Vitamin D Total (VitD) - Serum
Siemens ADVIA Centaur XP Systems

Technical Procedure 3268

For In Vitro Diagnostic Use Only

Principle

Intended Use

The Siemens Vitamin D Total (VitD) assay is a competitive immunoassay using direct chemiluminometric technology for the quantitative determination of total 25 (OH) vitamin D (D_2 and D_3) in human in serum or plasma using the ADVIA Centaur XP Immunoassay system.

Clinical Significance

Vitamin D is a steroid hormone involved in the intestinal absorption of calcium and the regulation of calcium homeostasis. Vitamin D is essential for the formation and maintenance of strong, healthy bones.

Vitamin D deficiency can result from inadequate exposure to the sun, inadequate alimentary intake, decreased absorption, abnormal metabolism, or vitamin D resistance.(1) Recently, many chronic diseases such as cancer (2,3,4), high blood pressure (5), osteoporosis (6,7) and several autoimmune diseases (9,10) have been linked to vitamin D deficiency. Whether consumed or produced, both forms of vitamin D (D_2 and D_3) are metabolized by the liver to 25(OH)D, and then converted in the liver or kidney into 1,25-dihydroxyvitamin D.(11) Vitamin D metabolites are bound to a carrier protein in the plasma and distributed throughout the body. The most reliable clinical indicator of vitamin D status is 25(OH)D because serum and plasma 25(OH)D levels reflect the body's storage levels of vitamin D, and 25(OH)D correlates with the clinical symptoms of vitamin D deficiency.(12)

Clinical Applications for Vitamin D Testing.(24)

Classical	Non-Classical
Osteoporosis	Cardiology - Hypertension
Recent falls in Elderly Patients	Autoimmunity – Autoimmune Disease
Pregnant Patients	Autoimmunity – High Risk for Autoimmune Disease
Chronic Kidney Failure Patients	Autoimmunity – Patients Starting or On Corticosteroids
Transplant Patients	Autoimmunity – Multiple Sclerosis
Patients with bone loss	Oncology – Cancer Patients Undergoing Treatment
Obese Patients	Oncology – Colon Cancer
Diabetes	Oncology – Prostate Cancer
Hospitalized Patients	Oncology – Breast Cancer
Patients with Bone/Muscle Aches	Oncology –Ovarian Cancer

Methodology

The ADVIA Centaur VitD assay is a one-pass, 18-minute antibody competitive immunoassay that uses an anti-fluorescein monoclonal mouse antibody covalently bound to paramagnetic particles (PMP), an anti-25(OH) vitamin D monoclonal mouse antibody labeled with acridinium ester (AE), and a vitamin D analog labeled with fluorescein.

The system automatically performs the following steps:

1. Dispenses 20 µL of sample into a cuvette, and incubates for 15 seconds.
2. Dispenses 200 µL of Ancillary Pack Reagent, and incubates for 4.5 minutes at 37°C.
3. Dispenses 50 µL of Lite Reagent, and incubates for 5.5 minutes at 37°C.

4. Dispenses 100 µL of Solid Phase reagent, and 50 µL of ancillary well reagent, and incubates for 2.75 minutes at 37°C.
5. Separates the Solid Phase from the mixture, and aspirates the unbound reagent.
6. Washes the cuvette with Wash 1.
7. Dispenses 300 µL each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
8. Reports results according to the selected option, as described in the system operating instructions or in the online help system.
9. The time to first result is 18 minutes with a throughput of 240 tests per hour.

An inverse relationship exists between the amount of vitamin D present in the patient sample and the amount of relative light units (RLU) detected by the system.

Specimen Collection

Acceptable Sample Containers

13 x 75 SST and Red Top BD tubes
SST and Red Top BD microtainers
Optimum volume: 1.0 mL, Minimum volume: 0.4 mL

Unacceptable Samples

All hemolyzed samples
Grossly lipemic samples

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI):(13)

1. Collect all blood samples observing universal precautions for venipuncture. Handle all samples as if capable of transmitting disease.
2. Human serum and plasma (EDTA, lithium-heparin, sodium-heparin) are the recommended sample types for this assay.
3. Allow serum samples to clot adequately before centrifugation.
4. Keep tubes stoppered and upright at all times.
5. Test samples as soon as possible after collecting.
6. Do not use samples that have been stored at room temperature for longer than 24 hours.
7. Do not use specimens with obvious microbial contamination.

