25 OH Vitamin D

Purpose

This procedure provides instructions for performing 25 OH VITAMIN D on the DIASORIN LIAISON.

Policy Statements

This procedure applies to all laboratory technical staff responsible for performing Vitamin D testing on the DiaSorin Liaison.

Principle

The Liaison 25 OH Vitamin D Total Assay uses direct competitive chemiluminescent immunoassay (CLIA) technology for the quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites in human serum, or plasma, useful in the assessment of Vitamin D sufficiency. Results should be used in conjunction with other clinical or laboratory data to assist the clinician in making patient management decisions.

Clinical Significance

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, primarily salmon and mackerel. Vitamin D_2's nutrition sources are from some vegetables, yeast, and fungi. The vegetarian diet is abundant in Vitamin D.

Vitamin D (D_3, D_2 and metabolites) is converted to 25-hydroxyvitamin D in the liver. The measurement of 25-OH vitamin D concentration in serum or plasma is the best indicator of vitamin D nutritional status.

Recent studies have identified an inactive 3-epimer of 25-OH vitamin D, which may be present in the serum of infants under the age of one year. Thus it is important that the assay avoids measuring the inactive 3-epi form and only measure the active D_3 and D_2 forms equally.

Instrument

DiaSorin LIAISON®, DiaSorin, Inc. Stillwater, MN
Sunquest Method Code: **LIAS**

Sunquest Test Code

VDT: Vitamin D in Serum

Sample

Serum (no gel) collected by venipuncture. Capillary samples are not recommended. Fasting samples are recommended, but not required. Refer to specimen collection procedures.

Minimum volume: 0.250 mL

Stability: 2-8 °C / 5 days, 45 days at -20 ºC or colder

Rejection criteria: Unlabeled tube

Preparation:

- Whole blood specimens should be centrifuged as soon as clotted, according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
- Clarify samples having particulate matter, turbidity, lipemia, or erythrocyte debris
- Transfer serum to a properly labeled tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
- If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freeze-thaw cycles. Check for and remove air bubbles before assaying

Materials

Reagents	Supplies	Equipment
LIAISON® 25 OH Vitamin D TOTAL Assay Integral, supplied ready to use Materials required but not provided LIAISON® Module (part # 319130) LIAISON® Cleaning Kit (part # 310990) LIAISON® Starter Kit (part # 319102) LIAISON® Light Check 12 (REF 319150) LIAISON® Wash/System Fluid (part # 319100) LIAISON® Waste Bags (part # 450003) LIAISON® 25 OH Vitamin D TOTAL Control Set (part # 310601) Maine Standards Validate® Vit D Calibration Verification / Linearity Test Kits.	• Transfer Pipet capable of delivering 250 µL • Glass or polypropylene sample tubes	DiaSorin Liaison System

Materials (cont)

Re-suspension of Magnetic Particles

Magnetic particles must be completely re-suspended before the integral is placed on the instrument.

- Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the color of the suspension has changed to brown (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have re-suspended.
- Alternatively, the LIAISON® Xcelerator (Cat. #A0090) may be used to aid in re-suspension.

Foaming of Reagents

- Visually inspect the reagents, calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present. 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 for a minimum of 30 minutes.

Storage and Stability of the Reagent Integral

- Store the reagent integral in the dark in an upright position.
- Stored unopened, protected from light, and kept upright, the reagents are stable at 2-8°C until the expiration date. Do not freeze. Do not use past the expiration date.
- Once opened, the reagent integral is stable for 4 weeks, sealed with the tape provided and stored at 2-8°C in the dark.

Special Safety Precautions

- Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands at the end of each assay.
- Some reagents contain sodium azide as a preservative. Because sodium azide may form explosive lead or copper azide in plumbing, it is recommended that drains be thoroughly flushed with water after disposal of solutions containing sodium azide.

Calibration

The predefined Master Curve is adjusted to an instrument-specific curve using the two calibrators supplied in the Reagent Integral.

Recalibration is required:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- Every 7 days.
- After servicing the LIAISON® Analyzer.
- If quality controls are out of your acceptable range.

Analytical Measuring Range**.** 4.0 - 150 ng/mL.

Analytical Measuring Range (AMR)

Verification of AMR is accomplished once every 6 months with Maine Standards Validate® Vit D Calibration Verification / Linearity Test Kits. (Order number: 506)

- Each test kit consists of one bottle each of Levels 1 through 5 plus a Base Matrix.
- Each bottle of 1.5 milliliters contains 25-hydroxyvitamin D in a human serum-based matrix.
- There exists a linear relationship in Levels 1 through 5.

