SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k102432

B. Purpose for Submission:

New device

C. Measurand:

25-hydroxyvitamin D

D. Type of Test:

Quantitative chemiluminescent assay

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

1. Diazyme 25-Hydroxy Vitamin D Microplate Assay Kit
2. Diazyme 25-Hydroxy Vitamin D Assay Calibrator Set
3. Diazyme 25-Hydroxy Vitamin D Assay Control Kit

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 Vitamin D Test System	Clinical Chemistry (75)
JIS	Class II	21 CFR 862.1150 Calibrator	Clinical Chemistry (75)
JJX	Class I, reserved	21 CFR 862.1660 Quality Control Material	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   Refer to indication for use below.

2. Indication(s) for use:

   The Diazyme 25-hydroxy Vitamin D Assay is designed for the quantification of total 25-hydroxy Vitamin D in human serum and plasma. The assay results are to be used in parallel with other clinical data to assess the Vitamin D status of a patient. For in vitro diagnostic use only.

   The 25-hydroxy Vitamin D Assay Calibrator set is intended for use in the calibration of the Diazyme 25-OH Vitamin D Assay Kit only. For in vitro diagnostic use only.

   The 25-hydroxy Vitamin D Assay Control kit is intended for use as quality controls for the Diazyme 25-OH Vitamin D Assay kit only. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use.

4. Special instrument requirements:

   96-well microplate reader that reads at 560 nm.

I. Device Description:

1. Diazyme's 25-Hydroxy Vitamin D Assay Kit is provided in microplate configuration (96 tests). The content of the kit includes:

   - 8-well Microtube Strips (12)
   - Microtube Rack (1)
   - 96-well Microplate (1)
   - Microplate Sealing Film (3)
   - EX: Acetonitrile-based extraction solution (1x 30 mL)
   - R1a: Lyophilized enzyme acceptor, Vitamin D binding protein and phosphate salts (1)
   - R1: Reconstitution Buffer containing phosphate salts and preservatives (1x 8 mL)
   - R2a: Lyophilized enzyme donor (1 vial)
   - R2: Reconstitution Buffer containing phosphate salts and preservatives (1x 6 mL)
   - R3a: Lyophilized substrate (CPRG) (1 vial)
   - R3: Reconstitution buffer containing phosphate salts and preservatives (1x 12.5 mL)
   - STOP: Sodium carbonate stop solution (1x 8 mL)

2. Diazyme's 25-hydroxy Vitamin D Assay Calibrator set is sold separately and contains:

   Six level calibrators in Human serum supplied in aliquots of 1.0 mL. The exact concentration (ng/mL) of each calibrator is indicated on the included certificate of analysis. 6x1 mL vials are provided per kit.

3. Diazyme's 25-hydroxy Vitamin D Control Pack is sold separately and contains:

Two Level controls in human serum supplied in aliquots of 1.0 mL. Control level 1 has a concentration of 25-OH Vitamin D that corresponds to a deficient patient. Control level 2 has a concentration of Vitamin D that corresponds to a normal patient. 2x1 mL vials are provided per kit.

4. The sponsor stated in the labeling that all donor units of serum used in the preparation of Calibrators and Controls were tested by FDA-approved method and found negative for HIV (HIV I/II antibody), HBV (HBsAg) and HCV (antibody).

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Immunodiagnostic Systems (IDS) 25-OH Vitamin D EIA assay

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

   k021163

3. Comparison with predicate:

Items	Diazyme 25-OH Vitamin D Assay (Candidate Device)	IDS 25-OH Vitamin D EIA assay (Predicate Device)
Similarity		
Intended use /Indication for use	For the quantitative determination of 25-hydroxyvitamin D (25-OH D) and other hydroxylated metabolites in human serum or plasma. Results are to be used in conjunction with other clinical and laboratory data to assist the clinician in the assessment of vitamin D sufficiency in adult populations. For *in vitro* diagnostic use only.	Same
Sample type	Serum and plasma	Same
Test format	96-well microplate assay	Same
Assay procedure	Manual	Same
Instrument	Microplate reader	Same
Approximate assay time	3.5 hours	Same
Antigen used in calibrators	25-OH Vitamin D3	Same
Difference		
Test methodology	The assay consists of a sample extraction step during which Vitamin D is irreversibly dissociated from its serum transporter. Extracted samples are then analyzed on a microplate reader after the sequential addition of	Samples are diluted with dissociation buffer containing proprietary reagent for dissociating Vitamin D and biotin labeled 25-OH Vitamin D. The diluted samples are incubated in microtitre wells which are coated with sheep 25-OH Vitamin D

