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

A. 510(k) Number:

k161831

B. Purpose for Submission:

New device

C. Measurand:

Total 25-hydroxyvitamin D

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Immunodiagnostic Systems Limited

F. Proprietary and Established Names:

IDS-iSYS 25VitD^{S}

IDS-iSYS 25VitD^{S} Control Set

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.1825, Vitamin D Test System

   21 CFR 862.1660, Quality Control Material

2. Classification:

   Class II

   Class I, reserved

3. Product code:

MRG, Vitamin D Test System

JJX, Single (specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

   Clinical Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   The IDS-iSYS 25 VitD^{S} Assay is intended for the quantitative determination of total 25-hydroxyvitamin D [(25(OH)D] in human serum or plasma on the IDS-iSYS Multi-Discipline Automated System. 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.

   The IDS-iSYS 25 VitD^{S} Control Set is used for quality control of the IDS-iSYS 25 VitD^{S} assay on the IDS-iSYS Multi- Discipline Automated System.

3. Special conditions for use statement(s):

   For in vitro diagnostic use only.

   For prescription use only.

4. Special instrument requirements:

   For use on the IDS-iSYS Multi- Discipline Automated System.

I. Device Description:

The IDS-iSYS 25VitD^{S} assay consists of a reagent cartridge (with multiple components) and one set of calibrators.

Reagent Cartridge:
MPV1 – Magnetic particles coated with streptavidin in phosphate buffer with sodium azide (<0.1%) as preservative (1 bottle, 2.5 mL)
NaOH – Sodium hydroxide solution (<0.5 M) (1 bottle, 13.0 mL)
25D ACR – 25D labelled with an acridinium ester derivative, in buffer containing bovine serum albumin with sodium azide (<0.1 %) as preservative (1 bottle, 9.0 mL)

Ab-BIOT - Anti-25(OH)D sheep polyclonal antibody labelled with an biotin, in buffer containing bovine, sheep and mouse proteins with sodium azide (<0.1 %) as preservative (1 bottle, 9.0 mL)
BUF - Assay buffer containing proprietary displacing compounds, methanol (>10 % but <20%) and sodium azide (<0.1 %) as preservative (1 bottle, 23.0 mL)

Calibrators:
Kit Calibrators A and B (CAL A & CAL B) (1 bottle of each, 1.5 mL per bottle) contain human serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide (<0.1%) as preservative.

Control Set:
Controls 1 and 2 (CTL 1 & CTL 2) (3 bottles of each, 2.5 mL per bottle) contain human serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide (<0.1%) as preservative. The target concentrations are 17 ng/mL and 90 ng/mL.

Statement in labeling regarding the use of human blood-based materials in calibrators and controls:
Human material used in the preparation of this product has been tested by FDA recommended assays for the presence of antibody to Human Immunodeficiency Virus (HIV I and II), Hepatitis B surface antigen, antibody to Hepatitis C, and found negative.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   IDS-iSYS 25-Hydroxy Vitamin D^S Assay
   IDS-iSYS 25-Hydroxy Vitamin D^S Control Set

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

   k140554

3. Comparison with predicate:

Assay: Similarities		
Item	Candidate Device IDS-iSYS 25VitD^S	Predicate IDS-iSYS 25-Hydroxy Vitamin D^S (k140554)
Intended Use	Same	For the quantitative determination of 25-hydroxyvitamin D in human blood samples.
Analyte	Same	25-Hydroxy Vitamin D (25(OH)D)

Assay: Similarities		
Item	Candidate Device IDS-iSYS 25VitD^S	Predicate IDS-iSYS 25-Hydroxy Vitamin D^S (k140554)
Reagent Storage	Same	2-8 °C
Sample preparation (pre-treatment)	Same	Performed on-board the analyzer
Sample volume	Same	10µL
Method of detection (Test methodology)	Same	Chemiluminescent immunoassay using magnetic-particle solid phase and acridinium label
Automation	Same	Fully automated assay
Calibration procedure	Same	User-initiated 2 point calibration to adjust the batch related master curve. The system stores the calibration for the interval specified in the kit IFU.
Traceability/ Standardization	Same	Traceable to the isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC-/MS/MS) 25(OH)D Reference Method Procedure (RMP) which was used in assigning the target value for the VDSP samples. The ID-LC-MS/MS RMP is traceable to the National Institute of Standards and Technology Standard Reference Material (SRM) 2972.
On board the analyzer reagent stability	Same	21 days

