LIAISON®
25 OH Vitamin D TOTAL Assay
Average Vitamin D in adults (>18 years) around the world

Adapted from Wahl et al. Arch Osteoporos (2012) 7:155–172

The history of DiaSorin Vitamin D test

1985
DiaSorin (INCSTAR) introduced first 3H-based RIA.

1996
DiaSorin’s RIA Vitamin D was the first FDA-cleared.

2004
LIAISON® 25 OH Vitamin D was the first fully automated CLIA assay FDA cleared.
What is Vitamin D?

Vitamin D is a steroid hormone that is produced in the skin upon UV-B exposure or is ingested via the diet. The 25-hydroxyvitamin D (25 OH Vitamin D) is the most common form of the hormone in the circulation and is the inactive precursor of the active form, 1,25-dihydroxyvitamin D (1,25 (OH)₂ Vitamin D). Because of its long half-life and high concentration, 25 OH Vitamin D is commonly measured for monitoring the Vitamin D status in the patient.

Vitamin D is maintained in the body thanks to two mechanisms, one endogenous, with the production of the hormone thanks to sun exposure and one exogenous, with the introduction of vitamin D with the food. The chemical structures of these types of Vitamin D are different and are called Vitamin D2 (ergocalciferol) and Vitamin D3 (cholecalciferol). D2 is present in some foods and food supplements. Vitamin D3, on the other hand, is produced by the body at the skin level under the influence of solar radiation. In Vitamin-based pharmaceutical preparations the two forms of Vitamin are indifferently present. The two forms of Vitamin D2 and D3 have the same effect and therefore it is important to know the sum of their quantities or the total concentration of 25 OH Vitamin D.

The concentration of 25 OH Vitamin D is used to estimate the reserve of Vitamin D.

Sources and forms of Vitamin:

VIT D2 (ERGOCALCIFEROL)  
EXOGENOUS

25 HYDROXYVITAMIN D

VIT D3 (COLECALCIFEROL)  
ENDOGENOUS - EXOGENOUS

1,25 DIHYDROXYVITAMIN D
Implication of Vitamin D in some pathologies

Chronic Kidney Disease

The KDIGO guidelines for the management of mineral and bone disorders (MBD) of CKD suggest to measure 25 OH Vitamin D in any patients with a CKD stage 3b and more (eGFR <45 mL/mn/1.73 m²), and to correct Vitamin D deficiency and insufficiency. Observational studies show that mineral disorders (i.e. hypocalcemia, high bone remodeling markers, high PTH, low 1,25 (OH)₂ Vitamin D are more frequent in CKD patients with Vitamin D deficiency than in those with a normal values. Low 25 OH Vitamin D serum concentrations are associated with a faster decline in eGFR in non dialysis CKD patients and renal transplant recipients, higher serum PTH levels in non-dialysis and dialysis patients, higher risk of cardiovascular and all-cause mortality in non-dialysis and dialysis patients, and lower bone mineral density at both spine and femoral neck.

Many intervention studies showed that supplementation with Vitamin D or 25 OH Vitamin D decreases PTH in non-dialysis and dialysis patients. Furthermore, Vitamin D supplementation showed to improve endothelial dysfunction in non-dialysis CKD and to decrease albuminuria in stage 3-4 CKD patients.

Pregnancy

Measuring Vitamin D status during pregnancy is important, as low Vitamin D level has been associated with an increased risk of preeclampsia, gestational diabetes, low birth weight and premature birth. New data show also the relationship between Vitamin D status or supplementation and the risk of asthma in the offspring.

Cancer

Recent meta-analysis showed association between low serum/plasma levels of Vitamin D and some cancers (colorectum cancer, breast cancer in post-menopausal women, bladder cancer, lung cancer).

Type-2 Diabetes

Subjects with increased risk of type 2 diabetes have low 25 OH Vitamin D serum concentration. Meta-analyses suggest that Vitamin D supplementation might have modest but significant reducing effect on HbA1C, fasting glycemia and insulin resistance, specially in patients with poor HbA1C at baseline.

Conversion factors: ng/mL = nmol/L/2.496

Health Based Reference Values as per LIAISON® Vitamin D Total IFU

Deficiency Insufficiency

0 10 30
**Muscle Health**

Muscle weakness is a well-known feature of rickets/osteomalacia due to severe Vitamin D deficiency, with recovery of muscle function after correction of Vitamin D deficiency. Vitamin D supplementation is able to slightly improve proximal muscle strength in Vitamin D deficient elderly people\textsuperscript{29} and seems to improve postural balance\textsuperscript{30}. It decreases falls in institutionalized patients\textsuperscript{31} and elderly subjects with low 25 OH Vitamin D serum concentration\textsuperscript{32}, but bolus doses showed to increase the risk\textsuperscript{33}.

**Skeletal System**

The main effect of the active Vitamin D is on the regulation of phosphaetemia and calcaemia, whose values are fundamental for bone mineralization. Vitamin D deficiency causes bone diseases due to lack of mineralization, rickets in the children and osteomalacia in adults. Vitamin D supplementation is able to prevent such kind of diseases. In case of less severe Vitamin D deficiency, the hypocalcaemia, due to the reduction in calcium intestinal absorption, causes the increase in PTH levels, stimulating bone remodelling that leads in the long term to osteoporosys in elderly people.

