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

A. 510(k) Number:

k123131

B. Purpose for Submission:

New assay

C. Measurand:

25-hydroxy Vitamin D

D. Type of Test:

Quantitative immunoassay

E. Applicant:

Tosoh Biosciences, Inc.

F. Proprietary and Established Names:

ST AIA-PACK 25-OH Vitamin D
ST AIA-PACK 25-OH Vitamin D Calibrator Set
AIA-PACK 25-OH Vitamin D Control Set

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	II	862.1825 Vitamin D Test System	Chemistry (75)
JIT	II	862.1150 Calibrator	Chemistry (75)
JJX	I, reserved	862.1660 Quality control material (assayed and unassayed)	Chemistry (75)

H. Intended Use:

1. Intended use(s):

   See indications for use.

2. Indication(s) for use:

   ST AIA-PACK 25-OH Vitamin D is designed for in vitro diagnostic use only for the quantitative measurement of total 25-hydroxyvitamin D (25-OH vitamin D) in human serum, Na-heparinized or EDTA plasma on TOSOH AIA System Analyzers. The Tosoh ST AIA-PACK 25-OH Vitamin D is intended as an aid in the determination of Vitamin D sufficiency.

   The ST AIA-PACK 25-OH Vitamin D Calibrator Set is intended for in vitro diagnostic use only for the calibration of the ST AIA-PACK 25-OH Vitamin D assay.

   The AIA-PACK 25-OH Vitamin D Control Set is intended for in vitro diagnostic use only for performing quality control procedures with the ST AIA-PACK 25-OH Vitamin D assay.

3. Special conditions for use statement(s):

   For prescription use only.

   Do not use hemolyzed sample. Hemolyzed sample will give erroneous results.

4. Special instrument requirements:

   TOSOH AIA-2000 System Analyzer

I. Device Description:

1. Tosoh's ST AIA-PACK 25-OH Vitamin D kit is provided as:

   -5 trays of 20 plastic test cups containing twelve magnetic beads coated with lyophilized anti-25-OH vitamin D sheep monoclonal antibody with sodium azide as a preservative.

   -5 vials containing 5 mL 25-OH vitamin D conjugated to bovine alkaline phosphatase.

2. Tosoh's ST AIA-PACK 25-OH Vitamin D Calibrator set is sold separately and contains:

   Six assigned concentrations of 25-OH Vitamin D in human serum (described on each vial) with sodium azide as preservative in aliquots of 1.0 mL, comes in lyophilized form, with vitamin D at target values of 0 (base material),10, 20, 40, 80, and 165 ng/mL.

3. Tosoh's AIA-PACK 25-OH Vitamin D Control Set is sold separately and contains:

   Two level controls (approximately 20 and 80 ng/mL) in human serum supplied lyophilized in 1 mL vials.

4. Tosoh's AIA-PACK 25-OH Vitamin D Pretreatment Set is sold separately and contains:

   Six empty vials for performing pretreatment of test samples, four 4 mL bottles of ST AIA-PACK 25-OH Vitamin D Pretreatment Set (a buffered solution containing no detectable concentration of 25-OH Vitamin D with sodium azide as a preservative), one 32 mL bottle of ST AIA-PACK 25-OH Vitamin D Pretreatment-1 (an aqueous solution containing sodium hydroxide), and six 5mL bottles of ST AIA-PACK 25-OH Vitamin D Pretreatment-2 (a buffered solution containing surfactant)

   The calibrator and control materials has been tested by FDA-approved methods and found negative for the presence of HBsAg, antibody to HIV-1/2 and HCV.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   DiaSorin 25-Hydroxyvitamin D ^{125}I RIA Kit

2. Predicate 510(k) number(s):
   k983617

3. Comparison with predicate:

Similarities and Differences		
Items	ST AIA-PACK 25-OH Vitamin D (Candidate Device)	DiaSorin 25-Hydroxyvitamin D ^{125}I RIA Kit (Predicate Device)
Intended Use/Indications for Use	Device is designed for in vitro diagnostic use only for the quantitative measurement of total 25-hydroxyvitamin D in serum or plasma. Vitamin D is intended as an aid in the determination of Vitamin D sufficiency.	Same
Matrix	EDTA plasma, Na-heparinized plasma, or serum	Plasma or serum
Assay Principle	Fluorescence Immunoassay	Radioimmunoassay
Reference Range	10.83 to 54.75 ng/mL	9 to 37.6 ng/mL
Assay Range	4 – 120 ng/mL	4.8 – 100 ng/mL