Assay: Differences		
Item	Candidate Device IDS-iSYS 25VitD^{S}	Predicate IDS-iSYS 25-Hydroxy Vitamin D^{S} (k140554)
Kit Calibrator matrix	Human serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide as a preservative.	Equine serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide as a preservative.
Kit reagent components	Reagent cartridge (1 vial each of MPV1, NaOH, 25D-ACR, Ab-BIOT & BUF), two concentration levels of calibrators (A&B) (1 vial of each) & a mini CD	Reagent cartridge (1 vial each of MPV1, CONJ, NaOH & BUF), two concentration levels of calibrators (A&B) (1 vial of each) & a mini CD
Control Kit components	Two concentration levels of controls (3 vials of each)	Three concentration levels of controls (3 vials of each)
Kit reagent component volumes	Reagent cartridge (1 vial each): MPV1 (2.5mL), NaOH (13.0mL), 25D-ACR (9.0mL), Ab-BIOT (9.0mL) & BUF (23.0mL)	Reagent cartridge (1 vial each): MPV1 (2.0mL), CONJ (10.1mL), NaOH (13mL) & BUF (26.0mL)
Antibodies	Same, but with a different source of antibody pool	Anti-25 OH D Sheep Polyclonal IgG
Calibration interval	7 days	14 days
Range of assay	4.00 – 110 ng/mL	7 – 125 ng/mL
Sensitivity	LoB: 1.31 ng/mL LoD: 1.98 ng/mL LoQ: 3.53 ng/mL	LoB: 0.6 ng/mL LoD: 2.6 ng/mL LoQ: 7.0 ng/mL
Expected values	10.4 to 59.5 ng/mL	12.7 to 64.2 ng/mL
In use (after opening at 2-8°C) reagent stability	42 days	21 days
Sample type	Serum (standard sampling tubes or tubes containing serum separating gel) or plasma (K2 EDTA, lithium heparin, sodium heparin)	Serum

Controls: Similarities		
Item	Candidate Device IDS-iSYS 25VitD^{S}	Predicate IDS-iSYS 25-Hydroxy Vitamin D^{S} (k140554)
Intended Use	Same	The quality control of the 25-OH vitamin D assay on the IDS-iSYS.
Stability	Same	After opening at 2 - 8 °C: To the expiry date
Reagent storage	Same	2-8 °C

Controls: Differences		
Item	Candidate Device IDS-iSYS 25VitD^{S}	Predicate IDS-iSYS 25-Hydroxy Vitamin D^{S} (k140554)
Control matrix	Human serum buffer matrix with two defined concentrations of 25-OH D and sodium azide as a preservative.	Equine serum buffer matrix with three defined concentrations of 25-OH D and sodium azide as a preservative.
Stability	On board the analyzer: 4 hours	On board the analyzer: 2.5 hours
Control levels	Level 1: 17 ng/mL Level 2: 90 ng/mL	Level 1: 12.0-18.0 ng/mL Level 2: 26.4-39.6 ng/mL Level 3: 59.0-86.0 ng/mL

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

- CLSI EP05-A2 Evaluation of Precision performance of Quantitative Measurement Methods; Approved Guidline.
- CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline.
- CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline.
- CLSI EP09-A3, Measurement Procedure Comparison and Bias Estmination Using Patient Samples; Approved Guideline.
- CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline.
- CLSI C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline.

