510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K192524

B Applicant

Fujirebio Diagnostics, Inc.

C Proprietary and Established Names

Lumipulse G CA15-3

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
MOI	Class II	21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

New device

B Measurand:

Cancer antigen 15-3

C Type of Test:

Quantitative assay, automated chemiluminescent enzyme immunoassay (CLEIA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Lumipulse G CA15-3 is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of CA 15-3 in human serum or plasma (sodium heparin, lithium heparin, or dipotassium EDTA) on the LUMIPULSE G System.

The assay is to be used as an aid in the management of patients previously diagnosed with stage II and III breast cancer. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods used for monitoring breast cancer.

WARNING: The concentration of CA 15-3 in a given specimen, as determined by assays from different manufacturers, can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay for CA 15-3 used. Values obtained with different assay methods cannot be used interchangeably. If, in the course of monitoring a patient, the assay method used for determining serial levels of CA 15-3 is changed, the laboratory must perform additional serial testing to confirm baseline values. Prior to changing assays, the laboratory MUST confirm baseline values for patients being serially monitored. Lumipulse G CA15-3 should not be used for cancer screening or diagnosis.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

D Special Instrument Requirements:

LUMIPULSE G1200 System (K142895)

IV Device/System Characteristics:

A Device Description:

Reagents

The Lumipulse G CA15-3 Immunoreaction Cartridges consists of 3 × 14 tests. Each kit contains the following

1) Antibody-Coated Particle Solution (250 µL/Immunoreaction Cartridge) contains 150 µg/mL anti-CA 15-3 monoclonal antibody (mouse)-coated particles, protein stabilizers (bovine and mouse) 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 (350 µL/Immunoreaction Cartridge) contains 0.2 µg/mL alkaline phosphatase (ALP: calf)-labeled anti-CA 15-3 monoclonal antibody (mouse), protein stabilizers (bovine and calf) and chemical stabilizers in 0.1 M sodium chloride/MES buffer. Preservative: sodium azide.

Materials required but provided separately:

1) Lumipulse G CA15-3 Calibrators: Calibrator 1 and Calibrator 2

2) LUMIPULSE G Substrate Solution
3) LUMIPULSE G Wash Solution
4) LUMIPULSE G Specimen Diluent 1
5) Sampling tips for LUMIPULSE SYSTEM
6) Soda lime for LUMIPULSE SYSTEM
7) LUMIPULSE G Dilution Cartridges

B Principle of Operation:

CA 15-3 in specimens specifically binds to anti-CA 15-3 monoclonal antibody (mouse) on the particles, and antigen-antibody immunocomplexes are formed. The particles are then washed and rinsed to remove unbound materials. Alkaline phosphatase (ALP; calf)-labeled anti-CA 15-3 monoclonal antibody (mouse) specifically binds to CA 15-3 of the 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. Adamantyl-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 CA 15-3 in specimen.

V *Substantial Equivalence Information:*

A Predicate Device Name(s):
Architect CA15-3 Assay

B Predicate 510(k) Number(s):
K042732

C Comparison with Predicate(s):

Device & Predicate Device(s):	K192524	K042732
Device Trade Name	Lumipulse G CA15-3	ARCHITECT CA15-3
General Device Characteristic Similarities		
Analyte	Cancer Antigen 15-3	Same
Intended Use/Indications For Use	Lumipulse G CA15-3 is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of CA 15-3 in human serum or plasma (sodium heparin, lithium heparin, or dipotassium EDTA) on the LUMIPULSE G System. The assay is to be used as an aid in the management of patients	The Architect CA15-3 assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of DF3 defined antigen in human serum and plasma on the Architect i systems. The Architect CA15-3 assay is to be used as an aid in the management of stage II and III

