



DiaSorin Inc.
1951 Northwestern Ave – Stillwater, MN 55082 – USA
Tel. 1.651.439.9710 – Fax 1.651.351.5669



Changes: § 5
Deletions: §

LIAISON® XL 1,25 Dihydroxyvitamin D ([REF] 310980)

1. INTENDED USE

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 (1,25(OH)₂D) in serum, EDTA and Lithium Heparin plasma. Assay results should be used in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions in adult populations.

The test is to be performed on the LIAISON® Analyzer Family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is derived from 2 sources: exogenous (dietary) and endogenous (biosynthesis, regulated by exposure to ultraviolet light). The exogenous or nutritional source includes foods containing naturally low levels of vitamin D₂ (e.g. milk, butter, cereals supplemented with vitamin D₂), nutritional supplements in the form of readily available over-the-counter vitamins, and therapeutic formulations of the D₂ vitamins.¹ Vitamin D is not inherently active as it enters the circulation by either dietary or photochemical routes. Biological activity is gained following a complex series of metabolic steps.²

It is now known that the metabolic activation of vitamin D is an intricately controlled process, subject to extensive alteration by variables including dietary calcium and phosphorus, degree of vitamin D deficiency, genetic deficiencies, parathyroid hormone concentrations, exposure to ultraviolet light, and degree of renal function.³ The biosynthesis of the dihydroxylated forms of vitamin D₃ begins with the action of solar ultraviolet light on 7-dehydrocholesterol to form vitamin D₃ in the skin. Once Vitamin D₃ enters the circulation it is rapidly taken up by the liver where it is metabolized to 25-hydroxyvitamin D₃ (25-OH-D₃). The liver will also hydroxylate dietary vitamin D₂ to 25-hydroxyvitamin D₂ (25-OH-D₂).^{2,4}

Following hepatic hydroxylation, 25-OH-D is transported in association with vitamin D binding protein to the kidney where further hydroxylation takes place. The addition of a hydroxyl at position 1 yields 1,25 dihydroxyvitamin D (1,25 (OH)₂ D). 1,25 dihydroxyvitamin D is the most potent naturally occurring vitamin D metabolite discovered so far, and its production is tightly regulated through concentrations of serum calcium, phosphorus, and parathyroid hormone. During times of calcium stress, 1,25 (OH)₂ D is the most important vitamin D metabolite produced by the kidney.^{5,6} This is due to its essential role in the efficient active absorption of calcium and phosphorus, as well as their normal metabolism.

In secondary hyperparathyroidism the parathyroid glands become enlarged and hyperactive. Kidney failure is a common cause of secondary hyperparathyroidism and usually occurs as a complication of renal disease where the kidney is unable to remove the phosphorus produced by the body and is also unable to produce enough of the active form of vitamin D (1,25 (OH)₂ D). The build up of phosphorus leads to low levels of calcium in the blood, which in turn stimulates the parathyroid glands to increase PTH production, causing the parathyroid glands to enlarge. As the disease progresses, the parathyroid glands no longer respond normally to calcium or vitamin D.

The clinical practice guidelines such as the Kidney Disease Outcomes Quality Initiatives (KDOQI), and the Kidney Disease: Improving Global Outcomes (KDIGO) recommend activated vitamin D therapeutic regimens for chronic kidney disease (CKD) patients.^{7,8} Consequently, the measurement of 1,25 (OH)₂ D is rapidly becoming an efficient tool in the research of diseases and conditions that affect the normal metabolism of phosphorus and calcium.^{9,10,11}

3. PRINCIPLE OF THE PROCEDURE

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.

During the first incubation, calibrators, controls or patient samples are incubated with the recombinant protein (binding agent) and assay buffer. Following this incubation the solid phase containing the specific monoclonal antibody is added and allowed to bind with the complex from the first incubation. After the second incubation, a wash cycle is performed to remove any unbound material. The third step consists of adding the conjugate and incubating. The unbound material is removed with a second wash step. The starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is proportional to the concentration of 1,25 (OH)₂ D present in the calibrators, controls and patient samples.

