Intended Use

For In Vitro Diagnostic Use

The IDS-iSYS 1,25-Dihydroxy Vitamin D assay is intended for the determination of 1,25-dihydroxyvitamin D (1,25D) levels in human serum or plasma on the IDS-iSYS Multi-Discipline Automated System [System]. Results of the 1,25-Dihydroxy Vitamin D assay are used in the assessment of vitamin D sufficiency.

Summary and Explanation

There are two forms of vitamin D: vitamin D₃ and vitamin D₃. Vitamin D₃, cholecalciferol, is the naturally occurring vitamin D that is produced in the skin after the exposure of 7-dehydrocholesterol to solar ultraviolet radiation. Vitamin D₃ is manufactured through the ultraviolet irradiation of ergosterol from yeast. Both are used in vitamin D supplements. The vitamin D compound is biologically inactive but enters the circulation and is hydroxylated in the liver to 25-hydroxyvitamin D (25D), which is used to determine a patient’s vitamin D status.

In the kidney, 25D is further hydroxylated to produce the biologically active metabolite, 1,25D. 1,25D is one of the major regulators of calcium and phosphate metabolism, stimulating intestinal calcium absorption and increasing bone resorption. 1,25D also inhibits parathyroid hormone (PTH) production both by direct action on the parathyroid glands and indirectly by raising serum calcium levels. 1,25D production is itself stimulated by parathyroid hormone (PTH), thus providing an effective control loop.

In secondary hyperparathyroidism, a disease outside of the parathyroid glands causes the parathyroid glands to become enlarged and hyperactive. It is usually caused by kidney failure, a problem where the kidney is unable to cleanse the blood of phosphorus produced by the body and unable to make enough vitamin D, specifically 1,25D - the active form of vitamin D. The build-up of phosphorus leads to low levels of calcium in the blood, which stimulates the parathyroid glands to increase parathyroid hormone (PTH) production leading to enlargement of the glands. As the disease progresses, the parathyroid glands no longer respond normally to calcium and vitamin D. The rationale for direct activated vitamin D therapy in CKD is to slow the progression of secondary hyperparathyroidism. Clinical practice guidelines such as the ‘Kidney Disease Outcomes Quality Initiative (KDQI)’ and ‘Kidney Disease: Improving Global Outcomes (KDIGO)’ recommend activated vitamin D therapeutic regimens for CKD patients.

Method Description

The IDS-iSYS 1,25-Dihydroxy Vitamin D kit is a complete test system involving immunopurification of 1,25-dihydroxyvitamin D in human serum or plasma followed by quantitative determination of 1,25D on the IDS-iSYS System. 150 μL of delipidated sample is added to an Immunocapsule which contains a gel containing a monoclonal anti-1,25D antibody. Samples in the Immunocapsules are rotated for 90 minutes to allow the binding of 1,25D to the monoclonal antibody gel. The gel is washed to remove potential interfering substances and the 1,25D eluted with ethanol. Eluates are then evaporated under a gentle flow of nitrogen at 40°C and reconstituted with 200 μL of assay buffer.

The reconstituted immunopurified samples are placed into the IDS-iSYS sample rack prior to loading the rack onto the System. During the assay, 120 μL of the reconstituted immunopurified samples are incubated with biotinylated sheep anti-1,25D antibody. The 1,25D-Accidinium conjugate is then added to compete for antibody binding sites. Streptavidin coated magnetic particles are then added and, following a further incubation step, the particles are washed to remove unbound materials. The addition of Trigger Reagents initiates a chemiluminescent reaction. The resulting light signal is measured by the photomultiplier as Relative Light Units (RLU) and is inversely proportional to the amount of 1,25D present in the sample.

Warnings and Precautions

The IDS-iSYS 1,25-Dihydroxy Vitamin D kit 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 the Instructions for Use (IFU). Do not use kit components beyond the expiry date and do not mix immunoextraction reagents from different kit lots. IDS Limited will not be held responsible for any loss or damage (except as required by statute) however 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

Controls contain sodium azide (NaN₃) >0.1% (w/w) (<1%).

Classification under CLP:

Acute Tox. 4.

Aquatic Chronic 3.