Similarities and Differences		
Item	ST AIA-PACK 25-OH Vitamin D Calibrator Set	25-OH D3 Calibrators for Diasorin^{125}I RIA Kit (Predicate Device k983617)
Intended use	For *in vitro* diagnostic use in calibration of Vitamin D assay	Same
Matrix	Lyophilized human serum	Liquid human serum
Number of calibrators	6	Same
Range	0-165 ng/mL	0-100 ng/mL
Storage temperature	2-8°C	same

Similarities and Differences		
Item	AIA-PACK 25-OH Vitamin D Control Set	25-OH-D Controls for Diasorin^{125}I RIA Kit (Predicate Device k983617)
Intended Use	For *in vitro* diagnostic for performing quality control procedures of the Vitamin D assay	Same
Matrix	Lyophilized human serum	Liquid human serum
Number of vials	2	Same
Levels	2 levels at approximately 20 and 80 ng/mL of 25-OH Vitamin D	Two levels at 9 and 37.6 ng/mL of 25-OH Vitamin D
Storage temperature	2-8°C	same

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

CLSI C28-A3, Defining, Establishing, and verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition

CLSI EP6-A, Evaluation of the Linearity of Quantitative Measurement Procedures

CLSI EP07-A2, Interference Testing in Clinical Chemistry; Approved Guideline - Second Edition

CLSI EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantitation

CLSI EP21-A, Estimation of Total Analytical Error for Clinical Laboratory Methods

L. Test Principle:

The ST AIA-PACK 25-OH Vitamin assay consists of a pretreatment step to release 25-OH Vitamin D from its serum transporter. Pretreated samples are then added to a ST AIA-PACK 25-OH Vitamin D test cup that contain 25-OH vitamin D-specific monoclonal antibody immobilized on magnetic beads to which 25-OH Vitamin D binds. Next, 25-OH vitamin D conjugated to bovine alkaline phosphatase is added to the reaction mixture, which competes with 25-OH vitamin D for binding to the antibody on magnetic beads. Subsequently, the magnetic beads are washed to remove the unbound enzyme labeled 25-OH vitamin D and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled 25-OH vitamin D that binds to the beads is inversely proportional to the 25-OH vitamin D concentration in the test sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision of the ST AIA-PACK 25-OH Vitamin D assay was evaluated according to CLSI EP5-A2. Three reagent lots and three analyzers were utilized in the study. Two replicates of each of the three levels of pooled and spiked Na-heparinized plasma, EDTA plasma and serum specimens were tested on two separate occasions per day for 20 non-consecutive days, leading to the generation of 80 data points for each sample type. Mean, within-run CV and total precision CV are summarized in the table below.

Sample	Mean (ng/mL)	Within-run		Total	
		SD	%CV	SD	%CV
Serum 1	16.0	1.2	7.3	1.2	7.4
	36.0	1.0	2.7	1.3	3.7
	94.1	1.5	1.6	2.2	2.3
Serum 2	21.8	0.7	3.3	0.8	3.8
	44.4	1.6	3.6	1.5	3.3
	98.0	1.9	2.0	2.0	2.0
Serum 3	21.8	0.9	4.3	1.3	5.8
	46.5	1.8	3.9	1.8	3.9
	103.7	2.1	2.0	2.4	2.3
Na-Heparin Plasma 1	15.7	0.8	4.8	1.0	6.4
	32.9	0.8	2.4	1.4	4.1

