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

A. 510(k) Number:

k123364

B. Purpose for Submission:

New device

C. Measurand:

25-hydroxy Vitamin D

D. Type of Test:

Quantitative, competitive enzyme immunoassay

E. Applicant:

DIAsource ImmunoAssays

F. Proprietary and Established Names:

25OH Vitamin D Total ELISA Test

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 – Vitamin D Test System	75

H. Intended Use:

1. Intended use(s):

   See Indications for use below

2. Indication(s) for use:

The DIAsource 25 OH Vitamin D Total ELISA Test is intended for the quantitative measurement of 25-hydroxy vitamin D2 and D3 (25 OH-D2 and 25 OH-D3) in human serum. The results are to be used in conjunction with other clinical and laboratory findings to assess the Vitamin D status of a patient.

3. Special conditions for use statement(s):

- For prescription use only

4. Special instrument requirements:

ELISA plate reader at 450nm.

I. **Device Description:**

The kit contains the following:

Microtiter-Plate consisting of 96 wells with Monoclonal anti-25OH Vitamin D_2 and D_3

Calibrator 0, 1 vial lyophilized, biological matrix (human plasma) with gentamycin and proclin

Calibrators 1-5, 1 vial each lyophilized with horse serum with gentamycin and proclin

Controls 1-2, 1 vial each lyophilized with human serum and proclin

Incubation Buffer, 20 ml, ready to use

Conjugate Concentrate 100x, 0.4ml. 25OH Vitamin D Concentrated Conjugate

HRP Conjugate 200x, 0.2ml. Concentrated HRP

Conjugate Buffer, 30ml. ready to use.

Wash Solution Concentrate 200x, 10ml

Chromogenic solution TMB, 12ml

Stop Solution, 12 ml, contains 1.5N HCl.

The human blood components in the kit have been tested by FDA-approved methods

and found negative for the presence of HBsAg, anti-HIV-1/2 and anti-HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   IDS 25-Hydroxy Vitamin D EIA

2. Predicate K number(s):

   k021163

3. Comparison with predicate

IDS 25OH Vitamin D ELISA (Predicate)	The DIAsource 25OH Vitamin D Total ELISA (Candidate)
Intended Use - For the quantitative determination of 25-hydroxyvitamin D (25-OH D) in human serum. The 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.	Same
Platform - ELISA plate read on a plate reader	Same
Microtiter Plate - 96 wells coated with sheep polyclonal anti 25OH D	**Microtiter Plate** - 96 wells coated with mouse monoclonal anti 25OH D_2 and D_3
Wash Solution Concentrate – 20x	**Wash Solution Concentrate** – 200x
Incubation Buffer – Proprietary buffer for dissociating vitamin D	Same
Conjugate Concentrate – None	100x
HRP Concentrate –Ready to Use	**HRP Conjugate** – 200x
Conjugate Buffer – None	Use to dilute Conjugate and HRP Concentrate
Substrate – Tetramethylbenzidine (TMB)	Same
Stop Solution – Acid mixture	Same
Calibrators 0-6 Ready to Use	**Calibrators 0-5** - Lyophilized
Controls 1-2 – Lyophilized	Same
Interpretation of Results – Standard Curve	Same
Expected Values: Deficient <10 ng/ml; Insufficient 10-29 ng/ml; Sufficient 20-100 ng/ml; Potential Toxicity >100ng/ml	Same

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

- *CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures*
- CLSI EP17-A, *Protocols for Determination of Limits of Detection and Limits of Quantitation*.

L. Test Principle:

The DIAsource 25 OH Vitamin D Total ELISA Test is an enzyme linked immunosorbent assay to detect total 25OH Vitamin D (D2 and D3) present in human serum. During the first incubation at room temperature, 25OH Vitamin D is dissociated from binding serum proteins to fix on binding sites of a specific monoclonal antibody. After washing, a fixed amount of 25OH Vitamin D-labeled with biotin in presence of horseradish peroxidase (HRP) compete with unlabeled 25OH Vitamin D2 and 25OH vitamin D3 present on the binding sites of the specific monoclonal antibody. After another incubation at room temperature, the microtiterplate is washed to stop the competition reaction. A chromogenic solution (TMB) is added and then stopped with a Stop Solution after the last incubation period. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is inversely proportional to the total 25OH Vitamin D concentration.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   The inter-assay and intra-assay precision were performed in two separate studies to cover the entire claimed range. Precision was calculated by running four serum samples in singlicate at the given n value for 20 days using 3 different lots. The results are summarized in the table below:

