K072536

MAY - 7 2008

510(k) SUMMARY

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

The assigned 510(k) number is:

Submitter:	ESA Biosciences Inc. 22 Alpha Road Chelmsford, MA 01824 USA Phone: 978-250-7000 Fax: 978-250-7090
Contact Person:	Harold Asp Quality Assurance Manager
Date of Summary Preparation:	May 05, 2008
Device Name:	ESA Biosciences Inc. Vitamin D HPLC Test
Classification Name:	Vitamin D Test System 21CFR 862.1825 Product Code: MRG
Predicate Device:	LIASON® 25 OH Vitamin D Assay 510(k) 032844
Statement of Intended 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.

Device Description

The ESA method is a complete kit for measurement of Total 25(OH)D by HPLC with electrochemical (EC) detection. Specific reagents and solid phase extraction columns are included for sample preparation and are employed with user-supplied standard laboratory equipment (centrifuge, test tubes, pipettes, etc.). A 200µL volume of sample (serum or plasma) is mixed with a precipitation reagent, which contains internal standard (IS).

The internal standard is a stable vitamin D analogue 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 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 isocratic 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). Analysis requires a specific guard and analytical column, mobile phase and calibration reagents to allow rapid quantitative analysis.

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. The dual coulometric EC cell is a rugged detector that provides much higher selectivity and sensitivity than commonly used absorbance detectors. This allows the use of lower sample volumes than are typically required with HPLC-UV methods and is less susceptible to interferences. Analytical run time is less than 12 minutes and Total 25(OH)D sample concentration is automatically determined by single-point internal standard quantitation.

Summary of Performance

Precision

Four 'neat' (i.e., not spiked or pooled) samples consisting of 2 sera and 2 EDTA plasma were used for this study, which followed CLSI EP5-A. 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. Analysis of variance calculations were used to estimate within-run and within-device imprecision.

Precision Performance

Assay	Mean	Within-run		Within-device		N
	ng/mL	SD (ng/mL)	% CV	SD (ng/mL)	% CV	
Total 25(OH)D						
Sample #1 (Plasma)	22.1	0.63	2.82	1.34	6.05	79
Sample #2 (Plasma)	21.8	0.50	2.31	1.19	5.48	80
Sample #3 (Serum)	25.5	0.65	2.55	1.44	5.66	80
Sample #4 (Serum)	25.6	0.55	2.16	1.39	5.43	78

Precision performance was also evaluated using plasma pools supplemented with 25(OH)D at 2 concentration levels. The protocol followed CLSI EP5-A. Five replicates of each level were individually prepared (i.e., taken through all pre-analytical and analytical steps) and analyzed in a single run each day. Data from a total of 20 runs, performed at two sites were included in the

precision evaluation (300 total observations). 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. Analysis of variance calculations were used to estimate within-run and total imprecision.

Precision Performance

Assay	Mean	Within-run		Total		N
	ng/mL	SD (ng/mL)	% CV	SD (ng/mL)	% CV	
Total 25(OH)D						
Level 1	43.8	4.6	10.6	5.5	12.6	100
Level 2	117.2	8.0	6.8	9.9	8.4	100

Limit of Detection (LoD)

The LoD was determined consistent with the guidelines in CLSI EP17A. Blank matrix was bovine serum albumin in phosphate buffered saline. Patient samples with low 25(OH)D concentrations, as determined by the LCUV reference method, were used to prepare low level pools The results were analyzed using the nonparametric method. The LoQ (limit of quantitation) was set to the concentration at which the % CV from low level samples was ≤ 20%.