Specimen Storage

1. Tightly cap and refrigerate specimens at 2° to 8°C up to 7 days if the assay is not completed within 24 hours. Specimens may be stored on the clot up to 6 days.(14)
2. Freeze samples at or below -20°C if the sample is not assayed within 7 days.(14) Frozen serum samples are stable up to one year.
3. Freeze samples up to 4 times, and mix thoroughly after thawing.(14)
4. Do not store in frost-free freezer.

Before placing samples on the system ensure that:
• Samples are free of fibrin or other particulate matter.
• Samples are free of bubbles.

Vitamin D Total (VitD) - Serum
Siemens ADVIA Centaur XP Systems

Technical Procedure 3268

Reagents and Supplies

Reagent Pack Kits

Ref	Description	Number of Tests
10631021	5 ReadyPack primary reagent packs containing ADVIA Centaur VitD Lite Reagent, Solid Phase, and Ancillary Well Reagent 5 ReadyPack ancillary packs containing ADVIA Centaur VitD Ancillary Reagent ADVIA Centaur VitD Master Curve card	500
or		
10491994	1 ReadyPack primary reagent pack containing ADVIA Centaur VitD Lite Reagent, Solid Phase, and Ancillary Well Reagent 1 ReadyPack ancillary pack containing ADVIA Centaur VitD Ancillary Reagent ADVIA Centaur VitD Master Curve card	100

Reagent Contents

Reagent Pack	Description	Storage	Stability
ADVIA Centaur VitD Ready Pack primary reagent pack	Lite Reagent 5.0 mL/reagent pack anti-VitD (monoclonal mouse) antibody labeled with acridinium ester (~0.8 µg/mL) in buffer with bovine serum albumin, mouse IgG, and sodium azide (< 0.1%)	2° - 8°C Protect reagent packs from all heat and light sources.	until the expiration date on the pack label. Onboard stability - 28 days
	Solid Phase 10.0 mL/reagent pack anti-fluorescein (monoclonal mouse)-coated para-magnetic particles (PMP) (~0.60 mg/mL) in buffer with bovine serum albumin, surfactant, and sodium azide (< 0.1%)	2° - 8°C Protect reagent packs from all heat and light sources.	until the expiration date on the pack label. Onboard stability - 28 days
	Ancillary Well Reagent 5.0 mL/reagent pack vitamin D-analog conjugated to fluorescein (~0.2 µg/mL) and 1-anilinonaphthalene-8-sulfonic acid in buffer with bovine serum albumin and sodium azide (< 0.1%)	2° - 8°C Protect reagent packs from all heat and light sources.	until the expiration date on the pack label. Onboard stability - 28 days
ADVIA Centaur VitD Ready Pack ancillary reagent pack	VitD Ancillary Pack Reagent 25.0 mL/reagent pack releasing agent in buffered saline with sodium azide (< 0.1%) and stabilizers	2° - 8°C Protect reagent packs from all heat and light sources.	until the expiration date on the pack label. Onboard stability - 28 days
ADVIA Centaur VitD diluent ancillary pack	VitD Diluent Ancillary Reagent Pack 25.0 mL/reagent pack phosphate buffer with BSA, cholesterol and sodium azide (< 0.1%)	2° - 8°C Protect reagent packs from all heat and light sources.	until the expiration date on the pack label. or 28 consecutive days after access-ing the ancillary reagent pack.

Vitamin D Total (VitD) - Serum
Siemens ADVIA Centaur XP Systems

Technical Procedure 3268

Calibrator Kit Required but not Provided

Ref	Contents
10630911	6 vials of lyophilized Low Calibrator (CAL L) 6 vials of lyophilized High Calibrator (CAL H) Calibrator Assigned Value card bar-code labels
or	
10493589	2 vials of lyophilized Low Calibrator (CAL L) 2 vials of lyophilized High Calibrator (CAL H) Calibrator Assigned Value card bar-code labels

Calibrator Contents

Calibrator Vial	Ingredients	Storage	Stability
2.0 mL/vial	2.0 mL/vial un-reconstituted lyophilized vial	2° - 8°C	Until the expiration date on the vial label.
	After reconstitution, low or high levels of 25 (OH) Vitamin D in buffered, defibrinated human plasma with bovine serum albumin, cholesterol, preservatives, and sodium azide (< 0.1%) (after reconstitution)	≤ -20°C	Reconstituted–120 days; freeze-thaw 4 times
		2°- 8°C	Reconstituted–28 days
		18°- 25°C	Reconstituted–24 hours; or onboard–10 hours.