*(LIAISON® XL and LIAISON® XS)

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	[SORB]	Magnetic particles coated with mouse monoclonal antibody, phosphate buffers, BSA, Tween 20, 0.09% sodium azide
TCEP (4.5 mL)	[BUFA]	TCEP (TRIS (2-carboxyethyl) phosphine), Citrate buffer and 0.09% sodium azide
Conjugate (14.0 mL)	[CONJ]	Mouse monoclonal antibody* conjugated to an isoluminol derivative in phosphate buffer, Tween 20, 0.01% gentamicin sulfate and 0.2% ProClin® 300
Assay Buffer (14.0 mL)	[BUFAS]	Phosphate buffer with blockers, mouse IgG, BSA, Tween 20, 0.01% gentamicin sulfate, and 0.2% ProClin® 300
Number of tests		100

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated Company of Dow.

Additional components not on the Reagent Integral

Calibrator 1 (2 vials) Lyophilized	[CAL1]	Human serum and Phosphate buffer spiked with low level 1,25(OH) ₂ D , 0.2% ProClin® 300, 0.01% gentamicin sulfate Reconstitute with 2.0 mL of distilled or deionized water.
Calibrator 2 (2 vials) Lyophilized	[CAL2]	Human serum and Phosphate buffer, spiked with high level 1,25(OH) ₂ D, 0.2% ProClin® 300, 0.01% gentamicin sulfate Reconstitute with 2.0 mL of distilled or deionized water.
Binding Agent (4 vials) Lyophilized	[PROT]	Recombinant fusion protein*, TRIS buffer with mannitol and detergent. Reconstitute with 5.0 mL of binding agent reconstitution buffer.
Binding Agent reconstitution buffer (4 x 5.5 mL)	[BUFB]	Water with 0.09% sodium azide

* European patent application No. 13152851

* International patent application No. PCT/EP2014/051482

Standardization: The calibrator concentrations (pg/mL) are referenced to an in-house standard preparation.

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® XS Analyzer
LIAISON® Wash/System Liquid ([REF] 319100)	LIAISON® EASY Wash Buffer ([REF] 319301)
-	LIAISON® EASY System Liquid ([REF] 319302)
LIAISON® XL Waste Bags ([REF] X0025)	LIAISON® EASY Waste ([REF] X0054)
LIAISON® XL Cuvettes ([REF] X0016)	LIAISON® Cuvettes on Tray ([REF] X0053)
LIAISON® XL Starter Kit ([REF] 319200) or LIAISON® EASY Starter Kit ([REF] 319300)	LIAISON® EASY Starter Kit ([REF] 319300) -
LIAISON® XL Disposable Tips ([REF] X0015) or LIAISON® Disposable Tips ([REF] X0055)	LIAISON® Disposable Tips ([REF] X0055) -
-	LIAISON® EASY Cleaning Tool ([REF] 310996)

Additional required materials

LIAISON® XL 1,25 Dihydroxyvitamin D Controls ([REF] 310984)

Additional recommended materials

LIAISON® XL 1,25 Dihydroxyvitamin D Diluent ([REF] 310982)
--

5. WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.

General Safety:

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Do not pipette solutions by mouth.
- Avoid direct contact with all potentially infectious materials by wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country.
- Do not use kits or components beyond the expiration date given on the label.

Chemical Hazard and Safety Information: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and applicable European Union directives (see Material Safety Data Sheet for additional information).