Provided below are estimates of LoD, limit of blank (LoB) and LoQ based on 133 determinations in 11 runs, with 67 blank and 66 low-level samples with less than 5% false negative and less than 5% false positives:

LoD, LoB, and LoQ for Vitamin D

	ng/mL			
Assay	LoB	LoD	LoQ	N
Total 25(OH)D	2.5	5.0	7.0	133

Linearity

Linearity was studied according to CLSI EP6 on two HPLC systems. Low and high level plasma were mixed proportionally to obtain a total of 13 equally -spaced concentrations spanning the intended assay range (7.0 - 200 ng/mL) for 25(OH)D. Three aliquots of each sample concentration were taken through all pre-analytical steps of the assay and analyzed in random order in a single run. Performance was assessed using the polynomial evaluation as described in CLSI EP-6A.

For total 25(OH)D by the ESA Vitamin D HPLC Test, the method has been demonstrated to be linear from the LoQ (7.0 ng/mL) to 200 ng/mL based on goals of ≤ 3ng/mL bias due to non-linearity for concentrations of ≤ 20 ng/mL and ≤ 7.5% bias due to non-linearity at concentrations of 21-200 ng/mL.

Recovery

Sample pools: EDTA plasma (5 subjects) and serum (6 subjects) were each spiked with 25HOD concentrate to increase the individual concentrations of 25(OH)D by 0, 5, 10, 20, 50, 100 and 200 ng/mL. Concentration levels of total 25(OH)D in unspiked pools were less than 10 ng/mL. Three replicates of each sample were taken through all pre-analytical and analytical steps of the assay. Each pool and corresponding spiked samples were analyzed in random order in a single run. Samples were analyzed on two different HPLC systems.

Percent recovery was determined by: [(Measured concentration - Endogenous (unspiked) concentration)/Spiked Concentration] x 100.

For each sample matrix, mean recovery (n = 6, each level = 3 replicates on 2 systems) are summarized below:

Recovery 25(OH)D

Sample	Spiked Concentration (ng/mL)	% Recovery Mean (n=6)	Standard Deviation % (n=6)
Plasma	10	90	20
	20	100	10
	40	100	11
	100	99	6.8
	200	107	7.2
Serum	10	93	20
	20	94	13
	40	96	8.9
	100	96	5.5
	200	105	3.8

Method Comparison and Bias Estimation

Method comparison studies followed CLSI EP9-A2. The studies were performed at two external sites. Serum samples from 85 individual patients were collected at the University of Wisconsin Hospitals and Clinics (UWHC). These were chosen from samples submitted routinely to UWHC for 25(OH)D analysis and represent a wide population with respect to medical, dietary and therapeutic conditions. Fifteen additional samples were prepared by augmenting aliquots of a single serum pool (prepared from approximately 50 individual patient samples) with varying amounts of 25(OH)D.

All 100 samples were run in duplicate by a LCUV reference method[1] at UWHC. Fifty of these patient samples were run in duplicate by the ESA LCEC test method at one external site and the other fifty patient samples were run in duplicate by the LCEC test method at UWHC. Samples were run on 12 separate days, by 3 different technologists over a 7 week period with no more than 10 patient samples run per day. Calibration and control materials used for the LCUV reference method were independent of the LCEC test method.

For this analysis, data from both sites were pooled and the first replicate of the Y (ESA LCEC) results were analyzed against the average of the X (LCUV) results. No outliers were removed. Five samples with values outside the measuring range, LOQ (7.0 ng/mL) to 200 ng/mL, of the ESA assay were excluded from this analysis.

