

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY  
ASSAY ONLY TEMPLATE**

**A. 510(k) Number:**

k110619

**B. Purpose for Submission:**

New assay

**C. Measurand:**

25-OH Vitamin D

**D. Type of Test:**

Quantitative, Automated Chemiluminescent immunoassay

**E. Applicant:**

Biokit S.A.

**F. Proprietary and Established Names:**

ARCHITECT 25-OH Vitamin D assay, ARCHITECT 25-OH Vitamin D  
Calibrators and ARCHITECT 25-OH Vitamin D Controls

**G. Regulatory Information:**

1. Regulation section:

21 CFR 862.1825, Vitamin D Test System;

862.1150, Calibrator;

862.1660, Quality Control Material

2. Classification:

Class II, II and I, reserved

3. Product code:

MRG, JIT, JJX

4. Panel:

Chemistry (75)

**H. Intended Use:**

1. Intended use(s):

See indications for use.

2. Indication(s) 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.

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

3. Special conditions for use statement(s):

For prescription use only.

4. Special instrument requirements:

Abbott Architect I

**I. Device Description:**

The device consists of the following reagents to be used on the Abbott Architect I Instrument:

Anti-human vitamin D IgG (sheep, polyclonal) coated microparticles in TRIS buffer, with preservatives (ProClin); 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.

Preservative: sodium azide; Assay Diluent containing acetic acid buffer with EDTA. Preservatives: ProClin 300, ProClin 950; Pre-Treatment containing

triethanolamine methanol buffer and 8-anilino-1-naphtalensulfonic acid (ANSA); Pre-trigger solution containing 1.32% (w/v) hydrogen peroxide; Trigger solution containing 0.35N sodium hydroxide; Wash buffer containing phosphate buffered saline solution. Preservatives: antimicrobial agents.

Calibrator and control materials:

Calibrators A - F and control materials contain PBS buffer with heat inactivated horse serum, and preservatives (Proclin). Calibrators B – F and control materials also contain 25-OH vitamin D.

The calibrators have the following target concentrations:

ng/mL	nMol/L
0	0
4	10
10	25
30	75
75	187.5
160	400

Controls materials are at target concentrations of 20, 40, and 75 ng/mL

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

LIAISON® 25 OH Vitamin D TOTAL Assay

2. Predicate 510(k) number(s):

k071480

3. Comparison with predicate:

Feature	Device	Predicate
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	Same

	assessment of vitamin D sufficiency.	
Platform	ARCHITECT <i>i</i> System	LIAISON® Analyzer
Methodology	Chemiluminescent immunoassay	Same
Components	<b>Microparticles:</b> Anti-human vitamin D IgG (sheep, polyclonal) coated microparticles. <b>Conjugate:</b> biotinylated vitamin D anti-Biotin IgG (mouse, monoclonal) acridinium-labeled conjugate complex.	<b>Magnetic particles:</b> Magnetic particles coated with antibody against 25 OH Vitamin D. <b>Conjugate (4.5 mL):</b> 25 OH Vitamin D conjugated to an isoluminol derivate.
Specimen type	Human serum or plasma	Same.

Sensitivity	LoB: 1.9 ng/mL LoD: 3.1 ng/mL LoQ: 8.0 ng/mL	Functional Sensitivity ≤ 4.0 ng/mL
Calibration Curve Type	6-point	2-point
Measuring interval	13.0 ng/mL to 96.0 ng/mL	4.0 ng/mL to 150.0 ng/mL

Feature	Device	Predicate
Intended Use	The ARCHITECT 25-OH Vitamin D Calibrators are for the calibration of the ARCHITECT <i>i</i> System when used for the quantitative determination of 25-hydroxyvitamin D (25-OH Vitamin D) in human serum and plasma.	Same

