74	47.34	50.5	52.83	2.3	4.6%
66.89	61.53	65.7	67.1	1.4	2.1%
82.66	75.73	80.91	80.5	-0.4	-0.5%
90.36	89.92	96.11	93.01	-3.1	-3.2%
101.55	104.12	111.31	104.65	-6.7	-6.0%
118.31	118.31	126.52	115.41	-11.1	-8.8%

Reagent Lot 3, Calibrator Lot 1 Regression

Linear fit:
Observed= -0.206003 + 1.071098*Expected
R^2=0.9499

Polynomial fit degree 2:
Observed =-0.983542 + 1.2095602*Expected -0.0021785*(Expected-7.59221) ^2
R^2=0.99921

Polynomial fit degree 3:
Observed= - 0.961495 + 1.2049377*Expected – 0.019661*(Expected-7.59221) ^2-1.7236e-6(Expected-7.59221) ^3
$R^{2=}$0.999203

The linearity study data supports the sponsor's claims that the measuring range of the Vitamin D assay is 7.0-120 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.

This assay has been standardized 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.

In accordance with the recommendations of the program, the traceability of Access 25 (OH) Vitamin D Total Calibrators for the Access 2 analyzer was verified using a panel of forty 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. Standardization of total 25OHD in serum was verified through method comparison and bias estimation between the reference method and the Access 25(OH) Vitamin D Total assay. This value assignment has aligned the candidate device to the RMP.

The relationship between the Access 25 (OH) Vitamin D Total for use on the Access 2 analyzer and ID-LC/MS/MS 25-OH vitamin D RMP is described using Deming regression:

y=1.01x-2.87 ng/mL, r=0.99

Value Assignment:

Primary reference calibrators are prepared from 25(OH) Vitamin D and human serum. A stock is prepared by volumetrically mixing the 25(OH) Vitamin D to a known concentration based on the ID-LC-MS/MS reference method. The stock is diluted to designated (assigned) concentrations using a serum based matrix. Primary working calibrators are prepared at six calibrator levels; zero, and approximately 7, 18, 35, 74 and 167 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.

Stability:

The sponsor claims the calibrators are stable until the expiration date indicated when stored unopened at -15-30°C. Once opened, the sponsor claims the calibrators are stable for 56 days when stored at 2-8°C. The sponsor recommends the use of commercially available controls for use with this assay in the labeling.

d. Detection limit:

The Limit of Blank (LoB), limit of detection (LoD) and the limit of Quantitation (LoQ) were determined following CLSI EP17-A2 guidelines.

The LoB was determined as the 95% non-parametric upper distribution limit of the result concentration. LoB was performed by analyzing 154 replicates of the S0 calibrator over 12 runs using three Access 2 Immunoassay Systems, three reagent lots and two calibrator lots.

The LoD determination was performed by analyzing 5 very low Vitamin D concentration serum samples between the anticipated LoB and 10 times the anticipated LoB in triplicate over 12 runs (180 replicates in total) using 3 reagent packs and three Access 2 Immunoassay Systems over 4 days.

The LoQ determination was performed by analyzing 3 replicates of 10 patient samples within 22 runs using multiple Access 2 Immunoassay Systems over multiple days. LoQ was defined as the lowest concentration which met the design requirements of total imprecision of ≤ 20%CV.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
0.55 ng/mL	1.0 ng/mL	3.0 ng/mL

The Access 25 (OH) Vitamin D assay has a measuring range of 7.0-120 ng/mL

e. Analytical specificity:

An interference study was conducted per CLSI EP7-A2. Serum samples containing Vitamin D concentrations of approximately 20, 40 and 100 ng/ml were used in the study. Potential interferents were spiked individually into each patient sample in up to four different concentrations. Results of the spiked patient samples were compared to results of the unspiked sample (control). Samples at Vitamin D concentration of ~30 ng/mL were tested in replicates of 8 and samples at ~40 and ~150 ng/mL were tested in replicates of five. The highest concentration at which no interference (defined by the sponsor as ≤10% bias relative to the control) was seen is shown in the table below.

