Alinity i 25-OH Vitamin D (Vit D 25OH)-02

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BIENNIAL REVIEW:

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SUPERSEDES: Procedure titled _____

INTENDED USE

The Alinity i 25-OH Vitamin D assay is a chemiluminescent microparticle immunoassay (CMIA) used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma on the Alinity i analyzer.

The Alinity i 25-OH Vitamin D assay is to be used as an aid in the assessment of vitamin D sufficiency.

SUMMARY AND EXPLANATION OF THE TEST

Vitamin D is a fat-soluble steroid prohormone mainly produced photochemically in the skin

from 7-dehydrocholesterol. Two forms of vitamin D are biologically relevant - vitamin D3 (Cholecalciferol) and vitamin D2 (Ergocalciferol). Both vitamins D3 and D2 can be absorbed from food, with vitamin D2 being an artificial source, but only an estimated 10-20% of vitamin D is supplied through nutritional intake.*1* Vitamins D3 and D2 can be found in vitamin supplements. Vitamin D is converted to the active hormone 1,25-(OH)2-vitamin D (Calcitriol) through two hydroxylation reactions. The first hydroxylation converts vitamin D into 25-OH vitamin D and occurs in the liver. The second hydroxylation converts 25-OH vitamin D into the biologically active 1,25-(OH)2-vitamin D and occurs in the kidneys as well as in many other cells of the body. Most cells express the vitamin D receptor and about 3% of the human genome is directly or indirectly regulated by the vitamin D endocrine system.*1*

The major storage form of vitamin D is 25-OH vitamin D and is present in the blood at up to 1000 fold higher concentration compared to the active 1,25-(OH)2-vitamin D. 25-OH vitamin D has a half-life of 2-3 weeks vs. 4 hours for 1,25-(OH)2-vitamin D. Therefore, 25-OH vitamin D is the analyte of choice for determination of the vitamin D status.*2*, *3*

Epidemiological studies have shown a high global prevalence of vitamin D insufficiency and deficiency.*4* Risk factors for vitamin D deficiency include low sun exposure, malnutrition, some malabsorption syndromes, and liver or kidney diseases.*10* The measurement of vitamin D status provides opportunities for preventive and therapeutic interventions.*5*, *6*, *7*

Vitamin D deficiency is a cause of secondary hyperparathyroidism and diseases resulting in impaired bone metabolism (like rickets, osteoporosis, osteomalacia).*2*, *8*, *9*

The Alinity i 25-OH Vitamin D assay is standardized against **NIST SRM 2972** (National Institute of Standards & Technology Standard Reference Material 2972).

BIOLOGICAL PRINCIPLES OF THE PROCEDURE

This assay is a delayed one-step immunoassay for the quantitative determination of 25-OH vitamin D in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.

Sample, anti-vitamin D coated paramagnetic microparticles, and assay diluent are combined and incubated. The 25-OH vitamin D present in the sample is displaced from the vitamin D binding protein and binds to the anti-vitamin D coated microparticles. Vitamin D acridinium-labeled conjugate is added to create a reaction mixture. The reaction mixture is incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.

The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of 25-OH vitamin D in the sample and the RLUs detected by the system optics.

For additional information on system and assay technology, **refer to the Alinity ci-series Operations Manual, Section 3.**

REAGENTS

Kit Contents

Alinity i 25-OH Vitamin D Reagent Kit 08P45

Volumes (mL) listed in the table below indicate the volume per cartridge.

REF	08P4522	08P4532
Tests per cartridge	100	500
Number of cartridges per kit	2	2
Tests per kit	200	1000
MICROPARTICLES	6.6 mL	27.0 mL
CONJUGATE	6.1 mL	26.5 mL
ASSAY DILUENT	10.4 mL	47.1 mL

MICROPARTICLES Anti-vitamin D IgG (rabbit monoclonal) coated microparticles in MES Buffer. Minimum concentration: 0.04% solids. Preservative: ProClin 300.