	75.4	1.2	2.2	1.6	2.9
Na-Heparin Plasma 2	21.5	1.0	4.5	1.0	4.4
	47.0	1.2	2.6	1.5	3.3
	102.8	1.9	2.7	1.9	2.7
Na-Heparin Plasma 3	21.8	1.1	5.2	1.1	5.2
	50.0	1.6	3.1	2.0	4.0
	108.9	1.8	1.7	3.3	3.0
EDTA Plasma 1	13.7	0.7	4.9	0.9	6.6
	34.7	0.9	2.7	1.3	3.9
	71.1	1.2	1.7	1.9	2.7
EDTA Plasma 2	24.4	0.8	3.3	1.0	4.1
	47.4	0.9	2.0	1.4	2.9
	105.2	1.5	1.4	2.3	2.2
EDTA Plasma 3	25.6	1.1	4.2	1.2	4.7
	50.3	1.0	1.9	1.6	3.2
	110.9	1.3	1.2	2.8	2.5

Precision of the ST AIA-PACK 25-OH Vitamin D Control Set was evaluated according to CLSI EP5-A2. The protocol consisted of testing 2 levels of controls in 2 replicates per run, 2 runs per day for 20 non-consecutive days on one analyzer generating a total of 80 data points for each control level. Mean, within-run CV and total precision CV are summarized in the table below.

Sample	**Mean (ng/mL)**	**Within-run**		**Total**	
		SD	**%CV**	**SD**	**%CV**
Control Level 1	20.0	0.79	3.9	1.16	5.8
Control Level 2	79.1	1.63	2.1	2.22	2.8

b. Linearity/assay reportable range:

Linearity was evaluated according to CLSI guideline EP6-A. Samples were prepared by mixing a high spiked serum sample with a low serum sample to obtain 11 concentrations across the range 2.6 ng/mL to 158.7 ng/mL, with each sample being assayed in 4 replicates. The data showed the following linear regression: $Y= 1.0366X + 0.1327$, $r= 0.9975$

The sponsor claimed that the assay range is 4 to 120 mg/dL.

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

Traceability

The calibrators for use with the ST AIA-PACK 25-OH Vitamin D are prepared gravimetrically. The commercially available stock of 25-hydroxycholecalciferol is used to make the internal standards by gravimetrical dilution into vitamin-free serum. The initial value for the internal material is assigned using the candidate device compared to the predicate device.

Value Assignment

ST AIA-Pack 25-OH Vitamin D Calibrator Set contains 6 levels of different concentrations of 25-hydroxycholecalciferol in steroid free human serum devoid of detectable vitamin D at target values of 0 (base material),10, 20, 40, 80, and 165 ng/mL.

AIA-PACK 25-OH Vitamin Control Set contains 2 levels of 25-hydroxycalciferol in steroid free human serum at target values of 20 and 80 ng/mL.

Production lots of kit calibrators and control materials are value assigned against internal standards using 3 reagent lots, 5 runs on two different instruments for a total of 30 replicates. The assigned value is the mean of the replicates. The controls are labeled with the assigned values as mean ±20%.

Shelf-life Stability

Real-time stability studies were performed for the control materials and the calibrators. The stability study protocol and the acceptance criteria have been reviewed and found to be acceptable. The real time stability study supports a stability of 6 months when materials are stored at 2-8^{o}C.

Open-Vial Stability

The stability study protocol and the acceptance criteria to determine open-vial stability of the control materials and the calibrators have been reviewed and found to be acceptable. The reconstituted controls were stable for 1 day when stored at 18-25° C, for 14 days when stored at 2-8° C, 60 days when stored at -20° C. Reconstituted calibrators were stable for 1 day when stored at 2-8° C.

For the calibration frequency, the sponsor claimed that the calibration is stable for up to 90 days.

d. *Detection limit:*

The sponsor performed a detection limit study to support their low end measuring range according to the CLSI guideline EP17-A.

For LoB determination, three blank samples were measured in three independent runs with 60 replicates per run giving 180 determinations in total. The LoB is calculated as the mean of the 57th and 58th highest values for the blanks. The LoB was estimated to be 1.6 ng/mL.

For LoD determination, six low level samples were measured in 3 independent runs with 10 replicates per run generating 180 measurements. The LoD samples were made by diluting commercial serum samples to yield concentrations ranging from 1.23-6.38 ng/mL. The LoD was calculated to be 2.6 ng/mL.

For LoQ determination, the test results from the LoD determination were used to calculate the functional sensitivity of the assay. Sponsor defines the LoQ as the interassay precision ≤ 20% CV. The LoQ was determined to be 2.9 ng/mL.

