

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

A. 510(k) Number:

k142351

B. Purpose for Submission:

Modification of traceability for standardization and modification of antibody pools of the previously cleared device, k021163

C. Measurand:

25-hydroxyVitamin D and other hydroxylated metabolites of Vitamin D

D. Type of Test:

Quantitative Enzyme Immunoassay (EIA)

E. Applicant:

Immunodiagnostic Systems Limited

F. Proprietary and Established Names:

25-Hydroxy Vitamin D^S EIA

G. Regulatory Information:

1. Regulation section:

21 CFR 862.1825, Vitamin D Test System

2. Classification:

Class II

3. Product code:

MRG - Vitamin D Test System

4. Panel:

Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

See Indications for use below

2. Indication(s) for use:

The 25-Hydroxy Vitamin D^S EIA kit is intended 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 an adult population.

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

The ID^S 25-Hydroxy Vitamin D^S EIA requires the use of a Microplate colorimeter reader (capable of reading at 450 nm).

I. Device Description:

The IDS 25-Hydroxy Vitamin D^S EIA kit consists of the following items:

Microtiter-plate:

Microplate with 25(OH) D sheep polyclonal antibody linked to the inner surface of the polystyrene wells, 12 x 8 well strips in a foil pouch with desiccant.

Enzyme Conjugate:

Phosphate buffered saline containing avidin linked to Horseradish peroxidase, protein, enzyme stabilizers and preservative, 22 mL per bottle.

Substrate:

An aqueous formulation of tetramethylbenzidine (TMB) and hydrogen peroxide, 28 mL per bottle.

Biotin Concentrate:

Lyophilized buffer containing 25(OH) D labelled with Biotin, and proprietary stabilizers, 1 mL per bottle.

Buffer:

Proprietary reagent for dissociating 25(OH) D from binding proteins, 50 mL per bottle.

Stop Solution:

0.5M Hydrochloric Acid, 13 mL per bottle, 1 bottle per kit

Wash Concentrate:

Phosphate buffered saline containing Tween, 50 mL per bottle.

Kit Calibrators, 7 levels:

Buffered human serum containing 25(OH) D and 0.09% sodium azide. The exact value of each Calibrator is printed on the QC Report, 1 mL per bottle, 7 bottles per kit.

The calibrator target values are as follows:

Calibrator	ng/mL
Cal A	0
Cal B	5.8
Cal C	11.6
Cal D	19.3
Cal E	33.2
Cal F	54.8
Cal G	98.0

Kit Quality Controls, 2 levels:

Human serum containing 25(OH) D and 0.09% sodium azide.
1 mL per bottle, 1 bottle per control level.

The control ranges are as follows:

QC	Mean ng/mL	Range ng/mL
1	16.3	11.4-21.2
2	60.8	46.4-75.6

Sponsor has the following statement in the labeling:

“Human serum is used in preparation of the calibrators and controls in this product. The material has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV 1 and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative. As no test can offer complete assurance that infectious agents are absent, the reagent should be handled in accordance of Biosafety Level 2.”

J. Substantial Equivalence Information:

1. Predicate device name(s):

Immunodiagnostic Systems Limited, OCTEIA 25-Hydroxy Vitamin D EIA

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

k021163

3. Comparison with predicate:

Similarities		
Item	Predicate Device OCTEIA 25-hydroxy vitamin D EIA (k021163)	New Device 25-Hydroxy Vitamin D ^s EIA
Intended Use	Assay for the quantitative measurement of 25-Hydroxy vitamin D in serum and plasma.	Same
Method	EIA	Same
Analyte	25-hydroxy vitamin D	Same
Reagent storage	2-8°C	Same
Capture Antibody	Antibody coated micro plate	Same
Calibrator	Low charcoal stripped human serum containing 25-hydroxy vitamin D and sodium azide as a preservative	Same
Calibration	Full standard curve to be run with all assays, per assay run	Same
Sample volume	25µL	Same
Quality Control	Two (2) controls provided	Same

