25-OH-Vitamin D (25-Hydroxyvitamin D)

Diagnostic reagent for determination of Vitamin D concentration.
Liquid. Dual Reagents (Ratio: R1/R2: 4/1). Store at +2/+8°C. For in Vitro Diagnostic Use (IVD). **Do not freeze**.

Ref No	Package
MH-500	100 mL

Changes made in the instructions for use are marked as grey.

INTENDED USE

This test is for in vitro diagnostic use in the quantitative determination of Vitamin D concentration in human serum and plasma by automated methods of biochemistry analyzers. It aids in the diagnosis and monitoring of Vitamin D deficiency-related conditions, such as rickets in children, osteomalacia in adults, and hypocalcemia. The test is indicated for individuals with malabsorption disorders or those using anticonvulsant medications, which may impair Vitamin D absorption or metabolism. Vitamin D levels are also known to serve as a supportive indicator in the clinical evaluation of conditions such as cardiovascular diseases, type 2 diabetes, autoimmune disorders, and certain cancers, particularly in individuals at risk of vitamin D deficiency. The Vitamin D test is also used for regular monitoring of calcium levels in individuals receiving 1-hydroxylated Vitamin D derivatives.

GENERAL INFORMATION

Vitamin D is crucial as a hormone for regulating calcium and phosphorus metabolism and maintaining bone health. Vitamin D comprises two structurally related compounds (both secosterols): ergocalciferol (D_2) and cholecalciferol (D_3). The compound initially called vitamin D_1 was found to be a mixture of ergocalciferol and lumisterol, and this term is now obsolete.[1]

Approximately 90% of the body's cholecalciferol is synthesized in the skin from 7-dehydrocholesterol through a UV-light-driven photochemical reaction. Vitamin D_3 lacks biological activity on its own but is transformed by liver and kidney enzymes into 1,25-dihydroxycholecalciferol, a hormone that controls intestinal calcium absorption and calcium levels in the kidneys and bones.[2]

Vitamin D_2 (ergocalciferol) is a commercially produced compound created by UV irradiation of yeast ergosterol. Vitamin D_2 is structurally comparable to D_3, with minor alterations in the side chain linked to the sterol D ring. Both exert identical biological effects, and D_2 is frequently incorporated into milk and butter as a nutritional supplement. Similar to steroid hormones, the metabolite of vitamin D, 1,25-dihydroxycholecalciferol, modulates gene expression, such as activating the production of an intestinal calcium-binding protein.[2]

Ergocalciferol is a semisynthetic vitamin form added to specific foods as a dietary supplement and is also found in certain fungi. Cholecalciferol is found in fatty fish, dairy products, and egg yolks. Ergocalciferol and cholecalciferol function similarly in the body;[1] thus, the term vitamin D in this chapter encompasses both compounds.

In plasma, vitamin D is transported bound to a specific carrier protein. It is further hydroxylated in the kidneys to produce 1,25-dihydroxyvitamin D (calcitriol), a potent hormone that regulates calcium levels. The 1-hydroxylation process is promoted by parathyroid hormone and low phosphate levels (hypophosphatemia) and suppressed by its product.[1]

Calcitriol promotes the production of calbindins, proteins that facilitate calcium absorption in the intestine, and enhances phosphate absorption in the gut. In the liver and kidneys, it induces the creation of inactive forms of 25-hydroxyvitamin D. Its role in promoting bone mineralization is facilitated by regulating extracellular calcium levels. While it has been shown to induce osteolysis in vitro, the physiological significance of this finding remains unclear.[1] The vitamin D receptor, which binds calcitriol and facilitates its effects, is found in numerous tissues, and vitamin D seems to contribute to cellular differentiation, especially in the immune system.[1]

At higher latitudes, the limited ultraviolet light reaching the earth's surface is inadequate for substantial vitamin D synthesis. Even in areas with sufficient ultraviolet light, various factors may lead to insufficient endogenous production, and the advised dietary intake is 600 IU/day (15 mg/day).[1,3]

