SIEMENS

ADVIA Centaur® XP
ADVIA Centaur® XPT
Immunoassay Systems

Vitamin D Total (VitD)

Current Revision and Date[a]	Rev. 08, 2022-10	
Product Name	ADVIA Centaur® VitD (100 tests)	REF 10699201
	ADVIA Centaur VitD (500 tests)	REF 10699533
Systems	ADVIA Centaur XP system ADVIA Centaur XPT system	
Materials Required but Not Provided	ADVIA Centaur Wash 1 (2 x 1500 mL)	REF 01137199
	ADVIA Centaur Wash 1 (2 x 2500 mL)	REF 03773025
Optional Materials	ADVIA Centaur VitD quality control material	REF 10699200
	ADVIA Centaur VitD Diluent, 2-pack	REF 10494100
	ADVIA Centaur VitD Diluent, 1 bottle	REF 10632114
	ADVIA Centaur VitD Master Curve Material (MCM)	REF 10699746
Specimen Types	Serum, EDTA plasma, lithium heparin plasma, sodium heparin plasma	
Sample Volume	20 µL	
Assay Range	4.2–150 ng/mL (10.5–375 nmol/L)	

[a] A vertical bar in the page margin indicates technical content that differs from the previous version.

Intended Use

The ADVIA Centaur® Vitamin D Total (VitD) assay is for *in vitro* diagnostic use in the quantitative determination of total 25(OH)vitamin D in human serum and plasma (EDTA, lithium heparin, sodium heparin) using the ADVIA Centaur® XP and ADVIA Centaur® XPT systems. The ADVIA Centaur VitD assay is intended as an aid in the determination of vitamin D sufficiency.

Summary and Explanation

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,[2-4] high blood pressure,[5] osteoporosis,[6,7] and several autoimmune diseases[8-10] 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)vitamin 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)vitamin D because serum and plasma 25(OH)vitamin D levels reflect the body's storage levels of vitamin D, and 25(OH)vitamin D correlates with the clinical symptoms of vitamin D deficiency.[12]

Principles of the Procedure

The ADVIA Centaur VitD assay is an 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.

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.

Reagents

Reagent	Description	Storage	Stability
ADVIA Centaur VitD Lite Reagent; ReadyPack® primary reagent pack	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%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	**Unopened**: Stable until the expiration date on the pack label. **On-system**: 28 days.
ADVIA Centaur VitD Solid Phase; ReadyPack primary reagent pack	10.0 mL/reagent pack: anti-fluorescein (monoclonal mouse)-coated paramagnetic particles (PMP) (~0.60 mg/mL) in buffer with bovine serum albumin, surfactant, and sodium azide (< 0.1%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	**Unopened**: Stable until the expiration date on the pack label. **On-system**: 28 days.
ADVIA Centaur VitD Ancillary Well Reagent; ReadyPack primary reagent pack	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%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	**Unopened**: Stable until the expiration date on the pack label. **On-system**: 28 days.
ADVIA Centaur VitD Calibrator vials	2 x 2.0 mL/vial 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%).	Store the reagents upright at 2–8°C. Protect vials from all heat and light sources.	**Unopened**: Stable until the expiration date on the pack label. **Reconstituted**: 28 days at 2–8°C 24 hours at 18–25°C 120 days at ≤ -20°C
ADVIA Centaur VitD Ancillary Pack Reagent; ReadyPack ancillary reagent pack	25.0 mL/reagent pack: releasing agent in buffered saline with sodium azide (< 0.1%) and stabilizers	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	**Unopened**: Stable until the expiration date on the pack label. **On-system**: 28 days.

