ARCHITECT 25-OH Vitamin D

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REF 5P02-35
REF 5P02-30

en
25-OH Vitamin D
5P02
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B5P020

Read Highlighted Changes: Revised August 2016.

Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

NAME

ARCHITECT 25-OH Vitamin D

INTENDED USE

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

The ARCHITECT 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 1,000 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.[5-7]

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

The ARCHITECT 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

The ARCHITECT 25-OH Vitamin D assay is a quantitative delayed one-step competitive immunoassay to determine the presence of vitamin D in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex.

1. Sample, assay diluent and paramagnetic anti-vitamin D coated microparticles are combined. 25-OH vitamin D present in the sample is displaced from the vitamin D binding protein and binds to anti-vitamin D coated microparticles, forming an antigen-antibody complex.
2. After incubation, a conjugate containing acridinium-labeled vitamin D is added to the reaction mixture and binds to unoccupied binding sites of the anti-vitamin D coated microparticles.
3. After further incubation and washing, Pre-Trigger and Trigger Solutions are added to the reaction mixture.
4. 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 ARCHITECT iSystem optics.

   Results are calculated automatically based on the previously established calibration curve.

For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3.

REAGENTS

Kit Contents

ARCHITECT 25-OH Vitamin D 5P02

NOTE: Some kit sizes are not available in all countries or for use on all ARCHITECT iSystems. Please contact your local distributor.

REF	5P02-25	5P02-35	5P02-30
Σ	100	500	2000
MICROPARTICLES	1 x 6.6 mL	1 x 27.0 mL	4 x 27.0 mL
CONJUGATE	1 x 5.9 mL	1 x 26.3 mL	4 x 26.3 mL
ASSAY DILUENT	1 x 10.0 mL	1 x 50.9 mL	4 x 50.9 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.

Other Reagents

PRE-TRIGGER SOLUTION ARCHITECT Pre-Trigger Solution containing 1.32% (w/v) hydrogen peroxide.

TRIGGER SOLUTION ARCHITECT Trigger Solution containing 0.35 N sodium hydroxide.

WASH BUFFER ARCHITECT Wash Buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

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.[11-14]

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.

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.

Safety Data Sheets are available at www.abbottdiagnostics.com or contact your local representative.

For a detailed discussion of safety precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 8.

Reagent Handling

- Do not use reagent kits beyond the expiration date.
- **Do not pool reagents within a kit or between kits.**
- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. For microparticle mixing instructions, refer to the **PROCEDURE, Assay Procedure** section of this package insert.
- **Septums MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. Reliability of assay results cannot be guaranteed if septums are not used according to the instructions in this package insert.**
  - To avoid contamination, wear clean gloves when placing a septum on an uncapped reagent bottle.
  - Once a septum has been placed on an open reagent bottle, **do not invert the bottle** as this will result in reagent leakage and may compromise assay results.

For a detailed discussion of handling precautions during system operation, refer to the ARCHITECT System Operations Manual, Section 7.

Reagent Storage

Do not freeze.

May be used immediately after removal from 2-8°C storage.

When stored and handled as directed, reagents are stable until the expiration date.

	Storage Temperature	Maximum Storage Time	Additional Storage Instructions
Unopened/ Opened*	2-8°C	Until expiration date	Store in upright position.
On board	System temperature	21 days	Discard after 21 days. For information on tracking onboard time, refer to the ARCHITECT System Operations Manual, Section 5.

* Reagents may be stored on or off the ARCHITECT iSystem. If reagents are removed from the system, store them at 2-8°C (with septums and replacement caps) in an upright position. For reagents stored off the system, it is recommended that they be stored in their original trays and boxes to ensure they remain upright. **If the microparticle bottle does not remain upright (with a septum installed) while in refrigerated storage off the system, the reagent kit must be discarded.** For information on unloading reagents, refer to the ARCHITECT System Operations Manual, Section 5.

Indications of Reagent Deterioration

When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary. For troubleshooting information, refer to the ARCHITECT System Operations Manual, Section 10.

