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Immunoassay

REF CMS0401 / CMS0402 / CMS0403 / CMS0404 / CMS0405

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

25-OH Vitamin D CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative determination of 25-OH Vitamin D in human serum or plasma (EDTA or heparin).

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	contains sufficient for <n> tests
IVD	*in vitro* diagnostic medical device		temperature limitation
REF	catalogue number		consult instructions for use
EC REP	authorized representative in the European Community		

EC REP

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Introduction

Vitamin D is a group of fat-soluble steroid hormone precursor responsible for increasing intestinal absorption of calcium,[1] magnesium, and phosphate, and multiple other biological effects, which is mainly produced in the skin by exposure to sunlight. Vitamin D from the skin synthesis is biologically inactive; hydroxylation in the liver and kidney is required for activation.[2]

In humans, the most important compounds in this group are vitamin D_3 and vitamin D_2, both of them can be ingested from the diet and from supplements. Only a few foods contain vitamin D. The major natural source of the vitamin is synthesis of vitamin D_3 in the skin from cholesterol through a chemical reaction that is dependent on sun exposure.[3, 4]

Vitamin D is transported to the liver in combination with a binding protein in the bloodstream, converted to 25-hydroxyvitamin D in the liver, and then converted to 1,25-hydroxyvitamin D in the kidney. This is an active ingredient in which vitamin D functions. The 1,25 hydroxy vitamin D content in the circulation is extremely low, with a half-life of only 4 h. This primary circulating form of vitamin D (25-OH) is biologically inactive with levels approximately1000-fold greater than the circulating1,25-dihydroxy vitamin D. The half-life of circulating vitamin D (25-OH) is 3 weeks.[5]

Vitamin D can regulate the balance of calcium and phosphorus metabolism and bone formation, and is closely related to cardiovascular disease, autoimmune diseases, diabetes and hypertension etc.[6]

Measurement Principle

This assay is based upon the one-step competitive method. The sample, 25-OH Vitamin D antibody coated microparticles and enzyme labeled 25-OH Vitamin D derivant are combined. During the incubation, enzyme labeled 25-OH Vitamin D derivant and 25-OH Vitamin D antigen present in the sample compete for binding to the 25-OH Vitamin D antibody coated on microparticles. After washing, a complex is generated between the solid phase and enzyme-linked derivant by immunological reactions. The complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is inversely proportional to the amount of 25-OH Vitamin D in the samples.

Materials provided

1. Calibrators

6 vials lyophilized calibrator A through F. The matrix is containing PBS (phosphate buffered saline) containing BSA (bovine serum albumin). Contains a selection of preservatives.

Reconstitute each lyophilized calibrator with 1.0 mL of distilled water. Allow the reconstituted material to stand for at least 10 minutes. Then invert the calibrator to mix it completely.

2. Reagent pack

Reagent pack provided ready to use.

Σ	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2
Dissociation Solution	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Contains of 25-OH Vitamin D antibody coated microparticles in PBS buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Contains of horseradish-peroxidase labeled 25-OH Vitamin D derivant in Tris-NaCl buffer containing Casein. Contains a selection of preservatives.

- Dissociation Solution

Contain DMSO buffer.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample tubes(s) or cup(s) for sample containing
4. Chemiluminescent Substrate
5. System Wash for washing the pipetting needles
6. Wash Buffer used in the washing procedure
7. Distilled or deionized water

Metrological Traceability of Calibrators

This method has been standardized against LC-MS/MS, which in turn has been standardized to the NIST standard.[7]

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this instruction for use.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Some reagents contain ProClin 300®may cause sensitization by skin contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are re-suspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.
16. Do not use reagents beyond the labeled expiry date.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 7 days.

Sample

1. Collect serum samples in accordance with correct medical practices.
2. Samples collected in tubes containing EDTA or heparin have no notable interference to this assay.
3. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
4. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃up to7 days. Or freeze the samples that need to be stored or transported for more than 7 days at -20℃. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
12. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials

- Verify adequate volume of consumable materials is present prior to running the test.
- Refer to the Assay Analyzer's operation manual.

2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cups or tubes on the sample rack, 50 μL of samples for each test. But consider the sample container and 150 μL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
  - Moves the sample to the set point
  - Loads a reaction vessel into the process path
  - Aspirates and transfers sample into the reaction vessel
  - Adds Microparticles Solution, Enzyme Conjugate and Dissociation Solution to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of 25-OH Vitamin D in the sample
  - Discards the used reaction vessel
  - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - Controls are out of range after repeated measurements
  - A reagent kit and substrate solution with new batch code is used
  - Beyond the expiration date of a calibration curve
  - Important parts of the analyzer are replaced or repaired
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a 25-OH Vitamin D value exceeding 150 ng/mL may be diluted manually. Human serum with a low analyte concentration is used to dilute the samples. After dilution, multiply the result by the dilution factor.

Measurement Results

The sample test results are determined automatically by the system software . The amount of 25-OH Vitamin D in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing sample results.

The default unit for this assay is ng/mL.

Conversion formula: ng/mL× 2.5= nmol/L

Control Procedure

Controls for the various concentration ranges should be run individually 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.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Performance of this test has not been established with neonatal samples.
5. This test measures concentrations within the range of 4.5-150 ng/mL. If 25-OH Vitamin D concentrations above the measuring range to be expected, it is recommended to dilute samples with human serum with a low analyte concentration. The recommended dilution is 1:3 of this test, allowing samples to be up approximately to 600ng/mL.
6. As International Endocrine Society recommended, the serum 25-OH Vitamin D levels are as follows[8]:

Vitamin D Status	25-OH Vitamin D Concentration Range (ng/ml)
Deficient	<20
Insufficient	20 to <30
Sufficient	30-100
Upper Safety Limit	>100

Biological Reference Interval

A normal range of 7.86 ng/mL to 45.5 ng/mL(central 95% interval) was obtained by testing serum samples from 457 individuals defined as normal by clinician. As the 25-OH Vitamin D in the human body is affected by seasonal changes, ultraviolet radiation, human species, diet and other factors, it is recommended that each laboratory establish a normal reference interval according to their actual conditions and contact with the population.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (ng/mL)	Within-run	Total
			%CV	%CV
1	80	16.17	4.56	7.50
2	80	41.06	3.21	5.22
3	80	65.56	3.36	4.71

*Representative data; results in individual laboratories may vary from these data.

2. Analytical Sensitivity

Limit of Blank: 2.0 ng/mL.

Limit of Detection: 3.5 ng/mL.

Limit of Quantitation: 4.5 ng/mL with a coefficient of variation of 20%.

3. Analytical Specificity

Cross Reaction: The following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration (ng/mL)
vitamin D_2	1000
vitamin D_3	1000
Rocalirol	5
Paricalcitol	2

Interference: No interference with 125 mg/dL of haemoglobin, 80 mg/dL of Bilirubin, 1000 mg/dL of Triglyceride.

4. Measurement Accuracy by Correlation

A comparison of the 25-OH Vitamin D CLIA Microparticles with a commercially available assay using clinical samples gave the following correlations (ng/mL):

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	383	-0.5281	1.016	0.9406

Literature References

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