510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

k142895

B. Purpose for Submission:

New device and instrument

C. Measurand:

Cancer Antigen 125 (CA 125)

D. Type of Test:

Fully automated, quantitative, chemiluminescent enzyme immunoassay (CLEIA)

E. Applicant:

Fujirebio Diagnostics, Inc.

F. Proprietary and Established Names:

Lumipulse ***G*** CA125II Immunoreaction Cartridges
Lumipulse ***G*** CA125II Calibrators
LUMIPULSE ***G***1200 System

G. Regulatory Information:

1. Regulation section

   21 CFR § 866.6010: Tumor-associated antigen immunological test system
   21 CFR § 862.1150: Calibrator
   21 CFR § 862.2160: Discrete photometric chemistry analyzer for clinical use

2. Classification

   Class II (Assay and Calibrators)
   Class I (Instrument)

3. Product code

   LTK: Test, epithelial ovarian tumor-associated antigen (CA 125)

JIT: Calibrator, secondary
JJE: Analyzer, chemistry (photometric, discrete), for clinical use

4. Panel

Immunology (82)
Chemistry (75)

H. Intended Use:

1. Intended use(s)

*a. Lumipulse **G** CA125II Immunoreaction Cartridges*

The Lumipulse ***G*** CA125II is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of CA125 in human serum and plasma (sodium heparin, lithium heparin, or dipotassium EDTA) on the LUMIPULSE ***G*** System.

The assay is to be used as an aid in monitoring recurrence or progressive disease in patients with ovarian cancer. Serial testing for patient CA125 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.

b. *Lumipulse **G** CA125II Calibrators*

The Lumipulse ***G*** CA125II Calibrators are for use in the calibration of the LUMIPULSE ***G*** 1200 System for the quantitative measurement of CA125 in human serum or plasma (sodium heparin, lithium heparin, or dipotassium EDTA).

c. *LUMIPULSE **G**1200 System*

LUMIPULSE ***G***1200 is intended for in vitro diagnostic use, and is designed to perform automated chemiluminescence immunoassays of specimens using LUMIPULSE ***G*** reagents, conducting various processes such as dispensing, agitation, and photometric measurement.

2. Indication(s) for use

Same as Intended Use

3. Special conditions for use statement(s)

For prescription use only

4. Special instrument requirements

LUMIPULSE ***G***1200 System

I. Device Description:

1. Lumipulse ***G*** CA125II Immunoreaction Cartridges

The Lumipulse ***G*** CA125II Immunoreaction Cartridge consists of 3 × 14 tests. Each test contains the following:

a. Antibody-Coated Particle Solution: (Liquid when used, 250 µL/Immunoreaction cartridge); contains mouse anti-human CA 125 monoclonal antibody coated particles, bovine and mouse proteins, and chemical stabilizers in Tris buffer with preservative. This solution contains gelatin and turns into gel at or below 15° C.

b. Enzyme-Labeled Antibody Solution: (Liquid, 350 µL/Immunoreaction Cartridge); contains alkaline phosphatase (ALP: calf) labeled mouse anti-human CA 125 monoclonal antibody, bovine, calf and mouse proteins, and chemical stabilizers in Tris buffer with preservative.

Materials required but not provided:

a. Lumipulse ***G*** Specimen diluent: 0.15M NaCl in Tris buffer with bovine protein and chemical stabilizers.

b. Lumipulse ***G*** Substrate Solution: AMPPD as substrate in diethanolamine buffer with chemical stabilizer.

c. Lumipulse ***G*** Wash solution (concentrate): 342 mM NaCl in Tris buffer with detergent.

2. Lumipulse ***G*** CA125II Calibrators

   Lumipulse ***G*** CA125II Calibrators: Liquid, two concentrations: CAL 1: 0 U/mL CA125 calibrator (1 × 1.5 mL); CAL 2: 1000 U/mL CA125 calibrator (1 × 1.5 mL); with bovine protein stabilizer in Tris buffer with preservative.

3. LUMIPULSE ***G***1200 System

   LUMIPULSE ***G***1200 is intended for in vitro diagnostics use, and is designed to perform automated chemiluminescence immunoassays of specimens using LUMIPULSE ***G*** reagents, conducting various processes such as dispensing, agitation, and photometric measurement. The chemiluminescent enzyme immunoassay system is carried out using the ferrite particle coated with antigen or antibody and conjugate with alkaline phosphatase and the chemical luminescent substrate. The luminescence which is produced by the chemiluminescent enzyme immunoassay is measured by photometric detector.

