BECKMAN COULTER

ACCESS
Immunoassay Systems

Instructions For Use

Access 25(OH) Vitamin D Total
25(OH) vitamin D

REF B24838

FOR PROFESSIONAL USE ONLY

Rx Only

FOR USE ON ACCESS 2 IMMUNOASSAY SYSTEMS† WITH TEST NAME: VitdA

ANNUAL REVIEW

Reviewed by	Date	Reviewed by	Date

PRINCIPLE

INTENDED USE

The Access 25(OH) Vitamin D Total assay is a paramagnetic particle, chemiluminescent immunoassay for the quantitative determination of total 25-hydroxyvitamin D [25(OH) vitamin D] levels in human serum and plasma using the Access 2 Immunoassay Systems. Results are to be used as an aid in the assessment of vitamin D sufficiency.

† Access 2 and UniCel DxC 600i

SUMMARY AND EXPLANATION

Vitamin D is a lipid-soluble steroid hormone that is produced in the skin through the action of sunlight or is obtained from dietary sources.[1]

The role of vitamin D in maintaining homeostasis of calcium and phosphorus is well established.[2] Chronic severe vitamin D deficiency in infants and children causes bone deformation commonly known as rickets, while in adults, proximal muscle weakness, bone pain and osteomalacia may develop.[3,4] Less severe vitamin D inadequacy may lead to secondary hyperparathyroidism, increased bone turnover, and progressive bone loss, increasing the risk of osteoporosis.[4,5] The presence of the vitamin D receptor in other tissues and organs suggests that vitamin D may also be important in non-skeletal biological processes.[2,6]

Vitamin D exists in two primary forms, vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). Vitamin D_3 is produced from the conversion of 7-dehydrocholesterol in the epidermis and dermis in humans upon exposure to sunlight, and can be found in oil-rich fish (e.g. salmon, mackerel, and herring), egg yolks, and from foods supplemented with vitamin D.[7] Vitamin D_2 is found in certain plants and mushrooms.

Prescription or over-the-counter dietary supplements are also a major source of vitamin D for many people.[2,7] Factors such as latitude, time of the day, aging, increased skin pigmentation, ethnic origin, application of sunscreen and season of the year can dramatically affect the production of vitamin D_3 in the skin and thus the levels of vitamin D in the blood.[2,7]

Vitamin D originating from the skin or the diet is biologically inactive. It enters the circulation bound to vitamin D binding protein (DBP), and is transported to the liver to undergo a hydroxylation to produce 25(OH) vitamin D.[1] 25(OH) vitamin D also circulates as a complex with DBP. It is further metabolized in the kidneys by the enzyme 25-hydroxy vitamin D-1α-hydroxylase to its biologically active form, 1,25-dihydroxyvitamin D.[8] 1,25-dihydroxyvitamin D circulates at levels 1000 times lower than 25(OH) vitamin D and its renal production is tightly regulated by plasma parathyroid hormone levels and serum calcium and phosphorus levels.[7,8]

Serum 25(OH) vitamin D is the major circulating metabolite of vitamin D in the body and reflects vitamin D inputs from cutaneous synthesis and dietary intake. For this reason, serum concentration of 25(OH) vitamin D is considered the standard clinical measure of vitamin D status.[7] Because serum 25(OH) vitamin D will be a mixture of the D_2 and D_3 forms, both the vitamin D_2 and vitamin D_3 forms of vitamin D must be measured to accurately assess total 25(OH) vitamin D levels.

METHODOLOGY

The Access 25(OH) Vitamin D Total assay is a two-step competitive binding immunoenzymatic assay. In the initial incubation, sample is added to a reaction vessel with a DBP releasing agent and paramagnetic particles coated with sheep monoclonal anti-25(OH) vitamin D antibody. 25(OH) vitamin D is released from DBP and binds to the immobilized monoclonal anti-25(OH) vitamin D on the solid phase. Subsequently, a 25(OH) vitamin D analogue-alkaline phosphatase conjugate is added which competes for binding to the immobilized monoclonal anti-25(OH) vitamin D.