Differences		
Item	Predicate Device OCTEIA 25-hydroxy vitamin D EIA (k021163)	New Device 25-Hydroxy Vitamin D ^s EIA
Traceability	Traceable to manufacturer's internal primary reference calibrators.	Traceable to the ID-LC/MS/MS 25 (OH) vitamin D Reference Measurement Procedure
Antibodies	Sheep anti 25-hydroxy Vitamin D	Same, but with a different source of antibodies pool
Reference range/ Expected	19.2-57.6 ng/mL	12.3 to 49.0 ng/mL

Differences		
Item	Predicate Device OCTEIA 25-hydroxy vitamin D EIA (k021163)	New Device 25-Hydroxy Vitamin D^s EIA
Values		
Measuring Range	2-152 ng/mL	6.5-100 ng/mL
Sample Type	Serum or Plasma (EDTA or Heparin)	Serum or plasma (EDTA, lithium heparin, sodium heparin or sodium citrate)

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

Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition (CLSI EP5-A2)

Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline (CLSI EP6-A)

How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline – Second Edition (CLSI C28-A2)

Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline (CLSI EP17-A)

Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline – Second Edition (CLSI EP9-A2)

Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition (CLSI EP7-A2)

L. Test Principle:

The IDS 25-Hydroxy Vitamin D^s assay is an enzyme immunoassay for the quantitation of 25(OH) D and other hydroxylated metabolites in serum or plasma. All patient samples, controls, and calibrators are run in duplicate and the mean results are reported in this assay procedure. 25µL of each calibrators, controls and samples are diluted with biotin labelled 25(OH) D. The diluted samples are incubated in microtitre wells which are coated with a highly specific sheep 25(OH) D at room temperature before aspiration and washing. Enzyme (horseradish peroxidase) labelled avidin, is added and binds selectively to complexed biotin and, following a further wash step, color is developed using a chromogenic substrate (TMB). The absorbance of the stopped reaction mixtures are read in a microtitre plate reader, color intensity developed being inversely proportional to the concentration of 25(OH) D.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was evaluated in accordance with a modified protocol based on CLSI EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods. Ten serum samples including 3 QC samples (ranging from approximately 12 ng/mL to approximately 80 ng/mL) were assayed using 3 lots of reagents in duplicate, twice per day for a minimum of 20 days ($n \geq 80$ replicates per sample). The within run, between run, between day and total precision results are summarized in the table below using one representative lot of reagent:

25(OH)D			Within Run		Between Run		Between Day		Total	
Sample	n	Mean (ng/mL)	SD	%CV	SD	%CV	SD	%cv	SD	%CV
QC1	88	19.1	0.4	1.9%	0.6	3.2%	0.1	0.4%	0.7	3.7%
QC2	88	43.7	0.8	1.9%	0.9	4.3%	0.0	0.0%	2.0	4.6%
QC3	88	62.1	2.3	3.5%	4.0	6.2%	1.7	2.6%	4.9	7.6%
1	88	11.7	0.4	3.4%	1.3	11.0%	0.0	0.0%	1.4	11.5%
2	88	24.5	0.6	2.5%	1.3	5.3%	0.0	0.0%	1.4	5.8%
3	88	40.7	1.0	2.5%	2.1	5.1%	0.0	0.0%	2.3	5.7%
4	88	73.2	2.7	3.7%	4.0	5.5%	0.6	0.8%	4.9	6.6%
5	88	21.7	0.5	2.4%	0.9	4.1%	0.0	0.0%	1.0	4.7%
6	88	51.0	1.9	3.7%	3.4	6.6%	0.0	0.0%	3.9	7.6%
7	88	28.3	0.8	2.7%	1.7	6.2%	0.0	0.0%	1.9	6.7%

b. *Linearity/assay reportable range:*

The linear range of the assay was determined following a protocol based on the CLSI guidance EP6-A, Evaluation of the linearity of quantitative measurement procedures: a statistical approach. Samples were prepared by diluting a high serum sample with a low serum sample to obtain eleven (11) concentration levels through the measuring range. Samples were run in replicate of six, using one reagent lot with sample range tested between 6.5 to 115 ng/mL. The observed concentrations of high and low samples were used to calculate the expected concentrations of the 11 evenly spaced dilution samples.