INSTRUMENT PROCEDURE

The ARCHITECT 25-OH Vitamin D assay file must be installed on the ARCHITECT iSystem from an ARCHITECT iSystem Assay CD-ROM prior to performing the assay.

For detailed information on assay file installation and viewing and editing assay parameters, refer to the ARCHITECT System Operations Manual, Section 2.

For information on printing assay parameters, refer to the ARCHITECT System Operations Manual, Section 5.

For a detailed description of system procedures, refer to the ARCHITECT System Operations Manual.

Alternate Result Units

The default result unit for the ARCHITECT 25-OH Vitamin D assay is ng/mL. The corresponding SI result unit is nmol/L. The conversion factor used by the system is 2.5.

SPECIMEN COLLECTION AND PREPARATION FOR ANALYSIS

Specimen Types

Verified specimen types to be used with this assay:

Specimen Types	Collection Tubes
Serum	Serum
	Serum separator tubes (SST)
Plasma	Dipotassium EDTA
	Tripotassium EDTA
	Sodium heparin
	Lithium heparin powder
	Plasma separator tubes (PST) - lithium heparin gel

- Other specimen collection tube types have not been tested with this assay.
- Performance has not been established for the use of cadaveric specimens or the use of body fluids other than human serum or plasma.
- Liquid anticoagulants may have a dilution effect resulting in lower concentrations for individual patient specimens.
- The instrument does not provide the capability to verify specimen type. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.

Specimen Conditions

- Do not use specimens with the following conditions:
  - heat-inactivated
  - pooled
  - grossly hemolyzed (> 500 mg/dL hemoglobin)
  - obvious microbial contamination
  - 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.
- Mix thawed specimens thoroughly by low speed vortexing or by inverting 10 times. Visually inspect the specimens. If layering or stratification is observed, continue mixing until specimens are visibly homogeneous.

  Avoid more than 4 freeze/thaw cycles.
- To ensure consistency in results, specimens must be transferred to a centrifuge tube and centrifuged for a minimum of 30,000 g-minutes before testing if
  - they contain fibrin, red blood cells, or other particulate matter,
  - they were previously frozen.
- Examples of acceptable time and force ranges that meet this criterion are listed in the table below. Centrifugation time using alternate Relative Centrifugal Force values (RCF) can be calculated using the following formula:

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

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

- 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.
- Inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

Specimen Storage

Specimen Type	Storage Temperature	Maximum Storage Time
Serum/Plasma	Room temperature	≤ 72 hours
	2-8°C	≤ 12 days

Remove serum or plasma from the clot, red blood cells, or separator gel if stored longer than the maximum room temperature storage time.

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]

Specimen Shipping

- Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.
- Do not exceed the storage limitations listed above.

PROCEDURE

Materials Provided

5P02 ARCHITECT 25-OH Vitamin D Reagent Kit

Materials Required but not Provided

- ARCHITECT 25-OH Vitamin D Assay file obtained from the ARCHITECT iSystem e-Assay CD-ROM found on www.abbottdiagnostics.com.
- 5P02-01 ARCHITECT 25-OH Vitamin D Calibrators
- 5P02-10 ARCHITECT 25-OH Vitamin D Controls
- 5P02-02 ARCHITECT 25-OH Vitamin D Calibrators (for use in USA only)
- 5P02-12 ARCHITECT 25-OH Vitamin D Controls (for use in USA only)
- ARCHITECT Pre-Trigger Solution
- ARCHITECT Trigger Solution
- ARCHITECT Wash Buffer
- ARCHITECT Reaction Vessels
- ARCHITECT Sample Cups
- ARCHITECT Septum
- ARCHITECT Replacement Caps
- Pipettes or pipette tips (optional) to deliver the volumes specified on the patient or control order screen.

For information on materials required for maintenance procedures, refer to the ARCHITECT System Operations Manual, Section 9.