CONJUGATE Acridinium-labeled vitamin D in MES Buffer and surfactant. Minimum concentration: 12 ng/mL labeled vitamin D. Preservative: sodium azide.

ASSAY DILUENT Citrate buffer with EDTA, Methanol, 8-anilino-1-naphthalenesulfonic acid (ANSA), and surfactant. Preservative: ProClin 300.

Warnings and Precautions

- IVD
- For *In Vitro* Diagnostic Use
- Rx ONLY

Safety Precautions

CAUTION: This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.[11], [12], [13], [14]

The following warnings and precautions apply to: MICROPARTICLES

	
WARNING	Contains methylisothiazolones.
H317	May cause an allergic skin reaction.
Prevention	
P261	Avoid breathing mist / vapors / spray.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: CONJUGATE	
Contains sodium azide.	
EUH032	Contact with acids liberates very toxic gas.
P501	Dispose of contents / container in accordance with local regulations.

The following warnings and precautions apply to: ASSAY DILUENT	
	
WARNING	Contains methanol and methylisothiazolones.
H371	May cause damage to organs.

H317	May cause an allergic skin reaction.
Prevention	
P260	Do not breathe mist / vapors / spray.
P264	Wash hands thoroughly after handling.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves / protective clothing / eye protection.
Response	
P302+P352	IF ON SKIN: Wash with plenty of water.
P308+P311	IF exposed or concerned: Call a POISON CENTER / doctor.
P333+P313	If skin irritation or rash occurs: Get medical advice / attention.
P362+P364	Take off contaminated clothing and wash it before reuse.
Disposal	
P501	Dispose of contents / container in accordance with local regulations.

Safety Data Sheets are available at www.abbottdiagnostics.com or/and SDS folder.

For a detailed discussion of safety precautions during system operation**, refer to the Alinity ci-series Operations Manual, Section 8.**

Reagent Handling

- Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.
  - Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.
- After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.
- If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.

- Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.

For a detailed discussion of reagent handling precautions during system operation, **refer to the Alinity ci-series Operations Manual, Section 7.**

Reagent Storage

- Do not freeze.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened	2-8°C	Until expiration date	Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.
Onboard	System Temperature	21 days	
Opened	2 to 8°C	Until expiration date	Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and potential to compromise reagent performance.

Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.

For information on unloading reagents, **refer to the Alinity ci-series Operations Manual, Section 5.**

Indications of Reagent Deterioration

Deterioration of the reagents may be indicated:

- A calibration error occurs
- A control value is out of the specified range.

Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.

For troubleshooting information, refer to the **Alinity ci-series Operations Manual, Section 10**.

INSTRUMENT PROCEDURE

The Alinity i 25-OH Vitamin D assay file must be installed on the Alinity i analyzer prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the Alinity ci-series Operations Manual, Section 2.

For information on printing assay parameters, refer to the Alinity ci-series Operations Manual, Section 5.

For a detailed description of system procedures, refer to the Alinity ci-series Operations Manual.

Alternate Result Units

Edit assay parameter "Result Units" to select an alternate unit.

Conversion formula:

(Concentration in Default result unit) x (Conversion factor) = (Concentration in Alternate result unit)

Default Result Unit	Conversion Factor	Alternate Result Unit
ng/mL	2.5	nmol/L

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

The specimen types listed below were verified for use with this assay.

Other specimen types and collection tube types have not been verified with this assay.

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator
Plasma	Dipotassium EDTA
	Tripotassium EDTA
	Sodium heparin
	Lithium heparin powder
	Lithium heparin plasma separator

Specimen Conditions

- Do not use:
  - heat-inactivated specimens
  - pooled specimens
  - grossly hemolyzed specimens
  - specimens with obvious microbial contamination
  - specimens with fungal growth
- For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.
- To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.

Preparation for Analysis

- Follow the tube manufacturer's processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.
- Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross-contamination.