Intra-Assay					Inter-Assay			
Sample	**N**	**<X> ± SD (ng/ml)**	**CV (%)**		**Sample**	**N**	**<X> ± SD (ng/ml)**	**CV (%)**
A	24	5.6 ± 0.4	7.8		A	42	17.7 ± 1.3	7.4
B	35	27.4 ± 1.5	5.5		B	10	26.3 ± 1.3	4.9
C	35	43.0 ± 1.2	2.7		C	10	42.0 ± 1.9	4.5
D	24	81.2 ± 2.0	2.5		D	42	85.4 ± 4.0	9.4

The reproducibility of the assay was done by testing 60 serum samples each at 3 concentrations in duplicate for five days, twice a day, at three sites with two technicians per site. The mean results are summarized in the table below:

Sample	**n**	**ng/ml**		**Within-Run**	**Between-Run**	**Between-Day**	**Between-Tech**	**Between-Site**	**Total**
1	60	25.5	SD	0.217	0.611	0.975	1.537	2.206	2.593
			CV	0.3%	0.9%	3.8%	6.0%	8.7%	10.2%
2	60	52.9	SD	0.638	1.571	1.108	2.285	4.310	5.192
			CV	0.9%	2.3%	2.1%	4.3%	8.2%	9.8%
3	60	124.8	SD	1.00	1.735	1.834	3.391	4.906	6.190
			CV	1.4%	2.5%	1.5%	2.7%	3.9%	5.0%

b. *Linearity/assay reportable range:*
Two samples with known concentrations (96.7 and 122.9 ng/mL) were tested at equidistant dilutions to determine the linear range of the assay. A linear regression analysis was performed. The data showed the following linear regression: Y= 1.005X + 0.435, r= 0.99.

The sponsor claimed that the assay range is 7.7 to 122.9 ng/dL.

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

Traceability and value assignment of calibrator:

The master calibrator stock is an ethanolic solution prepared in house by weighing 25OH Vitamin D3. The value assignment for the calibrator stock is made by UV absorbance at 254 nm using a molar extinction coefficient of 18000 L mol^{-1} cm^{-1}. The stock solution of 25OH Vitamin D is then used to make calibrators by diluting into horse serum. Calibrator values for all six levels have been assigned by using native serum samples that have been

assayed by LC/MS-MS. The LC/MS-MS method has been validated against a reference method.

Value assignment for 2 levels of controls:

The mean values of multiple replicates obtained from 10 independent runs wer assigned as the control target values. The controls target ranges were assigned using the mean with specified precision values.

Stability:

Open Vial Stability – Calibrators and Controls are provided in lyophilized form. Once reconstituted with DI water, they were stored at 4ºC for 7 days, and frozen at -20ºC for one month, two months and three months with one thawing. The study protocol and acceptance criteria have been reviewed and found to be acceptable. The sponsor claims that the calibrators and controls can be stored for 7 days at 4ºC or up to three months at -20ºC.
Closed vial stability and kit stability:
The shelf life studies were based on accelerated studies that supported the sponsor's claim of is 18 months at 4ºC. Real time studies are ongoing.

d. Detection limit:

Limit of Detection:
The Limit of Blank (LoB), Limit of Detection (LoD), and the Limit of Quantitation (LoQ), were determined in accordance with the CLSI guideline EP17-A.

The LoB was calculated by measuring the blank several times and calculating the 95^{th} percentile of the distribution of the test values. The LoB was calculated to be 1.69 ng/ml.

The LoD was calculated by using the formula LoD = LoB + 1.65 x SDs (from the CLSI EP17-A) where SDs is the standard deviation of a low value serum tested 50 times. The LoD was calculated to be 2.81 ng/ml.

For LoQ determination, 5 samples with concentrations above the LoD were tested 10 times. The LoQ was calculated to be 4.32 ng/mL based on an inter-assay precision of 20% CV.

e. Analytical specificity:

Interfering Substances

The effect of potential interfering substances on samples using the DIAsource 25 OH Vitamin D Total ELISA test was evaluated. Different levels of Hemoglobin, Bilirubin, Triglyceride, Vitamin C, Bilirubin Conjugate and Unconjugated and Zemplar in serum samples were tested on samples with different 25OH Vitamin D Concentration. The sponsor defined the acceptance criteria of < 10% difference to be non significant. The labeling states that hemolyzed samples should not be used.
Results are summarized in the table below:

