25 HYDROXY VITAMIN D

ELISA

fast & accurate

no sample preparation
short assay time
LC-MS/MS matching results
automatable

DBC
Diagnostics Biochem Canada

DBC 25-Hydroxyvitamin D ELISA

OVERVIEW

The worldwide aging and chronically ill population is increasing rapidly. It is forecasted that the Global Vitamin D Testing Market will grow at a Compound Annual Growth Rate (CAGR) of 32% over the period 2013–2018.

The market has been witnessing also an increase in the number of suppliers of Vitamin D tests. Strict regulatory requirements however, pose a challenge for both suppliers and distributors.

Diagnostics Biochem Canada Inc. (DBC) has more than 40 years of experience in the immunoassay market. With dozens of products that are registered with the FDA, Health Canada and that bear the CE mark, DBC exports to more than 70 countries and has solid reputation in new product development, approval and commercialization.

Answering the increasing demand for Vitamin D Tests, DBC has launched a new ELISA for Vitamin D analysis.

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 D_2 and vitamin D_3) or produced in the skin by sun exposure (vitamin D_3).

The body stores both vitamin D_2 and vitamin D_3 mainly in the form of 25-hydroxyvitamin D_2 or 25-hydroxyvitamin D_3 respectively, therefore the best approach to assess the physiological levels of vitamin D is to analyze the total concentration of both hydroxylated forms.

PRINCIPLE OF THE TEST

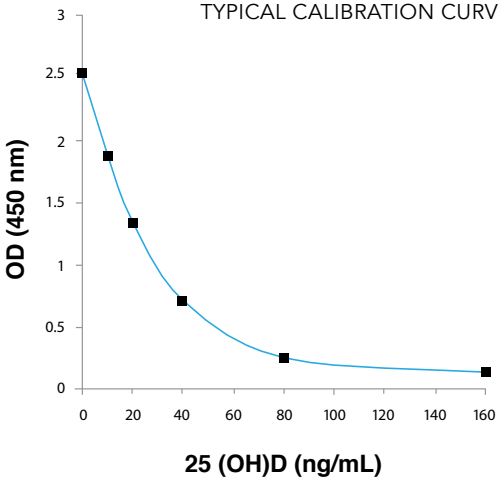
This kit measures the total concentration of both 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 (25(OH)D). The results are expressed in ng/mL.

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, unlabeled 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.

DBC 25(OH)D ELISA Method in 4 easy steps:

1. Add sample and incubation buffer into microplate wells. Incubate for 1 hour.
2. Wash microplate and add conjugates. Incubate for 30 minutes.
3. Wash microplate and add enzyme substrate. Incubate for 10–15 minutes.
4. Add stopping solution and read at 450 nm.

