

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

A. 510(k) Number:

k071480

B. Purpose for Submission:

New device

C. Measurand:

25-hydroxyvitamin D (25-(OH)-D₂/D₃) 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

LIAISON® 25 OH Vitamin D TOTAL Control Set

LIAISON® 25 OH Vitamin D TOTAL Specimen Diluent Set

G. Regulatory Information:

1. Regulation Section

21 CFR 862.1825 – Vitamin D Test System

21 CFR 862.1660 - Quality Control Material (Assayed and Unassayed)

2. Classification

Class II

Class I

3. Product Code

MRG - Vitamin D Test System

JJX – Single (specified) Analyte Controls (Assayed and Unassayed)

4. Panel

Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for use, below.

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, EDTA-plasma or lithium heparin plasma 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.

The LIAISON® 25 OH Vitamin D TOTAL Control Set is intended for use as assayed quality control samples to monitor the accuracy and precision of the DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay.

The LIAISON 25 OH Vitamin D TOTAL Specimen Diluent Set may be used to dilute specimens with values greater than 150 ng/mL by the LIAISON 25 OH Vitamin D TOTAL Assay.

3. Special conditions for use statement(s):

For prescription use only.

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.

The performance characteristics of this assay have not been established in a pediatric population.

4. Special instrument requirements:

For use on the LIAISON Analyzer

I. Device Description:

Assay contains magnetic particles coated with antibody against 25 OH Vitamin D, protein, buffer, and sodium azide; Assay Buffer with 10% ethanol, surfactants and preservatives; conjugate with 25 OH Vitamin D conjugated to an isoluminol derivative; and two human serum-based calibrators with buffer, sodium azide and 25 OH Vitamin D.

Control Set consists of human serum-based controls provided in vials with buffer salts and sodium azide.

All human source material was tested by FDA-approved methods for the presence of antibodies to Human Immunodeficiency Virus Type 1 (HIV-1) and Type 2 (HIV-2), Hepatitis B Surface Antigen (HBsAg) and antibodies to Hepatitis C Virus (HCV) and found to be negative/non-reactive.

J. Substantial Equivalence Information:

1. Predicate device name(s):

DiaSorin 25-hydroxyvitamin D ¹²⁵I RIA Assay, LIAISON 25 OH Vitamin D Kit

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

k983617, k032844

3. Comparison with predicates:

Assay:

Similarities		
	Predicate device (k983617)	Proposed device
Intended Use	Quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites to be used in the assessment of vitamin D sufficiency.	Same
Antisera	Polyclonal to 25-OH-D ₂ /D ₃	Same

Differences		
	Predicate device (k983617)	Proposed device
Assay principle	Quantitative RIA	Chemiluminescent immunoassay
Sample type	Serum and EDTA Plasma	Serum, EDTA Plasma, Li Heparin Plasma, Serum separator tubes
Functional sensitivity	1.5 ng/ml	4 ng/ml
Assay buffer	Buffer with 10% acetonitrile, surfactants and preservatives.	Buffer with 10% ethanol, surfactants and preservatives.

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

STANDARDS
• Stability Testing of In Vitro Diagnostic Reagents (13640)
• How to Define and Determine Reference Intervals in the Clinical Laboratory; CLSI Approved Guideline - Second Edition (C28-A2)
• Interference Testing in Clinical Chemistry; CLSI Approved Guideline (EP 7-A)
• Method Comparison and Bias Estimation Using Patient Samples; CLSI Approved Guideline (EP9-A2)
• Preliminary Evaluation of Quantitative Clinical Laboratory Methods; CLSI Approved Guideline (EP10-A2)
• Evaluation of Precision Performance of Quantitative Measurement Methods; CLSI Approved Guideline-Second Edition (EP5-A2)

L. Test Principle:

The LIAISON® 25 OH Vitamin D assay is a direct, competitive chemiluminescent immunoassay (CLIA) for quantitative determination of total 25 OH vitamin D in serum or plasma. 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 an additional 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. 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):1. Analytical performance:a. *Precision/Reproducibility:*

Nine serum specimens (with concentrations ranging from 7.2 – 128 ng/mL) and four plasma specimens (with concentrations ranging from 5.9 – 62.7 ng/mL) were assayed in duplicate in 2 separate runs per day for 20 days on a single instrument using a single lot of reagents. Extraction was included in this study.

