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25-Hydroxy Vitamin D Assay
For Automated Chemistry Analyzer

Configuration

The Diazyme 25-Hydroxy (25-OH) Vitamin D Assay is provided in the following configuration (80 tests):

Automated Chemistry Analyzer	REF	Kit size
Roche Modular P	DZ688C-K	Diluent: 1 x 17 mL Reagent R1: 1 x 8.5 mL Reagent R2: 1 x 17 mL Reagent R3: 1 x 8.5 mL Calibrators: 5 x 1 mL

Intended Use

The Diazyme 25-OH Vitamin D Assay is intended for use in clinical laboratories for the quantitative determination of total 25-OH Vitamin D in human serum and plasma on automated chemistry analyzer. Measurement of 25-hydroxy Vitamin D (25-OH Vitamin D) is for the assessment of vitamin D sufficiency. For *in vitro* diagnostic use only.

Clinical Significance

Vitamin D is a steroid hormone involved in the active intestinal absorption of calcium and in the regulation of its homeostasis. Vitamin D has two forms: Vitamin D2 and Vitamin D3. Vitamin D2 is obtained from dairy products whereas Vitamin D3 is produced in the skin after exposure to ultraviolet light. In the liver, Vitamin D is hydroxylated at its carbon 25 to form 25-OH Vitamin D. This metabolite is the predominant circulating form of Vitamin D and is considered to be an accurate indicator of the general Vitamin D status of an individual. Vitamin D deficiency has been linked to many diseases including osteoporosis, rickets, and osteomalacia[1]. Both dietary supplements of Vitamin D that are currently available in the market (Vitamin D2 and Vitamin D3) are converted to 25-OH Vitamin D in the liver. The sum of the concentrations of 25-OH Vitamin D2 and 25-OH Vitamin D3, in serum or plasma, is referred to as "Total 25-OH Vitamin D". Accurate monitoring of total 25-OH Vitamin D level is critical in clinical settings[1-5].

Assay Principle

The test is based on the principle of α-complementation of the enzyme β-galactosidase and the competition between an enzyme donor-25-OH Vitamin D conjugate, an anti-Vitamin D antibody and the 25-OH Vitamin D content of a serum sample. Samples with higher 25-OH Vitamin D concentrations produce higher β-galactosidase activities and *vice versa*. A nitro-phenyl-ß-galactoside derivative (NPG) is used as the enzyme substrate. The reaction's product has maximum absorbance at 415 nm. The 25-OH Vitamin D concentration of a sample is proportional to the measured β-galactosidase activity.

Reagents

DILUENT: 10mM sodium phosphate, 2.7mM KCl, 137mM NaCl, 0.1% sodium azide, < 0.1mg/mL Vitamin D sheep monoclonal antibody.
REAGENT 1: <3% acetic acid/sodium acetate, <10 mg/mL NPG substrate and 0.05% polysorbate 20.
REAGENT 2: 38mM sodium phosphate, 0.1%sodium azide.
REAGENT 3: 50mM Tris-HCl, 200mM sodium chloride, 10% glycerol, 0.1% sodium azide, <1mg/mL enzyme acceptor.
CALIBRATOR 1 – 5: Human serum containing specific amounts of 25-OH Vitamin D and 0.1% sodium azide.

Reagents Storage and Stability

Calibrators and Controls

CALIBRATOR and CONTROL are serum-based solutions and are stable when stored at 2-8°C until the expiration date on the label. Mix vials well before assaying.

Diluent

This solution is ready to use and is stable when stored at 2-8°C until the expiration date on the label. After use, the solution should be kept capped in its reagent bottle.

Reagents 1, 2 and 3

The REAGENT are ready to use and are stable when stored at 2-8°C until the expiration date on the label. After use, the REAGENT should be kept capped in their corresponding reagent bottles.

Specimen Collection and Handling

Serum, K_3-EDTA plasma or Li-heparin plasma samples can be used for the assay. Method comparison of K_3-EDTA plasma samples versus serum samples yielded a regression equation of y = 0.9948x - 0.7057 and an R^2 = 0.9866. Method comparison of Li-heparin plasma samples versus serum samples yielded a regression equation of y = 0.9657x – 0.6596 and R^2 = 0.9736.

For plasma, mix the sample by gentle inversion prior to centrifugation. Centrifuge and separate serum or plasma as soon as possible after collection. The specimens may be refrigerated at 2-8°C for two weeks. For long term storage, they can be stored at -20°C. Avoid repeated freeze-thaw cycles. Do not use hemolysed serum or plasma samples. Samples showing clear signs of hemolysis should not be used because excess hemoglobin (> 100 mg/dL) may interfere with the assay's results. Allow the refrigerated or frozen-thawed samples to equilibrate to room temperature for 30 minutes before use; samples must be mixed well before analysis.

