25(OH) Vitamin D Total (VitdD) – Serum, Plasma Technical Procedure 3232
Beckman UniCel DxI Systems

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 UniCel DxI Immunoassay Systems. Results are to be used as an aid in the assessment of vitamin D sufficiency.

Clinical Significance

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 Lumi-Phos* 530 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

Acceptable Sample Containers

13 x 75 SST and PST BD tubes
SST and PST BD microtainers

Unacceptable Specimens

Hemolyzed samples should not be assayed. Hemoglobin concentrations greater than 50 mg/dL may lead to falsely elevated results.

Grossly lipemic samples should not be assayed.

Specimen Storage and Stability

Tubes of blood are to be kept closed at all times and in a vertical position. Allow serum samples to clot in an upright position before centrifugation. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

Separated serum or plasma should not remain at room temperature longer than 72 hours. If assays are not completed within 72 hours, serum or plasma should be stored at 2°C to 10°C. If assays are not completed within 7 days, or the separated sample is to be stored beyond 7 days, samples should be frozen at -20°C or colder. Frozen samples can be stored up to one year at -20°C before testing.

Frozen samples should be thawed no more than 3 times. Mix gently by inversion, and centrifuge after thawing prior to sample analysis.

Ensure residual fibrin, cellular matter, and bubbles have been removed prior to analysis. Aliquoted samples must be centrifuged at 2200 RCF for 1 minute prior to analysis.

Sample Volume

Optimum volume: 0.5 mL, Minimum volume: 0.25 mL

Reagents

Access 25(OH) Vitamin D Total Reagent Pack

Cat. No. A98856: 100 determinations, 2 packs, 50 tests/pack
Provided ready to use.

Store upright and refrigerate at 2°C to 10°C.

Reagent packs must have been stored at 2°C to 10°C for a minimum of two hours before use on the instrument.

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

Stable at 2°C to 10°C for 28 days after initial use.

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.

Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range. If the reagent pack is damaged (i.e., broken elastomer), discard the pack.

Reagent Preparation

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 DxI800 REAGENT PACKS USING A VORTEX MIXER.

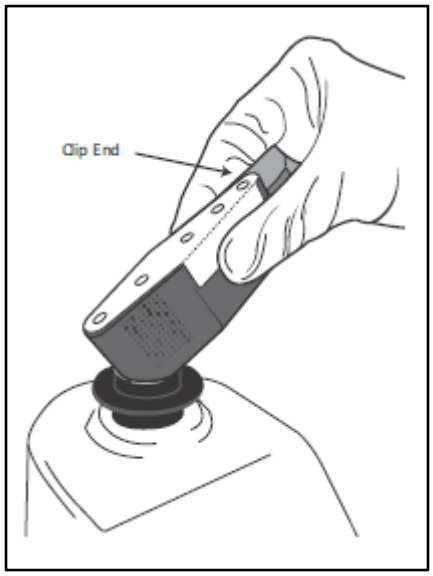
To mix:

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. 2500 to 3200 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.



Contents

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

Reagent Warnings and Precautions

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.

Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.(9)

ProClin 300 is a potential skin sensitizer. Avoid spilling or splashing this reagent on skin or clothing. In case of contact with the reagent, flush thoroughly with soap and water.

	R 43: May cause sensitization by skin contact. S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

Safety Data Sheets (SDS) are available online.

Access 25(OH) Vitamin D Total Calibrators

Catalog Reorder No. A98857: S0–S5, 1.4 mL/vial

S0	Human serum, < 0.1% sodium azide, and 0.1% ProClin* 300.
S1, S2, S3, S4, S5:	Human Serum with 25(OH) vitamin D levels of approximately 6, 17, 37, 87, and 210 ng/mL, < 0.1% sodium azide and 0.1% ProClin* 300
Calibration Card:	1

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

Refer to calibration card and or vial labels for exact concentrations.

