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

A. 510(k) Number:

k140554

B. Purpose for Submission:

Modification of traceability for standardization, and modification of antibody pools of the previously cleared device, k091849

C. Measurand:

25-hydroxyvitamin D and other hydroxylated metabolites of vitamin D

D. Type of Test:

Quantitative chemiluminescent immunoassay

E. Applicant:

Immunodiagnostic Systems Limited

F. Proprietary and Established Names:

IDS-iSYS 25-Hydroxy Vitamin D^S Assay

IDS-iSYS 25-Hydroxy Vitamin D^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-Hydroxy Vitamin D^S Assay (IDS-iSYS 25OHD^S) is intended for the quantitative determination of 25-hydroxy vitamin D (25OHD) and other hydroxylated metabolites in human serum 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-Hydroxy Vitamin D^S (25OHD^S) Control Set is used for quality control of the IDS-iSYS 25-Hydroxy Vitamin D^S assay on the IDS-iSYS Multi-Discipline Automated System

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

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

I. Device Description:

The IDS-iSYS 25-Hydroxy Vitamin D^S Assay consists of a reagent cartridge and one set of calibrators.
The reagent cartridge contains multiple reagents:
MPV1 - Magnetic particles coated with 25-OHD in a phosphate buffer containing methanol with sodium azide (<0.1%) as preservative (1 vial, 2.0 mL)
CONJ - Anti-25-OH D sheep polyclonal antibody labeled with an acridinium ester derivative, in buffer containing bovine, sheep, rabbit and mouse proteins with sodium azide (<0.1%) as preservative (1 vial, 10.1 mL)
NaOH - Sodium hydroxide solution <0.5 M (1 vial, 5.2 mL)

BUF - Assay buffer containing proprietary displacing compounds, methanol, and sodium azide (<0.1%) as preservative (1 vial, 26.0 mL)

Calibrators:
Kit Calibrators A and B (CAL A & CAL B) (1 vial of each, 2.5 mL per vial) contain horse serum in a buffer matrix with two defined concentrations of 25-OHD and sodium azide (<0.1%) as a preservative.
The Calibrator target values are as follows:
Kit Calibrator A 5.5 ng/mL
Kit Calibrator B 64.5 ng/mL

IDS-iSYS 25-Hydroxy Vitamin D^S Control Set (sold separately):
The IDS-iSYS 25-Hydroxy Vitamin D^S Control Set (3 vials of each level, 2.5 mL per vial) contains horse serum in a buffer matrix with three defined concentrations of 25-OH D and sodium azide (<0.1%) as a preservative.
The control ranges are as follows:
CTL1 range: 12.0 – 18.0 ng/mL
CTL2 range: 26.4 – 39.6 ng/mL
CTL3 range: 59.0 – 86.0 ng/mL

J. Substantial Equivalence Information:

1. Predicate device name(s):

   IDS-iSYS 25-Hydroxy Vitamin D

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

   k091849

3. Comparison with predicate:

   Assay:

Similarities		
Item	Predicate device IDS-iSYS 25-Hydroxy Vitamin D (k091849)	Candidate device IDS-iSYS 25-Hydroxy Vitamin D^S Assay
Intended Use	Quantitative determination of 25-Hydroxy Vitamin D and other hydroxylated metabolites in human serum.	Same

Similarities		
Item	Predicate device IDS-iSYS 25-Hydroxy Vitamin D (k091849)	Candidate device IDS-iSYS 25-Hydroxy Vitamin D^S Assay
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
Analyte	25-Hydroxy Vitamin D (25-OHD)	Same
Sample Type	Human serum	Same
Reagent Storage	2-8 °C	Same
Test Methodology	Chemiluminescent immunoassay using magnetic-particle solid phase and acridinium label	Same
Kit reagent components	Reagent cartridge (1 vial each of MPV1, CONJ, NaOH & BUF), two concentration levels of calibrators (A&B) (1 vial of each)	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 instructions for use.	Same

Differences		
Item	Predicate device IDS-iSYS 25-Hydroxy Vitamin D(k091849)	Candidate device IDS-iSYS 25-Hydroxy Vitamin D^S Assay
Kit reagent component volumes	Reagent cartridge (1 vial each): MPV1 (2.6mL), CONJ (7.1mL), NaOH (5.2mL) & BUF (26.0mL)	Reagent cartridge (1 vial each): MPV1 (2.0mL), CONJ (10.1mL), NaOH (5.2mL) & BUF (26.0mL)
Traceability/ Standardization	Traceable to U.V. quantification	Traceable to the isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC-/MS/MS) 25(OH) vitamin 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.
Antibodies	Anti-25 OHD Sheep Polyclonal IgG	Same, but with a different source of antibody pool
Calibration interval	7 days	14 days
Calibrator shelf life	8 months	6 months
Measuring range	6 – 126 ng/mL	7 – 125 ng/mL
Reference range	Non-parametric reference interval: 7.9 – 57.8 ng/mL (n=150)	Non-parametric reference interval: 12.7 – 64.2 ng/mL (n=275)
On board the analyzer reagent stability	7 days	21 days