J. Substantial Equivalence Information:

1. Predicate device name(s)

   Siemens ADVIA Centaur CA 125 II

2. Predicate 510(k) number(s)

   k020828

3. Comparison with predicate

Similarities		
Item	Assay	Siemens ADVIA Centaur CA 125 II Assay (k020828)
Intended Use	Quantitative determination of CA 125 as an aid in monitoring recurrence or progressive disease in patients with ovarian cancer.	Same
Antibodies	M11 and OC125 mouse monoclonal	Same
Analyte	Human CA 125	Human CA 125
Item	LUMIPULSE ***G***1200 System	Siemens ADVIA Centaur and ADVIA Centaur XP
Assay format	Automated	Same
Controls	Tumor Marker controls 1 and 2, not included	Same

Differences		
Item	Assay	Siemens ADVIA Centaur CA 125 II Assay (k020828)
Assay range	2.5–1000 U/mL	2–600 U/mL
Calibrators	Two levels (0, 1000 U/mL), ready to use liquid	Two levels, low and high, lyophilized
Specimen	Human serum or Na+ heparin, Li+ heparin, or K2-EDTA	Human Serum
Shelf-life Stability	10 months	9 months
Instrument	LUMIPULSE ***G***1200 system	ADVIA Centaur XP
Calibrator matrix	Tris Buffer with animal protein stabilizer	Buffered human serum albumin
Item	LUMIPULSE G1200 System	Siemens ADVIA Centaur and ADVIA Centaur XP
Principle of Operation	Chemiluminescent Enzyme Immunoassay (CLEIA)	Chemiluminescent Immunoassay (CLIA)

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

1. CLSI EP05-A2 - Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition
2. CLSI EP07-A2 - Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition
3. CLSI C28-A3c - Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition
4. CLSI EP06-A - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
5. CLSI EP09-A3 – Measurement Procedure Comparison and Bias Estimation Using Patient Samples; approved Guideline – Third Edition
6. FDA Guidance Document for the Submission of Tumor Associated Antigen Premarket Notifications, [510(k)] to FDA
7. FDA Guidance Document - Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices (May 11, 2005)

L. Test Principle:

CA 125 is an antigen recognized by the monoclonal antibody OC125, produced using cultured cells derived from ovarian serous cystadenoma as an immunogen. This is the second-generation CA 125 assay reagent, consisting of the monoclonal antibodies OC125 and M11, used as the solid phase antibody and the labeled antibody, respectively. CA 125 can be detected in more than 80% of patients with surgically demonstrable epithelial ovarian cancer and levels of CA 125 showed a significant correlation with clinical course in ovarian cancer patient. Determining blood CA 125 levels is thus useful as an aid in monitoring the course of disease and assessment of the response to therapy in ovarian cancer patients.
This CA 125 assay reagent, consisting of the monoclonal antibodies OC125 and M11, is used as the solid phase antibody and the labeled antibody, respectively. The latter antibody was obtained using the antigen purified with the OC125. Lumipulse ***G*** CA125II is an immunoassay reagent for the quantitative measurement of CA 125 in specimens, based on CLEIA (Chemiluminescent Enzyme Immunoassay) technology by a two-step sandwich immunoassay method on the Lumipulse ***G***1200 System as follows:

1. First reaction step: CA 125 in specimens specifically binds to anti-CA 125 monoclonal antibody (mouse) on the particles and antigen-antibody immunocomplexes are formed.
2. Washing step: The particles are washed and rinsed to remove unbound materials.
3. Second reaction step: Alkaline phosphatase (ALP; calf)-labeled anti-CA 125 monoclonal antibody (mouse) specifically binds to CA 125 of the immunocomplexes on the particles and additional immunocomplexes are formed.
4. Washing step: The particles are washed and rinsed to remove unbound materials.
5. Enzyme reaction step: Substrate Solution is added and mixed with the particles. AMPPD

(AMPPD: 3-(2'-spiroadamantane)-4-methoxy-4-(3"-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt) contained in the Substrate Solution is dephosphorylated by the catalysis of ALP indirectly conjugated to particles.

6. Luminescence measurement step: 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 125.

M. Performance Characteristics (if/when applicable):

1. Analytical performance

a. Precision/Reproducibility:

i. Total Imprecision

Panel composition: All panel levels were comprised of patient samples. Each panel level went through one freeze-thaw cycle.

Panel Level	Panel Composition	CA 125 (U/mL)
1	Pooled Ovarian Cancer Patient Serum Samples	22.5
2*	Pooled Ovarian Cancer Patient Serum Samples	39.3
4	Pooled Ovarian Cancer Patient Serum Samples	119.3
5	Pooled Ovarian Cancer Patient Serum Samples spiked with OC125 Defined Antigen	269.9
6	Pooled Ovarian Cancer Patient Serum Samples spiked with OC125 Defined Antigen	610.3
7	Pooled Apparently Healthy Human Serum Samples	10.6
8	Pooled High CA 125 Serum samples and apparently healthy human serum samples	440.0
9	Pooled high CA 125 serum samples, apparently healthy human serum samples and OC125 defined antigen	803.5

* There is no Panel 3

All eight of the sample pools described above and both levels of the suggested Tumor Marker Control (TMC) were tested at one internal site using three lots of Lumipulse ***G*** CA125II Immunoreaction Cartridges and Calibrators and one calibrator curve. Samples were tested in two runs per day, two replicates per run, runs separated by at least two hours, for twenty non-consecutive days per CLSI EP05-A2.