Substance	25OH Vitamin D Concentration (ng/ml)	Concentration of Interferent (mg/dL)	Mean Percent Variation
Hemoglobin	7.6	250	-0.5%
		500	
	29.3	250	
		500	
	42.5	250	
		500	
Bilirubin Conjugated	6.0	50	-3.5%
		100	
	21.5	50	
		100	
	38.6	50	
		100	
Bilirubin Unconjugated	7.6	50	2.5%
		100	
	29.3	50	
		100	
	42.5	50	
		100	
Triglyceride	7.6	7.5	-4.3%
		125	
		250	

		500	
	29.3	7.5	
		125	
		250	
		500	
	42.5	7.5	
		125	
		250	
		500	
Vitamin C	6.0	1	4.5%
		10	
		100	
	21.5	1	
		10	
		100	
	38.6	1	
		10	
		100	
Biotin	8.7	0.2	4.6%
		2	
		4	
	19.8	0.2	
		2	
		4	
	36.1	0.2	
		2	
		4	
Zemplar	17.6	$1.25x10^{-3}$	-4.3%
		$2.5x10^{-3}$	
		$5.0x10^{-3}$	
	33.5	$1.25x10^{-3}$	
		$2.5x10^{-3}$	
		$5.0x10^{-3}$	

Cross Reactivity

Cross reactivity of the 25OH Vitamin D Total ELISA assay was determined by testing sera with spiked and unspiked cross reactants. The results are summarized in the following table:

Compound and Concentration	Spiked Vitamin D (ng/ml)	Unspiked Vitamin D (ng/ml)	% Cross Reaction
1,25$(OH)_2$-Vitamin D_3 at 200 ng/mL	57.3	16.7	20.3
1,25$(OH)_2$-Vitamin D_2 at 690 ng/mL	29.9	16.7	1.9
Vitamin D_3 at 200 ng/mL	22.5	16.7	2.9
Vitamin D_2 at 200 ng/mL	19.3	16.7	1.3
24,25$(OH)_2$-Vitamin D_3 at 20 ng/mL	87.9	16.7	>100
25,26$(OH)_2$-Vitamin D_3 at 4 ng/mL	31.1	16.7	>100
3-epi-25 hydroxy Vitamin D_3 at20 µg/mL	31.58	16.7	0.07
25 OH Vitamin D_3 at 10 ng/mL	26.7	16.7	100
25 OH Vitamin D_2 at 10 ng/mL	25.0	16.7	83

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

The performance of the DIAsource 25OH Vitamin D Total ELISA test was determined by conducting a correlation study tested at three different sites using a total of 359 serum patient samples. The samples were tested on both the DIAsource 25OH Vitamin D Total ELISA test and a commercially available 25OH Vitamin D ELISA test manufactured by ImmunoDiagnostics System (IDS). The results ranged from 8.0 ng/mL to 123.0 ng/mL,

The correlation coefficient between the two methods was 0.917, with the 95% confidence interval of 87.3% to 93.6%, slope of 0.954 and y-intercept of 3.05.

b. Matrix comparison:

Not applicable, serum is the only sample type indicated

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:

Reference Range:

To determine the reference range, the total 25OH Vitamin D of 150 apparently healthy individuals were measured. The individual patient serum samples used were obtained from a certified commercial source (Dx Biosamples, San Diego, CA.) and were collected from an FDA Licensed Donor Center with informed consent. 50 samples were from Northern US (Pennsylvania), 50 samples were from Central US (Tennessee), and 50 samples were from Southern US (Florida).

All samples met the following criteria:
Age between 21-90 years old
Subjects from three different geographical locations
Samples collected in the Winter season (January, February, and March)
Subjects were not taking any vitamin D supplements
Subjects were of different skin tones
Subjects had no family history of parathyroid, or calcium regulatory disease
Subjects had no history or Kidney, Liver, Parathyroid, Calcium related disease or bariatric surgery.
Subjects were not taking any medications known to affect absorption or catabolism of Vitamin D.

The following results were obtained:
Ages between 20-62 years old.
Population consisted of 75 light skin (50%) and 75 dark skin (50%).

No subjects were taking vitamin D supplements.

No subjects had a family history of parathyroid, or calcium regulatory disease.

No subjects had a history of Kidney, Liver, Parathyroid, Calcium related disease or bariatric surgery.

No subjects were taking any medications known to affect absorption or catabolism of Vitamin D.

	Florida	Tennessee	Pennsylvania	Overall
Highest Conc. (ng/mL)	88.6	71.7	54.6	88.6
Lowest Conc. (ng/mL)	6.1	4.9	5.9	4.9
Median Conc. (ng/mL)	20.8	15.9	14.3	17.2

Central 95% (2.5% - 97.5%) of the results observed were used. The labeling states that the performance of this assay has not been established in a pediatric population.

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.