



BIOMED Vitamin D Assay

Intended Use

The BIOMED Vitamin D assay is intended for use in clinical laboratories for the quantitative determination of 25-OH vitamin D (vitamin D) in human serum and plasma, using automated & Semi automated chemistry analyzers. Measurement of vitamin D is used for the assessment of the 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. 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 settings¹⁻⁵.

Assay Principle

The BIOMED Vitamin D Assay is a direct particle-enhanced immuno-turbidimetric assay. The assay's proprietary reagents are designed to dissociate vitamin D from vitamin D binding proteins, found in serum or plasma specimens, while particles coated with anti-vitamin D antibodies bind to the dissociated vitamin D, thereby causing agglutination. This agglutination is detected as an absorbance change (630 nm), with the magnitude of the change being proportional to the quantity of total vitamin D in the sample. Specimen concentrations are determined by interpolation from a 5-point calibration curve prepared from calibrators of known concentrations.

Reagents

REAGENT 1: Phosphate buffer solution (< 100 mM), 0.1% sodium azide.

REAGENT 2: Suspension of latex particles (< 0.5%) coated with anti-vitamin D antibodies, ready to use.

CALIBRATOR 1 – 5: Human serum containing specific amounts of 25-OH Vitamin D and 0.1% sodium azide.

Materials Required but not Provided

An analyzer capable of dispensing two reagents and measuring absorbance at around 630 nm with temperature control (37°C).

Reagent Handling

The BIOMED Vitamin D Assay **REAGENTS** are liquid stable, ready-to-use reagents. Mix by inverting at least 10 times before use.

Reagent Stability and Storage

The **REAGENTS** are stable when stored at 2-8°C until the expiration date on the label. Do not mix **REAGENTS** of different lots. **DO NOT FREEZE**.

Specimen Collection and Handling

Serum, K₂-EDTA plasma, K₃-EDTA plasma or Li-heparin plasma samples can be used for the assay. Method comparison of K₂-EDTA plasma samples versus serum samples yielded a regression equation of $y = 1.0198x - 0.4985$ and $R^2 = 0.996$. Method comparison of K₃-EDTA plasma samples versus serum samples yielded a regression equation of $y = 1.0378x - 1.2959$ and $R^2 = 0.9944$. Method comparison of Li-heparin plasma samples versus serum samples yielded a regression equation of $y = 1.0475x - 1.3749$ and $R^2 = 0.9947$.

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 up to one week. For long term storage, they can be stored at -20°C or below. Avoid repeated freeze-thaw cycles (up to three cycles are acceptable). Do not use highly turbid or highly hemolyzed serum or plasma samples. 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. For *in vitro* diagnostic use only. Rx Only.
2. Caution: Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by laws of the State in which he/she practices, to use or order the use of the device.
3. As with any laboratory test, results should be interpreted considering all other test results and the status of the sample. Do not use the **REAGENT**, **CALIBRATOR**, and **CONTROL** after the expiration date labeled on the outer box.

4. Assay calibration frequency is dependent on instrument used. Additionally, the assay should be recalibrated and controls run with each new lot of reagents.
5. Avoid ingestion and contact with skin and eyes. See Safety Data Sheet (SDS).
6. 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 (HHS Publication Number [CDC] 93-8395).
7. **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.

Warnings

The **REAGENT**, **CALIBRATORS** and **CONTROLS** contains < 0.1% sodium azide, NaN₃, as preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azide. On disposal, flush with a large volume of water to prevent sodium azide buildup.

Assay Procedure

Method : Fixed time

Wave length : 630 nm

Delay time : 20 sec

Read time : 300 sec

Method of calibration : Multi standard non-linear(Line graph)

Number of calibrator : 5 calibrator

NOTE : Reagent blank using calibrator 1

	Reagent blank	Calibrator	Sample
R1	200 µl	200 µl	200 µl
Cal or sample	10 µl cal 1	10 µl	10 µl
Incubate for 300 sec			
R2	50 µl	50 µl	50 µl
Aspirate and read A1 at 20 sec & read A2 at 300 sec N.B work each tube separately			

Calibration

5 levels of the BIOMED Vitamin D calibrators are provided .

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

Following C28-A3 Approved Guideline-Third Edition, reference range of the BIOMED Vitamin D assay was determined by measuring the 25-OH vitamin D serum concentrations of a USA population of 145 apparently healthy adults, 21-67 years old, during the months of April and May (spring season). Individuals were from three different geographical locations: 47 from Pennsylvania (Northern US), 49 from Tennessee (Central US) and 49 from Texas (Southern US). All 145 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 central 95% of the population was found to have a 25-OH vitamin D concentrations ranging between 7.2 and 41.6 ng/mL, with a mean concentration of 20.1 ng/ml.

