

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

A. 510(k) Number:

k133410

B. Purpose for Submission:

New Device

C. Measurand:

25-hydroxyvitamin D [25(OH) Vitamin D]

Type of Test:

Quantitative, direct competitive colorimetric immunoassay

D. Applicant:

Diazyme Laboratories

E. Proprietary and Established Names:

Diazyme 25-OH Vitamin D Assay

Diazyme 25-OH Vitamin D Control set

G. Regulatory Information:

1. Regulation section:

Product Code	Classification	Regulation Section	Panel
MRG	II	862.1825 Vitamin D Test System	Chemistry (75)
JJX	I, reserved	862.1660 Quality control material (assayed and unassayed)	Chemistry (75)

H. Intended Use:

1. Intended use(s):

See indications for use below.

2. Indication(s) for use:

The Diazyme 25-OH Vitamin D Assay is intended for use in clinical laboratories for the quantitative determination of 25-hydroxyvitamin D (25-OH-D) in human serum and plasma on automated chemistry analyzer. Measurement of 25-hydroxyvitamin D (25-OH-D) is for assessment of vitamin D sufficiency. For *in vitro* diagnostic use only.

The 25-OH Vitamin D Control Set is intended for use as quality controls for Diazyme 25-OH Vitamin D Assay Kit only. For *in vitro* diagnostic use only.

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

For use on the Roche Modular P chemistry analyzer

I. Device Description:

The 25-Hydroxy Vitamin D Assay kit consists of:

1. Diluent: 10 mM sodium phosphate, 2.7 mM KCl, 137 mM sodium chloride, 0.1 % sodium azide, < 0.1 mg/mL sheep monoclonal antibody for 25-OH Vitamin D.
Reagent 1: <3 % acetic acid/sodium acetate, <10 mg/mL NPG substrate and 0.05% polysorbate 20. Dissociation solution, substrate, and stabilizers.
2. Reagent 2: 38 mM sodium phosphate, 0.1% sodium azide. Enzyme Donor-Vitamin D conjugate and stabilizers.
3. Reagent 3: 50 mM Tris-HCl, 200 mM sodium chloride, 10% glycerol, 0.1% sodium azide, <1 mg/mL enzyme acceptor. Enzyme Acceptor and stabilizers.
4. Calibrator 1-5: Human serum containing specific amounts of 25-OH Vitamin D and 0.1% sodium azide.

Controls (supplied separately)

The 25-OH Vitamin D Control Set is a two-level set that is supplied in liquid form. The controls are manufactured from human serum and are ready to use.

Reactive components: Pooled human serum and additives. The approximate target values (23.1 ng/mL and 45.7 ng/mL)

Non-reactive components: Sodium Azide (NaN₃) <0.1%.

Calibrator and Control contain human source material. Each donor unit of serum in the preparation of these materials were tested by FDA-approved methods and found negative for the Human Immunodeficiency Virus Antibody (HIV I/II Ab), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus Antibody (HCV).

J. Substantial Equivalence Information:

1. Predicate device name(s):

DiaSorin Inc. LIAISON 25-OH Vitamin D TOTAL Assay Kit

DiaSorin Inc. LIAISON 25-OH Vitamin D TOTAL Control Set

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

k112725

k071480

3. Comparison with predicate:

Assay

Similarities		
Item	DiaSorin Inc. LIAISON 25-OH Vitamin D TOTAL Assay Kit (Predicate Device)	Diazyme 25-OH Vitamin D Assay (Candidate Device)
Intended Use	For in vitro quantitative determination of 25-Hydroxyvitamin D to be used in the assessment of Vitamin D sufficiency.	Same

Differences		
Item	Predicate Device	Proposed Device
Test Methodology	Direct competitive chemiluminescence immunoassay (CLIA)	Direct competitive colorimetric immunoassay
Antibody	Goat anti-25 OH Vitamin D	Sheep anti-25 OH Vitamin D
Measuring Range	4.0 – 150 ng/mL	7.6 – 147.8 ng/mL
Sample type	Serum only	Serum or EDTA plasma
Instrument platform	DiaSorin Liaison analyzer	Roche Modular P analyzer

