REF IS-2700S	IDS-iSYS 25-Hydroxy Vitamin D^S	
Instructions for Use	*IN VITRO DIAGNOSTIC*	CE

Intended Use

For *In Vitro Diagnostic* Use

The IDS-iSYS 25-Hydroxy Vitamin D^S Assay (IDS-iSYS 25OHD^S) is intended for the quantitative determination of 25-hydroxyvitamin D (25OHD) and other hydroxylated metabolites in human serum on the IDS-iSYS Multi-Discipline Automated System (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.

Summary and Explanation

Vitamin D is a commonly used collective term for a family of closely related seco-steroids. Upon exposure to sunlight, 7-dehydrocholesterol, located deep in the actively growing layers of the epidermis, undergoes photolytic cleavage of the "B" ring to yield pre-vitamin D_3 which is isomerised to vitamin D_3 (cholecalciferol). Vitamin D_3 and vitamin D_2 (ergocalciferol) may also be obtained by dietary supplementation or from a limited number of foods. Vitamin D_2 is metabolised in a similar way to vitamin D_3[1]. Vitamin D is stored in adipose tissue and enters the circulation bound to vitamin D binding protein (VDBP) and albumin[2].

In the liver, vitamin D is hydroxylated to give 25OHD which also circulates as a complex with VDBP. A small proportion of the 25OHD is further hydroxylated in the kidney, under direct regulation by parathyroid hormone and ionised calcium levels, to form the biologically-active calcitropic hormone 1,25-dihydroxyvitamin D. Further hydroxylation and metabolism of vitamin D produces compounds that are water soluble and readily excreted. Hepatic vitamin D 25-hydroxylase activity is not tightly regulated and changes in cutaneous production of vitamin D_3, or ingestion of vitamin D (D_3 or D_2), result in changes in circulating levels of 25OHD[3].

Serum concentration of 25OHD is considered to be the most reliable measure of overall vitamin D status and thus can be used to determine whether a patient is vitamin D sufficient[4].

Method Description

The assay is based on chemiluminescence technology. 10µL of samples are subjected to a pre-treatment step to denature the VDBP. The treated samples are then neutralised in assay buffer and a specific anti-25OHD antibody labelled with acridinium is added. Following an incubation step, magnetic particles linked to 25OHD 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 25OHD in the original sample.

Warnings and Precautions

The IDS-iSYS 25OHD^S is for *In Vitro Diagnostic Use* only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in these Instructions for Use (IFU). IDS Limited will not be held responsible for any loss or damage (except as required by statute), howsoever caused arising out of non-compliance with the instructions provided.

CAUTION: This kit contains material of animal origin. Handle kit reagents as if capable of transmitting an infectious agent.

Appropriate precautions and good laboratory practice must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Sodium Azide

Some reagents in this kit contain sodium azide <0.1 % (w/w) which may react with lead, copper or brass plumbing to form highly explosive metal azides. On disposal, flush with large volumes of water to prevent azide build up.

Magnetic Particles

Magnetic Particles contain methanol at >3 %, but <10 %.
Acute Tox. 4.
STOT SE 1.
EYE Irrit 2.
Skin Irrit 2.

H302	Harmful if swallowed
H315	Causes skin irritation
H319	Causes serious eye irritation
H370	Causes damage to organs
P280	Wear protective gloves/protective clothing/eye protection/face protection.

Assay Buffer

Assay buffer contains methanol at >10%, but <20 %.
Acute Tox. 4.
STOT SE 1.
EYE Irrit 2.
Skin Irrit 2.

H302	Harmful if swallowed
H315	Causes skin irritation
H319	Causes serious eye irritation
H370	Causes damage to organs
P280	Wear protective gloves/protective clothing/eye protection/face protection.

Sodium Hydroxide Solution

Solution contains sodium hydroxide (<0.5M),0.5 %≤ C<2.0 %.
EYE Irrit 2.
Skin Irrit 2.

H315	Causes skin irritation
H319	Causes serious eye irritation
P280	Wear protective gloves/protective clothing/eye protection/face protection.

Shelf Life and Storage of Reagents

Store the cartridge and calibrators in an **upright** position in the dark at 2 to 8°C. Do not freeze the cartridge or the calibrators.

Reagent shelf life	Cartridge	Calibrators
Before opening at 2 - 8 °C	To the expiry date	To the expiry date
After opening at 2 - 8 °C	21 Days	To the expiry date
On board the System*	21 Days	2.5 Hours

***** Continuous on board stability.

Sample Collection and Storage

The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel).

