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

A. 510(k) Number:
k072536

B. Purpose for Submission:
New device

C. Measurand:
25-Hydroxyvitamin D

D. Type of Test:
Quantitative HPLC assay

E. Applicant:
ESA Biosciences, Inc.

F. Proprietary and Established Names:
Vitamin D HPLC Test

G. Regulatory Information:
1. Regulation section:
   21 CFR 862.1825
2. Classification:
   II
3. Product code:
   MRG
4. Panel:
   Chemistry (75)

H. Intended Use:
1. Intended use(s):
   See Indications for use below.

2. Indication(s) for use:
   The ESA Biosciences Inc. Vitamin D HPLC test is for the quantitative determination of 25-hydroxyvitamin D in human serum or EDTA-plasma to be used in the assessment of vitamin D sufficiency. Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions in an adult population.

3. Special conditions for use statement(s):
   Assay results should be used in conjunction with other clinical laboratory data to assist the clinician in making patient management decisions in an adult population.

   This assay should not be used for measurement in samples from patients less than 1 year of age.

   This assay is for prescription use.

4. Special instrument requirements:
   For use with the ESA high performance liquid chromatography (HPLC) system
   with an ESA CouloChem III (k903231) or CoulArray EC Detector (k963938).

I. Device Description:
   The Vitamin D HPLC Test system includes:
   Vitamin D Calibration Stock (includes alcohol and 1 µg/mL 25(OH)D); Vitamin
   D Internal Standard Stock (includes acetonitrile and 1 µg/mL 25(OH)D); Vitamin
   D Mobile Phase (includes acetonitrile, propanol and supporting electrolyte);
   Vitamin D Reagent A and Vitamin D Reagent B (both include acetonitrile);
   Vitamin D Reagent C (includes alcohol) and a Vitamin D SPE column.

J. Substantial Equivalence Information:
   1. Predicate device name(s):
      DiaSorin LIAISON Vitamin D Assay
   2. Predicate K number(s):
      k032844
   3. Comparison with predicate:

Similarities		
Item	Predicate Device (k032844)	Proposed Device
Intended Use	Quantitative determination of 25-Hydroxyvitamin D to be used in the assessment of Vitamin D sufficiency.	Same
Matrix	Human serum or plasma	Same
Limit of Quantitation	7.0 ng/mL	Same

Differences		
Item	Predicate Device (k032844)	Proposed Device
Test principle	Chemiluminescent immunoassay	HPLC with electrochemical detection
Calibrators	Derived from serum-based standards, extracted identically to controls and patient samples	Working single point calibrator is an aqueous calibration stock solution and internal stock solution
Reference range: 2.5th to 97.5th percentile (n)	9.5 – 52.0 ng/mL (98)	5.7 – 34.8 ng/mL (150)
Total precision (% CV)	6% - 13%	5.43% – 12.6%

K. Standard/Guidance Document Referenced (if applicable):
  • *User Evaluation of Precision Performance of Clinical Chemistry Devices: Approved Guideline* (EP5-A2)
  • *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline* (EP6-A2)
  • *Interference Testing in Clinical Chemistry: Approved Guideline* (EP7-A2)
  • *Method Comparison and Bias Estimation using Patient Samples: Approved Guideline* (EP9-A2)
  • *Protocols for Determination of Limits of Detection and Limits of Quantitation* (EP17-A3)
  • *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline (C28-A2)*

L. Test Principle:
  The ESA method is a kit for measurement of total 25(OH) Vitamin D by HPLC with electrochemical (EC) detection. Specific reagents and solid phase extraction columns are included for sample preparation. A 200 uL volume of sample (serum or plasma) is mixed with a precipitation reagent, which contains internal standard. The internal standard is a stable vitamin D analog that is used to correct for variability in extraction recovery and analytical sample volume. After centrifugation, supernatant is poured onto a pre-conditioned SPE column for rapid extraction of 25(OH)D and the IS. SPE columns are washed with 2 different reagents and analytes are eluted with a third reagent. The resulting eluent is diluted before analysis. The prepared sample is analyzed with an ESA HPLC system using an ESA EC detector (Coulochem III or CoulArray) equipped with a dual coulometric EC cell. Calibration is accomplished by direct HPLC analysis of authentic standard solutions (i.e. not taken through the extraction step). 25(OH)D and IS are separated chromatographically followed by EC detection. A dual EC cell is used with the first, upstream, cell maintained at a specific potential to oxidatively screen possible interfering sample components. The second, downstream cell is maintained at a potential that is optimized for selective 25(OH)D detection. Total 24(OH)D sample concentrations are determined by single-point internal standard quantitation.