Siemens Vitamin D Control Kit

to be used for troubleshooting QC problems

Ref	Contents
10632229	3 vials of lyophilized Level 1 (Control 1) 3 vials of lyophilized Level 2 (Control 2) Expected Value Card and bar-code labels

Siemens Vitamin D Control Kit Description

Volume	Ingredients	Storage	Stability
2.0 mL/vial	2.0 mL/vial un-reconstituted lyophilized vial	2° - 8°C	Until the expiration date on the vial label.
	After reconstitution, low or high levels of 25 (OH) Vitamin D in buffered, defibrinated human plasma with bovine serum albumin, cholesterol, preservatives, and sodium azide (< 0.1%) (after reconstitution)	≤ -20°C	Reconstituted–120 days; freeze-thaw 4 times
		2°- 8°C	Reconstituted–28 days
		18°- 25°C	Reconstituted–24 hours; or onboard–10 hours.

Materials Required But Not Provided

Ref	Description	Contents	Storage	Stability
10493257	ADVIA Centaur VitD MCM	5 levels	2° - 8°C	
03773025	ADVIA Centaur Wash 1	2 x 2500 mL/pack	18°- 25°C	Until the expiration date on the bottle label or onboard - 28 days
10310026	Acid/Base Kit (~1000 test bottles)	1 each/box	18°- 25°C	Until the expiration date on the bottle label or onboard - 28 days
10309546	Cuvettes	3000/case		
07413317	Pipet Tips (360/box)	18 boxes/case		
10310041	Cleaning Solution	12 bottles/case	2° - 8°C	Until the expiration date on the bottle label or prepared solution - 7 days
078-K137-01	Sample Cups	1500/box		

Vitamin D Total (VitD) - Serum
Siemens ADVIA Centaur XP Systems

Technical Procedure 3268

CAUTION: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

NOTE: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

Loading Reagents

1. Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.
2. Reagent Pack Kits contain primary reagent packs and VitD Ancillary Packs. When loading a **VitD Reagent Pack**, also always load a **VitD Ancillary Pack**.
3. Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, refer to *Appendix C, Handling Reagents* in the *Siemens ADVIA Centaur XP Immunoassay System Operator's Guide*. **MIX REAGENT PACKS END OVER END AT LEAST 20 TIMES UNTIL ALL PARA-MAGNETIC PARTICLES ARE IN SOLUTION**.
4. Document lot numbers on Reagent Log.
5. Date and initial packs.
6. Load the ReadyPack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. For detailed information about loading reagents, refer to the system operating instructions or to the *online* help system.
7. Load the ancillary pack in the ancillary reagent entry with the barcoded side facing outward. For detailed information about loading ancillary reagents, refer to the *Siemens ADVIA Centaur XP Immunoassay System Operator's Guide* or to the *online* help system.

Warnings and Precautions

1. Safety data sheets (SDS) are available at *www.siemens.com/diagnostics*.

CAUTION
The reagents contain material of animal origin and should be handled as a potential carrier and transmitter of disease.

2. Some components of this product contain sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.
3. Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

Onboard Reagent Stability and Calibration Interval

Onboard Stability	Calibration Interval
28 days	28 days

Additionally, the ADVIA Centaur VitD assay requires a two-point calibration:

- when changing lot numbers of primary reagent packs
- when replacing system components
- when quality control results are repeatedly out of range

NOTE:

- Discard the primary reagent packs at the end of the on-board stability interval.
- Do not use reagents beyond the expiration date.
- **When a primary reagent pack is discarded, the ancillary pack initially loaded at the same time must also be discarded.**

Equipment

This test is performed on the Siemens Advia Centaur XP systems; Siemens Healthcare Diagnostics, Tarrytown, New York. For technical assistance, call the Siemens Healthcare Diagnostics Technical Support Hotline: 1-877-229-3711, press 11, then 1.

Refer to the Siemens Advia Centaur XP systems *Quick Reference Guide* and/or *Operator's Guide* for detailed instructions.

Calibration

The ADVIA Centaur VitD assay requires a Master Curve calibration when using a new reagent lot number. For each new lot number of Lite Reagent and Solid Phase, use the bar-code reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering Master Curve values, refer to the system *Operator's Guide* or to the *online* help system.

Calibrate the assay at the end of the 28-day calibration interval. Additionally, this assay requires a two-point calibration when:

- Changing lot numbers of primary reagent packs.
- Replacing system components.
- Quality control results are repeatedly out of range.

