



Food and Drug Administration
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FUJIREBIO DIAGNOSTICS, INC.
STACEY DOLAN
MANAGER, REGULATORY AFFAIRS
201 GREAT VALLEY PARKWAY
MALVERN, PA 19355

April 15, 2016

Re: K153361

Trade/Device Name: Lumipulse G 25-OH Vitamin D, Lumipulse G 25-OH Vitamin D
Calibrators

Regulation Number: 21 CFR 862.1825

Regulation Name: Vitamin D test system

Regulatory Class: II

Product Code: MRG, JIT

Dated: March 17, 2016

Received: March 18, 2016

Dear Stacey Dolan:

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. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. 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); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address

<http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to

<http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address

<http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>.

Sincerely yours,


Katherine Serrano -S

FOR: Courtney H. Lias, Ph.D.
Director
Division of Chemistry and Toxicology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known)
k153361

Device Name

Lumipulse G 25-0H Vitamin D
Lumipulse G 25-0H Vitamin D Calibrators

Indications for Use (Describe)

Lumipulse G 25-0H Vitamin D
For in vitro diagnostic use.

Lumipulse G 25-0H Vitamin D is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of 25-hydroxyvitamin D (25-0H 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 25-0H 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 25-0H Vitamin D Calibrators

Lumipulse G 25-0H Vitamin D Calibrators are for in vitro diagnostic use in the calibration of Lumipulse G 25-0H Vitamin D on the LUMIPULSE G System.

Type of Use (Select one or both, as applicable)

Prescription Use (Part 21 CFR 801 Subpart D)

Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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Section 5

510(k) SUMMARY

Lumipulse **G** 25-OH Vitamin D
K153361

Date: April 13, 2016

Submitter: Fujirebio Diagnostics, Inc.
201 Great Valley Parkway
Malvern, PA 19355

Contact Person: Stacey Dolan
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Device Name: Lumipulse **G** 25-OH Vitamin D
Classification: Class II
MRG
75, Clinical Chemistry
21 CFR § 862.1825

Device Name: Lumipulse **G** 25-OH Vitamin D Calibrators
Classification: Class II
JIT
75, Clinical Chemistry
21 CFR § 862.1150

Predicate Device: k071480
DIASORIN, INC.
LIAISON® 25 OH Vitamin D TOTAL Assay

According to the requirements of 21 CFR 807.92, the following information provides sufficient detail to understand the basis for a determination of substantial equivalence.

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. 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 antigen-antibody immunocomplexes are formed. The particles are washed and rinsed to remove unbound materials. Alkaline phosphatase (ALP: calf)-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. 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.

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 **REF** 235089

The Lumipulse **G** 25-OH Vitamin D Immunoreaction Cartridges consists of 3 × 14 tests. Each kit contains the following:

1) Antibody-Coated Particle Solution

(Liquid when used, 250 µL/Immunoreaction Cartridge)

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 °C or lower. Preservative: sodium azide.

2) Enzyme-Labeled Antibody Solution

(Liquid, 320 µL/Immunoreaction Cartridge)

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.

Lumipulse **G** 25-OH Vitamin D Calibrators **CAL** 235096, Liquid 1x6 concentrations

Each calibrator kit contains one bottle each of Calibrators 1 – 6. The calibrator kit is packaged separately.

CAL 1	0 ng/mL (0 nmol/L) 25-OH Vitamin D calibrator (1 × 1.5 mL)
CAL 2	10 ng/mL (25 nmol/L) 25-OH Vitamin D calibrator (1 × 1.5 mL)
CAL 3	20 ng/mL (50 nmol/L) 25-OH Vitamin D calibrator (1 × 1.5 mL)
CAL 4	50 ng/mL (125 nmol/L) 25-OH Vitamin D calibrator (1 × 1.5 mL)
CAL 5	100 ng/mL (250 nmol/L) 25-OH Vitamin D calibrator (1 × 1.5 mL)
CAL 6	150 ng/mL (375 nmol/L) 25-OH Vitamin D calibrator (1 × 1.5 mL)

Contains calcifediol in 0.1 M sodium chloride in HEPES buffer with protein stabilizer (bovine). Preservative: sodium azide

Device Intended 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 Lumipulse **G** 25-OH Vitamin D on the LUMIPULSE **G** System.