The manufacturer's acceptance criterion was that the total % CV must be ≤ 10% for all controls and panels for all of the studies. The % CV for the 20-day

precision study was ≤ 2.6%, which met the manufacturer's acceptance criterion. The results for the 20-day precision study are below:

FDI 20-day Precision Study		Within-Run		Between-Run Within Day		Between-Day		Total	
Sample	**Mean (U/mL)**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**	**SD**	**%CV**
P1	21.1	0.36	1.7	0.31	1.5	0.00	0.0	0.48	2.3
P2	37.5	0.54	1.4	0.24	0.6	0.00	0.0	0.60	1.6
P4	112.2	1.47	1.3	0.90	0.8	0.50	0.4	1.80	1.6
P5	254.8	3.74	1.5	1.31	0.5	0.00	0.0	3.96	1.6
P6	577.9	10.90	1.9	0.00	0.0	1.54	0.3	11.01	1.9
P7	10.9	0.21	1.9	0.11	1.0	0.09	0.8	0.26	2.4
P8	454.7	6.51	1.4	5.26	1.2	2.64	0.6	8.78	1.9
P9	798.6	13.04	1.6	0.00	0.0	4.36	0.5	13.74	1.7
TMC1	20.2	0.47	2.3	0.00	0.0	0.23	1.1	0.52	2.6
TMC2	292.5	4.23	1.4	2.84	1.0	4.19	1.4	6.60	2.3
P = Panel sample (serum), TMC = Tumor Marker Control									

ii. Site-to-Site Study

The site-to-site precision study was performed at three sites using the above described sample pools. In addition to testing at FDI, panels 1, 2, 4, 5, 6, and the Tumor Marker Controls (TMCs) were also tested for 10 days at two external sites using one lot of Lumipulse ***G*** CA125II Immunoreaction Cartridges and Calibrators.
Site One was Fujirebio (FDI); the first ten days of the 20-day study in (i) were used for the site-to-site evaluation. The overall precision for Site One was 2.6%. The overall % CV for Site Two was ≤ 4.7%, which met the acceptance criterion. The total precision of Lumipulse ***G*** CA125II for the five panels in the study ranged from 1.6%–2.6%. The total precision of Lumipulse ***G*** CA125II for the two controls ranged from 2.1%–4.7%.

The overall precision for Site Three was ≤ 8.4%, which met the manufacturer's acceptance criterion. The total precision of Lumipulse ***G*** CA125II for the five panels ranged from 5.1%– 8.4%. The total precision of Lumipulse ***G*** CA125II for the two controls ranged from 6.9%–7.4%.

Combined site-to-site imprecision from the three sites met the manufacturer's

acceptance criterion.

Site-to-Site		Between-Site		Between-Day Within-Site		Between-Run/ Within-Day/ Within-Lot		Within-Run		Total	
Sample	Mean (U/mL)	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
P1	21.4	0.18	0.9	0.83	3.9	0.61	2.8	0.40	1.9	1.12	5.2
P2	38.0	0.79	2.1	1.50	3.9	0.00	0.0	0.62	1.6	1.81	4.8
P4	112.5	0.74	0.7	3.81	3.4	0.68	0.6	2.04	1.8	4.44	3.9
P5	254.8	4.22	1.7	5.22	2.0	5.17	2.0	3.27	1.3	9.08	3.6
P6	580.5	4.58	0.8	11.79	2.0	11.02	1.9	9.85	1.7	19.44	3.4
TMC1	21.2	0.73	3.4	0.9	4.3	0.24	1.1	0.62	2.9	1.35	6.4
TMC2	301.8	10.62	3.5	12.63	4.2	3.21	1.1	3.71	1.2	17.21	5.7

iii. Lot-to-lot study

The lot-to-lot study was performed using data from the first 10 days of the 2 x 2 x 20 study above and two 10-day studies using two additional lots, all run at FDI. The % CV was ≤ 5.2%, which met the manufacturer's acceptance criterion.

Lot-to-Lot		Between-Lot		Between-Day Within-Lot		Between-Run Within-Day Within-Lot		Within-Run		Total	
Sample	Grand Mean	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
P1	22.1	0.96	4.3	0.00	0.0	0.40	1.8	0.43	1.9	1.12	5.1
P2	39.1	1.59	4.1	0.48	1.2	0.63	1.6	0.64	1.6	1.88	4.8
P4	118.4	5.39	4.6	0.86	0.7	2.33	2.0	1.53	1.3	6.13	5.2
P5	268.0	11.16	4.2	3.81	1.4	3.01	1.1	3.78	1.4	12.75	4.8
P6	599.3	18.27	3.0	4.72	0.8	5.69	0.9	10.75	1.8	22.45	3.7
TMC1	21.1	0.72	3.4	0.36	1.7	0.33	1.5	0.52	2.5	1.01	4.8
TMC2	305.0	12.46	4.1	5.41	1.8	3.57	1.2	4.06	1.3	14.61	4.8