M. Performance Characteristics (if/when applicable):
  1. Analytical performance:
     a. *Precision/Reproducibility:*
        Precision studies were performed using CLSI Document EP5-A as a guideline. Five replicates of three concentration levels of analyte in EDTA plasma pools that were individually prepared and analyzed in a single run each day, repeated over 20 days. Five days were performed at an external site. Three different HPLC-EC systems, four operators, two reagent lots, five analytical columns (3 lots), three lots of SPE columns and four analytical cells were used in these studies. Three samples were prepared by spiking EDTA plasma, pooled from 5 individual blood bank donors, with assayed, commercially procured 25(OH)D.

Sample level	Mean ng/mL	Within-run		Total		N
		SD (ng/mL)	% CV	SD (ng/mL)	% CV	
1	43.8	4.6	10.6	5.5	12.6	100
2	117.2	8.0	6.8	9.9	8.4	100

Two serum and two EDTA plasma samples were acquired from adult human subjects. Four replicates of each sample were individually prepared (taken through all pre-analytical and analytical steps of the procedure) and analyzed in a single run each day, repeated over 20 days. Samples were run on a single instrument, by two operators and using two lots of reagents and extraction columns. Three replicates were lost during pre-analytical sample processing.

Sample # (matrix)	Mean ng/ml	Within-run		Within-device		N
		SD (ng/ml)	% CV	SD (ng/ml)	% CV	
1 (plasma)	22.1	0.63	2.82	1.34	6.05	79
2 (plasma)	21.8	0.50	2.31	1.19	5.48	80
3 (serum)	25.5	0.65	2.55	1.44	5.66	80
4 (serum)	25.6	0.55	2.16	1.39	5.43	78

b. *Linearity/assay reportable range:*
To establish the linearity of the assay, a study design was used based on CLSI protocol EP6-A: *Evaluation of the Linearity of Quantitative Measurement Procedures: a Statistical Approach: Approved Guideline.* The claimed measuring range of this device is 7 - 200 ng/mL.

A volume of low level EDTA plasma was spiked with assayed 25(OH)D concentrate. The low and high level samples were mixed to obtain 13 samples with concentrations spanning the measuring range. Three aliquots of each sample concentration were taken through the pre-analytical step for the assay. Upon completion of this step, extracts were divided into 2 aliquots and one aliquot was analyzed by an HPLC-Coulochem III system and the other was analyzed by an HPLC-CoulArray system.

Linear regression statistics:
HPLC-Coulochem III system
$y = 1.08x - 4.46$
$r^2 = 0.994$

HPLC-CoulArray system
$y = 1.05x - 2.46$
$r^2 = 0.995$

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

Calibrator:

Value assignment: An Internal Standard Stock and Master Product Calibrator Stock are created in-house (there is no primary calibrator, recognized reference material or reference measurement procedure for this Measurand at this time). Value assignment of both Stocks is made by UV absorbance spectrometry calibrated with NIST SRM 935a, based on NIST recommended molar extinction coefficients. A working Internal Standard is created by ten-fold volumetric dilution of the Internal Standard Stock. The working Calibrator is a mixture of equivalent volumes of the Master Product Calibration Stock and Internal Standard Stock, followed by dilution to 100 ng/mL of 25(OH)D. Each lot of product calibrator and internal standard are assayed by HPLC to confirm purity and between-lot consistency.