Hazard Statements

EUH032: Contact with acids liberates very toxic gas.

H302: Harmful if swallowed.

H412: Harmful to aquatic life with long lasting effects.

Precautionary statements:

P264: Wash hands thoroughly after handling.

P270: Do not eat, drink or smoke when using this product.

P273: Avoid release to the environment.

P301+310: IF SWALLOWED: Immediately call a POISON CENTER or doctor.

P330: Rinse mouth.

P501: Dispose of contents/container to hazardous or special waste collection point.

Some reagents in this kit contain sodium azide 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.

Elution Reagent

Elution reagent contains ethanol.

Classification under CLP:

Acute Tox. 4

Aquatic Chronic 3

Flam. Liq. 2

Hazard statements:

EUH032: Contact with acids liberates very toxic gas.

H225: Highly flammable liquid and vapour.

H302: Harmful if swallowed.

H412: Harmful to aquatic life with long lasting effects.

Precautionary statements:

P243: Take precautionary measures against static discharge.

P264: Wash hands thoroughly after handling.
P270: Do not eat, drink or smoke when using this product.
P273: Avoid release to the environment.
P280: Wear protective gloves/protective clothing/eye protection/face protection.
P301+310: IF SWALLOWED: Immediately call a POISON CENTER or doctor.
P303+361+353: IF ON SKIN (or hair): Remove immediately all contaminated clothing. Rinse skin with water.
P330: Rinse mouth.
P360: Rinse mouth.
P403: Store in a well-ventilated place.

Handling Precautions
Apart from the lyophilised calibrators, the reagents provided in the kit are ready to use. Refer to the calibrator section of the procedure for reconstitution methodology.

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. Should this occur, store the cartridge in an upright position in the dark at 2 to 8°C until foaming has dissipated; it could take up to 4 days for the reagents to settle.

Sheel 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.

<table>
<thead>
<tr>
<th>Reagent shelf life</th>
<th>Cartridge</th>
<th>Calibrators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before opening at 2 - 8 °C</td>
<td>To the expiry date</td>
<td>N/A</td>
</tr>
<tr>
<td>Cartridge, After opening at 2 - 8 °C</td>
<td>56 Days (8 Weeks)</td>
<td>N/A</td>
</tr>
<tr>
<td>On board the IDS-iSYS</td>
<td>49 Days (7 Weeks)</td>
<td>3 Hours</td>
</tr>
<tr>
<td>Calibrators, After reconstitution at 2-8°C</td>
<td>N/A</td>
<td>6 Hours</td>
</tr>
<tr>
<td>Calibrators, After reconstitution at -20 °C or lower</td>
<td>N/A</td>
<td>28 Days</td>
</tr>
<tr>
<td>Calibrators, Freeze/thaw cycle(s)</td>
<td>N/A</td>
<td>2</td>
</tr>
</tbody>
</table>

* Continuous on board stability.

Sample Collection and Storage
The assay should be performed using serum (standard sampling tubes or tubes containing serum separating gel) or plasma (lithium heparin, sodium heparin or potassium EDTA) samples. Samples should be separated as soon as possible after collection.

### Samples Storage Stability

<table>
<thead>
<tr>
<th>Room temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 - 8°C</td>
<td>24 Hours</td>
</tr>
<tr>
<td>-20°C or lower</td>
<td>7 Days</td>
</tr>
<tr>
<td>Freeze/thaw cycles</td>
<td>2.5 Months</td>
</tr>
</tbody>
</table>

To reduce possible evaporation effects, reconstituted calibrators should be measured within 3 hours and reconstituted immunopurified samples within 90 minutes after being placed on the System.