Storage and Stability

- Store at -10° to -25°C.
- ***Do NOT store in a frost-free freezer.***
- Test kits are stable until the expiration date printed on the bottle and storage container when handled according to instructions.
- **A maximum of four (4) freeze-thaw cycles is recommended.**

Preparation and Use

1. Prior to use, remove the **VALIDATE** VIT D Calibration Verification / Linearity Test Kit from storage and allow to come to room temperature (18° to 25ºC).
2. Invert gently several times before dispensing.
3. Minimize exposure to room temperature to maximize stability.
4. Tightly cap opened bottles and return to -10° to -25ºC immediately after dispensing.
5. Discard any solutions that appear to have gross bacterial contamination.
6. Analyze each level in triplicate; use a random analytical sequence to assay each level.
7. Enter results in EP Evaluator or submit data to Maine Standards for analysis.
8. If 25 OH Vitamin D TOTAL Calibration Verifiers lie outside the expected ranges, calibration of the LIAISON® 25 OH Vitamin D TOTAL Assay should be repeated and Calibration Verifiers should be retested.

Quality Control

DiaSorin LIAISON® 25 OH Vitamin D TOTAL Control Set

4 vials (2 mL each), containing human serum with buffer salts and <0.1% sodium azide as a preservative.

- Control Level 1 (2 each)
- Control Level 2 (2 each)
- Bar Code Labels for Controls 1 and 2

Frequency: Day of use or daily. Load one vial of each level of control into the “C” rack, or transfer 150 µL of each level to a tube. Affix the appropriate bar code label to the tube and place onto the LIAISON®

Stability: Stable until the date on vial when stored at 2-8°C. Open vial stability is 4 weeks when properly stored. Indications of possible deterioration include the presence of particulate matter in the liquid or significant deviation from previous results.

Sunquest Control names:
Level 1 = C-VTD1, Level 2 = C-VTD2 in LIS

Acceptable ranges: Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. Do not report patient results until control results are within expected ranges.

Interpretation

Vitamin D status	25 OH Vitamin D
Deficiency	< 10 ng/mL
Insufficiency	10-30 ng/mL
Sufficiency	30-100 ng/mL
Toxicity	>100 ng/mL

Dilutions

Do not dilute. See result Reporting.

Reference Intervals

30-100 ng/mL

Limitations

Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results.

The antibody utilized in this assay will demonstrate cross-reactivity to many dihydroxylated metabolites of Vitamin D in humans, these compounds are naturally present in picomolar concentrations.

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.

Interfering Substances

- Gross hemolysis falsely elevates Vitamin D using this assay. Append the comment "HP" (Hemolysis present, may affect results) to moderate to grossly hemolyzed samples.
- Controlled studies of potentially interfering substances showed that the assay performance was not affected by hemolysis (up to 200 mg/dL hemoglobin), lipemia (up to 549 mg/dL triglycerides), bilirubinemia (up to 20 mg/dL bilirubin) or cholesterolemia (up to 259 mg/dL cholesterol).

Result Reporting

- Confirm all results below 15 ng/mL by repeat analysis
- Results between 4 – 150 ng/mL without error messages are released
- Results below 4.0 that have been confirmed by repeat testing are reported as <4.0 ng/mL
- Results > 150 ng/mL without error messages are reported as > 150 ng/mL
- Append the comment "HP" (Hemolysis present, may affect results) to moderate to grossly hemolyzed samples

Alternate Methods

When test performance does not meet quality standards, refer testing to Mayo Medical Laboratory. Order test 25HDN, and submit 0.5 mL of serum, 0.25 mL minimum

References

1. LIAISON 25 OH Vitamin D TOTAL Assay (310600) Directions for Use, 01/2009, DiaSorin, Inc, 1951 Northwestern Ave, Stillwater, MN
2. LIAISON® 25 OH Vitamin D TOTAL Control Set (310601) IFU, us310601; 35810 4/11, DiaSorin, Inc, 1951 Northwestern Ave, Stillwater, MN
3. For Customer Service in the US call toll free: 1-800-328-1482 or 651.439.9710

Appendices

Specificity

Steroid	% Cross-reactivity
25 OH Vitamin D_2	104%
25 OH Vitamin D_3	100%
Vitamin D_2	<1%
Vitamin D_3	<1%
1,25-$(OH)_2$ Vitamin D_2	40%
1,25-$(OH)_2$ Vitamin D_3	17%
3-epi-25 OH Vitamin D_3	<1%

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	Linda Lichty	January 17, 2011	Initial Version
2.	Linda Lichty	April 17, 2013	Revised AMR material
3.	Linda Lichty	March 17, 2014	Revised Control stability
4.	Linda Lichty	November 9, 2015	Retest results <10.0
5.	Linda Lichty	July 7, 2016	Retest results <15.0