DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
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November 15, 2016

IMMUNODIAGNOSTIC SYSTEMS LTD
MICK HENDERSON
REGULATORY AFFAIRS OFFICER
10 DIDCOT WAY, BOLDON BUSINESS PARK
BOLDON NE35 9PD
GREAT BRITAIN

Re: K161831

Trade/Device Name: IDS-iSYS 25VitD^{S}, IDS-iSYS 25VitD^{S} Control Set
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D test system
Regulatory Class: II
Product Code: MRG, JJX
Dated: September 30, 2016
Received: October 4, 2016

Dear Mick Henderson:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See *PRA Statement below.*

510(k) Number *(if known)*
k161831

Device Name
IDS-iSYS 25VitD^S
IDS-iSYS 25VitD^S Control Set

Indications for Use *(Describe)*
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.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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510(k) SUMMARY

510k Number k161831

Introduction According to the requirements of 21CFR807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence.

Submitter

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Date prepared: 11th November 2016

Device Name

Proprietary names:	IDS-iSYS 25VitD^S IDS-iSYS 25VitD^S Control Set
Common names:	As above
Classification:	21CFR862.1825 Vitamin D Test System, Class II 21CFR862.1660 Quality Control Material (Assayed and Unassayed), Class I, reserved
Product Code:	MRG JJX

Predicate Device

The IDS-iSYS 25 VitD^S is substantially equivalent to other products in commercial distribution intended for similar use. We claim equivalency to the currently marketed IDS-iSYS 25-Hydroxy Vitamin D^S (k140554).

Device Description

IDS-iSYS 25 VitD^S

The IDS-iSYS 25 VitD^S reagent kit consists of one (1) Immunoassay Cartridge, one (1) vial each of 2 concentration Calibrator levels and a mini-CD containing the Instructions For Use (IFU), CRY files and Certificate of Analysis.

IDS-iSYS 25 VitD^S Cartridge, sufficient for 100 tests, consists of reagents provided in individual compartment within a plastic container called the Cartridge. IDS-iSYS 25 VitD^S Cartridge contains the following ready to use reagents:

- Magnetic particles coated with streptavidin in a phosphate buffer containing sodium azide.
- Sodium hydroxide solution.
- 25(OH)D labelled with an acridinium ester derivative, in buffer containing bovine serum albumin with sodium azide.
- Anti-25(OH)D sheep polyclonal antibody labelled with an biotin, in buffer containing bovine, sheep and mouse proteins and sodium azide.
- Assay buffer containing proprietary displacing compounds, methanol and sodium azide.

The IDS-iSYS 25 VitD^S Calibrators are included in the reagent kit. The ready to use calibrators contains human serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide as preservative (<0.1 %), 1 bottle per concentration level.

IDS-iSYS 25VitD^S Control Set

The IDS-iSYS 25VitD^S Control Set contains human serum in a buffer matrix with two defined concentrations of 25(OH)D and sodium azide as a preservative; 3 bottles per concentration level.

The intended use and composition of the calibration verifiers to be used with this assay are identical to the ones previously cleared in k111650 and will not form any part of this 510(k).
However they will be now known as IDS-iSYS 25 VitD^S Calibration Verifiers and not IDS-iSYS 25 Hydroxy Vitamin D Calibration Verifiers.

Indications 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.

Conditions for use: For in vitro diagnostic use only.
Rx Only

Special instrument Requirements:
IDS-iSYS Multi-Discipline Automated System (k091849)

Comparison Tables

Similarities compared to the chosen (FDA cleared; marketed) predicate device (k140554)

Assay

Item	**Predicate Device IDS-iSYS 25-Hydroxy Vitamin D^{S} (k140554)**	**Candidate Device IDS-iSYS 25 VitD^{S}**
Indications for use	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.	Same
Intended Use	For the quantitative measurement of total 25-hydroxyvitamin D	Same
Analyte	25-hydroxyvitamin D [25(OH)D]	Same
Reagent storage	2-8 °C	Same
Sample preparation (pre-treatment)	Performed on-board the system	Same
Sample volume	10µL	Same
Method of detection (Test methodology)	Chemiluminescent immunoassay using magnetic-particle solid phase and acridinium label	Same
Automation	Fully automated assay	Same
Calibration procedure	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.	Same
Traceability/ Standardization	Traceable to the isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC-/MS/MS)	Same