For the IDS-iSYS 1,25D assay, the following tube types gave the following correlations:

- **Serum Separator Tubes:**
  \[ Y = 0.99 \times (\text{serum}) + 1.54 \frac{\text{pg/mL}}{}; \ R^2 = 0.99 \]
- **Dipotassium EDTA Plasma Tubes:**
  \[ Y = 1.01 \times (\text{serum}) + 0.24 \frac{\text{pg/mL}}{}; \ R^2 = 0.99 \]
- **Lithium Heparin Plasma Tubes:**
  \[ Y = 1.00 \times (\text{serum}) + 0.99 \frac{\text{pg/mL}}{}; \ R^2 = 0.99 \]
- **Sodium Heparin Plasma Tubes:**
  \[ Y = 1.00 \times (\text{serum}) - 0.72 \frac{\text{pg/mL}}{}; \ R^2 = 0.99 \]

**NOTE:**

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

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

iii. The specimens’ storage and stability information stated above are general recommendations for use in a variety of settings of laboratories. Each laboratory should follow the guidelines or requirements of local, state, and/or federal regulations or accrediting organizations to establish its own specimens handling and storage stability. For guidance on appropriate practices, please refer to the CLSI GP44-A4, Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests; Approved Guideline - Fourth Edition.

### Procedure

#### Materials Provided

**Immunoeextraction Kit**

- **SORB** Immunocapsules containing a monoclonal antibody to 1,25D linked to solid phase particles in suspension with a vitamin D binding protein inhibitor, 100 Immunocapsules.

- **REAG 1** Delipidation reagent, a solution of dextran sulphate and magnesium chloride, 1 bottle, 6 mL per bottle.

- **REAG 2** Elution reagent, ethanol, 2 bottles, 25 mL per bottle.

- **BUF** Assay buffer, a MOPS buffer containing bovine serum albumin with 0.01% sodium azide, 1 bottle, 22 mL per bottle.

**Reagent Cartridge**

- **MP** Magnetic particles coated with streptavidin in a phosphate buffer containing bovine serum albumin and sodium azide as a preservative (<0.1%), 1 cartridge vial, 2.6 mL per vial.

- **CONJ** Conjugate, 1,25D labelled with an acridinium ester derivative, in a phosphate buffer containing bovine serum albumin with sodium azide as preservative (<0.1%), 1 cartridge vial, 9.6 mL per vial.

- **Ab-BIOT** Antibody-Biotin, anti-1,25D polyclonal antibody labelled with biotin in a phosphate buffer containing sheep proteins with 0.01% sodium azide as preservative, 1 cartridge vial, 13 mL per vial.

- **BUF D** Wash buffer, 1% proprietary detergent in PBS azide, 1 cartridge vial, 35 mL per vial.

**Calibrators**

- **CAL A** Lyophilised MOPS buffer containing bovine serum albumin, 1,25D and sodium azide as preservative (<1.0%), 2 each of 2 concentration levels, 1.2 mL.
MINI CD
Contains the IFU for IDS-iSYS 1,25D reagents and CRY files.

MATERIALS REQUIRED BUT NOT PROVIDED
IDS-iSYS Multi-Discipline Automated System: IS-310400
IDS-iSYS 1,25D Dihydroxy Vitamin D Control Set: IS-2430
IDS-iSYS Diluent A: IS-10DA
IDS-iSYS Cyvettles: IS-CC100
IDS-iSYS System Liquid (Syst L): IS-CS100
IDS-iSYS Wash Solution (Wash S): IS-CW100
IDS-iSYS Trigger Set: IS-CT100
IDS-iSYS Cartridge Check System (CCS): IS-6010

DISPOSABLE 12 x 75 mm or 13 x 75 mm tubes
Disposable polypropylene 2 mL. 10.8 mm diameter, conical skirted base, screw cap micro tubes, and screw cap with O-ring [Sarstedt 72.664 and 65.716 or equivalent.]

PREPARATION

1. Allow reagents to reach room temperature before use.
2. Prepare the Extraction Controls according to the IDS-iSYS 1,25D Control Set. (IS-2430) Instructions for Use (IFU).
3. Label glass/plastic tubes, one for each sample/Extraction Control.

DO NOT DEPILEDICATE CALIBRATORS/ASSAY CONTROLS.

4. Pipette 500 µL of sample/Extraction Control to appropriately labelled tubes.
5. Add 50 µL of delipidation reagent to each tube. Vortex all tubes.
6. Centrifuge all tubes at 2000 x g for 15 minutes.

NOTE:
Do not disturb the pellet when handling delipidated samples. If the pellet becomes suspended or the sample is not clear, repeat the centrifugation.