b. Linearity/assay reportable range:

i. Linearity

Four sample pools were created: a High serum and a High K2-EDTA plasma pool, and a Low serum and a Low K2-EDTA plasma pool. The high pools were supplemented with antigen purified from pooled patient samples to concentrations of CA 125 of 1198.4 U/mL and 1166.8 U/mL in serum and plasma, respectively. The low pools were prepared by using patient samples stripped of antigen to a concentration of CA 125 of 0.1 U/mL in both low pools. Sixteen dilutions of the high pools by the low pools were prepared covering the AMR of the assay (2 U/mL–1000 U/mL). Neat high and low pools of each matrix type were tested as sample 1 and sample 16 in the dilution series. The samples were assayed in quadruplicate on a LUMIPULSE ***G***1200 instrument and the % recovery and linear regression were evaluated.

Linearity: Serum Samples				
Sample ID	**Dilution Factor**	**Measured Concentration Average (U/mL)**	**Expected Value (U/mL)**	**Percent Recovery**
Serum 1	Neat High	1155.8	NA	NA
Serum 2	1.11	1045.4	1041.4	100
Serum 3	1.25	920.8	924.6	100
Serum 4	1.43	807.9	807.9	100
Serum 5	1.67	696.8	692.3	101
Serum 6	2.01	576.9	575.6	100
Serum 7	2.52	455.1	458.9	99
Serum 8	3.37	341.1	343.3	99
Serum 9	5.09	223.8	226.5	99
Serum 10	10.42	108.0	111.0	97
Serum 11	20	54.3	57.8	94
Serum 12	40	28.5	28.9	99
Serum 13	80	14.9	15.0	99
Serum 14	360	3.5	3.5	100
Serum 15	640	2.5	2.3	109
Serum 16	Neat low	0.1	0.0	100

Linearity: Plasma Samples				
Sample ID	**Dilution Factor**	**Mean Measured Concentration (U/mL)**	**Expected Value (U/mL)**	**% Recovery**
Plasma 1	Neat High	1213.3	NA	NA
Plasma 2	1.11	1069.5	1093.2	98
Plasma 3	1.25	958.1	970.7	99
Plasma 4	1.43	835.9	848.1	99
Plasma 5	1.67	708.4	640.6	111
Plasma 6	2.01	584.8	604.3	97
Plasma 7	2.52	457.5	481.7	95
Plasma 8	3.37	345.9	360.4	96
Plasma 9	5.09	232.3	237.9	98
Plasma 10	10.42	112.2	116.6	96
Plasma 11	20	55.1	60.8	91
Plasma 12	40	26.9	30.4	89
Plasma 13	80	14.6	15.9	92
Plasma 14	360	3.9	3.7	105
Plasma 15	640	2.5	2.5	100
Plasma 16	Neat low	0.1	0.1	100

Weighted and unweighted linear regression was performed as the variance was not constant across the range of the analyte. The equation for the unweighted linear regression lines were $y = 0.9973x+1.5017$, $R^2 = 0.9999$ for the serum samples and $y = 1.0147 + 3.9653$, $R^2 = 0.9996$ for the plasma samples.

These data demonstrate that the assay is sufficiently linear throughout the assay measuring range of 2.5 to 1000.0 U/mL.

ii. Spike recovery study

A spike recovery study was performed by spiking known concentrations of purified antigen into patient samples (serum and plasma) and assayed in triplicate on a LUMIPULSE ***G***1200 instrument. All samples met the manufacturer's acceptance criterion of recovery of 100 ±15%.

Sample S = serum P = plasma	**Spike Level (U/mL)**	**Measured Concentration (U/mL) (n=3)**	**Expected Concentration (U/mL)**	**% Recovery**	**Grand Mean % Recovery**
	100	102.7	103.2	100	
	300	304.6	296.1	103	104

Sample S = serum P = plasma	Spike Level (U/mL)	Measured Concentration (U/mL) (n=3)	Expected Concentration (U/mL)	% Recovery	Grand Mean % Recovery
S1	500	500.9	502.9	100	
	700	710.2	697.2	102	
	950	1087.2	945.1	115	
S2	100	121.9	119.9	102	101
	300	318.3	312.8	102	
	500	506.8	519.6	98	
	700	704.9	713.9	99	
	950	981.6	961.8	102	
S3	100	104.6	103.7	101	100
	300	301.3	296.6	102	
	500	490.1	503.4	97	
	700	679.9	697.7	97	
	950	952.4	945.6	101	
P1	100	103.9	101.9	102	100
	300	293.6	294.8	100	
	500	492.8	501.6	98	
	700	680.0	695.9	98	
	950	944.4	943.8	100	
P2	100	105.5	110.0	96	100
	300	307.7	302.9	102	
	500	507.9	509.7	100	
	700	719.5	704.0	102	
	950	973.8	951.9	102	
P3	100	106.5	105.2	101	99
	300	291.2	298.1	98	
	500	503.5	504.9	100	
	700	684.2	699.2	98	
	950	922.3	947.1	97	

iii. Assay measuring range

Assay	Calibration Range (U/mL)	Measuring Range (U/mL)
Lumipulse ***G*** CA125II	0–1000	2.5–1000.0