	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 system reagent stability	21 days	Same

Kit Controls similarities

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

Differences compared to the chosen predicate device (k140554)

Assay

Item	**Predicate Device IDS-iSYS 25-Hydroxy Vitamin D^S (k140554)**	**Candidate Device IDS-iSYS 25 VitD^S**
Calibrator matrix	Equine serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide as a preservative.	Human 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, CONJ, NaOH & BUF), two concentration levels of calibrators (A&B) (1 vial of each) & a mini CD	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
Quality Control	Requires three controls to validate the calibration	Requires two controls to validate the calibration
Kit reagent component volumes	Reagent cartridge (1 vial each): MPV1 (2.0mL), CONJ (10.1mL), NaOH (13mL) & BUF (26.0mL)	Reagent cartridge (1 vial each): MPV1 (2.5mL), NaOH (13mL), 25D-ACR (9.0mL), Ab-BIOT (9.0mL) & BUF (23.0mL)
Antibodies	Anti-25 OH D Sheep Polyclonal IgG	Same, but with a different source of antibody pool
Calibration interval	14 days	7 days
Range of assay	7 – 125 ng/mL	4– 110 ng/mL
Sensitivity	LoB: 0.6 ng/mL LoD: 2.6 ng/mL LoQ: 7.0 ng/mL	LoB: 1.31 ng/mL LoD: 1.98 ng/mL LoQ: 3.53 ng/mL
Expected values	12.7 to 64.2 ng/mL	10.4 to 59.5 ng/mL
Reference range	Overall: 8.2 to 91.9 ng/mL	Overall: 10.4 to 59.5 ng/mL
In use (after opening at 2-8°C) reagent stability	21 days	42 days

Method comparison	Against ID-LC-MS/MS Reference Method Procedure (n = 99) Passing-Bablok regression: IDS-iSYS = 0.95(x) + 0.8 ng/mL Correlation coefficient (r) = 0.925	Against ID-LC-MS/MS Reference Method Procedure (n = 136) Passing-Bablok regression: IDS-iSYS = 0.99(x) - 0.51 ng/mL Correlation coefficient (r) = 0.97
Sample matrix	Serum	Serum (standard sampling tubes or tubes containing serum separating gel) or plasma (K2 EDTA, lithium heparin, sodium heparin)
Sample tube type	Secondary tube	Primary or Secondary tube

Kit Controls differences:

Item	**Predicate device IDS-iSYS 25-Hydroxy Vitamin D^S Control Set (k140554)**	**Candidate device IDS-iSYS 25 VitD^S Control Set**
Control matrix	Equine serum buffer matrix with three defined concentrations of 25(OH)D and sodium azide as a preservative.	Human serum buffer matrix with two defined concentrations of 25(OH)D and sodium azide as a preservative.
Control Kit components	Three concentration levels of controls (3 vials of each) & a mini CD	Two concentration levels of controls (3 vials of each) & a mini CD
Quality Control	Requires three buffer based controls for daily Quality Control	Requires two serum based controls for daily Quality Control
Stability	On board the system: 2.5 hours	On board the system: 4 hours

Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was determined in accordance with CLSI EP5-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods". Assessment was made for the following variables: within run precision, total precision.

Nine serum based samples at different 25(OH)D concentration levels, ranging from approximately 13.9 ng/mL to approximately 103 ng/mL, to ensure that the assay range of the IDS-iSYS 25 VitD^S was covered were used to assess precision of the IDS-iSYS 25 VitD^S kit.

