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

A. 510(k) Number:

k153361

B. Purpose for Submission:

New device

C. Measurand:

25-hydroxyvitamin D and other hydroxylated vitamin D metabolites

D. Type of Test:

Quantitative assay, automated chemiluminescent enzyme immunoassay (CLEIA)

E. Applicant:

Fujirebio Diagnostics, Inc.

F. Proprietary and Established Names:

Lumipulse ***G*** 25-OH Vitamin D
Lumipulse ***G*** 25-OH Vitamin D Calibrators

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
MRG	II	862.1825, Vitamin D Test System	75-Clinical Chemistry
JIT	II	862.1150, Vitamin D Calibrator	75-Clinical Chemistry

H. Intended Use:

1. Intended use(s):

   See indications for use below.

2. Indication(s) for use:

   Lumipulse ***G*** 25-OH Vitamin D

   For in vitro diagnostic use.

   Lumipulse ***G*** 25-OH Vitamin D is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of 25-hydroxyvitamin D (25-OH vitamin D) and other hydroxylated vitamin D metabolites in human serum and plasma (sodium heparin, lithium heparin, or dipotassium EDTA) on the LUMIPULSE ***G*** System.

   Lumipulse ***G*** 25-OH Vitamin D is to be used as an aid 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.

   Lumipulse ***G*** 25-OH Vitamin D Calibrators
   Lumipulse ***G*** 25-OH Vitamin D Calibrators are for in vitro diagnostic use in the calibration of the Lumipulse ***G*** 25-OH Vitamin D on the LUMIPULSE G System.

3. Special conditions for use statement(s):

   Prescription use only.

4. Special instrument requirements:

   LUMIPULSE ***G***1200 System

I. Device Description:

Lumipulse ***G*** 25-OH Vitamin D is an assay system, including a set of immunoassay reagents, for the quantitative measurement of 25-OH Vitamin D in specimens based on CLEIA technology by a two-step sandwich immunoassay method on the LUMIPULSE ***G*** System.

Lumipulse ***G*** 25-OH Vitamin D Immunoreaction Cartridges consists of 3 x 14 tests. Each kit contains the following:
1) Antibody Coated Particle Solution (250 μL/Immunoreaction Cartridge, liquid when used) contains 200 μg/mL anti-25-OH vitamin D monoclonal antibody (sheep)-coated particles, protein stabilizers (bovine and sheep) and chemical stabilizers in 0.15 M sodium chloride/Tris buffer. This solution contains gelatin and turns into gel at 15^0C or lower. Preservative: sodium azide
2) Enzyme-labeled antibody solution (320 μL/Immunoreaction Cartridge, liquid when used) contains 3 μg/mL alkaline phosphatase (ALP: calf)-labeled anti-(25-OH vitamin D/anti-25-OH vitamin D monoclonal antibody immunocomplexes) recombinant chicken monoclonal antibody, protein stabilizers (bovine and calf) and chemical stabilizers in 0.15 M sodium chloride/MES buffer. Preservative: sodium azide.

Each Lumipulse ***G*** 25-OH Vitamin D Calibrator kit contains one bottle each of the following Calibrator 1 – 6. The calibrator kit is packaged separately.
1) CAL 1 0 ng/mL (0 nmol/L) 25-OH Vitamin D calibrator (1 x 1.5 mL)
2) CAL 2 10 ng/mL (25 nmol/L) 25-OH Vitamin D calibrator (1 x 1.5 mL)
3) CAL 3 20 ng/mL (50 nmol/L) 25-OH Vitamin D calibrator (1 x 1.5 mL)
4) CAL 4 50 ng/mL (125 nmol/L) 25-OH Vitamin D calibrator (1 x 1.5 mL)
5) CAL 5 100 ng/mL (250 nmol/L) 25-OH Vitamin D calibrator (1 x 1.5 mL)
6) CAL 6 150 ng/mL (375 nmol/L) 25-OH Vitamin D calibrator (1 x 1.5 mL)
Each solution contains 0.1 M sodium chloride in HEPES buffer with protein stabilizers (bovine). Preservative: sodium azide.