	three reagents. The assay is based on the principle of α-complementation of the enzyme β-galactosidase and the competition between an enzyme donor-25-hydroxy Vitamin D conjugate, Vitamin D binding protein and the 25-hydroxy Vitamin D content of a serum sample. The 25-hydroxy Vitamin D concentration of a patient sample is proportional to the measured β-galactosidase activity.	antibody for 2 hours at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labeled avidin, is added and binds selectively to complexed biotin and, following a further wash step, color is developed using a chromogenic substrate (TMB). Color intensity is inversely proportional to the concentration of 25-OH Vitamin D.
Measuring wavelength	560 nm	450 nm
Antibody	Not used	Sheep 25-OH Vitamin D antibody
Sample volume	90 µL	25 µl
Assay range	5.9-120 ng/mL	2.4-144 ng/mL
Specificity	25-OH Vitamin D	25-OH Vitamin D and other hydroxylated metabolite (24, 25-Dihydroxyvitamin D3, 100% cross reactivity)

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

- CLSI Guideline EP5-A2
- CLSI Guideline EP6-A
- CLSI Guideline EP7-A2
- CLSI Guideline EP17-A

L. Test Principle:

The assay consists of a sample extraction step during which Vitamin D is irreversibly dissociated from its serum transporter. Extracted samples are then analyzed on a microplate reader after the sequential addition of three reagents. The assay is based on the principle of α-complementation of the enzyme β-galactosidase and the competition between an enzyme donor-25-hydroxy Vitamin D conjugate, Vitamin D binding protein and the 25-hydroxy Vitamin D content of a serum sample. The 25-hydroxy Vitamin D concentration of a patient sample is proportional to β-galactosidase activity measured by absorbance at 560 nm.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Study Protocol:
Two replicates of each of 4 pooled human serum samples (with or without spike) and 5 patient serum samples were tested on 2 separate occasions per day for 10 different days using 2 lots of reagents. The spiked samples were allowed to mix gently at 4°C for 18 hours to ensure that all spiked 25-OH Vitamin D3 is captured by the serum transporter

VDBP. Samples ranged in value from 7.8-101.2 ng/mL. Mean, within-run CV and total precision CV are summarized in the table below.

Result Summary:

Precision:

Sample	1 Pooled Human Serum (PHS)	2 Spiked PHS	3 Spiked PHS	4 Spiked PHS	5 Patient Sample	6 Patient Sample	7 Patient Sample	8 Patient Sample	9 Patient Sample
Data points	40	40	40	40	40	40	40	40	40
Mean (ng/mL)	24.4	38.4	61.7	101.2	7.8	19.6	19.0	33.0	42.9
Within-run CV (%)	4.2	5.3	6.5	6.5	15.3	4.8	5.7	4.6	3.6
Total CV (%)	7.9	9.7	9.6	9.7	16.7	9.4	9.1	8.4	8.6

b. Linearity/assay reportable range:

Study Protocol:
Linearity was evaluated following CLSI guideline EP6-A. Samples were prepared by diluting a high patient sample with a sample diluent to obtain eleven concentrations across the measuring range, ranging from 0 to 130 ng/mL. Each sample was assayed in triplicate. The resulting mean concentrations of each sample were compared to predicted concentrations and yielded a linear regression of Y=1.021 X + 0.00.

Conclusion:
Based on the results of the linearity study and Limit of detection study, the sponsor claimed that the assay is linear from 5.9 to 120 ng/mL.

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

Traceability and value assignment:

Calibrators

The master calibrator batch stock was prepared in house gravimetrically in DMSO. Value assignment of stocks is made by UV absorbance spectrometry calibrated with NIST SRM 935a, based on NIST recommended molar extinction coefficients. The DMSO stock of 25-OH Vitamin D3 was used to build calibration levels by dilution into pooled human serum.