After a second incubation, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is inversely proportional to the concentration of 25(OH) vitamin D in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

SPECIMEN

SPECIMEN COLLECTION AND PREPARATION

1. Serum (gel and no gel) and plasma (lithium heparin) are the recommended samples.
2. Do not dilute patient samples as this could lead to incorrect vitamin D results.
3. Observe the following recommendations for handling, processing, and storing blood samples:[9,10,11]
   - Collect all blood samples observing standard precautions for venipuncture.
   - Allow serum samples to clot completely before centrifugation in an upright position. Clotting may be slowed at cooler temperatures, or if patient is on anticoagulant therapy.
   - Keep tubes stoppered at all times.
   - Physically separate serum or plasma from contact with cells as soon as possible.
   - Store samples tightly stoppered at room temperature (15 to 30°C) for no longer than 72 hours.
   - If the assay will not be completed within 72 hours, refrigerate the samples at 2 to 10°C.
   - If the assay will not be completed within 7 days, freeze at -20°C or colder.
   - Frozen specimens can be stored up to one (1) year at -20°C before testing.
   - Thaw samples no more than 3 times.
4. Use the following guidelines when preparing specimens:
   - Ensure residual fibrin and cellular matter have been removed prior to analysis.

- Follow blood collection tube manufacturer's recommendations for centrifugation.

5. Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
6. Do not assay grossly lipemic or hemolyzed samples.

REAGENTS

PRODUCT INFORMATION

Access 25(OH) Vitamin D Total Reagent Pack (for use on Access 2 Immunoassay Systems)

Cat. No. B24838: 100 determinations, 2 packs, 50 tests/pack

- Provided ready to use.
- Store upright and refrigerate at 2 to 10°C.
- Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument.
- To prevent light-induced degradation of the vitamin D molecule, the Access 25(OH) Vitamin D Total assay is provided in an opaque, brown reagent pack.
- To ensure that the paramagnetic particles in the reagent pack are fully suspended, **mix the pack using a vortex mixer immediately before loading the reagent pack on the instrument for the first time.** The requirement to mix the reagent pack by using a vortex mixer is unique to the Vitamin D assay. **Do not mix other Access reagent packs using a vortex mixer.**
- To mix:
  - Start the vortex mixer in the continuous "On" mode (i.e. not 'Auto' or 'Touch' mode), and set it to its maximum speed (i.e. 2,500 to 3,200 rpm).
  - Hold the pack upright by the clip end and place the base of the particle well (R1a) on the vortex pad at a slight downward angle (See Figure 1).
  - Mix the reagent pack continuously (**do not pulse**) for 20 to 30 seconds.
  - It is not necessary to remix packs after loading. Do not mix a punctured pack.

Figure 1

- Stable until the expiration date stated on the label when stored at 2 to 10°C.

- Stable at 2 to 10°C for 28 days after initial use
- Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.
- If the reagent pack is damaged (e.g., broken elastomer), discard the pack.

R1a:	Dynabeads* Paramagnetic particles coated with sheep monoclonal anti-25(OH) vitamin D antibody suspended in TRIS buffered saline, goat IgG, bovine serum albumin (BSA),< 0.1% sodium azide, and 0.1% Proclin** 300
R1b:	Formic Acid, Poly (vinyl alcohol) and 0.1% ProClin 300
R1c:	Formic Acid, Poly (vinyl alcohol) and 0.1% ProClin 300
R1d:	Vitamin D analog-alkaline phosphatase conjugate, ACES, < 0.1% sodium azide, and 0.1% ProClin 300.

*Dynabeads is a registered trademark of Dynal A.S., Oslo, Norway.

**ProClin™ is a trademark of The Dow Chemical Company (“Dow”) or an affiliated company of Dow.

WARNING AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- For hazards presented by the product refer to the following sections: REACTIVE INGREDIENTS and GHS HAZARD CLASSIFICATION.