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

i. Calibrators

The master calibration data for each lot is recorded in a two-dimensional bar code

on the Lumipulse ***G*** CA125II Immunoreaction Cartridge case. The calibration curve is created based on recorded master calibration data and the calibration data from each calibration cycle. The CA 125 concentration of a specimen is automatically calculated from the calibration curve. The result of the calculation is reported in units/mL (U/mL).

Calibration of the Lumipulse ***G*** CA125II is traceable to in-house reference calibrators, whose values have been assigned to correlate to Fujirebio Diagnostics, Inc.'s (FDI) CA 125 II Radioimmunoassay (RIA).
Primary and secondary calibrator preparations are prepared by spiking purified CA 125 antigen into buffer. The amount spiked is determined gravimetrically. Dilutions at 1000, 500, 100, 50, and 20 U/mL are produced for the primary calibrator. The secondary calibrator preparation is similarly prepared from the stock and compared with the primary calibrator and the values adjusted to match. The tertiary calibrators (included in the kit) are produced by spiking the purified antigen into buffer at 1000 U/mL; the 0 calibrator is buffer alone. The values are compared with the secondary calibrator and adjusted to match.

ii. Controls

The Quality Control material recommended by the manufacturer is the Fujirebio Diagnostics Tumor Marker Control (TMC) (k101809) (sold separately). The ranges established for the lots of TMC used in the studies presented here are 17.5–32.5 U/mL and 280–520 U/mL for the low and high levels, respectively.

iii. Antigens

The CA 125 II antigen used in the preparation of the calibrators and controls is purified from cell culture supernatant of a proprietary human ovarian carcinoma cell line maintained by Fujirebio Diagnostics, Inc. The antigen is purified through a series of steps and the purified antigen is characterized by several QC steps.

iv. Stability

a. Shelf life: The sponsor provided data demonstrating a shelf-life stability of 10 months at 2–10° C.

b. On board: The sponsor provided data demonstrating stability of 10 months when cartridges and calibrators were stored open under on-board conditions (12° C). Labeling recommends calibrator storage for 30 days.

c. Transport: The sponsor provided data demonstrating the transport stability of the instrument cartridge at 25° C, 37° C, and −20° C and the transport stability of the calibrators at 2–10° C, 20 ± 2° C, and −20° C.

d. Calibration curve: The sponsor provided data demonstrating that the calibration curve is stable for 30 days.

e. Sample Stability: The sponsor provided data demonstrating that samples

collected in all five matrices (Red top serum tube, SST, Li-heparin, Na-heparin, K2-EDTA) were stable in that 88% of the samples (22/25) demonstrated < 10% difference from Day 0 and (12 % (3/25) of the samples demonstrated a difference of ≤ 16 % from Day 0 when stored for 10 days at 2–10° C. 100% of the samples demonstrated < 10% difference from Day 0 when stored at −20°C ± 10° C for 10 days.

f. Freeze-thaw: The sponsor provided data demonstrating that measured concentration of CA 125 in serum and plasma samples was < 5% different from unfrozen controls for up to ten freeze-thaw cycles when stored at -20^0 C and thawed at room temperature. The recommendation on the package insert is to avoid more than six freeze-thaw cycles.

v. Hook effect

The hook effect was evaluated by spiking very high levels of purified antigen into a pool of normal serum and diluting this with a pool of serum from which CA 125 had been depleted. The CA 125 concentrations ranged from 275,000 U/mL through the bottom of the measuring range. All concentrations above the top of the measuring range had RLU measurements above the top calibrator, and no hook effect was seen at concentrations > 200,000 U/mL. There was a plateau in the response > 20,000 U/mL; therefore, the sponsor included a statement that results > 20,000 should be interpreted with caution.

vi. Instrument dilution study

Five patient samples with concentrations ranging from 1072.7 to 1353.0 U/mL were diluted in Sample Diluent either 1:10 or 1:100 manually or using the LUMIPULSE ***G***1200 auto-dilute functions. The observed concentrations from each sample set were compared with the expected concentrations, and the manufacturer's acceptance criterion was that the calculated concentration needed to be < 10% different from the expected concentration. In addition, the difference observed versus expected differences between the manual and the auto-dilute methods needed to be <10% as well. Both the manual and auto-dilute methods met the manufacturer's acceptance criteria for 1:10 dilution for all samples; however, the samples diluted with the auto-dilute function failed to meet the acceptance criterion for the 1:100 dilution; therefore, manual dilution may be used for either manual dilution is 1:10 or 1:100; however, auto-dilution may only be used for a 1:10 dilution. This limitation is explicitly stated in the package insert.