Controls:

Similarities		
Item	Predicate device IDS-iSYS 25-Hydroxy Vitamin D Control Set (k091849)	Candidate device IDS-iSYS 25-Hydroxy Vitamin D^S Control Set
Intended Use	For quality control of the IDS-iSYS 25-Hydroxy Vitamin D assay on the IDS-iSYS Multi-Discipline Automated System.	Same
Number of levels	3	Same
Matrix	Horse serum in buffer	Same
Packaging	Provided separately	Same
Storage temperature	2-8°C	Same

Differences		
Item	Predicate device IDS-iSYS 25-Hydroxy Vitamin D Control Set (k091849)	Candidate device IDS-iSYS 25-Hydroxy Vitamin D^S Control Set
Standardization/Traceability	Traceable to U.V. quantification	Traceable to the ID-LC/MS/MS 25 (OH) vitamin D Reference Measurement Procedure

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

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

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

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

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

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

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

L. Test Principle:

The assay is based on chemiluminescence technology. Samples are subjected to a pre-treatment step to denature the Vitamin D Binding Protein (VDBP). The treated samples are then neutralized in assay buffer and a specific anti-25-OHD antibody labelled with acridinium is added. Following an incubation step, magnetic particles linked to 25-OHD are added. Following a further incubation step, the magnetic particles are "captured" using a magnet. After a washing step and addition of trigger reagents, the light emitted by the acridinium label is inversely proportional to the concentration of 25-OHD in the original sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

A study was performed in accordance with CLSI EP5 A2 where six (6) serum samples (ranging from 12.0 ng/mL to 116.5 ng/mL) were assayed using three (3) lots of reagents in duplicate (n=2), twice per day for 20 days on three (3) analyzers. Each analyzer/reagent lot produced 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 (ng/mL)	SD	CV%	SD	CV%
Serum1	80	13.2	0.8	6.4	1.4	10.6
Serum 2	80	27.2	1.5	5.4	2.5	9.0
Serum 3	80	38.9	2.3	5.8	3.5	9.1
Serum 4	80	54.5	3.2	5.8	5.0	9.1
Serum 5	80	77.2	4.0	5.2	6.6	8.5
Serum 6	80	119.9	6.1	5.1	8.6	7.2

b. Linearity/assay reportable range:

Linearity was evaluated based on CLSI EP-6A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach. Samples were prepared by diluting a serum sample with a high 25-OH vitamin D concentration with a serum sample with a low 25-OH vitamin D concentration to obtain eleven (11) concentration levels across the measuring range. Samples were run in duplicate, using

one reagent lot with sample range tested between 5.8 to 152.9 ng/mL. The linear regression of the observed mean concentrations versus the expected concentration was calculated.

The resulting linear regression equation was y = 0.96x+ 2.1 ng/mL, R^2 = 1.00

The results of the linearity study support the sponsor's claim that the assay is linear from 7-125 ng/mL.

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

Standardization and Traceability

The IDS-iSYS 25-Hydroxy Vitamin D^S Assay was originally cleared under 510(k) k091849. IDS has modified the assay by standardizing the cleared vitamin D assay 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 vitamin 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.

To achieve standardization against the VDSP recognized Reference Measurement Procedure (RMP), the IDS-iSYS 25-Hydroxy Vitamin D^S assay master calibration parameters were aligned to the CDC VDSP by using 70 human serum samples from the CDC VSDP program which were value assigned by the RMP and traceable to NIST SRM2972.

Calibrator traceability and value assignment

This assay is traceable to the isotope dilution-liquid chromatography/tandem mass spectrometry (ID-LC-/MS/MS) 25-OH Vitamin D Reference Method Procedure (RMP) that is traceable to the National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 2972. The calibrator traceability and value assignment are prepared according to an internal procedure.

Kit calibrators are lot specific for each assay kit. Master calibrators and kit calibrators A and B are prepared gravimetrically from the stock solution or an intermediate stock solution. For value assignment, the kit calibrators are tested as unknowns in a minimum of 20 assay runs using one analyzer. Each run is calibrated using master calibrators. The values are then adjusted to ensure batch-to-batch consistency where necessary. The final assigned values obtained for the kit calibrators are verified on three additional analyzers. The values must fall within specified acceptable ranges. The calibrator target values are listed below:

Calibrator A target value: 5.5 ng/mL
Calibrator B target value: 64.5 ng/mL

The IDS-iSYS 25-Hydroxy Vitamin D^S assay (candidate device) has passed the certification process for the CDC VDSP and a certificate has been provided by the CDC. Please see, http://www.cdc.gov/labstandards/hs.html for more information on the CDC VDSP certification program.