Components	<b>Calibrators A – F:</b> 6 bottles (4.0 mL each). Calibrators A - F contain PBS buffer with heat inactivated horse serum. Calibrators B to F also contain 25-OH Vitamin D.	<b>Calibrator 1 (1.0 mL):</b> Human serum, Tris buffer, and 25 OH Vitamin D. <b>Calibrator 2 (1.0 mL):</b> Human serum, Tris buffer, <0.1% sodium azide and 25 OH Vitamin D. The calibrator concentrations (ng/mL) are referenced to standard preparations containing highly purified 25 OH Vitamin D.
Calibration Range/Levels	Cal A: 0.0 ng/mL Cal B: 4.0 ng/mL Cal C: 10.0 ng/mL Cal D: 30.0 ng/mL Cal E: 75.0 ng/mL Cal F: 160.0 ng/mL	The predefined Master Curve is adjusted to a new instrument-specific curve using the two calibrators supplied in the Reagent Integral via the bar codes on the reagent integral label.

Key word	Device	Predicate
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 <i>i</i> System when used for the quantitative determination of 25-hydroxyvitamin D (25-OH Vitamin D) in human serum and plasma.	Same
Control Levels	3 levels	2 levels
Concentration/Ranges	Control L: 20.0 ng/mL (13.0 - 27.0) Control M: 40.0 ng/mL (26.0 - 54.0) Control H: 75.0 ng/mL (48.8 - 101.3)	The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

**K. Standard/Guidance Document Referenced (if applicable):**

CLSI guidelines:

EP5 - Evaluation of Precision Performance of Quantitative Measurement Methods

EP7 - Interference Testing in Clinical Chemistry

EP17- Protocols for Determination of Limits of Detection and Limits of Quantitation

C28 – How to Define and Determine Reference Intervals in the Clinical Laboratory

**L. Test Principle:**

The ARCHITECT 25-OH Vitamin D assay is a delayed one-step immunoassay including a sample pre-treatment for the quantitative determination of vitamin D in human serum and plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex. Sample and pre-treatment reagents are combined. An aliquot of the pre-treated sample is combined with assay diluent and paramagnetic anti-vitamin D coated microparticles to create a reaction mixture. Vitamin D present in the sample binds to anti-vitamin D coated microparticles. After incubation a biotinylated vitamin D anti-Biotin acridinium-labeled conjugate complex is added to the reaction mixture and binds to unoccupied binding sites of the anti-vitamin D coated microparticles. After washing, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). An indirect relationship exists between the amount of vitamin D in the sample and the RLUs detected by the ARCHITECT i System optics.

**M. Performance Characteristics (if/when applicable):**

Performance shown below was evaluated on the Architect i2000SR.

1. Analytical performance:

*a. Precision/Reproducibility:*

A study was performed with the ARCHITECT 25-OH Vitamin D assay based on the CLSI Protocol EP5-A2. Six samples consisting of 3 ARCHITECT 25-OH Vitamin D Controls and 3 serum based samples (Low, medium and high 25-OH vitamin D concentration) were assayed, using two lots of reagents, on one i 2000SR, in replicates of two at two separate times per day for 20 days.

In addition 2 serum based samples (Ultra low and ultra high vitamin D concentration) were assayed, using one lot of reagents, on one i 2000SR, in

replicates of two at two separate times per day for 20 days.

Results are shown below:

Sample	Reagent Lot	n	Mean Conc. (ng/mL)	Within Run		Within Laboratory (total)	
				SD	%CV	SD	%CV
Low Control	1	80	19	0.709	3.7	0.712	3.8
	2	80	19.5	0.589	3	0.889	4.6
Medium Control	1	80	38.5	0.873	2.3	1.142	3
	2	80	38	0.879	2.3	1.062	2.8
High Control	1	80	78.4	1.47	1.9	2.201	2.8
	2	80	76.3	1.485	1.9	2.034	2.7
Serum Panel 1	1	80	23	0.714	3.1	0.912	4
	2	80	22.4	0.548	2.4	0.78	3.5
Serum Panel 2	1	80	42.5	1.095	2.6	1.346	3.2
	2	80	40.1	0.668	1.7	1.274	3.2
Serum Panel 3	1	80	75.4	1.088	1.4	2.064	2.7
	2	80	71.3	1.242	1.7	1.869	2.6