Serum samples:

Within-assay precision	S1	S2	S3	S4	S5	S6	S7	S8	S9
No. of tests	80	80	80	80	80	80	80	80	80
Mean (ng/mL)	7.2	14.7	21.7	35.0	73.0	62.7	93.6	115	128
SD (ng/mL)	0.40	0.63	0.86	1.01	2.33	1.95	3.03	4.79	6.08
CV (%)	5.5	4.2	4.0	2.9	3.2	3.1	3.2	4.2	4.8
Between-assay precision									
No. of tests	80	80	80	80	80	80	80	80	80
Mean (ng/mL)	7.2	14.7	21.7	35.0	73.0	62.7	93.6	115	128
SD (ng/mL)	0.93	1.12	1.72	2.22	4.95	4.04	6.19	7.50	9.33
CV (%)	12.9	7.7	7.9	6.3	6.8	6.4	6.6	6.5	7.3

Plasma:

Within-assay Precision	P1	P2	P3	P4
No. of tests	80	80	80	80
Mean (ng/mL)	5.91	45.9	52.6	62.7
SD (ng/mL)	0.48	1.51	1.71	3.25
CV (%)	8.1	3.3	3.2	5.2

Between-assay precision				
No. of tests	80	80	80	80
Mean (ng/mL)	5.91	45.9	52.6	62.7
SD (ng/mL)	0.75	3.19	3.65	4.92
CV (%)	12.7	6.9	6.9	7.9

b. *Linearity/assay reportable range:*

The DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay has a measuring range of 4.0 to 150 ng/mL.

In order to test linearity, a set of five high clinical samples or serum samples spiked with concentrated 25 OH Vitamin D, two of which had values above the upper limit of the assay range, were diluted with the recommended diluent and tested. Samples were diluted to several expected concentrations and all samples were assayed in a single assay with 2-4 replicates each. Samples tested covered the claimed measuring range of the assay. The data from the study showed the following regression equation:

$$Y = 0.9893X + 0.0904$$

$$R^2 = 0.9878$$

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

Calibrators are traceable to concentrations determined by UV spectrophotometric analysis. The antigen concentration of the ethanolic stock is spectrophotometrically calculated in triplicate using the extinction coefficient at 265 nm in the equation;

$$\text{Conc. } (\mu\text{g/mL}) = \frac{(\text{O.D.}_{265}) \times (400.65 \text{ mg/mmol}) (1 \text{ cm}) \times 1000 \mu\text{g/mg}}{18,300 \text{ mL mmol}^{-1} \text{ cm}^{-1}}$$

The ethanolic stock of antigen is used to build an intermediate stock volumetrically by dilution into vitamin-free serum based on the spectrophotometric reading of the stock. The intermediate stock is used in the manufacture of Master Calibrators. Master Calibrators are manufactured gravimetrically. For each manufacturing build, calibrators are built gravimetrically from the intermediate stock. The Master Calibrators serve as an anchor to confirm future manufacturing builds of calibrators. The calibrator levels for each manufacturing build are confirmed when run as unknowns using a minimum of 3 LIAISON® instruments with 2 approved 25-OH Vitamin D kits with Master Calibrators. Replicates of 6 are obtained and assays must pass specifications. Concentrations must fall within the specified range. Once confirmation of the calibrator values is established, the new calibrators are tested again in bulk and final.

Testing of the Controls and assignment of values involves 5 assays, 2 instruments, 3 approved Reagent Integrals and 20 reps of each control. The assigned values for each control must be 15 ng/mL ($\pm 15\%$) and 50 ng/mL ($\pm 15\%$).

Stability testing protocols for both calibrators and controls were reviewed and determined to be adequate.

d. Detection limit:

LoD: The Limit of Detection study was performed according to CLSI EP17-A. The zero calibrator is assayed as 10 replicates in duplicate and a low calibrator (2 ng/mL) is assayed as 5 replicates in duplicate. Testing was performed on three separate instruments.

The mean signal and standard deviation are determined for the zero calibrator and for the low calibrator on each instrument.

The limit of detection (as per CLSI EP17-A) was determined in three runs on three separate instruments to be 0.60, 0.56 and 1.28 ng/mL.

LoQ: The Limit of Quantitation (LoQ) study was performed according to CLSI EP17-A.