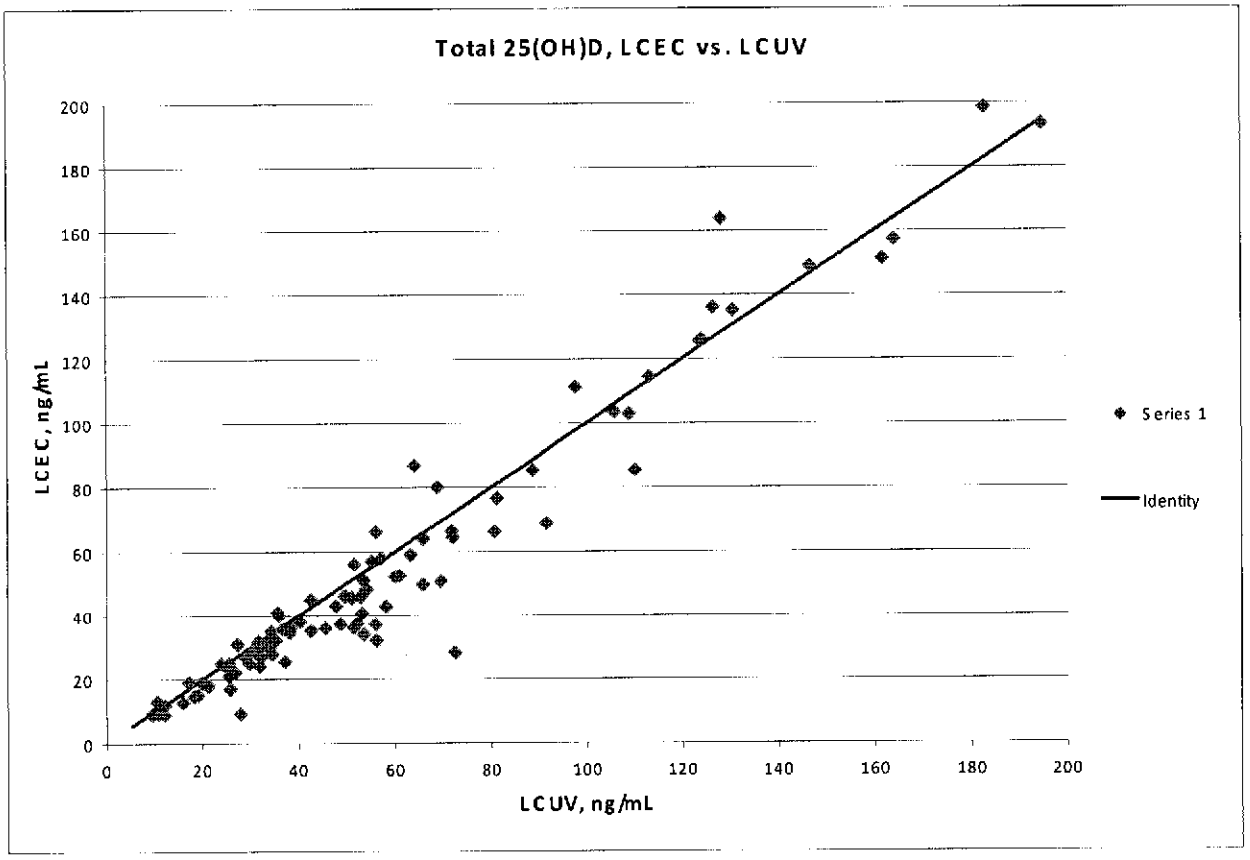
The following table and figure provide a summary of data analysis for total 25(OH)D. All data analyses were performed as described in EP9-A2 and include the following:

- Number of points used in the regression and range of X data.
- Slope and intercept of fitted ordinary least squares and Deming regression lines.
- From least squares regression: correlation coefficient, standard error of estimate (calculated in the vertical (Y) direction) and confidence intervals for the slope and intercept.
- Scatter plots with the line of identity.
- Predicted bias and confidence intervals calculated at various X values from the partitioned bias method.

Regression Summary – Total 25(OH)D

25(OH)D	
Equation: Least Squares	LCEC = 1.027(LCUV) - 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	LCEC = 1.056(LCUV) - 6.9

Total 25(OH)D - *Scatter Plot of First LCEC Replicate vs. Average of Reference Method with Line of Identity.*



Total 25(OH)D First LCEC Rep vs. Avg. LCUV

Because the correlation coefficient (r) fails the adequate range test (i.e., $r < 0.975$), the partitioned bias method was used.