To ensure consistency in results, recentrifuge specimens prior to testing if

- they contain fibrin, red blood cells, or other particulate matter.

NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low speed vortex or by inverting 10 times prior to recentrifugation.

Prepare frozen specimens as follows:

- Frozen specimens must be completely thawed before mixing.
- Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.
- Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.
- If specimens are not mixed thoroughly, inconsistent results may be obtained.
- Recentrifuge specimens.

Recentrifugation of Specimens

- Transfer specimens to a centrifuge tube and centrifuge at a minimum of 30 000 g-minutes.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below.

  Centrifugation time using alternate RCF values can be calculated using the following formula:

$$\text{Minimum Centrifugation time (minutes)} = \frac{30\,000 \text{ g-minutes}}{\text{RCF}}$$

Recentrifugation Time (Minutes)	RCF (x g)	g-Minutes
10	3000	30 000
15	2000	30 000
20	1500	30 000

$$RCF = 1.12 \times r_{max} (rpm/1000)^2$$

RCF -	The relative centrifugal force generated during centrifugation.
rpm -	The revolutions per minute of the rotor on which the specimens are being spun (usually the digital readout on the centrifuge will indicate the rpm).
Centrifugation Time -	The time should be measured from the time the rotor reaches the required RCF or rpm to the time it begins decelerating.
r_{max} -	Radius of the rotor in millimeters. NOTE: If custom tube adapters (i.e., adapters not defined by the centrifuge manufacturer) are used,

	then the radius (rmax) should be manually measured in millimeters and the RCF calculated.
g-minutes -	The unit of measure for the product of RCF (× g) and centrifugation time (minutes).

· Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.

Specimen Storage

Specimen Type	Temperature	Maximum Storage Time	Special Instructions
Serum/Plasma	Room temperature	72 hours	Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum room temperature storage time.
	2 to 8°C	12 days	Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum 2-8°C storage time and store frozen.

Storage of frozen serum samples at -20°C for up to one year has been reported to cause no loss in vitamin D metabolites.[15] Other studies showed sample stability for longer periods than one year.[16]

Avoid more than 4 freeze/thaw cycles.

Specimen Shipping

Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.

PROCEDURE

Materials Provided

08P45 Alinity i 25-OH Vitamin D Reagent Kit

Materials Required but not Provided

- Alinity i 25-OH Vitamin D assay file
- 08P4501 Alinity i 25-OH Vitamin D Calibrators
- 08P4510 Alinity i 25-OH Vitamin D Controls or other commercially available controls
- 08P4512 Alinity i 25-OH Vitamin D Controls (for use in USA only)
- Alinity Trigger Solution
- Alinity Pre-Trigger Solution
- Alinity i-series Concentrated Wash Buffer

For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.

For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.

Assay Procedure

For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.

- If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.
- To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.
- Maximum number of replicates sampled from the same sample cup: 10
  - Priority:
    - Sample volume for first test: 60 µL
    - Sample volume for each additional test from same sample cup: 10 µL
  - ≤ 3 hours on the reagent and sample manager:
    - Sample volume for first test: 150 µL
    - Sample volume for each additional test from same sample cup: 10 µL
  - > 3 hours on the reagent and sample manager:
    - Replace with a fresh aliquot of sample.
- Refer to the Alinity i 25-OH Vitamin D calibrator package insert and/or Alinity i 25-OH Vitamin D control package insert for preparation and usage.

- For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.
- For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.

Sample Dilution Procedures

Samples with a 25-OH vitamin D value exceeding 154.2 ng/mL (385.5 nmol/L) are flagged with the code "> 154.2 ng/mL" (> 385.5 nmol/L) and may be diluted with the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: **1:2**

Add 100 µL of the sample to 100 µL of Alinity i 25-OH Vitamin D Calibrator A.

The operator must enter the dilution factor in the Specimen or Control tab of the Create Order screen. The system will use this dilution factor to automatically calculate the concentration of the sample and report the result.