	previously diagnosed with stage II and III breast cancer. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods used for monitoring breast cancer.	breast cancer patients. Serial testing for patient CA 15-3 assay values should be used in conjunction with other clinical methods for monitoring breast cancer.
Classification	II	Same
Product Code	MOI	Same
Sample Type	Human serum or plasma (sodium heparin, lithium heparin, dipotassium EDTA)	Human serum or plasma (sodium heparin, lithium heparin, tri-potassium EDTA)
Traceability	Traceable to in-house reference calibrators	Reference preparation maintained by the applicant
General Device Characteristic Differences		
Instrument	LUMIPULSE G System	ARCHITECT i System
Principles of operation	Quantitative Chemiluminescent Enzyme Immunoassay (CLEIA)	Chemiluminsecent Microparticle Immunoassay (CMIA)
Capture antibody	anti-CA 15-3 monoclonal antibody	115D8 monoclonal antibody
Detection antibody	Alkaline phosphatase-labeled anti-CA 15-3 monoclonal antibody	Acridinium-labeled anti CA 15-3 monoclonal antibody
Assay Range	1.7–400 U/mL	0.5–800 U/mL

VI Standards/Guidance Documents Referenced:

CLSI EP05-A3 3rd Edition, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline

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

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 2nd Edition, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

CLSI EP28-A3c 3rd Edition, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline

CLSI EP34 1st Edition, Establishing and Verifying an Extended Measuring Interval Through Specimen Dilution and Spiking

CLSI EP37 1st Edition, Supplemental Tables for Interference Testing in Clinical Chemistry

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

All results presented below met the manufacturer's pre-determined acceptance criteria.

1. Precision/Reproducibility:

Precision testing was performed in accordance with CLSI Guideline EP05-A3. Six level panels (at six analyte concentrations) of native serum sample were run in triplicate for the 5-day studies at three sites using three lots of reagents and in duplicate for the 20-day study. Tumor Marker Controls (TMC) Level 1 (13.0–24.2 U/mL) and Level 2 (115–214 U/mL) were run in singlicate and used for validity. For each run, all replicates for each panel were obtained from the same sample cup. All panels were tested within two hours of being placed on-board the LUMIPULSE G1200 System.

a) *20-day precision*: The studies were performed at a single site and utilized one lot (Lot A) of Immunoreaction Cartridges (IC) and Calibrators, duplicate per sample per run, two runs daily over the course of 20 working days (n=80 for each sample). The data were analyzed for within-run, between-run, between-day, and total precision. The mean U/mL and percent coefficient of variation (%CV) are summarized in table below.

Sample	Mean (U/mL)	Within-Run (Repeatability)		Between Run		Between Day		Within-Laboratory (Total)	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel 1	2.5	0.1	2.7%	0.0	0.0%	0.0	2.0%	0.1	3.3%
Panel 2	21.0	0.4	2.0%	0.3	1.6%	0.4	1.7%	0.6	3.1%
Panel 3	35.9	0.7	2.0%	0.3	0.9%	0.4	1.2%	0.9	2.5%
Panel 4	101.8	1.8	1.8%	1.2	1.2%	1.1	1.1%	2.4	2.4%
Panel 5	232.7	4.2	1.8%	4.2	1.8%	0.9	0.4%	6.0	2.6%
Panel 6	369.3	7.2	1.9%	6.0	1.6%	2.5	0.7%	9.7	2.6%

b) *Lot-to-Lot Reproducibility*: A 5-day study was performed at a single site using three different lots (Lot A, B and C) of ICs and Calibrators. Each sample was tested in triplicate per run, two runs per day for five days. The combined results from each lot, day and run were used to calculate the repeatability (within-run), between-run, between-day, between-lot and total precision (n=90 for each sample) and are summarized in table below.

