For in vitro diagnostic use only.

25-Hydroxy Vitamin D (25-OH VD) Assay Reagent Kit(CMIA) Package Insert

INTENDED USE

The 25-hydroxy vitamin D(25-OH VD) Assay Reagent Kit(CMIA) is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of 25-hydroxy vitamin D (25-OH VD) in human serum or plasma.

PACKING SIZE

24 Device/Kit,30Device/Kit,48 Device/Kit,60 Device/Kit.

SUMMARY

Vitamin D is a group of fat-soluble secosteroids. In humans, vitamin D is unique both because it functions as a prohormone and because the body can synthesize it (as vitamin D3) when sun exposure is adequate (hence its nickname, the "sunshine vitamin"). Several forms (vitamers) of vitamin D exist. The two major forms are vitamin D2 or ergocalciferol, and vitamin D3 or cholecalciferol, vitamin D without a subscript refers to either D2 or D3 or both. These are known collectively as calciferol. Vitamin D2 was chemically characterized in 1932. In 1936, the chemical structure of vitamin D3 was established and resulted from the ultraviolet irradiation of 7-dehydrocholesterol. Vitamin D2 is a derivative of ergosterol, a membrane sterol named for the ergot fungus, which is produced by some organisms of phytoplankton, invertebrates, and fungi. The vitamin ergocalciferol (D2) is produced in these organisms from ergosterol in response to UV irradiation. D2 is not produced by land plants or vertebrates, because they lack the precursor ergosterol. The biological fate for producing 25(OH) D from vitamin D2 is expected to be the same as for D3. Low blood calcidiol (25-hydroxy-vitamin D) can result from avoiding the sun. Deficiency results in impaired bone mineralization, and leads to bone softening diseases including: We often use dose of 5000IU / month to 50000IU / week of vitamin D3 (or D2) to treat the Vitamin D deficiency, fortified foods and nutritional supplements may contain some form of VD, in order to ensure accurate assessment of the total content of vitamin D, vitamin D must be including all forms of vitamin D3, D2 and metabolites measured. Recent studies have confirmed that children's serum under 1 year old may exist 25-OH vitamin D in non-active 3 – epimer form, the test kit should be one of the important properties for the detection of only active ingredients, such as 25-OH vitamin D D3 and D2, but not inactive 3 - epimer of interference.

PRINCIPLE OF TEST

The 25-Hydroxy Vitamin D Reagent Kit is a two-step immunoassay for the quantitative measurement of 25-OH vitamin D in human serum or plasma using CMIA technology, with flexible assay protocols.

In the first step, sample and anti-25-OH VD coated paramagnetic microparticles are combined. 25-OH VD present in the sample binds to the anti-25-OH VD coated microparticles. After washing, ALP-labeled 25-OH VD antigen conjugate is added to create a reaction mixture in the second step. Following another wash cycle, substrates are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). A direct relationship exists between the amount of 25-OH VD in the sample and the RLUs detected by the system optics.

REAGENTS

The device is pre-dispensed with buffer needed for single use.

The device is constituted with Buffers described below is the main reagent

Object	Content
Micro-particles Buffer	Anti-25-OH VD (mouse, monoclonal) coated Micro-particles in TRIS buffer with protein (bovine) stabilizer. Minimum concentration: 0.1% solid. Dissociation agent. Preservative: ProClin-300.
Conjugate Buffer	25-OH VD antigen alkaline phosphatase (ALP) labeled conjugate in TRIS buffer with protein (bovine) stabilizer. Preservative: ProClin-300.
Wash buffer	TRIS buffer with surfactant. Preservative: ProClin-300.
Substrate buffer	AMPPD,Enhancer,surfactant,ProClin-300.

Reagent Handing

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

MATERIALS PROVIDED

·25-OH VD Test Device
·Product Insert
·Calibration Solution (optional)
·Control Solution (optional)

MATERIALS REQUIRED BUT NOT PROVIDED

·Analyzer

STORAGE AND STABILITY

▪Store at 2-8℃.
▪Do not freeze.
▪Store the reagent kit upright prior to use.
▪Expiration date: up to the stated expiration date.