*b. Linearity/assay reportable range:*

Linearity was determined using serum samples. by mixing a spiked high concentration of 125.9 ng/mL and a low of 8.3 ng/mL 25-OH vitamin D concentration in different ratios to create multiple levels of concentrations. The created high/low dilutions were tested in replicates of four. Both samples were pre-screened for Vitamin D binding protein and the high and low 25-OH vitamin D concentrations exhibited a difference in Vitamin D binding protein of 6%. The table below shows results in terms of deviation from linearity, according to CLSI guideline EP6.

Vitamin D concentration measured (ng/mL)	Percent deviation (%) from linearity
96.2	6
81.1	3
68.2	0
58.2	-1
46.3	-2

36.8	-2
27	-0
21.5	-2
17.1	5
13.1	10

Linear regression yielded  $y=0.91x - 1.5$ .  $R^2 = 0.9949$

Based on the results obtained the linear range is 13 to 96 ng/mL.

*c. Traceability, Stability, Expected values (controls, calibrators, or methods):*

Control materials:

Target ranges for control materials are: Low: 20.0 ng/mL (14.0 - 26.0 ng/mL); medium 40.0 ng/mL (28.0 - 52.0 ng/mL); high 75.0 ng/mL (52.5 - 97.0 ng/mL). The package insert states: Each laboratory should establish its own concentration ranges for new control lots at each control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days. Sources of variation that can be expected should be included in this study in order to be representative of future system performance.

Assay traceability and value assignment:

The ARCHITECT 25-OH Vitamin D is traceable to a manufacturer's internal standard (Primary Calibrator), which is anchored against Absorbance at 264 nm. Target concentrations are: Calibrator A: 0.0 ng/mL; Calibrator B: 4.0 ng/mL; Calibrator C: 10.0 ng/mL; Calibrator D: 30.0 ng/mL; Calibrator E: 75.0 ng/mL; Calibrator F: 160.0 ng/mL. The estimated uncertainty in assigned calibrator values is shown in terms of levels below:

<b>Conc. Level (ng/mL)</b>	<b>Conc. Range with 95% CI (ng/mL)</b>
20	±1.25
40	±1.10
75	±2.12

Stability of calibrators and controls:

Studies to support the claimed stability for opened and closed vials includes testing of calibrator held at the recommended 2-8 degrees C, relative to calibrator held at -70 degrees C. Criteria and results were reviewed and found to be adequate.

*d. Detection limit:*

A study was conducted to verify the limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) for the ARCHITECT 25-OH Vitamin

D assay.

Limit of Blank: Four different lots of Calibrator A (free of 25-OH Vitamin D) were tested in 4 replicates during 5 days (n = 80 data points in total). The observed value for the Limit of Blank for the ARCHITECT 25-OH Vitamin D assay is 1.9 ng/mL on the i 2000SR platform. (This was based on the highest value obtained within the two lots of reagents).

Limit of Detection: For each reagent lot / instrument combination, 4 different serum samples with low 25-OH Vitamin D concentrations (at the approximate target concentration of 4 ng/mL) were tested in 12 replicates during 5 days (n=60 per sample). The Limit of Detection determined for the ARCHITECT 25-OH Vitamin D assay is 3.6 ng/mL on the i 2000SR platform. (This was based on the highest value obtained within the two lots of reagents).

Performance at lower limit of reportable range: For each reagent lot / instrument combination, 4 different serum samples with low 25-OH Vitamin D concentrations targeted to 6.6, 7.1, 7.3 and 9.5 ng/mL were tested in 12 replicates during 5 days (n=60 per sample). At a concentration below 13 ng/mL (which is the lower limit based on linearity), CV was within 10%. In addition, deviations from linearity at 13 ng/mL are within 10% (see linearity section, above).

The assay has a measuring range of 13 to 96 ng/mL.