The primary diseases caused by vitamin D deficiency are rickets in children and osteomalacia in adults. In both conditions, insufficient bone mineralization results in deformities (particularly in children) and a higher fracture risk, often accompanied by myopathy. Hypocalcemia may occur but is frequently mitigated by elevated parathyroid hormone production (secondary hyperparathyroidism). Vitamin D deficiency is especially common in very young individuals (notably premature infants, particularly if their mothers were deficient, as vitamin D crosses the placenta only in the final trimester), older adults, individuals with darker skin, and those with limited sun exposure (e.g., those living in institutions). Individuals with malabsorption disorders and those using anticonvulsant medications, which can disrupt vitamin D absorption or metabolism, are also at risk.[1]

Substantial observational data suggest that vitamin D deficiency may be linked to various conditions, such as cardiovascular disease, type 2 diabetes, autoimmune disorders, and certain cancers,[4,5] but a causal connection

has not been proven, and there is little evidence supporting supplementation benefits for these conditions in at-risk individuals,[6,7] except in the case of falls in elderly individuals.[7]

Rickets may also result from impaired synthesis or function of calcitriol. Impaired synthesis is commonly observed in chronic kidney disease patients, but there are also two forms of vitamin D-resistant rickets caused by an inherited 1-hydroxylase enzyme deficiency. These should not be mistaken for the group of disorders known as vitamin D-dependent rickets, which involve an inherited renal tubular phosphate loss.[1]

Overconsumption of vitamin D, possible only through supplement misuse or use of 1-hydroxylated vitamin D derivatives, may lead to bone mineral loss and hypercalcemia. New evidence associates potential adverse effects with elevated levels, especially above 150 nmol/L.[8] Individuals receiving 1-hydroxylated vitamin D derivatives should undergo regular calcium level monitoring.[1]

Vitamin D levels are evaluated by measuring the 25-hydroxylated metabolites of D_2 and D_3, either combined using most immunoassays or individually via liquid chromatography-mass spectrometry. Results from different immunoassays can vary significantly,[9] and liquid chromatography-mass spectrometry is the preferred method.[1]

Since many seemingly healthy individuals may have subclinical vitamin D deficiency, it is preferable to use reference intervals based on the lowest risk of adverse levels (low or high) rather than general population values.[1]

TEST PRINCIPLE

End-point Method

The 25-hydroxy vitamin D in the sample binds to latex particles coated with anti-25-hydroxy vitamin D antibodies in the reagent, forming an insoluble immune complex. The turbidity of the reaction mixture increases proportionally with the 25-hydroxy vitamin D concentration, leading to an elevated absorbance. By comparing this absorbance to a standard, the 25-hydroxy vitamin D concentration in the sample can be determined.

REAGENT COMPONENTS

R1:

Sodium citrate	: 100mmol/L
NaCl	: 0.15mol/L
Preservative	: 0.03%

R2:

Sodium citrate	: 100mmol/L
BSA	: 1%
Tween-20	: 1%
Latex particles coated with anti-25-OH VD antibody	: 1%

REAGENT PREPARATION

Reagent is ready for use.

REAGENT STABILITY AND STORAGE

Reagents are stable at +2/+8˚C till the expiration date stated on the label which is only for closed vials.

Once opened vials are stable for 30 days at +2/+8°C in optimum conditions. On board stability is strongly related to auto analyzers' cooling specification and carry-over values.

Reagent stability and storage data have been verified by using Clinical and Laboratory Standards Institute (CLSI) EP25-A protocol.[10]

SAMPLE REQUIREMENTS

Serum or plasma (EDTA, heparin, anticoagulation) can be used and are collected according to the standard procedures. Multiple sample freezing and thawing should be avoided. The sample should be homogenized before testing.

Vitamin D activity stability in serum and plasma:

2 days at +2/+8ºC
6 months at -20ºC

Unit Conversion:

$$1\ \text{ng/mL} = \frac{1\ \text{nmol/L}}{2{,}5}$$

CALIBRATION AND QUALITY CONTROL

Calibration: The assay requires the use of a 25-OH-Vitamin D Calibrator Set. We recommend:

25-OH-Vitamin D Calibrator Set
Ref.No: VT-056

Calibration stability depends on the application characteristics and cooling capacity of the autoanalyzer used. Calibration stability is 21 days.