The resulting weighted linear regression equation of the observed concentrations versus the expected concentrations is:

$$Y = 1.02x + 0.23 \text{ ng/mL}, R^2 = 1.00$$

Maximum deviation from linearity; -8.8% for samples > 20 ng/mL and 2.37 ng/mL for samples < 20 ng/mL

The results of the linearity study support the sponsor's claim that the assay is linear from 6.5-100 ng/mL.

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

Standardization and Traceability:

The 25-Hydroxy Vitamin D^S EIA assay is traceable to the isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC-MS/MS) 25(OH)D Reference Method Procedure (RMP) which is traceable to the National Institute of Standards and Technology Standard Reference Material (SRM) 2972 in accordance with the Vitamin D Standardization Program (VDSP). The VDSP is an international collaborative effort to standardize the laboratory measurement of serum 25(OH) D. This collaboration involves the coordinated efforts of the National Institutes of Health, Office of Dietary Supplements (ODS), the Centers for Disease Control and Prevention (CDC), the National Institutes for Standards and Technology (NIST), Ghent University, and other institutions. Please refer to <http://ods.od.nih.gov/Research/VitaminD.aspx> for more information on the VDSP program.

The reference calibrators were initially value assigned by using the in-house internal standards with multiple kit lots over multiple runs. These values were further adjusted to align against the ID-LC-MS/MS by method comparison using a panel of 68 single donor samples from VDSP, over multiple runs with multiple kit lots. The results summary below shows correlation of the final calibrator adjustment to the original assigned calibrator values.

Deming regression (n =68):

$$y=1.02x- 0.36(\text{ng/mL}), r = 1.00$$

The 25-Hydroxy Vitamin D^S EIA assay (candidate device) has passed the certification process for the CDC VDSP and a certificate has been provided by the CDC. Please see the certification process at <http://www.cdc.gov/labstandards/hs.html>.

Stability:

Shelf life stability: The sponsor performed an accelerated stability study on the reagent kit. The accelerated stability study demonstrated an 8 month shelf life for the microtitre plates when stored at 2-8° C. The stability study protocols and acceptance criteria were reviewed and found to be acceptable. Current accelerated shelf life studies support the assigned 8 month shelf-life, with real time studies ongoing.

Open Vial stability: Open vial stability study has been conducted and found adequate. The stability data supports the sponsor's claims that the kit components are stable for 8 weeks after opening when stored at 2-8°C. The stability study protocols and acceptance criteria were reviewed and found to be acceptable. All kit components, except for the microtiter plates, have the same shelf life as stated below.

The following chart is the summary of the open-vial stability:

Kit Components	After opening or preparation(open-vial stability)
Biotin 25(OH)D Solution	8 weeks after reconstitution Store at 2-8°C in the dark immediately after use
Ab. coated plate strip	8 weeks Store at 2-8°C in foil pouch with desiccant sachet
Calibrators, Controls	8 weeks Store at 2-8°C after opening
Wash Solution	8 weeks Store at room temperature (18-25 °C) after preparation

Value assignments:

For value assignment, a set of reference standards were value assigned and verified from the CDC aligned reference standards. The kit calibrators were tested as unknowns in a minimum of 10 assay runs using multiple operators. For each lot, multiple replicates were assayed, the concentration of the kit calibrators were calculated from the reference standards, and the mean result was used as the assigned calibrator concentration.

Target values for Calibrators:

Calibrators	Target value (ng/mL)
Cal A	0
Cal B	5.8
Cal C	11.6
Cal D	19.3
Cal E	33.2
Cal F	54.8
Cal G	98.0

For the two levels of QC, for each lot, 10 replicates were tested, mean and SD were calculated and the QC range is +/- 2 SD from the calculated mean. The kit controls have the following target range.

Target range for Quality Control:

Control Set	Target range (ng/mL)
Level 1	11.4-21.2
Level 2	46.4-75.5

d. Detection limit:

The Limit of Blank (LoB), Limit of Quantitation (LoQ) and Limit of Detection (LoD) were determined based on guidance from CLSI EP17-A, Protocols for the Determination of Limits of Detection and Limits of Quantitation.