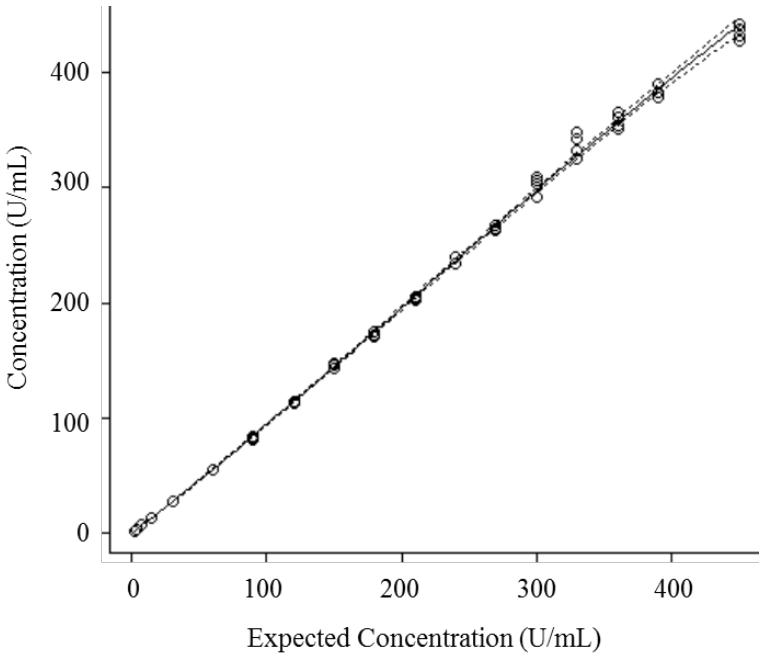
Sample	Mean (U/mL)	Within-Run (Repeatability)		Between Run		Between Day		Between Lot		Within-Laboratory (Total)	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel 1	2.5	0.1	2.5%	0.1	2.2%	0.0	0.3%	0.1	4.8%	0.1	5.8%
Panel 2	20	0.4	1.9%	0.2	1.0%	0.2	1.1%	0.6	3.2%	0.8	4.0%
Panel 3	34.2	0.5	1.6%	0.3	0.9%	0.4	1.1%	1.1	3.2%	1.3	3.9%
Panel 4	96.7	1.7	1.8%	0.6	0.6%	0.3	0.3%	2.9	3.0%	3.5	3.6%
Panel 5	224.4	4.6	2.0%	3.4	1.5%	0.0	0.0%	4.4	1.9%	7.2	3.2%
Panel 6	360.1	6.1	1.7%	5.7	1.6%	0.0	0.0%	6.9	1.9%	10.8	3.0%

c) *Site to Site Reproducibility*: A 5-day study was performed at three sites using singe lot (Lot A) of reagents. Each sample was tested in triplicates per run, two runs per day for five days. The combined results with the factors of site, day and run were used to calculate the repeatability (within-run), between-run, between-day, between-site and reproducibility (n=102 for each panel), and are summarized in table below:

Sample	Mean (U/mL)	Within-Run (Repeatability)		Between Run		Between Day		Between Site		Reproducibility (Total)	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Panel 1	2.4	0.1	2.2%	0.1	4.1%	0.0	0.0%	0.1	4.9%	0.2	6.7%
Panel 2	19.9	0.3	1.7%	0.5	2.7%	0.2	1.1%	0.4	2.0%	0.8	3.9%
Panel 3	33.7	0.5	1.5%	1.0	2.8%	0.0	0.0%	0.6	1.8%	1.3	3.7%
Panel 4	96.0	1.7	1.7%	1.8	1.8%	1.0	1.1%	0.9	1.0%	2.8	2.9%
Panel 5	219.2	4.2	1.9%	4.4	2.0%	3.2	1.4%	4.2	1.9%	8.0	3.7%
Panel 6	348.1	5.1	1.5%	6.8	2.0%	7.0	2.0%	3.1	0.9%	11.5	3.3%

2. Linearity:

a) Linearity studies were conducted in accordance with CLSI guideline EP6-A. High serum sample pools were created using patient serum samples that contained naturally expressed CA 15-3 (450 U/mL). This high CA 15-3 concentration patient serum pool was diluted at 19 levels of dilution with a low CA 15-3 concentration patient serum sample pool (0.1 U/mL). The samples were tested in replicates of four and all members of a sample set were run together. The fitted polynomial and its 95% confidence interval are shown in figure below.



Based on a ±10% deviation from linearity, the data supports the analytical measuring range of 1.7 to 434.8 U/mL for Lumipulse G CA15-3 with the following correlation:

Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
1.7–400	0.965 (0.956, 0.974)	-0.226 (-0.316, -0.136)	0.999

b) *Spiking and Dilutional Recovery Studies*:

i) Spike Recovery:

Known amounts of CA 15-3 were added to three normal human serum (NHS) samples to prepare intermediate stock solutions at concentrations between 350 and 3750 U/mL. The concentrations of each intermediate stock solution were confirmed by testing in triplicate at 1:10 dilution in NHS. The calculated percent recovery ranged between 92 to 104%.