Stability

The calibrator and control shelf-life and open-vial stability testing protocols and acceptance criteria were reviewed and found to be adequate. Once opened, calibrators and controls are stable for up to 2.5 hours on board the analyzer. Current accelerated shelf life studies support the assigned six-month shelf life, with real-time studies on-going.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantification (LoQ) studies were performed according to CLSI EP-17A.

To establish the Limit of Blank (LoB), the zero calibrator was assayed in replicates of 10 on 10 separate days giving a total of 100 measurements. The Limit of Blank was determined by calculating the concentration corresponding to the mean zero calibrator minus two standard deviations from the calibration curve. The LoB claim is 0.6ng/mL.

The Limit of Detection (LoD) study was performed by assaying 10 samples (native and/or diluted) with very low vitamin D concentrations (ranging from 1.9ng/mL to 8.0ng/mL) in duplicate in 12 runs spanning multiple days giving a total of 240 data points. The LoD claim is 2.6 ng/mL.

The Limit of Quantification (LoQ) was determined by measuring 13 low samples (native and /or diluted) in a concentration range of 2.8 – 24.6ng/mL in duplicate over 7 individual runs spanning multiple days giving a total of 182 data points. The LoQ claim is 7.0ng/mL and was calculated by interpolating the concentration of the analyte from the regression curve at 20% precision CV.

Detection limits results:

Limit of Blank	Limit of Detection	Limit of Quantitation
0.6 ng/mL	2.6 ng/mL	7.0 ng/mL

The reportable range of the assay is 7-125 ng/mL.

e. Analytical specificity:

An endogenous interference study was performed based on CLSI EP7-A2 guideline to assess common or known substances that could interfere with IDS-iSYS 25-

Hydroxy Vitamin D^S assay. The potential interferents listed below were spiked into human serum samples with different levels of 25-OH vitamin D. Each sample was tested in replicates of 26. The 25-OH vitamin D values of the spiked samples were compared to the reference samples containing no interferent. Significant interference was defined as greater or equal to ±10% difference from the expected concentration. The interference study results are summarized in the following table:

Potential Interferent	Sample 25OHD concentration (ng/mL)	Highest Tested Concentration at which no significant interference (≤10%) was observed
Bilirubin, conjugated	18.0 and 65.7	30 mg/dL
Hemoglobin	19.2 and 71.7	40 mg/dL
Triglycerides	50.0 and 87.9	500 mg/dL
Red blood cells	15.5 and 65.8	0.2%
Biotin	17.0 and 66.2	300 nmol/L
HAMA	18.2 and 72.8	500 ng/mL
Vitamin D binding protein	18.9 and 76.0	2000 ng/mL

The package insert contains the following limitation: The presence of hemoglobin at concentrations >40 mg/dL might lead to falsely depressed values. Do not use hemolyzed samples.

Rheumatoid Factor: Interference from rheumatoid factor (RF) was assessed using a recovery study. Serum samples containing 18.7 ng/mL and 21.4 ng/mL of 25OHD were spiked with1500 IU/mL of RF and tested in replicates of 4. The results were compared to results from samples not containing RF. Non-significant interference is defined as a recovery of between 90-110%. The highest tested concentration of RF at which no significant interference was observed was 1500 IU/mL.

Cross reactivity studies:

To evaluate the cross reactivity potential of substances with a similar chemical structure as 25(OH) vitamin D, two separate studies were performed. Literature describes problems with assessing % cross reactivity using exogenous spiked samples due to the hydrophobic nature of vitamin D molecules and changes in sample matrix with addition of organic solvents which may not reflect the actual % recovery of the endogenous 25-OH Vitamin D2 and D3 isoforms in the tested sample. Therefore, the % cross reactivity of the 25-OH Vitamin D2 and D3, and 24, 25 $(OH)_2$ D3 isoforms were tested mimicking endogenous conditions by adding endogenous 25(OH) vitamin D metabolite to serum samples. Endogenous 25(OH) vitamin D metabolites were spiked into vitamin D serum samples and analyzed with the IDS-iSYS 25-Hydroxy Vitamin D^S assay. The concentration of un-spiked sample and spike concentration were determined by a LC-MS/MS 25(OH)D method. The spiked sample was measured with the IDS-iSYS 25-

Hydroxy Vitamin D^S assay. The % cross-reactivity was calculated based on following equation:

$$\frac{\text{Mean conc. of spiked sample – mean conc. of unspiked sample}}{\text{Spiked concentration}} \times 100\%$$