*e. Analytical specificity:*

Cross-reactivity testing was performed on one ARCHITECT *i* 2000SR using two lots of reagents for spiked cross-reactants and on one ARCHITECT *i* 2000SR using one lot of reagents for the endogenous (non-spiked) serum samples to determine 25-OH vitamin D<sub>2</sub> % cross-reactivity. Results are shown below:

25-OH Vitamin D <sub>3</sub> (100 ng/mL)	102%
Vitamin-D <sub>2</sub> (Ergocalciferol) (1000 ng/mL)	0%
Vitamin-D <sub>3</sub> (Cholecalciferol) (1000 ng/mL)	0.3%
24,25-(OH) <sub>2</sub> Vitamin D <sub>3</sub> (20 ng/mL),	114%
1,25-(OH) <sub>2</sub> -Vitamin D <sub>3</sub> (100 ng/mL)	11.5%
3-epi 25-OH Vitamin D <sub>3</sub> (100 ng/mL)	3.1%
Paricalcitol (Zemplar) (24 ng/mL)	1.7%

Cross-reactivity for 25-OH Vitamin D<sub>2</sub> was determined from analysis of patient samples by LC-MS/MS. Results from 2 runs were:

	Mean cross-reactivity	Lower 95% CI	Upper 95% CI
Run 1	75.37	55.86	94.88
Run 2	87.71	65.51	109.92
Mean of runs	81.54	60.95	102.13

Interference was evaluated according to CLSI document EP7-A2. No significant interference (i.e., defined by the sponsor as  $\leq 10\%$  change in recovery) was observed in serum upon addition of :

- Hemoglobin up to 200 mg/dL
- Bilirubin at 20 mg/dL
- Triglyceride at 500 mg/dL
- Biotin at 30 ng/mL
- HAMA at 1000 ng/mL
- Red blood cells at 0.4% (v/v)
- Protein up to 12 g/dL final concentration
- Rheumatoid Factor up to 400 IU/mL
- Cholesterol up to 370 mg/dL

For hemoglobin with concentrations between 200 mg/dL and 500 mg/dL the % Recovery ranged from 90% to 62%.

For rheumatoid factors with concentrations between 400 IU/mL and 800 IU/mL the % Recovery ranged from 107% to 118%. The package insert includes the following statement: Specimens with hemoglobin greater than 200 mg/dL may generate falsely low results. Specimens with rheumatoid factor greater than 400 IU/mL may generate falsely high results.

*f. Assay cut-off:*

Not applicable – this is a quantitative assay.

## 2. Comparison studies:

*a. Method comparison with predicate device:*

A study was conducted to verify the correlation of the ARCHITECT 25-OH Vitamin D Reagent on the ARCHITECT i System to the predicate device (DiaSorin Liaison 25-OH Vitamin D TOTAL Assay) and to an LC-MS/MS method. Three lots of reagents were used. Patient samples were individual random banked serum specimens. A total of 131 samples were tested. Results shown below are based on single measurements of each sample.

Methods	n	Correlation coefficient	Passing-Bablok slope	Passing-Bablok intercept	Architect concentration range
Architect vs. Liason	131	0.93 [0.91 to 0.95]	0.97 [0.91 to 1.04]	-1.07 [-3.42 to 1.38]	13 to 96 ng/mL

*b Matrix comparison:*

Five plasma tube types and two serum tube types (without additives and SST) were collected from 39 individuals 4 of which were spiked to obtain the needed concentrations to span the assay range. These fresh (less than 24 hours from the time of draw) blood specimens with endogenous 25-OH vitamin D concentration sourced from a commercial source were analyzed with one lot of ARCHITECT 25-OH Vitamin D reagent on the ARCHITECT *i* System (*i* 2000SR).