Final claims data reported is representative of one manufacture batch (MB3) where 20 days were available for analysis. Results from one representative lot:

Sample	Mean Conc.		Within Run SD		Within Run %CV	Total SD		Total %CV
	ng/mL	nmol/L	ng/mL	nmol/L		ng/mL	nmol/L	
1	13.9	34.8	0.7	1.8	5.0	1.0	2.5	7.4
2	16.9	42.3	0.7	1.8	3.9	1.2	3.0	7.2
3	28.6	71.5	1.1	2.8	3.9	1.9	4.8	6.5
4	37.0	92.5	1.3	3.3	3.5	1.8	4.5	4.9
5	49.8	125	2.0	5.0	4.0	2.9	7.3	5.8
6	61.1	153	2.4	6.0	3.9	3.7	9.3	6.0
7	62.1	155	2.8	7.0	4.4	4.0	10.0	6.4
8	93.0	233	3.5	8.8	3.8	5.8	14.5	6.2
9	103	258	4.8	12.0	4.7	6.3	15.8	6.1

b. *Linearity/assay reportable range:*

A linearity study was conducted based on a modified version of CLSI EP6-A. A high human serum sample (pool of two endogenous spiked serum samples) and a low human serum sample (low serum sample diluted in zero matrix) were analyzed in addition to 9 evenly spaced dilutions which were created by mixing the high and low sample to cover the range of the assay (4 - 110 ng/mL) as indicated below:

Sample	Dilution	Dilution Factor (%)
1:	Low (L)	0
2:	0.90L + 0.10H	10
3:	0.80L + 0.20H	20
4:	0.70L + 0.30H	30
5:	0.60L + 0.40H	40
6:	0.50L + 0.50H	50
7:	0.40L + 0.60H	60
8:	0.30L + 0.70H	70
9:	0.20L + 0.80H	80
10:	0.10L + 0.90H	90
11:	High (H)	100

Observed = 1.00 x (Expected) + 3.75 ng/mL

Observed = 1.00 x (Expected) + 9.38 nmol/L

Regression coefficient R^2: 1.00

The IDS-iSYS 25 VitD^S assay is linear over the range tested, 3.58 to 136 ng/mL.

The measuring (reportable) range of the assay is from 4 to 110 ng/mL. Any value that read below 4 ng/mL (10 nmol/L) should be reported as "<4 ng/mL" or "<10 nmol/L".

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

Calibrator traceability and value assignment

An internal stock solution (Top Dose) is prepared by reconstituting a vial of 25-hydroxyvitamin D_3 with ethanol. The concentration of the Top Dose when used in the assay is then assigned by performing dilutions of the Top Dose low charcoal stripped lipid stripped human serum (calibrator serum) or normal non lipid stripped human serum (kit control serum) and testing in an approved kit combination, for example a previously QC released kit. The concentration of the Top Dose is calculated back to the stock concentration by correction of the dilution with serum. This figure is an accurate determination and is assigned to the CDC VDSP aligned calibrators.

A set of secondary standards (Internal reference calibrators, IRs) were manufactured using the same materials as those used in the kit calibrators, however these cover a wider assay range (0 – 168 ng/mL). Kit Calibrators are manufactured and value assigned using the CDC VDSP aligned secondary standards.

Kit Controls are manufactured and value assigned using at least one approved kit combination over three IDS-iSYS instruments using the kit calibrators that were assigned against CDC VDSP aligned internal references.

For kit calibrator value assignment, the kit calibrators are tested as unknowns in a minimum of 20 assay runs on one IDS-iSYS instrument. Each run uses secondary standards (IRs), and the concentrations of the Kit Calibrators are calculated from these secondary standards (IRs) by using Excel data reduction for outliers followed by Prism to establish the curve parameters following an issued QC procedure. 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.

For kit control value assignment, the kit controls are tested as unknowns in a minimum of 21 assay runs using multiple systems. Each run uses an approved kit combination e.g. QC previously released kit, and the concentrations of the Kit Controls are calculated as unknown samples using the IDS-iSYS instrument on-board software following an issued QC procedure. The established values are then verified in a single assay in an approved kit combination. The values must fall within specified acceptable ranges.