Reagent	Description	Storage	Stability
ADVIA Centaur VitD Diluent; ancillary reagent pack	25.0 mL/reagent pack: phosphate buffer with BSA, cholesterol and sodium azide (< 0.1%)	Store the reagents upright at 2–8°C. Protect reagent packs from all heat and light sources.	**Unopened**: Stable until the expiration date on the pack label or **On-system**: 28 consecutive days after accessing the ancillary reagent pack.
ADVIA Centaur Wash 1[a]	1500 mL/pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant	Store the reagents upright at 2–25°C.	**Unopened**: Stable until the expiration date on the vial. **On-system**: 1 month.
ADVIA Centaur Wash 1[a]	2500 mL/pack phosphate-buffered saline with sodium azide (< 0.1%) and surfactant	Store the reagents upright at 2–25°C.	**Unopened**: Stable until the expiration date on the vial. **On-system**: 1 month.

[a] See *Materials Required but Not Provided*.

Warnings and Precautions

For Prescription Use Only.

For Professional Use.

CAUTION
Federal (USA) law restricts this device to sale by or on the order of a licensed healthcare professional.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com.

Warning! Potential Biohazard
Contains human source material.

No known test method can ensure that products derived from human source materials will not transmit infection. These materials should be handled using good laboratory practices and universal precautions.[13–15]

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

H412	Harmful to aquatic life with long lasting effects.
P273, P501	Avoid release to the environment. Dispose of contents and container in accordance with all local, regional, and national regulations. **Contains:** Sodium azide; ADVIA Centaur VitD Calibrator

Contains 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 buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

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.

For *in vitro* diagnostic use.

Preparing Reagents

Reagents are liquid and ready to use. Remove all of the reagents from the refrigerator, and 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 the system operating instructions.

Prepare calibrators using the following steps:

1. Add 2.0 mL of reagent water 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.

Storing and Stability

Store unused reagent packs upright at 2° to 8°C away from heat and light sources. Reagents are stable at 2° to 8°C until the expiration date on the pack label.

Store ADVIA Centaur VitD Calibrator at 2° to 8°C away from heat and light sources. Calibrators are stable at 2° to 8°C until the expiration date on the pack label.

For onboard stability, refer to *On-System Stability*.

Specimen Collection and Handling

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI):[16]

Collecting the Specimen

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

Storing the Specimen

- 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.[17]
- Freeze samples at or below -20°C if the sample is not assayed within 7 days.[17]
- Freeze samples up to 4 times, and mix thoroughly after thawing.[17]
- Do not store in frost-free freezer.

The purpose of handling and storage information is to provide guidance to users. It is the responsibility of the individual laboratory to use all available references and/or its own studies when establishing alternate stability criteria to meet specific needs.

Procedure

Materials Provided

REF	Contents	Number of Tests
10699201	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 1 vial ADVIA Centaur VitD Low Calibrator CAL L 1 vial ADVIA Centaur VitD High Calibrator CAL H ADVIA Centaur systems VitD Master Curve card ADVIA Centaur systems VitD Calibrator Assigned Value Card	100
10699533	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 2 vials ADVIA Centaur VitD Low Calibrator CAL L 2 vials ADVIA Centaur VitD High Calibrator CAL H ADVIA Centaur systems VitD Master Curve card ADVIA Centaur systems VitD Calibrator Assigned Value Card	500

Materials Required but Not Provided

Item	Description	
01137199 (112351)	ADVIA Centaur Wash 1	2 x 1500 mL/pack WASH 1
03773025	ADVIA Centaur Wash 1[a]	2 x 2500 mL/pack WASH 1

[a] For use with systems with 2500 mL capacity.

Optional Materials

Item		Description	
REF	10699200	ADVIA Centaur VitD quality control material	3 x 2.0 mL/vial CONTROL 1 3 x 2.0 mL/vial CONTROL 2
REF	10494100	ADVIA Centaur VitD diluent	2 x 25 mL ancillary reagent packs VITD DIL
REF	10632114	ADVIA Centaur VitD diluent	1 x 25 mL/bottle VITD DIL
REF	10699746	ADVIA Centaur VitD MCM	5 x 2.0 mL levels of lyophilized master curve material

Assay Procedure

The ADVIA Centaur XP and ADVIA Centaur XPT systems automatically perform 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.

For detailed instructions on performing the procedure, refer to the system operating instructions.

