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

A. 510(k) Number:

k172992

B. Purpose for Submission:

New Device

C. Measurand:

25-hydroxyvitamin D

D. Type of Test:

Quantitative immunoturbidimetric assay

E. Applicant:

Diazyme Laboratories

F. Proprietary and Established Names:

Diazyme EZ Vitamin D Assay

G. Regulatory Information:

Product Code	Classification	Regulation	Panel
MRG	II	862.1825 Vitamin D Test System	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The Diazyme EZ 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 analyzers. Measurement of 25-hydroxyvitamin D (25-OH-D) is used for the assessment of the vitamin D sufficiency. For in vitro diagnostic use only.

3. Special conditions for use statement(s):

   For prescription use only.

4. Special instrument requirements:

   All performance testing was conducted on the Beckman AU680 analyzer.

I. Device Description:

The Diazyme EZ Vitamin D Assay kit consists of the following:
Reagent 1 (1 x 40 mL): Phosphate buffer solution (< 100 mM), 0.1% sodium azide.
Reagent 2 (1 x 10 mL): Suspension of latex particles (< 0.5%) coated with anti-vitamin D antibodies, ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Diazyme 25-OH Vitamin D Assay

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

   k133410

3. Comparison with predicate:

Similarities and Differences		
Item	Diazyme EZ Vitamin D Assay Candidate Device: k172992	Diazyme 25-OH Vitamin D Assay Predicate Device: k133410
Intended Use	For the quantitative determination of 25-hydroxyvitamin D (25-OH-D) in human serum and plasma. Measurement of 25-hydroxyvitamin D is used in the assessment of vitamin D sufficiency.	Same

Similarities and Differences		
Item	Diazyme EZ Vitamin D Assay Candidate Device: k172992	Diazyme 25-OH Vitamin D Assay Predicate Device: k133410
Storage	2-8^{o}C	Same
Measuring Range	7.6 - 147.8 ng/mL	Same
Test Methodology	Direct particle-enhanced immuno-turbidimetric assay	Direct competitive colorimetric immunoassay
Sample type	Serum, K2-EDTA, K3-EDTA, and lithium heparin plasma	Serum, K3-EDTA, and lithium heparin plasma

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

- CLSI EP05-A2: Evaluation of Precision Performance of Quantitative Measurement Methods Approved Guideline-Second Edition
- CLSI EP6-A: Evaluation of Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP07-A2: Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition
- CLSI EP09-A3: Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline-Third Edition
- CLSI EP17-A2: Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline-Second Edition
- CLSI EP28-A3c: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition

L. Test Principle:

The Diazyme EZ Vitamin D Assay is a direct particle-enhanced immuno-turbidimetric assay. The assay's reagents dissociate vitamin D from vitamin D binding proteins, found in serum or plasma specimens, while particles coated with anti-vitamin D antibodies bind to the dissociated vitamin D, thereby causing agglutination. This agglutination is detected as an absorbance change at 700 nm, with the magnitude of the change being proportional to the quantity of total vitamin D in the sample. Specimen concentrations are determined by interpolation from a 5-point calibration curve prepared from calibrators of known concentrations.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision testing was performed in accordance with CLSI EP05-A2 guideline. The precision study was performed using 11 samples, including 2 serum-based quality control materials and 9 human serum samples. Each sample was tested in duplicate on 2 runs per day over 20 days using 3 reagent lots, for a total of 80 replicates per

sample per lot. All three lots of reagent yielded similar precision results. Results of data analysis of the combined 3 lots (n=240) are summarized below:

Sample	Mean (ng/mL)	Within Run		Total	
		SD	%CV	SD	%CV
QC 1	21.7	0.9	3.9%	1.3	6.2%
QC 2	42.5	1.0	2.4%	1.7	3.9%
Serum 1	11.1	0.9	8.3%	1.8	16.6%
Serum 2	18.2	0.9	4.9%	1.6	8.7%
Serum 3	22.1	0.8	3.8%	1.2	5.6%
Serum 4	42.8	0.9	2.0%	1.3	3.1%
Serum 5	59.5	1.0	1.7%	1.6	2.7%
Serum 6	80.2	1.3	1.6%	2.0	2.5%
Serum 7	99.5	1.8	1.8%	2.7	2.8%
Serum 8	117.6	2.2	1.9%	3.7	3.2%
Serum 9	139.2	2.7	1.9%	4.1	2.9%

b. *Linearity/assay reportable range:*

A linearity study was conducted according to CLSI EP6-A guideline. Eleven levels were prepared by diluting a high-spiked serum sample (averaging 153.0 ng/mL 25-OH vitamin D) with a low 25-OH vitamin D serum sample (averaging 4.7 ng/mL 25-OH vitamin D). The samples were measured in triplicate using one lot of reagent.

The results of the linear regression analysis are summarized below:

$y = 0.9816x + 1.7094$, $R^2 = 0.9994$

The results of the linearity study supports the claimed measuring range of 7.6 to 147.8 ng/mL.

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

The Diazyme EZ Vitamin D calibrators are traceable to the NIST SRM972a 25-OH vitamin D reference material.

d. *Detection limit:*

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) studies were performed according to the CLSI EP17-A2 guideline.

The LoB study was performed on a serum based depleted 25-OH vitamin D tested using three lots of the reagents. The samples were tested in 12 replicates per day over 5 days. The LoB was determined to be 1.2 ng/mL.

The LoD study was performed with five commercial serum samples diluted with 25-OH vitamin D-depleted diluent and tested using three lots of the Diazyme EZ Vitamin D reagents. Samples were tested in replicates of 12. The LoD was calculated as the LoB + (1.645 * SD of LoD samples). The LoD was determined to be 2.9 ng/mL.

The LoQ study was performed using five commercial serum samples that were diluted with 25-OH vitamin D-depleted serum to concentrations ranging from about 1 to 8 times the claimed LoB. The diluted serum samples were then tested in 8 replicates per day over 5 days using three lots of reagents. The sponsor defines LoQ as the lowest concentration which meets an imprecision of <20%. The LoQ was determined to be 7.6 ng/mL.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
1.2 ng/mL	2.9 ng/mL	7.6 ng/mL

The claimed measuring range of the assay is 7.6 to 147.8 ng/mL

e. *Analytical specificity:*

Interference studies were conducted according to the CLSI EP7-A2 guideline. Serum pools with approximate vitamin D concentrations of 20, 40, and 80 mg/dL were spiked with the potentially interfering substance and tested in triplicate. The sponsor defines significant interference as less than 10% bias between the spiked sample and the control. The following substances were tested up to the levels indicated and demonstrated no significant interference:

Substance	Highest concentration of substance tested that did not demonstrate significant interference
Bilirubin	40 mg/dL
Conjugated Bilirubin	40 mg/dL
Hemoglobin	600 mg/dL
Total Protein	12.0 g/dL
Triglyceride	1000 mg/dL
Rheumatoid factor (RF)	200 IU/mL
Acetaminophen	20 mg/dL
Acetyl Salicylic Acid	60.0 mg/dL
Ampicillin	5.3 mg/dL
Ascorbate	3.0 mg/dL
Biotin	100 ng/mL
Carbamazepine	3.0 mg/dL
Cefotaxime	180.0 mg/dL
Chloramphenicol	5.0 mg/dL

Substance	Highest concentration of substance tested that did not demonstrate significant interference
Creatinine	30.0 mg/dL
Digoxin	6.1 ng/mL
Ethanol	400.0 mg/dL
Ethosuximide	25.0 mg/dL
Furosemide	6.0 mg/dL
HAMA	350 ng/mL
Heparin	3.0 U/mL
Ibuprofen	50.0 mg/dL
Lidocaine	1.2 mg/dL
Lithium Acetate	2.2 mg/dL
Noraderenalin	4.0 µg/mL
Rifampicin	5.0mg/dL
Theophylline	4.0 mg/dL
Urea	300 mg/dL
Uric acid	20 mg/dL
Valproid Acid	50 mg/dL
Vancomycin	10 mg/dL