If the operator does not enter the dilution factor, the result must be manually multiplied by the appropriate dilution factor before reporting the result. If a diluted sample result is less than the lower value of the measuring interval of 3.5 ng/mL (8.8 nmol/L), do not report the result. Rerun using an appropriate dilution.

For detailed information on ordering dilutions, refer to the Alinity ci-series Operations Manual, Section 5.

Calibration

For instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.

Each assay control must be tested to evaluate the assay calibration.

Once a calibration is accepted and stored, it may be used for 30 days. During this time, all subsequent samples may be tested without further calibration unless:

- A reagent kit with a new lot number is used.
- Daily quality control results are outside of quality control limits used to monitor and control system performance.

This assay may require recalibration after maintenance to critical parts or subsystems or after service procedures have been performed.

Quality Control Procedures

A single sample of each control level 25-OH Vitamin D assay is to be tested once every day testing performed.

To establish statistically-based control limits run a minimum of 20 replicates over several (3-5) days and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. Sources of variation that should be included in this study in order to be representative of future system performance include:

- Multiple stored calibrations
- Multiple reagent lots
- Multiple calibrator lots
- Multiple processing modules (if applicable)
- Data points collected at different times of the day

Refer to published guidelines for information or general control recommendation, for example Clinical and Laboratory Standards Institute (CLSI) Document C24-A3 or other published guidelines, for general quality control recommendations.*17*

- If more frequent control monitoring is required, follow the laboratory quality control procedures.
- If quality control results do not meet the acceptance criteria defined by laboratory, sample results may be suspect. Follow quality control procedures to troubleshoot. Recalibration may be necessary. **For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.**
- Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

Commercial controls should be used according to the guidelines and recommendations of the control manufacturer. Concentration ranges provided in the control package insert should be used only for guidance.

For any control material in use, the laboratory should ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.

Quality Control Guidance

Refer to “Basic QC Practices” by James O Westgard, Ph.D. for guidance on laboratory quality control practices.*18*

Verification of Assay Claims

For protocols to verify package insert claims, refer to Verification of Assay Claims in the Alinity ci-series Operations Manual.

RESULTS

Calculation

The Alinity i 25-OH Vitamin D assay utilizes a 4 Parameter Logistic Curve fit data reduction method (4PLC, Y-weighted) to generate a calibration and results.

For information on alternate result units, refer to the INSTRUMENT PROCEDURE, Alternate Result Units section of this package insert.

Flags

Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.

Measuring Interval

Measuring interval is defined as the range of values in ng/mL (nmol/L) which meets the limits of acceptable performance for linearity, imprecision, and bias.

The measuring interval of the Alinity i 25-OH Vitamin D assay is **3.5 to 154.2** ng/mL (8.8 to 385.5 nmol/L).

LIMITATIONS OF THE PROCEDURE

- When testing samples from patients whose predominant form of vitamin D is vitamin D2, such as patients receiving vitamin D2 supplementation, results that are subtherapeutic should be confirmed with another method, such as LC-MS/MS, before being used for patient management.
- Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.
- If the 25-OH vitamin D results are inconsistent with clinical evidence, additional testing is recommended.
- Specimens from patients who have received preparations of rabbit monoclonal antibodies for diagnosis or therapy may contain human anti-rabbit antibodies (HARA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as Alinity i 25-OH Vitamin D that employ rabbit monoclonal antibodies. Additional information may be required for diagnosis.*19*
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. 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.*20*
- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Additional information may be required for diagnosis.*20*
- The Alinity i 25-OH Vitamin D assay is susceptible to interference effects from

triglycerides at > 500 mg/dL. A triglyceride concentration of 800 mg/dL resulted in -13.8%, -10.2%, and -17.5% bias in results for 25-OH vitamin D concentration at approximately 20 ng/mL, 30 ng/mL, and 40 ng/mL 25-OH vitamin D, respectively.

EXPECTED VALUES

This study was performed on the ARCHITECT i System.