TYPICAL CALIBRATION CURVE



DBC 25-Hydroxyvitamin D ELISA

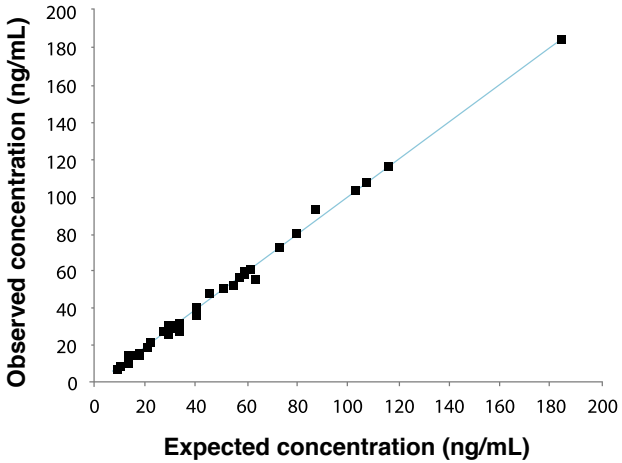
PERFORMANCE

Parameter	DBC	Competitor 1	Competitor 2	Competitor 3	Competitor 4
Total assay time, h	< 2	3	3	3	2
Sample pre-treatment	NONE	YES	YES	YES	YES
Reporter	HRP, colorimetric	^{125}I, radioactive	^{125}I, radioactive	HRP, colorimetric	HRP, colorimetric
Sample size, µL	25	50	50	20	25
Sample type	serum, plasma	serum, plasma	serum, plasma	serum, plasma	serum, plasma
Precision CV%					
Repeatability	2.4–5	—	—	—	—
Within Lab	8.1–10.3	—	—	—	—
Inter-assay	—	8.6–12.5	5.3–6.1	5	8.84
Intra-assay	—	8.2–11	7.3–8.2	7.8	12.7
Range, ng/mL	0–160	0–100	0–160	0–120	0–130

PERFORMANCE

LINEARITY

Combined results from ten samples diluted to three levels output the following correlation between observed and expected concentration values.



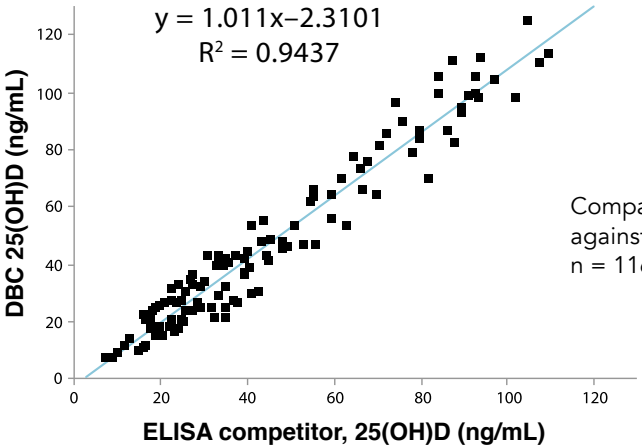
PRECISION

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 on the table below:

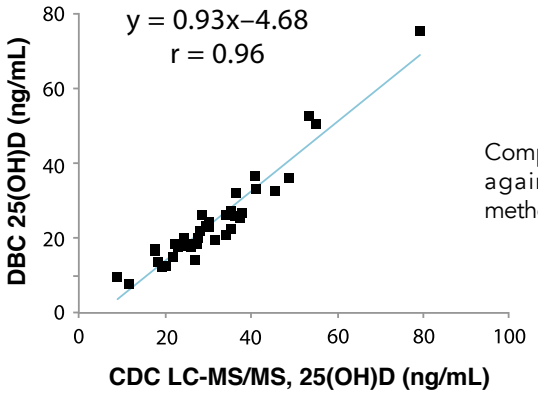
Sample	Mean (ng/mL)	Repeatability SD	Repeatability CV%	Within Lab SD	Within Lab CV%
1	21.87	1.09	5.0	1.77	8.1
2	36.57	1.01	2.8	3.17	8.7
3	45.01	1.07	2.4	4.45	9.9
4	60.25	2.82	4.7	6.21	10.3

PERFORMANCE

COMPARATIVE STUDIES



Comparison of DBC 25(OH)D ELISA against a leading ELISA competitor, n = 116 serum samples



Comparison of DBC 25(OH)D ELISA against LC-MS/MS standardized method from CDC, 40 serum samples

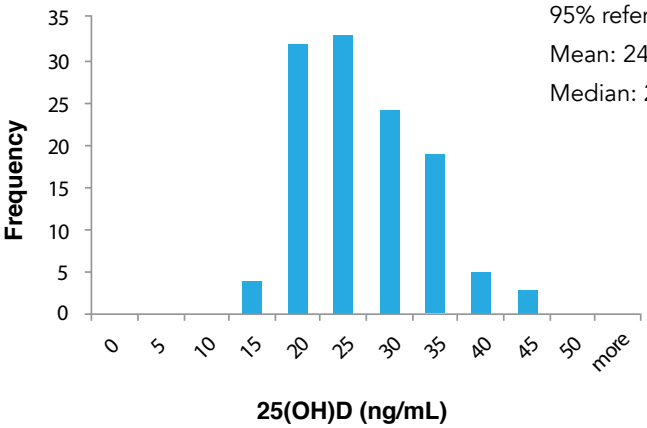
PERFORMANCE

Comparison of DBC 25(OH)D ELISA against LC-MS/MS results: International controls and pooled samples.