Preparing the System

Ensure that the system has sufficient primary and ancillary reagent packs. Load the primary reagent packs in the primary reagent area. You can use the arrows on the end label as a placement guide. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. Load the ancillary reagent pack in the ancillary reagent entry.

Note The Ancillary Reagent provided in this kit is matched to the ReadyPack primary reagent pack. Do not mix Ancillary Reagent lots with different lots of reagent packs.

For detailed information about loading reagents, refer to the system operating instructions.

For detailed information about preparing the system, refer to the system operating instructions.

Preparing the Samples

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container 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 the system operating instructions.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation at 1000 x g for 10 to 15 minutes.
- Samples are free of bubbles or foam.

Dilutions

Dilute and retest samples with total 25(OH)vitamin D levels greater than 150 ng/mL (375 nmol/L), to obtain accurate results.

Patient samples can be automatically diluted by the system, or prepared manually. Use ADVIA Centaur Vitamin D Diluent to dilute patient samples. Ensure that results are mathematically corrected for dilution. If a dilution factor is entered when scheduling the test, the system automatically calculates the result. For detailed information about dilutions, refer to the system operating instructions.

For automatic dilutions, ensure that ADVIA Centaur VitD Diluent is loaded on the system. Set the dilution factor to 2 and the dilution point to 150 ng/mL (375 nmol/L). Manually dilute the patient samples when patient results exceed the linearity of the assay using automatic dilution, or when laboratory protocol requires manual dilution.

On-System Stability

Reagent packs loaded on the system are protected from light.

Discard reagent packs at the end of the 28-day onboard stability interval. Do not use reagents beyond the expiration date.

ADVIA Centaur VitD Calibrators are stable on the system for 10 hours. Dispose of any calibrator remaining in the sample cups after 10 hours.

Performing Calibration

For calibration of the ADVIA Centaur VitD assay, use ADVIA Centaur VitD Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Note The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

Calibration Procedure

Perform the calibration procedure using the following steps:

1. Ensure that the appropriate master curve and calibration assigned values are entered on the system. Refer to *Master Curve and Calibration Values*.
2. Ensure that the required reagents are loaded for the assay.
3. Schedule the ADVIA Centaur VitD Calibrators to the worklist.
4. Label two sample cups with ADVIA Centaur VitD Calibrator barcode labels: one cup for the low calibrator and another cup for the high calibrator.

Note Place the barcode label on the sample cup with the readable characters oriented vertically.

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

5. Gently mix the low and high calibrators and dispense at least 200 µL of each calibrator into the appropriate sample cups. Avoid bubbles.

Note This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

6. Load the sample cups in a rack.
7. Place the rack in the sample entry queue.
8. Start the entry queue, if required.

Note Dispose of any calibrator remaining in the sample cups after 10 hours. Do not return any calibrators back into the vials after calibration because evaporation can occur, which may affect performance. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Master Curve and Calibration Values

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 barcode 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 operating instructions.

Each calibrator is packaged with a lot-specific Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the barcode scanner or the keyboard. The Calibrator Assigned Value card provided in this kit is matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagents. For detailed information about entering calibrator values, refer to the system operating instructions.

Calibration Frequency

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.
- When replacing system components.
- When quality control results are repeatedly out of range.

Performing Quality Control

Additional quality control material can be used at the discretion of the laboratory. Use the quality control material in accordance with the quality control instructions for use.

In addition, perform quality control:

- Following a valid calibration
- With use of a new lot of reagent
- When troubleshooting test results that do not match clinical conditions or symptoms

Follow government regulations or accreditation requirements for quality control frequency. Individual laboratory quality control programs and procedures may require more frequent quality control testing.

Acceptable performance is achieved when the analyte values obtained are within the expected control interval for the system, as indicated by the manufacturer of the control material or within the interval determined by an internal laboratory quality control procedure.

Follow your laboratory's quality control procedures if the results obtained do not fall within the acceptable limits. For information about entering quality control definitions, refer to the system online help.