For detailed information about entering calibration values, refer to the system *Operator's Guide* or to the *online* help system.

Using the Calibrator Assigned Value Card

Each lot of calibrators contains a Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. For detailed information about entering calibrator values, refer to the system *Operator's Guide* or to the *online* help system.

Using Barcode Labels

NOTE: Calibrator barcode labels are lot number specific. Do not use barcode labels from one lot of calibrators with any other lot of calibrators.

Affix the Low and High Calibrator bar-code labels to the calibrator sample cups so that the system recognizes the sample as a calibrator. Place the bar-code label on the sample cup with the readable characters oriented vertically.

Preparing Calibrators

1. Add 2.0 mL of reagent water(25) into each calibrator vial using a volumetric or precision pipet.
2. Let the calibrators stand for 30 minutes at room temperature (18° to 30°C) to allow the lyophilized material to dissolve.
3. Gently swirl and invert the vials until homogeneous.
4. Document lot number on Reagent Log.
5. The lyophilized calibrators are stable until the expiration date on the vial when stored at 2° to 8°C and for 28 days after reconstitution when stored at 2° to 8°C.

Warnings and Precautions for Calibrators

1. **R22**, **R52/53**, Harmful if swallowed. Harmful to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Contains: sodium azide.
2. **CAUTION! POTENTIAL BIOHAZARD**: Contains human source material. While each human serum or plasma donor unit used in the manufacture of this product was tested by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV), and antibody to HIV-1/2, all products manufactured using human source material should be handled as potentially infectious. Because no test method can offer complete assurance that hepatitis B or C viruses, HIV, or other infectious agents are absent, these products should be handled according to established good laboratory practices.(26,27,28)

Scheduling the Calibrators

The calibration interval for the ADVIA Centaur VitD assay is 28 days.

1. Schedule the calibrators to the worklist:
   a. Schedule VitD Calibrator for the Vitamin D.
2. Label the sample cups, one with a Low Calibrator barcode label and another with a High Calibrator barcode label. Because calibrator barcode labels are lot number specific, use barcode labels that correspond to the lot number of the calibrator used.
3. Gently mix the Low and High Calibrators.
4. Pipet at least 0.5 mL of Low and High Calibrator into the labeled sample cups.
   The sample cups are marked at 0.5 mL intervals to assist in filling.
5. Load the calibrator sample cups on the system.
   The Low Calibrator cup position must precede the High Calibrator cup position.
6. After successful calibration, schedule controls.
7. Print and save a copy of the calibration in the binder for the Centaur that calibration was performed on.

Limitations

1. Do not return any calibrators back into the vials after calibration because evaporation can occur, which may affect performance.
2. Dispose of any calibrator remaining in the sample cups after 4 hours.
3. Do not refill calibrator sample cups when the contents are depleted. If required, dispense fresh calibrators.

Vitamin D Total Assay Standardization

The ADVIA Centaur Vitamin D Total assay is standardized using internal standards which are traceable to LC/MS/MS. The relationship between the ADVIA Centaur Vitamin D assay (y) and liquid chromatography coupled with tandem mass spectrometry method (LC/MS/MS) (x) is described using linear regression as:

ADVIA Centaur VitD = 1.01 (LC/MS/MS) + 8.9 ng/mL, r=0.99.

Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, at least two levels of quality control material should be assayed daily that samples are analyzed. Quality control samples should also be assayed after performing a two-point calibration. Treat all quality control samples the same as patient samples.

Siemens Healthcare Diagnostics recommends the use of commercially available quality control materials with at least 2 levels (low and high). A satisfactory level of performance is achieved when the analyte values obtained are within the Acceptable Control Range for the system or within your range, as determined by an appropriate internal laboratory quality control scheme.

The following controls should be used in accordance with the package instructions for use inserts. Copies of these inserts can be found in the *Control IFUs* folder on the S drive (*S:\APS\ClinLab\PoliciesandProcedures\1000-8999CLINICALPATHOLOGY\3000-3999Chemistry\3000-3499AutomatedChemistry\Control IFUs*). 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 Centaur XP Reagent Log sheets.

If the quality control results do not fall within the Expected Values or within the established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.

• Rerun the assay with fresh quality control samples.
• Recalibrate assay if controls are still out of control.
• If necessary, contact your local technical support provider or distributor for assistance.

Quality Control Material

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.