Precautions

1. **DO NOT INGEST**. Avoid contact with skin and eyes.
2. REAGENT contain sodium azide, which may react with lead or copper plumbing to form explosive compounds. Flush drains with copious amounts of water when disposing of reagents.
3. Specimens containing human sourced materials should be handled as if potentially infectious, using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories, 5th Ed (HHS Publication Number [CDC] 21-1112).
4. CALIBRATOR and CONTROL contain human source material. Each donor unit of serum in the preparation of these materials were tested by FDA-approved methods and found negative for the Human Immunodeficiency Virus Antibody (HIV I/II Ab), Hepatitis B Surface Antigen (HBsAg), and Hepatitis C Virus Antibody (HCV). Because no method can offer complete assurance as to the absence of infectious agents, this material and all samples should be handled as though capable of transmitting infectious disease and such biohazardous material should be disposed of according to relevant local, state or federal regulations.
5. Additional safety information concerning storage and handling of this product is provided within the Material Safety Data Sheet for this product. To obtain an MSDS, please contact our customer service department at 858-455-4768 or email support@diazyme.com.

Materials Provided

Please see "Reagents" section.

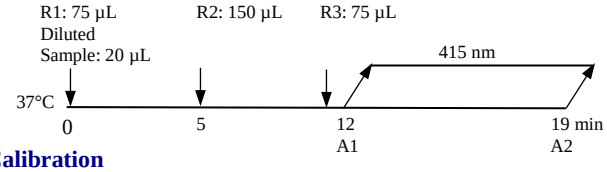
Materials Required but not Provided

The Diazyme 25-OH Vitamin D Control Set (REF DZ688C-CON) contains human serum containing specific amounts of 25-OH Vitamin D. CONTROL material is supplied in aliquots of 1.0 mL. CONTROL 1 has a concentration of 25-OH Vitamin D that corresponds to an insufficient sample. CONTROL 2 has a concentration of Vitamin D that corresponds to a sufficient sample. The Control Set is sold as 2 x 1 mL vials.

Assay Procedure

The assay procedure for the Roche Modular P chemistry analyzer is shown below:

- Specimen (CALIBRATOR, CONTROL and samples) are first diluted on-board: 20 µL of serum are diluted with 155 µL of Diluent. 20 µL of the diluted specimen is then used for analysis.
- Schematic:



Calibration

The Diazyme 25-OH Vitamin D Calibrator Set (REF DZ688C-CAL) is a five calibrator set that is provided in the kit.

Calibration Frequency: The CALIBRATOR should be used to calibrate the assay before each run.

Quality control

We recommend that each laboratory use the Diazyme 25-OH Vitamin D Control Set to validate the performance of REAGENT. These controls can be purchased separately. Follow federal, state, and local guidelines for testing quality control materials.

Results

Results are expressed in ng/mL. Note: Samples with values greater than 147.8 ng/mL should be reported as >147.8 ng/mL. Samples with values less than 7.6 ng/mL should be reported as <7.6 ng/mL.

Reference Range

Reference range of the Diazyme 25-OH Vitamin D assay was determined by measuring the 25-OH Vitamin D serum concentrations of a USA population of 157 apparently healthy adults, 21-80 years old, during the months of October and November (fall season). Individuals were from three different geographical locations: 47 from Pennsylvania (Northern US), 56 from Tennessee (Central US) and 54 from Texas (Southern US). All 157 individuals did not have kidney disease, GI disease, liver disease, calcium-levels related disease, thyroid disease, parathyroid disease, seizures, chronic disease or bariatric surgery.

The 2.5th to 97.5th percentile range was 15.0 to 45.9 ng/mL. The median concentration was 25.6 ng/mL.

Limitations

1. The assay is designed for use with human serum and plasma samples only.
2. Samples suspected of containing analyte values greater than 147.8 ng/mL should be reported as >147.8 ng/mL.
3. As with any diagnostic test it is possible that technical, procedural errors as well as substances and factors not listed may interfere with the proper functioning of the test kit.
4. Any visibly hemolyzed samples should not be used.
5. Heterophilic antibodies in human serum can react with reagent immunoglobulins or other reagent material, interfering with in vitro immunoassays. 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 obtained. 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.

Performance Characteristics

The following performance data was tested on Roche Modular P.

Sensitivity

The Limit of detections were determined according to the CLSI EP17-A guideline.

Limit of Blank LoB

Vitamin D-depleted serum was assayed with the Diazyme Vitamin D assay in three independent runs with 20 replicates per run. The LoB was calculated as the mean of the 57th and 58th highest values for the blanks. The LoB of the assay was 2.0 ng/mL.

Limit of Detection LoD

Five very low Vitamin D serum samples were measured in three independent runs, with 4 replicates per run. The LoD was defined as LoD = LoB + (1.645 * Standard Deviation of Low samples). The LoD of the assay was 3.5 ng/mL.