Stability:

Kit components: The shelf-life, on-board and open stability testing protocols for kit components and the acceptance criteria were described and found to be acceptable. Current shelf life studies support a six-month shelf life, with real-time studies on-going to support twelve months.

Samples: To evaluate the stability of samples stored at different conditions, two sample levels were prepared by spiking freshly drawn EDTA plasma with 25(OH)D. Three aliquots of each sample were processed immediately and analyzed within one hour of processing. Results from these aliquots were used as the basis for comparison to samples that were stored for various time periods and storage conditions before processing. On board stability of processed samples was also assessed.

Sample	Storage conditions	Stable for at least
Processed sample	20 – 23°C (on-board)	24 hours
Sample	-15 to -25°C	3 freeze-thaw cycles
Sample	2 - 8°C	7 days
Sample	20 - 23°C	6 hours

d. *Detection limit:*

The Limit of the Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for the ESA Vitamin D HPLC test was determined in accordance with the CLSI guideline EP17-A: *Protocols for Determination of Limits of Detection and Limits of Quantitation*. One low level sample was prepared by spiking blank matrix (bovine serum albumin in phosphate buffered saline) with 25(OH)D. Two additional low samples were prepared by pooling patient samples. Six replicates of the blank matrix and three replicates each of the low level samples were analyzed in each run. Each replicate was taken through all pre-analytical and analytical steps of the assay. Twelve runs were performed with two analytical systems, 3 EC cells, 3

analytical columns, 2 lots of SPE columns, 2 reagent lots and two operators. The non-parametric method (CLSI EP17-A) was used to calculate the LoB. The LoB was calculated to be 2.51 ng/ml and the LoD was calculated to be 5.3 ng/ml. The Limit of Quantitation (LoQ) was calculated to be 7.0 ng/ml and was defined as the concentration of the analyte demonstrated imprecision less than or equal to 20% CV.

e. *Analytical specificity:*
The ESA Vitamin D HPLC test was evaluated for interference consistent with CLSI Guideline EP7-A2.

The following substances were added to two EDTA plasma samples (with 25(OH)D levels of 43 ng/mL and 105 ng/mL) and were found not to interfere with the Vitamin D HPLC test (a bias exceeding 10% is considered interference):

Substance	Test concentration
Triglycerides	1571 mg/dL
Albumin	12.7 g/dL
Bilirubin	60 mg/dL
Hemoglobin	500 ng/dL
Paricalcitrol (vitamin D analog)	100 ng/mL

Cholesterol, tested at a level of 500 mg/dL, was found to produce a bias of more than 10%. (The actual bias seen was 10.1%.) In addition, 25(OH)EpiD3, a vitamin metabolite, was found to interfere with the Vitamin D HPLC test. This compound is found only in infants 0 – 1 year of age. As noted in the limitations section of the package insert, this assay should therefore not be used for measurement in samples from patients less than 1 year of age. (The sponsor did not test 25(OH)EpiD2, also found only in infants 0-1 year of age, which is known to interfere with 25(OH)D assays, but referred the user to literature in the product insert for further information.)

f. *Assay cut-off:*
Not applicable.

2. Comparison studies:
a. *Method comparison with predicate device:*
The ESA Vitamin D HPLC Test was compared to a reference method using CLSI Document EP9-A2 as a guideline for the method comparison study.

The Vitamin D HPLC Test was compared to a reference HPLC method using serum samples from 85 individual patients and fifteen additional samples prepared by augmenting aliquots of a single serum pool (prepared from approximately 50 individual patient samples) with varying amounts of 25(OH)D. Results from 95 specimens with analyte values that spanned the

measuring range of the device (tested over 6 non-consecutive days) were
reported. The correlation was found to be:

Equation: Least Squares	y = 1.027x - 5.3
Slope, 95% Confidence Interval	0.976 to 1.077
Intercept, 95% Confidence Interval (ng/mL)	-8.7 to -1.8
Correlation Coefficient, R	0.973
N	95
Standard Error (ng/mL, y-direction)	9.91
Range (ng/mL)	9.6 – 195
Equation: Deming	y = 1.056x - 6.9

b. *Matrix comparison:*
    19 neat and 28 spiked samples were used to demonstrate equivalent
    performance between paired serum and EDTA-plasma sample types over the
    claimed measuring range. Samples were spiked after serum/plasma separation
    using volumetric technique.
    y = 1.01x + 0.24
    r = 0.999