Sample Volume

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container (~400 µL) or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to *Sample Volume Requirements* in the *Siemens ADVIA Centaur XP Immunoassay System Operator's Guide* or to the *online* help system.

Assay Procedure

For detailed procedural information, refer to the *Siemens ADVIA Centaur XP Immunoassay System Operator's Guide* or to the *online* help system.

Effect of Light on Results

The ADVIA Centaur Vitamin D Total assay is sensitive to light. Protect the Reagent packs from all heat and light sources. The vitamin D molecule is known to be light sensitive and the Lite Reagent (containing the antibody) and the Ancillary Well Reagent (containing the conjugate) within the ReadyPack are light sensitive. Take all precautions to prevent and limit the exposure of the ReadyPacks to ambient light. Store the packs in dark, sealed containers and ensure that the reagent is not stored in a refrigerator with glass doors which may increase light exposure if the packs are not properly covered during storage. If light exposure is suspected, discard the ReadyPack and suggest recalibration with new ReadyPack, ensuring that controls are within range prior to continuing. Light exposure will impact results by showing pack to pack differences. Reagent packs stored in the original packaging box or loaded on the system are protected from light. Store unused reagent packs at 2°-8°C away from heat and light.

Store the reagents upright at 2° - 8°C and protect from light and heat. Mix all primary reagent packs 20 times end over end by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. Discard reagent packs at the end of the 28-day on-board stability interval. Do not use reagents beyond the expiration date. When discarding used primary reagent packs, also discard the corresponding ancillary pack that was loaded at the same time.

Results

Results should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings. The system reports VitD results in ng/mL (common units) or nmol/L (SI units), depending on the units defined when setting up the assay. The conversion formula is 1 ng/mL = 2.5 nmol/L. Vitamin D results at UCDMC will be reported in ng/ml.

For detailed information about how the system calculates results, refer to the *Siemens ADVIA Centaur XP Immunoassay System Operator's Guide* or to the *online* help system.

Dilutions

None.

Vitamin D results greater than 150 ng/mL will be reported as **≥ 150 ng/mL**. No dilutions will be required.

Limitations

1. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.(15) Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
2. Samples containing fluorescein may show interference with this assay. Evidence suggests that patients undergoing fluorescein dye angiography can retain small amounts of fluorescein in the body for up to 48 to 72 hours post-treatment. In the cases of patients with renal insufficiency, retention could be much longer. Samples containing fluorescein can produce falsely elevated Vitamin D values. With fluorescein interference, observed Vitamin D values can be as high as >150 ng/mL (>375 nmol/L). Samples should be resubmitted post fluorescein clearance to ensure there is no interference with Vitamin D test results.
3. **Do not use hemolyzed samples. Hemoglobin at concentrations > 155 mg/dL will cause falsely depressed values**.

Expected Results

From a review of the available literature,(1,16,17,18) the recommendations for 25(OH)D levels are as follows:

Vitamin D Status	Range
Deficiency	< 20 ng/mL (50 nmol/L)
Insufficiency	20 - 29 ng/mL (50 - 72 nmol/L)
Sufficiency	30 - 100 ng/mL (75 - 250 nmol/L)
Toxicity	> 100 ng/mL (250 nmol/L)

Reference Interval

Data using the ADVIA Centaur VitD assay was obtained on serum samples collected from 542 adults: 258 adults not taking supplements containing vitamin D, and 284 adults taking supplements containing vitamin D. The samples were collected in different seasons and different geographical regions of the United States. Samples with abnormal values for PTH, calcium, magnesium, phosphorus, and TSH were excluded from this study. Based on the 95% confidence interval, the following values were established following CLSI guideline C28-A2.(19)

The following values were obtained:

Observed Values	
Median 25 OH Vitamin D	21.1 ng/mL (52.8 nmol/L)
Observed Range 2.5th to 97.5th Percentile	10.6 - 43.4 ng/mL (26.5 - 108.5 nmol/L)

UCDMC In-house Study

An in-house study of medical and nursing personnel samples collected in February 2011 showed for n=118, a 100% range of 6 - 67 ng/mL and the following(19:

Observed Values	
Median 25 OH Vitamin D	15 ng/mL (37.4 nmol/L)
Observed Range 2.5th to 97.5th Percentile	6 - 37 ng/mL (15 - 92.4 nmol/L)

Performance Characteristics

Analytical Measurement Range

The ADVIA Centaur VitD assay measures 25(OH) vitamin D from concentrations of 4.2 to 150 ng/mL (10.5 to 375 nmol/L). The low end of the assay range is defined by the limit of quantitiation (LoQ).