Assay Procedure

- Before loading the reagent kit on the system for the first time, the microparticle bottle requires mixing to resuspend microparticles that may have settled during shipment. After the first time the microparticles have been loaded, no further mixing is required.
  - **Invert the microparticle bottle 30 times.**
  - Visually inspect the bottle to ensure microparticles are resuspended. If microparticles are still adhered to the bottle, continue to invert the bottle until the microparticles have been completely resuspended.
  - **If the microparticles do not resuspend, DO NOT USE. Contact your local Abbott representative.**
  - Once the microparticles have been resuspended, place a septum on the bottle. For instructions about placing septums on bottles, refer to the **Reagent Handling** section of this package insert.
- Load the reagent kit on the ARCHITECT iSystem.
  - Verify that all necessary reagents are present.
  - Ensure that septums are present on all reagent bottles.
- Order calibration, if necessary.
  - For information on ordering calibrations, refer to the ARCHITECT System Operations Manual, Section 6.
- Order tests.
  - For information on ordering patient specimens and controls and for general operating procedures, refer to the ARCHITECT System Operations Manual, Section 5.
- Minimum sample cup volume is calculated by the system and printed on the Orderlist report. 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 board:

    Sample volume for first test: 150 µL

    Sample volume for each additional test from same sample cup: 10 µL
  - > 3 hours on board: additional sample volume is required. For information on sample evaporation and volumes, refer to the ARCHITECT System Operations Manual, Section 5.
  - If using primary or aliquot tubes, use the sample gauge to ensure sufficient patient specimen is present.
- Prepare ARCHITECT 25-OH Vitamin D Calibrators and Controls.
  - Mix calibrator(s) and controls by gentle inversion before use.
  - Hold bottles **vertically** and dispense recommended volumes into each respective sample cup.
  - Recommended volumes:

    for each calibrator: 4 drops

    for each control: 4 drops
- Load samples.
  - For information on loading samples, refer to the ARCHITECT System Operations Manual, Section 5.
- Press RUN.
- For additional information on principles of operation, refer to the ARCHITECT System Operations Manual, Section 3.
- For optimal performance, it is important to perform routine maintenance as described in the ARCHITECT System 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 155.9 ng/mL (389.8 nmol/L) may be diluted using the Manual Dilution Procedure.

Manual Dilution Procedure

Suggested dilution: 1:2

1. Add 100 µL of the sample to 100 µL of ARCHITECT 25-OH Vitamin D Calibrator A.
2. The operator must enter the dilution factor in the Patient or Control order screen.

The system will use this dilution factor to automatically calculate the concentration of the sample before dilution and report the result.

For detailed information on ordering dilutions, refer to the ARCHITECT System Operations Manual, Section 5.

Calibration

- Test Calibrators A-F in duplicate. The calibrators should be priority loaded.

  A single replicate of each control level must be tested to evaluate the assay calibration.

  Ensure that assay control values are within the ranges specified in the respective control package insert.
- Calibration Range: 0.0 - 160.0 ng/mL (0.0 - 400.0 nmol/L).
- Once an ARCHITECT 25-OH Vitamin D calibration is accepted and stored, all subsequent samples may be tested without further calibration unless:
  - **The calibration is older than 30 days.**
  - 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.

    The ARCHITECT 25-OH Vitamin D assay may also need to be recalibrated after specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the assay.

For detailed information on how to perform an assay calibration, refer to the ARCHITECT System Operations Manual, Section 6.

Quality Control Procedures

- The recommended control requirement for the ARCHITECT 25-OH Vitamin D assay is that a single replicate of each control level be tested:
  - Once every 24 hours each day of use
  - After performing calibration
  - After instrument service procedures or maintenance that may affect assay performance have been performed.

  If the quality control procedures in your laboratory require more frequent use of controls to verify test results, follow your laboratory-specific procedures.
- Additional controls may be tested in accordance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.
- Each laboratory should establish control ranges to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.
- To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying 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 (ranges) 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
- Data points collected at different times of the day

- These results should be applied to your laboratory's quality control practices. In addition, the laboratory must ensure that the matrix of the control material is suitable for use in the assay per the assay package insert.
- Unless specified, target values and ranges provided with the commercial control product insert are guidelines only and should not be used for quality control purposes.
- Refer to Clinical and Laboratory Standards Institute (CLSI) Document C24-A3, or other published guidelines for general quality control recommendations.[17]

Verification of Assay Claims

For protocols to verify package insert claims, refer to the ARCHITECT System Operations Manual, Appendix B.