Serum Samples Storage Stability	Duration
Room temperature	3 days
2 - 8°C	3 days
Freeze/thaw cycles	3

For the IDS-iSYS 25OHD^S assay, the following tube types gave the following correlations:

- Serum Separator Tubes:
  Y = 1.00 x (serum) + 0.4 ng/ml; R^2: 0.97
  Y = 1.00 x (serum) + 1.0 nmol/l; R^2: 0.97
- Lithium Heparin Plasma Tubes:
  Y = 0.84 x (serum) - 2.7 ng/ml; R^2: 0.99
  Y = 0.84 x (serum) - 6.8 nmol/l, R^2: 0.99
- Sodium Heparin Plasma Tubes:
  Y = 0.83 x (serum) - 2.1 ng/ml; R^2: 0.99
  Y = 0.83 x (serum) - 5.3 nmol/l; R^2: 0.99

NOTE:

i. EDTA plasma cannot be used with the IDS-iSYS 25OHD^S assay.

ii. Specimens should be separated as soon as possible after collection. For long term storage, store at -20°C or below. Avoid repeated freeze-thaw of samples.

iii. Samples containing particulate matter must be centrifuged before performing the assay.

iv. Do not use heat-inactivated samples.

v. To minimise possible evaporation effects, samples, calibrators, and controls should be measured within 2.5 hours after being placed on the System.

vi. Some sample collection tubes that are commercially available might affect the results of testing in particular cases.

vii. It is recommended to follow the instructions of the tube manufacturer especially when processing samples in primary tubes.

Procedure

Materials Provided

Reagent Cartridge

MPV1

Magnetic particles coated with 25OHD in phosphate buffer containing methanol (>3 % but <10 %) with sodium azide as preservative (<0.1 %), 1 bottle, 2.0 mL.

CONJ

Anti-25OHD sheep polyclonal antibody labelled with an acridinium ester derivative, in buffer containing bovine, sheep, rabbit and mouse proteins with sodium azide as preservative (<0.1 %), 1 bottle, 10.1 mL.

NaOH

Sodium hydroxide solution (<0.5 M), 1 bottle, 5.2 mL.

BUF

Assay buffer containing proprietary displacing compounds, methanol (>10 % but <20 %) and sodium azide as preservative (<0.1 %), 1 bottle, 26 mL.

Calibrators

CAL A
CAL B

Equine serum buffer matrix containing 25OHD and sodium azide as preservative (<0.1 %), 1 each of 2 concentration levels, 2.5 mL.

Mini CD

Contains the IFU for IDS-iSYS 25OHD^S reagents and CRY files.

Materials Required But Not Provided

IDS-iSYS Multi-Discipline Automated System:	IS-310400.
IDS-iSYS 25OHD^S Control Set:	IS-2730S.
IDS-iSYS Cuvettes Cube:	IS-CC100.
IDS-iSYS System Liquid:	IS-CS100.
IDS-iSYS Wash Solution:	IS-CW100.
IDS-iSYS Triggers Set:	IS-CT100.
IDS-iSYS Cartridge Check System:	IS-6010.
IDS-iSYS Sample Cups (500 µL):	IS-SC105.

Materials Available on Request

Certificate of Analysis

Assay Procedure

Reagent Cartridge

The reagents provided in the kit are ready to use.
Before a new cartridge is loaded onto the System, mix the magnetic particles container by a brisk rotation motion. This will resuspend the magnetic particles that have settled during shipment and storage. Ensure that there is no foam formation in the cartridge reagents.

The barcode is read when the cartridge is loaded on the reagent tray. If the label cannot be read by the System's barcode reader, a manual procedure exists to enter the barcode data (see the IDS-iSYS User Manual).

Once the cartridge is on the reagent tray, the System automatically performs the mixing of magnetic particles to maintain homogeneity. Load the cartridge on the reagent tray and wait for at least 40 minutes before starting the assay. If the cartridge is removed from the reagent tray, store the cartridge vertically at 2 – 8 °C in the dark.

Calibrators

The IDS-iSYS 25OHD^S calibrators are ready to use. Leave the calibrators at room temperature for 10 minutes and **gently mix the vials by hand**. Avoid the formation of foam. Pipette approximately 200 µL of calibrators into sample cups and place onto the System.