HAMA was tested at 0, 100 and 350 ng/mL on samples with 25-OH vitamin D concentrations of around 30 and around 50 ng/mL in triplicate. Results showed that the Diazyme EZ Vitamin D Assay does not have any significant interference with HAMA when present at concentrations up to 350 ng/mL.

Cross-reactivity of the Diazyme EZ Vitamin D Assay was determined by testing serum pools that were spiked with each of the cross-reactants to the effective concentrations listed in the table below and analyzed in triplicates.

Cross-reactivity results are summarized in the table below:

Cross-reactant	Concentration tested	Cross Reactivity %
25-OH Vitamin D3	100 ng/mL	100.0%
25-OH Vitamin D2	100 ng/mL	106.9%
Vitamin D3	100 ng/mL	-0.8%
Vitamin D2	100 ng/mL	-1.7%
1,25-dihydroxy Vitamin D3	580 pg/mL	0.2%
1,25-dihydroxy Vitamin D2	580 pg/mL	-0.5%
24R, 25(OH)2 Vitamin D3	100 ng/mL	118.8%*
3-epi 25-hydroxy Vitamin D3	100 ng/mL	33.0%*
3-epi 25-hydroxy Vitamin D2	100 ng/mL	36.5%*
Zemplar/paricalcitrol	5 ng/mL	9.5%

*The high level of cross reactivity of the assay with 24R, 25(OH)2 Vitamin D3, 3-epi 25-hydroxy vitamin D3, and 3-epi 25-hydroxy vitamin D2 is attributed to the high concentration of each substance spiked into the test sample which is significantly above the normal physiological concentration. The low levels of the substances found at normal physiological concentrations are not expected to significantly cross react with this assay.

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed in accordance to CLSI EP09-A3 guideline. 171 human serum samples (161 native and 10 contrived) were tested in singlet with the candidate device on Beckman AU680 analyzer and compared to the predicate device (Diazyme 25-OH Vitamin D Assay).

The results of linear regression are presented in the following table:

n	171
Slope	1.062
Intercept	-3.03
Correlation Coefficient R	0.9785
Range of values	7.6-144.2 ng/mL

b. Matrix comparison:

Studies were performed to compare the performance of the Diazyme EZ Vitamin D with different sample matrices. A total of 49 matched samples obtained from a commercial vendor were tested by single measurements on Beckman AU680 analyzer. The results of the linear regression analysis are presented below:

Tube Type	Regression	R^2	Test range ng/mL
Lithium-heparin plasma vs serum	y = 1.0475x – 1.3749	0.995	8.6 - 126.3
K_2 EDTA plasma vs serum	y = 1.0198x 0.4985	0.996	8.4 - 126.0
K_3EDTA plasma vs serum	y = 1.0378x – 1.2959	0.994	9.2 - 123.0

The study data supports the sponsor's claim that the Diazyme EZ Vitamin D assay is suitable for use with human specimens consisting of serum, K2-EDTA, K3-EDTA and lithium heparin plasma.

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 EZ Vitamin D assay, the 25-OH vitamin D serum concentrations of a US population of 145 apparently healthy male and female individuals ranging from 21 to 67 years of age. To represent a broad spectrum of UV light exposure in the intended use population, the blood samples tested were collected from subjects residing in diverse U.S. geographical locations. All subjects did not have a history of kidney, GI, parathyroid or calcium regulatory disease and did not take vitamin D supplements. The results of the study indicate that the central 95% of the population was found to have 25-OH vitamin D concentrations between 7.2 ng/mL and 41.6 ng/mL as measured by the Diazyme EZ Vitamin D assay.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.