Cross reactivity results

Cross Reactant	Spike Conc. (ng/mL)	Un-spiked sample value (ng/mL)	Spiked sample value (ng/mL)	% Cross Reactivity	Mean % Cross Reactivity
$25(OH)D_3$	10.0 20.0	20.2 14.3	29.2 35.1	90% 104%	97%
$25(OH)D_2$	10.0 20.0	16.1 11.1	28.5 34.4	124% 117%	120%
$24,25(OH)_2D_3$	5.0	66.2	72.4	124%	124%

Due to the naturally low concentrations which exist endogenously, the other vitamin D metabolites are not expected to cause clinically significant cross-reactivity with the assay. Therefore, exogenous spiking was used to test the cross-reactivity of the vitamin D metabolites listed below. Exogenous synthetic 25(OH) vitamin D metabolites were spiked into vitamin D serum samples. The un-spiked and spiked samples were measured with the IDS-iSYS 25- Hydroxy Vitamin Ds assay. The % cross reactivity was calculated based on the following equation:

$$\frac{\text{Mean conc. of spiked sample – mean conc. of unspiked sample}}{\text{Spiked concentration}} \times 100\%$$

Cross reactivity results

Cross Reactant	Spiked conc. (ng/mL)	Sample Native conc. (ng/mL)	% Cross reactivity	Mean % Cross reactivity
3-epi-25(OH)D3	100.0 100.0	9.4 36.4	0% 3%	1%
3-epi-25(OH)D2	100.0 100.0	8.6 33.4	0% 2%	1%
1,25-(OH)2 D3	2.0 2.0	7.1 31.2	19% -65%	-23%
1,25-(OH)2 D2	20.0 20.0	8.6 37.9	13% 5%	9%
Vitamin D3 (Cholecalciferol)	1000.0 1000.0	6.9 30.8	0% 0%	0%

Vitamin D2 (Ergocalciferol)	100.0 100.0	7.4 37.3	1% -2%	0%
Paricalcitol	100.0 100.0	8.2 33.4	0% 2.9%	0%

f. Assay cut-off:

Not applicable

2. Comparison studies:

a. Method comparison with predicate device:

A method comparisons study was performed to compare the modified (standardized) IDS-iSYS 25 Hydroxy Vitamin D^S assay to the non-standardized IDS-iSYS 25 Hydroxy Vitamin D assay (k091849). Following CLSI EP9- guidelines, an internal method comparison study was performed with a total of 283 native serum samples tested in singlicate on the IDS-iSYS 25-Hydroxy Vitamin D^S Assay and on the predicate device. Passing-Bablok regression analysis was performed to produce a summary of results for each study, as shown below.

Passing Bablok	y= 0.96x + 1.1
Slope, 95% Confidence Interval	0.91 to 1.01
Intercept, 95% Confidence Interval	-0.3 to 2.3 ng/mL
Correlation Coefficient, r	0.94
n	283
Range tested	7.3-115.1 ng/mL

An additional method comparison study was performed to demonstrate the accuracy of the newly aligned IDS-iSYS 25 Hydroxy Vitamin D^s assay. With the aligned assay master calibration parameters, 99 independent samples value assigned by ID-LC-MS/MS RMP (9.0 ng/ml to 98.6 ng/mL), were used to assess the IDS-iSYS 25 Hydroxy Vitamin Ds assay traceability against the ID-LC-MS/MS RMP. The test result range on the candidate device was 10.5 to 96.1 ng/mL. The correlation between the IDS-iSYS (y) and the ID-LC-MS/MS RMP (x) is described using Passing-Bablok regression & Deming regression:

Passing Bablok regression:
IDS-iSYS = 0.95 x (ID-LC-MS/MS RMP) +0.8ng/mL
95 % CI of the slope: 0.86to 1.04
95 % CI of the intercept: -1.32 to 3.08ng/mL

Deming regression:
IDS-iSYS = 0.94x (ID-LC-MS/MS RMP) +1.34ng/mL
95 % CI of the slope: 0.86 to 1.01
95 % CI of the intercept: -0.78 to 3.45ng/mL

Correlation coefficient, r: 0.925

b. Matrix comparison:

Not applicable, only serum may be used.

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 values study was performed according to the non-parametric method in CLSI C28-A2 guideline. Samples from 275 apparently healthy light skin and dark skin male (71.6%) and female (28.4%) adults , aged 21-77 years, living in geographical diverse regions of the United States to represent a broad spectrum of UV light exposure in the intended use population, were collected. The samples were tested for 25-OH vitamin D concentrations using the IDS-iSYS 25-Hydroxy Vitamin D^S Assay. The 95% reference interval was calculated by a non-parametric method following C28-A2. The following range was obtained:

Normal Adults: 12.7 – 64.2 ng/mL (n=275)

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.