Clinical Reportable Range

The ADVIA Centaur VitD assay measures 25(OH) vitamin D from concentrations of 4.2 to 150 ng/mL (10.5 to 375 nmol/L). The reportable range will be 4 to 150 ng/mL. Results less than 4 ng/mL will reported as **< 4 ng/mL** and results greater than 150 ng/ml will be reported as **> 150 ng/mL**.

Specificity

The ADVIA Centaur VitD Total assay shows high specificity for 25(OH) vitamin D_2 and 25(OH) vitamin D_3. The following compounds were tested with total 25(OH) vitamin D concentrations of 35 and 115 ng/mL. Percent change is calculated as:

Percent cross-reactivity = (corrected assay value / amount of compound spiked) x 100

The following results were obtained:

Compound	Concentration (ng/mL)	Cross-reactivity(%)
1, 25 $(OH)_2$ Vitamin D_2	100	4.0
1, 25 $(OH)_2$ Vitamin D_3	100	1.0
25 OH Vitamin D_2	30	104.5
25 OH Vitamin D_3	30	100.7
Paricalcitol	24	0.1
3-epi-25-OH Vitamin D_3	100	1.1
Vitamin D_2	1000	0.5
Vitamin D_3	1000	0.3

Sensitivity

The limit of blank (LoB), limit of detection (LoD), and the limit of quantitation (LoQ) were determined as described in CLSI Document EP17-A.(20) The ADVIA Centaur VitD assay had an LoB of 1.7 ng/mL (4.3 nmol/L), an LoD of 3.20 ng/mL (8.0 nmol/L), and an LoQ of 4.2 ng/mL (10.5 nmol/L). The LoD is defined as the lowest concentration of 25(OH) vitamin D that can be detected with 95% probability.

Linearity

Linearity was evaluated according to the CLSI protocol EP6-A.(21) A sample containing high levels of total 25(OH) vitamin D was mixed in various proportions with a sample containing low levels of total 25(OH) vitamin D. The resulting sample mixtures were assayed for total vitamin D. On the ADVIA Centaur system, the VitD assay is linear from 4.2 to 150 ng/mL.

Linearity/Reportable Range Verification determined by UCDMC

Centaur XP-IRL96830930

Level	N	Mean	%CV	Target
1	3	0.0	0.0	5.0
2	3	17.5	10.90	16.9
3	3	41.0	2.37	38.6
4	3	79.9	3.91	73.8
5	3	125.3	1.57	121.0

Centaur XP-IRL13981026

Level	N	Mean	%CV	Target
1	3	0.0	0.0	5.0
2	3	16.2	4.62	16.9
3	3	41.3	4.73	38.6
4	3	78.1	6.67	73.8
5	3	124.4	0.85	121.0

Precision

As determined by Siemens

Precision was evaluated according to the CLSI protocol EP5-A2.(22) Six samples were assayed twice a day in replicates of 4, over 20 days (n = 160 replicates per sample) using the ADVIA Centaur VitD assay. The following results were obtained:

Mean	Within-run		Total	
(ng/mL)	SD	%CV	SD	%CV
11.7	0.81	7.0	1.30	11.1
18.0	1.20	6.6	1.74	9.6
32.4	1.87	5.8	3.17	9.8
49.9	2.22	4.5	4.07	8.2
55.8	2.66	4.8	4.38	7.8
132.1	3.53	2.7	6.33	4.8

Precision as determined at UCDMC

Centaur XP-IRL96830930

Type of Precision	Sample Type	n	Mean	SD	%CV
Within-run	Siemens Level 1	20	17.8	0.9	5.28
	Siemens Level 2	20	84.8	2.5	2.95
Between-run	MAS OMNI Level 1	20	16.8	2.3	13.7
	MAS OMNI Level 2	20	120.0	4.4	3.7

Centaur XP-IRL13981026

Type of Precision	Sample Type	n	Mean	SD	%CV
Within-run	Siemens Level 1	20	21.1	2.0	9.65
	Siemens Level 2	20	92.1	5.1	5.56
Between-run	MAS OMNI Level 1	20	15.7	2.8	18.0
	MAS OMNI Level 2	20	116.7	6.5	5.5