Reagents Containing Human Source Material:

Warning – Treat as potentially infectious. Each serum/plasma donor unit used in the preparation of this product has been tested by an U.S. FDA approved method and found non-reactive for the presence of the antibody to Human Immunodeficiency Virus 1 and 2 (HIV 1/2), the Hepatitis B surface antigen (HBsAg), and the antibody to Hepatitis C (HCV). While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source diseases for which there is no approved test. Because no known test method can offer complete assurance that HIV, Hepatitis B Virus (HBV) and HCV or other infectious agents are absent, all products containing human source material should be handled following universal precautions; and as applicable in accordance with good laboratory practices as described in the Centers for Disease Control and the National Institutes of Health current manual, Biosafety in Microbiological and Biomedical Laboratories (BMBL); or the World Health Organization current edition, Laboratory Biosafety Manual.

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	CONJ BUFAS CAL1 CAL2	SORB BUFA BUFB
Classification:	Skin sensitization, Category 1 Aquatic Chronic, Category 3	None required
Signal Word:	Warning	None required
Pictogram:	 GHS07 – Exclamation mark	None required
Hazard Statements:	H317 – May cause an allergic skin reaction. H412 – Harmful to aquatic life with long lasting effects.	None required
Precautionary Statements:	P261 – Avoid breathing mist or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P273 – Avoid release to the environment. P280 – Wear protective gloves and clothing, and eye protection.	None required

Reagents Containing Sodium Azide: Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

6. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

6.1 Re-suspension of Magnetic Particles

Magnetic particles must be completely re-suspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the color of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have re-suspended.
- Repeat as necessary until the magnetic particles are completely re-suspended.
- After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

6.2 Foaming of Reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

- Visually inspect the reagents, to ensure there is no foaming present before using the integral. If foam is present after re-suspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

6.3 Loading of integral into the reagent area

LIAISON[®] XL Analyzer and LIAISON[®] XS Analyzer

- LIAISON[®] XL analyzer and LIAISON[®] XS Analyzer is equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
 - a. Insert the reagent integral into the dedicated slot.
 - b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

7. STORAGE AND STABILITY OF THE REAGENT INTEGRAL

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles. See Reagent Integral Preparation for resuspension instructions. When the Reagent Integral is stored unopened, the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral must not be used past the expiration date indicated on the kit and Reagent Integral labels. After opening and each use, the Reagent Integral should be sealed with the tape provided with the kit and stored upright at 2-8°C in the dark. Open use is 4 weeks when properly stored. Undue exposure to light should be avoided.

7.1 Preparation, storage and stability of Binding Agent

Proper reconstitution of Binding Agent is essential. The Binding Agent is supplied lyophilized. Upon receipt, store Binding Agent at 2-8°C. Reconstitute 1 vial of Binding Agent with 5 mL of Binding Agent Reconstitution Buffer. **Let sit for 15 minutes at room temperature, then mix by gentle inversion 7 times.** Load Binding Agent onto Ancillary rack and slide onto LIAISON[®] XL Analyzer or LIAISON[®] XS Analyzer within 1 hour of reconstitution. Refer to the analyzer operator's manual for instructions on use of ancillary rack.

After opening and each use, cap vial and return to storage at 2-8°C. Once opened and reconstituted, Binding Agent is stable for 2 days.

The Binding Agent is kit lot specific and must be used only with the matched Reagent Integral lot. Correct lot matching between the Reagent Integral and Binding Agent is automatically checked by the LIAISON[®] XL Analyzer or LIAISON[®] XS Analyzer.

8. SPECIMEN COLLECTION AND PREPARATION

Human Serum, SST serum, EDTA plasma and Lithium Heparin plasma may be used in this assay.

Blood should be collected aseptically by venipuncture. Serum samples should be allowed to clot, and the serum separated from the clot as soon as possible. Plasma samples should be centrifuged and removed from the cells immediately after centrifugation. Samples having particulate matter, turbidity, lipemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. **Proper sample handling is crucial to ensuring the integrity of the sample. Samples are stable at room temperature for up to 48 hours. If the assay is performed within 14 days of sample collection, the samples may be kept at 2-8°C, otherwise they should be dispensed in aliquots and stored deep-frozen (-20°C or below).** If samples are stored frozen, mix thawed samples well before testing. Samples may be frozen-thawed **4** times. Self-defrosting freezers are not recommended for sample storage.