REACTIVE INGREDIENTS

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Vitamin D PMP (Compartment R1a) — WARNING

H316	Causes mild skin irritation.
H317	May cause an allergic skin reaction.
P261	Avoid breathing vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.

	P280	Wear protective gloves, protective clothing and eye/face protection.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P332+P313	If skin irritation occurs: Get medical advice/attention.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
	P501	Dispose of contents/container in accordance with local/national regulations
		Tris(hydroxymethyl)– aminomethane 1 - 5%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Vitamin D Dissociation Buffer (Compartment R1b)	WARNING	
		
	H315	Causes skin irritation.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P337+P313	If eye irritation persists: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Formic Acid 1 - 3%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Vitamin D Dissociation Buffer (Compartment R1c)	WARNING	
		
	H315	Causes skin irritation.
	H317	May cause an allergic skin reaction.
	H319	Causes serious eye irritation.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.

	P337+P313	If eye irritation persists: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		Formic Acid 1 - 3%
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%
Vitamin D Conjugate (Compartment R1d)	WARNING	
		
	H317	May cause an allergic skin reaction.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at techdocs.beckmancoulter.com

MATERIALS NEEDED BUT NOT SUPPLIED WITH REAGENT KIT

1. Access 25(OH) Vitamin D Total Calibrators (for use on Access 2 Immunoassay Systems)
   Provided at zero and approximately 7, 18, 35, 74 and 167 ng/mL.
   (18, 45, 88, 185 and 418 nmol/L).
   Cat. No. B24839
2. Quality Control (QC) materials: commercial control material.
3. Access Substrate
   Cat. No. 81906
4. Access Wash Buffer II, Cat. No. A16792
5. Vortex mixer with a continuous 'On' mode (i.e. not 'Auto' or 'Touch' mode) and a maximum speed between 2,500 and 3,200 rpm.

EQUIPMENT AND MATERIALS

R1	Access 25(OH) Vitamin D Total Reagent Packs

CALIBRATION

CALIBRATION INFORMATION

Run the Access 25(OH) Vitamin D Total Calibrator S0 in quadruplicate, the calibrator S1 in triplicate, and the calibrator S2-S5 in duplicate.

An active calibration curve is required for all tests. For the Access 25(OH) Vitamin D Total assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

QUALITY CONTROL

Quality control materials simulate the characteristics of patient samples and are essential for monitoring the system performance of immunochemical assays. Because samples can be processed at any time in a "random access" format rather than a "batch" format, quality control materials should be included in each 24-hour time period.[12] Include commercially available quality control materials that cover at least two levels of analyte. More frequent use of controls or the use of additional controls is left to the discretion of the user, based on good laboratory practices or laboratory accreditation requirements and applicable laws. Follow manufacturer's instructions for reconstitution and storage. Each laboratory should establish mean values and acceptable ranges to assure proper performance. Quality control results that do not fall within acceptable ranges may indicate invalid test results. Examine all test results generated since obtaining the last acceptable quality control test point for this analyte. Refer to the appropriate system manuals and/or Help system for information about reviewing quality control results.

TESTING PROCEDURE(S)

PROCEDURAL COMMENTS

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.
2. Refer to 'Product Information' section for Vitamin D specific instructions for reagent pack handling.
3. Do not invert open (punctured) packs.
4. Use thirty (30) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.
5. The system default unit of measure for sample results is ng/mL. To change sample reporting units to the International System of Units (SI units), nmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply ng/mL by multiplication factor 2.5.

PROCEDURE

Refer to the appropriate system manuals and/or Help system for information on managing samples, configuring tests, requesting tests, and reviewing test results.