Materials required but not provided:
LUMIPULSE ***G***1200 System- analyzer was previously cleared in k142895
LUMIPULSE wash solution
LUMIPULSE Specimen diluent
LUMIPULSE substrate solution

J. Substantial Equivalence Information:

1. Predicate device name(s):

   LIAISON 25 OH Vitamin D TOTAL Assay

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

   k071480 (for both Vitamin D assay and calibrator)

3. Comparison with predicate:

   Lumipulse G 25-OH Vitamin D

Similarities and Differences		
Item	Predicate Device: LIAISON 25 OH Vitamin D TOTAL Assay (k071480)	Candidate Device: Lumipulse ***G*** 25-OH Vitamin D (k153361)
Intended use	For the quantitative measurement of 25-OH vitamin D and other hydroxylated vitamin D metabolites.	Same
Principle of Operation	Automated Quantitative Chemiluminescent Immunoassay	Same
Assay Range	4.0 – 100 ng/mL	6.9-150 ng/mL
Instrument system	LIAISON	Lumipulse ***G*** System
Assay type	Competitive immunoassay based on chemiluminescent technology	Direct sandwich immunoassay based on chemiluminescent technology

Similarities and Differences		
Item	Predicate Device: LIAISON 25 OH Vitamin D TOTAL Assay (k071480)	Candidate Device: Lumipulse ***G*** 25-OH Vitamin D (k153361)
Sample volume	20 μL	Same
Specimen	Human serum or plasma (lithium heparin or dipotassium EDTA)	Human serum or plasma (sodium heparin, lithium heparin, or dipotassium EDTA)
Calibrators	2	6
Analyte	25-hydroxyvitamin D and other hydroxylated vitamin D metabolites	Same

Lumipulse ***G*** 25-OH Vitamin D Calibrator

Similarities and Differences		
Item	Predicate Device: LIAISON 25 OH Vitamin D TOTAL Assay (k071480)	Candidate Device: Lumipulse ***G*** 25-OH Vitamin D (k153361)
Number of Calibrators	2 calibrators, 1.0 mL each	6 calibrators, 1.5 mL each
Standardization/ Traceability	Calibrators are traceable to concentrations determined by UV spectrophotometric analysis.	The calibrators for use with Lumipulse ***G*** 25-OH Vitamin D are prepared gravimetrically and are traceable to internal reference calibrator concentrations determined by UV spectrophotometric analysis and verified by Reference Method Procedure (University of Ghent).
Calibration Curve	Master calibration curve	Full calibration curve

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

ISO 17511:2003 Measurement of Quantities in Biological Samples – Metrological Traceability of Values Assigned to Calibrator and Control Materials

CLSI EP5-A3 - Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition

CLSI EP7-A2 - Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition

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

CLSI EP17-A2 - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline Second Edition

CLSI EP6-A - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP9-A3 – Measurement Procedure Comparison and Bias Estimation Using Patient Samples; approved Guideline – Third Edition

CLSI EP25-A – Evaluation of Stability of *In Vitro* Diagnostic Reagents: Approved Guideline

L. Test Principle:

Lumipulse G 25-OH Vitamin D is an assay system, including a set of immunoassay reagents, for the quantitative measurement of 25-OH Vitamin D in specimens based on CLEIA technology by a two-step sandwich immunoassay method on the LUMIPULSE G System. 25-OH Vitamin D Calibrator or specimen is initially auto-diluted with Specimen Diluent 1 in the system. 25-OH vitamin D in specimens is separated from vitamin D binding protein by the extraction agent and specifically bound to anti-25-OH vitamin D monoclonal antibody (sheep) on the particles, and form immunocomplexes. The particles are washed and rinsed to remove unbound materials. Alkaline phosphatase (calf ALP)-labeled anti-(25-OH vitamin D/anti- 25-OH vitamin D monoclonal antibody immunocomplexes) recombinant chicken monoclonal antibody specifically binds to 25-OH vitamin D immunocomplexes on the particles, and additional immunocomplexes are formed The particles are washed and rinsed to remove unbound materials. Substrate Solution is added and mixed with the particles. Chloro-5-substituted adamantly-1,2-dioxetane phosphate(AMPPD) contained in the Substrate Solution is dephosphorylated by the catalysis of ALP indirectly conjugated to particles. Luminescence (at a maximum wavelength of 477 nm) is generated by the cleavage reaction of dephosphorylated AMPPD. The luminescent signal reflects the amount of 25-OH vitamin D.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

   A precision study was performed internally using one lot of reagents and calibrators on one LUMIPULSE ***G***1200 instrument. Precision study was performed in accordance with CLSI EP5 A2 in which five human serum-based samples (all native except panel 5 was spiked to achieve high concentration), and three controls were assayed in replicates of two at two separate times of the day for 20 days (n=80 for each sample). The precision results are summarized below:

		Within Run		Between Run Within Day		Between Day		Total	
Sample	Mean (ng/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Control Level 1	10.3	0.23	2.2%	0.24	2.3%	0.32	3.1%	0.46	4.5%
Control Level 2	34.0	0.36	1.1%	0.48	1.4%	0.54	1.6%	0.81	2.4%
Control Level 3	70.6	0.68	1.0%	0.72	1.0%	1.07	1.5%	1.46	2.1%
Panel 1	7.7	0.21	2.8%	0.20	2.6%	0.28	3.6%	0.40	5.2%
Panel 2	20.7	0.33	1.6%	0.24	1.2%	0.32	1.6%	0.52	2.5%
Panel 3	41.2	0.53	1.3%	0.45	1.1%	0.67	1.6%	0.97	2.3%
Panel 4	61.0	0.57	0.9%	0.05	0.1%	0.77	1.3%	0.96	1.6%
Panel 5	120.6	1.36	1.1%	0.00	0.0%	1.32	1.1%	1.89	1.6%

b. Linearity/assay reportable range:

Linearity was evaluated based on CLSI EP6-A. Two human serum specimen pools and two K2EDTA plasma specimen pools with high 25-Hydroxyvitamin D levels were diluted with low 25-Hydroxyvitamin D levels throughout the range of the assay. The linearity was tested in the range of 4.0 to 150.0 ng/mL for serum and in the range of 6.9 to 150.0 ng/mL for plasma. Lumipulse ***G*** 25-OH Vitamin D results observed were correlated with expected concentrations with the following regression results:

Serum: y= 1.0382 (x) + 1.251; R-squared: 0.9978
K2EDTA Plasma: y= 1.0151 (x) + 6.7669; R-squared: 0.9936

The results of the linearity study support the sponsor's claim that the assay's measuring range is 6.9 - 150 ng/mL.

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

Standardization and Traceability

The standardization of Lumipulse ***G*** 25-OH Vitamin D is traceable to in-house reference calibrators, whose values have been assigned to correlate to Liquid Chromatography-tandem Mass Spectrometry (LC/MS/MS). To achieve standardization against the CDC Vitamin D Standardization Program (VDSP) recognized Reference Measurement Procedure (RMP), samples value assigned by RMP were tested against the Lumipulse ***G*** 25-OH Vitamin D. The RMP is further traceable to the NIST SRM 2972 material. Please refer to https://ods.od.nih.gov/Research/VitaminD.aspx for more information on the VDSP program. The Lumipulse ***G*** 25-OH Vitamin D has passed the certification process for the CDC VDSP and a certificate has been provided by the CDC. Please see the certification process at http://www.cdc.gov/labstandards/hs.html.

Value assignment

The Lumipulse ***G*** 25-OH Vitamin D Calibrator kit contains six different levels of 25-OH Vitamin D concentration. Value assignment of the calibrators was based on an internal procedure and results had been verified by testing calibrators in multiple replicates. All results must meet the internal acceptance criteria before released.

1) CAL 1 0 ng/mL
2) CAL 2 10 ng/mL
3) CAL 3 20 ng/mL
4) CAL 4 50 ng/mL
5) CAL 5 100 ng/mL
6) CAL 6 150 ng/mL

Shelf life stability

The long term stability data were tested at 2–10°C and the study is still on going. The real-time stability study protocol and acceptance criteria has been reviewed and found to be acceptable. The current test results support a shelf life of 12 months at 2–10°C for Lumipulse ***G*** 25-OH Vitamin D Immunoreaction Cartridges and the Lumipulse ***G*** 25-OH Vitamin D Calibrators.

On-board stability

On-board stability study and acceptance criteria has been reviewed and found to be acceptable. The stability data supports the sponsor's claim that Lumipulse ***G*** 25-OH Vitamin D Immunoreaction Cartridges can be stored on-board the LUMIPULSE ***G***1200 for a maximum of 30 days.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) studies are performed according to the CLSI EP17-A2 guideline. LoB study is performed using a blank sample with 60 replicates per lot of reagent for a total of 120 replicates, over 3 days using 2 lots of reagents. LoD study is performed using 7 low serum samples with multiple replicates over 3 days using 2 lots of reagents (N=420, 7x 60 replicates each). The LoQ is defined as the lowest analyte concentration at which concentration has an inter-assay precision of <10%CV.