The minimum volume required for testing on the analyzer is 225 µL per specimen [75 µL specimen for testing + 150 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)].

9. CALIBRATORS 1 AND 2

The LIAISON® XL 1,25 Dihydroxyvitamin D calibrators are supplied lyophilized. Reconstitute each vial with 2.0 mL of distilled or deionized water. Allow vials to stand for **15** minutes at room temperature and mix by gentle inversion. Transfer a minimum of 400 µL (triplicate calibration) to a glass or plastic sample tube. Affix the appropriate bar code label to the tube and place onto the LIAISON® XL Analyzer or LIAISON® XS Analyzer. Calibrate the assay as described in the Analyzer Operator's Manual.

LIAISON® XL 1,25 Dihydroxyvitamin D calibrators are stable for **6 hours** at room temperature on the LIAISON® XL Analyzer or LIAISON® XS Analyzer, or for **14 days** when stored at 2-8°C. Calibrators may also be aliquoted into a minimum 400 µL and stored frozen at -20°C. Calibrators are stable through **4** freeze thaw cycles. Mix by gentle inversion after freeze thaw cycle prior to use.

Calibrator and Reagent Integral lot number are lot specific. Do not use calibrators matched with a different reagent lot in the same assay.

10. CALIBRATION

Individual LIAISON® XL 1,25 Dihydroxyvitamin D Reagent Integrals contain specific information for calibration of the particular Reagent Integral lot. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibrator vial allows for **5** calibrations to be performed. Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:

- A new lot of Reagent Integral or Starter Reagent is used.
- The previous calibration was performed more than **14** days prior.
- If Quality Control results are out of the expected ranges.
- The analyzer has been serviced.

Refer to the analyzer operator's manual for calibration instructions.

Measuring range: The LIAISON® XL 1,25 Dihydroxyvitamin D assay measures between 5.0 and 200 pg/mL.

The lowest reportable value is 5.0 pg/mL. Values below 5.0 pg/mL should be reported as < 5.0 pg/mL. The highest reportable value without dilution is 200 pg/mL.

Samples that read above the assay range may be diluted with the LIAISON® XL 1,25 Dihydroxyvitamin D Diluent ([REF] 310982) and retested.

Suggested dilution: 1 part sample and 2 parts specimen diluent.

11. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Analyzer.

LIAISON® XL Analyzer and LIAISON® XS Analyzer: Each test parameter is identified via information encoded in the Reagent Integral Radio Frequency Identification transponder (RFID Tag). In the event the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the Reagent Integral; contact your local DiaSorin Technical Support for instruction. For details, refer to the analyzer operator's manual.

The analyzer operations are as follows:

1. Dispense assay buffer and TCEP.
2. Dispense calibrators, controls or specimens.
3. Dispense binding agent
4. Incubate.
5. Dispense coated magnetic particles.
6. Incubate.
7. Wash with Wash/System liquid.
8. Dispense conjugate
9. Incubate
10. Wash with Wash/System liquid
11. Add the Starter reagents and measure the light emitted.

12. QUALITY CONTROL

Quality control is required to be performed once per day of use, or according to the guidelines or requirements of local regulations or accredited organizations. It is recommended that the user refer to CLSI C24-A3, and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® XL 1,25 Dihydroxyvitamin D Controls ([REF] 310984) is intended to monitor for substantial reagent failure. LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should be established for all quality control materials used.

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

13. INTERPRETATION OF RESULTS

The Analyzer automatically calculates the concentration of 1,25 (OH)₂ D in the samples. This concentration is expressed in pg/mL. To convert results to SI units: pg/mL x 2.4 = pmol/L.

