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

A. 510(k) Number:

k112725

B. Purpose for Submission:

Modifications to a previously cleared device

C. Measurand:

25-OH Vitamin D and other hydroxylated Vitamin D metabolites

D. Type of Test:

Quantitative Chemiluminescent immunoassay

E. Applicant:

DiaSorin Inc.

F. Proprietary and Established Names:

LIAISON® 25 OH Vitamin D TOTAL Assay

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1825, Vitamin D Test System

2. Classification:

   Class II

3. Product code:

   MRG

4. Panel:

   Chemistry (75)

H. Intended Use:

1. Intended use(s):
   See indications for use.

2. Indication(s) for use:

   The LIAISON® 25 OH Vitamin D TOTAL Assay uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites in human serum to be used in the assessment of vitamin D sufficiency. Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions in an adult population.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   For use on the Liaison Analyzer

I. Device Description:

The device consists of:

- Magnetic particles coated with antibody against 25 OH Vitamin D, protein, phosphate buffer, < 0.1% sodium azide.
- Assay Buffer with 10% ethanol, surfactants and 0.1% ProClin® 300.
- 25 OH Vitamin D conjugated to an isoluminol derivative, in phosphate buffer with 10% ethanol, EDTA, surfactant and preservatives.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   LIAISON® 25 OH Vitamin D TOTAL Assay

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

   k071480

3. Comparison with predicate:

The intended use and assay principle are the same as for the predicate device. Changes include modifications to the magnetic particle surface and buffer

formulation. The predicate device was indicated for serum and plasma. This device is only for use with serum.

Feature	New device	Predicate device
Intended Use	Same	For the quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites to be used in the assessment of vitamin D sufficiency using the LIAISON® Analyzer family.
Matrix	Serum only	Serum and plasma
Methodology	Same	Chemiluminescent immunoassay

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

EP5-A2; Evaluation of Precision Performance of Quantitative Measurement Methods
EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures
EP7-A2: Interference Testing in Clinical Chemistry.

L. Test Principle:

The LIAISON® 25 OH Vitamin D assay is a direct competitive chemiluminescence immunoassay (CLIA) for quantitative determination of total 25 OH vitamin D in serum. During the first incubation, 25 OH Vitamin D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 minutes the tracer, (vitamin D linked to an isoluminol derivative) is added. After a second 10 minute incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25 OH vitamin D present in calibrators, controls, or samples.

M. Performance Characteristics (if/when applicable):

Performance was evaluated on the LIAISON® Analyzer.

1. Analytical performance:

   a. *Precision/Reproducibility:*
   A twenty day reproducibility/precision validation study was performed at DiaSorin Inc. and 2 external sites, on one LIAISON® Analyzer per site. Samples included a coded panel of 2 low, 2 medium, 1 moderately high, and 1 high Vitamin D concentration serum samples. The LIAISON® 25 OH Vitamin D TOTAL serum controls (2 levels) also were included in the study. Samples and kit controls were tested on two lots of modified

LIAISON® 25 OH Vitamin D TOTAL Assay at each of three sites in 2 replicates per run, 2 runs per day for 20 operating days. A total of 160 replicate results were generated per sample at each site. The mean, standard deviation, and coefficient of variation (%CV) of the results were computed by Nested Analysis of Variance for each of the tested specimens. Results of this analysis across three sites are shown below:

Mean	**Intra-Run**		**Run-to-Run**		**Day-to-Day**		**Total/Within-Lot/Within-Site**		**Total/Across-Lots/Across-Sites**	
(ng/mL)	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
7.9	0.6	7.7%	0.2	3.2%	0.7	9.3%	1.0	12.5%	1.0	12.6%
12.0	0.7	5.8%	0.4	2.9%	1.0	8.7%	1.3	10.8%	1.3	11.1%
18.0	0.9	5.0%	0.6	3.1%	1.3	7.4%	1.7	9.5%	1.7	9.7%
20.4	1.0	5.0%	0.5	2.2%	1.6	7.8%	1.9	9.5%	2.1	10.4%
24.3	1.2	5.0%	0.5	2.1%	1.8	7.5%	2.2	9.2%	2.6	10.6%
56.8	2.9	5.0%	1.3	2.3%	3.9	6.8%	5.0	8.8%	5.8	10.3%
61.8	3.0	4.9%	1.6	2.6%	4.2	6.8%	5.4	8.8%	5.8	9.5%
112.1	5.4	4.8%	3.0	2.7%	9.3	8.3%	11.1	9.9%	12.2	10.8%

Results within one representative site are shown below:

Lot 1			Lot 2		
	Within-run	Total		Within-run	Total
Mean (ng/mL)	%CV	%CV	Mean	%CV	%CV
8.2	6.1	11.1	7.7	5.9	12.3
12.3	5.3	9.9	11.7	3.6	9.6
18.4	5.4	9.5	17.4	2.8	10.7
21.0	4.8	8.0	19.8	3.6	9.6
25.4	4.2	9.3	23.3	4.2	8.5
58.8	3.3	8.4	54.1	3.3	8.0
62.5	3.3	8.7	58.6	2.5	8.8
115.5	5.2	9.2	106.0	3.6	8.9

b. Linearity/assay reportable range:

A linearity study was performed using samples slightly above the upper measuring limit of the modified LIAISON® 25 OH Vitamin D TOTAL Assay, following CLSI EP6-A. Separate serum and SST (serum separator tubes) pools that met this criterion were diluted with the assay's Specimen Diluent to yield multiple dilutions with Vitamin D levels spanning the

assay's full measuring range of 4 – 150 ng/mL. Each dilution was analyzed in replicates of 4 with one lot of the modified Vitamin D Assay on one LIAISON® Analyzer.

