Vitamin D testing with the LIAISON® 25 OH Vitamin D TOTAL kit
UV light → skin → Vitamin D₃ → nutritional status → active form
Skin synthesis of Vitamin D

SUN

LUMISTEROL / TACHYSTEROL

7-DEHYDROCHOLESTEROL → PRE-VITAMIN D₃

SKIN TEMPERATURE

VITAMIN D₃

SKIN

BLOOD

DBP → DBP-D₃
**Vitamin D metabolites**

**Vitamin D₃** (cholecalciferol)
- synthetized in skin
- animal origin
- contained in supplements

**Vitamin D₂** (ergocalciferol)
- vegetable origin
- contained in supplements

Vit D₂ and Vit D₃ are hydroxilated to 25 OH Vitamin D in the liver

**TOGETHER Vit D₂ and Vit D₃ ARE the BEST indicator of vitamin D nutritional status**
1,25 dihydroxyvitamin D

The most potent Vitamin D metabolite
its synthesis is stimulated by PTH in the kidney

- regulates bone mineral metabolism

- important role in
  - cancer
  - inflammation
  - and immunity

Biological Actions of Vitamin D

**Intestine**
Enhances the intestinal absorption of calcium and phosphate

**Bone**
Promotes bone remodeling (both formation and resorption)

**Kidney**
Proximal tubular calcium reabsorption
In general:

- All, when limited exposure to the sun is supposed
- All, when malabsorption syndromes are supposed
- All, when malnutrition is supposed
- All, when limited too low supplementation is supposed

- Children for the correct development of bones to prevent rickets
- People aged >50 yrs for the osteoporosis management
- Homebound Elderly Persons for a better survival
A number of studies have identified widespread Vitamin D insufficiency in apparently healthy populations world-wide.

Seasonal Variation of 25 OH D levels

25 OH D (ng/mL) vs. UV dose (mJ/cm²)

Year, 1978

25 OH Vitamin D, measurement issues
Exposure time and skin colour effects on 25 OH Vitamin D production

White skin

Very Dark skin

Same capacity for vit D, different exposure-time requirements

Yield of vitamin D

20 min

120 min
Circulating 25(OH)D as a Function of Oral Vitamin D Intake

- 10,000 IU/d
- 5,000 IU/d
- 1,000 IU/d
- 400 IU/d

25 OH Vitamin D, measurement issues
Recent literature has suggested the following ranges:

**Deficiency:**  
<10 ng/mL  
(0 - 25 nmol/L)

**Insufficiency:**  
10 - 30 ng/mL  
(25 - 75 nmol/L)

**Sufficiency:**  
30 - 100 ng/mL  
(75 - 250 nmol/L)

**Toxicity:**  
> 100 ng/mL  
(> 250 nmol/L)
Vitamin D Supplementation

either D₃ and D₂ therapies are considered

A.I. for Adults is 400-600 IU/day
Studies Show Elderly May Need More

700 IU/day (Dawson-Hughes et al., 1995)
Protection against bone loss

800 IU/day (Chapuy et al., 1987)
Reduced biochemical indexes of secondary hyperparathyroidism

800 IU/day (Chapuy et al., 1992)
Reduced risk of hip fracture
Vitamin D measurement

Vitamin D itself is rarely measured

The two metabolites measured are:

25 Hydroxyvitamin D (25 OH D)
gives an indication of Vitamin D stores obtained by sun exposure, diet, supplementation defines Vitamin D
deficiency insufficiency sufficiency toxicity

Optimal levels > 30 ng/mL

1, 25 Dihydroxyvitamin D (1, 25(OH)₂D)

25 OH Vitamin D measurement

25 OH Vitamin D
UV Quantitation following HPLC

25 OH Vitamin D
LC-MS Quantitation

25 OH Vitamin D
Radioimmunoassay (RIA)
25 OH Vitamin D measurement

25 OH Vitamin D Radioimmunoassay (RIA)

DiaSorin RIA kit to test 25 OH Vitamin D stays as the golden standard for clinical evaluation of deficiency and insufficiency.
LIAISON® 25 OH Vitamin D TOTAL

direct, competitive chemiluminescence immunoassay in serum or EDTA plasma.

• 1\textsuperscript{st} incubation, 10’
25 OH Vitamin D dissociated from its binding protein binds to the specific antibody on the solid phase.

• 2\textsuperscript{nd} incubation, 10’
Tracer 25 OH vitamin D linked to an isoluminol derivative added.

