DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
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ABBOTT LABORATORIES
JUDITH WALLACH
REGULATORY AFFAIRS PROJECT MANAGER
DEPT 09AA, BLDG. AP8-1
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August 12, 2016

Re: K153375
Trade/Device Name: ARCHITECT 25-OH Vitamin D 5P02,
ARCHITECT 25-OH Vitamin D 5P02 Calibrators,
ARCHITECT 25-OH Vitamin D 5P02 Controls
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D test system
Regulatory Class: II
Product Code: MRG, JIT, JJX
Dated: July 8, 2016
Received: July 11, 2016

Dear Ms. Judith Wallach:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Katherine Serrano -S

For: Courtney H. Lias, Ph. D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
k153375

Device Name
ARCHITECT 25-OH Vitamin D 5P02
ARCHITECT 25-OH Vitamin D 5P02 Calibrators
ARCHITECT 25-OH Vitamin D 5P02 Controls

Indications for Use *(Describe)*
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.

The ARCHITECT 25-OH Vitamin D Calibrators are for the calibration of the ARCHITECT iSystem when used for the quantitative determination of 25 hydroxyvitamin D (25-OH vitamin D) in human serum and plasma.

The ARCHITECT 25-OH Vitamin D Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT iSystem when used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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K153375

510(k) Summary (Summary of Safety and Effectiveness)

This summary of the 510(k) safety and effectiveness information is being submitted in accordance with the requirements of SMDA 1990 and 21 CFR 807.92.

I. Applicant Name

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II. Device Name

ARCHITECT 25-OH Vitamin D 5P02
ARCHITECT 25-OH Vitamin D 5P02 Calibrators
ARCHITECT 25-OH Vitamin D 5P02 Controls

Reagents

Trade Name: ARCHITECT 25-OH Vitamin D 5P02
Device Classification: Class II
Classification Name: Vitamin D Test System
Governing Regulation: 862.1825
Code: MRG

Calibrators

Trade Name: ARCHITECT 25-OH Vitamin D 5P02 Calibrators
Device Classification: Class II
Classification Name: Calibrator
Governing Regulation: 862.1150
Code: JIT

Controls

Trade Name: ARCHITECT 25-OH Vitamin D 5P02 Controls
Device Classification: Class I, reserved
Classification Name: Quality Control Material (assayed and unassayed)
Governing Regulation: 862.1660
Code: JJX

III. Predicate Device

Reagents

ARCHITECT 25-OH Vitamin D (k110619)

Calibrators

ARCHITECT 25-OH Vitamin D Calibrators (k110619)

Controls

ARCHITECT 25-OH Vitamin D Controls (k110619)

IV. Description of Device

Reagents

The ARCHITECT 25-OH Vitamin D reagent kit contains:

- **Microparticles:** (1 bottle x 6.6 mL per 100-test / 1 bottle x 27.0 mL per 500-test / 4 bottles x 27.0 mL per 2000-test) Anti-vitamin D IgG (rabbit monoclonal) coated microparticles in MES Buffer. Minimum concentration: 0.04 % solids. Preservative: ProClin 300.

- **Conjugate:** (1 bottle x 5.9 mL per 100-test / 1 bottle x 26.3 mL per 500-test / 4 bottles x 26.3 mL per 2000-test). Acridinium-labeled vitamin D in MES Buffer and surfactant. Minimum concentration: 12 ng/mL labeled vitamin D. Preservative: Sodium Azide.

- **Assay Diluent:** (1 bottle x 10.0 mL per 100-test / 1 bottle x 50.9 mL per 500-test / 4 bottles x 50.9 mL per 2000-test). Citrate buffer with EDTA, Methanol, 8-anilino-1-naphthalenesulfonic acid (ANSA), and surfactant. Preservative: ProClin 300.

Calibrators

The ARCHITECT 25-OH Vitamin D calibrators contain:

- 6 Bottles (4.0 mL each) of ARCHITECT 25-OH Vitamin D Calibrators. Calibrators A-F contain PBS buffer and human serum. Calibrators B–F also contain different concentrations of 25-OH vitamin D. Preservatives: ProClin 950, Sodium Azide.

Calibrators cover the calibration range of the assay (0.0–160.0 ng/mL, 0.0-400.0 nmol/L). The calibrators are at the following 25-OH vitamin D concentrations:

Calibrator	25-OH Vitamin D Concentration (ng/mL)	25-OH Vitamin D Concentration (nmol/L)
A	0.0	0.0
B	4.0	10.0
C	10.0	25.0
D	30.0	75.0
E	75.0	187.5
F	160.0	400.0

Standardization Statement

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

Controls

The ARCHITECT 25-OH Vitamin D controls contain:

- 1 Bottle (8.0 mL) Low Control contains 25-OH vitamin D prepared in PBS buffer with human serum. Preservatives: ProClin 950, Sodium Azide.
- 1 Bottle (8.0 mL) Medium Control contains 25-OH vitamin D prepared in PBS buffer with human serum. Preservatives: ProClin 950, Sodium Azide.
- 1 Bottle (8.0 mL) High Control contains 25-OH vitamin D prepared in PBS buffer with human serum. Preservatives: ProClin 950, Sodium Azide.