Expected concentrations for the dilutions were determined by (Vitamin D value of high pool) x (dilution factor).

The results were analyzed by simple linear regression (slope, intercept and R^2 value) of observed results versus expected results, recovery versus expected concentrations, and the linear, second degree and third degree polynomial fits of the data. Results are shown below for the three analyses:

Linear regression equation: y=0.997x-0.411
Recoveries (serum samples are shown below; similar results were shown for SST samples)

Expected concentration (ng/mL)	Observed concentration (ng/mL)	%recovery (observed/expected)
151	151	100%
132	130	98%
113	110	97%
94.3	95.2	101%
75.4	78.4	104%
56.6	56.5	100%
37.7	36.2	96%
18.9	17.0	90%
9.43	8.49	90%
3.77	3.84	102%

A polynomial regression fit of the serum data yielded statistically insignificant second and third order terms

These results support the sponsor's claimed measuring range of 4 to 150 ng/mL.

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

Calibrators and control materials were cleared under k071480

d. *Detection limit:*

The DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay measures

between 4.0 and 150 ng/mL. The lowest reportable value is 4.0 ng/mL which is based on an inter-assay precision ≤ 20% CV. Values below 4.0 ng/mL are reported as < 4.0 ng/mL.,

The sponsor performed a detection limit study to support their low end measuring range claim. For LoB and LoD determination, study was performed on 4 reagent lots on each of the 2 analyzers and 30 replicates of zero calibrator and 20 replicates of Calibrator A were evaluated. The LoD was calculated to be 2 ng/mL. For LoQ determination, 8 samples with concentrations above the LoD were tested on three reagent lots and 2 analyzers, with 5 replicates per sample i.e., n=30). The LoQ was calculated to be 4 ng/mL based on an inter-assay precision of ≤ 20% CV.

e. Analytical specificity:

Cross-reactivity:
Aliquots of samples of low, moderate and high Vitamin D were spiked with 25 OH D metabolites. Vitamin D concentrations were near 10, 30, and 70 ng/mL. Metabolite concentrations of 30 ng/mL and 100 ng/mL were spiked into the vitamin D samples and analyzed. Percent Cross-Reactivity was determined based on:

(Measured Value/Concentration Spiked)*100

Cross-reactivity results are shown below:

25 OH D_2	100%
25 OH D3	100%
Vitamin D2	1.9%
Vitamin D3	1.9%
1,25-(OH)2-Vitamin D2	6.7%
1,25-(OH)2-Vitamin D3	9.3%
3-epi-25OH Vitamin D3	1.3%

Interference:
Vitamin D samples containing concentrations of 30 ng/mL and 60 ng/mL were spiked with hemoglobin, triglycerides, cholesterol, and bilirubin, uric acid, albumin, and IgG. Replicate samples (n=6 for each test and control sample) were tested and results were compared to control samples without the potentially interfering substances added. The highest concentrations at which no interference, or minimal interference (defined by the sponsor as ≤ 10% bias relative to the control) are shown below.

300 mg/dL cholesterol
590 mg/dL triglycerides
20 mg/dL uric acid

12.9 g/dL human serum albumin
12.9 g/dL IgG
40 mg/dL Bilirubin (conjugated and unconjugated tested separately)
200 mg/dL hemoglobin

The sponsor has the following limitations in the labeling:

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 that hemolyzed or grossly lipemic samples should not be used.

f. Assay cut-off:

Not applicable – this is a quantitative assay.

2. Comparison studies:

a. Method comparison with predicate device:

Serum samples from a reference laboratory spanning the assay range (N=587) were tested with the modified LIAISON® 25 OH Vitamin D TOTAL Assay and the DiaSorin 25-hydroxyVitamin D 125I RIA (k983617). The samples, ranging in concentration from 4 to 150 ng/mL spanned the claimed measuring range of the modified assay. Only neat patient samples (no spiked samples) were used in the study.

Results of regression analysis are presented in the following table.

N	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient	range (ng/mL)	Mean Bias	SD of differences (ng/mL)
587	1.047 (1.02 – 1.07)	2.41 (1.43 – 3.40)	0.936	4-150	4.083	7.212

b Matrix comparison:
One hundred thirty seven (137) matched sets of Serum and SST samples were tested in singlet across 5 LIAISON® Analyzers and 2 lots of the modified assay. In order to obtain analyte values across the reportable range of the assay (4 to 140 ng/mL), it was necessary to dilute one sample and to spike 19 samples with Vitamin D.