Total 25(OH)D – Bias and Confidence Intervals Using Partitioned Bias Method

Average LCUV (ng/mL)	Range of LCUV (ng/mL)	N	Bias (ng/mL)	Bias (%)	Lower 95% CI Bias* (ng/mL)	Upper 95% CI Bias* (ng/mL)
22.9	9.6 – 34.4	32	-2.6	-11.2	-17.4	-4.9
45.4	34.5 – 56.1	31	-5.6	-12.3	-18.4	-6.1
100.3	57.0 – 195	32	-3.3	-3.3	-8.5	2.0

* Confidence intervals calculated at the indicated average LCUV concentration

Interference Studies

This assay is susceptible to interference from 25(OH)epiD2 and 25(OH)epiD3, which are endogenous metabolites present in neonates[2]. This assay should therefore not be used for measurement in samples from patients < 1 year of age. A 10% positive bias was observed for samples with a cholesterol level of 500mg/dL. Estimated bias for lower concentrations of cholesterol was acceptable (<10%). The following provides additional detail of interference susceptibility testing.

Interference susceptibility testing was performed according to CLSI EP7-A. Substances chosen for study were based on consideration of chemical structure determinants for electrochemical response and chromatographic retention, common medications in the intended population, therapeutic vitamin D analogs and matrix effects / conditions. The following table lists substances that were initially screened by direct HPLC-EC analysis along with the observed retention times.

Compounds Analyzed by Direct HPLC Analysis

Compound / Class	Alternate and Commercial Names	Retention time (min.) for peaks observed on analytical channel
Lipid-soluble vitamins		
Retinyl Acetate		25.06
Retinol		11.5
α-Tocopherol		None
Coenzyme Q10		None
β-Carotene		None
Lutein		None
γ–Tocopherol		None
δ-Tocopherol		None
Lycopene		None
Vitamin D Compounds / Analogues		
D3	Cholicalciferol	None
D2	Ergocalciferol	None
1α,25(OH)$_2$D3	Calcitriol, Rocaltrol, Calcijex	4.08
19-nor-1α,25(OH)$_2$D2	Paricalcitol, Zemplar	3.74
Dihydrotachysterol		None
1α,25(OH)$_2$D2		4.7
1α(OH)D3	One-alpha, Alfarol	33.9
1α(OH)D2	Doxercalciferol, Hectorol	34
25(OH)epiD3		8.46

From Table 9-1, 19-nor-1α,25(OH)$_2$D2 (paricalcitol) and 25(OH)epiD3 were the only compounds that were identified as possible interfering substances. These substances and several endogenous compounds were characterized to determine the degree of interference as a function of interferent concentration (i.e., dose-response). Serum was prepared with high and low (normal) concentrations of each possible interferent (see below). High and low samples were mixed proportionally to produce a total of 5 equally-spaced levels of each possible interfering substance. Three replicates of each sample were individually prepared and analyzed in random order a single run.

The results of these studies are described below:

The following endogenous substances, when tested according to CLSI EP7-A, in a serum sample containing 25(OH)D2 at 67 ng/mL and 25(OH)D3 at 53 ng/mL, did not interfere at, or below, the interferent test concentration indicated. Bias, calculated from regression analysis of the dose-response data, exceeding 10% is considered interference.

Substance	High Test Concentration	Comments
Triglycerides	1571 mg/dL	Test Material Triolein
Albumin	12.7 g/dL	
Bilirubin	60 mg/dL	
Hemoglobin	500 mg/dL	Gross hemolysis

The following vitamin D analog, when tested according to CLSI EP7-A, in a serum sample containing 25(OH)D2 at 67 ng/mL and 25(OH)D3 at 53 ng/mL, did not interfere at the interferent test concentration indicated. Bias, calculated from regression analysis of the dose-response data, exceeding 10% is considered interference.

Substance	High Test Concentration	Comments
Paricalcitrol	100 ng/mL	Approx 100 times therapeutic range for this compound.