The controls are at the following proposed target 25-OH vitamin D concentrations and ranges:

Control	25-OH Vitamin D Target Concentration		25-OH Vitamin D Control Range	
	(ng/mL)	(nmol/L)	(ng/mL)	(nmol/L)
Low	20.0	50.0	14.0–26.0	35.0–65.0
Medium	40.0	100.0	28.0–52.0	70.0–130.0
High	75.0	187.5	52.5–97.5	131.3–243.8

The target concentrations for the Low Control and the Medium Control were chosen to bracket the lower and upper medical decision points of 20.0 ng/mL and 30.0 ng/mL, respectively. The target concentration for the High Control was chosen to be near or above the upper end of the expected ranges for 25-OH vitamin D.

The ARCHITECT 25-OH Vitamin D Controls are prepared in PBS (Phosphate Buffered Saline) combined with heat-treated vitamin D depleted human serum (50% for Low Control and 75% for Medium and High Controls).

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.

V. Indications for Use of the Device

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.

The ARCHITECT 25-OH Vitamin D Calibrators are for the calibration of the ARCHITECT iSystem when used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma.

The ARCHITECT 25-OH Vitamin D Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT iSystem when used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma.

VI. Comparison of Technological Characteristics

The ARCHITECT 25-OH Vitamin D assay (candidate assay) utilizes a chemiluminescent microparticle immunoassay (CMIA) methodology for the quantitative *in vitro* determination of 25-OH vitamin D and is intended for use on the ARCHITECT iSystem.

The similarities and differences between the candidate assay and the predicate assay are presented in the following tables.

Reagent: Similarities

Characteristics	Candidate Device ARCHITECT 25-OH Vitamin D (List No. 5P02)	Predicate Device ARCHITECT 25-OH Vitamin D (k110619, List No. 3L52)
Platform	ARCHITECT i2000SR System	Same
Methodology	Chemiluminescent Microparticle Immunoassay (CMIA)	Same
Assay Protocol	Delayed 1-step	Same
Calibration Curve Type	6-point	Same
Intended Use and Indications for 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.	Same
Specific Analyte Detected	25-OH vitamin D	Same
Specimen Type	Serum or plasma	Same

Reagent: Differences

Characteristics	Candidate Device ARCHITECT 25-OH Vitamin D (List No. 5P02)	Predicate Device ARCHITECT 25-OH Vitamin D (k110619, List No. 3L52)
Tube Types	Serum: • Serum • Serum separator tubes (SST) Plasma: • Dipotassium EDTA • Tripotassium EDTA • Sodium heparin • Lithium heparin powder • Plasma separator tubes(PST) – lithium heparin gel	Serum: • Serum • SST Plasma: • Sodium heparin • Lithium heparin powder • PST – lithium heparin gel
Components	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.	Microparticles – Anti-human vitamin D IgG (sheep, polyclonal) coated microparticles in TRIS buffer. Minimum concentration: 0.05% solids. Preservatives: ProClin 300, ProClin 950. Conjugate – biotinylated vitamin D anti-biotin IgG (mouse, monoclonal) acridinium-labeled conjugate complex in BIS-TRIS HCl buffer with protein stabilizers (bovine gamma globulin) and detergent. Minimum concentration: 1.2 µg/mL anti-biotin IgG and 0.1 µg/mL vitamin-D-biotin. Preservative: sodium azide. Assay Diluent – Assay Diluent containing acetic acid buffer with EDTA. Preservatives: ProClin 300, ProClin 950. Pre-Treatment 1 – Pre-Treatment 1 containing triethanolamine methanol buffer and 8-anilino-1-naphtalensulfonic acid (ANSA). Pre-Treatment 2 – Pre-Treatment 2 containing triethanolamine methanol buffer and 8-anilino-1-naphtalensulfonic acid (ANSA).