**Cardiovascular Health**

Low serum 25 OH Vitamin D in humans is associated with more cardiovascular events and diseases, while the active Vitamin D exerts beneficial effects on the vasculature and vascular cells\textsuperscript{32} and is a negative regulator of the renin-angiotensin system\textsuperscript{33}. Different observational studies showed a negative correlation between serum 25 OH Vitamin D level and blood pressure in humans\textsuperscript{34}.

**Respiratory infection**

Vitamin D plays an important role also in the immuno response. It has been demonstrated that immuno cells (like monocytes, macrophages) are able to activate 25 OH Vitamin D to stimulate the production of antibacterial peptides such as cathelicidin, when in contact with tuberclosis bacillus\textsuperscript{37}.

Meta-analysis of 25 RCT and more than 10,000 patients showed that Vitamin D supplementation reduces slightly but significantly the risk of acute respiratory infections, and that the risk is considerably more decreased in Vitamin D treated patients that are severely deficient at baseline\textsuperscript{38}.

In addition, 1-25 (OH)\textsubscript{2} Vitamin D suppresses the T-cell driven inflammation and enhance the effect of Treg cells\textsuperscript{39}.
Assay performance characteristics

**Functional Sensitivity**
- **Guidelines**: evaluated according to CLSI EP17-A
- **Definition**: dose concentration at which the % CV exceeds 20%
- **Sample concentration**: 2-14 ng/mL
- **Assays**: multiple runs performed
- **Result**: Functional Sensitivity: ≤ 4.0 ng/mL

**Assay precision**
- **Guidelines**: evaluated according to CLSI EP5-A2
- **# samples**: 6 samples + 2 controls

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<tr>
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<th>Intra Run Across Lots and Sites</th>
<th>Total Across Lots and Sites</th>
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<tbody>
<tr>
<td></td>
<td>SD</td>
<td>% CV</td>
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<tr>
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<tr>
<td>LIAISON® XL</td>
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**Trueness**
- **Guidelines**: Evaluated according to CLSI EP6-A
- **Method**: Verified by the dilution and recovery tests
- **Sample concentration**: 3.8 to 151 ng/mL
- **Assays**: 2 sample pools diluted
- **Result**: Regression equation $R^2=0.995$

**Cross-reactivity**
- **Guidelines**: Evaluated according to CLSI EP7-A2
- **Sample concentration**: spiking up to 100 ng/mL of the potential cross-reactant
- **Molecules**
  - 25 OH Vitamin D2: 100.0%
  - 25 OH Vitamin D3: 100.0%
  - Vitamin D2: 1.9%
  - Vitamin D3: 1.9%
  - 1,25 (OH)2 Vitamin D2: 6.7%
  - 1,25 (OH)2 Vitamin D3: 9.3%
  - 3-epi-25 OH Vitamin D3: 1.3%

**Recovery**
High concentration samples were mixed with low concentration samples in ratios of 1:2, 1:1 and 2:1. The observed values were then compared to the expected values to determine the % recovery. The mean recovery is 93%.
• A fully automated immunoassay for the direct measurement of 25 OH Vitamin D total levels in human serum, EDTA plasma and lithium heparin plasma.

• Allows quick and reliable determination of Vitamin D status and effective therapy monitoring.

• Uses advanced chemiluminescence technology with magnetic microparticle separation to achieve the best assay sensitivity and precision.

Dynamic range: 4.0 – 150 ng/mL

Time to first result: 35 min

Throughput: 170 tests/hour (LIAISON® XL)

The scatter plot generated with a Passing & Bablock regression obtained a fit of $y = 1.04x - 1.32$ vs the VDSP assigned mean, showing an excellent correlation to the Diasorin 25 OH Vitamin D CLIA assay.

DiaSorin LIAISON® 25 OH Vitamin D TOTAL Assay succeeded for three consecutive years.

Ease of Use

• No solvent extraction
• Barcode identification of primary sample tubes and reagents
• Continuous reloading of samples and reagents

• Ready-to-use reagents, calibrators included
• Two-point calibration, stored master curve
DiaSorin: The Diagnostic Specialist in Vitamin D

> 45 billion 25 OH Vitamin D tests sold worldwide since 2004

References

1. KDIGO guidelines *Kidney Int* 2009;76 (Suppl 113)
27. Mirhosseini N et al. *J Clin Endocrinol Metab* 2017; 102(9):3097-3110
34. Villanueva-A et al. *Atherosclerosis* 2006; 186(2):203-208
38. Martineau AR et al. *BMJ* 2017; 356:i6583

Ordering information

LIAISON® 25 OH Vitamin D TOTAL Assay (code 310600)
LIAISON® 25 OH Vitamin D TOTAL Control Set (code 310601)
LIAISON® 25 OH Vitamin D TOTAL Specimen Diluent Set (code 310602)

AVAILABLE ON LIAISON® SYSTEMS

The Diagnostic Specialist