Clinical background of 1,25 Dihydroxyvitamin D

1,25 Dihydroxyvitamin D is the active form of Vitamin D, its production is tightly regulated through concentration of serum calcium, phosphorus and PTH.

- Low levels can be found in CKD, Vit D dependant rickets type 1, hypophosphatemic rickets, hypoparathyroidism
- High levels in Vit D dependant rickets type 2, sarcoidosis, RA, IBD, primary hyperparathyroidism

Challenges in 1,25 Dihydroxyvitamin D measurement

Until now, all assays required a long, manual, operator dependant pre-analytical step due to the following facts:

- The molecule circulates in low amounts (pg/mL concentration vs ng/mL concentrations)
- Similarity with its metabolic precursor, 25-OH Vitamin D

Novel Assay format

The LIAISON® XL 1,25 Dihydroxyvitamin D assay is a modified 3 step sandwich assay that uses a recombinant fusion protein for capture of the 1,25(OH)₂ D molecule and a murine monoclonal antibody which specifically recognizes the complex formed by the recombinant fusion protein with the 1,25(OH)₂ D molecule.
Specific performance characteristics

PATIENT CORRELATION/METHOD COMPARISON
A total of 173 samples spanning the assay range were tested by the LIAISON® XL 1,25 Dihydroxyvitamin D and LC-MS/MS comparator assay following CLSI EP9-A3. The study yielded the following Passing & Bablok regression analysis: LIAISON® XL 1,25 Dihydroxyvitamin D = 0.9811x – 1.37; R² = 0.9588

Precision
Two lots of kit controls and 6 serum samples spanning the range of the assay were tested twice per day in duplicate, over twenty days using 2 reagent lots on 2 XL Analyzers at DiaSorin Inc. The testing was performed according to CLSI EP5-A2.

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>N</th>
<th>MEAN 1,25(OH)₂ D (pg/mL)</th>
<th>BETWEEN-LOT</th>
<th>TOTAL (ACROSS LOTS)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>SD</td>
<td>%CV</td>
</tr>
<tr>
<td>Kit Control 1</td>
<td>160</td>
<td>30.9</td>
<td>0.84</td>
<td>2.7%</td>
</tr>
<tr>
<td>Kit Control 2</td>
<td>160</td>
<td>122.9</td>
<td>6.09</td>
<td>5.0%</td>
</tr>
<tr>
<td>Prec Serum 1</td>
<td>160</td>
<td>23.3</td>
<td>0.05</td>
<td>0.2%</td>
</tr>
<tr>
<td>Prec Serum 2</td>
<td>160</td>
<td>38.9</td>
<td>0.63</td>
<td>1.6%</td>
</tr>
<tr>
<td>Prec Serum 3</td>
<td>160</td>
<td>52.7</td>
<td>0.64</td>
<td>1.2%</td>
</tr>
<tr>
<td>Prec Serum 4</td>
<td>160</td>
<td>76.0</td>
<td>1.33</td>
<td>1.7%</td>
</tr>
<tr>
<td>Prec Serum 5</td>
<td>160</td>
<td>137.4</td>
<td>1.91</td>
<td>1.4%</td>
</tr>
<tr>
<td>Prec Serum 6</td>
<td>160</td>
<td>193.4</td>
<td>5.53</td>
<td>2.9%</td>
</tr>
</tbody>
</table>
Sample Equivalence - Linearity

Fifty-one (51) matched patient sets of serum, SST serum, EDTA plasma, and Lithium Heparin plasma samples were tested to determine if these sample types provide equivalent results on the LIAISON® XL 1,25 Dihydroxyvitamin D assay. The results were analyzed by regression of Observed 1,25(OH)₂ D Concentration (serum, SST Serum, EDTA plasma or Lithium Heparin plasma) versus expected 1,25(OH)₂ D Concentration (serum).

The resulting equations for each sample type are:

- Serum: Observed 1,25(OH)₂ D = 1.014x - 1.936; R² = 0.9829
- SST Serum: Observed 1,25(OH)₂ D = 1.011x - 0.285; R² = 0.9908
- EDTA plasma: Observed 1,25(OH)₂ D = 1.010x + 0.321; R² = 0.9975
- Lithium Heparin plasma: Observed 1,25(OH)₂ D = 1.000x + 0.100; R² = 0.9957

Expected Values

It is recommended that each laboratory establishes its own range of expected values.

To assess the expected reference range for the LIAISON® XL 1,25 Dihydroxyvitamin D a study was performed with samples from 123 apparently healthy adults aged 21-75 years of age from mixed ethnic backgrounds (48% dark-skinned and 52% light-skinned). Samples were collected in the winter (48.8%) and summer (51.2%) from subjects with normal Total Calcium, TSH and PTH values from the northern, central, and southern regions of the U.S.

Based on the 95% Reference Interval, the following values were established following CLSI guideline EP28-A3C.

<table>
<thead>
<tr>
<th>U.S. SUBJECTS</th>
<th>MEDIAN 1,25(OH)₂ D</th>
<th>OBSERVED RANGE 2.5th TO 97.5th PERCENTILE</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 123</td>
<td>47.8 pg/mL</td>
<td>19.9 – 79.3 pg/mL</td>
</tr>
</tbody>
</table>

- First fully automated, extraction free
- First result in just 65 minutes
- Low sample volume (75 μL)
- More test from the same patient tube (eg 25-OH Vitamin D, PTH)
The LIAISON® XL assay removes the necessity of manual sample extraction, by performing all assay steps on-board the analyser.

Time to first result within 65 minutes.
Bone & Mineral

LIAISON® 1,25 Dihydroxyvitamin D

The DiaSorin LIAISON® XL 1,25 Dihydroxyvitamin D is an in vitro chemiluminescent immunoassay (CLIA) intended for the quantitative determination of 1,25 Dihydroxyvitamin D in serum, EDTA and Lithium Heparin plasma.

- LIAISON® XL 1,25 Dihydroxyvitamin D (code 310980)
- LIAISON® XL 1,25 Dihydroxyvitamin D Controls (code 310984)
- LIAISON® XL 1,25 Dihydroxyvitamin D Specimen Diluent (code 310982)

The LIAISON® Bone & Mineral panel also includes:

- LIAISON® 25 OH Vitamin D TOTAL Assay (Code 310600)
- LIAISON® 1-84 PTH (Code 310630)
- LIAISON® N-TACT® PTH Gen II (Code 317910)
- LIAISON® BAP OSTASE® (Code 310970)
- LIAISON® Osteocalcin (Code 310950)

Product availability subject to required regulatory approval.