Controls

Similarities and differences		
Item	DiaSorin, Inc. LIAISON 25-OH Vitamin D Control Set Predicate Device	Diazyme 25-OH Vitamin D Control Set (Candidate Device)
Intended Use	It is intended for use as quality controls for Diazyme 25-OH Vitamin D Assay	Same
Controls	Serum based, ready to use	Same
Format	Liquid stable	Same
Levels	2 levels	Same
Stability	12 months at 2 - 8°C	Same
Preparation	Ready to use	Same

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

CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods

CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures

CLSI EP07-A2: Interference Testing in Clinical Chemistry

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation

L. Test Principle:

The assay is based on the principle of α -complementation of the enzyme β -galactosidase and the competition between an enzyme donor-25-OH vitamin D conjugate, an anti-Vitamin D antibody and the 25-OH Vitamin D content of a serum sample. Samples with higher 25-OH Vitamin D concentrations produce higher galactosidase activities and *vice versa*. A nitro-phenyl- β -galactoside derivative (NPG) is used as the enzyme substrate. The reaction's product has maximum absorbance at 415 nm. The 25-OH Vitamin D concentration of a specimen is proportional to the measured galactosidase activity. Five calibration levels are needed for each run. Calibrators are treated exactly the same as patient samples.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

All performance testing were conducted on the Roche Modular P chemistry analyzer

a. *Precision/Reproducibility:*

The precision of Diazyme 25-OH Vitamin D Assay was evaluated according to the CLSI EP5-A guideline. A total of 11 samples (2 serum controls and 9 patient

samples) were used in the study.

The study protocol consisted of testing each control and human serum sample in duplicate, 2 runs per day for 20 days, for 80 data points per sample. Three reagent lots and one instrument were used in testing. Within-run and total precision were calculated. All three lots of reagent yielded similar precision results. The precision study results are summarized in the following tables:

25-OH Vitamin D (ng/mL)			Within-run		Between-run		Total	
Sample	n	Mean (ng/mL)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)	SD (ng/mL)	CV (%)
Control #1	80	23.1	1.47	6.4	1.04	4.5	1.68	7.3
Control #2	80	45.7	2.06	4.5	1.67	3.7	2.12	4.6
Sample #1	80	22.6	1.19	5.3	1.11	4.9	1.45	6.4
Sample #2	80	31.7	1.42	4.5	1.59	5.0	1.81	5.7
Sample #3	80	40.6	1.42	3.5	1.59	3.9	1.66	4.1
Sample #4	80	48.6	2.32	4.8	1.71	3.5	2.41	4.9
Sample #5	80	55.8	2.14	3.8	1.73	3.1	2.34	4.2
Sample #6	80	65.4	2.03	3.1	1.79	2.7	2.42	3.7
Sample #7	80	69.7	2.02	2.9	1.99	2.9	2.55	3.7
Sample #8	80	92.8	2.52	2.7	2.02	2.2	3.40	3.7
Sample #9	80	134.6	2.97	2.2	2.69	2.0	3.87	2.9
Very low Sample #1	80	9.4	1.22	13.0	0.98	10.4	1.31	14.0
Very low Sample #2	80	11.2	1.58	14.2	0.88	7.9	0.88	13.9

b. Linearity/assay reportable range:

Linearity samples were prepared by diluting a serum sample containing 147.8 ng/mL of 25-OH Vitamin D with Vitamin D-depletion serum (0 ng/mL), resulting in eleven equally-spaced concentrations which spanned the assay's measuring range. The samples were run in triplicate using one lot of Diazyme 25-OH Vitamin D reagent on the Roche Modular P analyzer. The data from the study showed the following regression equation:

$$Y = 1.0004x - 1.2327, R^2 = 0.9986$$

The results of the study support the sponsor's claim that the assay is linear across the measuring range of 7.6 ng/mL to 147.8 ng/mL.

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

Traceability: The master calibrator stock solution is prepared and tested on a NIST calibrated spectrophotometer. The 25-OH Vitamin D3 concentration of the prepared DMSO stock solution was spectrophotometrically calculated in triplicate using the extinction coefficient at 265 nm using the equation given below:

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

The master stock of 25-OH Vitamin D3 is prepared then used to build the calibration levels by dilution into pooled human serum.