		LC-MS/MS ng/mL		DBC 25(OH)D, ng/mL
		AVE	Range	
Assigned value controls (Bio-Rad)	Level 1	7.42	4.54–10.3	8.30 ± 2.6
	Level 2	13.1	8.99–17.2	13.2 ± 4.0
	Level 3	33.6	24.1–43.1	26.9 ± 5.0
	Level 4	91.9	67.1–117	84.6 ± 15
Assigned value controls (Fujerebio)	Level 1	12.3	8.6–16	10.7 ± 2.3
	Level 2	34.1	23.9–44.4	33.4 ± 5.6
	Level 3	76.2	53.3–99	76.4 ± 9.9
Pooled serum samples	Men	35.3		39.4 ± 6.2
	Women Post-menopausal	30.0		31.6 ± 49
	Women Post-menopausal	30.9		31.4 ± 5.3

PERFORMANCE

Distribution of 25(OH)D values in 120 putatively normal individuals from Florida, mixed races, between 18 and 65 years old.



95% reference range: 12.6–42.3 ng/mL

Mean: 24.6 ng/mL

Median: 23.5 ng/mL

IMPORTANT

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) during the month of August from putatively healthy Black, White and Hispanic individuals of both genders, between 20 and 60 years old. Population reference ranges for 25(OH)D vary widely depending on age, ethnic background, geographic place 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.

RECOMMENDED LEVELS

The Institute of Medicine at Washington DC concluded that the levels of vitamin D can be associated with health conditions as in the following table:

25(OH)D, ng/mL	Health Status
< 12	Vitamin D deficiency leading to rickets in infants and children and osteomalacia in adults.
12–20	Generally considered inadequate for bone and overall health in healthy individuals.
≥ 20	Generally considered adequate for bone and overall health in healthy individuals.
> 60	Emerging evidence links potential adverse effects to such high levels.

Another source reports the following threshold levels:

25(OH)D, ng/mL	Health Status
< 10	Severe deficiency. Could be associated with osteomalacia or rickets.
10–19	Mild to moderate deficiency. May be associated with increased risk of osteoporosis or secondary hyperparathyroidism.
20–50	Optimum levels in the healthy population; patients with bone disease may benefit from higher levels within this range.
51–80	Increased risk of hypercalciuria. Sustained levels > 50 ng/mL 25OH-VitD along with prolonged calcium supplementation may lead to hypercalciuria and decreased renal function.
> 80	Toxicity possible. 80 ng/mL is the lowest reported level associated with toxicity in patients without primary hyperparathyroidism who have normal renal function. Most patients with toxicity have levels > 150 ng/mL. Patients with renal failure can have very high 25(OH)D levels without any signs of toxicity, as renal conversion to the active hormone 1,25(OH)D is impaired or absent.

LITERATURE

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25 HYDROXY VITAMIN D ELISA

at a glance

DBC 25(OH)D ELISA REF CAN-VD-510

Sample: 25 µL of human serum or plasma

No sample preparation

Total assay time: less than 2 hours

Number of calibrators: 6

Number of supplied internal controls: 2

Sensitivity: 5.5 ng/mL

Cross-reactivity: 100% 25(OH)D_2
100% 25(OH)D_3
< 1.0% Vitamin D_2
< 1.0% Vitamin D_3

Results match LC-MS/MS

Automatable

FOR MORE INFORMATION, PLEASE CONTACT DBC AT:

DIAGNOSTICS BIOCHEM CANADA
384 NEPTUNE CRESCENT • LONDON, ONTARIO, CANADA • N6M 1A1
TEL: 519-681-8731 • FAX: 519-681-8734 • dbc@dbc-labs.com • www.dbc-labs.com

Diagnostics Biochem Canada

384 Neptune Crescent
London, Ontario, Canada N6M 1A1
Tel: 519-681-8731
Fax: 519-681-8734
dbc@dbc-labs.com
www.dbc-labs.com

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