NHS Sample 1 (9.6 U/mL)		
Expected (U/mL)	Measured (U/mL)	% Recovery
Neat	9.6	n/a
48.9	49.7	102%
56.2	57.8	103%
105.7	101.7	96%
186.5	172.3	92%
367.9	357.9	97%
NHS Sample 2 (11.2 U/mL)		

Neat	11.2	n/a
50.4	51.1	101%
57.7	59.8	104%
107.2	105.7	99%
188.0	175.6	93%
369.4	355.5	96%
NHS Sample 3 (20.7 U/mL)		
Neat	20.7	n/a
58.9	59.5	101%
66.2	68.7	104%
115.7	114.8	99%
196.5	182.9	93%
377.9	360.2	95%

ii) Dilution Recovery:

A study was completed to assess the automated and manual dilution (1:10 and 1:100) for the Lumipulse G CA15-3 assay according to CLSI guideline EP34. Panels from 10 apparently healthy normal human serum (NHS) samples within the target measurement range of the assay (2.0–400.0 U/mL) and 10 high, naturally expressing CA 15-3 serum sample pools exceeding the target measurement range of the assay (>400.0 U/mL) were prepared. The panels were tested as neat specimens in duplicate using the auto-dilution feature of the assay parameter with dilution cartridges at 1:10 and 1:100. In addition, two users each prepared three (3) separate dilutions for each panel in LUMIPULSE G Specimen Diluent 1 (LPSD1) to capture the variability of manual dilution preparation. The within-lot, between-run, between-day and between-instrument %CVs were all ≤ 10%. The calculated percent recovery is summarized in table below.

	% Recovery	
Dilution Set	Manual Dilution	Automated Dilution
1:10	94% to 113%	93% to 112%
1:100	92% to 105%	90% to 102%

c) *High Dose Hook Effect*:

Hook effect was evaluated using samples prepared by adding an CA 15-3 analyte to one apparently healthy normal human serum sample to 9,000 U/mL. The sample was serially diluted with the Low Serum Sample Pool throughout the measuring range of the assay (1.7 U/mL to 400 U/mL) and tested in triplicate with all members of a sample set run together. No high dose hook effect was observed when samples up to approximately 9,000 U/mL of CA 15-3 were assayed.

3. Analytical Specificity/Interference:

Interference study was performed according to CLSI EP07-A2 and EP37 guidelines to determine the effect of various endogenous and exogenous substances on the Lumipulse G CA15-3 assay. Three native human serum pools with a target concentration of 30–35 U/mL, 80–100 U/mL and 300–350 U/mL were supplemented with potentially interfering compounds, and the percent bias was determined by comparing the result of sample with interferent to a control sample without the interferent.

a) *Endogenous Substance Interference*:

The following endogenous substances were tested using Lumipulse G CA15-3 assay. No significant interference was found for each substance at the concentrations listed below.

Endogenous Interferences	Concentration
Free Bilirubin (unconjugated)	60 mg/dL
Conjugated Bilirubin	60 mg/dL
Hemoglobin	1,000 mg/dL
Total Protein (Human Serum Albumin)	15 g/dL
Triglycerides (Intralipid, 20% Emulsion)	3,000 mg/dL
Immunoglobulin G (IgG)	5 g/dL
Biotin	19.7 mg/dL
Human Anti-Mouse Antibodies (HAMA IgG)	1,000 ng/mL
Rheumatoid Factor (RF) IgM	1,000 IU/mL

b) *Exogenous Substance Interference*:

The following exogenous substances were tested using Lumipulse G CA15-3 assay. No significant interference was found for each substance at the concentrations listed below.

