

 CUSTOMISED SOLUTIONS FOR YOUR LABORATORY	Vitamin D (100 test) Instructions for use	
	R1: 1x 15,5 mL R2: 1x 2,8 mL	REF R000000005

Intended Use

The Vitamin D assay is intended for use in clinical laboratories for the quantitative determination of 25-OH vitamin D (vitamin D) in human serum and plasma, using automated chemistry analyzers. Measurement of vitamin D is used for the assessment of the vitamin D sufficiency.

For Professional in Vitro Diagnostic use only.

Clinical significance

Vitamin D is a steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. Vitamin D has two forms: Vitamin D2 and Vitamin D3. Vitamin D2 is obtained from dairy products whereas Vitamin D3 is produced in the skin after exposure to ultraviolet light. In the liver, Vitamin D is hydroxylated at its carbon 25 to form 25-OH Vitamin D. This metabolite is the predominant circulating form of Vitamin D and is considered to be an accurate indicator of the general Vitamin D status of an individual. Vitamin D deficiency has been linked to many diseases including osteoporosis, rickets, and osteomalacia. Both dietary supplements of Vitamin D that are currently available in the market (Vitamin D2 and Vitamin D3) are converted to 25-OH Vitamin D in the liver. The sum of the concentrations of 25-OH Vitamin D2 and 25-OH Vitamin D3, in serum or plasma, is referred to as "Total 25-OH Vitamin D". Accurate monitoring of total 25-OH Vitamin D level is critical in clinical setting.

Assay Principle

The Vitamin D assay is a direct particle-enhanced immunoturbidimetric assay. The assay's proprietary reagents are designed to dissociate vitamin D from vitamin D binding proteins, found in serum or plasma specimens, while particles coated with anti-Vitamin D antibodies bind to the dissociated vitamin D, thereby causing agglutination. This agglutination is detected as an absorbance change (700 nm), with the magnitude of the change being proportional to the quantity of total vitamin D in the sample. Specimen concentrations are determined by interpolation from a 5-point calibration curve prepared from calibrators of known concentrations.

Reagents Provided

	Volume	Format
Reagent 1	1 x 15.5 mL	Ready to Use
Reagent 2	1 x 2.8 mL	Ready to Use

Reagent 1:
Phosphate buffer solution (<100 mM), 0,1 % sodium azide
Reagent 2:
Suspension of latex particles (0,5%) coated with anti-vitamin D antibodies, ready to use

Reagents required but not supplied

1. Calibrators and Controls*

Key Reference	Description
R0000000006	Vitamin D calibrator
R0000000007	Vitamin D control

Materials required but not provided

An analyser capable of dispensing two reagents and measuring absorbance at around 700 nm with temperature control (37°C).

Reagent handling

The Vitamin D assay reagents are liquid stable, ready to use reagents. Mix by inverting at least 10 times before use.

Reagent stability and storage

The reagents are stable when stored at 2-8°C until the expiration date on the label. Do not mix reagents of different lots. **DO NOT FREEZE.**

Sample collection and handling

Serum, K2-EDTA plasma, K3-EDTA plasma or Li-heparin plasma samples can be used for the assay.

For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. The specimens may be refrigerated at 2-8°C for up to one week. For long term storage, they can be stored at -20°C or below. Avoid repeated freeze-thaw cycles (up to three cycles are acceptable). Do not use highly turbid or highly hemolyzed serum or plasma samples. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed well before analysis.

Precautions

- For *in vitro* diagnostic use only. Rx Only.
- As with any laboratory test, results should be interpreted considering all other test results and the status of the sample. Do not use the reagent, calibrator and control after the expiration date labeled on the outer box.
- Assay calibration frequency is depending on instrument used. Additionally the assay should be recalibrated and controls run with each new lot of reagents.
- Avoid ingestion and contact with skin and eyes. See safety data sheet.
- Specimens containing human sourced materials should be handled as if potentially infectious using safe laboratory procedures.
- Calibrators and controls contain human source material. Each donor unit of serum in the preparation of these materials were tested by approved methods and found negative Hepatitis B surface antigen (HBsAg), and Hepatitis C Virus antibody (HCV). Because no method can offer complete assurance as to the absence of infectious agents, this material and all samples should be handled as though capable of transmitting infectious disease and such biohazardous material should be disposed of according to relevant local or state regulations.
- Additional safety information concerning storage and handling of this product is provided within the MSDS for this product.