RESULTS INTERPRETATION

Patient test results are determined automatically by the system software. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

REPORTING RESULTS

EXPECTED RESULTS

OBSERVED REFERENCE VALUES

1. In one study, 25(OH) vitamin D concentrations were measured in serum samples collected from 367 apparently healthy adults using the Access 25(OH) Vitamin D Total assay on the Access 2 Immunoassay System. To represent a broad spectrum of UV light exposure, the study population included subjects from three geographically diverse regions of the United States that were sampled during different seasons, and were representative of the overall United States population in terms of sex, race and ethnicity. Individuals who were pregnant, had a history of bone metabolism disease (e.g., hypocalcemia), cancer, kidney disease, or abnormal serum calcium, magnesium, phosphorus, PTH or TSH levels were excluded from the study. Subjects ranged in age from 21 to 89 years of age and 20% of subjects reported taking vitamin D supplements. The observed range of 25(OH) vitamin D concentrations, established according to CLSI guideline EP28-A3c, is summarized in Table 1.0 below.[13]

Table 1.0 Observed values for the Access 25(OH) Vitamin D Total assay on the Access 2 Immunoassay System

Unit	N	Median	Observed Range	
			2.5th Percentile (95% CI)	97.5th Percentile (95% CI)
ng/mL	367	24.9	11.9 (10.2 - 13.3)	43.6 (42.4 - 50.5)
nmol/L	367	62.3	29.7 (25.6 - 33.3)	109.1 (106.1 - 126.4)

2. Vitamin D levels may vary according to factors such as geography, season, or the patient's health, diet, age, ethnic origin, use of vitamin D supplementation or environment.[7] To assure proper representation of specific populations, each laboratory should establish its own reference intervals.

EXPECTED VALUES

There is currently debate over the optimal values of 25(OH) Vitamin D in serum. In 2011, the Clinical Guidelines Subcommittee of the Endocrine Society Task Force established the guidelines below for recommended serum 25(OH) vitamin D levels.[7] Other clinical reference citations may show different values.

Vitamin D Status	25 (OH) Vitamin D Concentration Range (ng/mL)	25 (OH) Vitamin D Concentration Range (nmol/L)
Deficient	< 20	< 50
Insufficient	20 to < 30	50 to < 75
Sufficient	30 - 100	75 - 250
Upper Safety Limit	>100	> 250

PROCEDURAL NOTES

LIMITATIONS

1. This product is for use on Access 2 Immunoassay Systems only. It is not compatible with UniCel DxI Immunoassay Systems.

2. The reportable measuring range of the assay is 7.0 to 120 ng/mL (17.5 to 300 nmol/L). The lower end of the measuring range is defined by the Limit of Quantitiation (LoQ). Values outside of this measuring range should be reported as < 7.0 ng/mL or > 120 ng/mL, respectively. Do not dilute patient samples, as this could lead to incorrect Vitamin D results.
3. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies (e.g. human anti-sheep antibodies) may be present in patient samples.[14,15] Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.
4. Do not assay hemolyzed samples. Hemoglobin concentrations greater than 50 mg/dL may lead to falsely elevated results.
5. Falsely elevated results may occur in patients being treated with Paricalcitol (Zemplar). Vitamin D levels should not be tested in patients who have received Paricalcitol within 24 hours of obtaining the sample.[16]
6. Other potential interferences in the patient sample could be present and may cause erroneous results in immunoassays. Some examples that have been documented in literature include rheumatoid factor, endogenous alkaline phosphatase, fibrin, and proteins capable of binding to alkaline phosphatase.[17] Carefully evaluate the results of patients suspected of having these types of interferences.
7. The Access 25(OH) Vitamin D Total results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests, and other appropriate information.
8. The role of preanalytical factors in laboratory testing has been described in a variety of published literature.[18,19] Following blood collection tube manufacturers' specimen collection and handling recommendations is essential to reduce preanalytical errors.