Therapeutic Drug Interferences	Concentration
Abemaciclib	24 mg/dL
Acetaminophen	20 mg/dL
Acetylsalicylic Acid	100 mg/dL
Acetylcysteine	166 mg/dL
Albumin-bound paclitaxel	27 mg/dL
Alpelisib	18 mg/dL
Ampicillin-Na	100 mg/dL
Ascorbic Acid	30 mg/dL
Atezolizumab	72 mg/dL
β-Estradiol	0.67 mg/dL
Capecitabine	128 mg/dL
Carboplatin	100 mg/dL
Cefoxitin	660 mg/dL
Cisplatin	17.5 mg/dL

Therapeutic Drug Interferences	Concentration
Cyclophosphamide	80.0 mg/dL
Cyclosporine	0.5 mg/dL
Diethylstilbestrol	2.5 mg/dL
Docetaxel	10 mg/dL
Doxorubicin HCl	5.0 mg/dL
Doxycycline	3mg/dL
Epirubicin	12 mg/dL
Eribulin	0.14 mg/dL
Etoposide	1.0 mg/dL
Everolimus	1.2 mg/dL
Exemestane	12 mg/dL
5-Fluorouracil	28.0 mg/dL
Flutamide	1.0 mg/dL
Fulvestrant	51 mg/dL
Gemcitabine	128 mg/dL
Heparin	5000 U/L
Ibuprofen	50 mg/dL
Lapatinib	90 mg/dL
Levodopa	2 mg/dL
Liposomal doxorubicin	5 mg/dL
Megestrol acetate	3.96 mg/dL
Methotrexate	45.0 mg/dL
Methyldopa	2.25 mg/dL
Metronidazole	12.3 mg/dL
Mitomycin	7.5 mg/dL
Olaparib	18 mg/dL
Paclitaxel	0.35 mg/dL
Palbociclib	8 mg/dL
Pertuzumab	50 mg/dL
Phenylbutazone	40 mg/dL
Ribociclib	36 mg/dL
Rifampicin	6 mg/dL
Talazoparib	0.06 mg/dL
Tamoxifen	6.0 mg/dL
Testosterone	3.3 mg/dL
Theophylline	10 mg/dL
Vinblastine sulfate	0.13 mg/dL
Vincristine	0.15 mg/dL

Therapeutic Drug Interferences	Concentration
Vinorelbine	3 mg/dL
Herceptin	40.0 mg/dL

c) *Cross-Reactivity*:

Cross-reactivity of the following cancer marker antigens in the Lumipulse G CA15-3 assay was tested—the results are summarized in the following table.

Interferent (Antigen)	Test Concentration	Highest Observed % Cross Reactivity
CA19-9	11,000 U/mL	0.003
CA125	11,000 U/mL	0.024
AFP	500 ng/mL	-0.063
CEA	5,000 ng/mL	8.246

4. Assay Reportable Range:

The claimed Lumipulse G CA15-3 Analytical Measuring Interval (AMI) is 1.7 to 400.0 U/mL. The device allows auto-dilution of samples—the Extended Measuring Interval (EMI) (The ULOQ to the maximum dilution concentration) is 400.0 to 40,000 U/mL. The Reportable Range (the lower limit of the AMI to the maximum dilution concentration) is 1.7 to 40,000 U/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

a) Traceability:

There is no recognized standard for CA 15-3. Calibration of the Lumipulse G CA15-3 is traceable to in-house reference calibrators, whose values have been assigned to correlate to Fujirebio Diagnostics' CA 15-3 Radioimmunoassay.

b) Kit and On-board Stability:

The shelf-life studies for Lumipulse G CA15-3 Immunoreaction Cartridges showed the cartridges were stable under the following conditions: (i) during transportation when shipped at 2–10°C; (ii) for shelf-life at 2–10°C for 12 months; (iii) on-board the LUMIPULSE G System for 30 days.

c) Sample Stability:

The sample stability was performed according to CLSI EP25-A guidelines. The stability of serum, K_2 EDTA, lithium heparin and sodium heparin plasma samples at 2–10°C or at -20°C ±10°C was determined to be 14 Days, and up to four freeze/thaw cycles.

6. Detection Limit:

CLSI guideline EP17-A2 were followed to determine the Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) for Lumipulse G CA15-3.

