I. PRINCIPLE

The role of vitamin D in bone and mineral metabolism was recognized from its first identification as a factor that could cure rickets. However, vitamin D is now recognized as a prohormone which has multiple roles in maintaining optimal health.

Vitamin D$_3$ (cholecalciferol) and Vitamin D$_2$ (ergocalciferol) are the most abundant forms of Vitamin D in the body. Vitamin D$_3$ is synthesized in the skin from 7-dehydrocholesterol in response to sunlight. The best nutrition sources of D$_3$ are oily fish primary salmon and mackerel. Vitamin D$_2$’s nutrition sources are from some vegetables, yeast, and fungi.

Vitamin D (D$_3$, D$_2$, and metabolites) is converted to 25-hydroxy vitamin D in the liver. The measurement of 25-OH vitamin D concentration in the serum or plasma is the best indicator of vitamin D nutritional status.

The LIASION 25 OH Vitamin D assay is a direct competitive chemiluminescence immunoassay (CLIA) for quantitative determination of total 25 OH vitamin D in serum. During the first incubation, 25 OH Vitamin D is dissociated from its binding protein and binds to the specific antibody on the solid phase. After 10 minutes the tracer, (vitamin D linked to an isoluminol derivative) is added. After a second 10 minute incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added to initiate a flash chemiluminescent reaction. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of 25-OH vitamin D present in calibrators, controls, or samples.

II. POLICY/SCOPE

This is intended for the China Basin Chemistry section of the Clinical Laboratories and intended for testing by licensed Clinical Laboratory Scientists and Clinical Laboratory staff.

III. TEST AVAILABILITY (n/a)

IV. SPECIMEN REQUIREMENTS

a. Serum from SST or red top tubes. Any other tube type is NOT acceptable.

b. Allow complete clot formation to take place in serum samples prior to centrifugation.

c. Samples should be free of fibrin, red blood cells, or other particulate matter. Centrifuge samples containing fibrin, red blood cells, or particulate matter.

d. Samples should be free of bubbles.

e. Specimen storage and stability:

i. If testing will be delayed more than 24 hours, remove serum or plasma from the clot, serum separator or red blood cells.

ii. Serum or plasma may be stored for up to 5 days at 2-8°C.
iii. Serum or plasma stored at -20°C or colder is stable for 6 months.

iv. Multiple freeze-thaw cycles of samples should be avoided. Samples must be mixed thoroughly after thawing by low speed vortexing or by gentle inversion and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results.

v. Minimum volume: 200 uL serum

V. EQUIPMENT, REAGENTS, AND SUPPLIES

a. LIAISON 25 OH Vitamin D Reagent Kit (100 tests), Catalog # 310600
   i. Storage and Stability:
      1. Store reagents in the dark at 2-8°C.
      2. Unopened: Stable until the expiration date on the carton.
      3. Opened: Stable for a maximum of 4 weeks.

   ii. Preparation:
      1. Remove all seals from the reagent integral and remove any bubbles or foam that is visible on the reagent openings.
      2. Insert the reagent integral into the accelerator located on the right-side of the instrument and let stand for at least 30 seconds.
      3. Manually stir the magnetic particles for 1 minute before placing onto the instrument. Take precaution to not create bubbles.
      4. Place the reagent integral on the instrument and let it stand for 15 minutes before using.

b. LIAISON 25 OH Vitamin D TOTAL Specimen Diluent, Catalog # 310602
   i. Storage and Stability:
      1. Store the diluent at 2-8°C until expiration date on bottle.

VI. WARNINGS AND PRECAUTIONS

Caution: This product requires the handling of human specimens. It is recommended that all human sourced materials are considered potentially infectious and be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.

VII. CALIBRATION

a. Two-point calibration is performed:
   i. Every 7 days.
   ii. When changing lot numbers of primary reagent packs.
   iii. When changing lot numbers of starter reagents.
   iv. When quality control results are repeatedly out of range.
b. Refer to “Liaison XL Operating Procedure” for instructions on checking calibration status, performing calibration, and reviewing calibration data.

VIII. QUALITY CONTROL

   i. Preparation: Controls are stored frozen. Thaw before use.
   ii. Stability: 30 days when stored at 2-8°C.

b. See posted QC chart for acceptability limits.

c. Frequency:
   i. Analyze all levels of QC on each day that samples are analyzed.
   ii. Analyze all levels of QC every time a two-point calibration is performed.
   iii. Analyze all levels of QC on every primary reagent pack used for sample analysis.

d. Refer to “Liaison XL Operating Procedure” for instructions on programming QC on the instrument.

IX. PROCEDURE

Refer to “Liaison XL Operating Procedure” for instructions on scheduling and loading patient samples.

X. RESULTING/REPORTABLE RANGE

a. Analytical Measuring Range (AMR)
   i. The AMR for the Liaison XL 25 OH Vitamin D assay is 4 to 130 ng/mL.

b. Reportable Range
   i. The reportable range for the Liaison XL 25 OH Vitamin D assay is 4 to 130 ng/mL.
   ii. Values below 4 ng/mL are reported as “<4 ng/mL”.
   iii. Values greater than 130 ng/mL are reported as “>130 ng/mL”.
   iv. Results are reported in whole numbers.

c. Autoverification
   i. Results between 4 to 80 ng/mL will autoverify.
   ii. Results <4 and >80 ng/mL needs to be repeated and verified before reporting.

