

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

A. 510(k) Number:

k123660

B. Purpose for Submission:

New Assay

C. Measurand:

25-hydroxyvitamin D and other hydroxylated metabolites

D. Type of Test:

Quantitative, competitive enzyme immunoassay

E. Applicant:

EUROIMMUN US

F. Proprietary and Established Names:

25-OH Vitamin D ELISA

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 Vitamin D Test System	Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for Use

2. Indication(s) for use:

EUROIMMUN's 25-OH Vitamin D ELISA is intended for the quantitative determination of 25-OH Vitamin D and other hydroxylated vitamin D metabolites in human serum and

plasma (EDTA, Li-heparin). 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.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Microwell plate reader capable of measuring OD at 450nm and at 620nm for dual wavelength readings.

I. Device Description:

EUROIMMUN's 25-OH Vitamin D ELISA consists of a microwell ELISA plate coated with anti-25-OH vitamin D antibodies (sheep), 6 calibrators, 2 controls, Biotin concentration solution, sample buffer, enzyme conjugate, wash buffer concentrate, TMB chromogen/substrate solution and stop solution.

Six levels of calibrators (level 1 to 6) containing various concentrations of 25OHD come with the kit. Each bottle contains a horse serum based with active ingredients of 0.09% ProClin 950 and 0.09% sodium azide.. Calibrators are 25-OH vitamin D3 spiked and are supplied in 1 mL bottle for each level and are liquid material ready to use.

Two levels of controls (level 1 and 2) containing a low and a high concentration of 25OHD come with the kit. Each bottle contains a horse serum based with active ingredients of 0.09% ProClin 950 and 0.09% sodium azide.. Controls are supplied in 1 mL bottle for each level and are liquid material ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):

IDS 25-Hydroxy Vitamin D EIA

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

k021163

3. Comparison with predicate:

Item	Candidate device- 25-OH Vitamin D ELISA k123660	Predicate device- IDS 25-Hydroxy Vitamin D EIA k021163
Intended Use/Indication for use	For the quantitative determination of 25-OH Vitamin D and other hydroxylated vitamin D 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.	same
Sample type	Serum and plasma	same
Test format	96-well microplate assay	same
Assay components	Micro-titer plate; Calibrators; Biotin labeled 25-OH Vitamin D; Avidin-Conjugate; Assay buffer; Wash buffer; Substrate; Stop solution	same
Instrument	ELISA plate reader	same
Measuring wavelength	450/620nm	same
Approximate assay time	3.5 hours	same
Antigen used in calibrators	25-OH Vitamin D3	same
Assay principle	Competitive immunoassay	same
Reagent storage temperature	2-8 °C	same
Interpretation of results	Standard curve	same
Traceability	Standardized using UV quantification of 25-OH vitamin	same
Specificity	25-OH Vitamin D and other hydroxylated vitamin D metabolites	same

Antibody Calibrators	Monoclonal sheep anti-25-OH-Vitamin D IgG antibody	Polyclonal sheep anti-25-OH-Vitamin D IgG antibody
Assay range	4.0 – 120 ng/mL	2.4 – 144 ng/mL
Sample Volume	20 µl	25 µl

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

CLSI EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods
 CLSI EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach
 CLSI EP7-A2 Inference Testing in Clinical Chemistry
 CLSI EP9-A Method Comparison and Bias Estimation Using Patient Samples
 CLSI EP17-A Protocols for Determination of Limits of Detection and Limits of Quantification
 CLSI C28-A3: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition
 EN 13612:2002: Performance evaluation of in vitro diagnostic medical devices
 EN 13640:2002: Stability testing of in vitro diagnostic reagents

L. Test Principle:

The method for quantitative determination of 25-OH Vitamin D is a direct, competitive immunosorbent assay (ELISA). In the first analysis step, the calibrators and patient samples are diluted with biotin-labeled 25-OH vitamin D and added to microplate wells coated with monoclonal sheep anti-25-OH Vitamin D antibodies. During the incubation an unknown amount of 25-OH vitamin D in the patient sample and a known amount of biotin labeled 25-OH vitamin D compete for the antibody binding sites in the microplate wells plate. Unbound 25-OH vitamin D is removed by washing. For the detection of bound biotin-labeled 25-OH vitamin D, a second incubation is performed using peroxidase-labeled streptavidin. In a third incubation using the peroxidase substrate tetramethylbenzidine (TMB) the bound peroxidase promotes a color reaction. The color intensity is inversely proportional to the 25-OH vitamin D concentration in the sample. Results for the samples can be calculated directly using a standard curve.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

The precision/reproducibility of the test was investigated following CLSI standard EP05-A2. Intra-assay and inter-assay coefficients of variation (CV) were determined using 8 natural human serum samples with different concentrations of 25OHD. Two of the 8 serum samples were spiked to achieve higher levels of 25OHD. The intra-assay and inter-assay CVs are based on 40 determinations, with inter-assay performed in 10 different runs over 5 different days (with 4 replicates per run). The following precision results were obtained:

Intra-assay precision								
n = 40	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean value (ng/mL)	4.1	16.8	24.6	28.8	42.9	46.3	68.7	93.3
Standard deviation	0.5	0.9	1.7	2.1	1.8	2.8	3.5	6.3
Coeff. of variation (CV,%)	12.4	5.5	6.9	7.4	4.2	6.0	5.1	6.7

Inter-assay precision								
n = 40	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean value (ng/mL)	5.8	16.6	22.3	34.8	43.5	55.3	67.8	94.4
Standard deviation	0.9	1.3	1.8	3.1	3.0	3.7	5.8	7.8
Coeff. of variation (CV,%)	16.2	7.8	8.1	8.8	7.0	6.7	8.6	8.3

The lot-to-lot reproducibility of the test was investigated following CLSI standard EP05-A2. Inter-lot coefficients of variation (CV) were determined using 8 natural human serum samples with different concentrations of 25OHD. Two of the 8 serum samples were spiked to achieve higher levels of 25OHD. The inter-lot CVs are based on 32 determinations performed in 8 different runs on 4 different lots. The following reproducibility results were obtained:

Lot to lot reproducibility								
n = 32	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Mean value (ng/mL)	7.3	18.5	24.8	37.4	47.6	58.0	74.3	97.3
Standard deviation	0.89	1.89	1.81	2.29	4.25	4.07	6.59	9.26
Coeff. of variation (CV,%)	12.2	10.2	7.3	6.1	8.9	7.0	8.9	9.5

b. Linearity/assay reportable range:

The linearity of the test was investigated following CLSI standard EP6-A. Sample preparations were prepared by mixing of natural low patient sample (2 ng/mL) and high 129 ng/mL patient blood samples to create 9 different inter-mixture concentrations. The sample preparations covered the concentration range of 2 to 129 ng/mL. All samples were tested in double determinations per sample preparation, the mean of the two determinations for each sample was calculated and polynomial regression was performed of observed results vs. expected results. The linear regression generated was: $Y=0.98X - 0.57$, $r^2 = 0.999$

Based on the results of the linearity study sponsor claimed that the candidate assay is linear from 2.0 to 129 ng/mL.

The 25-OHD assay has a measuring range of 4.0 to 120 ng/mL.

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

Traceability

Calibrators and controls are traceable to concentrations determined by UV spectrophotometric analysis. An in-house stock solution is prepared gravimetrically by reconstituting a vial of 25-OH vitamin D3 with ethanol. The antigen concentration of the ethanolic stock is spectrophotometrically calculated using the OD coefficient of 18.2 at 264 nm to calculate the concentration from the absorbance value. The ethanolic stock of antigen is used to build an intermediate stock volumetrically by dilution into horse serum based on the spectrophotometric reading of the ethanolic stock solution. The intermediate stock is used in the manufacturing of lot specific calibrators and controls volumetrically.

Value assignments

Target ranges for control materials are: C1 (low): 15 ng/mL (10-25 ng/mL) and C2

(high): 40 ng/mL (25-60 ng/mL). Target values are confirmed using two commercially available devices and a HPLC method. Testing of the controls and assignment of values involves multiple replicates with multiple operators to generate a total of 40 values. The mean obtained values are assigned as control values. The controls are labeled with the assigned values as mean $C1 \pm 50\%$ and $C2 \pm 30\%$.

Calibrator value assignments are based on an internal procedure. The calibrator levels for each batch are confirmed by running a minimum of eight quality control sera covering the whole concentration range of the calibration curve measured in multiple replicates with multiple operators over days. The initial value assignment for calibrators was performed using two commercially available devices and a HPLC method. The final calibrators values are then verified & assigned by adjusting their initial values to meet the specified ranges when tested against the commercially available assays. Once confirmation of the calibrator values is established, the new calibrators are tested again in bulk and final. The target ranges of the calibrators are as follows:

Calibrator 1 = 0 ng/mL, Calibrator 2 = 4 ng/mL, Calibrator 3 = 10 ng/mL,
Calibrator 4 = 25 ng/mL, Calibrator 5 = 60 ng/mL, Calibrator 6 = 120 ng/mL,

Stability

Stability studies were conducted using accelerated stability study. The predicted shelf-life, based on results of an accelerated testing at 37°C, is at least 12 months at 2-8°C for the control materials and the calibrators. Real-time stability testing is ongoing to support the predicted shelf-life. Open-vial stability is 3 months when stored at 2-8°C. Biotin was found to be stable for at least 2 weeks when diluted to working strength. Stability study protocol has been reviewed and found to be adequate.

d. Detection limit:

Limit of blank (LoB), limit of detection (LoD) and limit of quantification (LoQ) were investigated following CLSI standard EP17-A. LoB, LoD, and LoQ studies were performed with three lots of reagents. LoB was determined using the mean of 60 replicates for calibrator 1 (0 ng/mL) and the mean of 20 replicates for calibrator 2 (4 ng/mL). LoB was found to be 0.54 ng/mL. LoD was determined as the LoB + 1.645 times the standard deviation of 200 determinations from 5 samples in the low range of 2 to 10 ng/mL, measured in 5 independent runs with 8 replicates per run. The mean LoD was determined to be 2.1 ng/mL. LoQ is defined as the lowest concentration at which the curve line crosses the 20% CV line and was determined from a plot of the mean concentrations (X-axis) vs. % CVs (Y-axis). LoQ was determined to be 4.0 ng/mL.