Calibrator value assignment:

The serum-based calibrators prepared are used in conjunction with the Diazyme 25-OH Vitamin D assay reagents to test the NIST 972 25-OH Vitamin D standards. Using regression analysis, the calibrator values are adjusted by testing Diazyme controls, third party controls and a library of serum samples in addition to the NIST 972 standards. The library of serum samples was previously tested with the LIAISON Vitamin D total Assay to establish the expected values.

The target values of the calibrators are:

Calibrator 1	0.0 ng/mL
Calibrator 2	14.5 ng/mL
Calibrator 3	35.0 ng/mL
Calibrator 4	75.0 ng/mL
Calibrator 5	160.0 ng/mL

Control value assignment:

Normal (>30.0 ng/mL) and abnormal (<29.9 ng/mL) 25-OH Vitamin D controls prepared in human serum are tested with released Diazyme 25-OH Vitamin D reagents and calibrators and obtained from 3 independent runs in 20 replicates. The obtained mean values are assigned as control target values. The controls target range is assigned as mean $\pm$ 25%.

The approximate target values for the controls are 23.1 ng/mL and 45.7 ng/mL.

Calibrator and control stability

Studies were performed by the manufacturer to support the storage claim of up to 8 months for unopened vials and 4 weeks for opened vials stored at 2-8 °C for both the calibrator and controls. Stability protocols and acceptance criteria were reviewed and found to be adequate.

d. *Detection limit:*

The limit of Blank (LoB), the Limit of Detection (LoD), and Limit of Quantitation (LoQ) of the Diazyme 25-OH Vitamin D Assay were determined according to CLSI17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation.

LoB: To calculate the limit of blank (LoB) of the Diazyme 25-OH Vitamin D assay, aliquots of Vitamin D-depleted serum were used. This serum contains undetectable levels of 25-OH Vitamin D as measured by LCMS/MS (<1 ng/mL). The Vitamin D-depleted serum was assayed with the Diazyme Vitamin D assay in three independent runs with 20 replicates per run. The LoB was calculated as the mean of the 57th and 58th highest values for the blanks.

LoD: To calculate the limit of detection (LoD) of the Diazyme 25-OH Vitamin D assay, five very low Vitamin D serum samples were measured in three independent runs, with four replicates per run. The LoD is defined with the following equation: $LoD = LoB + (1.645 * STDEV \text{ of Low samples})$.

LoQ: Five specimens with mean measured concentration ranging from 6.62 to 22.43 ng/mL were assayed. Each sample was assayed in replicates of 40 in 5 separate runs for 200 data points. A curve was fit to estimate the relationship between mean and CV. LoQ is defined as the point where the upper 95% confidence interval for the curve has a CV of 20%. The following are the limits determined with the Diazyme 25-OH Vitamin D Assay.

LoB	LoD	LoQ
2.0ng/mL	3.5 ng/mL	7.6 ng/mL

The measuring range of this assay is 7.6 – 147.8 ng/mL.

e. *Analytical specificity:*

Interference Study:

An interference study was performed according to the CLSI EP7-A2 protocol. For each concentration of interfering substance, five serum samples at two different 25-OH Vitamin D concentrations were measured in duplicate, with two independent runs to ensure precise results. The sponsor defines non-significant interference as a difference of less than or equal to 10% between the spiked and the control samples. Results of non-significant interference are summarized in the table below.

Substance	Highest Concentration tested which demonstrated no significant interference.
Ascorbic Acid	176 mg/dL
Free Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Triglycerides	750 mg/dL
Hemoglobin	100 mg/dL
Uric Acid	20 mg/dL
Biotin	2 mg/dL
Human Serum Albumin	9 g/dL
N-Acetyl Cysteine Amide	1663 ng/mL
Ampicillin	1000 ng/mL
Cyclosporine C	105 ng/mL
Cefoxitin	660 ng/mL
Acetylsalicylic Acid	1000 ng/mL
Rifampicin	64 ng/mL
Acetaminophen	200 ng/mL
Ibuprofen	500 ng/mL
Theophylline	100 ng/mL

Because assay results are affected by hemoglobin, the sponsor has the following limitation in their labeling:

“Any visibly hemolyzed sample should not be used.”

