



**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT**

I Background Information:

A 510(k) Number

K221817

B Applicant

Immunostics Inc.

C Proprietary and Established Names

ALFIS Vitamin D, ALFIS-3 Analyzer

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MRG	Class II	21 CFR 862.1825 - Vitamin D Test System	CH - Clinical Chemistry
KHO	Class I	21 CFR 862.2560 - Fluorometer for clinical use	CH - Clinical Chemistry

II Submission/Device Overview:

A Purpose for Submission:

New Device

B Measurand:

Total 25-hydroxyvitamin D (25-OH Vitamin D)

C Type of Test:

Quantitative Immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

ALFIS Vitamin D in conjunction with ALFIS-3 Analyzer is an enzyme-linked fluorescence immunoassay intended for in vitro diagnostic use at clinical laboratories for the quantitative measurement of Total 25-hydroxy Vitamin D (25-OH Vitamin D) in human serum, lithium heparin plasma and sodium heparin plasma.

ALFIS Vitamin D is indicated to be used as an aid in the determination of Vitamin D sufficiency in adults.

ALFIS-3 Analyzer is a fluorescence-scanning instrument using magnetic beads and alkaline phosphatase enzyme system for in vitro diagnostic use in conjunction with various ALFIS immunoassays intended for measuring the concentration of designated analytes in human blood and other specimens.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

ALFIS-3 Analyzer

IV Device/System Characteristics:

A Device Description:

ALFIS-3 Analyzer

The ALFIS-3 Analyzer is an automated, fluorometric analyzer intended to perform in vitro diagnostic tests on clinical specimens. The system is intended for quantitative analysis of body fluids.

ALFIS Vitamin D

The ALFIS Vitamin D test kit is comprised of 24 ALFIS Vitamin D test cartridges, 1 ALFIS Vitamin D test ID Chip, 24 pipette tips, and 24 magnetic tips.

ALFIS Vitamin D Test Cartridge

The ALFIS Vitamin D Test Cartridge is a disposable, self-contained, unitized device which houses the magnetic bead, antibody-alkaline phosphatase-conjugator, sample diluent, diethanolamine, 4-Methylumbelliferyl phosphate, and washing buffer. Cartridges are provided in individually sealed aluminum pouches. The magnetic bead well contains one lyophilized magnetic bead which is conjugated with streptavidin and Vitamin D antibody-biotin complex.

ALFIS Vitamin D Test ID Chip

ALFIS Vitamin D Test ID Chip is a lot-specific electronic memory device. Each lot-specific Vitamin D Test ID chip contains an encoded master calibration curve data/equation which is meant to compensate for the lot-to-lot variation in ALFIS Vitamin D test cartridges on test results. Apart from the lot-specific calibration curve the ALFIS Vitamin D test ID chip also contains other information such as name of the test, test cartridge, lot number, expiry date, reportable ranges etc.

Materials required, but not supplied: ALFIS-3 Analyzer, ALFIS Vitamin D Calibrators, ALFIS Vitamin D Controls.

B Principle of Operation:

The ALFIS Vitamin D assay is an enzyme-linked fluorescence immunoassay. To quantitatively measure total 25-hydroxyvitamin D in human serum or plasma, a magnetic tip and pipette tip provided with the test kit are inserted into the designated sites on the analyzer. The sample (100 µL) is added to the sample well. The test cartridge is inserted into the analyzer and the following steps are performed automatically within the instrument: an antibody is labeled with an alkaline phosphatase enzyme and other reagents are conjugated with a magnetic bead. Upon interaction between antigen and enzyme-labeled antibody, a substrate is added, and fluorescence product is formed by catalytic action of enzyme. The obtained fluorescence intensity of a substrate is proportional to the amount of analyte. ALFIS-3 uses an amplified photodetector (APD) module for the measurement of UV light intensity as a light detector. The fluorescent light is collected with together the scattered UV light. Pure fluorescence is filtered from the scattered UV and fluorescent light. The exact fluorescence intensity is measured by APD module and converted into the relative fluorescence ratio (RFR) which is correlated with the concentration of target analytes in the sample. The on-board microprocessor computes the concentration of the analyte in the test sample based on proprietary software. Computed and converted test result are displayed on the display screen of the ALFIS-3.

C Instrument Description Information:

1. Instrument Name:
ALFIS-3 Analyzer

2. Specimen Identification:
Human serum, Lithium heparin and Sodium heparin samples are identified by the lot specific barcode that identifies the lot number of the ALFIS Vitamin D test cartridge while it undergoes scanning in the ALFIS-3 analyzer.

Specimen Identification can be done by writing patient ID on the space labeled Patient ID on the sample cartridge. The lot specific barcode signifies the identity and lot number of the ALFIS Vitamin D test cartridge while it undergoes scanning in the ALFIS-3 analyzer.