PERFORMANCE CHARACTERISTICS

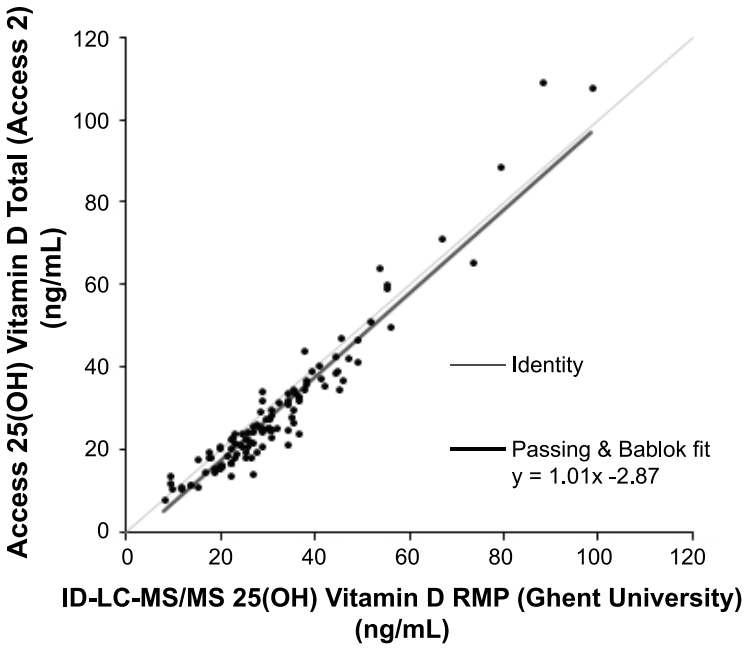
PERFORMANCE CHARACTERISTICS

METHODS COMPARISON (MEASUREMENT PROCEDURE COMPARISON)

A comparison of 110 serum samples evaluated with the Access 25(OH) Vitamin D Total assay on the Access 2 Immunoassay System and an ID-LC-MS/MS reference measurement procedure (RMP) developed at Ghent University[20,21] gave the following results using Passing-Bablok regression and Spearman correlation:

N	Slope (95% CI)	Correlation Coefficient (r)	Unit = ng/mL		Unit = nmol/L	
			Range of Observations†	Intercept (95% CI)	Range of Observations†	Intercept (95% CI)
110	1.01 (0.94 - 1.10)	0.95	8.0 - 98.6	-2.87 (-5.44 to -0.88)	20.0 - 246.5	-7.17 (-13.60 to -2.19)

† Observed concentration range of the RMP.



A comparison of 45 paired serum (no gel) and plasma samples (lithium heparin) using the Access 25(OH) Vitamin D Total assay on the Access 2 Immunoassay system gave the following results (Passing-Bablok regression and Spearman correlation):

N	Slope (95% CI)	Correlation Coefficient (r)	Unit = ng/mL		Unit = nmol/L	
			Range of Observations (Serum)	Intercept (95% CI)	Range of Observations (Serum)	Intercept (95% CI)
45	1.05 (1.02 - 1.07)	0.99	8.27 - 87.81	0.11 (-0.83 - 0.88)	20.68 - 219.52	0.26 (-2.07 - 2.19)

A comparison of 45 paired serum (no gel) and serum (gel) samples using the Access 25(OH) Vitamin D Total assay on the Access 2 Immunoassay system gave the following results (Passing-Bablok regression and Spearman correlation):

N	Slope (95% CI)	Correlation Coefficient (r)	Unit = ng/mL		Unit = nmol/L	
			Range of Observations (no gel)	Intercept (95% CI)	Range of Observations (no gel)	Intercept (95% CI)
45	1.02 (1.00 - 1.06)	0.99	8.27 - 87.81	0.55 (-0.24 - 1.25)	20.68 - 219.52	1.37 (-0.61 - 3.12)

LINEARITY

In a study based on CLSI EP6-A[22], the Access 25(OH) Vitamin D Total assay demonstrated clinically acceptable linearity throughout the analytical measuring range of 7.0 to 120 ng/mL (17.5 to 300 nmol/L).