Limit of Blank (LoB): 5 different lots of calibrator buffer were used as the blank to determine the LoB, by running 60 replicates using 3 reagent lots. LoB was calculated as: $LoB = \text{mean} + (1.645 \times SD)$.

Limit of Detection (LoD): To determine the LoD, 10 samples with low concentrations of 25-OH vitamin D were run in duplicate. LoD was calculated from a total of 6 assay runs tested over 4 days for a total of 118 results for each lot by using 3 lots of reagent (355 samples in total). $LoD = LoB + 1.645 \times \text{pooled SD}$

Limit of Quantitation (LoQ): Human serum samples were diluted in calibrator buffer to achieve low levels of 25-OH D in the sample. For calculation of LoQ, 10 different low samples were run in duplicate, with 3 reagent lots, for a total of 120 results. The sponsor determines LoQ based on functional sensitivity. LoQ is determined by the concentration at which 20%CV was obtained (using the upper 95% confidence interval).

Results of Detection Limit claims:

LoB	LoD	LoQ
1.3 ng/mL	2.7 ng/mL	4.8 ng/mL

The claimed measuring range of the assay is 6.5-100 ng/mL.

e. Analytical specificity:

To determine potential interference, two serum samples at two different concentrations of 25(OH) D from pooled serum spiked with the potential interferent. The mean of 26 replicate (except RF has 6 replicates), for both spiked and control samples, were then compared. One reagent lot was used for interference testing. The study results demonstrate that the following substances have non-significant interference in the 25-Hydroxy Vitamin D^s EIA assay when the concentrations presented in the following table

are below the stated threshold, based on the sponsors predefined acceptance criteria of non-significant interference of < 10% bias between the test and control samples.

Potentially interfering Substance	Highest tested concentration of Substance without significant interference	Sample 25(OH)D Conc.(ng/mL)
Triglycerides	475 mg/dL	24.3
		53.3
Bilirubin, conjugated	20 mg/dL	30.4
		75.6
Hemoglobin	400 mg/dL	11.6
		25.2
Human Anti Mouse Antibody (HAMA)	1000mg/dL	29.7
		71.8
Red Blood Cells	0.40%	11.8
		29.6
Vitamin D Binding Protein (GC Globulin)	2000ng/mL	31.4
		74.8
Total Protein	9.2 g/dL	11.3
		38.5
Cholesterol Total	500 mg/dL	31.5
		73.8
Biotin	200 nmol/L	27.4
		46.3
Rheumatoid factor	800 IU/mL	21.0
		66.0

Cross Reactivity Studies:

Endogenous 25(OH) D metabolites and exogenous synthetic 25(OH) D metabolites were spiked into vitamin D serum samples and analyzed with the 25-Hydroxy Vitamin D^S assay. The cross-reactivity was calculated based on following equation:

$$\% \text{ cross reactivity} = \frac{(\text{Mean conc. of spiked sample} - \text{mean conc. of unspiked sample})}{\text{Spike concentration}} \times 100\%$$

Endogenous Cross reactivity:

Analyte	Spike Concentration(ng/mL)	% Cross reactivity	Mean % Cross Reactivity
25(OH)Vitamin D ₃	10.0	99%	95%
	20.0	91%	
25(OH)Vitamin D ₂	10.0	124%	109%
	20.0	95%	
24,25(OH) ₂ Vitamin D ₃	4.0	90%	91%
	6.0	91%	

Exogenous Cross reactivity:

Cross reactant	Spike Concentration (ng/mL)	% Cross Reactivity
1,25-(OH) ₂ D ₃	2.0	5%
1,25-(OH) ₂ D ₂	20.0	84%
3-epi-25(OH)D ₃	100	-1.0%
3-epi-25(OH)D ₂	100	-1.0%
Paricalcitol	100	17%
Vitamin D ₃ (Cholecalciferol)	1000	0%
Vitamin D ₂ (Ergocalciferol)	100	6%

The sponsor has the following limitation added in the labeling: “Paricalcitol interferes with the 25-Hydroxy Vitamin DS EIA test. Paricalcitol, when tested, caused a positive bias in sample result.”