• Washing
• Starter reagents
• Flash chemiluminescent reaction

The light signal measured in relative light units is inversely proportional to the level of the analyte.
On-line extraction procedure with ethanol
Highly specific antibody to identify 25 OH Vitamin D
Fully correlated to the DiaSorin RIA method
Specificity: 100% 25 OH Vitamin D$_2$ and 25 OH Vitamin D$_3$

Functional sensitivity: $< 4 \text{ ng/mL}$
Dynamic range: $4 - 150 \text{ ng/mL}$
Two step incubation time

Time to first result: 35’
Throughput: $> 100 \text{ tests/hour}$
Precision was evaluated following CLSI EP5-A2. Samples containing different concentrations of analyte were assayed in duplicate, two assays per day, over 20 operating days, to determine the repeatability and reproducibility of the assay (i.e. within- and between-assay variability).
The Bland-Altman plot indicates that the LIAISON® 25 OH Vitamin D TOTAL assay has a slight tendency to give lower results below ~25 ng/mL or 63 nmol/L, but no bias in higher values.
A total of 109 samples was tested by LIAISON® 25 OH Vitamin D TOTAL and by a radioimmunoassay method (RIA). The resulting regression equation was:

\[
\text{LIAISON} = 1.19 \times \text{RIA} + 0.31 \quad R = 0.95
\]
LIAISON® 25 OH Vitamin D TOTAL

Correlation to LC-MS
D₂ and D₃ specificity

Samples containing only 25-OH D₃ (n=64):
LIAISON® = 0.88(LC-TMS) + 1.9;
R = 0.90

Samples containing both 25-OH D₃ and 25-OH D₂ (n=46):
LIAISON® = 0.80(LC-TMS) + 3.6;
R = 0.78

All samples (n=110):
LIAISON® = 0.83(LC-TMS) + 2.7;
R = 0.87
The Bland-Altman plot shows no systematic bias in the results:

nor is there a systematically increasing bias in the results with increasing 25OH D2:

**LIAISON® 25 OH Vitamin D TOTAL**

**Correlation to LC-MS**

D2 and D3 specificity
125 serum samples from African Americans by three different assay methods: liquid chromatography-tandem mass spectrometry and LIAISON® 25 OH Vitamin D TOTAL

Distribution of DBP (Gc) Allele Frequencies

<table>
<thead>
<tr>
<th>Population</th>
<th>Gc-1F</th>
<th>Gc-1S</th>
<th>Gc-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Europe</td>
<td>0.06-0.20</td>
<td>0.51-0.61</td>
<td>0.24-0.41</td>
</tr>
<tr>
<td>Africa – North &amp; East</td>
<td>0.26-0.50</td>
<td>0.35-0.67</td>
<td>0.01-0.21</td>
</tr>
<tr>
<td>Africa – South &amp; West</td>
<td>0.68-0.88</td>
<td>0.08-0.12</td>
<td>0.02-0.16</td>
</tr>
<tr>
<td>USA – Whites</td>
<td>0.11-0.28</td>
<td>0.49-0.57</td>
<td>0.21-0.31</td>
</tr>
<tr>
<td>USA – Blacks</td>
<td>0.67-0.79</td>
<td>0.12-0.18</td>
<td>0.08-0.13</td>
</tr>
</tbody>
</table>

The form of DBP that is most common in African Americans has been reported to exhibit a higher affinity for vitamin D metabolites than the forms most common in Europeans (Braun A, et al. Electrophoresys 1990, Arnaud et al. Hum Genet 1993)
Other reports conclude that there is no difference in affinity between the various isoforms (Boutin B et al. J Ster Biochem 1989, Kawakami M et al Biochem J 1979)
Correlation to LC-MS in African American and Caucasian

Samples from African Americans

LIAISON® = 0.86(LC-TMS) + 1.7;  
R = 0.96

Samples from Mixed Population

LIAISON® = 0.82(LC-TMS) + 5.6;  
R = 0.94
External evaluation on DEQAS control sera run in two customer sites in Europe

<table>
<thead>
<tr>
<th>DEQAS</th>
<th>RIA</th>
<th>old LSN</th>
<th>TOTAL D site 1</th>
<th>TOTAL D site 2</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>nmol/L</td>
<td></td>
</tr>
<tr>
<td>286</td>
<td>44,4</td>
<td>34</td>
<td>43,3</td>
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<tr>
<td>287</td>
<td>46,1</td>
<td>34,1</td>
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<td>288</td>
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<td>289</td>
<td>66,1</td>
<td>51,5</td>
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<tr>
<td>290</td>
<td>63,2</td>
<td>55,7</td>
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<tr>
<td>301</td>
<td>90,3</td>
<td>95,2</td>
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<tr>
<td>310</td>
<td>75,3</td>
<td>70,1</td>
<td>84,5</td>
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</table>
External evaluation run in France
Blind correlation to results obtained with the DiaSorin RIA kit

\[ y = 1.0986x - 2.6432 \]

\[ R = 0.8953 \]

\[ n = 41 \]
External evaluation run in Danemark
Regional Hospital, Henring
correlation to tandem mass spect on routinary samples

XY-plot metodesammenligning
\[ y = 1.0303x - 4.6092 \]
\[ R^2 = 0.9474 \]

Bland Altman evaluation run on samples
< 100nmol/L
External evaluation run in Danemark
Regional Hospital, Henring
Limit of quantification