Control: Commercially available control material with established values determined by this method can be used. We recommend:

25-OH-Vitamin D Control Set
Ref.No: VT-057

At least two level controls must be run once in every 24 hours. Each laboratory should determine its own quality control scheme and procedures. If quality control results are not within acceptable limits, calibration is required.

REFERENCE INTERVALS / MEDICAL DECISION LEVELS

The use of health-based reference intervals is recommended; however, there is currently no universally accepted definition of optimal vitamin D status. Many experts argue that the commonly applied population-based reference values are insufficient. Therefore, it is recommended that health-based reference values be used instead of population-based values.[11]

The majority of experts agree that vitamin D deficiency should be defined as a 25-hydroxy vitamin D level of ≤ 20 ng/mL (≤ 50 nmol/L).[23] Vitamin D insufficiency has been defined as 21–29 ng/mL.[23] Similarly, the National Kidney Foundation in the United States classifies levels <30 ng/mL as insufficient or deficient.[24]

At present, many experts recommend that the preferred level for 25-hydroxy vitamin D is ≥ 30 ng/mL (≥ 75 nmol/L).[23,25-27] In apparently healthy individuals, 25-hydroxy vitamin D levels may vary depending on factors such as age, sex, season, geographical latitude, and ethnicity.[23,25]

Each laboratory should assess whether existing values are transferable to its patient population and, if necessary, establish its own reference intervals.

Population-based reference intervals should not be used as clinical thresholds for initiating or discontinuing vitamin D supplementation. Guidelines from the recent literature should be consulted for recommendations regarding supplementation.[23,25]

A reference interval study was conducted using samples obtained from apparently healthy donors in the United States. Samples were collected during both summer and winter from centers located in the southern, central, and northern regions of the country. Among the participants, male and female donors were approximately equal in number, and about 30% of the donors had dark skin. The ages of the donors ranged from 21 to 83 years. The values presented in this study are intended solely for informational purposes and may differ from other published data.

			Season			
	Total (n = 400)		Summer (n = 197)		Winter (n = 203)	
Unit	ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
Mean	25.7	64.3	28.9	72.3	22.6	56.5
2.5 percentile	7.61	19.0	11.1	27.8	5.65	14.1
97.5 percentile	55.5	139	60.3	151	52.3	131

PERFORMANCE CHARACTERISTICS

Measuring Interval

The determined analytic measuring interval for Vitamin D is 8 – *130* ng/mL.

Detection Capability

Limit of Detection (LoD): 2 ng/mL

Limit of Quantitation (LoQ): 8 ng/mL

Note: LoQ values are based on Coefficient of Variation Percentage (CV) ≤ 20%.

LoD and LoQ values have been verified by using CLSI EP17-A2:2012 protocol.[12]

Linearity

This method shows measurement linearity in the activities up to *130* ng/mL.

Autoanaylzer's auto-dilution system can be used if the concentrations have higher values. See device manual for further information.

For manual dilution procedure, dilute the sample 10-fold using 0.90% isotonic. After the dilution, multiply the result of rerun sample by the dilution factor. Do not report the sample result after dilution if it is marked as lower than the linear lower limit. Rerun with a suitable dilution.

Linearity Studies data have been verified by using CLSI EP06-A:2003 protocol.[13]

Precision

Running system has been developed according to 20x2x2 "The Single Site" protocol. Repeatability and Within-Laboratory Precision/Within-Device values have been obtained according to the running results.

According to the protocol in use, 2 separate runs per day have been made for 20 days (no obligation for being consecutive days). This protocol has been applied to each low and high samples separately and 80 results have been obtained for each one. Statistically, the results have been obtained using 2-factor Nested-ANOVA model.[14]

Repeatability (Within Run) and Repeatability (Day to Day) SD (standard deviation) and CV% values of Vitamin D have been given in the table 1 and 2 respectively.