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the modified (standardized) 25-Hydroxy Vitamin D^S EIA assay (candidate device) to the non-standardized 25-Hydroxy Vitamin D assay (predicate device), following CLSI EP-9A2 guideline. A total of 195 serum samples were tested on the candidate device and the predicate device. The relationship between the candidate device (y) and the predicate device (x) is described by using Passing Bablok regression, as shown below:

Passing Bablok regression:
 $y = 0.88x + 3.23 \text{ ng/mL}$
 95 % CI of the slope: 0.86 to 0.91
 95 % CI of the intercept: 2.7 to 3.8 ng/mL
 Pearson correlation coefficient, r : 0.97
 Sample range tested: 8.1 ng/mL to 79.6 ng/mL

The slope showed an approximate bias of -12% between the candidate device and the predicate device. Based on the raw data, there is a significant negative trend/bias with results above the medical decision point ($>30 \text{ ng/mL}$), but results below 30 ng/mL has a positive trend/bias. However, this shift is expected because the purpose of the device modification was to adjust the calibration to better align with the VDSP reference sample concentration target levels. Therefore, test results from the candidate device do not, and are not expected to, directly correlate with test results from the predicate device. Laboratories that use the IDS 25-Hydroxy Vitamin D^s assay should be aware of the significantly different performance of the modified assay.

An additional method comparison study was conducted to evaluate the accuracy between the candidate device and the RMP, University of Ghent's ID-LC/MS/MS method. The method comparison against the RMP was the basis of the substantial equivalence determination.

An additional method comparison study was performed to demonstrate the accuracy of the newly aligned IDS- 25 Hydroxy Vitamin D^s assay. A total of 109 independent native serum samples in the range of 7.3 to 93.4 ng/mL were used to compare the Ghent/CDC ID-LC-MS/MS 25(OH) Vitamin D Reference Method Procedure and the candidate device. Result of the method comparison is summarized below:

Deming Regression: $y = 0.97x - 0.84 \text{ ng/mL}$
 Slope, 95% Confidence Interval: 0.90 to 1.04
 Intercept, 95% confidence Interval: -2.75 to 1.08 ng/mL
 Correlation Coefficient, $r = 0.947$

c. Matrix comparison:

A matrix comparison study was performed using sample sets with 25(OH) D concentrations that ranged from 6.6 to 82.7 ng/mL . Some of the samples were spiked in order to cover the high end of the claimed measuring range of the assay. Passing Bablok regression was used to compare the results from the different tube types against serum. The summary of the results are shown in the table below:

	SST	EDTA	NA-Heparin	Li-Heparin	Citrate
Passing Bablok	$y = 0.99x - 0.18$ (N=28)	$y = 0.97x + 0.60$ (N=38)	$y = 1.07x - 0.47$ (N=28)	$y = 1.04x - 0.22$ (N=28)	$y = 1.03x - 0.70$ (N=28)
Correlation Coefficient	0.99	1.00	0.99	1.00	0.99

Based on the study data, the sponsor claims that serum, serum from serum separator tubes (SST), EDTA plasma, Sodium Heparin plasma, Lithium Heparin plasma and citrate plasma are acceptable specimen types for the candidate device.

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:

A reference range study was performed according to the non-parametric method in CLSI C28-A2 guideline. Samples from 280 apparently light skin and dark skin, healthy male (71.1%) and female adults (28.9%), ages 21-77 years living in geographical diverse regions of the United States to represent a broad spectrum of UV light exposure in the intended population were assayed by the 25-Hydroxy Vitamin D^S Assay. Samples were from individuals with normal values for intact PTH, calcium, phosphate, and TSH, and not taking any interfering medications.

The 95% reference interval was calculated and the following range was obtained:

Method	n	Median Conc.	95% Reference Range (2.5-97.5) percentile
IDS 25(OH) Vitamin D ^s	280	26.0 ng/mL	12.3-49.0 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.