L. Test Principle:

The assay is based on chemiluminescence technology. 10 µL of patient sample is subjected to a pre-treatment step to denature the vitamin D binding protein (VDBP). The treated samples are then neutralised in assay buffer and a specific anti-25(OH)D antibody labelled with biotin is added. Following an incubation step, acridinium labelled 25(OH)D is added. Following a further incubation step, the magnetic particles linked to streptavidin are added. After the final incubation step the complex is captured using a magnet and a wash step performed to remove any unbound analyte. Trigger reagents are added and the resulting light emitted by the acridinium label is inversely proportional to the concentration of 25(OH)D in the original sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A precision study was performed in accordance with CLSI EP5-A2. Nine serum samples (all native except Serum 9 that was spiked to achieve a high concentration) and two controls were assayed using three lots of reagents in duplicate, twice per day for 20 days on three to four analyzers. All three lots generated similar precision results. The within-run and total precision results are summarized in the table below using one representative lot of reagents:

			Within Run		Total	
	n	Mean Conc. ng/mL	SD	%CV	SD	%CV
Serum 1	80	13.9	0.7	5.0	1.0	7.4
Serum 2	80	16.9	0.7	3.9	1.2	7.2
Serum 3	80	28.6	1.1	3.9	1.9	6.5
Serum 4	80	37.0	1.3	3.5	1.8	4.9
Serum 5	80	49.8	2.0	4.0	2.9	5.8
Serum 6	80	61.1	2.4	3.9	3.7	6.0
Serum 7	80	62.1	2.8	4.4	4.0	6.4
Serum 8	80	93.0	3.5	3.8	5.8	6.2
Serum 9	80	103.0	4.8	4.7	6.3	6.1

The within-run and total precision results for the combined lots of reagents are shown in the table below:

			Within Run		Total	
	n	Mean Conc. ng/mL	SD	%CV	SD	%CV
Serum 1	244	14.1	0.7	5.2	1.3	9.4
Serum 2	244	17.2	0.7	4.2	1.5	8.8
Serum 3	244	29.1	1.2	4.0	2.2	7.4
Serum 4	244	37.6	1.2	3.2	2.6	6.8
Serum 5	244	50.8	1.7	3.3	3.4	6.6
Serum 6	244	61.0	2.3	3.7	4.0	6.6
Serum 7	244	62.9	2.2	3.4	4.1	6.6
Serum 8	244	93.4	3.1	3.3	6.0	6.4
Serum 9	244	102.0	5.0	4.9	8.7	8.6

b. *Linearity/assay reportable range:*

Linearity was evaluated based on CLSI EP6-A. A high human serum sample (pool of two endogenous spiked serum samples), a low human serum sample (low serum sample diluted in zero matrix), and nine evenly spaced dilutions (created by mixing the high and low sample) were analyzed in replicates of four with sample range tested between 3.6 to 135.9 ng/mL. The concentrations tested were as follows: 3.6, 16.8, 30.0, 43.3, 56.5, 69.8, 83.0, 96.2, 109.5, 122.7, and 135.9 ng/mL. Linearity curves of the observed concentration versus the expected concentration were plotted using unweighted linear regression.

The resulting linear regression equation was $y = 1.00x + 3.75$ ng/mL, $R^2 = 1.00$

The results of the linearity study support the sponsors claim that the assay is linear in the assay's reportable range of 4 to 110 ng/mL.

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

Standardization and Traceability

The IDS-iSYS 25 VitD^S assay is traceable to the Ghent University isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LCMS/MS) 25(OH)D Reference Method Procedure (RMP). The ID-LCMS/MS RMP is further traceable to the National Institute of Standards and Technology Standard Reference Material (NIST SRM) 2972.

This assay has been standardized in accordance with the Vitamin D Standarization Program (VDSP). Please refer to https://ods.od.nih.gov/Research/vdsp.aspx for more information on the VDSP program. To achieve standardization, the IDS-iSYS 25 VitD^S assay master calibration parameters were aligned to the VDSP by using 136 single-donor human serum samples from the VSDP program, which were value assigned using the ID-LCMS/MS RMP.