Cross Reactivity study:

Cross-reactivity of the Diazyme 25-OH Vitamin D Assay was evaluated by adding Vitamin D metabolites to serum pool samples. Based on the results in the table below, the assay did not cross react with Vitamin D2 and Vitamin D3 and the assay recovers both 25-OH Vitamin D2 and 25-OH Vitamin D3 similarly. Cross-reactivity with various Vitamin D metabolites is summarized in the table below:

Cross-reactant	Concentration of cross reactants tested (ng/mL)	Cross-reaction*
25-OH Vitamin D3	44.0	100%
25-OH Vitamin D2	44.0	92.3%
Vitamin D3	44.0	1.0%
Vitamin D2	44.0	2.9%
1,25-OH Vitamin D3	2.9	2.5%
1,25-OH Vitamin D2	2.9	-1.5
24R, 25-OH Vitamin D3	41.0	5.1%
3-epi-25-OH Vitamin D3	42.0	61.7%

3-epi-25-OH Vitamin D2	42.0	55.1%
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*% Cross-reactivity = (mean value spiked – mean value unspiked /Concentration Spiked)*100

No significant cross-reactivity (4.1%) was found for Paricalcitol (Zemplar®) up to 25ng/mL.

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

Human serum samples were tested with the Diazyme 25-OH Vitamin D Assay and the obtained results were compared to the predicate method. 98 unaltered serum samples were tested in the method comparison study. All the Diazyme 25-OH Vitamin D values were obtained from a single measurement using one reagent lot and one instrument (Roche Modular P chemistry analyzer). Results of the Deming Regression analysis are presented in the following table:

n	98
Slope	1.005 (0.969 to 1.041)
Intercept	-0.21 (-2.15 to 1.73)
Correlation Coefficient	0.984 (0.976 to 0.989)
Range of values	9.5 to 140.9

b. Matrix comparison:

To evaluate the effect of anticoagulants, the Diazyme 25-OH Vitamin D Assay was used to measure the 25-OH Vitamin D concentrations of matched sets of serum, K₃-EDTA plasma, and Lithium-Heparin plasma. The reported values for each sample and for each matrix were obtained from single measurements. The total numbers of matched sets tested were 66. In order to cover the claimed measuring range for each matrix, seven spiked patient samples were included in the study.

Linear regression of the Lithium-Heparin plasma versus serum data yielded the following results: $y = 0.9657x - 0.6596$ and $R^2 = 0.9736$

Linear regression of the K₃-EDTA plasma versus serum data yielded the following results: $y = 0.9948x - 0.7057$ and $R^2 = 0.9866$.

The results from the matrix comparison study support the sponsors claim that EDTA and Lithium Heparin samples are acceptable for this 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:

To determine a reference range for the Diazyme 25-OH Vitamin D Assay, the 25-OH Vitamin D serum concentrations of a US population of 157 apparently healthy individuals (21- 80 yrs) were measured with the Diazyme method. Forty seven (47) samples from Pennsylvania (Northern U.S.), fifty six (56) samples from Tennessee (Central U.S.) and fifty four (54) samples from Texas (Southern U.S.) were evaluated. All participating individuals met the following inclusion conditions:

- All samples were collected during the months of October and November (fall season).
- The studied population consisted of 72 light skinned individuals (46%) and 85 dark skinned individuals (54%).
- 155 individuals (98.7%) did not take any artificial Vitamin D supplements. Two individuals (1.3%) did take some Vitamin D supplements but did not exceed the daily dose of 2000 IU.
- All 157 individuals did not have any family history of parathyroid or calcium regulatory disease.
- All 157 individuals did not have any history of kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, and calcium related dis-ease, seizures, chronic disease, or bariatric surgery.
- All 157 individuals were not currently taking any medications that are known to affect absorption or catabolism of Vitamin D (including cholesterol absorption inhibitors such as Vytorin®, Inegy™ or Zetia; anticonvulsants such as Neurontin, Depakine® and Tri-leptal; glucocorticoids such as Cortisol, Prednisone and Dexamethasone; HAART (AIDS treatment) or antirejection medications.

Analysis of the reference range study data yielded the following results:

- Lowest 25-OH Vitamin D concentration: 12.6 ng/mL.
- Highest 25-OH Vitamin D concentration: 51.4 ng/mL.
- Median 25-OH Vitamin D concentration: 25.6 ng/mL
- Observed range (2.5th to 97.5th percentile): 15.0 to 45.9 ng/mL

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.