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

It is recommended that each laboratory determine its own reference range based upon its particular locale and population characteristics.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) C28-A3c.*21* Human serum specimens from apparently healthy individuals were collected during the summer (April to October) and winter (November to March). The specimens were collected from 3 different geographical locations in the 48 contiguous United States (north, south, and central states) from subjects with different skin tones (minimum 30% dark and 30% light) and ethnicities (African-American, Hispanic, and Caucasian). No more than 50% of the subjects were taking a vitamin D supplement. Of the 283 specimens collected, 141 were female and 142 were male.

The specimens were from subjects that met the following inclusion criteria: age between 21 and 90 years; no vitamin D supplementation of ≥ 2000 IU; no bone disease or rheumatism; not currently prescribed any medications known to affect vitamin D absorption (drugs that inhibit cholesterol absorption are known to affect vitamin D absorption) or catabolism (medications that are known to increase catabolism of vitamin D include anticonvulsants, glucocorticoids, HAART (AIDS treatment) and antirejection medications); no chronic disease (especially diabetes, renal failure, high cholesterol); no family history of parathyroid or calcium regulatory disease; no personal history of disease of the kidney, gastrointestinal tract, liver, thyroid, or parathyroid; no other chronic diseases; no history of seizures; and no bariatric surgery. In addition, specimens were excluded if results were outside of the expected values for calcium (2.15 to 2.50 mmol/L for specimens from individuals ≤ 60 years old, or 2.15 to 2.55 mmol/L for specimens from individuals > 60 years old), thyroid-stimulating hormone (0.35 to 4.94 µIU/mL), or intact parathyroid hormone (15.0 to 68.3 pg/mL). The observed values are summarized in the following table.

		25-OH Vitamin D Values (ng/mL)		
			Central 95% of Data[a]	
Season	**n**	**Mean**	**Lower Limit**	**Upper Limit**
Winter	129	16.8	6.2	45.5

Summer	154	19.3	7.0	53.2
Combined	283	18.2	6.6	49.9

[a] The central 95% of data represents the mean concentration ± 1.96 × SD.

A recommended target range of vitamin D in serum by one expert panel suggested a target range of at least **30 - 40 ng/mL** (75 - 100 nmol/L).[22]

SPECIFIC PERFORMANCE CHARACTERISTICS

Representative performance data are provided in this section. Results obtained in individual laboratories may vary.

The Alinity i analyzer and the ARCHITECT i System utilize the same reagents and sample/reagent ratios.

Unless otherwise specified, all studies were performed on the Alinity i analyzer.

Precision

Within-Laboratory Precision

A study was performed based on guidance from CLSI EP05-A2. Testing was conducted using 1 lot of the Alinity i 25-OH Vitamin D Reagent Kit, 1 lot of the Alinity i 25-OH Vitamin D Calibrators, and 1 lot of the Alinity i 25-OH Vitamin D Controls and 1 instrument. Three controls and 5 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.[23]

			Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
Sample	**n**	**Mean (ng/mL)**	**SD**	**%CV**	**SD**	**%CV**
Low Control	119	20.6	0.57	2.8	0.67	3.3
Medium Control	120	39.8	1.42	3.6	1.84	4.6
High Control	119	74.6	2.46	3.3	2.83	3.8
Panel 1	119	5.9	0.33	5.5	0.53	9.0
Panel 2	120	21.1	0.63	3.0	0.74	3.5
Panel 3	118	30.2	0.78	2.6	1.05	3.5
Panel 4	120	107.8	5.21	4.8	6.06	5.6

Sample	n	Mean (ng/mL)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Panel 5	119	149.4	10.38	6.9	12.33	8.3

[a] Includes within-run, between-run, and between-day variability.