Table 1. Vitamin D Repeatability (Within Run) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
23,7 ng/mL	0,85	3,57	80
42,9 ng/mL	0,57	1,32	80

Note: This working system has been named "Within-Run Precision" in the previous CLSI - EP05-A2 manual.[15]

Table 2. Vitamin D Repeatability (Day to Day) results obtained from samples in two different concentrations

Mean Concentration	SD	CV%	n
33,65 ng/mL	0,69	2,04	80
104,27 ng/mL	3,90	3,74	80

Note: This working system has been named "Total Precision" in the previous CLSI - EP05-A2 manual.[15]

Method Comparison

As a result of the statistical evaluation of the method comparison data:

Passing-Bablock equation:[16]
y= 1,021x – 1,185
r=0,98

Interference

Endogenous interferant and analyte concentrations that have been used in the Vitamin D scanning tests has been determined according to "CLSI EP37-ED1:2018" and "CLSI EP07-ED3:2018" manuals.[17,18]

The total acceptable error rate, which is going to be used to detect whether the observed differential value obtained from Vitamin D interference scanning test is appropriate, is determined as ±25%.[19]

In Vitamin D test results, no significant interaction has been observed in the determined endogenous interferant and analyte concentrations or between interferants and analyte.

Bilirubin	: ≤ 20 mg/dL
Hemoglobin	: ≤ 2 g/L
Triglyceride	: ≤ 240 mg/dL
Ascorbic acid	: ≤ 20 mg/dL

It should be noted that endogenous interferants, as well as various medicines and metabolites, anticoagulants (e.g. Heparin, EDTA, citrate, oxalate) and preservatives (e.g. sodium floride, iodoacetate, hydrochloride acide) such as additives, materials that may contact with samples during collection and processing (serum separator devices, sample collection containers and contents, catheters, catheter wash solutions, skin disinfectants, hand cleaners and lotions, glass washing detergents, powder gloves), dietary substances known to affect some specific tests (caffeine, beta-carotene, poppy seeds, etc.), or some substances present in a sample that cause foreign proteins (heterophilic antibodies, etc.), autoimmune response (autoantibodies, etc.), or due to malignancy (for example, interference by paraproteins with phosphate testing and indirect ion selective electrode methods) may show some negative effects that will cause various attempts and some misjudgements.[18]

These performance characteristics have been obtained using an autoanalyzer. Results may vary slightly when using different equipment or manual procedures.

WARNINGS AND PRECAUTIONS

IVD: For in Vitro Diagnostic use only.
Do not use expired reagents.
Reagents with two different lot numbers should not be interchanged.
For professional use.
Follow Good Laboratory Practice (GLP) guidelines.

CAUTION: Human source samples are processed with this product. All human source samples must be treated as potentially infectious materials and must be handled in accordance with OSHA (Occupational Safety and Health Administration) standards.

Danger

EUH032	:Releases a very toxic gas if contacts with acid.
H317	:May cause allergic skin reaction.

Precaution

P280	:Use protective gloves / clothes / glasses / mask.
P264	:Wash your hands properly after using.
P272	:Contaminated work clothes should not be allowed to be used outside of the workplace.

Intervention

P302+P352	:Wash with plenty of water and soap if it contacts with skin.
P333+P313	:Seek medical help if it irritates your skin or develops rash.
P362+P364	:Remove contaminated clothes and wash properly before using.

Disposal

P501	:Dispose the vials and contents according to the local regulations.

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SYMBOLS

IVD	In Vitro Diagnostic Medical Device
LOT	Lot Number
R1	Reagent 1
R2	Reagent 2
GTIN	Global Trade Item Number
REF	Reference Number
GLP	Good Laboratory Practices
FOR USE WITH	Identifies Products to Be Used Together
PRODUCT OF TURKEY	Product of Turkey
	Manufacturer
	Expiration Date
	Temperature Limits
	Consult Instructions for Use
	Caution
	Number of Tests

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