IMPRECISION

This assay exhibits total imprecision ≤ 10.0% CV at concentrations greater than 15.0 ng/mL (37.5 nmol/L), and total Standard Deviation (SD) ≤ 1.5 ng/mL (3.8 nmol/L) at concentrations ≤ 15.0 ng/mL. One study, using serum patient

samples generating a total of 40 assays, 2 replicates per assay, over 20 days provided the following data, calculated based on CLSI EP5-A2 guidelines.[23]

Table 2.0 Units = ng/mL

Sample	Mean (n=80)	Within-run		Between-run		Total (within-laboratory)	
		SD	%CV	SD	%CV	SD	%CV
Sample 1	13.3	0.5	3.8	0.9	6.7	1.0	7.7
Sample 2	24.6	0.5	2.2	1.8	7.2	1.8	7.5
Sample 3	49.8	1.1	2.1	3.5	7.0	3.6	7.3
Sample 4	110.5	1.6	1.5	7.3	6.6	7.5	6.8

Table 3.0 Units = nmol/L

Sample	Mean (n=80)	Within-run		Between-run		Total (within-laboratory)	
		SD	%CV	SD	%CV	SD	%CV
Sample 1	33.3	1.3	3.8	2.2	6.7	2.6	7.7
Sample 2	61.4	1.3	2.2	4.4	7.2	4.6	7.5
Sample 3	124.6	2.6	2.1	8.7	7.0	9.1	7.3
Sample 4	276.2	4.1	1.5	18.4	6.6	18.8	6.8

INTERFERENCES

Vitamin D samples containing concentrations of 20, 40 and 100 ng/mL (50, 100 and 250 nmol/L) were spiked with multiple concentrations of the substances below and run on a single Access 2 Immunoassay System. Values were calculated as described in CLSI EP7-A2.[24] Interference was determined by testing controls (no interfering substance added) and matched test samples (with interfering substance added). Of the compounds tested, none were found to cause a bias of > 10.0% using the highest test concentrations indicated in the table below.

Substance	Highest Concentration Added
Acetaminophen	20 mg/dL
Bilirubin (conjugated and unconjugated)	40 mg/dL
Biotin	180 ng/mL
Acetylsalicylic Acid	65 mg/dL
Ascorbic Acid	3 mg/dL
Hemoglobin	50 mg/dL
Cholesterol	500 mg/dL
Heparin (low molecular weight)	3 U/mL
Ibuprofen	30 mg/dL
Rheumatoid Factor	200 IU/mL
Protein (gamma globulin)	6 g/dL
Triglycerides	3280 mg/dL
Uric Acid	24 mg/dL

ANALYTICAL SPECIFICITY

Based on guidance from CLSI protocol EP7-A2,[24] a study was performed to evaluate the potential Cross Reactivity of the assay with other substances that are similar in structure to 25(OH) vitamin D. The substances shown in the following table were added into samples containing 25(OH) vitamin D concentrations of 20, 40 and 100 ng/mL and run on a single Access 2 Immunoassay System. Values for the Observed % Cross Reactivity were calculated using the following equation:

$$\text{Observed \% Cross Reactivity} = \frac{\text{value spiked (ng/mL)} - \text{value unspiked (ng/mL)}}{\text{concentration of cross-reactant added (ng/mL)}} \text{ X } 100$$

Substance	Concentration Added		Observed % Cross Reactivity		
	ng/mL	nmol/L	Concentration of 25(OH) vitamin D in sample:		
			20 ng/mL	40 ng/mL	100 ng/mL
3-epi-25(OH) vitamin D_3†	100	250	38	47	32
$1,25(OH)_2$ vitamin D_2††	9	20	796	913	1026
$1,25(OH)_2$ vitamin D_3††	25	60	175	186	147
$24,25(OH)_2$ vitamin D_3	104	250	6	1	-6
Vitamin D_3 (Cholecalciferol)	19,832	50,000	0	0	0
Vitamin D_2 (Ergocalciferol)	19,232	50,000	0	0	0
1αOH vitamin D_3 (alfacalcidol)	8,013	20,000	0	0	0
Paricalcitol (Zemplar)	24	60	172	147	131
25(OH) vitamin D_2	41	100	76	81	76

Due to the insufficient spike recovery in 25(OH) vitamin D immunoassays[25] the Observed % Cross Reactivity results obtained above were normalized by dividing by the Observed % Cross Reactivity of 25(OH) vitamin D_3 to obtain the final % Cross Reactivity values below:

Substance	Concentration Added		% Cross Reactivity		
	ng/mL	nmol/L	Concentration of 25(OH) vitamin D in sample:		
			20 ng/mL	40 ng/mL	100 ng/mL
3-epi-25(OH) vitamin D_3†	100	250	64	54	70
$1,25(OH)_2$ vitamin D_2††	9	20	1336	1043	2262
$1,25(OH)_2$ vitamin D_3††	25	60	293	212	324

Substance	Concentration Added		% Cross Reactivity		
	ng/mL	nmol/L	Concentration of 25(OH) vitamin D in sample:		
			20 ng/mL	40 ng/mL	100 ng/mL
24,25$(OH)_2$ vitamin D_3	104	250	9	2	-13
Vitamin D_3 (Cholecalciferol)	19,832	50,000	0	0	0
Vitamin D_2 (Ergocalciferol)	19,232	50,000	0	0	0
1αOH vitamin D_3 (alfacalcidol)	8,013	20,000	0	0	0
Paricalcitol (Zemplar)	24	60	282	264	288
25(OH) vitamin D_2†††	41	100	96	99	116
25(OH) vitamin D_3†††	20/40	50/100	100	100	100

†Concentrations tested were approximately 50-200 times the average endogenous levels reported for 3-epi-25(OH) vitamin D_3 in infant, pediatric and adult subjects; in these populations, the maximum 3-epi-25(OH) vitamin D_3 concentration found was 4.9 ng/mL.[26]

††Concentrations tested were 125 - 375 times the endogenous levels typically found for 1,25 $(OH)_2$ vitamin D.[27]

†††Data supporting the equimolar recognition of 25(OH) Vitamin D_2 and D_3 is available upon request. Contact Beckman Coulter Technical Support for more information.

LIMIT OF BLANK

The Access 25(OH) Vitamin D Total assay is designed to have a Limit of Blank (LoB) of 1.50 ng/mL (3.75 nmol/L). In one study, LoB was tested using a protocol based on CLSI EP17-A2.[28] A total of 154 replicates of a zero analyte sample (Access 25(OH) Vitamin D Total Calibrator S0) were measured in 12 runs using multiple reagent packs and two calibrator lots on multiple Access 2 Immunoassay Systems. This study determined the LoB for the Access 25(OH) Vitamin D Total assay to be 0.55 ng/mL (1.38 nmol/L), which supports the above claim of 1.50 ng/mL.

LIMIT OF DETECTION

The Access 25(OH) Vitamin D Total assay is designed to have a Limit of Detection (LoD) of 2.0 ng/mL (5.0 nmol/L). In one study, LoD was tested using a protocol based on CLSI EP17-A2.[28] Three replicates from five low-level samples were measured using multiple reagent packs and two calibrator lots in 12 runs on multiple Access 2 Immunoassay Systems. This study determined the LoD for the Access 25(OH) Vitamin D Total assay to be 1.0 ng/mL (2.5 nmol/L), which supports the above claim of 2.0 ng/mL.

LIMIT OF QUANTITATION

The Access 25(OH) Vitamin D Total assay is designed to have a Limit of Quantitation (LoQ) of 7.0 ng/mL (17.5 nmol/L). In one study, LoQ was tested using a protocol based on CLSI EP17-A2.[28] Three replicates of 10 samples were measured using multiple reagent packs and one calibrator lot in 22 runs on multiple Access 2 Immunoassay Systems. LoQ was determined as the lowest concentration which met the design requirements of total imprecision ≤ 20% CV. This study determined the LoQ for the Access 25(OH) Vitamin D Total assay to be 3.0 ng/mL (7.5 nmol/L), which supports the above claim of 7.0 ng/mL.

ADDITIONAL INFORMATION

REVISION HISTORY

Revision F

IFU updated to change copyright, add revision history and add patent statement.

SYMBOLS KEY

Glossary of Symbols is available at techdocs.beckmancoulter.com (document number C02724)

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