SPECTRUM

The Creative Approach to Bioscience

25-OH Vitamin D (FOR *TWIN D ANALYZER*)
Enzymatic Immunoassay

REF 399 001	50 test	REF 399 002	100 test
Diluent (D)	1 x 8.25 ml	Diluent (D)	1 x 16.5 ml
Reagent(R1)	1 x 4.25 ml	Reagent(R1)	1 x 8.5 ml
Reagent(R2)	1 x 8.0 ml	Reagent(R2)	1 x 16.0 ml
Reagent(R3)	1 x 4.25 ml	Reagent(R3)	1 x 8.5 ml
Calibrator 1	1 x 1 ml	Calibrator 1	1 x 1 ml
Calibrator 4	1 x 1 ml	Calibrator 4	1 x 1 ml
Calibrator Diluent	1 x 2 ml	Calibrator Diluent	1 x 2 ml
Control Level 1	1 x 1 ml	Control Level 1	1 x 1 ml
Control Level 2	1 x 1 ml	Control Level 2	1 x 1 ml

Intended Use

25-OH Vitamin D Assay is intended for use in clinical laboratories for the screening of total 25-OH Vitamin D in human serum . Measurement of 25-hydroxy Vitamin D is for the assessment of vitamin D sufficiency. For *IVD* use only.

Background

The testing of Vitamin D in serum is an important tool for physicians and individuals to determine whether or not one is deficient in the vitamin D
The role of vitamin D in regulating circulating levels of calcium and phosphorus to ensure normal bone mineralization is well known.
Emerging evidence correlates insufficient levels of vitamin D to an increased risk of developing non-skeletal pathologies: cardiovascular diseases, hypertension, cancer, diabetes, multiple Sclerosis, rheumatoid arthritis, infectious diseases. Weak immune system (colds and flu)
The diverse effects of vitamin D are mediated by receptors that regulate more than 200 genes. Besides the receptors present in the intestine and the bone, vitamin D Receptors have been identified in brain, prostate, breast, colon, immune cells, Vascular smooth muscle and cardiomyocytes plus 17 types of cancers and Alzheimer's and Depression.

Assay Principle

The test is based on the principle of α--complementation of the enzyme ß - 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 ß- galactosidase activities and *vice versa*.
A nitro-phenyl-ß-galactoside derivative (NPG) is used as the enzyme substrate
The 25-OH Vitamin D concentration of a sample is proportional to the measured ß -galactosidase activity.

Reagents

DILUENT (D): 10mM sodium phosphate, 2.7mM KCl, 137mM NaCl, 0.1% sodiumazide, < 0.1mg/mL Vitamin D sheep monoclonal antibody.
R1 : <3% acetic acid/sodium acetate,<10 mg/mL NPG substrate and 0.05%polysorbate 20.
R 2: 38mM sodium phosphate, 0.1%sodiumazide.
R 3: 50mM Tris-HCl, 200mM sodium chloride, 10% glycerol, 0.1% sodium azide, <1mg/mL enzyme acceptor.
Calibrator 4 (CAL4) pooled human serum and additives.
Calibrator 1 (CAL1) pooled human serum and additives.
Calibrator Diluent. (CALD)
Control Level1 (L1) pooled human serum and additives.
Control Level2 (L1) pooled human serum and additives.

Other Component provided with the kit:

Calibration Card: Smart card loaded with Vitamin D
Calibration curve to calibrate the Twin D analyzer before assaying Vitamin D. This Card is lot dependent.

Reagents preparation and stability

Diluent
This solution is ready to use and is stable when stored capped at 2-8°*C* until the expiration date on the label.

Reagents 1, 2 and 3
The unopened Reagents are ready to use and are stable when stored and capped at 2-8°*C* until the expiration date on the label..
Opened vials are stable for two months.

Calibrators, Calibrator Diluent and Controls
The unopened Vials are ready to use and are stable when stored and capped at 2-8°*C* until the expiration date printed on the label.
Opened vials are stable for 4 Weeks.

Precaution

1. **DO NOT INGEST**. Avoid contact with skin and eyes.
2. Reagents 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,

Specimen Collection and preservation

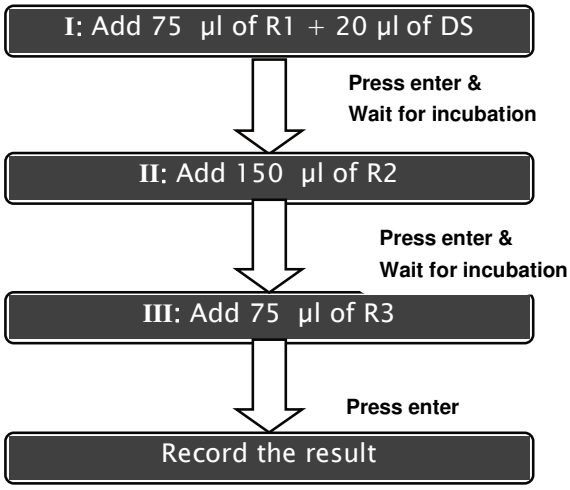
Centrifuge and separate serum as soon as possible after collection. Fresh sample is required. Do not use hemolysed serum samples. Samples showing clear signs of hemolysis should not be used.

Calibration

Use the calibration card provided with the kit
" Please see the instructions on the Twin D user manual".
Note: Once reagent bottles are opened, the calibration curve is valid for one month.

Assay Procedure

1. Specimens,calibrators and controls are first diluted as following:
20 µL of serum are diluted with 155 µL of Diluent (DS).
2. Mix carefully after each step of the following:



Quality control

Use spectrum 25-OH Vitamin D Control to validate the performance of reagents.

When Twin D analyzer need to be recalibrated?

If The quality control values are out of the assayed range manual recalibration is required. Please use Calibrator1, calibrator4 and calibrator diluent to construct calibration curve according to the 25-OH Vitamin D calibration sheet provided with the kit.

Expected values

Deficiency: < 20 ng/mL
Optimum levels: 25 - 100 ng/mL
Toxicity possible: > 100 ng/mL

Limitations

1. Samples with values less than 7.5 ng/mL should be reported as <7.5 ng/mL. Such samples are recommended to be retested by ELISA.
2. Samples suspected of containing analyte values greater than 160 ng/mL should be reported as >160 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.

Sensitivity

When run as recommended, The minimum Limit of the assay is 7.5 ng/mL.

Linearity

The assay is linear up to the value of the calibrator**4**.

Interference

Bilirubin up to 40mg/dL
Hemoglobin up to 50 g/dL
Triglyceride up to 750mg/dL
Uric Acid up to 20mg/dL

Accuracy

The performance of this assay was compared to the performance of 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

CV% within the run 2%-14 %, between-run 2.9-14%

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

References

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IFUFCC98 Rev. (3) 4/6/2015

ISO 13485

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