LoB: Four unique ultra-low CA 15-3 human serum samples (≤ 0.1 U/mL) were obtained by ultra-filtering with a 100 kDa membrane filter. The samples were tested for three days, two runs per day, 10 replicates per run, with two reagent lots (N=240). The LoB was calculated nonparametrically as the upper 95% point of the numerically recorded readings. This gave the LoB value 0.020 U/mL for Lot-A and 0.022 U/mL for Lot-B.

LoD: The ultra-low samples were combined with seven individual native serum samples with elevated CA 15-3 concentrations to prepare seven CA 15-3 serum sample panels at the targeted concentration range of 0.2 to 1.4 U/mL. The samples were tested for three days, two runs per day, 10 replicates per run, with two reagent lots (N=420). The LoD value was determined as 0.053 U/mL for Lot-A and 0.037 U/mL for Lot-B.

LoQ: The quantitative detection of a CA 15-3 in the samples (described in LoD above) with known measurement accuracy was determined as 0.138 U/mL for Lot-A and 0.0617 U/mL for Lot-B.

The claimed LoB was determined to be 0.022 U/mL, the claimed LoD was determined to be 0.053 U/mL and the claimed LoQ was calculated to be 0.138 U/mL.

7. Assay Cut-Off:

See Clinical Cut-off below.

B Comparison Studies:

1. Method Comparison with Predicate Device:

To compare values of the Lumipulse G CA15-3 and the predicate device and to assert accuracy by high correlation, 132 human serum samples, obtained from commercial vendors, were tested. The Lumipulse G CA15-3 measuring range (1.7–400.0 U/mL) was different from that of the predicate ARCHITECT CA 15-3 (0.5 – 800.0 U/mL). The samples tested ranged from 5.3 to 375.6 U/mL for Lumipulse G CA15-3 and 5.1 to 1004.4 U/mL for ARCHITECT CA15-3.

Lumipulse *G* CA15-3 Ranges	Number of Samples
5.3 – 10.0 U/mL	25
10.1 – 35.0 U/mL	32
35.1 – 200.0 U/mL	36
200.1 – 375.6 U/mL	24

>400.0 U/mL	15
Total	132

The comparison was performed on 117 specimens (93% from female subjects and 7% samples from male subjects), spanning the overlapping range of the Lumipulse G CA15-3 and the predicate device. Sample testing with Lumipulse G CA15-3 was performed on three LUMIPULSE G1200 Systems (on three sites) utilizing one lot of Immunoreaction Cartridges, two lots of Calibrators and one calibration curve at each site. Sample testing with predicate ARCHITECT CA15-3 was performed on one ARCHITECT i2000SR System utilizing one calibration curve, one lot of reagents and one lot of calibrators. The linear regression analysis obtained from Weighted Deming regression method comparing the performance of Lumipulse G CA15-3 to the ARCHITECT CA15-3 test results is shown below.

Comparative Method	n	Correlation Coefficient	Intercept (95% CI)	Slope (95% CI)
Lumipulse G CA15-3 vs. ARCHITECT CA15-3	117	0.854	0.463 (-0.32 to 1.24)	1.05 (0.97 to 1.14)

2. Matrix Comparison:

The Lumipulse G CA15-3 matrix comparison study was performed in accordance with CLSI EP09c using 55 matched sets to evaluate the difference across tube types, including Serum Separator Tube (SST), K2-EDTA, lithium heparin, and sodium heparin, versus the means of serum samples. A weighted Deming regression analysis was performed and the results summarized in the table below.

Tube Type	n	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	Cor Coef
SST	55	4.4–397.6	1.02 (1.00–1.04)	-0.82 (-2.15–0.52)	0.997
K2-EDTA	55	8.4–393.8	1.02 (1.00–1.04)	-1.11 (-2.21– -0.01)	0.998
Lithium Heparin	55	8.2–397.5	0.98 (0.93–1.02)	-0.52 (-1.98–0.94)	0.983
Sodium Heparin	55	8.5–395.4	0.98 (0.94–1.01)	0.133 (-2.04–2.31)	0.994

Cor Coef = Pearson Correlation Coefficient

C Clinical Studies:

The effectiveness of Lumipulse G CA15-3 as an aid in monitoring recurrence or progressive disease in patients with breast cancer who have detectable levels of CA 15-3 at some point in their disease process was determined by assessing changes in CA 15-3 levels in retrospectively collected serial serum samples from 112 patients compared to changes in disease status. The clinical information collected for each subject included age, gender, race, ethnicity, date of cancer diagnosis, histology, grade of diagnosis and stage of diagnosis. Samples were selected for age (range 33 years old to 92 years old; mean age 58 years), ethnicity and stage of disease (stage I through IV). The staging of the patients was done according to American Joint Committee on Cancer (AJCC) 7th edition. Changes in CA 15-3 concentrations and in disease status were

analyzed on a per visit basis. Patients were categorized as 'no evidence of disease' (NED), stable disease, progressive disease, responsive disease or recurrent disease by the attending physician based on the clinical information (imaging, physical examination, and other clinical investigations). A total of 566 pairs of observations was undertaken with an average number of 6.1 observations per patient. A positive change in CA 15-3 was defined as an increase in the value that was at least 21% greater than the previous value of the test. Twenty-five percent (25%) (14/57) of the patient samples with a positive change correlated with the disease progression, indicating a change in CA 15-3 value of <21% is associated with decreased likelihood of progression. The results are summarized below.

Primary Progression Performance: The primary analysis was of patients with Stage II (51 patients) and Stage III (19 patients) disease at diagnosis. The visits were classified by whether or not patients showed progression or recurrent disease, and whether or not the 'level of change' exceeded cut-off. No progression included NED, stable disease and responsive disease.

Change in Disease Status per sequential pair in Stages II and III patients

Change in CA 15-3 Concentration	Progression	No Progression	Total
≥21%	14	43	57
<21%	13	282	295
Total	27	325	352

Performance Measurement	Value	SE	Lower CI*	Upper CI*
Sensitivity	51.85 (14/27)	15.71	20.5	83.2
Specificity	86.77 (282/325)	2.02	82.7	90.8
PPV	24.56 (14/57)	7.38	9.9	39.3
NPV	95.59 (282/295)	1.97	91.7	99.5
1-NPV	4.41%	1.97	8.3	0.5
PLR	3.92	0.33	2.0	7.6
NLR	0.55	0.34	0.3	1.1
Prevalence (%)	7.7% (27/352)			

SE = standard error; *CI = 95% Confidence interval
PPV = positive predictive value; NPV = negative predictive value
PLR = positive likelihood ratio; NLR = negative likelihood ratio

Full Progression Performance (additional analysis): The analysis was of patients with 'all stages' disease at diagnosis (21 Stage I, 51 Stage II, 19 Stage III, 12 stage IV and 9 un-staged patients). The visits were classified by whether or not patients showed progression or recurrent disease, and whether or not the 'level of change' exceeded cut-off. No progression included NED, stable disease and responsive disease.

Change in Disease Status per sequential pair for all progression events

Change in CA 15-3 Concentration	Progression	No Progression	Total

≥21%	22	61	83
<21%	23	460	483
Total	45	521	566

Performance Measurement	Value	SE	Lower CI	Upper CI
Sensitivity	48.89 (22/45)	10.75	27.6	70.2
Specificity	88.29 (460/521)	1.47	85.4	91.2
PPV	26.51 (22/83)	6.16	14.3	38.7
NPV	95.24(460/483)	1.48	92.3	98.2
PLR	4.18	0.25	2.5	6.9
NLR	0.58	0.21	0.4	0.9

Lower prevalence of progression events was observed during the course of this study in all stages of disease, demonstrating similar test performance throughout the testing. This can be primarily explained by significant improvement in breast cancer treatment. Based on this information, an interpretation of results section will be added to the labeling according to the table below.

Change in CA 15-3 Value and associated interpretation for Stage II and Stage III patients:

Change in CA 15-3 Concentration	Interpretation (Likelihood of progression)
<21%	approximately 4%
≥21%	approximately 25%

Based on the results observed in this study, the likelihood of having progression when a positive result is present (change in CA 15-3 value ≥21%) is approximately 25%, while the likelihood of progression when a change in CA 15-3 value <21% is approximately 4%. However, the likelihood of having progression when a positive result is present (a change in CA 15-3 value ≥21%) is only 25%, indicating that 75% of women with CA 15-3 value change ≥21% do not have progression.