Interfering Substance	Highest Concentration in which no significant interference was seen.
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

A cross-reactivity study was conducted according to CLSI-EP7-A2 to evaluate the potential cross-reactivity of the assay with other substances that are similar in structure to 25(OH) Vitamin D. The potential cross-reactants were added to the Vitamin D samples with an approximate 25(OH) vitamin D concentration of 20, 40 and 100 ng/mL. Results from these cross-reactant spiked serum samples were evaluated against that of matched unspiked serum samples. Samples at 20 ng/ml were tested in replicates of eight. Samples at 40 and 100 ng/mL were tested in replicates of five. The substances shown in the following table were spiked into samples containing 25(OH) vitamin D and analyzed on the Access 2 Immunoassay system. Values for percent 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 of interfering substance tested ng/mL	Crossreactivity %		
		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	38	47	32
1,25(OH)2 vitamin D_2*	9	796	913	1026
1,25(OH)2 vitamin D_3*	25	175	186	147
24,25$(OH)_2$ vitamin D_3	104	6	1	-6
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	172	147	131
25(OH) vitamin D_2	40	76	81	76
25(OH) Vitamnin D_3	50	61	56	45

Due to the insufficient spike recovery in Vitamin D immunoassays[1] the % Cross Reactivity results obtained above were normalized. Without normalization, cross reactivity would appear artificially low therefore % cross reactants are normalized to the 25(OH) D_3 to reflect the endogenous cross-reactivity. This is achieved by dividing the observed % Cross reactivity (61, 56 and 45) of 25(OH) Vitamin D3 to obtain the final % Cross Reactivity values below:

Substance	Concentration of interfering substance tested ng/mL	Crossreactivity %		
		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	64	54	70
1,25(OH)2 vitamin D_2*	9	1336	1043	2262
1,25(OH)2 vitamin D_3*	25	293	212	324
24,25$(OH)_2$ vitamin D_3	104	9	2	-13
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	282	264	288
25(OH) vitamin D_2	40	96	99	116

*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.

The labeling contains the following limitations:

"Do not assay hemolyzed samples. Hemoglobin concentrations greater than 50 mg/dL may lead to falsely elevated results.

"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.

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 25-OH vitamin D concentrations of serum samples using the candidate device on the Access 2 analyzer and the University of Ghent ID-LC/MS/MS 25-OH vitamin D

Reference Measurement Procedure (RMP). 109 native independent patient samples with RMP assigned concentrations 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 first result obtained and the Passing-Bablok method. The results are summarized below:

N	Intercept (ng/mL) [95%CI]	Slope (ng/mL) [95% CI]	Correlation Coefficient	Sample range tested
109	-3.57 [-6.15 to- 1.30]	1.02 [0.95-1.12]	0.95 [0.92 to 0.96]	8.0-109.4 ng/mL

b. Matrix comparison:

A matrix comparison study was conducted using 45 matched sets of serum and plasma (lithium-heparin) samples which spanned the reportable range of the assay (8.3 to 87.9 ng/mL). The samples (40 neat and 5 spiked) were analyzed on the Access 2 analyzer. The results of the study using Passing Bablok analysis are as follows:

Sample Type	n	Intercept	Slope	95% CI	R
Serum (no gel) vs. Serum (gel)	45	0.97	1.01	0.96-1.06	0.99
Serum (no gel) vs. Lithium Heparin	45	-0.02	1.05	1.02-1.10	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, this is a quantitative assay.

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. These samples were collected during the cold and warm weather. Samples included subjects with Vitamin D supplements (20% of the participants) and without Vitamin D supplements. Samples were only included in this study if the samples had normal Calcium, Magnesium, Phosphorus, PTH and TSH values. The results of the study are as follows:

Method	N	Median Concentration	95% Reference Range (2.5-97.5) Percentile
Access 25(OH) Vitamin D Total (ng/mL)	367	24.9 ng/mL	11.9 ng/mL to 43.6 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 substantial equivalence decision.