Initial value for calibrators is assigned using the predicate assay. Subsequently, patient serum samples (at least 20) are tested with the Diazyme 25-OH Vitamin D assay reagents and calibrators and compared to the values obtained using the predicate assay. The final calibrators values are then assigned by adjusting their initial values to meet the following acceptance criteria for the two-method comparison: Slope = 1.0 ± 0.1; $r^2 \geq 0.9$. Finally, calibrator value assignment was verified by measuring the 25-

OH Vitamin D content of standard control level 1 and standard control level 2, provided by the National Institute of Standards and Technology (NIST SRM 972).

Controls

Normal (> 32.0 ng/mL) and Abnormal (< 31.9 ng/mL) 25-OH Vitamin D controls prepared in human serum are tested with released Diazyme 25-OH Vitamin D microplate kits, in 12 replicates, in three runs. The mean obtained values are assigned as control values. The controls are labeled with the assigned values as mean ±25%.

Stability:

- Shelf-life Stability:

  Real-time testing at 2-8^{o}C and accelerated testing at 37^{o}C were conducted for the control materials and the calibrators. The stability study protocol and the acceptance criteria have been reviewed and found to be acceptable. The predicted shelf-life, based on results of accelerated testing at 37^{o}C, is at least 12 months at 2-8^{o}C for the control materials and the calibrators. Real-time stability testing is on-going to support the predicted shelf-life of 12 months at 2-8^{o}C.

- Open-Vial Stability:

  The stability study protocol and the acceptance criteria to determine open-vial stability of the control materials and the calibrators have been reviewed and found to be acceptable. Diazyme 25-OH Vitamin D assay reconstituted reagents R1, R2, R3 and thawed calibrators and controls are stable for at least 11 days when stored at 2-8^{o}C.

- Sample Stability:

  Stability of samples stored at different conditions was evaluated. Two sample levels were prepared by spiking freshly drawn serum or EDTA plasma with 25-OH Vitamin D. Three aliquots of each sample were processed immediately and analyzed within one hour of processing. Results from these aliquots were used as the basis for comparison to samples that were stored for various time periods and at various storage conditions before processing. Each sample aliquot was measured in duplicates.

  Based on results summarized in the below table, the sponsor made the following recommendation in the labeling: "the specimens may be refrigerated at 2-8°C for two weeks. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles."

Sample	**Storage conditions**	**Stable for at least**	**% Value drift**
Processed sample	20 -23°C	5 hours	<7.9
Sample	-15 to -25°C	3 freeze-thaw cycles	<2.4
Sample	2 - 8°C	14 days	<11.9

Sample	20 - 23°C	18 hours	<15

d. Detection limit:

For LoB determination, saline supplemented with 9% Bovine Serum Albumin was measured in three independent runs with 20 replicates per run giving 100 determinations in total. The LoB is calculated as the mean of the 57th and 58th highest values for the blanks. The LoB claim is 1.0 ng/mL.

For LoD determination, five low serum samples were measured in 3 independent runs with 4 replicates per run. The LoD is defined as LoD = LoB + (1.645 * STDEV of Low samples). The five LoD samples were made by diluting 5 commercial serum samples 1:100 with saline supplemented with 9% Bovine Serum Albumin. The LoD claim is 3.6 ng/mL.

For LoQ determination, five low samples were tested. For each sample, 40 replicates were obtained from at least 5 independent runs. The level of imprecision used to accept the LoQ was within 20%. The LoQ claim is 5.9 ng/mL.

The sponsor claimed that the assay has a measuring range from 5.9 to 120 ng/mL.

e. Analytical specificity:

- Interference

  To determine the level of interference from the substances normally present in serum, the sponsor spiked each of the substances at different concentrations into each of the two unaltered patient serum samples (with 18.5 ng/mL and 65.0 ng/mL 25-OH D levels). The sponsor defines no significant interference as <10 difference between the spiked and the control samples. The interference substances examined and their concentrations tested are listed in the following table:

Interference Substances	Concentration tested			
Ascorbic acid (mM)	1.0	2.5	5.0	10.0
Bilirubin (mg/dL)	10	20	30	40
Bilirubin Conjugated (mg/dL)	10	20	30	40
Hemoglobin (mg/dL)	50	100	250	500
Triglycerides (mg/dL)	250	500	750	1000

The sponsor indicates in the labeling that the assay's results were not significantly affected by the presence of the following substances normally present in the serum:

Interference	Concentration
Ascorbic Acid	10 mM
Bilirubin	40 mg/dL

Bilirubin Conjugate	30 mg/dL
Triglyceride	500 mg/dL
Hemoglobin	100 mg/dL

The sponsor states the following in their labeling: "Do not use hemolysed samples."