XI. EXPECTED VALUES

20-50 ng/mL
25-OHD values < 20 are considered to be insufficient and values < 10 - 12 are associated with vitamin D deficiency and risk for osteomalacia. Although values of 20 or more are generally considered to be sufficient, values in the range of 20 - 30 may be insufficient in certain high risk patient subgroups. There is no known benefit of values > 50, and values > 100 should be avoided because of possible risk of vitamin D toxicity.


XII. LIMITATIONS OF PROCEDURE

a. Testing should be performed on serum only. Whole blood and plasma specimens should not be used.
b. A skillful and strict adherence to the instructions are necessary to obtain reliable results.
c. Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results.
d. Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays.
e. Patients routinely exposed to animals, animal serum products, or other immunogenic products that may elicit heterophilic antibody production against the assay’s reagents can be prone to this interference and anomalous values may be observed.
f. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions in an adult population.
g. Integrals may not be exchanged between analyzer types (LIAISON and LIAISON XL).

XIII. PERFORMANCE CHARACTERISTICS

a. SPECIFICITY

Cross-reactivity was tested as described in CLSI EP-7-A2. Data on the cross-reactivity of the antiserum used in this assay were obtained by spiking up to 100 ng/mL of the potential cross-reactant and assaying. The cross-reactivity of each compound, normalized to 25 OH-vitamin D_3, is listed below. The antibody utilized in this assay will demonstrate cross-reactivity to the many dihydroxylated metabolites of Vitamin D.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>% Cross-reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 OH Vitamin D₂</td>
<td>100.0%</td>
</tr>
<tr>
<td>25 OH Vitamin D₃</td>
<td>100.0%</td>
</tr>
<tr>
<td>Vitamin D₂</td>
<td>1.9%</td>
</tr>
<tr>
<td>Vitamin D₃</td>
<td>1.9%</td>
</tr>
<tr>
<td>1,25-(OH)₂ Vitamin D₂</td>
<td>6.7%</td>
</tr>
<tr>
<td>1,25-(OH)₂ Vitamin D₃</td>
<td>9.3%</td>
</tr>
<tr>
<td>3-epi-25 OH Vitamin D₃</td>
<td>1.3%</td>
</tr>
</tbody>
</table>
b. SENSITIVITY
   i. The Liaison 25 OH Vitamin D assay has an analytical sensitivity of 4 ng/mL.
   ii. The analytical sensitivity is defined as the concentration of 25 OH Vitamin D that is two standard deviations less than the mean concentration of 10 replicate determinations of 25 OH Vitamin D zero diluent (0 ng/mL).

c. PRECISION
   i. Intra-Precision study performed with BioRad Liquichek Specialty Immunoassay Controls Lot 57441, 57442, 57443.

   \[
   \begin{array}{|c|c|c|}
   \hline
   \text{Control} & \text{SP1} & \text{SP2} & \text{SP3} \\
   \text{Criteria} & <10\% & <10\% & <10\% \\
   \text{Results} & 4\% & 2\% & 2\% \\
   \hline
   \end{array}
   \]

   ii. Inter-Precision study performed with BioRad Liquichek Specialty Immunoassay Controls Lot 60201, 60202, 60203.

   \[
   \begin{array}{|c|c|c|}
   \hline
   \text{Control} & \text{SP1} & \text{SP2} & \text{SP3} \\
   \text{Criteria} & <10\% & <10\% & <10\% \\
   \text{Results} & 7\% & 7\% & 7\% \\
   \hline
   \end{array}
   \]

d. METHOD COMPARISON
   i. Laboratory performed study using 111 patient samples comparing Beckman DXI results to the Diasorin Liaison XL results.

   \[
   \begin{array}{|c|c|c|}
   \hline
   \text{Criteria} & \text{Slope} & \text{R value} & \text{Mean Bias} \\
   \text{Results} & 0.9-1.1 & > 0.9 & 10\% \\
   \hline
   \end{array}
   \]

e. INTERFERENCES

Interfering substances were tested as described in CLSI EP7-A2 using the Liaison 25 OH Vitamin D TOTAL Assay. The results are presented below.

Specimens That Are Demonstrated \leq 10\% change in results up to
Hemolyzed 200 mg/dL of hemoglobin
Lipemic 589 mg/dL of triglycerides
Icteric 40 mg/dL of conjugated/unconjugated bilirubin

Specimens That Contain Demonstrated \leq 10\% change in results up to
Cholesterol 301 mg/dL
Uric Acid 20 mg/dL
Total Protein 12 g/dL
XIV.  TECHNICAL NOTES (N/A)

XV.  ALTERNATE METHODS

In exceptional cases, when Chemistry is unable to run the assay on the Liaison XL platform, samples may be sent to an alternate laboratory such as SFGH or a commercial facility for testing.

XVI.  REFERENCES