The Access 25(OH) Vitamin D Total Calibrators are intended to calibrate the Access 25(OH) Vitamin D Total assay for the quantitative determination of total 25-hydroxyvitamin D levels in human serum and plasma using the Beckman Coulter UniCel DxI Immunoassay Systems.

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response.

The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

Traceability – references from Calibrator IFU

The measurand (analyte) in the Access 25(OH) Vitamin D Total Calibrators is traceable a Joint Committee for Traceability in Laboratory Medicine (JCTLM)-approved isotope dilution mass spectrometry (ID-LC-MS/MS) reference measurement procedure (RMP) developed at Ghent University.(10,11) This RMP is further traceable to the NIST SRM 2972.(11,12) Traceability process is based on EN ISO 17511.

The assigned values were established using representative samples from this lot of calibrator and are specific to the assay methodologies of the Access reagents. Values assigned by other methodologies may be different. Such differences, if present, may be caused by inter-method bias.

Access Substrate

Catalog Reorder No. 81906: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

Condition	Storage	Stability
Unopened	2°C to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15°C to 30°C (room temperature)	Minimum 18 hours, Maximum 14 days
In use (opened)	Internal substrate supply position	Maximum 14 days

Lumi-Phos* 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant). Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.

Access Wash Buffer II

UniCel DxI Wash Buffer II, Cat. No. A16793: 1 x 10 L

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15°C to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

R3 Wash Buffer II: TRIS buffered saline, surfactant, < 0.1% sodium azide, and 0.1% ProClin** 300.

Materials Needed But Not Supplied With Reagent Kit

Quality Control (QC) materials: commercial control material.

Vortex mixer with a continuous ’On’ mode (i.e. not ’Auto’ or ’Touch’ mode) and a maximum speed between 2500 and 3200 rpm

Equipment

This test is performed on the Beckman UniCel DxI800 systems; Beckman-Coulter , Brea, California. For technical assistance, call the Beckman-Coulter hotline: 1-800-854-3633.

Refer to the Beckman UniCel DxI systems *Instructions for Use* manual, *Reference Manual* and/or *Help System* for detailed instructions

Calibration

An active calibration curve is required. For the Access 25(OH) Vitamin D Total assay, calibration is required every 28 days. Refer to the UniCel DxI System *Instruction for Use* manual and/or *Help System* for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

Calibrator Required

Access 25(OH) Vitamin D Total Calibrator
Catalog Reorder No. A98857: S0–S5, 1.4 mL/vial

Provided ready to use. Store upright and freeze upon receipt at -15°C to -30°C. Stable until the expiration date stated on the label when stored unopened at -15° to -30°C.

Calibrator Preparation

Thaw at room temperature. Mix contents thoroughly by gently inverting before use. Avoid bubble formation.

Calibrator Storage and Stability

After removing from storage at -15°C to -30°C, the thawed vials are stable at 2°C to 10°C for 56 days. Label the vials with the date of expiration. Return calibrators to 2°C to 10°C after each use. Do not refreeze opened vials.

Signs of possible reagent pack deterioration are control values out of range. Discard the vials if there is evidence of microbial contamination or excessive turbidity in the calibrators.

Calibration Information

Assay calibration is required every 28 days.

Run the Access 25(OH) Vitamin D Total Calibrator S0 in quadruplicate (4 replicates), the Calibrator S1 in triplicate (3 replicates), and the Calibrator S2-S5 in duplicate (2 replicates).

Quality Control

Two levels of control material will be analyzed each day of patient testing.

In addition, controls should be run under the following circumstances:

Upon loading a new reagent cartridge.
Following each new calibration.
Following specific maintenance or troubleshooting procedures as detailed in the UniCel DxC800 System *Instructions For Use* manual.

More frequent use of controls or the use of additional controls is left to the discretion of the user based on workload and workflow.