14. LIMITATIONS OF THE PROCEDURE

1. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
2. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Proper reconstitution of Binding Agent is essential.
3. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested.
4. Bacterial contamination of samples may affect the test results.
5. Do not heat inactivate serum, or plasma.
6. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.
7. Integrals may not be exchanged between analyzer types (LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.
8. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON® XL or LIAISON® XS).

15. EXPECTED VALUES

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

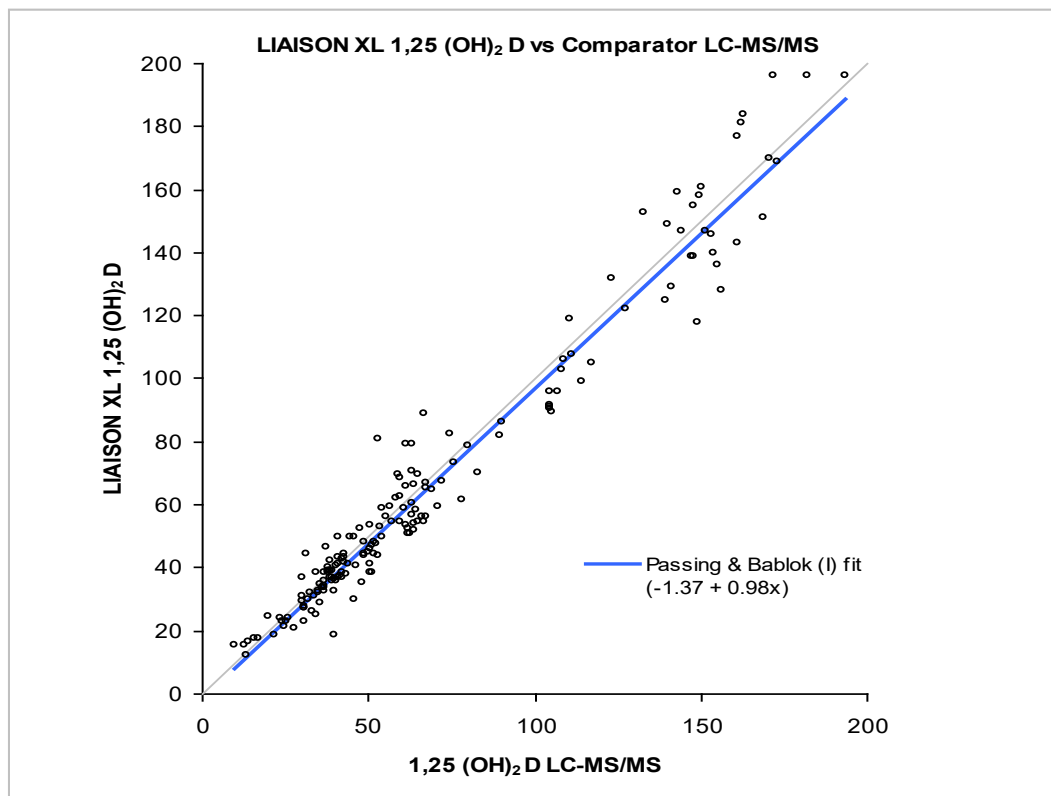
To assess the expected reference range for the LIAISON® XL 1,25 Dihydroxyvitamin D a study was performed with samples from 123 apparently healthy adults aged 21 – 75 years of age from mixed ethnic backgrounds (48% dark-skinned and 52% light-skinned). Samples were collected in the winter (48.8%) and summer (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.

U.S. Subjects	Median 1,25 (OH) ₂ D	Observed Range 2.5 th to 97.5 th Percentile
n = 123	47.8 pg/mL	19.9 – 79.3 pg/mL

16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1 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 = $0.9811x - 1.37$; $R^2 = 0.9588$



16.2 Precision

LIAISON® XL Analyzer

2 lots of kit controls and 6 serum samples spanning the range of the assay were tested twice per day in duplicate, over 20 days using 2 reagent lots on 2 LIAISON® XL Analyzers at DiaSorin Inc. The testing was performed according to CLSI EP5-A2.