Device Indications for Use:

Same as Intended Use

Substantial Equivalence:

Comparison between the Lumipulse **G 25-OH Vitamin D Assay and LIAISON® 25 OH Vitamin D TOTAL Assay**

Similarities and Differences		
Parameter	Lumipulse G 25-OH Vitamin D (Proposed Device)	LIAISON® 25 OH Vitamin D TOTAL Assay (Predicate Device) k071480
Device Type	<i>In vitro</i> diagnostic	Same
Classification	Class II	Same
Regulation Number	21CFR § 862.1825 Vitamin D test system.	Same
Principle of Operation	Automated Quantitative Chemiluminescent Immunoassay	Same
Specimen Collection Method	Routine Phlebotomy Techniques	Same
Assay Range	6.9 – 150 ng/mL	4.0 – 150 ng/mL
Intended 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.	The LIAISON 25 OH Vitamin D TOTAL Assay uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites in human serum, EDTA-plasma or lithium heparin plasma to be used in the assessment of vitamin D sufficiency. Assay results should be

Similarities and Differences		
Parameter	Lumipulse G 25-OH Vitamin D (Proposed Device)	LIAISON[®] 25 OH Vitamin D TOTAL Assay (Predicate Device) k071480
	Assay results should be used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions.	used in conjunction with other clinical or laboratory data to assist the clinician in making individual patient management decisions in an adult population.
Instrument System	LUMIPULSE G System	LIAISON
Assay Type	Direct sandwich immunoassay based on chemiluminescent technology	Competitive immunoassay based on chemiluminescent technology
Sample Volume	Sample Volume: 20 µL Minimum Volume: 120 µL for sample cups and 270 µL for sample tubes	Sample Volume: 25 µL Minimum Volume: 250 µL
Type of Specimen	Human serum or plasma (sodium heparin, lithium heparin, or dipotassium EDTA)	Human serum or plasma (lithium heparin or dipotassium EDTA)
Analyte Detected	25-OH vitamin D and other hydroxylated vitamin D metabolites	Same

Calibrator Set

Similarities and Differences		
Parameter	Lumipulse G 25-OH Vitamin D (Proposed Device)	LIAISON[®] 25 OH Vitamin D TOTAL Assay (Predicate Device) k071480
Number of Calibrators	6 calibrators, 1.5 mL each	2 calibrators, 1.0 mL each
Standardization/Traceability	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).	Calibrators are traceable to concentrations determined by UV spectrophotometric analysis.
Calibration Curve	Full Calibration Curve	Master Calibration Curve
Matrix	HEPES buffer with protein stabilizer (bovine)	Human serum and BSA

Performance Characteristics

Data were generated using the LUMIPULSE **G**1200 System.

1. Analytical performance:

a. *Precision/Reproducibility:*

Lumipulse **G** 25-OH Vitamin D is $\leq 10\%$ total (within-laboratory) CV (coefficient of variation). Lumipulse **G** 25-OH Vitamin D demonstrated precision $\leq 5.2\%$ (total %CV) in a study run according to the Clinical and Laboratory Standards Institute (CLSI) guideline EP5-A3. Five human serum-based samples (specimen pools), and three controls were assayed in replicates of two at two separate times of the day for 20 days (n=80 for each sample) using one LUMIPULSE **G**1200 System. Data from this study are presented 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:*

Lumipulse **G** 25-OH Vitamin D demonstrated linearity in a study consistent with the guidelines in the CLSI Guideline EP6-A. Two human serum specimen pools and two K2EDTA plasma specimen pools with high 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3 levels were diluted with one human serum specimen pool and one dipotassium EDTA plasma specimen pool with low 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3 levels throughout the range of the assay. The linearity was found in the range of 6.9 to 150.0 ng/mL for serum and plasma. Lumipulse **G** 25-OH Vitamin D correlated with expected concentrations according to the linear regression formulas:

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

Lumipulse **G** 25-OH Vitamin D has been verified to detect 25-OH vitamin D2 and vitamin D3 in equal parts to the true molar concentration. 25-OH vitamin D2 and 25-OH vitamin D3 were prepared in seven (7) different ratios targeting a final concentration of 50 ng/mL in a serum sample pool with a low endogenous 25-OH vitamin D level. The concentration of 25-OH vitamin D was determined using Lumipulse **G** 25-OH Vitamin D and the resulting percent recovery was calculated. Samples with varying amounts of 25-OH vitamin D2 and 25-OH vitamin D3 recover within $100 \pm 10\%$, ranging from 101% to 103%. The data are summarized in the following table

Ratio of vitamin D2 to vitamin D3	Expected Conc. (ng/mL)	Mean Measured Conc. (ng/mL) (n=3)	% Recovery
1to1	53.1	54.8	103
1to2	54.0	55.6	103
2to1	52.3	53.5	102
1to3	54.4	55.8	103
3to1	51.9	52.9	102
1to7	55.0	55.7	101
7to1	51.4	52.7	103

Lumipulse **G** 25-OH Vitamin D recovers known concentrations of supplemental equimolar 25-OH vitamin D2 and 25-OH vitamin D3. A study was performed where known concentrations of 25-OH vitamin D (25-OH vitamin D2 and 25-OH vitamin D3) were added to human serum and K_2 EDTA plasma samples with low endogenous 25-OH vitamin D levels. The concentration of 25-OH vitamin D was determined using the Lumipulse **G** 25-OH Vitamin D and the resulting percent recovery was calculated. 25-OH vitamin D recovery is $100 \pm 10\%$, ranging from 92% to 106%. The data are summarized in the following table.

Sample	Mean Measured Conc. (ng/mL) (n=3)	Expected Conc. (ng/mL)	% Recovery
Serum 1	29.5	31.0	95
	44.2	45.3	98
	57.7	57.9	100
	89.9	89.8	100
	112.9	112.4	100
	137.1	135.7	101
Serum 2	23.9	24.1	99
	38.0	38.4	99
	50.6	51.0	99
	83.2	82.9	100
	105.0	105.5	100
	136.3	128.8	106
Serum 3	29.3	29.0	101
	43.7	43.3	101
	56.1	55.9	100

Sample	Mean Measured Conc. (ng/mL) (n=3)	Expected Conc. (ng/mL)	% Recovery
	88.5	87.8	101
	113.1	110.4	102
	137.3	133.7	103
Plasma 1	26.7	26.5	101
	38.1	40.5	94
	52.1	56.0	93
	83.3	86.0	97
	110.2	114.8	96
	137.9	143.4	96
Plasma 2	23.1	23.9	97
	34.9	37.9	92
	49.0	53.4	92
	79.9	83.4	96
	106.9	112.2	95
	134.9	140.8	96
Plasma 3	20.7	21.8	95
	33.4	35.8	93
	48.3	51.3	94
	77.2	81.3	95
	103.8	110.1	94
	130.5	138.7	94

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For Lumipulse **G** 25-OH Vitamin D, no high dose hook effect was observed for samples containing up to 3,000 ng/mL of 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3.

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

Lumipulse **G** 25-OH Vitamin D Calibrators are for *in vitro* diagnostic use in the calibration of Lumipulse **G** 25-OH Vitamin D on the LUMIPULSE **G** System for the quantitative determination of 25-hydroxyvitamin D (25-OH Vitamin D) in human serum and plasma (sodium heparin, lithium heparin, or dipotassium EDTA).

6 Bottles (1.5 mL each) are supplied for the LUMIPULSE **G** 25-OH Vitamin D Calibrators. Preservative: Sodium azide. The calibrators are at the following concentrations:

Calibrator	Concentration (ng/mL)	Concentration (nmol/L)
CAL 1	0	0
CAL 2	10	25
CAL 3	20	50
CAL 4	50	125

CAL 5	100	250
CAL 6	150	375

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).

Shelf life

The shelf life for Lumipulse **G** 25-OH Vitamin D Immunoreaction Cartridges and the Lumipulse **G** 25-OH Vitamin D Calibrators is 12 months at 2–10°C.