Eight serum specimens at concentrations near the limit of detection of the assay were prepared. Specimen values ranged from 1.7 to 10.6 ng/mL. All specimens were tested in 7 assays in replicates of 6 for each assay (for at least 40 replicates) on three separate instruments.

For each instrument's assays, the sample mean, standard deviation, and % CV for each sample were calculated. The mean concentrations (X-axis) vs. % CVs (Y-axis) were plotted. LoQ is defined as the lowest concentration at which the polynomial regression line crosses the 20% CV line.

This study was run on three separate instruments and determined to be 3.9, 3.5 and 3.5 ng/mL. The LoQ claim is 4 ng/mL. Specimens with results under 4 ng/mL are reported as < 4 ng/mL.

e. Analytical specificity:

Interfering substances: An evaluation of potential assay interferences was performed to assess the performance of the LIAISON 25OH Vitamin D TOTAL assay when potential contaminants are present in a sample. Testing was done according to CLSI EP7-A2.

Three sample pools with 25OH Vitamin D at low, medium and high therapeutic concentrations were spiked with the interferants, extracted and tested. Assay performance was unaffected by hemolysis (up to 200 mg/dL hemoglobin), lipemia (up to 549 mg/dL triglycerides), bilirubinemia (up to 20 mg/dL bilirubin) or cholesterolemia (up to 259 mg/dL cholesterol).

Cross Reactivity: Three serum samples were each spiked with a cross reactant at a specific concentration, listed below. The samples were compared to the unadulterated samples and the percent cross reactivity was calculated.

Calculation:

$$\% \text{Cross-Reactivity} = (\text{Corrected Assay Value} / \text{Concentration Spiked}) * 100.$$

Cross reactant	Concentration tested	Cross reactivity
25 OH Vitamin D2	30 ng/mL	104%
25 OH Vitamin D3	30 ng/mL	100%
Vitamin D2	100 ng/mL	<1%
Vitamin D3	100 ng/mL	<1%
1,25 (OH)2 D2	100 ng/mL	40%
1,25 (OH)2 D3	100 ng/mL	17%
3-epi-25OHD3 metabolite	100 ng/mL	<1%

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:

a. *Method comparison with predicate device:*

Serum samples were obtained from clinical reference laboratories, previously obtained archived samples and ongoing specimen collection projects. A total of 155 samples were tested by the LIAISON® 25OH Vitamin D TOTAL Assay and a vitamin D radioimmunoassay (k983617).

The distribution of sample values (by radioimmunoassay result) across the assay measuring range of <4 – 150 ng/mL is shown in the following table.

Assay Range (ng/mL)	Number of Samples
0-10	12
10-20	39
20-40	44
40-100	51
100-150	9

The study results showed correlation between the two methods as follows:
 $y = 0.99x + 2.4$, $R = 0.97$

b. *Matrix comparison:*

A matrix comparison study comparing serum, SST serum, EDTA plasma, and lithium heparin plasma samples was performed. 52 patient samples and 11 spiked samples were tested that covered the claimed measuring range.

Performance of the SST serum and the plasma samples were compared to serum samples. The subset of the same set of samples was also tested after one freeze thaw cycle and each sample type was compared to the fresh

samples of the same type. There was no significant difference between fresh and frozen samples for all sample types tested.

Serum vs. EDTA plasma:

$$y = 0.9782x + 2.9716$$

$$R = 0.995$$

Serum vs. Li Heparin plasma:

$$y = 0.9729x + 5.4417$$

$$R = 0.995$$

Serum vs. SST:

$$y = 0.96x + 0.5$$

$$R = 0.96$$

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:

DiaSorin evaluated 136 apparently healthy adult subjects from centers in the United States. These results are given as an example only.

	Observed Reference Ranges	
Population (136)	Median 25 OH Vitamin D	Observed Range 2.5 th to 97.5 th Percentile
United States	23.6 ng/mL	4.8 ng/mL – 52.8 ng/mL

It is important for each laboratory to establish its own reference range, representative of its typical population.

A review of the literature suggests the following ranges for the classification of 25 OH Vitamin D status:

<u>Vitamin D Status</u>	<u>25 OH Vitamin D</u>
Deficiency:	< 10 ng/mL (< 25 nmol/L)
Insufficiency:	10-30 ng/mL (25-75 nmol/L)
Sufficiency:	30-100 ng/mL (75-250 nmol/L)
Toxicity:	>100 ng/mL (>250 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.