Limit of Quantitation LoQ

Five low Vitamin D samples were measured in 40 replicates obtained from five independent runs. The LoQ was measured as the lowest concentration with a CV of 20%. The LoQ of the assay was 7.6 ng/mL.

Accuracy

The performance of this assay was compared to the performance of a legally marketed 25-OH Vitamin D enzyme immunoassay. The results for 98 serum samples are shown in the table below:

Deming Regression Analysis	95% Confidence Interval
Slope	1.005 (0.969 to 1.041)
Intercept	-0.21 (-2.15 to 1.73)
Correlation Coefficient	0.984 (0.976 to 0.989)
Range	9.5-140.9

Precision

Precision was evaluated according to the CLSI EP5-A guideline. Controls and samples were measured daily over the span of 20 days, using three lots of reagents and one chemistry analyzer. 40 independent runs were performed on each specimen. Each run produced two measurements. 80 data points were obtained per specimen. Results are shown below:

25-OH Vitamin D (ng/mL)			Within-run		Between-run		Total	
Specimen	n	Mean	SD	%CV	SD	%CV	SD	%CV
Control #1	80	23.1	1.47	6.4	1.04	4.5	1.68	7.3
Control #2	80	45.7	2.06	4.5	1.67	3.7	2.12	4.6
Sample #1	80	22.6	1.19	5.3	1.11	4.9	1.45	6.4
Sample #2	80	31.7	1.42	4.5	1.59	5.0	1.81	5.7
Sample #3	80	40.6	1.42	3.5	1.59	3.9	1.66	4.1
Sample #4	80	48.6	2.32	4.8	1.71	3.5	2.41	4.9
Sample #5	80	55.8	2.14	3.8	1.73	3.1	2.34	4.2
Sample #6	80	65.4	2.03	3.1	1.79	2.7	2.42	3.7
Sample #7	80	69.7	2.02	2.9	1.99	2.9	2.55	3.7
Sample #8	80	92.8	2.52	2.7	2.02	2.2	3.40	3.7
Sample #9	80	134.6	2.97	2.2	2.69	2.0	3.87	2.9
Low Sample#1	80	9.4	1.22	13.0	0.98	10.4	1.31	14.0
Low Sample#2	80	11.2	1.58	14.2	0.88	7.9	1.55	13.9

Linearity

Eleven levels of linearity were prepared by diluting a high serum sample with Vitamin D-depleted serum. Linearity levels were prepared according to the CLSI EP6-A guideline. Measurements were done in triplicates. The assay was found to be linear between 7.6 and 147.8 ng/mL.

Interference

Interference studies were conducted according to the CLSI EP7-A2 guideline. The acceptance criterion was set at 10% or less deviation between the spiked sample and the control. The assay's results were not significantly affected by the following substances:

Substance	Concentration
Conjugated Bilirubin	40 mg/dL
Free Bilirubin	40 mg/dL
Hemoglobin	100 mg/dL
Ascorbic Acid	176 mg/dL
Triglycerides	750 mg/dL
Uric Acid	20 mg/dL
Biotin	2 mg/dL
Human Serum Albumin	9 g/dL
N-Acetyl Cysteine Amide	1663 ng/mL
Ampicillin	1000 ng/mL
Cyclosporine C	105 ng/mL
Cefoxitin	660 ng/mL
Acetylsalicylic Acid	1000 ng/mL
Rifampicin	64 ng/mL
Acetaminophen	200 ng/mL
Ibuprofen	500 ng/mL
Theophylline	100 ng/mL

Cross-reactivity of the Diazyme 25-OH Vitamin D Assay was determined by adding Vitamin D metabolites to serum pool samples. Based on the results in the table below, the assay did not cross react with Vitamin D2 and Vitamin D3 and the assay recovers both 25-OH Vitamin D2 and 25-OH Vitamin D3 similarly. Cross-reactivity with various Vitamin D metabolites is summarized in the table below:

Compound	Concentration tested	Cross-reactivity
25-OH Vitamin D3	44.0 ng/mL	100%
25-OH Vitamin D2	44.0 ng/mL	92.3%
Vitamin D3	44.0 ng/mL	1.0%
Vitamin D2	44.0 ng/mL	2.9%
1,25-(OH)2 Vitamin D3	2.9 ng/mL	2.5%
1,25-(OH)2 Vitamin D2	2.9 ng/mL	-1.5%
24R,25-(OH)2 Vitamin D3	41.0 ng/mL	5.1%
3-epi-25-OH Vitamin D3	42.0 ng/mL	61.7%
3-epi-25-OH Vitamin D2	42.0 ng/mL	55.1%

**% Cross-reactivity = (Corrected Assay Value /Concentration Spiked)*100*

No significant cross-reactivity (4.1%) was found for Paricalcitol (Zemplar®) up to 25 ng/mL.

References

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