The following endogenous substance, when tested according to CLSI EP7-A, in a serum sample containing 25(OH)D2 at 67 ng/mL and 25(OH)D3 at 53 ng/mL was found to produce a positive bias of 10% for both analytes at the high test concentration indicated. Acceptable bias (i.e., < 10%) was found for interferent concentrations < 375mg/dL.

Substance	High Test Concentration	
Cholesterol	500 mg/dL	

The C3 epimer of 25(OH)D, 25(OH)EpiD3, when tested according to CLSI EP7-A, in a serum sample containing 25(OH)D2 at 67 ng/mL and 25(OH)D3 at 53 ng/mL, was found to interfere with 25(OH)D. As reported by Singh *et al.*[2], 25(OH)D2 or D3 epimers may account for a significant proportion (8.7-61.1%) of the total 25(OH)D in infants, with concentrations ranging from 5-92ng/mL. No C3 epimers were detected in age groups greater than 0-1 year. Based on this report, this assay should not be susceptible to interference from C3 epimers for the intended adult patient population.

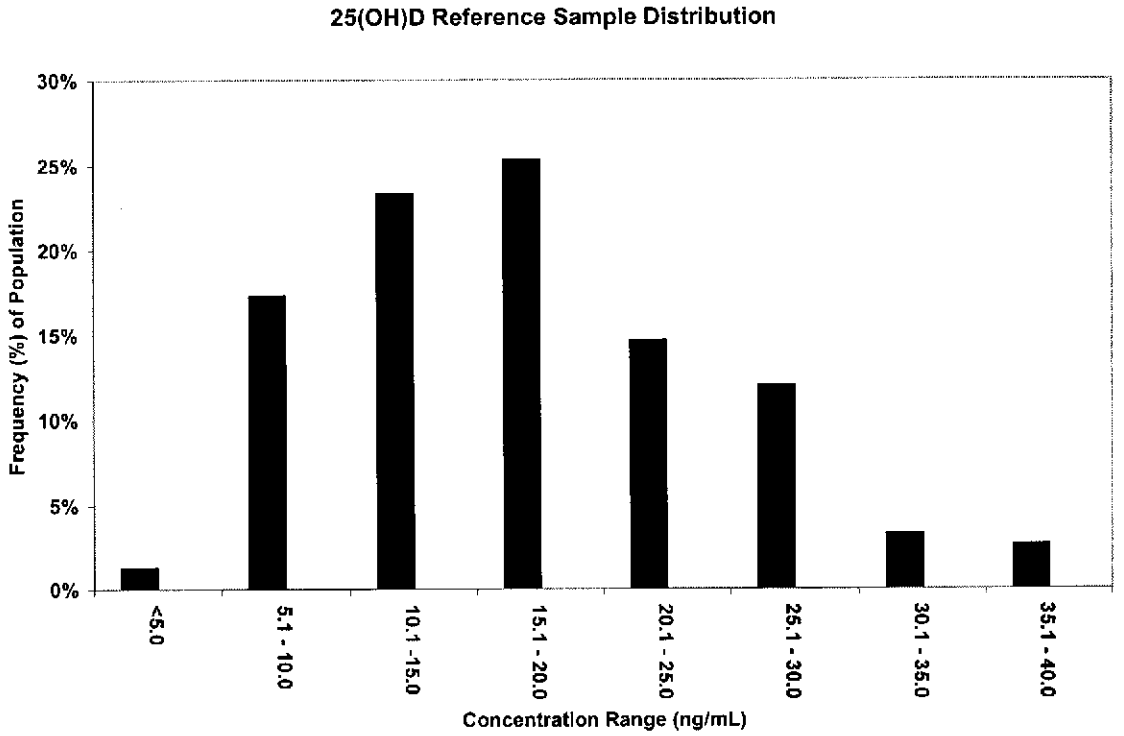
Reference Range

The following results were obtained specifically with the ESA Vitamin D HPLC Test from a reference sample group that is reflective of a typical sample population within the contiguous 48 US States. Note: These data are provided as an example. It is important for each laboratory to establish a reference range representative of its typical population.