3. Specimen Sampling and Handling:
This sample is loaded into the sample well of the ALFIS Vitamin D test cartridge. An opening at the right end of the cartridge housing acts as the “scanning well” as the sample loaded test cartridge undergoes scanning in ALFIS-3 analyzer after automated pipetting-mixing steps and reactions as part of the assay.

The sample handling system consists of the ALFIS vitamin D test cartridge, the magnetic tip, and the pipette tip. The pipette tip is inserted into the pipette tip hole and dispenses washing buffer from the washing buffer well, into the magnetic bead well.

4. Calibration:

Analyte levels of ALFIS Vitamin D Calibrators

ALFIS Vitamin D Calibrator	Approximate Assigned/Target 25(OH)D Level
ALFIS Vitamin D Calibrator Level 1	10 ng/mL
ALFIS Vitamin D Calibrator Level 2	40 ng/mL

Calibrators are prepared according to the reagent kit instructions. Calibration recommendations are described in the labeling.

5. Quality Control:

Analyte levels and reference ranges of ALFIS Vitamin D Controls

ALFIS Vitamin D Control	Approximate Assigned/Target 25(OH)D Level	Acceptable Range
ALFIS Vitamin D Control Level 1	15 ng/mL	13.5 – 16.5 ng/mL
ALFIS Vitamin D Control Level 2	35 ng/mL	31.5 – 38.5 ng/mL

Recommendations relating to Quality Control procedures are described in the labeling.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys Vitamin D total

B Predicate 510(k) Number(s):

k210901

C Comparison with Predicate(s):

Device & Predicate Device(s):	<u>K221817</u>	<u>K210901</u>
Device Trade Name	ALFIS Vitamin D with ALFIS-3 Analyzer	Elecsys Vitamin D total III
General Device Characteristic Similarities		
Intended Use/Indications For Use	In vitro quantitative determination of total 25-hydroxy vitamin D	Same
Sample Type	Serum or plasma	Same

General Device Characteristic Differences		
Detection Method	Enzyme-linked fluorescence	Electro-chemiluminescence
Analytical Measuring Interval	6-100 ng/mL	6-125 ng/mL
Associated Instrument	ALFIS-3 Analyzer	Cobas e 601 analyzer
Sample volume required per test	100 µL	20 µL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 Evaluation of Precision of Quantitative Measurement Procedures; approved guideline - Third edition

CLSI EP07 3rd Edition Interference Testing in Clinical Chemistry

CLSI EP09c 3rd Edition Measurement Procedure Comparison and Bias Estimation Using Patient Samples

CLSI EP17-A2: 2013 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures.

CLSI EP28-A3c Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline - Third Edition

IEC 60601-1-2 Edition 4.0 2014-02 Medical Electrical Equipment - Part 1-2: General Requirements for Basic Safety and Essential Performance - Collateral Standard: Electromagnetic Disturbances - Requirements and Tests

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

The precision performance of the ALFIS Vitamin D test system on the ALFIS-3 Analyzer was evaluated following the recommendations described in CLSI guideline EP05-A3.

Repeatability (within-run)

Three (3) serum samples were tested in duplicate each day for 20 days by one operator using one lot of ALFIS Vitamin D test cartridges on one ALFIS-3 analyzer for a total of 40 measurements per sample.

Mean (ng/mL)	Repeatability (within-run)	
	SD (ng/mL)	CV (%)
10.07	0.69	6.9
38.7	1.99	6.5
50.11	3.00	6.0

Within-Lab Precision

Three (3) serum samples were tested in replicates of five each day for 5 days by 3 operators using 3 lots of ALFIS Vitamin D test cartridges and 3 ALFIS-3 analyzers at one internal site for a total of 75 measurements per sample. Within-lab precision for all three lots combined is represented below.

Mean (ng/mL)	Within-Lab Precision	
	SD (ng/mL)	CV (%)
10.31	0.62	6.01
29.72	1.75	5.89
48.45	2.74	5.66

Reproducibility

Three (3) serum samples were tested over five days at three testing sites by 1 operator per site. Each sample was assayed in triplicate, in five runs per day, using 3 lots of ALFIS Vitamin D test cartridges and 3 ALFIS-3 analyzers for a total of 75 determinations.