Limitations

1. The assay is designed for use with human serum and plasma samples only.
2. 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.
3. 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

Sensitivity

The LOB, LOD, and LOQ of the assay were determined following CLSI EP17-A2 guideline.

Limit of Blank LoB

Vitamin D-depleted serum was assayed with the BIOMED Vitamin D assay in five independent (over 5 days) runs with 12 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 1.2 ng/mL.

Limit of Detection LoD

Five very low vitamin D serum samples were measured in four independent runs (over 4 days), with 3 replicates per run. The LoD was defined as $LoD = LoB + (1.645 * \text{Standard Deviation of Low samples})$. The LoD of the assay was 2.9 ng/mL.

Limit of Quantitation LoQ

Five low Vitamin D samples were measured in 40 replicates obtained from five independent runs (5 days). The LoQ was measured as the lowest concentration with a CV of 20%. The claimed LoQ of the assay was 7.6 ng/mL.

Method comparison

The method comparison of the assay was evaluated following CLSI EP9-A3 guideline. A total of 171 serum samples were tested in comparison with a legally marketed 25-OH Vitamin D enzyme

immunoassay. The results for 171 serum samples are shown in the table below:

Deming Regression Analysis	95% Confidence Interval
Slope	1.062 (1.028 to 1.095)
Intercept	-3.03 (-4.94 to -1.11)
Correlation Coefficient	0.9785 (0.970 to 0.9841)
Predicate Range (ng/mL)	8.4 to 146.8

Precision

Precision was evaluated according to the CLSI EP5-A2 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	21.7	0.9	3.9%	0.6	2.8%	1.3	6.2%
Control #2	80	42.5	1.0	2.4%	0.8	2.0%	1.7	3.9%
Sample #1	80	11.1	0.9	8.3%	0.5	4.4%	1.8	16.6%
Sample #2	80	18.2	0.9	4.9%	0.7	3.9%	1.6	8.7%
Sample #3	80	22.1	0.8	3.8%	0.8	3.8%	1.2	5.6%
Sample #4	80	42.8	0.9	2.0%	1.0	2.4%	1.3	3.1%
Sample #5	80	59.5	1.0	1.7%	0.7	1.2%	1.6	2.7%
Sample #6	80	80.2	1.3	1.6%	1.1	1.4%	2.0	2.5%
Sample #7	80	99.5	1.8	1.8%	1.5	1.6%	2.7	2.8%
Sample #8	80	117.6	2.2	1.9%	2.0	1.7%	3.7	3.2%
Sample #9	80	139.2	2.7	1.9%	2.6	1.8%	4.1	2.9%

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 endogenous substances:

Substance	Tolerance	Unit
Free bilirubin	40	mg/dL
Conjugated bilirubin	40	mg/dL
Hemoglobin	600	mg/dL
Total protein	12.0	g/dL
Triglycerides	1000	mg/dL
Rheumatoid Factor (RF)	200	IU/mL

The assay's results were also not significantly affected by the following exogenous substances:

Substance	Tolerance	Unit
Acetaminophen	20.0	mg/dL
Acetyl Salicylic Acid	60.0	mg/dL
Ampicillin	5.3	mg/dL
Ascorbate	3.0	mg/dL
Biotin	100.0	ng/mL
Carbamazepine	3.0	mg/dL
Cefotaxime	180.0	mg/dL
Chloramphenicol	5.0	mg/dL
Creatinine	30.0	mg/dL
Digoxin	6.1	ng/mL
Ethanol	400.0	mg/dL

Ethosuximide	25.0	mg/dL
Furosemide	6.0	mg/dL
HAMA	350	ng/mL
Heparin	3.0	U/mL
Ibuprofen	50.0	mg/dL
Lidocaine	1.2	mg/dL
Lithium Acetate	2.2	mg/dL
Noradrenalin	4.0	ug/mL
Rifampicin	5.0	mg/dL
Theophylline	4.0	mg/dL
Urea	300.0	mg/dL
Uric Acid	20.0	mg/dL
Valproic Acid	50.0	mg/dL
Vancomycin	10.0	mg/dL

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

Compound	Concentration tested	Cross-reactivity*
25-OH Vitamin D3	100.0 ng/mL	100%
25-OH Vitamin D2	100.0 ng/mL	106.9%
Vitamin D3	100.0 ng/mL	-0.8%
Vitamin D2	100.0 ng/mL	-1.7%
1,25-(OH) ₂ Vitamin D3	580.0 pg/mL	0.2%
1,25-(OH) ₂ Vitamin D2	580.0 pg/mL	-0.5%
24R,25-(OH) ₂ Vitamin D3	100.0 ng/mL	118.8%
3-epi-25-OH Vitamin D3	100.0 ng/mL	33.0%
3-epi-25-OH Vitamin D2	100.0 ng/mL	36.5%

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

Paricalcitol did not significantly cross-react with the assay when present at 5 ng/mL.

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