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:
    The reference range was determined according to the non-parametric method in
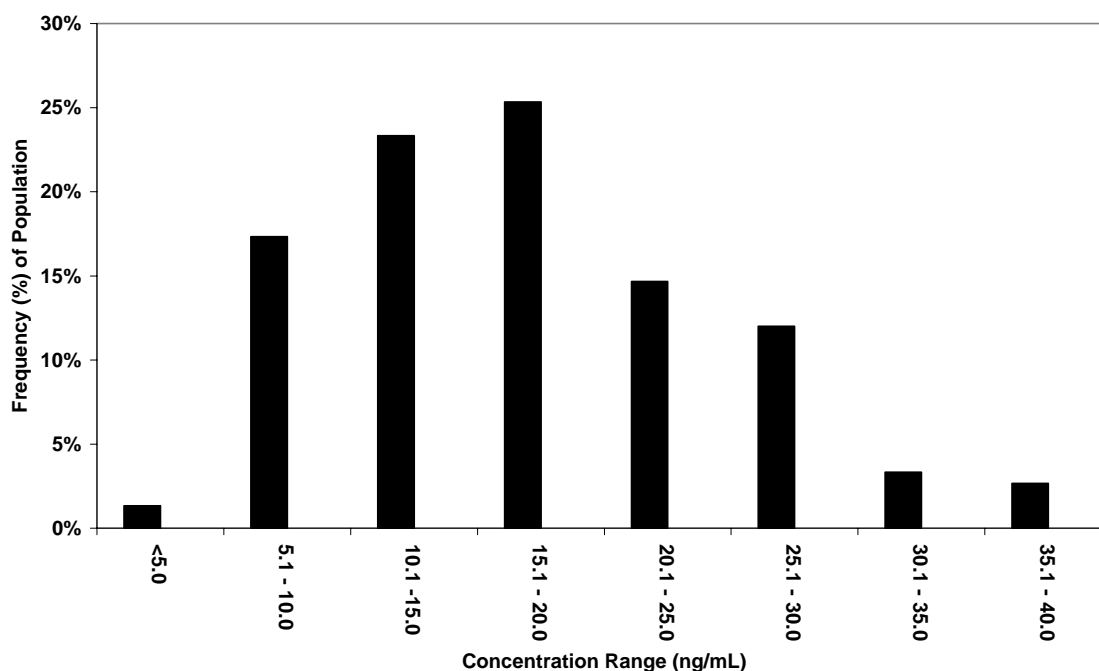   CLSI C28-A2.

    Samples from 150 apparently healthy adult human subjects were collected from 3
    locations to achieve even North-South geographical distribution within the
    latitudes of the contiguous 48 US States. (Specifically, samples were obtained
    from 75 individuals living in Massachusetts, 37 in Tennessee and 38 in Texas.)
    Inclusion and exclusion of individuals was based on a questionnaire. The
    designation of "apparently healthy adult human subjects" was based on the initial
    Normal Blood Donor intake questionnaire and by preliminary viral testing.
    Individuals taking prescription or over-the-counter vitamin D supplements were
    excluded.

Sample analysis was performed over a 2-day time frame using one analytical system, one lot of reagents and by one operator. Samples were collected in late winter (i.e., March). The population consisted of 71 (47%) females and 79 (53%) males ranging in age from 20 to 64 years [mean = 40 years, SD = 10 years] and comprised 109 (73%) Caucasian, 36 (24%) African-American and 5 (3%) Hispanic individuals.

Reference range and frequency distribution from the population are as indicated below:

Population (N)	Median 25(OH)D ng/mL	Observed Range ng/mL 2.5th to 97.5th Percentile
150	17.4	5.7 – 34.8

25(OH)D Reference Sample Distribution

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.