Sample ID	n	Mean 1,25 (OH) ₂ D (pg/mL)	Between-Lot		Total	
					(Across Lots)	
			SD	%CV	SD	%CV
Kit Control 1	160	30.9	0.84	2.7%	1.16	3.8%
Kit Control 2	160	122.9	6.09	5.0%	4.36	3.6%
Serum 1	160	23.3	0.05	0.2%	1.53	6.6%
Serum 2	160	38.9	0.63	1.6%	2.20	5.7%
Serum 3	160	52.7	0.64	1.2%	2.65	5.0%
Serum 4	160	76.0	1.33	1.7%	3.13	4.1%
Serum 5	160	137.4	1.91	1.4%	6.55	4.8%
Serum 6	160	193.4	5.53	2.9%	11.34	5.9%

LIAISON® XS Analyzer

2 kit controls and 6 samples containing concentrations of analyte prepared to span the range of the assay were prepared and tested at DiaSorin Inc. once per day in replicates of 6, over 5 operating days on 3 LIAISON® XS Analyzers, using 1 reagent lot of the LIAISON® XL 1,25 Dihydroxyvitamin D assay. The testing was performed according to CLSI EP15-A3.

Sample ID	Mean	Intra-Run		Total within site	
		SD	%CV	SD	%CV
Kit Control 1	30.459	0.722	2.4%	2.176	7.1%
Kit Control 2	92.821	1.820	2.0%	4.570	4.9%
Sample 1	20.382	0.488	2.4%	1.232	6.0%
Sample 2	35.359	0.615	1.7%	1.335	3.8%
Sample 3	45.472	1.117	2.5%	2.825	6.2%
Sample 4	67.861	1.584	2.3%	2.988	4.4%
Sample 5	99.699	2.048	2.1%	4.605	4.6%
Sample 6	146.133	4.092	2.8%	9.270	6.3%

16.3 LoB – Limit of Blank

Following the method from CLSI EP17-A2, the limit of blank for the LIAISON® XL 1,25 Dihydroxyvitamin D is ≤ 0.35 pg/mL.

Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity”.

16.4 LoD – Limit of Detection

Following the method from CLSI EP17-A2, the limit of detection for the LIAISON® XL 1,25 Dihydroxyvitamin D is 0.70 pg/mL.

16.5 LoQ – Limit of Quantitation

Following the method from CLSI EP17-A2, the limit of quantitation for the LIAISON® XL 1,25 Dihydroxyvitamin D is 5.0 pg/mL.

16.6 Linearity

1 sample pool of each type; serum, SST serum, EDTA plasma and Lithium Heparin plasma were diluted and analyzed by the LIAISON® XL 1,25 Dihydroxyvitamin D assay following CLSI EP6-A. The results were analyzed by regression of Observed 1,25 (OH)₂ D Concentration versus Expected 1,25 (OH)₂ D Concentration.

The resulting equations for each sample type are:

Serum: Observed 1,25 (OH)₂ D = $1.014x - 1.936$; $R^2 = 0.9829$

SST Serum: Observed 1,25 (OH)₂ D = $1.028x + 0.2179$; $R^2 = 0.9874$

EDTA plasma: Observed 1,25 (OH)₂ D = $1.02x + 4.807$; $R^2 = 0.9565$

Lithium Heparin plasma: Observed 1,25 (OH)₂ D = $1.023x - 2.441$; $R^2 = 0.9925$

16.7 Recovery

5 high concentration serum samples and 5 low concentration serum samples were analyzed neat. Recovery samples were then prepared by mixing defined ratios of the high and low samples and tested in replicates of 5. The mean results of the 5 replicates are provided in the table below.