Reagent: Differences

<table>
<tr><th>Characteristics</th><th>Candidate Device
ARCHITECT 25-OH Vitamin D
(List No. 5P02)</th><th>Predicate Device
ARCHITECT 25-OH Vitamin D
(k110619, List No. 3L52)</th></tr>
<tr><td>On-Board Storage</td><td>Maximum of 21 days</td><td>Maximum of 14 days</td></tr>
<tr><td>Expected Values</td><td>
<table>
<tr><th></th><th></th><th colspan="3">25-OH Vitamin D Values (ng/mL)</th></tr>
<tr><th></th><th></th><th></th><th colspan="2">Central 95% of Data[a]</th></tr>
<tr><th>Season</th><th>n</th><th>Mean</th><th>Lower Limit</th><th>Upper Limit</th></tr>
<tr><td>Winter</td><td>129</td><td>16.8</td><td>6.2</td><td>45.5</td></tr>
<tr><td>Summer</td><td>154</td><td>19.3</td><td>7.0</td><td>53.2</td></tr>
<tr><td>Combined</td><td>283</td><td>18.2</td><td>6.6</td><td>49.9</td></tr>
</table>
[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.</td><td>
<table>
<tr><th>n=137</th><th>Concentration (ng/mL)</th></tr>
<tr><td>Median</td><td>24.6</td></tr>
<tr><td>2.5th Percentile</td><td>< 13.0</td></tr>
<tr><td>97.5th Percentile</td><td>47.8</td></tr>
</table>
Representative data; results in individual laboratories and in different geographical areas may vary from these data.</td></tr>
<tr><td>Measuring Interval</td><td>3.4–155.9 ng/mL (8.5–389.8 nmol/L)

Note: Because the measuring interval of the predicate device does not extend the proposed range of the candidate device, the primary method comparison study was performed using the Isotope Dilution - Liquid Chromatography - Tandem Mass Spectrometry (ID-LC-MS/MS) as the comparator, which has a measuring interval of 0.6-160 ng/mL.</td><td>13.0–96.0 ng/mL
(32.5–240.0 nmol/L)</td></tr>
</table>

Calibrators: Similarities and Differences

Characteristics	Candidate Device ARCHITECT 25-OH Vitamin D (List No. 5P02)	Predicate Device ARCHITECT 25-OH Vitamin D (k110619, List No. 3L52)
Intended Use	The ARCHITECT 25-OH Vitamin D Calibrators are for the calibration of the ARCHITECT iSystem when used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma.	Same
Calibrator Level: Calibrator A Calibrator B Calibrator C Calibrator D Calibrator E Calibrator F	Concentration (ng/mL, nmol/L) 0.0 / 0.0 4.0 / 10.0 10.0 / 25.0 30.0 / 75.0 75.0 / 187.5 160.0 / 400.0	Same
Calibrator Material	Calibrators A–F contain PBS buffer and human serum. Calibrators B–F also contain different concentrations of 25-OH vitamin D. Preservatives: ProClin 950, Sodium Azide.	Calibrators A–F contain PBS buffer with heat inactivated horse serum. Calibrators B–F also contain 25-OH Vitamin D. Preservatives: ProClin 300, ProClin 950.
Standardization	The ARCHITECT 25-OH Vitamin D assay is standardized against NIST SRM 2972 (National Institute of Standards & Technology Standard Reference Material 2972).	The ARCHITECT 25-OH Vitamin D assay is standardized against internal reference material.
Calibration Storage	Maximum of 30 days	Maximum of 7 days

Controls: Similarities and Differences

Characteristics	Candidate Device ARCHITECT 25-OH Vitamin D (List No. 5P02)	Predicate Device ARCHITECT 25-OH Vitamin D (k110619, List No. 3L52)
Intended Use	The ARCHITECT 25-OH Vitamin D Controls are for the estimation of test precision and the detection of systematic analytical deviations of the ARCHITECT iSystem when used for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) in human serum and plasma.	Same
Control Level: Low Control Medium Control High Control	Target Concentration (ng/mL) (nmol/L) 20.0 50.0 40.0 100.0 75.0 187.5	Same
Control Material	25-OH vitamin D prepared in PBS buffer with human serum. Preservatives: ProClin 950, Sodium Azide.	25-OH Vitamin D in PBS buffer with heat inactivated horse serum. Preservatives: ProClin 300, ProClin 950.

VII. Summary of Nonclinical Performance

Expected Values (Reference Range)

A study was performed to determine the reference range / expected values for the ARCHITECT 25-OH Vitamin D assay on the ARCHITECT i2000SR System. The study was based on guidance from Clinical and Laboratory Standards Institute (CLSI) document C28-A3c.

Human serum specimens from apparently healthy individuals were collected during summer (April to October) and winter (November to March); a minimum of 120 specimens for each season were evaluated. The specimens were tested to determine calcium, TSH (thyroid-stimulating hormone), and intact PTH (parathyroid hormone) levels. Specimens with levels that were outside of the normal range 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), TSH (0.35 to 4.94 µIU/mL), or intact PTH (15.0 to 68.3 pg/mL) were excluded from this study. The study included specimens from male (n = 142) and female (n = 141) subjects 21 years or older from 3 different geographical locations in the 48 contiguous United States (north, south, and central).

Specimens from subjects with different skin tones (minimum 30% dark and 30% light) and ethnicities (African American, Hispanic, and Caucasian) were included. No more than 50% of the subjects were taking vitamin D supplement(s).