ALTERNATIVE SAMPLE PREPARATION
When the available sample volume is less than 500 µL.
1. Label conical-bottom plastic tubes or micro tubes, one for each sample.
2. Add sample (e.g. 300 µL) to appropriately labelled tubes.
3. Add 0.1 x sample volume of delipidation reagent (e.g. 30 µL) to each tube. Vortex all tubes.
4. Centrifuge all tubes at 2000 x g for 15 minutes.

IMMUNOE XTR A CTION PROCEDURE
1. Label Immunocapsules for each sample/Extraction Control. Do not immunopurify calibrators/Assay Controls.

NOTE:
DO NOT USE an Immunocapsule if it shows signs of leakage or incorrect volume.
2. Vortex Immunocapsules. Allow solid phase to settle. Stand Immunocapsules upright in the cardboard inlay provided for 3-5 minutes.
3. Remove each Immunocapsule’s screw cap. Add 150 µL of delipidated sample/Extraction Control to each Immunocapsule. Replace caps securely.
4. Place Immunocapsules in the cardboard inlay and rotate end-over-end at 5-20 revolutions per minute for 90 minutes at room temperature (18-25°C).
5. Stand Immunocapsules upright for 3-5 minutes allowing the gel to settle.
   a. Tap to dislodge any gel adhering to the screw caps.
   b. Allow the gel to settle for a further 1-2 minutes.
6. Remove the screw cap; break off (do not twist off) the bottom stopper from each Immunocapsule.
   a. Place each Immunocapsule in a plastic/glass tube.
   b. Centrifuge at low speed (500-1000 x g) for approximately 1 minute.
7. Add 500 µL of distilled or deionised water to each Immunocapsule. Add carefully to avoid any solid phase splashing out of the Immunocapsule.
   a. Centrifuge at low speed (500-1000 x g) for approximately 1 minute.
   b. Repeat the above step for a further two (2) times for a total of 3 wash cycles.
8. Label 2 mL polypropylene conical skirted base, screw cap micro tubes; one for each Immunocapsule. Transfer Immunocapsules to the appropriate, labelled micro tubes.
9. Add 150 µL of elution reagent to all Immunocapsules. Allow reagents to soak for 1 to 2 minutes.
   a. Centrifuge at low speed (500-1000 x g) for approximately 1 minute to collect the eluate.
   b. Repeat the above step a further two times. The total elution volume collected is 450 µL for each sample/Extraction Control.
10. Discard the Immunocapsules. Place micro tubes into a heating block or water bath set to 40°C.
11. Evaporate the eluates under a gentle flow, approximately 2-4 psi, of nitrogen gas. Avoid splashing of the eluates. Evaporation should take approximately 45 minutes.

NOTE:
Eluants must be completely dry.

ALTERNATIVE EVAPORATION DEVICES
Use of other evaporation equipment such as a vacuum centrifuge evaporator is viable for Step 11. The laboratory should validate the procedure to ensure optimal settings for their equipment.
12. Add 200 µL of assay buffer to each tube. Vortex for at least 5 seconds to dissolve residues. The immunopurified samples/Extraction Controls are now ready for assay.
13. Load the immunopurified samples/Extraction Controls onto the IDS-iSYS System.

NOTE:
Cap the immunopurified samples/Extraction Controls if they are not loaded onto and measured by the IDS-iSYS System within 90 minutes to reduce the possibility of evaporation.

IMMUNOPURIFIED SAMPLES STORAGE AND STABILITY
The immunopurified samples must be loaded onto and measured by the IDS-i SYS System within 90 minutes after reconstitution. Otherwise, cap the immunopurified sample's micro tubes and store in an upright position. Ensure that the immunopurified samples are at room temperature before loading onto the System.

IS-2400PL V10 03 June 2015 English GB
IDS-iSYS 1,25-Dihydroxy Vitamin D

**Immunoassay Sample Storage**

<table>
<thead>
<tr>
<th>Storage</th>
<th>Before reconstitution</th>
<th>Reconstituted</th>
</tr>
</thead>
<tbody>
<tr>
<td>On board the IDS-iSYS *</td>
<td>N/A</td>
<td>90 Minutes</td>
</tr>
<tr>
<td>2 - 8 °C</td>
<td>4 Hours</td>
<td>2 Days</td>
</tr>
<tr>
<td>-20 °C or lower</td>
<td>1 Day</td>
<td>2 Days</td>
</tr>
<tr>
<td>Freeze/Thaw cycle(s)</td>
<td>N/A</td>
<td>2</td>
</tr>
</tbody>
</table>

* Continuous on board stability.