The ARCHITECT 25-OH Vitamin D assay belongs to method group 2.

RESULTS

Calculation

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

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

The ARCHITECT iSystem calculates the Calibrator A through F mean chemiluminescent signal from two Calibrator A through F replicates, generates a calibration curve and stores the result.

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 ARCHITECT System 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 imprecision, bias, and linearity.

The measuring interval of the ARCHITECT 25-OH Vitamin D assay is 3.4 to 155.9 ng/mL (8.5 to 389.8 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 ARCHITECT 25-OH Vitamin D that employ rabbit monoclonal antibodies. Additional information may be required for diagnosis.[18]
- 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.
- Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Additional information may be required for diagnosis.[19]
- The ARCHITECT 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

It is recommended that each laboratory establish its own reference range, which may be unique to the population it serves depending upon geographical, season, patient, dietary, or environmental factors.

A study was performed based on guidance from Clinical and Laboratory Standards Institute (CLSI) C28-A3c.[20] 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.

Representative data; results in individual laboratories and in different geographical areas may vary from these data.

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).[21]

SPECIFIC PERFORMANCE CHARACTERISTICS

Data in the section **SPECIFIC PERFORMANCE CHARACTERISTICS** were generated using the ARCHITECT i2000SR System.

Assay results obtained in individual laboratories may vary from data presented.

Precision

A study was performed based on guidance from National Committee for Clinical Laboratory Standards (NCCLS) EP5-A2.[22] Testing was conducted using 3 lots of ARCHITECT 25-OH Vitamin D Reagents, 2 lots of ARCHITECT 25-OH Vitamin D Calibrators, and 1 lot of ARCHITECT 25-OH Vitamin D Controls, and 2 instruments. Three controls and 7 human serum panels were assayed in a minimum of 2 replicates at 2 separate times per day on 20 different days.

Sample	Instrument	Reagent Lot	n	Mean (ng/mL)	Within-Run SD	Within-Run %CV	Within-Laboratory (Total)[a] SD	Within-Laboratory (Total)[a] %CV
Low Control	1	1	119	20.0	0.45	2.2	0.60	3.0
		2	120	20.2	0.46	2.3	0.60	3.0
		3	119	20.6	0.44	2.2	0.61	3.0
	2	1	119	20.3	0.35	1.7	0.56	2.7
		2	120	20.8	0.46	2.2	0.72	3.5
		3	119	20.6	0.48	2.4	0.75	3.6
Medium Control	1	1	120	39.7	0.85	2.1	1.09	2.8
		2	120	40.2	0.83	2.1	1.25	3.1
		3	118	40.8	0.84	2.1	1.07	2.6
	2	1	119	40.3	0.73	1.8	1.07	2.7
		2	120	41.2	0.88	2.1	1.32	3.2
		3	119	40.3	0.84	2.1	1.29	3.2
High Control	1	1	120	75.6	1.44	1.9	1.81	2.4
		2	120	76.7	1.70	2.2	2.47	3.2
		3	120	78.3	2.21	2.8	3.19	4.1
	2	1	120	76.4	1.66	2.2	2.45	3.2
		2	120	78.4	1.89	2.4	2.65	3.4
		3	120	77.1	1.88	2.4	3.11	4.0
Panel A	1	1	120	5.3	0.21	3.9	0.29	5.5
		2	119	5.0	0.25	5.1	0.35	7.1
		3	120	5.2	0.24	4.7	0.32	6.2
	2	1	118	5.4	0.24	4.4	0.38	7.1
		2	120	5.3	0.24	4.6	0.37	7.0
		3	120	5.3	0.24	4.5	0.36	6.8
Panel B	1	1	120	10.0	0.25	2.5	0.37	3.7
		2	120	9.5	0.26	2.8	0.40	4.2
		3	119	9.7	0.30	3.1	0.38	3.9
	2	1	120	10.1	0.29	2.8	0.40	3.9
		2	120	10.0	0.24	2.4	0.44	4.4
		3	120	9.9	0.30	3.1	0.43	4.3
Panel C	1	1	118	20.8	0.47	2.3	0.54	2.6
		2	119	20.6	0.44	2.1	0.70	3.4
		3	120	20.9	0.44	2.1	0.66	3.2
	2	1	120	21.2	0.39	1.9	0.61	2.9
		2	120	21.3	0.41	1.9	0.73	3.4
		3	119	20.8	0.42	2.0	0.67	3.2
Panel D	1	1	120	29.9	0.55	1.8	0.69	2.3
		2	119	30.1	0.59	1.9	0.89	3.0
		3	120	30.4	0.70	2.3	0.88	2.9
	2	1	119	30.5	0.54	1.8	0.83	2.7
		2	120	31.1	0.68	2.2	1.01	3.3
		3	120	30.0	0.67	2.2	0.97	3.2
Panel E	1	1	120	69.4	1.32	1.9	1.64	2.4
		2	120	72.2	1.66	2.3	2.51	3.5
		3	119	73.4	1.77	2.4	2.46	3.4
	2	1	118	71.0	1.26	1.8	2.01	2.8
		2	120	74.0	1.56	2.1	2.11	2.8
		3	120	72.3	1.66	2.3	2.61	3.6
Panel F	1	1	120	104.5	2.40	2.3	3.26	3.1
		2	120	109.1	3.29	3.0	4.55	4.2
		3	120	113.5	3.00	2.6	4.40	3.9
	2	1	120	107.1	2.88	2.7	3.77	3.5
		2	120	112.7	3.05	2.7	4.47	4.0
		3	120	112.0	2.92	2.6	4.58	4.1
Panel G	1	1	120	147.2	4.38	3.0	4.82	3.3
		2	120	158.1	6.21	3.9	7.31	4.6
		3	119	158.3	5.58	3.5	6.79	4.3
	2	1	120	148.6	5.19	3.5	6.29	4.2
		2	120	152.6	5.78	3.8	6.36	4.2
		3	119	158.1	6.68	4.2	8.01	5.1