Calibrator and control value assignment

The IDS-iSYS 25VitD^S kit calibrators are value assigned using the internal reference calibrators through an internal procedure. The IDS-iSYS 25VitD^S internal reference calibrators are value assigned by assignment to samples with known levels of 25(OH)D as determined by the ID-LC-MS/MS reference method procedure. The kit calibrators are tested as unknowns in a minimum of 20 assay runs on one iSYS instrument. Each run uses secondary standards (IRs), and the concentrations of the Kit Calibrators are calculated from these secondary standards (IRs). Following assignment, the kit calibrator values are then verified over 3 assays using the full curve parameters in addition to the established calibrator values. The values must fall within specified acceptable ranges. The kit calibrator concentrations are reagent batch specific and linked together. The calibrator nominal ranges are listed below:

Calibrator A Nominal Range: 8.0 – 12.0 ng/mL
Calibrator B Nominal Range: 80.0 – 90.0 ng/mL

For kit control value assignment, the kit controls are tested as unknowns in a minimum of 21 assay runs using multiple systems. The established values are then verified in a single assay in an approved kit combination. The values must fall within specified acceptable ranges. The control nominal ranges are listed below:

Kit Control 1 Nominal Range: 12.0 – 18.0 ng/mL
Kit Control 2 Nominal Range: 65.0 – 85.0 ng/mL

Stability

The calibrator and control shelf-life and open-vial stability testing protocols and acceptance criteria were reviewed and found to be adequate. The open-kit stability study demonstrates that the calibrators and controls are stable for 42 days once opened and stored at 2 – 8°C in an upright position in the dark. Calibrators and controls are stable for up to 4 hours on board the analyzer. Real-time stability studies are on-going to support a kit calibrator and control minimum shelf life of 12 months.

d. Detection limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) were determined with guidance from CLSI EP17-A.

To establish the Limit of Blank (LoB), the zero calibrator was assayed in duplicate on three analyzers, with three reagent lots over multiple days for a total of 6 assays and 60 replicates per reagent lot. LoB was determined by the following equation:

LoB = mean(blank) + [1.645 x SD(blank)]
The LoB claim is 1.31 ng/mL.

The Limit of Detection (LoD) study was performed by assaying six serum samples with very low vitamin D concentrations in duplicate on three analyzers with three

reagent lots over multiple days for a total of 6 assays and 72 replicates per reagent lot. LoD was calculated using the following equation:

LoD = LoB + 1.645 x pooled SD
The LoD claim is 1.98 ng/mL.

The Limit of Quantification (LoQ) was determined by measuring ten serum samples with low vitamin D concentrations (ranging from 0.994 to 9.32 ng/mL) in duplicate on three analyzers with three reagent lots over multiple days for a total 120 replicates per reagent lot. The LoQ claim is 3.53 ng/mL and is defined as concentration interpolated from the regression curve where the upper 95% confidence interval for the curve has a 20% CV.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
1.31 ng/mL	1.98 ng/mL	3.53 ng/mL

The reportable range of the assay is 4 to 110 ng/mL.

e. *Analytical specificity:*

Interference
Interference testing was performed based on CLSI EP7-A2 to assess common or known substances that could interfere with the IDS-iSYS 25VitD^{S} assay. The potential endogenous interferents listed below were spiked into two human serum samples that contained two different concentrations of 25(OH)D. Each sample was tested in replicates of 26. The 25(OH)D values of the spiked samples were compared to the control samples containing no interferent. Significant interference was defined as greater than 10% difference from the expected concentration. The interference study results are summarized in the following table:

Potential endogenous interferent	**25(OH)D concentration of non-spiked sample (ng/mL)**	**Highest Test Concentration that demonstrated no significant interference**
Triglycerides (Intralipid)	31.4 and 61.4	500 mg/dL
Hemoglobin	33.2 and 54.8	500 mg/dL
Bilirubin, conjugated	30.3 and 59.4	20 mg/dL
Bilirubin, unconjugated	23.2 and 67.4	20 mg/dL
Total Protein	32.0 and 30.5	10 g/dL
Human Anti Mouse Antibody (HAMA)	33.8 and 65.6	1000 ng/mL
Red Blood Cells	29.8 and 57.7	0.2%
Vitamin D Binding Protein	30.0 and 61.7	2000 ng/mL

Potential endogenous interferent	25(OH)D concentration of non-spiked sample (ng/mL)	Highest Test Concentration that demonstrated no significant interference
Biotin	34.9 and 64.6	200 nM
Acetaminophen	34.7 and 60.2	200 µg/mL
Ibuprofen	32.6 and 66.7	140 µg/mL
Carbamazepine	28.3 and 67.4	30 µg/mL
Phenytoin	32.2 and 62.2	50 µg/mL