**Assay Procedure**

**IDS-iSYS Multi-Discipline Automated System Settings**

The IDS-iSYS System’s automatic validation of results feature should be disabled.

**Sample Identification (ID)**

To lessen the potential of measuring 1,25D in samples that have not been immunopurified, the laboratory might consider assigning a sample ID that is unique for 1,25D testing.

**Reagent Cartridge**

The reagents provided in the cartridge are ready to use. Before a new cartridge is loaded on board the System, mix the magnetic particles container by a brisk rotating motion. Ensure that there is no foam formation in the cartridge reagents. Should this occur, store the cartridge in an upright position in the dark at 2 to 8°C until foaming has dissipated; it could take up to 4 days for the reagents to settle.

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 IDS-iSYS User Manual).

Once the cartridge is on the reagent tray, the System automatically performs the mixing of magnetic particles to maintain homogeneity. The cartridge should be loaded on the reagent tray 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 1,25D calibrators are lyophilised. Reconstitute immediately before use. Add 1.2 mL of distilled or deionised water to one bottle of each level. Replace the stopper. Leave for 10 minutes to reconstitute. Vortex calibrators for 2-3 seconds; avoid the formation of foam. Pipette approximately 500 μL (120 μL per replicate) of calibrators into 2 mL polypropylene conical, skirted base micro tubes and place onto the instrument within 15 minutes of reconstitution. Proceed according to the instructions in the IDS-iSYS User Manual.

If calibrators are to be used more than once, they should be stored at -20°C or lower within 15 minutes of reconstitution in the original vial. When re-using frozen calibrator vials, thaw at room temperature and vortex for 2-3 seconds before use; avoid the formation of foam. Ensure that calibrators are at room temperature before pipetting approximately 500 μL of calibrators into 2 mL polypropylene conical, skirted based micro tubes and place onto the instrument. Thawed calibrators should be placed onto the System within 15 minutes of reaching room temperature.

**NOTE:**

i. **DISCARD** the material in the micro tubes after use.
ii. **DO NOT** return material to the calibrator vials.
iii. **DO NOT** immunopurify the calibrators.
iv. Store the unused calibrators in their original glass vial at -20°C or lower.
v. Vortex thawed calibrators for 2-3 seconds before use, avoiding the formation of foam.

**System Calibration**

Two 1,25D calibrators are required to perform the adjustment of the master curve. These calibrators are supplied with the kit; calibrators from another kit lot must not be used. All Assay Control levels of the IDS-iSYS 1,25D Control Set (IS-2430) 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 a 1,25D cartridge on the reagent tray and the availability of the cartridge master curve in the database. If the data for the calibrators lot are not available on board the System, load the data using the mini CD provided with the kit.

Start the immunoassay calibration on the IDS-iSYS Analyser according to the IDS-iSYS User Manual. The calibration is carried out in triplicate. One replicate may be removed to meet the calibration requirements. As stated above, please note that controls must also be run. Verify and approve the calibration according to the calibration status displayed in the calibration windows and discard the calibrator from the sample tray after use.

**Calibration**

Currently, there are no certified standard reference materials or an agreed reference measurement method for 1,25-dihydroxyvitamin D.

The IDS-iSYS 1,25D assay has been standardised against in-house reference standards.

**Calibration Frequency**

A new calibration is required:

- Each time a new cartridge lot 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 21 days has expired.
- After System service.

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

**Quality Control**

The IDS-iSYS 1,25-Dihydroxy Vitamin D Control Set (IS-2430) is required for quality control.

All Assay Control levels should be measured prior to loading the immunopurified samples/Extraction Controls onto the System. This is carried out to ensure the integrity of both the System and the reagent cartridge, both when the test is in use and during every calibration.