Sample	n	Mean (nmol/L)	Within-Run (Repeatability)		Within-Laboratory (Total)[a]	
			SD	%CV	SD	%CV
Low Control	119	51.4	1.42	2.8	1.68	3.3
Medium Control	120	99.4	3.56	3.6	4.60	4.6
High Control	119	186.4	6.15	3.3	7.08	3.8
Panel 1	119	14.9	0.82	5.5	1.34	9.0
Panel 2	120	52.9	1.57	3.0	1.84	3.5
Panel 3	118	75.4	1.96	2.6	2.64	3.5
Panel 4	120	269.5	13.03	4.8	15.15	5.6
Panel 5	119	373.6	25.95	6.9	30.83	8.3

[a] Includes within-run, between-run, and between-day variability.

Lower Limits of Measurement

A study was performed based on guidance from CLSI EP17-A2. Testing was conducted using 3 lots of the Alinity i 25-OH Vitamin D Reagent Kit on each of 2 instruments over a minimum of 3 days. The maximum observed Limit of Blank (LoB), Limit of Detection (LoD), and Limit of Quantitation (LoQ) values are summarized below.[24]

	ng/mL	nmol/L
LoB[a]	2.4	6.0
LoD[b]	3.5	8.8
LoQ[c]	**3.5**	8.8

[a] The LoB represents the 95th percentile from n ≥ 60 replicates of zero-analyte samples.

[b] The LoD represents the lowest concentration at which the analyte can be detected with 95% probability based on n ≥ 60 replicates of low-analyte level samples.

[c] The LoQ was determined from n ≥ 60 replicates of low-analyte level samples and is defined as the lowest concentration at which a maximum allowable precision of 20 %CV was met.

Linearity

A study was performed based on guidance from CLSI EP06-A.*25*

This assay is linear across the measuring interval of **3.5 to 154.2 ng/mL** (8.8 to 385.5 nmol/L).

Cross-Reactants

This study was performed on the ARCHITECT i System.

A study was performed based on guidance from CLSI EP07-A2. The cross-reactants listed below were evaluated to determine whether 25-OH vitamin D concentrations were affected when using the ARCHITECT 25-OH Vitamin D assay.*26*

Cross-Reactant	Cross-Reactant Concentration (ng/mL)	% Cross-Reactivity[d]
25-OH vitamin D3[a]	20 to 40	98.6% to 101.1%
25-OH vitamin D2[b]	26 / 68	80.5% / 82.4%
Vitamin D3 (Cholecalciferol)[c]	100	0.8%
Vitamin D2 (Ergocalciferol)[c]	100	0.4%
C-3-epimer of 25-OH vitamin D3[c]	100	1.3%
C-3-epimer of 25-OH vitamin D2[c]	100	0.8%
1,25-$(OH)_2$-vitamin D3[c]	100	0.1%
1,25-$(OH)_2$-vitamin D2[c]	100	0%
24,25-$(OH)_2$-vitamin D3[c]	20	101.9% to 189.2%

Cross-Reactant	Cross-Reactant Concentration (ng/mL)	% Cross-Reactivity[d]
24,25-$(OH)_2$-vitamin D2[c]	20	71.4% to 114.2%
Paricalcitol (Zemplar)[c]	24	0.6%

[a] Samples containing the cross-reactant were prepared at three 25-OH vitamin D3 concentrations (20, 30, and 40 ng/mL).

$$\% \text{ Cross-Reactivity} = \frac{\text{Mean Test Result}}{\text{25-OH vitamin D3 Concentration}} \times 100$$

[b] Cross-reactivity of the ARCHITECT 25-OH Vitamin D assay with 25-OH vitamin D2 was assessed by using endogenous (non-spiked) serum specimens. The specimens were analyzed with a chromatographic method (Liquid Chromatography - Tandem Mass Spectrometry [LC-MS/MS]) in order to determine 25-OH vitamin D2 and 25-OH vitamin D3 concentrations. The 25-OH vitamin D3 concentration of each specimen was below the LoQ of the LC-MS/MS method.

$$\% \text{ Cross-Reactivity} = \frac{\text{25-OH vitamin D total (ARCHITECT)}}{\text{25-OH vitamin D2 (LC-MS/MS)}} \times 100$$

[c] Samples containing the cross-reactant were prepared at three 25-OH vitamin D concentrations (approximately 20, 30, and 40 ng/mL). The highest observed value or range is shown.

$$\% \text{ Cross-Reactivity} = \frac{\text{Mean Test Result - Mean Reference Result}}{\text{Cross-Reactant Concentration}} \times 100$$

[d] % Cross-Reactivity < 0% is reported as 0% cross-reactivity.