The LoB, LoD and LoQ are summarized below:

LoB	LoD	LoQ
0.0 ng/mL	0.28 ng/mL	3.33 ng/mL

The measuring range for the assay is: 6.9 ng/mL – 150 ng/mL

e. Analytical specificity:

Potential interference from common endogenous substances was evaluated in accordance with CLSI EP7-A2. Human serum specimens with 25-OH vitamin D concentrations of approximately 20, 40 and 100 ng/mL were supplemented with potentially interfering compounds. Spiked samples with different interference substances were tested in replicate of three and the % difference between the spiked and unspiked (control) samples were calculated. The following compounds were tested and found not to interfere significantly with the test, based on the sponsors predefined acceptance criteria of non-significant interference of <10% bias between the test and control samples.

Endogenous Substances	Highest Test Concentration that demonstrated no significant interference
Free Bilirubin (unconjugated)	60 mg/dL
Conjugated Bilirubin	60 mg/dL
Triglycerides (Intralipid 20% Emulsion)	1000 mg/dL
Hemoglobin	500 mg/dL
Total Protein (Human Serum Albumin)	11 g/dL
Immunoglobulin G (IgG)	5 g/dL
Biotin	19.7 mg/dL
Uric Acid	24 mg/dL
Cholesterol	500 mg/dL
L-Ascorbic Acid	3 mg/dL
Human Vitamin D Binding Protein	200,000 ng/mL
Human Anti-Mouse Antibodies (HAMA)	1,000 ng/mL
Rheumatoid Factor (RF)	1,000 IU/mL
Acetaminophen	1455 µmol/L
Acetylsalicylic acid	3.65 mmol/L
Alendronate	350 mg/L
Ampicillin	344 µmol/L
Ascorbic Acid	375 µmol/L
Caffeine	309 µmol/L
Chloramphenicol	155 µmol/L
Digoxin	8.7 µmol/L
EinsAlpha (Alfacalcidol)	3633 µg/L
Hydrochlorothiazide	22.2 µmol/L
Ibandronate	52 mg/L
Ibuprofen	2486 µmol/L
Indomethacin	103 µmol/L
Lidocaine	57.9 µmol/L
Lovastatin	1932 µmol/L
Metoprolol	18.7 µmol/L
Naproxen	2247 µmol/L

Endogenous Substances	Highest Test Concentration that demonstrated no significant interference
Pamidron	90 mg/L
Risedronate	175 mg/L
Theophylline	243 µmol/L
Warfarin	37.5 µmol/L

Lumipulse ***G*** 25-OH Vitamin D on the LUMIPULSE ***G***1200 System was evaluated for cross-reactivity of the assay with other substances that are similar in structure to 25(OH) Vitamin D in a study consistent with the guidelines in the CLSI Protocol EP7-A2. Human serum specimens with 25-OH vitamin D concentrations of approximately 20, 40 and 100 ng/mL were supplemented with potentially cross-reacting compounds. The compounds were tested at the concentrations listed below and found to have the following percent cross-reactivity.

Substance	Test Concentration (ng/mL)	Mean % Cross-Reactivity	Range of % Cross-reactivity
3-epi-25(OH) vitamin D3	100	2	1% - 3%
3-epi-25(OH) vitamin D2	100	0	0% - 1%
1,25(OH)2 vitamin D2	10	143	139% - 146%
1,25(OH)2 vitamin D2	100	24	21% - 26%
1,25(OH)2 vitamin D3	100	39	39% - 40%
24,25(OH)2 vitamin D3	100	21	20% - 22%
Vitamin D3 (Cholecalciferol)	20,000	0	0% - 0%
Vitamin D2 (Ergocalciferol)	20,000	0	0% - 0%
1αOH Vitamin D3 (alfacalcidol)	8,000	0	0% - 0%
Paricalcitol (Zemplar)	25	-2	-6% - 1%

25-OH vitamin D2 & 25-OH vitamin D3 spiking recovery was performed using purified materials with three different serum pools (with different vitamin D concentration). The observed spiked results were calculated against the observed unspiked results and percent recovery was determined. The spiking recovery results are shown below:

Substance	Concentration tested (ng/mL)	Mean % Recovery	Range of % Recovery
25-OH vitamin D2	20	92	91% – 95%
25-OH vitamin D2	50	84	77% – 91%
25-OH vitamin D3	20	103	100% - 106%
25-OH vitamin D3	50	106	102% - 113%

f. Assay cut-off:

Not applicable.