The following controls should be used in accordance with the package instructions for use inserts. Quality control results should be evaluated and handled with respect to the Clinical Chemistry Quality Control Procedure #3000.T. Controls are compiled statistically in the LIS and reagent lot changes are documented on DxC Reagent Log sheets.

Control	Storage
MAS Omni-Immune1	2° to 8°C
MAS Omni-Immune 2	2° to 8°C

36 month shelf Life at -25° to -15°C; 30 days open-vial at 2° to 8°C.

Thaw controls at room temperature (18° to 25°C) on a rocker until liquid and then immediately store at 2° to 8°C. Thoroughly mix the control bottles before each use by mixing on a rocker for five minutes. Use immediately and return bottles to 2° to 8°C after use.

Testing Procedure

Refer to the UniCel DxI *Instructions for Use* manual and/or *Help System* for information on managing samples, configuring tests, requesting tests, and reviewing test results.

1. If necessary, load the reagent onto the system. Mix contents of new (unpunctured) reagent packs by vortexing as described in Reagent Preparation before loading on the instrument. Do not invert open (punctured) packs. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions. Date, initial cartridge and document in reagent log before loading each new cartridge.
2. After reagent load is completed, calibration may be required. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.
3. Program samples and controls for analysis. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.
4. After loading samples and controls onto the system, follow the protocols for system operation. Refer to the Beckman UniCel DxI systems *Instructions for Use* manual and/or *Help System* for detailed instructions.

Reporting Results

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 UniCel DxI *Instructions for Use* manual and/or *Help System* for complete instructions on reviewing sample results.

Reference Intervals

In one study performed by Beckman Coulter, 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 UniCel DxI 800 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.(17)

Observed Values for the Access 25(OH) Vitamin D Total assay on the UniCel DxI800 Immunoassay System

Sample Type	n	Median	2.5th Percentile (95% CI)	97.5th Percentile (95% CI)
Serum	367	27.4	14.0 ng/mL (13.0 – 14.8)	49.8 ng/mL 47.4 – 56.9)

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.

UCDHS Reference Intervals

The following reference intervals are from the Institute of Medicine (IOM) recommendations.

Intervals	Sample Type	Conventional Units
Literature	Serum or Plasma	20 to 50 ng/mL
UCDMC	Serum or Plasma	20 to 50 ng/mL

The Institute of Medicine (IOM) recommends a reference range (optimum level) of 20 – 50 ng/mL for the general healthy population. The lower end cut-off of 20 ng/mL is intended for use in the generally healthy population whereas a low end cut-off value of 30 ng/mL is considered to be appropriate for use in patients with disorders of bone and mineral metabolism per Endocrine Society guidelines. There is no known benefit to values > 50 ng/mL.

Procedural Notes

Limitations

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. (18,19)

Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

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.

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 UniCel DxI 800 Immunoassay System. Values were calculated as described in CLSI EP7-A2. 23 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 (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

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.(20)

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.(21)

Carefully evaluate the results of patients suspected of having these types of interferences.

Performance Characteristics

Analytical Measurement Range

Samples can be accurately measured within the analytical range of the lower limit of detection and highest calibrator value (approximately 7.0 to 120 ng/mL).

Clinical Reportable Range:

If a samples contains less than the lower limit of detection for this assay, report the results as "**< 7.0 ng/mL**".

If a sample contains more than the stated value of the highest Access 25(OH) Vitamin D Total Calibrator (S5), report the result as "**> 120 ng/mL**".

DO NOT DILUTE PATIENT SAMPLES, as this could lead to incorrect Vitamin D results.

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.

In one study, LoB was tested using a protocol based on CLSI EP17-A2.(31) A total of 156 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 UniCel DxI 800 Immunoassay Systems. This study determined the LoB for the Access 25(OH) Vitamin D Total assay to be 0.98 ng/mL, which supports the manufacturer's 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 2.00 ng/mL.