d. Detection limit:

i. Limit of Blank (LoB)

Sixty replicates of Lumipulse ***G*** Specimen Diluent were run on each of two LUMIPULSE ***G***1200 instruments. The mean and SD were calculated. The LoB was determined to be 0.1 U/mL.

ii. Limit of Detection (LoD)

A low-level panel set with concentrations ranging from 0.5 to 3.5 U/mL were prepared from normal serum pools diluted to the desired concentration with Lumipulse ***G*** Specimen Diluent. Thirty replicates of each were measured each of two LUMIPULSE ***G***1200 instruments using each of two lots, and mean and SD were calculated for each set and overall. As the distribution was not normal and the variances were unequal, the LoD was determined as the 5^{th} percentile of the observed concentrations and was 0.5 U/mL.

iii. Limit of Quantitation (LoQ)

The individual biases and mean biases between the target values and measured values of each replicate of each member of the low-level sample set per Lumipulse ***G*** CA125II Immunoreaction Cartridge lot were calculated, along with total error and percent total error. Because the percent total error in all cases is less than 30%, the LoQ was determined to equal the LoD, i.e., LoQ = 0.5 U/mL, and met the manufacturer's predetermined acceptance criteria of LoD $\leq$ 2 U/mL.

e. Analytical specificity:

i. Endogenous Interference

The study was modeled after CLSI EP07-A2 *Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition (2005*). Four sample pools with concentrations just above the normal range, moderately elevated, mid-range, and near the top of the measuring range were prepared in the assay matrices with the CA 125 concentrations shown below. These concentrations included a sample near what is considered the limit for a normal reading (35 U/mL).

The concentrations of each interferent are listed below.

Interferent	Final Interferent Test Conc.
Free Bilirubin (Unconjugated)	60 mg/dL
Conjugated Bilirubin	60 mg/dL
Triglycerides (Intralipid 20%)	3,000 mg/dL
Hemoglobin	500 mg/dL
Biotin	19.7 mg/dL
Immunoglobulin G	5 g/dL
Human Serum Albumin	12 g/dL

The manufacturer's acceptance criterion was < 10% difference between the spiked sample and the control sample; all samples met the criterion and therefore no interference was observed.

ii. Exogenous Interference

The same sample panel in (i) was used for measuring the effect of exogenous interferents. These were spiked into each of the sample pools at the concentrations below:

Interferent	Final Interferent Test Conc.
HAMA	1,000 ng/mL
Rheumatoid Factor (RF)	1,000 ng/mL
Carboplatin	600 µg/mL
Cisplatin	180 µg/mL
Clotrimazole	0.3 µg/mL
Cyclophosphamide	800 µg/mL
Dexamethasone	20 µg/mL
Doxorubicin	120 µg/mL
Erlotinib	150 µg/mL
Etoposide	10 µg/mL
5-Fluorouracil	900 µg/mL
Gemcitabine	100 µg/mL
Leucovorin	750 µg/mL
Magestrol Acetate	10 µg/mL
Melphalan	15 µg/mL
Methotrexate	450 µg/mL
Paclitaxel	3.5 ng/mL
Tamoxifen	60 µg/mL

The manufacturer's acceptance criterion was that there was < 10% difference between the spiked sample and the control sample; all samples met the criterion and therefore no interference was observed.

f. Assay cut-off:

See clinical cut-off.

2. Comparison studies

a. Method comparison with predicate device:
A method comparison study with 120 samples was performed on the LUMIPULSE ***G*** 1200. The concentrations of 102 of the samples spanned the range in common between the two devices of 2.0–600.0 U/mL. Because the range of the Lumipulse ***G*** CA125II assay is 2.5–1000.0 U/mL, whereas the range of the ADVIA Centaur only

rises to 600 U/mL, 18 samples with CA 125 concentrations above 600.0 U/mL also were analyzed, using dilution to bring them into range of assays. Fifteen of these were not diluted for the Lumipulse CA125II assay but were diluted for the predicate, and three were diluted to bring them into range for both assays.
The primary analysis was conducted on the undiluted samples in the common range of the two instruments, and the secondary analysis included the samples > 600 U/mL that fell outside the range of the predicate. No outliers were excluded.
The manufacturer's acceptance criteria for this study were that the Lumipulse ***G*** CA125II assay must have a Deming regression analysis slope whose 95% confidence interval lie entirely within the range of 0.9–1.1 and the correlation coefficient > 0.9 across the range of 2.0–600.0 U/mL when compared with the predicate device.