Mean (ng/mL)	N	Repeatability		Between-Day		Between-Site		Reproducibility	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
10.20	75	0.66	6.51	0.28	2.77	0.65	6.39	0.81	7.92
30.05	75	2.03	6.77	0.80	2.66	2.07	6.88	2.39	7.97
48.84	75	2.83	5.79	1.56	3.20	2.55	5.22	3.37	6.90

2. Linearity:

The linearity performance of the ALFIS vitamin D assay on the ALFIS-3 analyzer was established in a study conducted following the recommendations of CLSI guideline EP06-A. Two sample panels were prepared: in the first panel, 10 samples were prepared by intermixing a serum sample spiked with a high concentration of 25-hydroxyvitamin D with a depleted, low analyte serum sample to cover a range of 13 to 130 ng/mL. In the second panel, 9 serum samples were prepared by intermixing a serum sample spiked with a low concentration of 25-hydroxyvitamin D with a depleted, low analyte serum sample to cover a range of 1.3 to 11.7 ng/mL. Each sample was assayed in triplicate using one lot of ALFIS Vitamin D test cartridges and one ALFIS-3 analyzer. The results of the linearity study support the claimed measuring interval of 6-100 ng/mL.

3. Analytical Specificity/Interference:

Endogenous Interferences:

Potential interference from endogenous substances was evaluated. For each interfering substance, pooled native serum samples with concentrations representing low, middle, and high vitamin D were tested. An aliquot of each serum sample was spiked with the interfering substances. Each sample was measured in triplicate using one lot of ALFIS Vitamin D test cartridge on one ALFIS-3 analyzer. The following substances were tested up to the levels indicated and demonstrated no significant interference ($\leq 10\%$).

Substance	Highest concentration tested at which no significant interference was observed
Albumin	12 g/dL
Conjugated Bilirubin	40 mg/dL
Human Hemoglobin	1,000 mg/dL
Triglycerides	2,000 mg/dL
HAMA	800 ng/mL
Rheumatoid Factor	200 IU/mL
Biotin	3,500 ng/mL

Exogenous Interference:

Interference was assessed using serum samples representing low (~12-15 ng/mL), middle (~35-37 ng/mL), and high (~68-75 ng/mL) concentrations of vitamin D. Each of the samples (without any added interferent) acted as control samples. To evaluate potential interference, test samples for each interferent were prepared by adding specified concentration of the respective interferent to the serum samples. The results were analyzed as percent recovery between the test sample and the control sample. The substance was considered to have no significant interference if the recovery was 90-110%.

Exogenous Interferent/Drugs Tested	Highest concentration of spiked drug that demonstrated no significant interference
Acetaminophen	15.5 mg/dL
Acetylcysteine	15 mg/dL
Acetylsalicylic acid	3.0 mg/dL
Ampicillin-Na	7.5 mg/dL
Ascorbic acid	5.25 mg/dL
Cyclosporine	0.18 mg/dL
Doxycycline	1.8 mg/dL
Ibuprofen	22 mg/dL
Itraconazole	3.0 mg/dL
Levodopa	0.75 mg/dL
Methyldopa	2.25 mg/dL
Rifampicin	4.8 mg/dL

The following limitation statement is included in the labeling:

Endogenous alkaline phosphatase and exogenous alkaline phosphatase (e.g., asfotase alfa, Strensiq) are capable of binding to alkaline phosphatase and may cause erroneous results. Carefully evaluate results if the sample is suspected of having these types of interferences.

Cross-reactivity

A study was conducted to evaluate the potential cross-reactivity of the assay with other vitamin D metabolites. In the study, three test samples were prepared by adding specified concentrations of the respective cross-reactant to 25 hydroxyvitamin D-spiked serum samples

containing low, medium, and high concentrations of 25 hydroxyvitamin D. Control and test samples were tested in replicates of five with one lot of ALFIS Vitamin D test cartridge on one ALFIS- 3 analyzer. Cross-reactivity was evaluated in terms of percent cross-reactivity in presence of the concentrations of respective potential cross-reactants/structural analog of 25-hydroxyvitamin-D mentioned below. Percent cross-reactivity was determined using the following formula:

$$\text{Cross-reactivity (\%)} = \frac{[(\text{average of test sample measurements} - \text{average of control sample measurements}) / \text{concentration of the potential cross-reactants}] \times 100}{}$$

Cross-Reactant	Concentration of Cross-Reactant, ng/mL	%Cross Reactivity	Cross reactivity normalized to 25-hydroxyvitamin D3
25-hydroxyvitamin D3	50	100%	100%
25-hydroxyvitamin D2	50	98%	99%
3 epi-25-hydroxyvitamin D3	50	21%	21%
24,25-dihydroxyvitamin D3	50	122%	122%
1,25-dihydroxyvitamin D3	100	0.5%	0.5%
1,25-dihydroxyvitamin D2	100	0.2%	0.2%
Vitamin D3 (Cholecalciferol)	1000	0.1%	5.0%
Vitamin D2 (Ergocalciferol)	1000	0.1%	4.5%

The product labeling lists %cross-reactivity observed.