The measuring range of the ST AIA-PACK 25-OH Vitamin D assay is 4.0 to 120 ng/mL.

e. Analytical specificity:

Cross reactivity

To estimate cross reactivity of various Vitamin D metabolites with 25-OH Vitamin D, aliquots of samples of moderate Vitamin D levels were spiked with 25-OH Vitamin D metabolites.

Cross reactivity from compounds listed below were evaluated. Results are tabulated:

Cross-reactant	**Spiked (ng/mL)**	**% Cross Reactivity**
25-OH Vitamin D2	30	101
25-OH Vitamin D3	158.2	99.2
3-epi 25-OH Vitamin D2	30	131.3
3-epi 25-OH Vitamin D3	30	107
24,25-$(OH)_2$ Vitamin D2	100	5.2
24,25-$(OH)_2$ Vitamin D3	100	18
Vitamin D3	1000	<0.1
Vitamin D2	1000	0.5
1,25-(OH)2 Vitamin D3	1000 pg/mL	<0.1
1,25-(OH)2 Vitamin D2	1000 pg/mL	<0.1
Paricalcitol	2	<0.1

Interference

The sponsor performed studies to evaluate the effects of potential interferents on the performance of the ST AIA-25-OH Vitamin assay, following CLSI EP7-A2. Testing was done in the presence of 14 to 80 ng/mL 25-OH vitamin D and different concentrations of the listed compounds. All samples were run in triplicate. Percent recovery was calculated relative to control samples

containing vitamin D without spiked endogenous compounds. The highest concentrations at which non-significant interference (defined by the sponsor as ≤ 10% bias relative to the control) are shown below:

Compound	**Concentration above which significant interference observed**
Bilirubin (conjugated)	18.4 mg/dL
Bilirubin (unconjugated)	16.8 mg/dL
Triglycerides	357 mg/dL
Hemoglobin	11.4 mg/dL
Human serum albumin (protein)	8.5 g/dL
Ascorbic acid	20 mg/dL
EDTA-2K	10 mg/mL
Heparin	100 U/mL
Rheumatoid Factor	500 IU/mL

The sponsor has the following limitations in their labeling:

"Do not use hemolyzed sample. Hemolyzed sample will give erroneous results."

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed with the predicate device (Diasorin 25-Hydroxyvitamin D ^{125}I RIA Kit). A total of 156 unaltered human serum samples were measured in singlicate. The samples ranged in concentration from 6.2 ng/mL to 103.2 ng/mL.

Results of the Deming regression analysis are presented in the table below:

	Deming
Slope (95% CI)	0.934 (0.884 to 0.979)
Intercept (95% CI)	2.53 (0.86 to 4.34)
Correlation Coefficient	0.944
Bias	0.61

b. Matrix comparison:

115 matched sets of serum, Na-heparin-plasma, and EDTA-plasma were assayed in singlicate with one reagent lot on one AIA-2000 analyzer. Samples tested ranged from 11.0 to 112.8 ng/mL.

Results of Regression Analysis:

Matrix Y	Slope		Intercept		R
	Deming	Linear	Deming	Linear	
Na-Heparin plasma	0.993	0.988	-0.390	-0.20	0.995
EDTA plasma	1.041	1.034	0.91	1.14	0.994

Based on the study data, the sponsor claims that EDTA and sodium heparin are acceptable anti-coagulants for the vitamin D assay.

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   *b. Clinical specificity**:***

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

   Not applicable

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   To determine the reference range for the ST-AIA-PACK 25-OH Vitamin D assay, a total of 233 serum specimens form 50 females and 183 males (ages 19 to 80 years old) were measured. The specimens were obtained from the northern and southern regions of the United States from apparently healthy individuals who were not taking more than the normal Vitamin D supplements i.e. 2000 IU. Specimens with high parathyroid hormone, thyroid stimulating hormone, calcium, magnesium, and phosphorus were excluded from the data. Samples were collected in April and July 2012. The following results were obtained:

   Lowest 25-OH Vitamin D concentration: 7.23 ng/mL
   Highest 25-OH Vitamin D concentration: 56.70 ng/mL
   Observed central 95% range: 10.83 to 54.75 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.