Two lots of reagents and 1 lot each of calibrators and controls were used to test one replicate of each specimen that met the inclusion criteria. Testing was performed on 2 ARCHITECT i2000SR instruments. Approximately the same number of randomized specimens was tested with each reagent lot/instrument combination.

The reference range was determined to be from 6.6 ng/mL to 49.9 ng/mL. The results 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.

20-Day Precision (Within-Laboratory)

Precision was determined based on guidance from the National Committee for Clinical Laboratory Standards (NCCLS) document EP5-A2. Samples included 3 controls and 7 serum panels.

Testing was performed using 2 ARCHITECT i2000SR instruments, 3 reagent lots, 2 calibrator lots, and 1 control lot.

One calibrator lot was paired with one reagent lot; the second calibrator lot was paired with the other reagent lots. A calibration per reagent lot was performed on each instrument by testing the calibrators in replicates of 2.

The samples were tested in a minimum of 2 replicates (from separate sample cups) 2 times per day (separated by a minimum of 2 hours) for a total of 20 testing days.

The within-laboratory (total) imprecision (within-run, between-run, and between-day) across reagent lots on one representative instrument for the ARCHITECT 25-OH Vitamin D assay was:

			Within-Run		Between-Run		Between-Day		Within-Laboratory (Total)	
Sample	**N**	**Mean (ng/mL)**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**	**SD**	**CV%**
Low Control	358	20.6	0.44	2.1	0.17	0.8	0.49	2.4	0.68	3.3
Medium Control	358	40.6	0.82	2.0	0.38	0.9	0.84	2.1	1.23	3.0
High Control	360	77.3	1.81	2.3	0.53	0.7	2.00	2.6	2.75	3.6
Panel A	358	5.3	0.24	4.5	0.11	2.1	0.26	4.8	0.37	6.9
Panel B	360	10.0	0.28	2.8	0.13	1.3	0.29	2.9	0.42	4.2
Panel C	359	21.1	0.41	1.9	0.22	1.1	0.49	2.3	0.67	3.2
Panel D	359	30.5	0.63	2.1	0.47	1.6	0.51	1.7	0.94	3.1
Panel E	358	72.4	1.53	2.1	0.61	0.8	1.56	2.2	2.26	3.1
Panel F	360	110.6	2.97	2.7	0.00	0.0	3.09	2.8	4.29	3.9
Panel G	359	153.1	5.91	3.9	1.84	1.2	3.10	2.0	6.93	4.5

The ARCHITECT 25-OH Vitamin D assay demonstrated acceptable precision. Samples at concentrations less than 8.0 ng/mL met the requirement of an SD of ≤ 0.8 ng/mL. Samples at concentrations ≥ 8.0 ng/mL met the requirement of ≤ 10 %CV. Panel A results support the measuring interval lower limit of 3.4 ng/mL (8.5 nmol/L); Panel G results support the measuring interval upper limit of 155.9 ng/mL (389.8 nmol/L).

Linearity

Linearity was determined based on guidance from NCCLS document EP6-A.

A low-level 25-OH vitamin D sample with a concentration at or below the Limit of Quantitation (LoQ) was prepared by diluting human serum with a low concentration of analyte with Calibrator A (low sample).

A high-level 25-OH vitamin D sample with a concentration at or above the upper limit of the measuring interval was prepared by spiking normal human serum with a 25-OH vitamin D3 stock solution (high sample).

The sample set was prepared by combining low and/or high samples to obtain 12 sample pools which spanned the measuring interval of the assay.

The samples/pools were tested in a minimum of 2 replicates using 1 lot of reagents, calibrators, and controls on 1 ARCHITECT i2000SR. All samples were tested within a single run.

The weighted least squares linear regression of the observed mean concentrations versus the expected concentration was calculated and yielded the following linear regression equation:

$$y = 0.9435x + 0.4 \text{ ng/mL}, r = 1.00$$

The linear range for the ARCHITECT 25-OH Vitamin D assay was demonstrated to be from 3.4 to 155.9 ng/mL (8.5 to 389.8 nmol/L).

Limits of Blank (LoB), Detection (LoD), and Quantitation (LoQ)

The LoB, LoD, and LoQ study was performed based on guidance from the CLSI document EP17-A2.

Four zero-analyte samples (Calibrator A, 0 ng/mL) were obtained for the study. Fourteen low-level samples (2 samples at each of 7 unique target concentrations of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, and 3.5 ng/mL) were prepared gravimetrically by diluting normal human serum with Calibrator A.

The 4 zero-analyte samples were tested in a minimum of 5 replicates and the 14 low-analyte samples were tested in a minimum of 10 replicates. Testing occurred over a minimum of 3 days using a minimum of 2 lots of reagents and 1 lot each of calibrators and controls on 2 ARCHITECT i2000SR instruments. Each sample was tested using each reagent lot and instrument combination.