NOTE:

i. DISCARD the material in the sample cups after use.

ii. DO NOT return material to the calibrator vials.

System Calibration

Two 25OHD calibrators are required to perform the adjustment of the master curve. These calibrators are supplied with the kit and calibrators from another lot must not be used. All levels of IDS-iSYS 25OHD^S control **MUST** be measured in duplicate at the same time as the calibrators to perform a master curve adjustment.

Use calibrators A and B to adjust the master curve to the reagents on board the System. Check for the presence of an IDS-iSYS 25OHD^S cartridge on the reagent tray and the availability of the cartridge master curve in the database. If the data for the lot of calibrators are not available on board the System, load the data using the mini CD provided with the kit.

During calibration, the calibrators are measured in triplicate. RLU CVs of **> 7 %** will result in a failed calibration. One replicate may be removed to meet the calibration requirements. All levels of 25OHD control must be measured in duplicate to calibrate the System. Verify and approve the calibration according to the calibration status displayed in the calibration windows. Discard the calibrators and controls from the sample tray after use.

Calibration Frequency

A new calibration is required:

- Each time a new lot of cartridges is loaded onto the System.
- Each time a new lot of triggers or cuvettes are used.
- When the control values do not fall within the defined ranges.
- When the calibration interval of **14** days has expired.
- After System service.

Verification of the calibration is automatic and managed by the IDS-iSYS System.

Quality Control

The IDS-iSYS 25OHD^S Control Set (IS-2730S) is required for quality control. Controls should be tested minimally at least once every 24 hours when the test is in use and during every calibration in conformance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality procedure. It is recommended that labs testing the specimens in multiple shifts in a day should measure the controls during each shift.

Refer to the IDS-iSYS 25OHD^S Control Set Instructions for Use for preparation and handling procedures.

Determination of Sample 25OHD levels

Process samples according to the IDS-iSYS User Manual.

Calculation of Results

The 25OHD concentration of each sample is calculated automatically. The display of each concentrations (screen or printed) is produced as per the user settings.

To convert results to SI units:

nmol/l = ng/ml x 2.5

The IDS-iSYS 25OHD^S assay uses a 4-parameter logistic curve fit (4PL) to calculate the 25OHD concentrations.

Dilution

Samples with 25OHD concentrations above the reportable range should be diluted manually with a low concentration human serum sample in a ratio of 1 in 2. The results for diluted samples must be multiplied by the dilution factor 2 and corrected for the concentration of the low sample.

Limitations of Use

1. As in the case of any diagnostic procedure, results must be interpreted in conjunction with the patient's clinical presentation and other information available to the physician.
2. The performance characteristics of this assay have not been established in a paediatric population.
3. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays[5]. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
4. Do not use samples showing sign of haemolysis. Haemoglobin at concentrations > 40 mg/dL might cause to falsely depressed values.
5. The following substances do not interfere in the IDS-iSYS 25OHD^S assay when the concentrations presented in the following table are below the stated threshold.

Potentially Interfering Agent	Threshold Concentration
Triglycerides	500 mg/dl
Bilirubin	5 mg/dl
Haemoglobin	40 mg/dl
Biotin	300 nmol/l
HAMA	500 ng/ml
Rheumatoid Factor	1500 IU/ml
Red Blood Cells	0.2 %
Vitamin DBP	2000 ng/ml

Expected Values

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

In the case of 25OHD, 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[6-10].

From a review of the available literature[11-24], the recommendations for 25OHD levels are shown below:

Vitamin D Status	Range (ng/ml)	Range (nmol/l)
Deficient	<20	<50
Insufficient	20 - <30	50 - <75
Sufficient	30 - 100	75 - 250

Performance Data

Representative performance data are shown. Results obtained at individual laboratories may vary.

Measurement Range (Reportable Range)

The IDS-iSYS 25OHD^S Assay reportable range is 7 - 125 ng/ml (18 - 313 nmol/l). Any value that reads below 7 ng/ml (18 nmol/l) should be reported as "< 7 ng/ml (<18 nmol/l)".

Sensitivity

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

Sensitivity	25OHD levels
LoB	0.6 ng/ml (1.5 nmol/l)
LoD	2.4 ng/ml (6.0 nmol/l)
LoQ	7.0 ng/ml (17.5 nmol/l)

Precision

Precision was evaluated in accordance with a modified protocol based on CLSI EP-5A2, "Evaluation of Precision Performance of Quantitative Measurement Methods". 6 serum samples were assayed using 3 lots of reagents in duplicate, twice per day for 20 days (n = 80 replicates per sample) on 3 systems.