Quality Control Procedure

Perform the quality control procedure using the following steps:

1. Ensure that the quality control definitions are defined, and that the quality control values are entered on the system using the lot-specific Expected Value card provided.
2. Ensure that the required reagents are loaded for the assay.
3. Schedule the quality control samples to the worklist.
4. Label two sample cups with ADVIA Centaur VitD quality control barcode labels: one cup for the Level 1 control and another cup for the Level 2 control.

Note Place the barcode label on the sample cup with the readable characters oriented vertically.

Note Quality control barcode labels are lot-number specific. Do not use barcode labels from one lot of controls with any other lot of controls.

5. Gently mix the quality control materials and dispense at least 200 µL into the appropriate sample cups. Avoid bubbles.

Note This procedure uses control volumes sufficient to measure each control in duplicate.

6. Load the sample cups in a rack.
7. Place the rack in the sample entry queue.
8. Start the entry queue, if required.

Note Dispose of any quality control materials remaining in the sample cups after 10 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Quality Control Values

Siemens Healthcare Diagnostics recommends the use of VitD quality control material or equivalent commercially available control materials with at least two 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.

Refer to the Expected Value card for the suggested expected values specific for the lot number of the controls.

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

Quality Control Frequency

To monitor system performance and chart trends, as a minimum requirement, assay two levels of quality control material on each day that samples are analyzed. Assay quality control samples when performing a two-point calibration. Treat all quality control samples the same as patient samples.

Taking Corrective Action

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

1. Determine and correct the cause of the unacceptable control results:
    a. Verify that the materials are not expired.
    b. Verify that required maintenance was performed.
    c. Verify that the assay was performed according to the instructions for use.
    d. Rerun the assay with fresh quality control samples, and confirm that quality control results are within acceptable limits before running patient samples.
    e. If the quality control results are not within acceptable limits, recalibrate the assay, and repeat step d.
    f. If necessary, contact your local technical support provider or distributor for assistance.
2. Repeat testing of patient samples before reporting results.

Perform corrective actions in accordance with your established laboratory protocol.

Results

Calculation of Results

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.

For detailed information about how the system calculates results, refer to the system operating instructions.

Interpretation of Results

Results of this assay should always be interpreted in conjunction with the patient's medical history, clinical presentation, and other findings.

Limitations

The following information pertains to limitations of the assay:

- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays.[18] 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.
- Do not use samples which contain fluorescein. Fluorescein levels > 0.10 µg/mL can produce falsely elevated results in this assay. Interference testing with varying levels of sodium fluorescein as high as 3.13 µg/mL at clinically relevant concentrations of 25(OH)vitamin D (~ 25 – 50 ng/mL) can result in 25(OH)vitamin D concentrations to be >150 ng/mL. Evidence suggests that patients undergoing retinal fluorescein angiography can retain 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. Such samples can produce falsely elevated values when tested with this assay, and should not be tested.[19]

- Do not use hemolyzed samples. Hemoglobin at concentrations > 155 mg/dL will cause falsely depressed values.

Expected Values

A review of the available literature[1,20-23] suggests that the recommendations for 25(OH)vitamin D levels are summarized in the table below. Consult the listed references for discussion of vitamin D toxicity levels.[24,25]

Vitamin D Status	Range, Adult[1,20-22] ng/mL (nmol/L)	Range, Pediatric[23] ng/mL (nmol/L)
Deficiency	< 20 ng/mL (50 nmol/L)	< 15 ng/mL (37.5 nmol/L)
Insufficiency	20– < 30 ng/mL (50– < 75 nmol/L)	15– < 20 ng/mL (37.5– < 50 nmol/L)
Sufficiency	30–100 ng/mL (75–250 nmol/L)	20–100 ng/mL (50–250 nmol/L)

Observed Reference Values

Observed reference values were obtained for the ADVIA Centaur VitD assay using serum samples collected from adult and pediatric populations. The adult population consisted of 291 apparently healthy male and female subjects of light and dark skin types ranging in age from 21 - 93 years. The pediatric population consisted of 237 male and female subjects of light and dark skin types: 32 subjects between the ages of 1–3 years, 114 subjects between 3–12 years, and 91 subjects between 12 and up to 21 years.