- Cross-Reactivity

Study Protocol:
To estimate the cross-reactivity of various Vitamin D metabolites with 25-OH Vitamin D3, six identical serum aliquots (10 mL) were each spiked with one of the cross-reactant listed below, to a final concentration of 100 ng/mL. Cross-reactants stock solutions were made with 100% DMSO. A seventh serum aliquot of 10 mL was spiked with 100% DMSO (devoid of Vitamin D) and served as a negative control. All 7 spiked sera were then quantified in quadruplicates. % Cross-reactivity was calculated using the following the equation:

$$\% \text{ Cross-reactivity} = \frac{\text{Recovery of cross-reactant spiked sample}}{\text{Recovery of 25-OH Vitamin D3 spiked sample}} \times 100$$

Recovery = Measured Vitamin D concentration from spiked sample – Measured Vitamin D concentration from negative control

Conclusion:
Based on the results summarized in the below table, the sponsor concluded that the assay did not significantly cross react with Vitamin D2, Vitamin D3, 1,25-OH vitamin D3, and 1,25-OH Vitamin D2. The assay recovers both 25-OH Vitamin D3 and 25-OH vitamin D2.

Cross-Reactant	Vitamin D3	Vitamin D2	1,25-OH Vitamin D3	1,25-OH Vitamin D2	25-OH Vitamin D3	25-OH Vitamin D2
Spiked (ng/mL)	100.0	100.0	100.0	100.0	100.0	100.0
Cross Reactivity	-0.6%	2.9%	3.9%	2.6%	100.0%	92.2%

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Study Protocol:
To demonstrate comparable performance with the predicate device, a total of 80 serum samples (individual or pooled patient serum) were obtained from a certified commercial source and tested on the candidate device and the predicate device. To ensure that the tested concentrations of 25-OH Vitamin D are distributed across the reportable dynamic range, 12 samples in the set were spiked with 25-OH Vitamin D stock solution of 25-OH Vitamin D3.

Result of linear regression analysis:

	Serum Samples
n	79
Slope	1.039
Intercept	-2.481
Correlation coefficient	0.935
Range of values	7.9 ng/mL-120 ng/mL (Predicate)
	7.7 ng/mL-113.8 ng/mL (Candidate)

b. Matrix comparison:

Study Protocol:

Serum versus Lithium Heparin:

Matched set of serum, Li-Heparin plasma samples were obtained from 26 patients. The reported values for each sample and for each matrix were obtained from single measurements (one replicate). In order to cover the claimed measuring range for each matrix, samples from 2 of the 26 patients were spiked with 25-OH Vitamin D3 stock solution.

Serum versus EDTA plasma:

Matched set of serum and K3-EDTA plasma samples were obtained from 55 patients. The reported values for each sample and for each matrix were obtained from single measurements (one replicate). In order to cover the claimed measuring range for each matrix, samples from 7 of the 55 patients were spiked with 25-OH Vitamin D3 stock solution.

Results of linear regressions:

Matrix Y	Matrix X	Slope	Intercept	R^2	N	25-OH D range (ng/mL)
Lithium heparin plasma	Serum	0.992	-0.173	0.986	26	12.3-118.4
K3-EDTA plasma	Serum	0.908	1.749	0.981	55	8.9-118.4

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:

   To determine a reference range for the Diazyme 25-OH Vitamin D microplate assay, the 25-OH Vitamin D serum concentrations of a U.S. population of 150 apparently healthy individuals (age between 21 and 90 years old), were measured with the Diazyme method. These samples were collected from three different geographical locations, with 30 samples collected from Pennsylvania (Northern U.S.), 60 samples collected from Tennessee (Central US) and 60 samples collected from Texas (Southern US). All serum samples were collected during the fall season (October 2010-November 2010). The following results were obtained:

   Lowest 25-OH Vitamin D concentration: 9.9 ng/mL
   Highest 25-OH Vitamin D concentration: 65.8 ng/mL
   Median 25-OH Vitamin D concentration: 21.1 ng/mL
   Observed range (2.5th to 97.5th percentile): 11.3 to 41.4 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.