The IDS-iSYS 25 VitD^{S} assay is traceable to the isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LCMS/MS) 25(OH)D Reference Method Procedure (RMP) which was used in assigning the target value for the Vitamin D Standarization Program (VDSP) samples. The ID-LCMS/MS RMP is traceable to the National Institute of Standards and Technology Standard Reference Material (NIST SRM) 2972.

Stability

The kit stability was performed in which the kit calibrators were tested in combination with all kit combination reagents. The stability based on accelerated studies determined a minimum shelf life of 12 months. Real time stability studies are on-going and shelf life will be amended accordingly.

Kit control stability is based on accelerated studies where a minimum shelf life of 12 months was determined.

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, "Protocols for Determination of Limits of Detection and Limits of Quantitation". For the measurement of LoB, LoD and LoQ three manufacture batches (MB1, MB2B and MB3) were tested.

Each LoB sample was run in duplicate. Lot MB1 and MB2B each ran a total of 6 assays over 4 days and MB3 ran 6 assays over 5 days, for a total of 60 replicates per batch. Two operators were used and where two assays were performed in one day the set up time between assays start time was at least 2 hours. Each manufacture batch was tested on a different system.

The LoD was 6 very low 25(OH)D samples. Each LoD sample was run in duplicate. For MB1 a total of 6 assays were run over 3 days, MB2B ran 6 assays over 4 days and MB3 ran 6 assays over 5 days, for a total of 72 replicates for each manufacture batch. Two operators were used and where two assays were performed in one day the set up time between assays start time was at least 2 hours. Each manufacture batch was tested on a different system.

For calculation of LoQ 10 samples were used, these all had low levels of 25(OH)D in them. Each LoQ sample was run in duplicate. For MB1 a total of 6 assays were run over 3 days, MB2B ran 6 assays over 4 days and MB3 ran 6 assays over 5 days, for a total of 120 replicates for each manufacture batch. For MB1, 1 sample was miss-labelled and excluded from data analysis resulting in a total of 118 replicates being available. Two operators were used for the LoQ assays and where two assays were performed in one day the set up time between assays start time was at least 2 hours. Each manufacture batch was tested on a different system.

Sensitivity	Concentration
LoB	1.31 ng/mL (3.28 nmol/L)
LoD	1.98 ng/mL (4.95 nmol/L)
LoQ	3.53ng/mL (8.83 nmol/L)

e. Analytical specificity:

Interference and cross-reactivity studies were performed in accordance with the CLSI EP7-A2"Interference testing in clinical chemistry".

To determine potential interference in the specific detection of 25(OH)D, two serum samples at two different concentrations of 25(OH)D were spiked with the potential interferent. Control samples (blank) were spiked with a volume of Phosphate Buffer saline (PBS) (0 ng/mL) or relevant diluent equal to that of the spiked interferent. The mean of 26 replicate assays, for both spiked and control samples, were then compared. The differences observed between the mean spiked and control sample values were examined and assessed according to acceptance criteria of non-significant interference of <10% between the test and control samples.

% Interference was calculated using the formula below:

$$\% \text{ Interference} = \frac{(\text{mean spiked concentration} - \text{mean un-spiked concentration})}{\text{mean un-spiked concentration}} \times 100$$

Cholesterol interference was tested by recovery of 25(OH)D from a high serum pool with 25(OH)D at 107.3 ng/mL spiked into a serum sample with a known cholesterol levels. 10% and 20% of high 25(OH)D was spiked into the cholesterol sample. The percent recovery was calculated using the formula below:

Recovery value = Observed mean spiked value – Observed mean un-spiked value

% Recovery = (Recovery value / Spike value) x 100

Rf Interference was tested by spiking a sample with known levels of Rf into two serum base samples. The 25(OH)D in the base samples were calculated at 32.6 ng/mL and 54.9 ng/mL. The 25(OH)D in the Rf sample was calculated to be 30.7 ng/mL. The level of interference was calculated using the formula below:

$$\text{Interference \%} = \frac{\text{Observed concentration}}{(\text{Expected conc. from base sample} + \text{Expected conc. from Rf sample})}$$