The samples were collected in different seasons and from different geographical regions of the United States. Adult samples from subjects not taking supplements containing vitamin D >2000 IU per day, and with normal values for PTH, calcium, and TSH were included in this study.

Pediatric samples with normal values for PTH and TSH were included in this study. Based on the 95% confidence interval, the following values were established following CLSI guideline C28-A2.[26]

	Observed Values, Adult ng/mL (nmol/L)	Observed Values, Pediatric (12 months up to 21 years)
Median 25(OH)vitamin D	22.5 ng/mL (56.3 nmol/L)	23.8 ng/mL (59.5 nmol/L)
Observed Range 2.5th to 97.5th Percentile	7.4–44.0 ng/mL (18.5–110.0 nmol/L)	11.4–45.8 ng/mL (28.5–114.5 nmol/L)

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference range(s) for the diagnostic evaluation of patient samples.[26] Consider these values as guidelines only.

Performance Characteristics

Analytical Measuring 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 quantitation (LoQ).

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-A2.[27] 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. The LoQ is defined as the lowest concentration of 25(OH)vitamin D that can be detected at a total CV of 20%.

Precision

The ADVIA Centaur VitD assay is designed to have a Within-Run precision CV of ≤ 8%, and a Total CV of ≤ 12%, with samples > 20 ng/mL.

Precision was evaluated according to the CLSI protocol EP5-A2.[28] Six samples were assayed twice a day in replicates of 2, over 20 days (n = 80 replicates per sample) using the ADVIA Centaur VitD assay. The following results were obtained:

Mean	Within-Run Repeatability		Total Precision (Within-Lab)	
(ng/mL)	SD	%CV	SD	%CV
21.29	1.36	6.4	2.04	9.6
26.10	1.56	6.0	2.37	9.1
32.16	1.71	5.3	2.38	7.4
65.47	2.52	3.8	3.60	5.5
84.12	1.90	2.3	3.34	4.0
132.32	3.13	2.4	4.76	3.6

Method Comparison

For 126 samples (118 native, 8 contrived) in the range of 5.9–130.8 ng/mL (14.8–327.0 nmol/L), the relationship between the ADVIA Centaur VitD (y) and a comparable assay (x) is described using Deming regression:

ADVIA Centaur VitD (y) = 1.03 (x) + 0.85 ng/mL, r = 0.99

Specimen Equivalency

Specimen equivalency was determined with the weighted Deming regression model in accordance with CLSI Document EP09-A3.[29]

Tube (y) vs. Serum (x)	Regression Equation	Sample Interval	N[a]	r[b]
gel-barrier tube (serum)	y = 0.97x + 0.87	13.03–142.85 ng/mL	74	0.99
	(y = 0.97x + 2.18)	(32.57–357.11 nmol/L)		
dipotassium EDTA (plasma)	y = 0.97x + 0.64	13.03–142.85 ng/mL	74	0.99
	(y = 0.97x + 1.61)	(32.57–357.11 nmol/L)		
tripotassium EDTA (plasma)	y = 0.96x + 0.68	13.03–142.85 ng/mL	74	0.99
	(y = 0.96x + 1.70)	(32.57–357.11 nmol/L)		
lithium heparin (plasma)	y = 0.99x - 0.18	13.03–142.85 ng/mL	74	0.99
	(y = 0.99x - 0.44)	(32.57–357.11 nmol/L)		
sodium heparin (plasma)	y = 1.02x - 1.13	13.03–142.85 ng/mL	74	0.99
	(y = 1.02x - 2.83)	(32.57–357.11 nmol/L)		

[a] Number of samples tested.
[b] Correlation coefficient.

The assay is designed to have a correlation coefficient ≥ 0.95, a slope of 0.90–1.10, and an intercept ± 5 ng/mL.