Samples	Defined Concentration	Expected pg/mL	Observed pg/mL	% Recovery
High Sample 1 (HS1)	177.4			
2 HS1 : 1 LS1		128.0	121.0	95%
1 HS1 : 1 LS1		102.6	97.3	95%
1 HS1 : 2 LS1		77.2	73.0	95%
Low Sample 1 (LS1)	27.8			
High Sample 2 (HS2)	203.6			
2 HS2 : 1 LS2		151.7	141.6	93%
1 HS2 : 1 LS2		125.0	115.4	92%
1 HS2 : 2 LS2		98.2	89.8	91%
Low Sample 2 (LS2)	46.3			
High Sample 3 (HS3)	181.6			
2 HS3 : 1 LS3		134.2	127.0	95%
1 HS3 : 1 LS3		109.8	101.8	93%
1 HS3 : 2 LS3		85.4	81.7	96%
Low Sample 3 (LS3)	38.0			
High Sample 4 (HS4)	201.6			
2 HS4 : 1 LS4		143.0	137.6	96%
1 HS4 : 1 LS4		112.9	105.4	93%
1 HS4 : 2 LS4		82.7	77.4	94%
Low Sample 4 (LS4)	24.2			
High Sample 5 (HS5)	223.8			
2 HS5 : 1 LS5		158.3	148.4	94%
1 HS5 : 1 LS5		124.6	111.8	90%
1 HS5 : 2 LS5		90.8	85.1	94%
Low Sample 5 (LS5)	25.3			
Mean Recovery				94%

16.8 Interfering Substances

Controlled studies of potentially interfering substances performed in serum at two 1,25 (OH)₂ D levels (20 and 60 pg/mL) showed no interference in the LIAISON® XL 1,25 Dihydroxyvitamin D at the highest concentration for each substance listed below. The testing was based on CLSI EP7-A2.

Drug/Substance	Concentration Tested
Hemoglobin	300 mg/dL
Bilirubin (conjugated)	40 mg/dL
Bilirubin (unconjugated)	40 mg/dL
Triglycerides	3,000 mg/dL
Cholesterol	400 mg/dL
Albumin	12 g/dL
Uric Acid	20 mg/dL
HAMA	3774 ng/mL
Rheumatoid Factor	7310 IU/mL
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	65 mg/dL
Salicylic Acid	60 mg/dL
Ibuprofen	50 mg/dL
Biotin	0.1mg/dL
Ascorbic Acid	6 mg/dL
Metoprolol	1.2 mg/dL
Propanolol Hydrochloride	0.23 mg/dL
Hydrochlorothiazide (HCTZ)	0.6 mg/dL
Furosemide	6 mg/dL
Valproic Acid	57.6 mg/dL
Spirolactone	0.6 µg/mL
Nifedipine	43 µg/dL
Verapamil	216 µg/dL
Losartan Potassium	2.25 µg/mL
Valsartan	11 µg/mL
Tetracycline	15.1 µg/mL
Enalapril	42.4 µg/dL
Doxycycline	34.6 µg/mL
Lisinopril	32.7 µg/dL

16.9 Cross-reactants

Control Studies of potentially cross-reacting substances were performed on the LIAISON® XL 1,25 Dihydroxyvitamin D assay at the concentrations listed below. Testing was based on CLSI EP7-A2.

Cross-Reactant	Spiked Concentration	% Cross Reactivity
1,25 (OH) ₂ D ₃	100 pg/mL	100%
1,25 (OH) ₂ D ₂	100 pg/mL	104%
Zemplar	100 pg/mL	113%
25(OH)D ₃	100,000 pg/mL	<0.1%
25(OH)D ₂	50,000 pg/mL	<0.1%
24,25 (OH) ₂ D ₃	50,000 pg/mL	<0.1%
25,26 (OH) ₂ D ₃	50,000 pg/mL	<0.1%
3-epi 25 (OH)D ₃	50,000 pg/mL	<0.1%
Vitamin D ₂	50,000 pg/mL	<0.1%
Vitamin D ₃	50,000 pg/mL	<0.1%
Sensipar	50,000 pg/mL	<0.1%

16.10 High Dose Hook Effect

No high dose hook effect was observed for 1,25 Dihydroxyvitamin D concentrations up to 5000 pg/mL.