<p>| | |</p>
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<tr>
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<td>10.5</td>
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<tr>
<td>14</td>
<td>10.6</td>
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Mean: 12.35 nmol/L
SD: 1.287 nmol/L
CV: 10.4%

14 determinations every run
REMIND
Functional sensitivity is defined as the levels at which CV% is lower than 20%
External evaluation run in UK
Kings College Hospital
precision test, intra-assay evaluation

<table>
<thead>
<tr>
<th></th>
<th>Level 1 (ng/mL)</th>
<th>Level 2 (ng/mL)</th>
<th>Level 3 (ng/mL)</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
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<td>5.78</td>
<td>33.7</td>
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<tr>
<td>4</td>
<td>5.23</td>
<td>37.3</td>
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<td>7.32</td>
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<td>44.3</td>
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<td>49.4</td>
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<td>10</td>
<td>5.9</td>
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<td>46.8</td>
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<tr>
<td>Mean</td>
<td>5.95</td>
<td>35.47</td>
<td>45.96</td>
</tr>
<tr>
<td>S.D</td>
<td>0.79</td>
<td>1.70</td>
<td>2.88</td>
</tr>
<tr>
<td>% C.V</td>
<td>13.31</td>
<td>4.80</td>
<td>6.26</td>
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</table>
External evaluation run in UK
Kings College Hospital
precision test, Quality Control evaluation

<table>
<thead>
<tr>
<th></th>
<th>Low QC</th>
<th>High QC</th>
<th>ng/mL</th>
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<tbody>
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<td>2</td>
<td>16.70</td>
<td>44.70</td>
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<td>3</td>
<td>15.40</td>
<td>50.50</td>
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<td>4</td>
<td>17.10</td>
<td>45.20</td>
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<td>5</td>
<td>15.20</td>
<td>43.80</td>
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</tr>
<tr>
<td>6</td>
<td>16.90</td>
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<td>7</td>
<td>18.00</td>
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<td>17.10</td>
<td>50.00</td>
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<td>9</td>
<td>17.70</td>
<td>47.00</td>
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<td>10</td>
<td>17.80</td>
<td>47.60</td>
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<tr>
<td>Mean</td>
<td>16.72</td>
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<tr>
<td>S.D</td>
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<tr>
<td>%C.V</td>
<td>6.35</td>
<td>5.20</td>
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</table>
LIAISON® 25 OH Vitamin D TOTAL

direct, competitive chemiluminescence immunoassay in serum or EDTA plasma.

- 1st incubation, 10’
25 OH Vitamin D dissociated from its binding protein binds to the specific antibody on the solid phase

- 2nd incubation, 10’
Tracer 25 OH vitamin D linked to an isoluminol derivative added.
- Washing
- Starter reagents
- Flash chemiluminescent reaction

LIAISON® 1,25 dihydroxyvitamin D

Indirect competitive chemiluminescence immunoassay in serum or EDTA plasma

- Off-line extraction and purification of vitamin D metabolites through C18OH “Extra Clean” cartridges

- 1st incubation, 30’
Extracted sample binds to the polyclonal antibody on the solid phase

- 2nd incubation, 10’
Tracer 1,25-(OH)2 vitamin D linked to an isoluminol derivative added.
- Washing
- Starter reagents
- Flash chemiluminescent reaction
Method Comparison LIAISON® 1,25-(OH)$_2$ Vitamin D

25 clinical samples tested versus the 1,25-Dihydroxyvitamin D $^{125}$I RIA

Linear regression equation:  

$$\text{LIAISON} = 0.95 \times (\text{RIA}) - 3.0$$

$$R = 0.98$$
LIAISON® 25-OH Vitamin D TOTAL

CONCLUSIONS

 ✓ good performance characteristics, with excellent precision and sensitivity

 ✓ assay automated on the LIAISON® System assuring quick availability of the results for the clinicians

 ✓ 100% cross-reactivity versus Vitamin D₂ and Vitamin D₃ allows the optimal characterization of the nutritional status and of the 25 OH Vitamin D supplementation

 ✓ correlation of the LIAISON TOTAL D assay to the DiaSorin RIA demonstrates a very tight association of the two methods

 ✓ correlation of the LIAISON TOTAL D assay to the LC/LC-MS reference method in Caucasian and African American shows an optimized extraction technique from the binding protein
LIAISON Vitamin D assays

CONCLUSIONS

- Convenient methods for the routine measurement of Vitamin D metabolism with automated non-isotopic immunoassays

- With only one blood sample both 25 OH Vitamin D and 1,25(OH)₂ can be performed

- Fast analytical protocols allow the physicians to get results in 1 hour time from the drawing in order to better assess the patient treatment

- Close correlation to the gold standard methods assures full clinical consistency of the results obtained

- 100% recovery to D₂ and D₃ metabolites assures reliable follow-up of therapy for insufficiency and deficiency