- The LoB ranged from 1.1 to 1.6 ng/mL.
- The LoD ranged from 1.7 to 2.2 ng/mL.
- The LoQ ranged from 1.8 to 2.4 ng/mL.

The LoQ of the ARCHITECT 25-OH Vitamin D assay met the evaluation criteria.

The highest observed values will be reported in the package insert:

- LoB = 1.6 ng/mL
- LoD = 2.2 ng/mL
- LoQ at ≤ 20 %CV = 2.4 ng/mL

Specificity – Cross-Reactivity

Potential interference from the following potential cross-reactants was evaluated based on guidance from CLSI document EP7-A2:

- Vitamin D3 (cholecalciferol) at ≥ 100 ng/mL
- Vitamin D2 (ergocalciferol) at ≥ 100 ng/mL
- C-3-epimer- of 25-OH vitamin D3 (OHD3) at ≥ 100 ng/mL
- C-3-epimer- of 25-OH vitamin D2 (OHD2) at ≥ 100 ng/mL
- 1,25-(OH)2-vitamin D3 at ≥ 100 ng/mL
- 1,25-(OH)2-vitamin D2 at ≥ 100 ng/mL

- 24,25-(OH)2-vitamin D3 at ≥ 20 ng/mL
- 24,25-(OH)2-vitamin D2 at ≥ 20 ng/mL
- Paricalcitol (Zemplar) at ≥ 24 ng/mL

The potential cross-reactants were evaluated at three analyte levels (20 ng/mL, 30 ng/mL, and 40 ng/mL), bracketing the medical decision points.

Serum samples at these concentrations were obtained and each sample was divided into 2 aliquots for each potential cross-reactant: one aliquot for the test sample and one aliquot for the reference sample.

Each sample was tested in a minimum of 12 replicates using 1 lot each of reagents, calibrators, and controls on 1 ARCHITECT i2000SR instrument.

The % cross-reactivity across the three analyte levels (20 ng/mL, 30 ng/mL, and 40 ng/mL) was determined:

Cross-Reactant	**Concentration (ng/mL)**	**Maximum % Cross-Reactivity***
Vitamin D3 (Cholecalciferol)	100	0.8%
Vitamin D2 (Ergocalciferol)	100	0.4%
C-3-epimer- of 25-OHD3	100	1.3%
C-3-epimer- of 25-OHD2	100	0.8%
1,25-(OH)2-vitamin D3	100	0.1%
1,25-(OH)2-vitamin D2	100	-0.4%
Paricalcitol (Zemplar)	24	0.6%

Cross-Reactant	**Concentration (ng/mL)**	**% Cross-Reactivity**
24,25-(OH)2-vitamin D3	20	101.9% to 189.2%
24,25-(OH)2-vitamin D2	20	71.4% to 114.2%

Specificity – 25-OH Vitamin D3 Cross-Reactivity

A study was performed to evaluate the ARCHITECT 25-OH Vitamin D3 target analyte reactivity based on guidance from CLSI document EP7-A2.

The target analyte (25-OH vitamin D3) was tested at three analyte levels (20 ng/mL, 30 ng/mL, and 40 ng/mL), which bracketed the medical decision points.

* Maximum % cross-reactivity < 0% is reported as 0% cross-reactivity.

A 25-OH vitamin D3 stock solution (NIST SRM 2972) was obtained. The NIST SRM 2972 standard was added to:

- ARCHITECT 25-OH Vitamin D Calibrator A to obtain a sample at a concentration of 20 ng/mL.
- ARCHITECT 25-OH Vitamin D calibrator/control diluent (75%) to obtain samples at concentrations of 30 ng/mL and 40 ng/mL.

Each sample was tested in a minimum of 12 replicates using 1 lot each of reagents, calibrators, and controls on 1 ARCHITECT i2000SR instrument.

The target analyte reactivity for 25-OH vitamin D3 was determined at three analyte levels:

Spiked Analyte Level (ng/mL)	**% Cross-Reactivity (Target Analyte Reactivity)**
20.0	98.6%
30.0	101.1%
40.0	99.8%

The 25-OH vitamin D3 target analyte cross-reactivity met the evaluation criteria.

Specificity – 25-OH Vitamin D2 Cross-Reactivity

A study was performed to evaluate the 25-OH vitamin D2 cross-reactivity of the ARCHITECT 25-OH Vitamin D assay.

The cross-reactivity of the ARCHITECT 25-OH Vitamin D assay with 25-OH vitamin D2 (25OHD2) was assessed using endogenous (non-spiked) serum specimens. The specimens were obtained from approved vender(s) that met the following criteria:

- 25-OH vitamin D3 concentration below the LoQ of the validated LC-MS/MS method.
- endogenous 25 OH vitamin D2 concentrations close to 20 ng/mL and 60 ng/mL.