Rheumatoid Factor (Rf): Interference from Rf was assessed by spiking different amounts of a high Rf sample into two serum base samples containing two different concentrations of 25(OH)D. Each sample was tested in replicates of 4. The concentration of 25(OH)D in the Rf spike was determined in the high Rf sample (diluted to a level where Rf interference would not be expected to occur prior to 25(OH)D determination). The observed 25(OH)D values of the spiked samples were compared to the expected 25(OH)D values contributed by the base samples and high Rf sample. Significant interference was defined as greater than 10% difference from the expected concentration. The results are summarized in the following table:

Potential endogenous interferent	25(OH)D concentration of non-spiked sample (ng/mL)	Highest Test Concentration that demonstrated no significant interference
Rheumatoid Factor	32.6 and 54.9	600 IU/mL

Cholesterol, Total: Interference from total cholesterol was assessed using a recovery study. A serum sample containing 343.4 mg/dL cholesterol was spiked with 10% or 20% of a high serum pool containing 168.0 ng/mL 25(OH)D. Serum samples containing 392.6 mg/dL or 309.1 mg/dL cholesterol were spiked with 10% or 20% of a high serum pool containing 392.6 mng/mL 25(OH)D. Each sample was tested in replicates of 4. Recovery of 25(OH)D was assessed by comparing observed versus expected 25(OH)D values contributed by the 25(OH)D spike. Non-significant interference is defined as % recovery between 90-110%. The results are summarized in the following table:

Potential endogenous interferent	25(OH)D concentration of non-spiked sample(ng/mL)	Highest Test Concentration that demonstrated no significant interference
Cholesterol, Total	8.6	300 mg/dL

The Sponsor has the following limitations in the labeling based on the interference studies:

- The lowest Bilirubin level that does not significant interfere (<10% bias) with the assay is 20 mg/dL; greater than +10% bias was first observed when bilirubin is above 22 mg/dL.
- Rheumatoid factor at 800 IU/mL will cause a bias in 25(OH)D results up to -11%.
- Cholesterol at 343mg/dL will cause a bias in 25(OH)D results up to -19%.

Cross-reactivity

Two studies were conducted according to CLSI EP7-A2 to evaluate the potential cross-reactivity of the assay with endogenous cross-reactants ($25(OH)D_2$ and $25(OH)D_3$) and exogenous substances similar in chemical structure to 25(OH)D.

Endogenous cross-reactants $25(OH)D_2$ and $25(OH)D_3$ were spiked into serum samples, with intial concentrations of endogenous $25(OH)D_2$, $25(OH)D_3$, and 24, $25(OH)_2D_3$ determined using an ID-LCMS/MS method. Spiked and un-spiked sample concentrations were measured with the IDS-iSYS 25VitD^S assay. The % cross-reactivity was calculated based on following equation:

(Mean conc. of spiked sample – mean conc. of unspiked sample) × 100% Spiked concentration

Cross reactivity results

Potential Cross Reactant	Spike concentration (ng/mL)	% Cross Reactivity
$25(OH)D_3$	20	101
$25(OH)D_2$	20	105

Exogenous synthetic 25(OH)D metabolites were spiked into serum samples and spiked and un-spiked sample concentrations were measured with the IDS-iSYS 25VitD^S assay. The % cross reactivity was calculated based on the following equation:

(Mean conc. of spiked sample – mean conc. of unspiked sample) × 100% Spiked concentration

Cross reactivity results

Potential Cross Reactant	Spike concentration (ng/mL)	% Cross Reactivity
24,25 dihydroxyvitamin D_3	25	197
24,25 dihydroxyvitamin D_2	25	37
1,25 dihydroxyvitamin D_3	20	3
1,25 dihydroxyvitamin D_2	20	8
3-epi-25-OH vitamin D_3	100	0

Potential Cross Reactant	Spike concentration (ng/mL)	% Cross Reactivity
3-epi-25-OH vitamin D_2	100	2
Paricalcitol	100	-2
Alfacalcidol	500	0
25,26 dihydroxyvitamin D_3	10	54
Cholecalciferol (Vitamin D3)	1000	1
Ergocalciferol (Vitamin D2)	100	5