Sample	Mean (ng/ml)	Within-run		Total	
		SD	CV%	SD	CV%
1	12.0	0.7	6.2%	1.4	11.6%
2	25.3	1.3	5.3%	2.4	9.3%
3	36.2	2.0	5.5%	3.3	9.2%
4	52.1	2.5	4.8%	4.4	8.4%
5	74.2	3.2	4.3%	5.1	6.9%
6	116.5	5.4	4.6%	7.3	6.3%

Sample	Mean (nmol/l)	Within-run		Total	
		SD	CV%	SD	CV%
1	30.0	1.8	6.2%	3.5	11.6%
2	63.3	3.3	5.3%	6.0	9.3%
3	90.5	5.0	5.5%	8.3	9.2%
4	130.3	6.3	4.8%	11.0	8.4%
5	185.5	8.0	4.3%	12.8	6.9%
6	291.3	13.5	4.6%	18.3	6.3%

Linearity

Linearity was evaluated based on CLSI EP-6A, "Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach". Samples containing varying concentrations of 25-hydroxyvitamin D were assayed in duplicate. The resulting mean concentrations were compared to predicted concentrations. Samples were prepared by diluting a high patient sample with a low patient sample prior to assay. The linear regression of the Observed concentrations versus the expected concentrations is:

Observed = 1.03 x (Expected) + 0.7 ng/ml

Observed = 1.03 x (Expected) + 1.8 nmol/l

Regression coefficient R^2: 0.99

Traceability

The IDS iSYS 25OHDS assay is aligned 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 [25,26].

89 samples in the range of 7.3 to 145.1 ng/ml (18.3 to 362.8 nmol/l), by LC-MS/MS, were used to further assess the IDS-iSYS 25OHD^S traceability against an LC-MS/MS assay which is in turn standardised against the NIST SRM 2972 and ID-LC-MS/MS RMP through VDSP samples. The relationship between the IDS-iSYS (y) and the LC-MS/MS (x) is described using Passing-Bablok regression:

IDS-iSYS = 0.98 x (LC-MS/MS) + 1.4 ng/ml
IDS-iSYS = 0.98 x (LC-MS/MS) + 3.5 nmol/l
95 % CI of the slope: 0.89 to 1.08
95 % CI of the intercept: -1.1 to 4.4 ng/ml
-2.8 to 11.0 nmol/l
Pearson correlation coefficient, r: 0.93

Method Comparison

The IDS-iSYS 25OHD^S assay was compared against a recognised automated chemiluminescent immunoassay (CLIA) for the quantitative determination of 25OHD, following CLSI EP-9A2, “Method Comparison and Bias Estimation Using Patient Samples”. A total of 333 samples, selected to represent a wide range of 25OHD concentrations [7.0 - 102.7 ng/ml (17.5 - 256.8 nmol/l)], by CLIA, was assayed by each method. Passing-Bablok regression analysis was performed on the comparative data:

IDS-iSYS = 0.98 x (CLIA) + 0.6 ng/ml
IDS-iSYS = 0.98 x (CLIA) + 1.5 nmol/l
95 % CI of the slope: 0.94 to 1.03
95 % CI of the intercept: -0.9 to 1.8 ng/ml
-2.3 to 4.5 nmol/l
Pearson correlation coefficient, r: 0.95

Specificity

Analyte	Cross-Reactivity (%)
25-hydroxyvitamin D_3	102%
Cholecalciferol (D_3)	0%
Ergocalciferol (D_2)	1%
3-epi-25(OH)D_3	1%

Specificity for 25-hydroxyvitamin D_2 was determined from analysis of samples from patients supplemented with ergocalciferol (vitamin D_2). A total of 25 samples, containing a wide range of 25OHD_2 concentrations [2.0 – 120.4 ng/ml (5.0 – 301.0 nmol/l) by LC-MS/MS] was assayed by each method. Passing-Bablok regression analysis was performed on the comparative data:

IDS-iSYS = 0.95 x (LC-MS/MS) - 0.1 ng/ml
IDS-iSYS = 0.95 x (LC-MS/MS) - 0.3 nmol/l
Pearson correlation coefficient, r: 0.90

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