The expected values were calculated by taking into account the amount each sample was diluted by following the equation below:

$$\text{Expected concentration of 25(OH)D} = \frac{\text{25(OH)D concentration in neat sample}}{(\text{Total sample volume / volume Rf or base sample})}$$

The following table provides the levels of interferents tested and the design acceptance criteria for specificity of ≤10% bias between the test and control samples:

Potential interferent	Highest concentration tested that demonstrated no significant interference	25(OH)D concentration (ng/mL)	
		low control	high control
Triglycerides (Intralipid)	500 mg/dL	31.4	61.4
Hemoglobin	500 mg/dL	33.2	54.8
Bilirubin, conjugated	20 mg/dL	30.3	59.4
Bilirubin, unconjugated	20 mg/dL	23.2	67.4
Total Protein	10 g/dL	32.0	30.5
Human Anti Mouse Antibody (HAMA)	1000 ng/mL	33.8	65.6
Red Blood Cells	0.2%	29.8	57.7
Vitamin D Binding Protein (GC Globulin)	2000 ng/mL	30.0	61.7
Biotin	200nM	34.9	64.6
Acetaminophen	200 µg/mL	34.7	60.2
Ibuprofen	140 µg/mL	32.6	66.7
Carbamazepine	30 µg/mL	28.3	67.4
Phenytoin	50 µg/mL	32.2	62.2
Rheumatoid Factor	600 IU/mL	32.6	54.9
Cholesterol Total	300 mg/dL	8.6	N/A

Exogenous and Endogenous cross-reactants where tested:

Exogenous cross-reactants were prepared by manufacturing a 'top dose' of the relevant analyte (in ethanol typically) which was then diluted down to a range of stock concentrations in undetectable 25(OH)D matrix. The stock cross reactant or undetectable 25(OH)D matrix was spiked directly into zero serum (no measurable 25(OH)D), a low sample and a high sample. The cross reactivity was then determined using the following equation:

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

Where:

Mean concentration of spiked sample is determined by the candidate device
Mean concentration of un-spiked sample is determined by the candidate device
Spiked concentration is calculated by dilution of known concentration Top Dose

Endogenous cross-reactants ($25(OH)D_3$ and $25(OH)D_2$) were prepared by spiking samples with known levels of $25(OH)D_3$ and $25(OH)D_2$ analytes with sample also with known levels of $25(OH)D_3$ and $25(OH)D_2$ analytes. The samples which must be used for endogenous cross reactivity tests are supplied where the levels of $25(OH)D_3$ and

25(OH)D_2 have been established ID-LCMS/MS. The cross reactivity was then determined using the following equation:

% cross reactivity =

$$\frac{\text{(Mean concentration of spiked sample – mean concentration of un-spiked sample)}}{\text{Spike concentration}} \times 100\%$$

Where:

Mean concentration of spiked sample is determined by the candidate device
Mean concentration of un-spiked sample is determined by LC-MS/MS
Spiked concentration is determined by LC-MS/MS

Potential cross-reactant	**Spike concentration tested (ng/mL)**	**% Cross Reactivity**
25 (OH)D_3	20	101%
25 (OH)D_2	20	105%
24,25 dihydroxyvitamin D_3	25	197%
24,25 dihydroxyvitamin D_2	25	37%
25,26 dihydroxyvitamin D_3	10	54%
1,25 dihydroxyvitamin D_3	20	3%
1,25 dihydroxyvitamin D_2	20	8%
3-epi-25-OH vitamin D_3	100	0%
3-epi-25-OH vitamin D_2	100	2%
Alfacalcidol	500	0%
Cholecalciferol	1000	1%
Ergocalciferol	100	5%
Paricalcitol	100	-2%

2. Comparison studies:

Method comparison:

The IDS-iSYS 25 VitD^S assay was compared against the NIST/Ghent ID-LC-MS/MS 25(OH) Vitamin D Reference Method Procedure, following CLSI EP-9A2 “Method Comparison and Bias Estimation Using Patient Samples”. A total of 136 human serum samples, selected to represent a wide range of 25(OH)D concentrations (5.6 to 110 ng/mL, 14.0 to 275 nmol/L), were tested in on the IDS-iSYS 25 VitD^S assay.