Each specimen was tested in a minimum of 2 replicates using 1 lot each of reagents, calibrators, and controls on 1 ARCHITECT i2000SR instrument.

The % cross-reactivity was calculated using the following equation:

$$\%\ \text{Cross-Reactivity}_{25OHD2} = 100 \times \frac{\text{25-OH vitamin D total (ARCHITECT)}}{\text{25-OH vitamin D2 (LC-MS/MS)}}$$

The 25-OH vitamin D2 cross-reactivity of the ARCHITECT 25-OH Vitamin D assay was determined to be 80.5% and 82.4% at 25-OH vitamin D2 concentrations of 26 ng/mL and 68 ng/mL, respectively, and met the evaluation criteria.

Interference: Endogenous Substance

Potential interference was evaluated based on guidance from the CLSI document EP7-A2. Interference effects were assessed by comparing test samples containing potentially-interfering endogenous substances to reference samples.

Each sample was tested in a minimum of 12 replicates using 1 lot each of reagents, calibrators, and controls on 1 ARCHITECT i2000SR instrument.

The ARCHITECT 25-OH Vitamin D assay is within acceptable limits from potentially-interfering endogenous substances at the following concentrations:

- conjugated bilirubin (up to and including 30 mg/dL)
- unconjugated bilirubin (up to and including 30 mg/dL)
- hemoglobin (up to and including 500 mg/dL)
- total protein (up to and including 12 g/dL)
- triglycerides (up to and including 500 mg/dL)
- biotin (up to and including 30 ng/mL)
- cholesterol (up to and including 500 mg/dL)

For conjugated bilirubin (targeted to 66 mg/dL), the lower and upper one-sided 95% CL (Confidence Limits) around the % difference were as follows:

- Analyte level: 20 ng/mL: 0.7%, 4.4%
- Analyte level: 30 ng/mL: 0.7%, 3.2%
- Analyte level: 40 ng/mL: -1.9%, 0.9%

For unconjugated bilirubin (targeted to 33 mg/dL), the lower and upper one-sided 95% CL around the % difference were as follows:

- Analyte level: 20 ng/mL: -0.6%, 3.0%
- Analyte level: 30 ng/mL: -1.0%, 1.8%
- Analyte level: 40 ng/mL: -1.4%, 1.2%

For hemoglobin (targeted to 600 mg/dL), the lower and upper one-sided 95% CL around the % difference were as follows:

- Analyte level: 20 ng/mL: -8.4%, -6.1%
- Analyte level: 30 ng/mL: -9.5%, -6.3%

- Analyte level: 40 ng/mL: -7.2%, -4.8%

For total protein up to 12 g/dL, the lower and upper one-sided 95% CL around the % difference were as follows:

- Analyte level: 20 ng/mL: 1.0%, 4.4%
- Analyte level: 30 ng/mL: -0.1%, 2.5%
- Analyte level: 40 ng/mL: -4.3%, -0.8%

For triglycerides (targeted to 500 mg/dL), the lower and upper one-sided 95% CL around the % difference were as follows:

- Analyte level: 20 ng/mL: -10.0%, -6.6%
- Analyte level: 30 ng/mL: -8.8%, -6.6%
- Analyte level: 40 ng/mL: -10.0%, -7.4%

For biotin (targeted to 35 ng/mL), the lower and upper one-sided 95% CL around the % difference were as follows:

- Analyte level: 20 ng/mL: -2.1%, 0.8%
- Analyte level: 30 ng/mL: -2.4%, 0.5%
- Analyte level: 40 ng/mL: -2.8%, -0.3%

For cholesterol (targeted to 550 mg/dL), the lower and upper one-sided 95% CL around the % difference were as follows:

- Analyte level: 20 ng/mL: -1.6%, 1.4%
- Analyte level: 30 ng/mL: -3.7%, -0.9%
- Analyte level: 40 ng/mL: -2.3%, 1.0%

Triglyceride interference greater than ± 10% was observed for triglycerides at a concentration of > 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.

Interference: Rheumatoid Factor and Heterophilic Antibody

Potential interference from rheumatoid factor (RF) and heterophilic antibody (*i.e.*, goat anti-rabbit antibodies [GARA]) was evaluated based on guidance from the CLSI document EP7-A2.

A 25-OH vitamin D stock solution was prepared by dissolving and diluting 25-hydroxyvitamin D3 (monohydrate) in ethanol, and then diluting further in vitamin D depleted human serum to obtain a final concentration of 2,000 ng/mL.

Samples were prepared at three analyte levels (20 ng/mL, 30 ng/mL, and 40 ng/mL) which bracket the medical decision points. The 20 ng/mL sample was prepared by pooling normal human serum. The 30 ng/mL and 40 ng/mL samples were prepared by supplementing normal human serum pools with the 25 OH vitamin D stock solution.