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

Linearity

A study was performed based on guidance from NCCLS EP6-A.[23] Three dilution series were prepared as follows: a high 25-OH vitamin D sample was combined in specific ratios with a low 25-OH vitamin D sample. The 3 dilution series, including the low-level and high-level samples, were tested using the ARCHITECT 25-OH Vitamin D assay. The ARCHITECT 25-OH Vitamin D assay demonstrated linearity from 3.4 to 155.9 ng/mL (8.5 to 389.8 nmol/L).

Sensitivity

Limit of Blank and Limit of Detection

A study was performed based on guidance from CLSI EP17-A2.[24] Four zero-level samples (Calibrator A) and 14 low-level 25-OH vitamin D samples (2 samples at each of 7 unique target concentrations of approximately 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ng/mL) were tested over a minimum of 3 days using 2 reagent lots and 2 instruments. The highest observed Limit of Blank (LoB) value was 1.6 ng/mL (4.0 nmol/L), and the highest observed Limit of Detection (LoD) value was 2.2 ng/mL (5.5 nmol/L).

Limit of Quantitation

In the same study, the Limit of Quantitation (LoQ) was determined. The highest observed LoQ value at ≤ 20% CV was 2.4 ng/mL (6.0 nmol/L).

Specificity

Cross-Reactivity

A study was performed based on guidance from CLSI EP7-A2.[25] 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.

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%
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

A study was performed based on guidance from CLSI EP7-A2.[25] 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.

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.

A study was performed based on guidance from CLSI Document EP09-A3.[27] 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.