Vitamin D Total (VitD) - Serum
Siemens ADVIA Centaur XP Systems

Technical Procedure 3268

Specimen Collection Comparison

The ADVIA Centaur VitD assay was evaluated using different specimen matrices and tube collection types. A specimen collection study was performed using 231 matched specimens drawn in different tube types including serum red top, serum separator tube, EDTA, lithium heparin, and sodium-heparin. Vitamin D values ranged from 11.9 to 136.9 ng/mL (29.8 to 342.3 nmol/L). Linear regression analysis was performed using the following tube types:

- serum (x) vs. Serum Separator Tube (y1)
- serum (x) vs. EDTA (y2)
- serum (x) vs. lithium-heparin (y3)
- serum (x) vs. sodium-heparin (y4)

No significant difference between tube types was observed. The following results were obtained:

Tube Types*	Slope	Intercept	R
Serum vs Serum Separator Tube	1.01	-0.33	0.994
Serum vs EDTA	1.09	-0.17	0.993
Serum vs Lithium Heparin	1.04	0.18	0.992
Serum vs Sodium Heparin	1.04	0.90	0.992

* This study was performed using Becton Dickinson tubes.

Method Comparison

As determined by Siemens

For 195 samples in the range of 6.2 to 150 ng/mL (15.3 to 375 nmol/L), the relationship between the ADVIA Centaur VitD assay (y) and the IDS 25-Hydroxy Vitamin D EIA assay is described using Deming regression as:

ADVIA Centaur VitD = 1.00 (IDS 25-Hydroxy Vitamin D EIA) + 2.22 ng/mL, r = 0.96

As determined at UCDMC

Equivalency assessed by Deming regression analysis of patient samples to accepted clinical methods.

Advia Centaur XP(IRL13981026) vs Diasorin Liason
Serum (in the range of 8.3 to 84.8 ng/mL):

Y (Centaur XP-IRL13981026)	= 1.017X - 2.142
N	= 100
Average Difference	= -1.64
Average Difference (%)	= -5.4
CORRELATION COEFFICIENT (r)	= 0.823

Advia Centaur XP(IRL96830930) vs Advia Centaur XP(IRL13981026)
Serum (in the range of 5.2 to 129.0 ng/mL):

Y (Centaur XP-IRL96830930)	= 0.988X - 0.084
N	= 101
Average Difference	= -0.44
Average Difference (%)	= -1.5
CORRELATION COEFFICIENT (r)	= 0.987

Vitamin D Total (VitD) - Serum
Siemens ADVIA Centaur XP Systems

Technical Procedure 3268

Dilution Recovery

Eight serum samples in the range of 154 to 237 ng/mL (385 to 592.5 nmol/L) of total 25 (OH) vitamin D were diluted 1:2, with ADVIA Centaur VitD diluent and assayed for recovery and parallelism. The recoveries ranged from 91 to 109% with a mean of 99.6%.

The recoveries ranged from 60% to 118% with a mean of 93%

Sample	Dilution	Observed (ng/mL)	Expected (ng/mL)	Recovery %
1	1:2	76.9	77.2	100
2	1:2	93.0	95.6	97
3	1:2	83.5	89.0	94
4	1:2	124.8	118.6	105
5	1:2	126.2	116.0	109
6	1:2	96.3	93.1	103
7	1:2	84.2	85.6	98
8	1:2	71.9	79.0	91
Mean				**99.6**

Interferences

Interfering substances were tested as described in CLSI Document EP7-A2(23) using the ADVIA Centaur VitD assay.

Specimens That Are	Demonstrate ≤ 10% Change in Results Up to
Hemolyzed	155 mg/dL of hemoglobin
Lipemic	540 mg/dL of triglycerides
Icteric	40 mg/dL of conjugated bilirubin
Icteric	40 mg/dL of unconjugated bilirubin

Specimens That Contain	Demonstrate ≤ 10% Change in Results Up to
Cholesterol	350 mg/dL
Uric Acid	20 mg/dL
Human immunoglobulin	12 g/dL

Samples containing fluorescein may show interference with this assay. Evidence suggests that patients undergoing fluorescein dye angiography can retain small amounts of fluorescein in the body for up to 48 to 72 hours post-treatment. In the cases of patients with renal insufficiency, retention could be much longer. Samples containing fluorescein can produce falsely elevated Vitamin D values. With fluorescein interference, observed Vitamin D values can be as high as >150 ng/mL (>375 nmol/L). Samples should be resubmitted post fluorescein clearance to ensure there is no interference with Vitamin D test results.

