Diagnostics Biochem Canada

25-Hydroxyvitamin D [25(OH)D] ELISA

REF CAN-VD-510

ASSAY PROCEDURE

Bring kit components to room temperature. Prepare working solutions.

Pipette 25 µL of each calibrator, control and specimen sample. Pipette 150 µL of the incubation buffer into each well.

Tap the plate gently by hand for 10 seconds. Incubate at room temperature, in a dark place, for 60 minutes (no shaking).

Wash the wells 3 times with 300 µL/well of diluted wash buffer.

Pipette 150 µL of the working conjugate solution.

Tap the plate gently by hand for 10 seconds. Incubate at room temperature, in a dark place, for 30 minutes (no shaking).

Wash the wells 3 times with 300 µL/well of diluted wash buffer.

Pipette 150 µL of TMB substrate into each well.

Tap the plate gently by hand for 10 seconds. Incubate at room temperature, in a dark place, for 10–15 minutes (no shaking).

Pipette 50 µL of stopping solution to each well. Tap the plate gently by hand for 10 seconds.

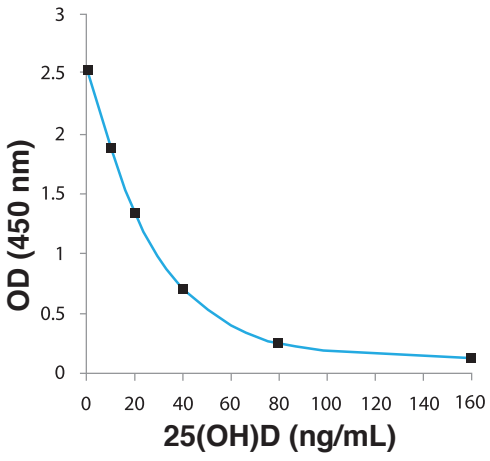
Read the plate on a microplate reader at 450 nm.

Vitamin D concentration in blood should be measured regularly to ensure that satisfactory physiological levels are maintained year round. Vitamin D is assimilated from food sources (both vitamin D2 and vitamin D3) or produced in the skin by sun exposure (vitamin D3). The body stores both vitamin D2 and vitamin D3 mainly in the form of 25-hydroxyvitamin D2 or 25-hydroxyvitamin D3 respectively. Therefore, the ***best approach*** to assess the physiological levels of vitamin D is to analyze the total concentration of ***both*** hydroxylated forms.

This kit measures the total concentration of both **25-hydroxyvitamin D2** and **25-hydroxyvitamin D3** (25(OH)D). The results are expressed in ng/mL.

PRINCIPLE OF THE TEST

DBC's immunoassay of 25(OH)D is a ***sequential competitive assay*** that uses ***two incubations***, with a total assay incubation time of less than two hours. During the first incubation, unlabelled 25(OH)D (present in the standards, controls, serum and plasma samples) is dissociated from binding proteins such as vitamin D binding protein and binds to the anti-25(OH)D antibody immobilized on the microplate wells. A washing step is performed next. During the next incubation, the complex of 25(OH)D-biotin conjugate and streptavidin-HRP conjugate competes with antibody-bound 25(OH)D for antibody binding sites. The washing and decanting procedures remove any unbound materials. The TMB substrate is added next which reacts with HRP to form a coloured product. The intensity of the colour is proportional to the amount of immobilized HRP. Stopping solution is added next which stops the colour development reaction. The optical density of each well is measured in a microplate reader. The absorbance values are inversely proportional to the concentration of 25(OH)D in the sample. A set of calibrators is used to plot a standard curve from which the concentrations of 25(OH)D in the samples and controls can be directly read.



Typical calibration curve

PERFORMANCE CHARACTERISTICS

SENSITIVITY

The limit of detection (LoD) was determined from the analysis of 64 samples of the blank and a low value sample and it was calculated as follows: LoD = μB + 1.645σB + 1.645σS, where σB and σS are the standard deviation of the blank and low value sample and μB is the mean value of the blank.