Each sample was divided into 2 aliquots: one aliquot for the test sample and one aliquot for the reference sample.

- The RF test sample was supplemented with RF stock solution with isotonic saline to a target RF concentration of ≥ 800 IU/mL. The RF reference sample was prepared by adding isotonic saline (equal to the volume of RF stock solution added to the test sample) to the reference aliquot.
- The GARA test sample was supplemented with GARA stock solution with ARCHITECT 25-OH Vitamin D Calibrator A to a target GARA concentration of ≥ 1 µg/mL. The GARA reference sample was prepared by adding ARCHITECT 25-OH Vitamin D Calibrator A (equal to the volume of GARA stock solution added to the test sample) to the reference aliquot.

Each sample was tested in a minimum of 12 replicates using 1 lot each of reagents, calibrators, and controls on 1 ARCHITECT i2000SR instrument.

Across the three 25-OH vitamin D analyte levels (20.0 ng/mL, 30.0 ng/mL, and 40.0 ng/mL), the lower and upper one-sided 95% CL around the % difference ranged from:

- RF = -2.3% to 1.6%
- GARA = -2.7% to 3.9%

The ARCHITECT 25-OH Vitamin D assay is not susceptible to interference effects from the RF at ≤ 800 IU/mL or GARA at ≤ 1 µg/mL.

Interference: Other Medical Conditions

Potential interference from other medical conditions (pregnant females and hemodialysis patients) was evaluated based on guidance from CLSI document EP09-A3. Potentially interfering other medical conditions were evaluated using serum samples.

Interference effects were assessed by comparing the investigational results to the results generated with a commercially-available device that is not susceptible to interference

from the medical conditions being evaluated (Liquid Chromatography – Tandem Mass Spectrometry [LC-MS/MS]). Specimens were sourced with concentrations that, at minimum, ranged from 20 ng/mL to 40 ng/mL 25-OH vitamin D.

For the investigational method, the specimens were tested internally using 2 lots of reagents, and 1 lot each of calibrators and controls on 2 ARCHITECT i2000SR instruments. For the comparator method, the specimens were tested at an external laboratory on 1 LC-MS/MS instrument.

Each specimen was tested in duplicate on the investigational method and at least once on the comparator method. Testing occurred over a minimum of 3 calendar days.

For specimens with 25-OH Vitamin D concentrations ranging from 20 ng/mL to 40 ng/mL, the mean % bias for each other medical condition is summarized in the following table.

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

The ARCHITECT 25-OH Vitamin D assay is not susceptible to interference effects from the following other medical conditions:

- Pregnant females, 1st trimester
- Pregnant females, 2nd trimester
- Pregnant females, 3rd trimester

The ARCHITECT 25-OH Vitamin D assay demonstrated a mean % bias of -15.3% with hemodialysis patient samples and therefore, is susceptible to interference effects from hemodialysis patients. **Note:** 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.[†]

[†] Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 Routine 25-Hydroxyvitamin D Assays; Influence of Vitamin D Binding Protein Concentration. *Clinical Chemistry* 2012; 58 (3):543-548.

Method Comparison

A method comparison study was performed based on guidance from the CLSI document EP09-A3.

A minimum of 100 human serum specimens were evaluated with the ARCHITECT 25-OH Vitamin D assay and the comparator method (Isotope Dilution - Liquid Chromatography - Tandem Mass Spectrometry [ID-LC-MS/MS]).

Normal human serum specimens were chosen to span the measuring interval of the assay, and no more than 10% of specimens were spiked with 25-OH vitamin D stock solution.

For the investigational method, the samples were tested internally using 2 lots of reagents and 1 lot each of calibrators and controls on 2 ARCHITECT i2000SR instruments. For the ID-LC-MS/MS method, the samples were tested at an external reference laboratory using a minimum of 1 ID-LC-MS/MS.

Each sample was tested in a minimum of 2 replicates for the investigational method. Testing occurred over a minimum of 3 calendar days.

The Passing-Bablok regression slope was 1.02, the intercept was -0.99, and the correlation coefficient (r-value) was 0.99 for samples across the measuring interval when evaluating the first replicate of the ARCHITECT 25-OH Vitamin D assay to the comparator method result.

The method comparison data for the investigational method, ARCHITECT 25-OH Vitamin D (List No. 5P02), and the comparator method, ID-LC-MS/MS, was acceptable.

Tube Type Equivalency

A study was performed based on guidance from CLSI document EP7-A2 to evaluate whether specific blood collection tube types are suitable for use with the ARCHITECT 25-OH Vitamin D assay.

Samples were obtained from a specimen vendor from a minimum of 40 donors in the control tube type (serum plastic) and in the following evaluation tube types:

- serum separator tubes (SST)
- dipotassium EDTA
- tripotassium EDTA
- sodium heparin
- lithium heparin powder

- plasma separator tubes (PST) – lithium heparin gel

The blood collection tubes from one individual constituted one donor set. The samples were processed according to the blood collection tube manufacturer's instructions.