Agreement of the specimen types may vary depending on the study design and sample population used. Results obtained at individual laboratories may vary from the data presented.

Dilution Recovery

Five serum samples in the range of 186.0–211.0 ng/mL (465.0–527.5 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 97.0%–109.0% with a mean of 101.0%.

Sample	Dilution	Observed ng/mL	Expected ng/mL	Recovery %
1	1:2	96.0	97.5	98.0
2	1:2	96.3	93.0	103.0
3	1:2	102.6	103.5	99.0
4	1:2	102.5	105.5	97.0
5	1:2	102.1	93.5	109.0
Mean				**101.0**

Interferences

Interfering substances were determined for the ADVIA Centaur VitD assay as described in CLSI Document EP7-A2.[30]

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
fluorescein	0.1 µg/mL

Linearity

Linearity was evaluated according to the CLSI protocol EP6-A.[31] 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 systems, the VitD assay is linear from 4.2 to 150 ng/mL.

Traceability

The internal standards for the ADVIA Centaur VitD assay are traceable to the ID-LC/MS/MS 25(OH)vitamin D RMP.[32,33] The ID-LC/MS/MS is traceable to the National Institute of Standards and Technology Standard Reference Material 2972.

To establish traceability, for 120 samples in the range of 5.6–153.2 ng/mL (14.0–383.0 nmol/L), the relationship between the ADVIA Centaur VitD assay (y) and the ID-LC/MS/MS 25(OH)vitamin D RMP (x) is described using Weighted Deming regression:

ADVIA Centaur VitD = 0.95 (ID-LC/MS/MS) - 0.18 ng/mL, r = 0.98

Technical Assistance

For customer support, contact your local technical support provider or distributor.

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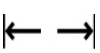
Trademarks

Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
	Manufacturer	5.1.1[a]		Authorized representative in the European Community	5.1.2[a]
	Use-by date	5.1.4[a]		Authorized representative in Switzerland	Proprietary
	Catalog number	5.1.6[a]		Batch code	5.1.5[a]
	Consult Instructions for Use	5.4.3[a]		Contains sufficient for <n> tests	5.5.5[a]
	Internet URL address to access the electronic instructions for use	Proprietary		Version of Instructions for Use	Proprietary
	*In vitro* diagnostic medical device	5.5.1[a]		Revision	Proprietary
	Prescription device (US only)	FDA[c]		Unique Device Identifier	5.7.10[b]
	CE Marking with Notified Body	EU IVDR[d]		CE Marking	EU IVDR[d]
	Temperature limit	5.3.7[a]		Keep away from sunlight	5.3.2[a]
	Upper limit of temperature	5.3.6[a]		Lower limit of temperature	5.3.5[a]
	Do not re-use	5.4.2[a]		Do not freeze	Proprietary
	Recycle	1135[e]		This way up	0623[e]
	Biological risks	5.4.1[a]		Caution	5.4.4[a]
	Common Units	Proprietary		Document face up[f]	1952[e]
YYYY-MM-DD	Date format (year-month-day)	N/A		International System of Units	Proprietary
	Target	Proprietary	YYYY-MM	Date format (year-month)	N/A
				Interval	Proprietary

Symbol	Symbol Title	Source	Symbol	Symbol Title	Source
	Handheld barcode scanner	Proprietary	CHECKSUM	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.	Proprietary
LOT DTL	Lot details	Proprietary	MC DEF	Master Curve definition	Proprietary
CAL LOT VAL	Calibrator lot value	Proprietary	CONTROL LOT VAL	Quality control lot value	Proprietary

a International Standard Organization (ISO). ISO 15223-1 Medical Devices- Symbols to be used with medical device labels, labelling and information to be supplied.

b ISO 15223-1:2020-04

c Federal Register. Vol. 81, No 115. Wednesday, June 15, 2016. Rules and Regulations: 38911.

d IVDR REGULATION (EU) 2017/746

e International Standard Organization (ISO). ISO 7000 Graphical symbols for use on equipment.

f Indicates Assay-eNote

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