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. Samples were collected in late winter. Individuals taking prescription or over-the-counter vitamin D supplements were excluded. The reference range was determined according to the non-parametric method in CLSI C28-A2.

Observed Reference Range and Frequency Distribution

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



Technical Characteristics Compared to Predicate:

Similarities		
Item	**Device**	**Predicate**
Intended Use	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.	For in vitro diagnostic use. Quantitative determination of 25-Hydroxyvitamin D (25-OH-D) and other hydroxylated vitamin D metabolites in human serum or plasma to be used in the assessment of vitamin D sufficiency. 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.
Indications for Use	Determination of Vitamin D sufficiency.	Same
Matrix	Human serum or plasma	Same
Differences		
Item	**Device**	**Predicate**
Analyte	25-Hydroxyvitamin D	Same
Methodology	High Performance Liquid Chromatography with Electro-Chemical Detection	Chemiluminescent immunoassay
Kit Controls	Recommend commercially available controls	Two levels, horse serum based, extracted identically to patient samples
Standards	Working single point calibrator is an aqueous calibration stock solution and internal stock solution	Stored Master Curve based on 10 points, derived from serum based standards extracted identically to controls and patient samples

Conclusion

Through the use of plasma and serum samples, augmented plasma and serum samples, standards and controls the performance and reliability of this assay has been validated over the measuring range of 7.0 ng/mL (LoQ) to 200 ng/mL. In so doing adequate sensitivity, precision, linearity, recovery, detection limits and immunity from interferences has been demonstrated.

Standards Referenced

1. EP9-A2 Method Comparison and Bias Estimation Using Patient Samples: Approved Guideline- Second Edition
2. C28-A2 How to define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline-Second Addition
3. EP6-A Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
4. EP7-A Interference testing in Clinical Chemistry; Approved Guideline
5. EP5-A2 Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second edition

Literature Referenced

1. Lensmeyer GL, Wiebe DA, Binkley N, Drezner MK. HPLC method for 25-hydroxyvitamin D measurement: comparison with contemporary assays. Clin Chem 2006;52:1120-6.
2. Singh RJ, Taylor RL, Reddy GS, Grebe SK. C-3 epimers can account for a significant proportion of total circulating 25-hydroxyvitamin D in infants, complicating accurate measurement and interpretation of vitamin D status. J Clin Endocrinol Metab 2006;91:3055-61.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

ESA Biosciences Inc.
c/o Mr. Harold Asp
Quality Assurance Manager
22 Alpha Road
Chelmsford, MA 01824

MAY - 7 2008

Re: k072536
Trade/Device Name: Vitamin D HPLC Test
Regulation Number: 21 CFR 862.1825
Regulation Name: Vitamin D Test system
Regulatory Class: Class II
Product Code: MRG
Dated: March 24, 2008
Received: March 26, 2008

Dear Mr. Asp:

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.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. 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); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (240) 276-0490. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). You may obtain other general information on your responsibilities under the Act from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (240) 276-3150 or at its Internet address at http://www.fda.gov/cdrh/industry/support/index.html.

Sincerely yours,

Jean M. Cooper, M.S., D.V.M.

Jean M. Cooper, M.S., D.V.M.
Director
Division of Chemistry and Toxicology
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

Indication for Use

510(k) Number (if known): k072536

Device Name: ESA Biosciences Inc. Vitamin D HPLC Test

Indication For Use: k072536

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.

Prescription Use ___X___ (21 CFR Part 801 Subpart D) And/Or Over the Counter Use ____. (21 CFR Part 801 Subpart C)

(PLEASE DO NOT WRITE BELOW THIS LINE; CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of In Vitro Diagnostic Device Evaluation and Safety (OIVD)

Division Sign-Off
Office of In Vitro Diagnostic Device
Evaluation and Safety

510(k) k072536