17. References

1. Haddad, J.G. and T.C.B. Stamp, "Circulating 25-Hydroxyvitamin D in Man" American Journal of Medicine, 57: 57, (1974).
2. Holick, M.F., "The Cutaneous Photosynthesis of Previtamin D₃: A Unique Photoendocrine System," Journal of Investigative Dermatology, 76: 51, (1981).
3. Harrison, J.E., A.J.W. Hitchman, G. Jones, C.S. Tam and J.N.M. Heersche, "Plasma Vitamin D Metabolite Levels in Phosphorus Deficient Rats During the Development of Vitamin D Deficient Rickets," Metabolism, 31 (11): 1121, (1982).
4. DeLuca, H.F., "The Vitamin D Systems: A View From Basic Science to the Clinic," Clinical Biochemistry, 14: 213, (1981).
5. Norman, A.W. and F.P. Roos, "Vitamin D Seco-Steroids: Unique Molecules with Both Hormone and Possible Membranophilic Properties," Life Science, 24: 759, (1979).
6. DeLuca, H.F. and H.K. Schnoes, "Metabolism and Mechanism of Action of Vitamin D," Annual Reviews Biochemistry, 45: 631, (1976).
7. K/DOQI: Kidney Disease Outcomes Quality Initiative; Clinical Practice Guidelines for Bone Metabolism and Disease in Chronic Kidney Disease
8. KDIGO: Kidney Disease: Improving Global Outcomes
9. Aarskog, D., L. Aksnes and T. Markestad, "Effect of Parathyroid Hormone on Vitamin D Metabolism in Osteoporosis," Pediatrics, 68 (1): 109, (1981).
10. Chesney, R.W., "Current Clinical Applications of Vitamin D Metabolite Research," Clinical Orthopedics and Related Research, 161: 285, (1981).
11. Sorensen, O.H., B. Lumholtz, B. Lund, I. Hjelmstrand, L. Mosekilde, F. Melsen, J.E. Bishop and A.W. Norman, "Acute Effects of Parathyroid Hormone on Vitamin D Metabolism in Patients with the Bone Loss of Aging," Journal of Clinical Endocrinology and Metabolism, 54 (6): 1258, (1982).
12. Clinical and Laboratory Standards Institute (CLSI) C24-A3, Vol.19, No.5, Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline - Third Edition
13. Clinical and Laboratory Standards Institute (CLSI) EP28-A3C, Vol.28, No.30; Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline – Third Edition
14. Clinical and Laboratory Standards Institute (CLSI) EP17-A2, Vol.32, No.8, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline June 2012 – Second Edition.
15. Clinical and Laboratory Standards Institute (CLSI) EP09-A3, Vol.33, No.11 Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline - Third Edition
16. Clinical and Laboratory Standards Institute (CLSI) EP5-A2, Vol.24, No.25, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition
17. Clinical and Laboratory Standards Institute (CLSI) EP6-A, Vol.23, No.16 Evaluation of Linearity of Quantitative Analytical Methods; Proposed Guideline - Approved Guideline
18. Clinical and Laboratory Standards Institute (CLSI) EP7-A2, Vol.25, No.27 Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition
19. Clinical and Laboratory Standards Institute (CLSI) EP15-A3, Vol.34, No.13, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Third Edition



DiaSorin Inc.
1951 Northwestern Avenue
Stillwater, MN 55082
USA

UK Responsible Person:
DiaSorin Italia S.p.A.
UK Branch
Central Road
Dartford Kent DA1 5LR
UK



DiaSorin **Italia** S.p.A.
Via Crescentino snc
13040 Saluggia (VC)
Italy