Note: The 25-OH VD Reagent Kit must be stored at 2-8℃ in an upright position and must be used immediately after removal from 2-8℃ storage or the device was opened.

SPECIMEN COLLECTION AND STORAGE

Specimen Types

Validated specimen types to be used with this assay:

Specimen Types	Collection Tubes
Human serum	Serum
	Serum separator tubes
Human plasma	Sodium heparin
	Lithium heparin
	Potassium EDTA
	Sodium EDTA

Other anticoagulants have not been validated for use with this assay.

The instrument does not provide the capability to verily specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  heat-inactivated
  pooled
  grossly hemolyzed
  obvious microbial contamination
- For optimal results, serum and plasma specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens especial those from patients receiving anticoagulant or thrombolytic therapy may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for specimen collection tubes.
- Specimens must be mixed THOROUGHLY after thawing, by LOW speed vortex, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum
Serum/Plasma	2-8℃	6 days

- If testing will be delayed more than 12 hours, remove serum or plasma from the clot, serum separator or red blood cells.
- If testing will be delayed more than 6 days, specimens should be frozen at -10℃ or colder.
- Specimens stored frozen at -10℃ or colder for 3 months showed no performance difference.
- Avoid more than 3 freeze/thaw cycles.

Specimen Shipping

- Before shipping specimens,it is recommended that specimens be removed from the clot, red blood cells, or separator gel.
- When shipping specimens, package and label specimens in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances.
- Specimens may be shipped ambient,at 2-8℃ (wet ice),or frozen (dry ice). Do not exceed the storage time limitations listed above.

INSTRUMENT

The 25-OH VD Test Device is designed for use on the REALY Analyzer System.

TEST PROCEDURE

Assay Procedure

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer specific assay instructions. Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the digit sequence of numbers. Bring the cooled reagents to approx. 20℃ and place on the reagent disk of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents.

For this test device, the transfer volume of specimens, calibrators or controls into the sample hole is 60 µL. (No less than 60 µL.)

Reagent strips should be left at room temperature between 20 and 25 ℃ for more than 30 minutes before use and kept away from light.

In order to avoid the magnetic beads adsorbed on the side wall and top due to the upside down and side placement of the reagent strip during transportation, the reagent strip should be mixed by shaking and mixing before use. The reagent strip should be

mixed upside down for about 30 seconds, and then the reagent strip should be mixed upward for about 30 seconds.The reagent strip was then gently shaken so that the magnetic beads fell completely to the bottom of the strip.

Calibration

Every Test Device has a barcode label containing specific information for calibration of the particular reagent lot. The pre-defined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed before new lot of device be used. Renewed calibration is recommended as follows:

- After 90 days (when using the same reagent lot on the analyzer).
- As required: e.g. quality control findings outside the defined limits.

Note: Refer to Instruction of Analyzer for the procedure of calibration.

Quality Control

For quality control, please use Control of REALY or Control Universal.

In addition, other suitable control material can be used. Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Specimen Dilution Procedures

Specimens with a 25-OH VD concentration greater than 160 ng/mL will be flagged as "> 160.00 ng/mL" and may be diluted using Manual Dilution Procedure. Use the 1:10 or greater dilution is recommended. The operator must enter the dilution factor in the Patient or Control order screen. The system will use this dilution factor to automatically calculate the concentration of the sample before dilution.

EXPECTED VALUES

Currently there is no standard definition of the optimal vitamin D status.

Many specialists consider the commonly used population based reference values too low. Health based reference values are recommended to replace population based reference values.

Most experts agree that vitamin D deficiency should be defined as vitamin D (25-OH) of ≤ 20 ng/mL (≤ 50 nmol/L). Vitamin D insufficiency is recognized as 21-29 ng/mL .