Hook/Prozone Effect

High-dose hook effect was assessed on the ALFIS Vitamin D test. Ten (10) serum samples were spiked to achieve 25-OH Vitamin D concentration of 1000 ng/mL. Each test sample was tested in triplicate on the same day by the same operator with one lot of ALFIS Vitamin D test cartridges using one ALFIS-3 analyzer. The study results show that there was no hook effect up to 1000 ng/mL of 25-hydroxyvitamin D concentration.

4. Assay Reportable Range:
See Linearity section A.2.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):
The device is traceable to internal calibrators prepared using Standard Reference Material (SRM) 2972a: 25-Hydroxyvitamin D Calibration Solutions supplied by National Institute of Standards and Technology (NIST).

6. Detection Limit:
Limit of Blank (LoB)
The LoB of the assay was evaluated using four (4) Vitamin D-depleted serum samples that were measured in replicates of six (6) with three lots of test cartridges over three successive days for a total of 72 blanks per lot. The LoB was calculated by the nonparametric analysis at the 95th percentile. The highest LoB of the three lots was found to be 1.82 ng/mL, supporting the claimed limit of blank of 1.82 ng/mL.

Limit of Detection (LoD)

The LoD was evaluated by testing 4 low level spiked serum samples using three lots of test cartridges on three analyzers (1 lot per analyzer) for 3 successive days. Each sample was measured in 5 replicates for a total of 60 measurements per lot. The LoD was calculated by parametric analysis which was the lowest concentration of which the analyte can be detected with 95% probability. The claimed LoD is 3.76 ng/mL.

Limit of Quantification (LoQ)

The LoQ was evaluated using 5 low level spiked serum samples using three lots of test cartridges on three analyzers (1 lot/analyzer). Each sample was tested in replicates of four across each lot on 3 successive days for a total of 36 replicates per sample. The data was visually inspected to find out the lowest concentration of analyte which has imprecision less than or equal to 20% CV. The LoQ of the assay was found to be 6.0 ng/mL with inter-assay CV < 20%.

7. Assay Cut-Off:

Not applicable

8. Accuracy (Instrument):

Not applicable

9. Carry-Over:

Not applicable

B Comparison Studies:

1. Method Comparison:

A study was conducted to compare the accuracy of the ALFIS Vitamin D on the ALFIS-3 analyzer to a reference method for Vitamin D. In the study, 120 native single donor patient serum samples were tested in singlicate on the candidate device and the reference method. Samples provided by the Vitamin D Standardization and Certification Program with assigned values by the reference method procedure (ID-LCMS/MS) at CDC Vitamin D Reference Laboratory. The results of the study were analyzed graphically for slope and intercept by weighted Deming regression analysis, and summarized as follows:

N	Sample Range Tested* (ng/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient (r)
120	6.45 – 83.33	0.997 (0.9449 – 1.049)	0.4725 (-1.006 – 1.951)	0.978

*As measured by reference method

2. Matrix Comparison:

A matrix equivalency study was conducted to support use of the ALFIS Vitamin D on the ALFIS-3 analyzer with lithium-heparin plasma and sodium-heparin plasma samples. In the study 45 samples of each matrix type were tested in singlicate with the same lot of ALFIS Vitamin D test cartridges and ALFIS-analyzer to span the claimed measuring interval of ALFIS Vitamin D test system. The results were analyzed by Passing Bablok regression analysis and are summarized as follows:

Sample matrices	N	Linear Regression Equation	Correlation Coefficient	Sample Interval (ng/mL)
Serum vs. Li-heparin Plasma	45	$y = 0.9797x + 0.3501$	0.9982	8-96
Serum vs. Na-heparin Plasma	45	$y = 0.98x + 0.3486$	0.9981	8-96

The results support the sponsor's claims that the assay is suitable for use with serum, lithium heparin and sodium heparin plasma samples.

C Clinical Studies:

1. Clinical Sensitivity:
Not applicable
2. Clinical Specificity:
Not applicable
3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):
Not applicable

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

A reference range study with the ALFIS Vitamin D assay on the ALFIS-3 Analyzer was conducted testing serum samples collected from healthy individuals from central, northern, and southern sites of the United States during summer (25%), winter (25%), and fall (50%). There were approximately equal numbers of males and females donors. Approximately 30% percent of the donors were African American and 30% Caucasian. The age range was 21 to 70 years. The results of the study are as follows:

N	Mean	Median	2.5 th – 97.5 th percentile
299	22.84 (ng/mL)	23 (ng/mL)	8.75 – 43.66

The reference interval for the total study population using the ALFIS Vitamin D assay was calculated using the 95% reference interval: 9.0-43.0 ng/mL.

F Other Supportive Instrument Performance Characteristics Data:

Not applicable

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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