Passing-Bablok regression analysis was performed on the comparative data:

Unit	n	Slope	95% CI	Intercept	95% CI	Correlation Coefficient (r)
ng/mL	136	0.99	0.94 to 1.05	-0.51	-1.93 to 0.75	0.97
nmol/L	136	0.99	0.94 to 1.05	-1.28	-4.83 to 1.88	0.97

3. Expected values/Reference range:

There is no universal agreement on the optimal concentration of 25(OH)D. Ranges should be based on clinical decision values that apply to both sexes of all ages rather than population based reference ranges for 25(OH)D.

In the case of 25(OH)D, there are also many other factors that may influence values: diet, time of day, sun exposure, season of year, geographic location, age, use of sunscreen and/or protective clothing and skin pigmentation.

The 25(OH)D concentration were measured in serum samples collected from three hundred and ninety-two (392) apparently healthy donors using the IDS-iSYS 25 VitD^S assay. To represent a broad spectrum of UV light exposure, the study population included 200 males and 192 females from three diverse regions of the United States (North, Central and South) that were sampled in the winter and summer and were representative of the overall United Stated population in term of gender, race and ethnicity. Individuals who were pregnant, had bariatric surgery, had personal history of kidney disease and GI disease, had family history of parathyroid or calcium regulatory disease, had abnormal serum calcium, phosphorus, PTH or TSH level were excluded from the study. The study cohort included subjects from 21 to 89 years of age and 40% reported taking vitamin supplements.

The observed ranges were established, according to CLSI C28-A3c "Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory" is summarised in the table below:

n	Mean Concentration	Median Concentration	Observed Range (2.5^{th} to 97.5^{th} percentile)
392	30.7 ng/mL	29.5 ng/mL	10.4 ng/mL to 59.5 ng/mL
392	76.8 nmol/L	73.8 nmol/L	26.0 nmol/L to 149 nmol/L

The above ranges should be considered as guidelines only. It is recommended that each laboratory to establish its own expected range, representative of its typical population.

4. Matrix Comparison Study:

The IDS-iSYS 25 VitD^S matrix comparison study was performed to evaluate the difference across tube types (serum separator tubes (SST), lithium heparin plasma, sodium heparin plasma and K_2 EDTA plasma) versus the control samples (red top serum, without additive) following the CLSI EP9-A3 guideline. Fifty seven (57) native samples

were tested with ten (10) treated (spiked or diluted) samples to cover the range of 4.8 to 108 ng/mL (serum without additives).

Passing-Bablok regression analysis was performed on the comparative data:

Sample Type	n	Slope	95% Cl.	Intercept (ng/mL)	95% Cl.	Corr. Coeff. (r)
SST	67	0.98	0.94 to 1.02	0.18	-0.31 to 1.12	0.99
EDTA	67	0.96	0.94 to 0.98	0.05	-0.31 to 0.59	1.00
Lithium Heparin	67	0.98	0.93 to 1.01	0.22	-0.52 to 0.86	1.00
Sodium Heparin	67	0.99	0.95 to 1.02	0.05	-0.66 to 0.71	0.99

The IDS-iSYS 25 VitD^S assay is suitable for use with human serum (red top serum without additive, serum separator tubes SST) and plasma (lithium heparin, sodium heparin and K2 EDTA).

Conclusion:

The IDS-iSYS 25 VitD^S assay and IDS-iSYS 25 VitD^S Control Set data presented and provided is complete and supports the basis for substantial equivalence to the predicate device.