Primary analysis: 2–600 U/mL (n=102)			
Pearson correlation coefficient=0.9745			
	Parameter	**Estimate**	**95% CI**
Weighted Deming	Intercept	−0.87	−6.32–4.58
	Slope	1.13	1.06–1.20
Passing-Bablok	Intercept	−3.10	−8.87–2.25
	Slope	1.12	1.07–1.18

Secondary analysis: 2–1000 U/mL (n=120)			
Pearson correlation coefficient=0.9829			
	Parameter	**Estimate**	**95% CI**
Weighted Deming	Intercept	1.74	−2.48–5.96
	Slope	1.08	1.04–1.12
Passing-Bablok	Intercept	5.92	−0.70–11.12
	Slope	1.02	0.99–1.07

Both analyses had regression correlation coefficients > 0.9; however, for both analyses the 95% CI of the slope of the Weighted Deming regression analysis fell outside the required range of 0.9 to 1.1 and therefore exceeded the acceptance criteria. This indicates that, according to the manufacturer's pre-determined criteria, there is a difference in the performance of the assays; however, this difference was deemed not to be clinically meaningful if the same assay is always used to monitor the patient. The sponsor included a warning in the package insert to inform the clinician that results are not interchangeable between assay methods and only one assay should be used for serial measurements.

b. Matrix comparison:

This study design was based on CLSI EP09-A3, *Measurement Procedure and Bias Estimation Using Patient Samples; Approved Guideline – Third Edition*. Five donors each provided five tubes of blood: two serum tubes (serum control (red-top) tubes and serum separating (SST) tubes) and three different plasma anti-coagulants (K2 EDTA, Li-heparin, and Na-heparin). The 25 tubes were spiked with one of eight concentrations of CA 125: 25 U/mL, 50 U/mL, 100 U/mL, 250 U/mL, 300 U/mL, 500 U/mL, 700 U/mL, and 950 U/mL, or left unspiked. This was repeated for each tested concentration, resulting in samples from 45 different donors. Each set of concentrations was tested in duplicate with all members of a sample set run together. Weighted Deming regression analyses were performed to compare the results from the different matrices to that of the samples collected in serum control tubes. The manufacturer's acceptance criterion was that the 95% CI for the slope must lie entirely with the range of 0.9–1.1 and the Pearson correlation coefficient must be > 1. The results below indicate that the results met the manufacturer's acceptance criterion.

Matrix comparison: Compared with Red Top (serum) tubes								
Tube Type	**N**	**U/mL range**		**Slope**		**Intercept**		**R**
		Min	**Max**	**Estimate**	**95% CI**	**Estimate**	**95% CI**	
SST	45	5.2	901	0.99	0.99–1.01	−0.12	−0.50–0.26	0.999
K2 EDTA	45	5.1	931.5	1.01	0.98–1.05	−0.31	−0.79–0.17	0.998
Li-heparin	45	5.2	923.1	1.00	0.99–1.01	−0.22	−0.51–0.08	0.999
Na-heparin	45	5.7	939.3	0.99	0.98–1.00	0.12	−0.01–0.26	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):

The clinical performance of the assay was evaluated using samples from 59 subjects. Samples were obtained from commercial brokers and had been obtained either prospectively with consent for future research use or were remnant samples from other studies. All samples were from women aged 16–84 (one patient was 16 at the time of the initial draw), with the majority postmenopausal (40/59, 67.8%)

and Caucasian (43/59, 72.9%).

Ethnicity	Frequency	Percent (%)
Caucasian	43	72.9
African American	1	16.9
Not available	3	5.1
Other	2	3.4
Unknown	1	1.7

All post-baseline visits were characterized by clinical disease status and by whether the patient's CA 125 was higher than at the previous visit. One cross tabulation was made: disease progression as defined by the physician was related to changes in the concentration of CA 125. From this data, the sensitivity and a specificity of the change in CA 125 was computed. The manufacturer's success criterion for the study was the sum of sensitivity and specificity for progression should exceed 125%.

A total of 348 evaluable samples from 59 subjects were used for the analysis, resulting in 289 pairs of observations. The mean number of draws per subject was 5.9, ranging from 3–20 draws. The length of time over which the subjects were monitored ranged from 29–2446 days (median = 229 days). The median interval between successive visits ranged from 7–1086 day (median = 35 days). The ability of the device to distinguish between progression and no progression was evaluated.

		Disease Progression		
		No Progression	Progression	Total
Lumipulse ***G*** CA125II Increase from previous reading	≤ 20 %	180	17	197
	< 20 %	57	35	92
	Total	237	52	289

Clinical performance evaluation			
Performance Measurement	**%**	**SE**	**95% CI**
Sensitivity	67.31	18.70	29.87–100
Specificity	75.95	7.45	61.04–90.86
Acceptance Criterion (Se+Sp> 125)	**143.26**	**19.58**	**104.07–182.45**
Total Concordance	74.39	6.74	60.91–87.88
PPV	38.04	10.57	16.88–59.21
NPV	91.37	8.96	73.44–100

Sensitivity + Specificity = 143.3, which exceeds the success criterion of 125.