D Clinical Cut-Off:

A positive change in CA 15-3 was defined as an increase in the value that was at least 21% greater than the previous value of the test for the same patient. This level of change considers the maximum imprecision of the assay within the reportable range, the intra-individual biological variation, and the 95% interval (z value for $p<0.05$). The percent change was derived by considering the published biological variation; within-subject biological variation (6.2%) was obtained from the literature.

E Expected Values/Reference Range:

Serum specimens obtained from apparently healthy males (22 years old to 67 years old) and females (22 years old to 93 years old) were tested using Lumipulse G CA15-3 per CLSI EP28-

A3c. The observed ranges are as follows (all Lumipulse G CA15-3 concentrations are presented in U/mL):

		N	Mean (SD)	Median	Range (min, max)	Reference Interval*
Healthy subjects		356	16.3 (6.8)	15.1	4.3, 41.4	6.5, 32.5
Apparently Healthy	Males	120	16.2 (6.1)	15.3	4.3, 40.0	6.2, 30.3
	Females	236	16.4 (7.1)	15.1	5.6, 41.4	6.5, 33.9

* = 2.5th Percentile, 97.5th Percentile
Healthy subjects = Apparently Healthy Males and Females (Combined)

In addition to the normal cohort, serum specimens obtained from subjects with benign conditions and subjects with malignant diseases were tested using Lumipulse G CA 15-3 per CLSI EP28-A3c. All Lumipulse G CA15-3 concentrations are presented in U/mL. The observed ranges are as follows:

Reference Intervals per Group (Apparently Healthy and Benign Subjects)

		N	Mean (SD)	Median	Range (min, max)	Reference Interval*
All subjects		591	17.0 (7.4)	15.7	4.3, 55.2	6.6, 34.2
Healthy subjects		356	16.3 (6.8)	15.1	4.3, 41.4	6.5, 32.5
Apparently Healthy Females	All	236	16.4 (7.1)	15.1	5.6, 41.4	6.5, 33.9
	Pre-menopausal	90	14.6 (6.8)	13.3	5.6, 34.2	60, 33.5
	Post-menopausal	119	16.9 (6.5)	15.9	6.4, 34.2	7.2, 31.0
Apparently Healthy Males		120	16.2 (6.1)	15.3	4.3, 40.0	6.2, 30.3
Benign Breast		75	18.4 (8.7)	18.2	6.1, 55.2	6.6, 44.5
Benign Ovarian		40	16.1 (7.6)	14.8	6.0, 33.1	6.0, 33.1
Urogenital		40	20.1 (9.1)	18.3	8.0, 39.5	8.0, 39.5
Pregnant		40	16.0 (6.4)	15.3	6.3,28.9	6.3, 28.9
Hypertension		40	18.6 (7.4)	17.0	7.4, 38.9	7.4, 38.7

* = Reference Interval 2.5th Percentile, 97.5th Percentile
Healthy subjects = Apparently Healthy Males and Females (Combined)
All subjects = Apparently Healthy and Benign (All)

Reference Intervals per Group (Subjects with Cancer)

	N	Mean (SD)	Median	Range (min, max)	Reference Interval*
Cancers (All)	368	31.1 (52.4)	20.1	3.8, 749.0	7.0, 136.7
All Stages**	130	24.4 (31.0)	19.7	3.8, 350.2	6.9, 58.3
Uterine/Endometrial	40	28.0 (32.9)	16.1	5.6, 190.0	5.6, 187.6
Ovarian	40	59.0 (123.9)	25.8	6.0, 749.0	6.0, 738.4
Lung	40	44.6 (55.1)	26.2	8.7, 283.1	8.7, 280.0
Colorectal	38	22.5 (30.6)	16.6	4.5, 200.2	6.5, 47.9

Pancreatic	40	28.0 (20.0)	22.9	9.2, 124.7	9.3, 123.4
Liver	40	25.3 (25.2)	18.1	8.3, 143.0	8.3, 141.9

* = Reference Interval 2.5th Percentile, 97.5th Percentile

** = All Stages (Treatment Naïve Breast)

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

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.