To verify the validity of sample results, all levels of Extraction Controls should be immunopurified and measured at the same time as the patient samples. All levels of immunopurification extraction control should be measured in every run containing patient samples or according to local, state and/or federal regulations or accreditation requirements and your laboratory’s quality procedure. The immunopurified Extraction Controls MUST be identified in a manner similar to other control materials according to the instructions provided in the IDS-iSYS User Manual.

Refer to the IDS-iSYS 1,25D Control Set (IS-2430) Instructions for Use for preparation and handling procedures.

**Determination of Sample 1,25D levels**

Proceed according to the instructions of the IDS-iSYS User Manual for assaying samples.

**Calculation of Results**

The 1,25D concentration of each sample is calculated automatically. The display of each concentration (screen or printed) is produced as per the user settings.

To convert results to SI units: pmol/L = pg/mL x 2.4
The IDS-iSYS 1,25D Assay uses a 4-parameter logistic curve fit (4PL) to calculate the 1,25D concentrations.

**Validation of Samples Results**
If the immunopurified Extraction Control values do not fall within the defined ranges, the samples results are invalid. In this case, repeat the delipidation and immunopurification of the samples and Extraction Controls prior to the determination of 1,25D levels using the assay procedure.

**Measurement Range (Reportable Range)**
The reportable range of the assay is 7.5 – 210.0 pg/mL (18.0 – 504.0 pmol/L). Any value that reads below 7.5 pg/mL (18.0 pmol/L) should be reported as “< 7.5 pg/mL” (“< 18.0 pmol/L”). The highest reportable value, without dilution, is 210.0 pg/mL (504.0 pmol/L).

**Sample Dilution**
Use the IDS-iSYS Diluent A is used for diluting samples when the results exceed 210 pg/mL (504.0 pmol/L). Refer to the IDS-iSYS Diluent A (IS-10DA) Instructions for Use for preparation and usage procedures.

**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. Haemolysed samples should not be used with this assay.
4. The following substances do not interfere in the IDS-iSYS 1,25D assay when the concentrations presented in the following table are below the stated threshold.

<table>
<thead>
<tr>
<th>Potentially Interfering Agent</th>
<th>Threshold Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides</td>
<td>1000 mg/dL</td>
</tr>
<tr>
<td>Haemoglobin</td>
<td>200 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>20 mg/dL</td>
</tr>
<tr>
<td>Albumin</td>
<td>9.1 g/dL</td>
</tr>
<tr>
<td>Red Blood Cells</td>
<td>0.4%</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>300 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>300 nM</td>
</tr>
<tr>
<td>HAMA</td>
<td>1000 mg/mL</td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>2079 IU/mL</td>
</tr>
</tbody>
</table>

**Expected Values**
1,25-dihydroxyvitamin D values of apparent healthy and vitamin D sufficient adult Caucasians from USA were determined using the IDS-iSYS 1,25D assay.
Each laboratory should determine ranges for their local population. The range below is provided for information only.
The 95% reference interval for the following group was calculated by a non-parametric method following the NCCLS guideline C28-A2, “How to Define and Determine Reference Intervals in the Clinical Laboratory”.

Normal Adults:
- 26.1 – 95.0 pg/mL (n = 119)
- 62.6 – 228 pmol/L (n = 119)

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

**Sensitivity**
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” using zero-matrix and 12 low level samples. The LoQ was defined as the concentration that achieved a CV of 20%.

<table>
<thead>
<tr>
<th></th>
<th>LoB</th>
<th>LoD</th>
<th>LoQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>pg/mL</td>
<td>2.8</td>
<td>5.5</td>
<td>7.5</td>
</tr>
<tr>
<td>pmol/L</td>
<td>6.7</td>
<td>13.2</td>
<td>18.0</td>
</tr>
</tbody>
</table>

**Precision**
Precision was evaluated in accordance with a modified protocol based on CLSI EP-5A2, “Evaluation of Precision Performance of Quantitative Measurement Methods”. Four serum samples and the kit controls (two Assay Controls and two Extraction Controls) were assayed using one lot of reagents in duplicate twice per day for 20 days on one instrument.