Results of linear regression are shown below. Recoveries for individual samples were also provided and found to be adequate.

Number of Samples	Linear Regression		
	Slope	Intercept	R^2 value
137 matched sets	0.99	-0.43	0.988

Only plain serum tubes and serum separator tubes (SST) are acceptable tube types for the modified assay, plasma samples are unacceptable for the modified assay and this information was stated clearly in the labeling.

3. Clinical studies:

   a. *Clinical Sensitivity:* Not applicable; Clinical sensitivity and specificity is not typically provided in 510(k)s for this type of assay.

   b. *Clinical specificity**:*** See a, above.

   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 new reference range study was performed for the modified assay in accordance with CLSI Guideline C28-A2. Participant enrollment took place at four sites in the contiguous US, representing a wide distribution of sun strength: northern, southern, and central US. Blood collection occurred over two seasons, with 136 collected from 3 sites during the winter months (February and March) and 260 collected from 4 sites during the summer months (June, July and August).

   The collection was conducted on adults (ages 21 – 90 years) in summer and winter, including light and dark skin individuals, and males and females. Three hundred and ninety six (396) samples met the inclusion/exclusion criteria and were tested with the modified LIAISON® 25 OH Vitamin D TOTAL Assay.

   The determination of apparently healthy for this study was based on the following definition:

   - Normal serum levels of:
     - Total calcium
     - Intact parathyroid hormone (PTH)
     - Thyroid stimulating hormone (TSH)

- No personal history of kidney, gastrointestinal or liver disease
- No personal history of parathyroid, thyroid, or chronic disease (defined by severity of condition which is linked to treatment and frequency as stated on subject's CRF)
- No personal history of seizures
- No bariatric surgery
- No family history of parathyroid or calcium regulatory disease

Other criteria were: at least 50% of subjects NOT taking ANY Vitamin D supplementation, and of the less than 50% of subject taking Vitamin D supplementation, level of supplementation must be <2000 IU/day. Subjects were not currently taking any medications known to affect absorption (drugs that inhibit cholesterol absorption) or increase catabolism, such as anticonvulsants, glucocorticoids, HAART (AIDS treatment) and anti-rejection medications

1. Current use of dietary or alternative therapies containing high concentrations of Vitamin D (e.g. adults ≥ 2,000 IU / day)
2. More than 50% of subjects are taking Vitamin D supplementation
3. Family history of parathyroid or calcium regulatory disease
4. Personal history of the following diseases: kidney, gastrointestinal, liver, thyroid, or parathyroid
5. Personal history of seizures
6. Bariatric surgery
7. Pregnancy or lactation

Site	**n**	**Females**	**Males**	**Light Skin**	**Dark Skin**	**Age Group ≥ 21 to ≤ 35 Yrs**	**Age Group >35 to ≤ 50 Yrs**	**Age Group >50 to ≤ 90 Yrs**
Northern Site Buffalo, NY	123	74	49	105	18	Total = 30 Female = 16 Male = 14	Total = 49 Female = 29 Male = 20	Total = 44 Female = 29 Male = 15
Central Site Oklahoma City, OK	116	67	49	73	43	Total = 33 Female = 18 Male = 15	Total = 38 Female = 23 Male = 15	Total = 45 Female = 26 Male = 19
Southern Site Orange City, FL	88	59	29	71	17	Total = 27 Female = 18 Male = 9	Total = 34 Female = 23 Male = 11	Total = 27 Female = 18 Male = 9
2nd	69	32	37	36	33	Total = 32	Total = 22	Total = 15

Site	n	Females	Males	Light Skin	Dark Skin	Age Group ≥ 21 to ≤ 35 Yrs	Age Group >35 to ≤ 50 Yrs	Age Group >50 to ≤ 90 Yrs
Southern Site Delray Beach, FL (Summer only)						Female = 19 Male = 13	Female = 6 Male = 16	Female = 7 Male = 8
TOTAL	396	232	164	285	111	Total = 122 Female = 71 Male = 51	Total = 143 Female = 81 Male = 62	Total = 131 Female = 80 Male = 51
Percent of Total		59%	41%	72%	28%	31%	36%	33%

Category	n	Range (ng/mL)	Mean (ng/mL)	Median (ng/mL)
Modified LIAISON® 25 OH Vitamin D TOTAL Assay				
All data	396	9.3 – 47.9	26.0	24.9
Northern	123	8.9 – 47.2	26.6	25.5
Central	116	8.0 – 44.3	22.1	20.9
Southern	157	11.0 – 56.1	28.3	27.1
Winter	136	8.1 – 47.2	23.4	22.4
Summer	260	10.4 – 49.3	27.3	26.1
Light skin	284	11.3 – 50.4	28.3	27.8
Dark skin	112	7.8 – 39.0	20.1	19.7

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.