On board the LUMIPULSE **G**1200

The Lumipulse **G** 25-OH Vitamin D Immunoreaction Cartridges are sealed unit dose stored at 2–10°C. To reduce risk for any misuse, the package insert states “The Lumipulse **G** 25-OH Vitamin D Immunoreaction Cartridges can be stored (refrigerated unit) on-board the LUMIPULSE **G** System for a maximum of 30 days”.

The package insert recommends calibrator curve storage on the LUMIPULSE **G**1200 for a maximum of 30 days.

Transport Conditions

Lumipulse **G** 25-OH Vitamin D Immunoreaction Cartridges and the Lumipulse **G** 25-OH Vitamin D Calibrators are shipped at 2-10°C.

Materials will be shipped to the end user using an insulated container and a predetermined configuration of gel (cold and/or frozen) packs to maintain the product for up to 72 hours when stored at ambient temperature.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) of Lumipulse **G** 25-OH Vitamin D on the LUMIPULSE **G**1200 is ≤ 4.0 ng/mL.

1) LoB and LoD

The LoB for Lumipulse **G** 25-OH Vitamin D was 0.0 ng/mL.

The LoD for Lumipulse **G** 25-OH Vitamin D on the LUMIPULSE **G**1200 was 0.28 ng/mL, determined consistent with the CLSI guideline EP17-A2. Seven low-level specimens were tested over 3 days using two LUMIPULSE **G**1200 Systems and two Lumipulse **G** 25-OH Vitamin D lots giving 120 determinations for each panel.

2) LoQ

The LOQ for Lumipulse **G** 25-OH Vitamin D on the LUMIPULSE **G**1200 was 3.33 ng/mL, determined consistent with the CLSI guideline EP17-A2. The LoQ is the analyte level at which the CV is 10%.

e. *Analytical specificity:*

The Lumipulse **G** 25-OH Vitamin D demonstrated an average interference of $\leq 10\%$ (for each compound) in a study consistent with the guidelines in the CLSI guideline 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. The following compounds were tested and found not to interfere with the test.

Endogenous Interferences	Test Concentration
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	0.200 mg/mL
Human Anti-Mouse Antibodies (HAMA)	1,000 ng/mL
Rheumatoid Factor (RF)	1,000 IU/mL

Therapeutic drug Interferences	Test Concentration
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
Pamidron	90 mg/L
Risedronate	175 mg/L
Theophylline	243 µmol/L
Warfarin	37.5 µmol/L
Zometa (Zoledronic Acid)	4 mg/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)	% Cross-Reactivity
3-epi-25(OH) vitamin D3	100	2
3-epi-25(OH) vitamin D2	100	0
1,25(OH)2 vitamin D2	10	143
1,25(OH)2 vitamin D2	100	24
1,25(OH)2 vitamin D3	100	39
24,25(OH)2 vitamin D3	100	21
Vitamin D3 (Cholecalciferol)	20,000	0
Vitamin D2 (Ergocalciferol)	20,000	0
1αOH Vitamin D3 (alfacalcidol)	8,000	0
Paricalcitol (Zemplar)	25	-2

f. Assay cut-off:

See Clinical Cutoff in 4. below.

2. Comparison studies:

a. Method Comparison - Liaison® 25 OH Vitamin D TOTAL

Lumipulse **G** 25-OH Vitamin D method comparison study was performed using specimens consistent with CLSI guideline EP9-A3. The weighted Deming regression method was used to compare Lumipulse **G** 25-OH Vitamin D to Liaison® 25 OH Vitamin D TOTAL. The data are summarized in the following table and presented graphically.