In one study, LoD was tested using a protocol based on CLSI EP17-A2.(31) Three replicates from five low-level samples were measured using multiple reagent packs and two calibrator lots in 12 runs on multiple UniCel DxI 800 Immunoassay Systems. This study determined the LoD for the Access 25(OH) Vitamin D Total assay to be 1.47 ng/mL, which supports the manufacturer's claim of 2.00 ng/mL.

Limit of Quantitation (Analytical Sensitivity)

The Access 25(OH) Vitamin D Total assay is designed to have a Limit of Quantitation (LoQ) of 7.0 ng/mL.

In one study, LoQ was tested using a protocol based on CLSI EP17-A2.(31) Three replicates of 10 samples were measured using multiple reagent packs and one calibrator lot in 22 runs on multiple UniCel DxI 800 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 4.4 ng/mL, which supports the manufacturer's claim of 7.0 ng/mL.

Analytical Specificity

Based on guidance from CLSI protocol EP7-A2, 23 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 UniCel DxI 800 Immunoassay System.

Values for the Observed % Cross Reactivity were calculated using the following equation:

$$\text{OBSERVED \% CROSS REACTIVITY} = \frac{\text{VALUE SPIKED (ng/mL)} - \text{VALUE UN-SPIKED(ng/mL)}}{\text{CONCENTRATION OF CROSS-REACTANT ADDED (ng/mL)}} \times 100$$

Substance Added	Concentration Added	Observed % Cross-Reactivity		
		Concentration of 25(OH) vitamin D in sample		
	ng/mL	20 ng/mL	40 ng/mL	100 ng/mL
3-epi-25(OH) vitamin D_3*	100	43	64	47
1,25$(OH)_2$ vitamin D_2**	9	974	1140	1278
1,25$(OH)_2$ vitamin D_3**	25	306	329	186
24,25$(OH)_2$ vitamin D_3	104	6	2	-11
Vitamin D_3 (Cholecalciferol)	19,832	0	0	0
Vitamin D_2 (Ergocalciferol)	19,232	0	0	0
1αOH vitamin D_3 (alfacalcidol)	8,103	0	0	0
Paracalcitol (Zemplar)	24	218	209	195
25(OH) vitamin D_2	41	57	69	80

Due to the insufficient spike recovery in 25(OH) vitamin D immunoassays (28), 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 Added	Concentration Added	Observed % Cross-Reactivity		
		Concentration of 25(OH) vitamin D in sample		
	ng/mL	20 ng/mL	40 ng/mL	100 ng/mL
3-epi-25(OH) vitamin D_3*	100	55	100	71
1,25$(OH)_2$ vitamin D_2**	9	1253	1797	1927
1,25$(OH)_2$ vitamin D_3**	25	393	518	281
24,25$(OH)_2$ vitamin D_3	104	7	3	-16
Vitamin D_3 (Cholecalciferol)	19,832	0	0	0
Vitamin D_2 (Ergocalciferol)	19,232	0	0	0
1αOH vitamin D_3 (alfacalcidol)	8,103	0	0	0
Paracalcitol (Zemplar)	24	483	389	293
25(OH) vitamin D_2***	41	86	86	103
25(OH) vitamin D_3***	20/40	100	100	100

*Concentrations tested were approximately 50-200 times the average endogenous levels reported for 3-epi-25(OH) vitamin D3 in infant, pediatric and adult subjects; in these populations, the maximum 3-epi-25(OH) vitamin D3 concentration found was 4.9 ng/mL. (29)
**Concentrations tested were 125 - 375 times the endogenous levels typically found for 1,25 (OH) 2 vitamin D. (30)
***Data supporting the equimolar recognition of 25(OH) Vitamin D2 and D3 is available upon request. Contact Beckman Coulter Technical Support for more information.