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%

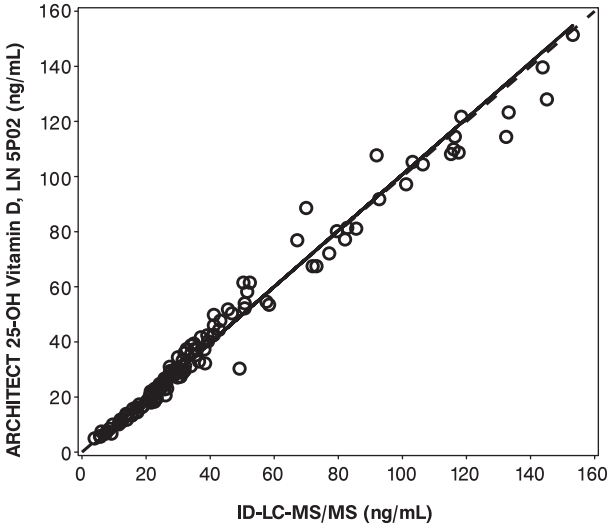
*Published data demonstrated that results from patients undergoing hemodialysis may show a negative bias when tested with various automated 25-OH vitamin D assays when compared to LC-MS/MS.[26]

Method Comparison

A correlation study using human serum specimens (n = 129) was performed based on guidance from CLSI Document EP09-A3.[27] The specimens were tested with the ARCHITECT 25-OH Vitamin D assay (LN 5P02) and compared to assigned reference values provided by the CDC (Centers for Disease Control and Prevention) or the University of Ghent, which were generated using a certified reference method, Isotope Dilution - Liquid Chromatography - Tandem Mass Spectrometry (ID-LC-MS/MS) for total 25-OH vitamin D. The results were evaluated using the Passing-Bablok[28] regression method. The data are summarized in the following table and graph.

Concentration Range (ng/mL)				
ARCHITECT 25-OH Vitamin D (LN 5P02)	ID-LC-MS/MS	Correlation Coefficient (r) (95% CI[a])	Slope (95% CI[a])	Intercept (95% CI[a])
4.9 - 151.3	4.0 - 153.2	0.99 (0.99, 0.99)	1.02 (0.99, 1.05)	-0.99 (-1.92, -0.24)

[a] CI = Confidence Interval



Tube Type Matrix Comparison

The following tube types are acceptable for use with the ARCHITECT 25-OH Vitamin D assay:

- Serum, including serum separator tubes (SST)
- Plasma: dipotassium EDTA, tripotassium EDTA, sodium heparin, lithium heparin powder, plasma separator tubes (PST) – lithium heparin gel

Each evaluation tube type was compared to the control tube type (serum), and the results were evaluated using the Passing-Bablok regression method. The equations and correlation coefficients (r) are summarized in the following table.

	Serum Specimen	Plasma Specimen				
	SST	Dipotassium EDTA	Tripotassium EDTA	Sodium Heparin	Lithium Heparin Powder	PST Lithium Heparin Gel
N	51	51	51	51	51	51
Passing-Bablok	y=0.99x +0.04	y=0.93x +0.76	y=0.92x +0.83	y=0.94x +0.84	y=0.94x +0.90	y=0.93x +1.09
r value	1.00	1.00	1.00	1.00	1.00	1.00

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Key to Symbols

	Consult instructions for use
	Manufacturer
	Sufficient for
	Temperature limitation
	Use by/Expiration date

ASSAY DILUENT	Assay Diluent
CONJUGATE	Conjugate
CONTAINS: AZIDE	Contains Sodium Azide. Contact with acids liberates very toxic gas.
CONTAINS: METHANOL	Contains Methanol
CONTROL NO.	Control Number
DISTRIBUTED IN THE USA BY	Distributed in the USA by
INFORMATION FOR USA ONLY	Information needed for United States of America only
IVD	*In Vitro* Diagnostic Medical Device
LOT	Lot Number
MICROPARTICLES	Microparticles
PRE-TRIGGER SOLUTION	Pre-Trigger Solution
PRODUCT OF IRELAND	Product of Ireland
REACTION VESSELS	Reaction Vessels
REAGENT LOT	Reagent Lot
REF	List Number
REPLACEMENT CAPS	Replacement Caps
Rx ONLY	For use by or on the order of a physician only (applicable to USA classification only).
SAMPLE CUPS	Sample Cups
SEPTUM	Septum
SN	Serial number
TRIGGER SOLUTION	Trigger Solution
WARNING: ORGAN DAMAGE	Warning: May cause damage to organs.
WARNING: SENSITIZER	Warning: May cause an allergic reaction.
WASH BUFFER	Wash Buffer

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