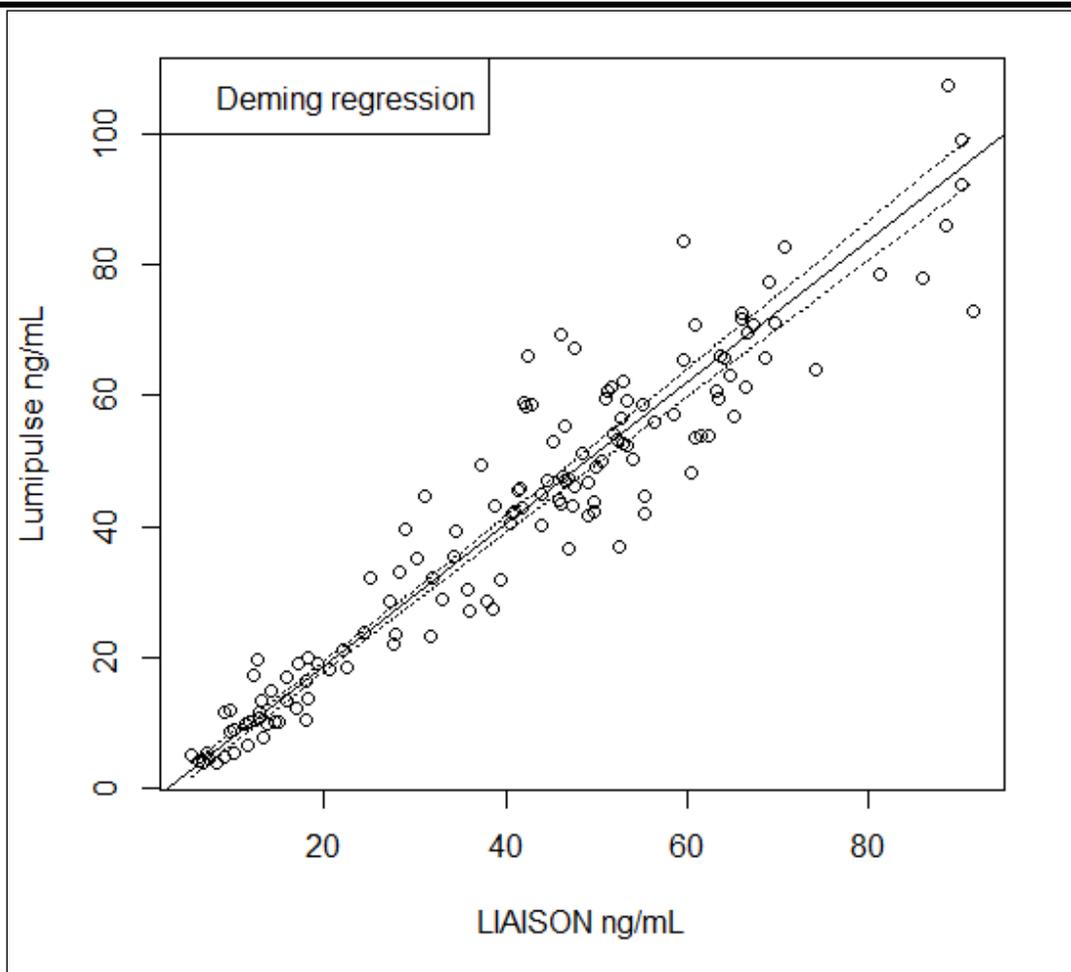
Range of samples:

4.0 to 107.3 ng/mL. (Lumipulse **G** 25-OH Vitamin D)

5.45 to 91.5 ng/mL (Liaison® 25 OH Vitamin D TOTAL)

Lumipulse **G 25-OH Vitamin D vs. Liaison® 25 OH Vitamin D TOTAL**

n	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)	Average Bias (ng/mL)
137	0.9476	-3.073 -4.357 – -1.790	1.09 1.04 – 1.13	0.423



b. Method Comparison – ID- HPLC-MS/MS

To evaluate accuracy of Lumipulse **G** 25-OH Vitamin D, an additional method comparison study was performed using specimens consistent with CLSI guideline EP9-A3. The weighted Deming regression method was used to compare Lumipulse **G** 25-OH Vitamin D to reference Vitamin D values determined by the CDC reference method, a validated isotope dilution high performance liquid chromatography tandem mass spectrometry (ID- HPLC-MS/MS) 25-OH Vitamin D. The data are summarized in the following table and presented graphically.