Interference

Potentially Interfering Substances

This study was performed on the ARCHITECT i System.

A study was performed based on guidance from CLSI EP07-A2. Potentially interfering substances were evaluated to determine whether 25-OH vitamin D concentrations were affected when using the ARCHITECT 25-OH Vitamin D assay. Samples containing the potential interferents were prepared at three 25-OH vitamin D concentrations (approximately 20, 30, and 40 ng/mL). The samples were assayed, and the 25-OH vitamin D concentrations of the spiked samples were compared to the reference samples.[26]

Potential Interferent	Interferent Concentration	Lower and Upper 95% CL[a] Around the % Difference[b]: Range Across Analyte Concentrations
Conjugated Bilirubin	30 mg/dL	-1.7% to 1.5%
Unconjugated Bilirubin	30 mg/dL	-1.4% to 3.0%
Hemoglobin	500 mg/dL	-9.5% to -4.8%
Total Protein	12 g/dL	-4.3% to 4.4%
Triglycerides	500 mg/dL[c]	-10.0% to -6.6%
Biotin	30 ng/mL	-2.8% to 0.8%
Cholesterol	500 mg/dL	-3.7% to 1.4%
Rheumatoid Factor	800 IU/mL	-2.3% to 1.6%
Goat Anti-Rabbit Antibodies	1 µg/mL	-2.7% to 3.9%

[a] CL = Confidence Limits

[b]

$$\% \text{ Difference} = \frac{\text{Mean Test Result - Mean Reference Result}}{\text{Mean Reference Result}} \times 100$$

[c] Samples containing triglycerides at > 500 mg/dL demonstrated interference. A triglyceride concentration of 800 mg/dL resulted in -13.8%, -10.2%, and -17.5% bias in results for 25-OH vitamin D concentration at approximately 20 ng/mL, 30 ng/mL, and 40 ng/mL 25-OH vitamin D, respectively. Refer to the **LIMITATIONS OF THE PROCEDURE** section of this package insert for further information.

Potentially Interfering Other Conditions

This study was performed on the ARCHITECT i System.

A study was performed based on guidance from CLSI EP09-A3. Specimens from pregnant females and hemodialysis patients were evaluated by comparing the ARCHITECT 25-OH Vitamin D results to the results generated using LC-MS/MS, which is not susceptible to interference from these specimens.*27*

Category	n	LC-MS/MS Concentration Range (ng/mL)	Mean % Bias
Pregnant Females (1st Trimester)	40	5.9 - 43.2	4.5%
Pregnant Females (2nd Trimester)	40	12.4 - 48.8	-2.2%
Pregnant Females (3rd Trimester)	40	10.4 - 44.8	0.1%
Hemodialysis Patients*	44	4.1 - 61.2	-15.3%

*Published data demonstrated that results from patients undergoing hemodialysis may show a negative bias when tested with various automated 25-OH vitamin D assays when compared to LC-MS/MS.*28*

Method Comparison

A study was performed based on guidance from CLSI EP09-A3 using the Passing-Bablok regression method. *27*

		Units	n	Correlation Coefficient	Intercept	Slope	Concentration Range
Alinity i 25-OH Vitamin D vs ARCHITECT 25-OH Vitamin D	Serum	ng/mL (nmol/L)	138	1.00	-1.05 (-2.60)	1.00	5.2 to 126.9 (12.9 to 317.3)

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