**Linearity**
Linearity was evaluated in adherence with CLSI EP-6A, “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach” procedure. Samples containing varying concentrations of 1,25D 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 extraction.

The linear regression of the observed concentrations versus the expected concentrations is:

\[
\text{Expected} = 1.01 \times \text{Observed} + 1.67; \ R^2 = 1.00.
\]

**Method Comparison**
The IDS-iSYS 1,25D assay was compared against the IDS 1,25-Dihydroxy Vitamin D (AA-54) radioimmunoassay for the quantitative determination of 1,25D, following CLSI EP-9A2, “Method Comparison and Bias Estimation Using Patient Samples”. A total of 121 samples, selected to represent a wide range of 1,25D concentrations [10.7 – 197.5 pg/mL (25.7 – 474.0 pmol/L)], was assayed by each method.
A Passing Bablok analysis was performed on the comparative data: 
IDS-iSYS = 1.00 x (IDS RIA) - 2.8. (95% CI of the slope and intercept were 0.93 to 1.07, and -6.3 to 0.4 respectively); correlation coefficient (r) = 0.95.

Specificity

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Cross-Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,25(OH)<em>{2}D</em>{3}</td>
<td>98%</td>
</tr>
<tr>
<td>1,25(OH)<em>{2}D</em>{2}</td>
<td>75%</td>
</tr>
<tr>
<td>1,24,25(OH)<em>{3}D</em>{3}</td>
<td>92%</td>
</tr>
<tr>
<td>25(OH)D_{3}</td>
<td>0.0015%</td>
</tr>
<tr>
<td>25(OH)D_{2}</td>
<td>0.0009%</td>
</tr>
<tr>
<td>Epi-25(OH)D_{3}</td>
<td>&lt; 0.0015%</td>
</tr>
<tr>
<td>24,25(OH)<em>{2}D</em>{3}</td>
<td>0.006%</td>
</tr>
<tr>
<td>24,25(OH)<em>{2}D</em>{2}</td>
<td>0.005%</td>
</tr>
<tr>
<td>Alfacalcidol</td>
<td>0.04%</td>
</tr>
</tbody>
</table>

Bibliography

### Delipidation Procedure

<table>
<thead>
<tr>
<th>Tube #</th>
<th>Description</th>
<th>Sample/Extraction Control</th>
<th>Delipidation Reagent</th>
<th>Centrifuge</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extraction Control 3</td>
<td>Pipette 500 μL</td>
<td>Add 50 μL</td>
<td>15 minutes at 2000 x g</td>
</tr>
<tr>
<td>2</td>
<td>Extraction Control 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Sample 1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>4</td>
<td>Sample 2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>Sample 3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sample 4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sample 5</td>
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</tr>
<tr>
<td>8</td>
<td>Etc.</td>
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</table>

### Immunoextraction Procedure

<table>
<thead>
<tr>
<th>Capsule #</th>
<th>Description</th>
<th>Immunoextraction Capsules</th>
<th>Sample/Extraction Control</th>
<th>DI water</th>
<th>Elution Reagent</th>
<th>Assay Buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Extraction Control 3</td>
<td>Vortex.</td>
<td>150 μL delipidated sample.</td>
<td>150 μL</td>
<td>500 μL</td>
<td>200 μL</td>
</tr>
<tr>
<td>2</td>
<td>Extraction Control 4</td>
<td>Allow gel to settle; 3-5 min.</td>
<td>Rotate end over end. 90 min. 5-20 rpm</td>
<td>Centrifuge 1 min. @ 500-1000 x g Transfer capsules to micro tubes</td>
<td>Soak 1-2 min. Centrifuge 1 min. @ 500-1000 x g Repeat 2 x.</td>
<td>Vortex 5 sec.</td>
</tr>
<tr>
<td>3</td>
<td>Sample 1</td>
<td></td>
<td>Allow gel to settle. Stand upright, 3-5 min.</td>
<td>Centrifuge 1 min. @ 500-1000 x g Repeat 2 x.</td>
<td>Discard capsules. Evaporate eluates under N₂ @40°C.</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Sample 2</td>
<td></td>
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<tr>
<td>5</td>
<td>Sample 3</td>
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