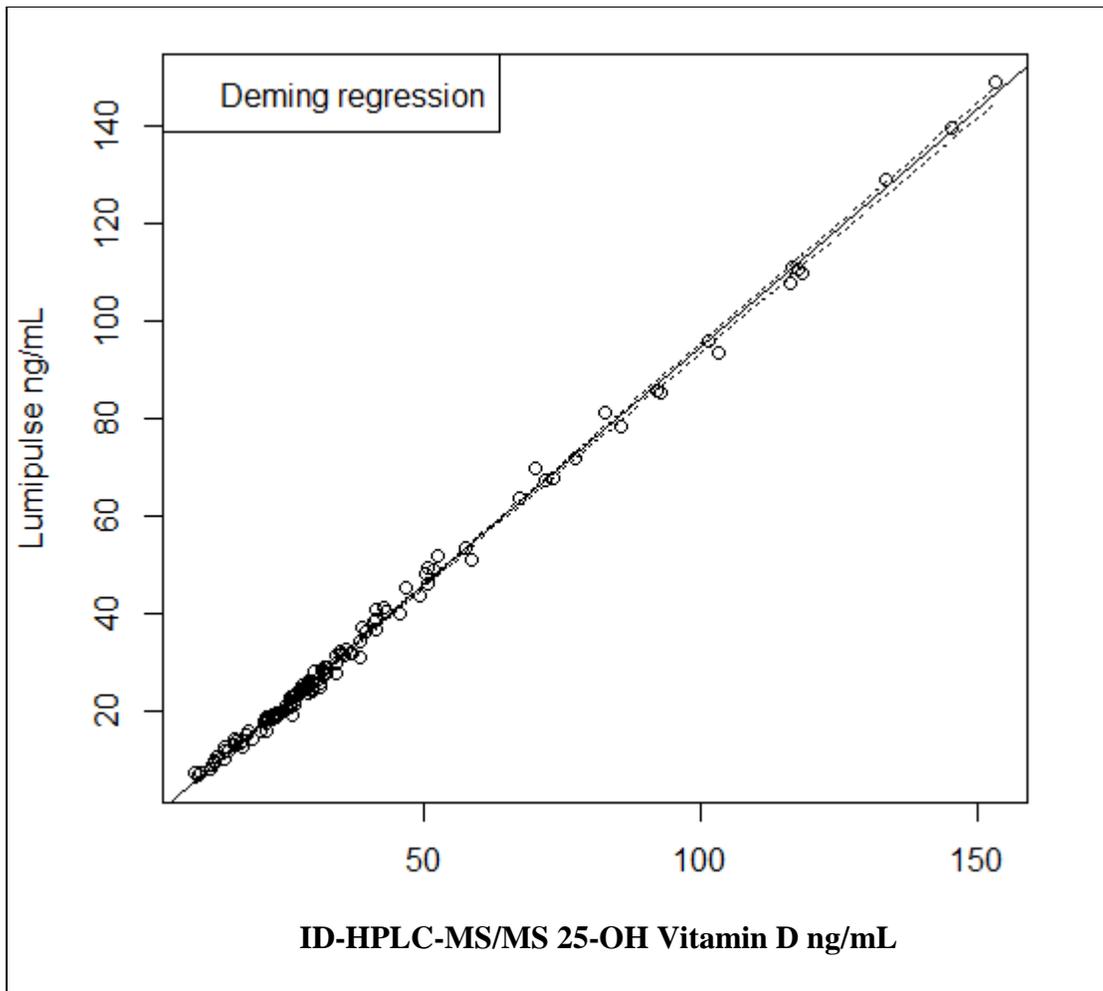
Range of samples:

7.2 to 149.0 ng/mL (Lumipulse **G** 25-OH Vitamin D)

8.65 to 153 ng/mL (ID- HPLC-MS/MS)

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

n	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)	Average Bias (ng/mL)
117	0.9986	-2.788 -3.225 – -2.351	0.97 0.96 – 0.99	-3.826



c. Matrix Comparison

Lumipulse G 25-OH Vitamin D on the LUMIPULSE G1200 System was evaluated for matrix differences by performing a study using fifty (50) matched sets of serum (red top and serum separator tubes (SST)) and plasma (sodium heparin, lithium heparin, K2EDTA) samples. The results demonstrated equivalency between matrices and are presented as regression equations in the following table:

Matrix Comparison	Sample Range (ng/mL)	Regression Equation and Correlation Coefficient
SST versus Red Top	8.6 – 113.7	$y = 1.0076(x) - 0.2178$; $r = 0.9981$
K ₂ EDTA versus Red Top	8.6 – 111.2	$y = 0.9673(x) + 0.0008$; $r = 0.9982$
Lithium Heparin versus Red Top	8.8 – 119.2	$y = 1.019(x) - 0.5969$; $r = 0.9964$
Sodium Heparin versus Red Top	8.8 – 116.3	$y = 1.0103(x) - 0.4566$; $r = 0.9983$

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:

See Expected Values below.

5. Expected values/Reference range:

It is recommended that each laboratory establish its own range, which may be unique to the population it serves depending upon geographical, patient, and environmental factors.

There is considerable discussion of the serum concentrations of 25-OH Vitamin D associated with deficiency, adequacy for bone health and optimal overall health. In the past, vitamin D deficiency was defined as serum 25-OH vitamin D below 10 ng/mL. The World Health Organization (WHO) defined vitamin D insufficiency as serum 25-OH vitamin D below 20 ng/mL. However, others recently started to define vitamin D deficiency as serum 25-OH vitamin D level below 20 ng/mL and vitamin D insufficiency as less than 30 ng/mL. A review of the available literature suggests the following recommendations for 25-OH vitamin D levels:

Level	Ranges according to World Health Organization	Ranges according to Endocrine Society Clinical Practice Guideline
Deficient	< 10 ng/mL	< 20 ng/mL
Insufficient	10 – 19 ng/mL	20 – 29 ng/mL
Sufficient	20 – 100 ng/mL	30 – 100 ng/mL
Potential Toxicity	>100 ng/mL	>100 ng/mL

Results from a total of 322 samples were used to determine the distribution of Lumipulse **G** 25-OH Vitamin D results in apparently healthy adults.

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 18 to 74 years (median age of 36) and were representative of the overall US population in terms of sex (46.9% male and 53.1% female) and ethnicity/race (60.0% White, 7.5% Black, 19.3% Hispanic, 8.1% Asian/Pacific Islander and 5.3% Other). The majority of the population was < 50 years old (231/322, 71.7%) with a median age of 28.

To represent a broad spectrum of UV light exposure, the study population included adult subjects from 2 geographically diverse regions of the US (North: Northern California and New Jersey and South: Southern California and North Carolina) that were sampled during spring/summer (April through September) and fall/winter (October through March) seasons. The majority of patients was from Northern sites (247/322, 76.7%) and sampled during the fall/winter season (241/322, 74.8%).

To represent a broad spectrum of skin types, the assessment of skin type was done using the Fitzpatrick Skin Test questionnaire, which was completed by each study participant. Subjects were classified into six categories of the Fitzpatrick scale. There were 265 (82.3%) lighter skinned subjects (Fitzpatrick scale Types I, II, III) and 57 (17.7%) darker skinned subjects (Fitzpatrick scale Types IV, V, VI). The majority of subjects (198/322, 61.5%) were Type III. Type I and Type VI had the smallest populations (0.6% and 0.3%, respectively).

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 table below.

n	Mean Conc.	Median Conc.	Observed Range (2.5th to 97.5 th percentile)
287	22.9 ng/mL	22.2 ng/mL	7.6 to 47.6 ng/mL

It is recommended that each laboratory establish its own reference value for the population of interest for Lumipulse **G** 25-OH Vitamin D on the LUMIPULSE **G**1200 System.

Standard/Guidance Document Referenced:

- 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
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff
- Test Principle: Guidance for Industry and Food and Drug Administration Staff - eCopy Program for Medical Device Submissions (December 31, 2012)
- Guidance for Industry and Food and Drug Administration Staff - Refuse to Accept Policy for 510(k)s (August 4, 2015)

Conclusion

The results of these analytical (nonclinical) and clinical studies demonstrate that the Lumipulse **G** 25-OH Vitamin D assay is substantially equivalent to the performance of the Liaison[®] 25 OH Vitamin D TOTAL assay.