Sample from each donor (in the control tube) was tested in replicates of 3 with the ARCHITECT 25-OH Vitamin D assay to determine the 25-OH vitamin D concentration.

The blood collection tubes (for no more than 20% of the donor sets) were supplemented with the 25-OH vitamin D stock solution to create samples that spanned the measuring interval of the assay.

The samples from each sample set were tested in a minimum of 2 replicates using 1 lot each of reagents, calibrators, and controls on 2 ARCHITECT i2000SR instruments.

The tube types under evaluation, when compared to the control tube type, had lower and upper one-sided 95% CL around the % difference within ± 10% across the measuring interval of the assay.

Each evaluation tube type was compared to the control tube type, 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

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

- serum plastic
- serum separator tubes (SST)
- dipotassium EDTA
- tripotassium EDTA
- sodium heparin

- lithium heparin powder
- plasma separator tubes (PST) – lithium heparin gel

Specimen Stability

A study was performed to evaluate serum and plasma specimens when subjected to various conditions (room temperature storage, 2 to 8°C storage, and freeze/thaw) and tested with the ARCHITECT 25-OH Vitamin D assay.

A total of 14 serum specimens and 14 plasma specimens were obtained from a specimen vendor in serum (plastic, no additive), serum separator (plastic, additive), and tripotassium EDTA (plastic) blood collection tubes.

The specimens in each of the tube types were tested in a minimum of 2 replicates using 1 lot each of reagents and calibrators, 2 lots of controls, and 2 ARCHITECT i2000SR instruments.

The ARCHITECT 25-OH Vitamin D assay had lower and upper one-sided 95% CL around the % difference of ± 10% when comparing baseline control specimens tested within 8 hours from draw to the same speciments stored at the following conditions:

- 2 to 8°C for ≥ 12 days (both on and off the clot)
- approximately 22°C or 30°C for ≥ 72 hours
- ≥ 4 freeze/thaw cycles after being stored at 2 to 8°C for ≥ 12 days off the clot

Specimens stored at 2 to 8°C (on or off the clot/cells) for up to 12 days, specimens stored at room temperature (approximately 22 to 30°C) for up to 72 hours, and specimens subjected to 4 freeze thaw cycles after being stored at 2 to 8°C off the clot for up to 12 days are acceptable for use in the ARCHITECT 25-OH Vitamin D assay.

Manual Dilution

A study was performed to demonstrate that the ARCHITECT 25-OH Vitamin D assay can recover manually-diluted samples.

A minimum of 13 unique donor specimens were obtained. The specimens were spiked with 25-OH vitamin D stock solution to create high samples with 25-OH vitamin D concentrations of approximately 180 ng/mL, 230 ng/mL, 280 ng/mL, and 320 ng/mL.

Test samples were prepared by diluting the high samples with ARCHITECT 25-OH Vitamin D Calibrator A (List No. 5P02A) at dilution factors of 1:2, 1:3, and 1:4.

The samples were tested in a minimum of 2 replicates using 1 lot each of reagents, calibrators, and controls on 1 ARCHITECT i2000SR instrument.

Using a first replicate concentration analysis, the mean (or median) % recovery value was 96.8% for the 1:2 dilution factor, 97.1% for the 1:3 dilution factor, and 103.7% for the 1:4 dilution factor.

The ability of the ARCHITECT 25-OH Vitamin D assay to recover manually-diluted samples was demonstrated.

Measuring Interval Determination

The measuring interval for the ARCHITECT 25-OH Vitamin D assay has been determined to be 3.4 to 155.9 ng/mL (8.5 to 389.8 nmol/L).

The measuring interval is based on the acceptable performance of imprecision, linearity, limit of quantitation, and bias (based on the method comparison with ID-LC-MS/MS) on the ARCHITECT i2000SR instrument.

Precision data, including samples ranging from 5.2 to 142.2 ng/mL, support the lower (3.4 ng/mL) and upper limits (155.9 ng/mL) of the measuring interval.

The linear range was demonstrated to be from 3.4 to 155.9 ng/mL.

The highest observed LoQ was 2.4 ng/mL, which is below the lower limit of the measuring interval (3.4 ng/mL).

Method comparison with ID-LC-MS/MS was performed with samples ranging from 4.0 to 153.2 ng/mL on the ID-LC-MS/MS, which supports the ARCHITECT 25-OH Vitamin D measuring interval of 3.4 to 155.9 ng/mL.

Conclusion Drawn from Nonclinical Laboratory Studies

The results presented in this 510(k) premarket notification demonstrate that the candidate assay (ARCHITECT 25-OH Vitamin D [List No. 5P02]) performance is substantially equivalent to the predicate assay (ARCHITECT 25-OH Vitamin D, k110619).