Sponsor claims that the candidate assay has a measuring range of 4.0 to 120 ng/mL.

e. *Analytical specificity:*

Cross reactivity study:

Cross reactivity was investigated to evaluate the potential cross-reactants. A 25-OH Vitamin D free sample was aliquoted and spiked with potential cross reacting Vitamin D metabolites at the concentrations listed below. The following cross-reactions were calculated based on the following equation and results summarized in the table below:

$$\text{Cross reactivity (\%)} = \frac{x \text{ (ng/mL)}}{c \text{ (ng/mL)}} * 100$$

with

x = observed value

c = spiked concentration of potential cross reactant

Potential cross reacting substance	Concentration spiked	Concentration observed	Cross reactivity
	ng/mL	ng/mL	%
25-OH Vitamin D3	10.0	10.0	100
25-OH Vitamin D2	25.0	24.3	100
24,25-OH Vitamin D3	100	0.3	0.3
Cholecalciferol (Vitamin D3)	10,000	3.4	0.03
Ergocalciferol (Vitamin D2)	10,000	5.1	0.05
1,25-OH Vitamin D3	10.0	4.3	45
1,25-OH Vitamin D2	10.0	19.8	212
3-epi-25-OH Vitamin D3	10.0	1.7	17

Interference study:

To investigate the influence from hemoglobin, triglycerides and bilirubin, 3 different sera at different 25-OH vitamin D concentrations (9 to 76 ng/mL) were spiked with potential interfering substances and were incubated with the test system according to the package insert. Non-significant interference is defined as < 10% bias between the spiked and unspiked sample. No significant interference was observed for concentrations of up to 750 mg/dL for hemoglobin, 2000 mg/dL for triglyceride, 40 mg/dL for bilirubin, 400 mg/dL for cholesterol, 1000 mg/dL for biotin, and 10.0 mg/mL ascorbic acid.

f. *Assay cut-off:*

N/A

2. Comparison studies:

a. *Method comparison with predicate device:*

The method comparison against the predicate device was performed following CLSI standard EP09-A2. 240 human serum samples were obtained from different sources for the method comparison study. The samples, ranging from 4.1 to 119.1 ng/mL, spanned the claimed measuring range of the candidate assay. To ensure that the tested concentrations of 25-OH Vitamin D are distributed across the reportable dynamic range, 36 samples (15%) in the set were spiked to represent difficult to achieve concentrations between 46.9 – 119.1 ng/mL.

Results of linear regression analysis are shown in the table below.

n	240
Concentration range (predicate)	4.1 – 110.4 ng/mL
Concentration range (candidate)	4.1 – 119.1 ng/mL
Regression equation (y = candidate, x = predicate)	$y = 1.08x - 0.78$
95% C.I. of Intercept	-0.06 – 1.63
95% C.I. of Slope	1.06 – 1.11
Correlation coefficient 95% C.I. of R	0.9858 0.9817 – 0.9890

b. *Matrix comparison:*

The usability of plasma was investigated using sample pairs each of serum and corresponding plasma (EDTA, and Li-heparin) from donors. To ensure that the tested concentrations of 25- OH Vitamin D are distributed across the reportable dynamic range, 3 sample pairs in the set were spiked with 25-OH Vitamin D stock solution of 25-OH Vitamin D3. Passing-Bablok regression was calculated for the comparison of serum (X) to plasma (Y) and results are summarized below:

	EDTA plasma	Li-heparin plasma
n	38	38
Concentration range (serum)	8.6 – 105.1 ng/ml	8.6 – 105.1 ng/ml
Concentration range (plasma)	8.8 – 104.1 ng/ml	8.5 – 104.1 ng/ml
Regression equation with (95% C.I. of intercept) (95% C.I. of slope)	$y = 0.99x + 0.29$ (-0.37 – 1.18) (0.93 – 1.02)	$y = 0.97x + 0.55$ (-0.65 – 1.33) (0.93 – 1.04)
Coefficient of determination R ²	0.996	0.993

3. Clinical studies:

a. *Clinical Sensitivity:*

N/A

b. *Clinical specificity:*

N/A

c. *Other clinical supportive data (when a. and b. are not applicable):*

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

The levels of 25-OH vitamin D were analyzed in a panel of 206 serum samples from healthy subjects (70 men and 139 women with an average age of 63 years; age range: 21 – 99 years) from a commercial source from the US. Samples are known to be from healthy blood donors from the mid-West region of the United States and drawn in October. The results are shown in the table below.

n = 206	25-OH Vitamin-D ELISA
	ng/mL
Minimum	< 4
Maximum	64.8
Mean	20.8
Median	19.4
2.5% percentile	5.4
97.5% percentile	47.0

The sponsor states that the data is provided for guidance only. It is important for each laboratory to establish its own reference ranges, representative of its typical population. Also, published studies representing the local population can be taken into consideration.

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.