Methods Comparison

As determined by Beckman

A comparison of 110 serum samples evaluated with the Access 25(OH) Vitamin D Total assay on the UniCel DxI 800 Immunoassay System and an ID-LC-MS/MS reference measurement procedure (RMP) developed at Ghent University (23,24) gave the following results using Passing-Bablok regression and Spearman correlation:

Number of samples	Slope (95% CI)	Correlation Coefficient (r)	Units = ng/mL	
			Range of Observations*	Intercept (95% CI)
110	0.99 (0.91 to 1.07)	0.94	8.0 to 98.6	-3.38 (-5.48 to -1.04)

* 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 UniCel DxI 800 Immunoassay System gave the following results (Passing-Bablok regression and Spearman correlation):

Number of samples	Slope (95% CI)	Correlation Coefficient (r)	Units = ng/mL	
			Range of Observations*	Intercept (95% CI)
45	1.04 (1.01 to 1.09)	0.99	12.00 to 102.44	-0.34 (-1.50 to -0.83)

A comparison of 45 paired serum (no gel) and serum (gel) samples using the Access 25(OH) Vitamin D Total assay on the UniCel DxI 800 Immunoassay System gave the following results (Passing-Bablok regression and Spearman correlation):

Number of samples	Slope (95% CI)	Correlation Coefficient (r)	Units = ng/mL	
			Range of Observations*	Intercept (95% CI)
45	1.01 (0.96 to 1.04)	0.99	12.00 to 102.44	0.87 (-0.16 to 2.01)

As determined by UCDMC

Serum (in the range of 5.3-112.3 ng/mL):

Y (Uni-Cel DxI800-049)	= 0.9751X – 6.0156
N	= 50
MEAN (Uni-Cel DxI800-049)	= 16.6
MEAN (Centaur XP3)	= 15.3
CORRELATION COEFFICIENT (r)	= 0.8244

Serum (in the range of 5.3-112.3 ng/mL):

Y (UniCel DxI800-053)	= 0.9901X-5.919
N	= 50
MEAN (UniCel DxI800-053)	= 15.6
MEAN (Centaur XP3)	= 15.3
CORRELATION COEFFICIENT (r)	= 0.8363

Serum (in the range of 4.2-132.4 ng/mL):

Y (UniCel DxI800-053)	= 0.9974X-0.9919
N	= 50
MEAN (UniCel DxI800-053)	= 15.6
MEAN (Uni-Cel DxI800-049)	= 16.6
CORRELATION COEFFICIENT (r)	= 0.9790

Linearity

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

Imprecision

This assay exhibits total imprecision ≤ 10.0% CV at concentrations greater than 15.0 ng/mL, and total Standard Deviation (SD) ≤ 1.5 ng/mL 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. (26)

	Mean (ng/mL)	Within-Run		Between-run		Total (within-laboratory)	
Sample	**n=80**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
Sample 1	15.6	0.7	4.6	1.3	8.1	1.5	9.3
Sample 2	26.0	1.2	4.7	1.5	5.7	1.9	7.4
Sample 3	53.0	1.6	3.0	3.5	6.5	3.8	7.2
Sample 4	110.9	3.3	3.0	6.5	5.9	7.3	6.6

Precision established at UCDHS

Analyzer	Type of Precision	Sample Type	n	Mean (pg/mL)	1 SD	CV (%)
DxI800-602049	Within-run	Low QC	20	71.1	3.1	4.4
		High QC	20	141.4	7.8	5.5
	Between-run	MAS Omni-Immune1	20	70.5	4.1	5.9
		MAS Omni-Immune2	20	144.0	6.7	4.6
DxI800-602053	Within-run	Low QC	20	69.9	4.3	6.1
		High QC	20	141.7	7.8	5.5
	Between-run	MAS Omni-Immune1	20	69.3	4.7	6.8
		MAS Omni-Immune2	20	138.9	6.6	4.7

Additional Information

For more detailed information on UniCel DxC Systems, refer to the *Instructions for Use* and *Reference* manual.

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Kathy Dagang	07/12/2016	Centaur Procedure #3268

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
06/30/2016	Replaces testing on Centaur XP	kdagang		