Human serum and plasma specimens were collected in the following tube types:

- serum no additive (glass)
- serum separator tube (SST)
- potassium EDTA plasma
- sodium-citrate plasma
- lithium heparin plasma powder
- lithium heparin plasma gel
- sodium heparin plasma

The difference in mean concentration (ng/mL) between each evaluation tube type and the control tube was calculated using the following formula: difference (mean concentration of the evaluation tube type – mean concentration of control tube type (serum without additives)) Sample concentrations ranged from near 20 to near 70 ng/mL.

Tube Type comparison	n	Deming Regression-Slope	Deming Regression-Intercept	Test Range (ng/mL)
Serum Separator Tube (SST)- Serum no additive	39	1.01	0.62	18.8 – 76.4
Li-Heparin powder plasma- Serum no additive	39	0.95	1.13	17.9 – 72.1

Li-Heparin gel plasma-Serum no additive	35	0.96	0.68	17.8 – 73.9
Na-Heparin plasma-Serum no additive	35	0.93	1.24	18.2 – 71.8

The % Difference between each evaluation tube type and the control tube for each donor with each matrix was also submitted. Summaries are shown below.

Tube Type comparison	Mean percent difference [95% CI]	SD of percent difference
Serum Separator Tube (SST)-Serum no additive	4.2 [3.6 to 4.8]	4.6
Li-Heparin powder plasma-Serum no additive	-2.2 [-3.2 to -1.3]	2.9
Li-Heparin gel plasma-Serum no additive	-1.5 [-2.5 to 0.5]	2.9
Na-Heparin plasma- Serum no additive	-2.8 [-4,2 to -1,3]	4.3

The labeling states that Sodium citrate plasma and potassium EDTA plasma tubes cannot be used with the ARCHITECT 25-OH Vitamin D assay.

3. Clinical studies:

- a. *Clinical Sensitivity:* Not applicable; Clinical sensitivity and specificity is not typically provided in 510(k)s for this type of assay.
- b. *Clinical specificity:* See a, above.
- c. Other clinical supportive data (when a. and b. are not applicable): Data regarding patient demographics and selection criteria were provided in the method comparison evaluation in the 510(k).

4. Clinical cut-off:

Not applicable; this is a quantitative assay.

5. Expected values/Reference range:

A study was conducted to establish a Reference Interval.

It is also 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.

The study was conducted based on guidance from the Clinical Laboratory and Standard Institute (CLSI), Protocol C28-A3. Two hundred serum specimens of apparently healthy individuals from the US (North, Central and South states), collected during summertime (June to July 2011) were evaluated (in replicates of one) using the ARCHITECT 25-OH Vitamin D assay. A medical questionnaire was used to select the reference sample group based on the following exclusion criteria: no family history of parathyroid or calcium regulatory disease, no personal history of disease of kidney, gastrointestinal, liver, thyroid or parathyroid, no history of seizures or bariatric surgery, individuals with Vitamin D supplementation of  $\geq 2,000$  IU/day, individuals currently prescribed any medications that include cholesterol absorption inhibitors, anticonvulsants, glucocorticoids, AIDS antiretroviral drugs and anti-rejection drugs. Individuals with results outside of the expected values for serum calcium (2.15 – 2.50 mmol/L for individuals < 60 years and 2.20 – 2.55 mmol/L for individuals >60 years), TSH (0.35 – 4.94  $\mu$ IU/mL), and intact PTH (15.0 – 68.3 pg/mL) were excluded. After applying the exclusion criteria the following groups were included in the calculation:

	N
Total Group	137
Male	75
Female	62
Northern USA	39
Central USA	50
Southern USA	48
Light Skin	74
Dark Skin	63
African American	60
Caucasian	74
Hispanic	3
Some Vit-D Supplementation (<2000 IU/day)	9

		All	female	male
n		137	62	75
2.5th	Percentile	<13	<13	<13

97.5th	Percentile	47.8	48.8	48.1
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The package insert also notes: “representative data; results in individual laboratories and in different geographical areas may vary from these data. There is currently debate over the recommended target range of vitamin D in serum, but the Endocrine Society Clinical Practice Guideline recently suggested a target level of at least 30 ng/mL.

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.