References

1. Holick MF. Vitamin D Deficiency. N Engl J Med. 2007;357:266–81.
2. Blutt SE, Weigel NL. Vitamin D and prostate cancer. Proc Soc Exp Biol Med. 1999; 221(2):89–98.
3. Feskanich D, Ma J, Fuchs CS, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidemiol Biomarkers Prev. 2004; 13(9):1502–1508.
4. Shin MH, Holmes MD, Hankinson SE, Wu K, Colditz GA, Willett WC. Intake of dairy products, calcium, and vitamin D and risk of breast cancer. J Natl Cancer Inst. 2002; 94(17):1301–1311.
5. Krause R, Buhring M, Hopfenmuller W, Holick MF, Sharma AM. Ultraviolet B and blood pressure. Lancet. 1998; 352(9129):709–710.
6. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001; 22(4):477–501.
7. Lips P, Hosking D, Lippuner K, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. J Intern Med. 2006; 260(3):245–254.
8. Hypponen E, Laara E, Reunanen A, Jarvelin MR, Virtanen SM. Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. Lancet. 2001: 358(9292):1500–1503.
9. Munger KL, Zhang SM, O'Reilly E, et al. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004; 62(1):60–65.
10. Merlino LA, Curtis J, Mikuls TR, Cerhan JR, Criswell LA, Saag KG. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum. 2004; 50(1)72–77.
11. Hollis BW, Horst RL. The Assessment of Circulating 25(OH)D and 1,25 (OH)2D: Where We Are and Where We Are Going. J Steroid Biochem Mol Biol. March 2007;103(3-5):473–76.
12. Hart GR. Medical Conditions Associated with Vitamin D Deficiency and the Clinical Consequences. Review Series Volume 1, Sept 2004;1–8.
13. Clinical and Laboratory Standards Institute (formerly NCCLS). Procedures for the Handling and Processing of Blood Specimens; Approved Guideline - Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document H18-A3.
14. Data on file at Siemens Healthcare Diagnostics.
15. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem. 1988;34:27–33.
16. Holick MF. MrOs is D-ficient. J Clin Endocrinol Metab. 2009;94(4):1092–3.
17. Rollins G. Vitamin D Testing–What's the Right Answer? Labs Grapple with Confusing Analytics, Evidence. Clinical Laboratory News. July 2009;35(7): 1,6.
18. Freeman R. Vitamin D: The sunshine hormone. How and when to treat deficiencies. Menopausal Medicine. May 2009; S8–11.
19. Clinical and Laboratory Standards Institute (formerly NCCLS). How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2000. NCCLS Document C28-A2.
20. Clinical and Laboratory Standards Institute (formerly NCCLS). Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP17A.
21. Clinical and Laboratory Standards Institute (formerly NCCLS). Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 2003. NCCLS Document EP-6A.

22. Clinical and Laboratory Standards Institute (formerly NCCLS). Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP5-A2.
23. Clinical and Laboratory Standards Institute (formerly NCCLS). Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. NCCLS Document EP7-A2.
24. Sourberbielle, JC., et al. Autoimmunity Reviews 2010;9:709-715.
25. Reagent Water Technical Bulletin. Siemens Healthcare Diagnostics, 107060.
26. Centers for Disease Control. 1988. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR, 37:377–382, 387, 388.
27. Clinical and Laboratory Standards Institute (formerly NCCLS). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline - Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. NCCLS Document M29-A3.
28. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030
29. Vitamin D Assay Information Sheet, 10699314_EN Rev. C, 2012-08. Siemens Healthcare Diagnostics Inc.
30. Bulletin Number: 078N0584-02, GPS-F-002-1 DCM Rev. 2.0 Effective: May, 24th, 2010, Related Procedure: GPS-P-002, DX Support Bulletin Communication, Proprietary Information of Siemens Healthcare Diagnostics Inc.
31. Vitamin D Calibrator Information Sheet, 10631095 Rev. B, 2011-01. Siemens Healthcare Diagnostics Inc.
32. Vitamin D Quality Control Information Sheet, 10632228 Rev. B, 2011-01. Siemens Healthcare Diagnostics Inc.
33. Recalibration of the Vitamin D Total Assay Beginning with Reagent Lot 134020, Customer Bulletin 10813470 Rev. A, 2012-08. Siemens Healthcare Diagnostics Inc.
34. Test Definition Enhancements, Customer Bulletin 10813306 Rev. A, 2012-12. Siemens Healthcare Diagnostics Inc.