LoD = **5.5 ng/mL** of 25(OH)D.

SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity using the Abraham method with 25(OH)D3 cross reacting at 100%:

Antigen	% Cross-Reactivity
25 (OH)D3	100
25 (OH)D2	100
1,25 $(OH)_2$D3	8.3
3-epi-25 (OH)D3	66
Vitamin D3	< 1.0
Vitamin D2	< 1.0

INTERFERENCE

Interference testing was performed according to the CLSI guideline EP7-A2. Serum samples with varying levels of 25(OH)D were spiked with potential interfering substances at recommended levels and analyzed. Results were compared to the same serum samples with no extra substances added to calculate the % interference. The following substances were tested and did not show significant interference in the assay up to concentrations more elevated than the highest occurring levels: hemoglobin up to 7.5 mg/mL; bilirubin conjugated and free up to 200 μg/mL; triglycerides up to 5.5 mg/mL; cholesterol up to 2.6 mg/mL; ascorbic acid up to 10 mg/mL, biotin up to 40 μg/mL and caffeine up to 10 μg/mL.

PRECISION

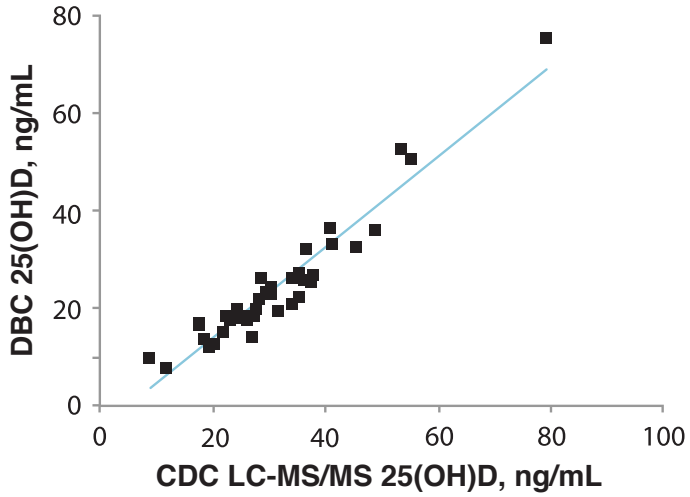
The precision study followed EP5-A3 and used a nested components -of-variance design with 21 testing days, two runs per testing day, and two replicate measurements per run (a 21 x 2 x 2 design) for each sample. Data was analyzed with a two-way nested ANOVA and summarized in the table below:

Sample	Mean (ng/mL)	Repeatability SD	Repeatability CV %	Within Lab SD	Within Lab CV %
1	21.87	1.09	5.0%	1.77	8.1%
2	36.57	1.01	2.8%	3.17	8.7%
3	45.01	1.07	2.4%	4.45	9.9%
4	60.25	2.82	4.7%	6.21	10.3%

COMPARATIVE STUDIES

The DBC 25(OH)D ELISA kit (y) was compared to a higher level test (LC-MS/MS) (x). The comparison of 40 serum samples yielded the following linear regression results:

y = 0.93x – 4.68, r = 0.96



REFERENCE VALUES (SERUM/PLASMA)

As for all clinical assays each laboratory should collect data and establish their own range of reference values. Data presented here are from samples collected in Florida (USA) from putatively healthy Black, White and Hispanic individuals of both genders and between 20 and 60 years old. Population reference ranges for 25(OH)D vary widely depending on age, ethnic background, geographic location and season. Population-based ranges correlate poorly with serum 25(OH)D concentrations that are associated with biologically and clinically relevant vitamin D effects and are therefore of limited clinical value.

N	25(OH)D Mean (ng/mL)	25(OH)D Median (ng/mL)	25(OH)D Range (2.5^{th}–97.5^{th} percentile) (ng/mL)
120	24.6	23.5	12.6–42.3

Results from the NHANES III study (1) yielded a mean of 30 ng/mL among 15,390 individuals.

Ordering Information: REF CAN-VD-510

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