Warnings

The reagent, calibrators and controls contains < 0.1 % sodium azide, NaN₃, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal flush with a large volume of water to prevent sodium azide buildup.



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Assay procedure

This Vitamin D kit is supplied ready to use on the Hemo One Analyzer and the protocol preloaded within the system database.

Please contact I.S.E Srl or your local authorised I.S.E Srl distributor in case of issues or missing protocols.

Calibration

5 level of the Vitamin D calibrator cod. R0000000006 are provided separately and ready to use.

Quality control

We recommend that each laboratory use Vitamin D controls to validate the performance of the Vitamin D assay reagents. A set of normal and abnormal ranges of Vitamin D controls are available cod. R0000000007 Vitamin D control high and cod. R0000000008 Vitamin D control low. The range of acceptable control limits should be established by individual laboratories.

Results

Results are expressed in ng/mL. Note: Samples with values greater than 147.8 ng/mL should be reported as > 147.8 ng/mL. Samples with values less than 7.6 ng/mL should be reported as < 7.6 ng/mL.

Reference range

Following C28-A3 approved guideline third edition, reference range of the Vitamin D assay was determined by measuring the 25-OH vitamin D serum concentrations of a USA population of 145 apparently healthy adults, 21-67 years old, during three different geographical locations: 47 from Pennsylvania (Northern USA), 49 from Tennessee (Central USA) and 49 from Texas (Southern USA). All 145 individuals did not have kidney disease, G1 disease, liver disease, calcium – levels related disease, thyroid disease, parathyroid disease, seizures, chronic disease or bariatric surgery. The central 95 % of population was found to have a 25-OH vitamin D concentrations ranging between 7.2 and 41.6 ng/mL, with a mean concentration of 20.1 ng/mL.

Limitations

- The assay is designed for use with human serum and plasma samples only.
- As with any diagnostic test it is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays. Patients routinely exposed to animals, animal serum products or other immunogenic products that may elicit heterophilic antibody production against the assay's reagents can be prone to this interference and anomalous values may be obtained. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions in an adult population.

Performance characteristics

Sensitivity

The LOB, LOD and LOQ of the assay were determined following CLSI EP17-A2 guideline.

Limit of Blank LoB

Vitamin D – depleted serum was assayed with the Vitamin D assay in five independent (over 5 days) runs with 12 replicates per run. The LoB was calculated as the mean of the 57th and 58th highest values for the blanks. The LoB of the assay was 1.2 ng/mL.

Limit of Detection LoD

Five very low vitamin D serum samples were measured in four independent runs (over 4 days), with 3 replicates per run. The LoD was defined as $LoD = LoB + (1.645 * \text{Standard Deviation of Low samples})$. The LoD of the assay was 2.9 ng/mL.

Limit of Quantitation LoQ

Five low Vitamin D samples were measured in 40 replicates obtained from five independent runs (5 days). The LoQ was measured as the lowest concentration with a CV of 20 %. The claimed LoQ of the assay was 7.6 ng/mL.

Method comparison

The method comparison of the assay was evaluated following CLSI EP9-A3 guideline. A total of 171 serum samples were tested in comparison with a legally marketed 25-OH Vitamin D enzyme immunoassay. The results for 171 serum samples are shown in the below table:

Deming Regression Analysis	95 % Confidence Interval
Slope	1062 (1028 to 1095)
Intercept	-3.03 (-4.94 to -1.11)
Correlation coefficient	0.9785 (0.970 to 0.9841)
Predicate Range (ng/mL)	8.4 to 146.8

Precision

Precision was evaluated according to the CLSI EP5-A2 guideline. Controls and samples were measured daily over the span of 20 days. Using three lots of reagents and one chemistry analyser. 40 independent runs were performed on each specimen. Each run produced two measurements. 80 data points were obtained per specimen. Results are shown below:

25-OH Vitamin D ng/mL			Within-run		Between run		Total	
Specimen	N	Mean	SD	%CV	SD	%CV	SD	%CV
Control #1	80	21.7	0.9	3.9%	0.6	2.8%	1.3	6.2%
Control #2	80	42.5	1.0	2.4%	0.8	2.0%	1.7	3.9%
Control #1	80	11.1	0.9	8.3%	0.5	4.4%	1.8	16.6%
Control #2	80	18.2	0.9	4.9%	0.7	3.9%	1.6	8.7%
Control #3	80	22.1	0.8	3.8%	0.8	3.8%	1.2	5.6%
Control #4	80	42.8	0.9	2.0%	1.0	2.1%	1.3	3.1%
Control #5	80	59.5	1.0	1.7%	0.7	1.2%	1.6	2.7%
Control #6	80	80.2	1.3	1.6%	1.1	1.4%	2.0	2.5%
Control #7	80	99.5	1.8	1.8%	1.5	1.6%	2.7	2.8%
Control #8	80	117.6	2.2	1.9%	2.0	1.7%	3.7	3.2%
Control #9	80	139.2	2.7	1.9%	2.6	1.8%	4.1	2.9%



Linearity

Eleven levels of linearity were prepared by diluting a high serum sample with vitamin D – depleted serum. Linearity levels were prepared according to the CLSI EP6-A guideline. Measurements were done in triplicates. The assay was found to be linear between 7.6 and 147.8 ng/mL.

Interference

Interference studies were conducted according to the CLSI EP7-A2 guideline. The acceptance criterion was set at 10 % or less deviation between the spiked sample and the control. The assay's result were not significantly affected by the following endogenous substances:

Substance	Tolerance	Unit
Free bilirubin	40	mg/dL
Conjugated bilirubin	40	mg/dL
Hemoglobin	600	mg/dL
Total protein	12.0	g/dL
Triglycerides	1000	mg/dL
Rheumatoid factor (RF)	200	IU/mL

The assay's results were also not significantly affected by the following exogenous substances:

Substances	Tolerance	Unit
Acetaminophen	20.0	mg/dL
Acetyl salicylic acid	60.0	mg/dL
Ampiellin	5.3	mg/dL
Ascorbate	3.0	mg/dL
Biotin	100.0	mg/dL
Carbamazepine	3.0	mg/dL
Cefotaxime	180.0	mg/dL
Chloramphenicol	5.0	mg/dL
Creatinine	30.0	mg/dL
Digoxin	6.1	mg/dL
Ethanol	400.0	mg/dL
Ethosuximide	25.0	mg/dL
Furosemide	6.0	mg/dL
HAMA	350	ng/mL
Heparin	3.0	U/mL
Ibuprofen	50.0	mg/dL
Lidocaine	1.2	mg/dL
Lithium acetate	2.2	mg/dL
Noradrenalin	4.0	Ug/mL
Rifampicin	5.0	mg/dL
Theophylline	4.0	mg/dL
Urea	300.0	mg/dL
Uric acid	20.0	mg/dL
Valpoid acid	50.0	mg/dL
Vancomycin	10.0	mg/dL

Cross-reactivity results are summarized in the table below:

Compound	Concentration tested	Cross-reactivity *
25-OH Vitamin D3	100.0 ng/mL	100 %
25-OH Vitamin D2	100.0 ng/mL	106.9 %
Vitamin D3	100.0 ng/mL	-0.8%
Vitamin D2	100.0 ng/mL	-1.7 %
1.25 –(OH)2 Vitamin D3	580.0 pg/mL	0.2 %

1.25 –(OH)2 Vitamin D2	580.0 pg/mL	-0.5 %
24R,25-(OH)2 Vitamin D3	100.0 ng/mL	118.8%
3-epi-25-OH Vitamin D3	100.0 ng/mL	31.0 %
3-epi-25-OH Vitamin D2	100.0 ng/mL	36.5 %

*% Cross reactivity = (corrected assay value / concentrations spiked)*100

Paricalcitol (Zemlar®) did not significantly cross-react with the assay when present at 5 ng/mL.

Disposal of reagent

Disposal of reagents must be performed in accordance with the EC regulations regarding waste, or the local national or regional legislation.

The product is in conformity with D.L: 8 September 2000, no. 332 "Actuation of the directive 98/79/EC regarding in vitro medical diagnostic devices".

Symbols on labels and packaging

IVD	In vitro diagnostic medical device
REF	Catalog Number
LOT	Lot number
	Manufacturer
	Expiry date
	Temperature limitation
	Consult Instructions for use

References

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Revision history

Rev. B	01/11/2018	Layout modification
Rev. C	01/02/2021	Heading modification
Rev. D	05/09/2023	General revision of the document Added revision history