Similarly, the US National Kidney Foundation considers levels < 30 ng/mL to be insufficient or deficient. The preferred level for vitamin D (25-OH) by many experts is now recommended to be ≥ 30 ng/mL (≥ 75 nmol/L).

nmol/L x 0.40 = ng/mL

ng/mL x 2.50 = nmol/L

INTERPRETATION OF RESULTS

As interpret the results, the patient's overall clinical situation, including symptoms, medical history and other related data, should be referred to.

LIMITATIONS

- Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful technique must be used to obtain valid results.
- If the 25-OH VD results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- For diagnostic purposes, results should be used in conjunction with other data; e.g., symptoms, results of other tests, clinical impressions, etc.
- Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Specimens containing HAMA may produce anomalous values when tested with assay kits such as REALY 25-OH VD that employ mouse monoclonal antibodies.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous results may be observed. Additional information may be required for diagnosis.
- Although the REALY 25-OH VD assay is specifically designed to minimize the effects of HAMA and heterophilic antibodies, assay results that are not consistent with other clinical observations may require additional information for diagnosis.

PERFORMANCE CHARACTERISTICS

Linearity

Linearity of the 25-Hydroxy Vitamin D Reagent Kit was determined by use 25-OH VD calibrator to prepare the 6 different specimens, measuring all these specimens follow the test instruction and then do linear fitting, the results show that the linear correlation coefficient(r) was better than 0.9900.

Precision/Reproducibility

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum. Repeatedly measured 20 times, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean (ng/mL)	SD (ng/mL)	CV
Level 1	7.6	0.56	7.37%
Level 2	29.5	2.12	7.19%
Level 3	80.76	5.56	6.88%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 30 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean (ng/mL)	SD (ng/mL)	CV
Level 1	8.1	0.41	5.39%
Level 2	31.2	1.89	6.41%
Level 3	79.95	6.50	8.13%

Analytical Sensitivity

The sensitivity is defined as the concentration of 25-OH VD equivalent to the mean RLU of 20 replicates of the zero standard minus two standard deviations corresponding to the concentration from the standard curve. The analytical sensitivity is typically less than 3.5ng/mL.

Specificity

The specificity of the 25-OH VD assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.

Compound	Concentration	Cross-reactivity
C3-epimer of 25-OH Vitamin D3	50 ng/mL	2.5%

Interfering Substances

The following compounds in both low-level specimen and high-level specimen with show no cross-reactivity when tested with the 25-Hydroxy Vitamin D Reagent Kit at a concentration show below:

Compound	Concentration
Bilirubin	66 mg/dL
Hemoglobin	2 g/L
Triglycerides	400mg/dL

Method Comparison

A comparison of the 25-Hydroxy Vitamin D Reagent Kit (y) with a commercially available 25-OH VD test (x) using clinical samples gave the following correlations (ng/mL):

Linear regression

y = 1.0288x - 0.176

r = 0.9869

Number of samples measured: 121

The sample concentrations were between about 5.65 and 126.38 ng/mL.

WARNINGS AND PRECAUTIONS

- For *In Vitro* Diagnostic Use.
- Do not use expired or clearly damaged kits.
- Operating according to the steps described, can make the risk of daily handling patients` samples and blood products into a minimum, however, no matter what the source of the products, handling mode or the previous proof, these potentially infectious substances were used shall be in accordance with the unified considerations and Good Laboratory Practice (GLP).
- Proper disinfectant should be used to eliminate pollution.
- Follow local rules and regulations to keep and dispose of these items and containers for these items.
- The ProClin-300 is a potential skin sensitizer. Avoid dumping or splashing this reagent on your skin and clothing. In case of contact with this reagent, wash thoroughly with soap and water.
- Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).
- Any modification of the procedure is likely to alter the results.
- Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

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SYMBOLS

Symbol	Meaning	Symbol	Meaning
IVD	In vitro diagnostic medical device		Storage temperature limit
	Manufacturer	EC REP	Authorized representative in the European Community /European Union
	Date of Manufacture		Use-by date
	Do not re-use		Consult instructions for use or consult electronic instructions for use
LOT	Batch code		Do not use if package is damaged and consult instructions for use
REF	Catalogue number		Contains sufficient for <n> tests

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