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed to compare the candidate device to the predicate device in accordance with CLSI EP9-A2. A total of 120 serum samples with a sample range of 7.3 to 112 ng/mL were tested in singlicate on the candidate and predicate devices. Of the 120 samples tested, 2 samples were altered (one sample was diluted and one sample was spiked) in order to cover the claimed measuring range. Passing-Bablok regression analysis was performed on the comparative data:

n	Slope (ng/mL) [95% CI]	Intercept (ng/mL) [95% CI]	Correlation Coefficient (r)	Sample range tested (ng/mL)
120	0.93 [0.86 to 1.01]	1.09 [-0.90 to 3.01]	0.95	7.3 to 112

The candidate device (IDS-iSYS 25 $VitD^S$ assay) is a modification of the predicate device (IDS-iSYS 25-Hydroxy Vitamin D^S assay (k140554)) and the main differences are the source of antibody pools and the calibrator matrix used. It is expected that the candidate device has a slightly different performance at the low end of the measuring range when compared against the predicate device because of the overall assay optimization. The slope showed an overall negative bias when compared against the predicate device. In addition, scattering above 60 ng/mL was also observed for this comparison study, but falls within the clinically insignificant range. The accuracy of the candidate device has been established through a method comparison study against the ID-LC-MS/MS 25(OH)D RMP and was the basis of the substantial equivalence determination.

An additional method comparison study was performed to compare the candidate device IDS-iSYS 25 $VitD^S$ assay to the ID-LC-MS/MS 25(OH)D RMP. A total of 136 independent serum samples value assigned by ID-LC-MS/MS RMP (NIST/Ghent University) were tested. Of these 136 samples, 7 samples with a high 25(OH)D

concentration were diluted in a zero 25(OH)D matrix down to target values falling within the high end of the measuring range. The remaining 129 samples were unaltered. Passing Bablok regression analysis was performed on the comparative data:

n	Slope (ng/mL) [95% CI]	Intercept (ng/mL) [95% CI]	Correlation Coefficient (r)	Sample range tested (ng/mL)
136	0.99 [0.94 to 1.05]	-0.51 [-1.93 to 0.75]	0.97	5.6-110

b. Matrix comparison:

A matrix comparison study using the IDS-iSYS 25 VitD^S assay was performed according to CLSI EP09-A3 to evaluate the difference between serum (serum without additives, SST) and plasma (lithium heparin, sodium heparin, K_2 EDTA). Sixty-seven (67) samples, ranging from 4.8 to 108 ng/mL, were tested with ten of these samples spiked in order to cover the measuring range. Passing-Bablok regression analysis was performed on the comparative data relative to serum with no additives:

Sample Type	Slope (ng/mL) [95% CI]	Intercept (ng/mL) [95% CI]	R^2
Serum – SST	0.98 [0.94 to 1.02]	0.18 [-0.31 to 1.12]	0.99
Plasma – K_2 EDTA	0.96 [0.94 to 0.98]	0.05 [-0.31 to 0.59]	1.00
Plasma – Lithium Heparin	0.98 [0.93 to 1.01]	0.22 [-0.52 to 0.86]	1.00
Plasma – Sodium Heparin	0.99 [0.95 to 1.02]	0.05 [-0.66 to 0.71]	0.99

The results of the matrix comparison study support the sponsor's claim that serum as well as K_2 EDTA, Lithium Heparin, and Sodium Heparin are acceptable sample types for this 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:

An expected value study was performed according to the non-parametric method in CLSI C28-A3c. Samples were collected from 392 apparently healthy male (51%) and female (49%) adults, aged 21-89 years, during winter and summer seasons and from geographically diverse regions of the United States. This overall diversity in, geographic location, race, and ethnicity represents a broad spectrum of UV light exposure in the intended use population. The samples were tested for 25(OH)D concentrations using the IDS-iSYS 25 VitD^{S} assay. The 2.5th to 97.5th reference interval is shown below:

25-OH vitamin D expected values for adults: 10.4 to 59.5 ng/mL (n = 392)

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.