2. Comparison studies:

a. Method comparison with predicate device:

Lumipulse ***G*** 25-OH Vitamin D method comparison study was performed using specimens consistent with CLSI guideline EP9-A3. A total of 137 serum samples were assayed using the candidate device (Lumipulse G assay) and the predicate device (LIAISON vitamin D). Sample range tested between 4.0 to 107.3 ng/mL. The weighted Deming regression method was used and results are summarized below:

n	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)
137	0.9476	-3.073 -4.357 to -1.79	1.09 1.04 to 1.13

The results showed a significant bias between the candidate device and the predicate device. However, this bias between the candidate device and the predicate device is expected due to different standardization of the two devices. Therefore, test results from the candidate device do not, and are not expected to, directly correlate with test results from the predicate device.

An additional method comparison study was performed to evaluate the accuracy between the candidate device and the RMP, CDC reference method. The method comparison against the RMP was the basis of the substantial equivalence determination.

A total of 119 serum samples were assayed using the candidate device (the Lumipulse G 25-OH assay) and the RMP. The weighted Deming regression method was used to compare Lumipulse **G** 25-OH Vitamin D to the RMP. The results are shown below:

Range of samples:
7.2 to 149.0 ng/mL (Lumipulse **G** 25-OH Vitamin D)
8.65 to 153 ng/mL (RMP)

Lumipulse G 25-OH Vitamin D vs. ID-HPLC-MS/MS (RMP)

n	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)
117	0.9986	-2.788 -3.225 - 2.351	0.97 0.96 – 0.99

c. Matrix comparison:

The Lumipulse ***G*** 25-OH Vitamin D matrix comparison study was performed to evaluate the difference across tube types (SST, K2EDTA, Lithium Heparin, and Sodium Heparin) versus the means of the control samples (Red top serum) analyzed per CLSI guideline EP9-A3. Fifty samples were tested with eight samples spiked in order to cover the measuring range.

Sample Type	n	Concentration range		Slope			Intercept			r
		Min	Max	Estimate	Lower 95%Cl	Upper 95%Cl	Estimate	Lower 95%Cl	Upper 95%Cl	
SST Serum	50	8.6	113.7	1.008	0.979	1.037	-0.218	-0.907	0.471	0.998
K2 EDTA Plasma	50	8.6	111.2	0.967	0.943	0.992	0.001	-0.552	0.554	0.998
Li Heparin Plasma	50	8.8	119.2	1.019	0.981	1.057	-0.597	-1.389	0.196	0.996
Na Heparin Plasma	50	8.8	116.3	1.010	0.980	1.040	-0.457	-1.162	0.248	0.998

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:

Results from a total of 287 samples were used to determine the distribution of Lumipulse ***G*** 25-OH Vitamin D results in apparently healthy adults to establish the reference range. Excluded from the study were individuals who had history of current use of dietary

supplements containing > 2,000 IU per day of Vitamin D, history of Vitamin D deficiency, any disease considered chronic, history of seizures, bariatric surgery, parathyroid and thyroid disease, were pregnant or lactating, had an active malignancy or were diagnosed with cancer other than basal/squamous cell skin cancer within 5 years, were receiving chemotherapy or radiation treatment, had a family history of parathyroid or calcium regulatory disease, were on medicines known to affect absorption or increase catabolism, or had abnormal serum levels for calcium PTH or TSH.

The subjects ranged in age from 21to 74 years (median age of 40) and were representative of the overall US population in terms of sex (48.4% male and 51.6% female) and ethnicity/race (61.3% White, 8.0% Black, 18.5% Hispanic, 7.7% Asian/Pacific Islander and 4.5% Other). The majority of the population was < 50 years old (196/287, 68.3%) with a median age of 31.

To represent a broad spectrum of UV light exposure, the study population included adult subjects from 2 geographically diverse regions of the US , the Northern states and the Southern states, that were sampled during spring/summer (April through September) and fall/winter (October through March) seasons. The observed range of 25(OH) vitamin D concentrations, established according to Clinical and Laboratory Standards Institute (CLSI) Protocol EP28-A3c is summarized in the tables below.

n	Mean Concentration	Median Concentration	Observed Range (2.5^{th} to 97.5^{th} percentile)
287	22.9 ng/mL	22.2 ng/mL	7.6 – 47.6 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.