The table below presents a summary of the CA 125 ratios (i.e., change from one measurement to the next) between successive samples broken down by clinical disease status. The ratio of 1.20, or 20% change, at the median is exceeded only for progressive disease.

Clinical Disease Status	n	CA 125 ratio (successive draws)				
		Minimum	1st Quartile	Median	3rd Quartile	Maximum
NED*	71	0.007	0.79	0.99	1.14	57.43
Stable	97	0.001	0.84	1.01	1.39	939.76
Responding	69	0.027	0.70	0.91	1.03	9.96
Progression	52	0.015	1.05	1.64	3.69	74.70

* NED- no evidence of disease

4. Clinical cut-off

A clinically meaningful change is defined as a reading at least 20% higher than the previous reading.

A CA 125 reading was classified as significantly elevated if the ratio of that result to the previous result exceeded the 95% point of the ratio of two estimates. This 95% point of this ratio is given by 1.645 * √2 * the total CV or 8.6 = 20%. The cut-off of > 20% was chosen to balance sensitivity and specificity.

An ROC curve for the diagnosis of progression from the ratio of successive CA 125II readings using the 20% change cut-off was calculated. The area under the AUC curve is 0.727 with SE 0.047.

5. Expected values/Reference range

Samples from women with cancer, other differential diagnoses or normal healthy donors were tested to establish the reference range for the study. The samples, obtained from commercial brokers, are listed below:

a. Normal and non-cancer subject demographics

	Apparently Healthy Females	**Benign Gynecologic**	**Benign Non-Gynecologic**	**Pregnant**	**Congestive Heart Failure**	**Hypertension**
N	240 (120 pre- and 120 post-menopausal)	260	40	40	40	40
Age range (years)	19–69	19–91	39–89	18–39	20–97	19–87
Results						
Mean	12.2	42.0	30.5	23.8	26.3	31.2
Median	10.4	14.9	11.4	17.0	18.9	16.9
SD	9.3	291.1	80.0	13.1	25.3	49.8
Min	2.9	4.3	4.5	9.2	5.3	5.2
Max	123.9	4677.0	493.2	54.7	145.9	238.4
2.5th Percentile	4.4	5.0	4.5	9.2	5.3	5.2
5th Percentile	5.0	6.0	5.6	11.0	6.5	5.4
95th Percentile	23.7	104.2	162.9	52.4	69.8	223.7
Proportion below ULN (95% CI)	97.5% (94.7%–98.8%)	86.5% (81.9%–90.2%)	92.5% (80.1%–97.4%)	72.5% (57.2%–83.9%)	75.0% (59.8%–85.8%)	80.0% (65.2%–89.5%)

b. Cancer subject demographics

	Treatment-Naïve Ovarian	Endometrial	Breast	Gastro-intestinal	Lung	Bladder
N	105	40	40	40	40	40
Age range (years)	18–84	34–92	34–70	33–83	25–88	30–91
Results						
Mean	360.8	50.2	96.0	45.5	124.5	19.7
Median	84.7	19.4	16.4	13.7	42.5	13.4
SD	768.3	131.3	417.5	152.3	242.5	14.4
Min	5.7	5.9	5.8	7.3	8.4	4.1
Max	3923.5	835.5	2660	976.8	957.9	69.0
2.5th Percentile	7.1	5.9	5.8	7.3	8.4	4.1
5th Percentile	10.3	6.6	6.3	8.9	8.9	5.4
95th Percentile	2525.7	159.6	174.6	107.9	937.9	43.1
Proportion below ULN (95% CI)	35.2% (26.8%– 44.7%)	72.5% (57.2%–83.9%)	67.5% (52.0%–79.9%)	77.5% (62.5%–87.7%)	40.0% (26.3%–55.4%)	77.5% (62.5%–87.7%)

In addition, the Upper Limit of Normal (ULN), defined as the investigational value that corresponded to the 97.5th cumulative percent for the apparently healthy subjects, is used for defining the ULN, i.e., 30.8 U/mL for Lumipulse ***G*** CA125II.

N. Instrument Name:

LUMIPULSE ***G***1200 System

O. System Descriptions:

1. Modes of Operation

Fully automated

2. Software

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes __X_____ or No _____

The LUMIPULSE ***G*** System software is the computer program that interprets system and assay information, calculates results, and provides the interface for controlling the system hardware. The software interface is the portion of the computer program which a user operates to make selections and enter information. The interface allows users to initiate commands or make choices by selecting icons, buttons, items from lists, etc. The LUMIPULSE ***G*** System software interface is designed to provide consistent and easy access to system information, software functions, and troubleshooting.

3. Specimen Identification

Patient samples are barcoded and barcodes are read by the instrument. The assay to be performed can be identified by manual entry of the patient ID number and associated information or information can be imported from a host computer.

4. Specimen Sampling and Handling

Sample containers are set in Sample Racks. Barcode IDs attached to sample container are read, along with the type of Sample rack and Rack ID. The operator follows the system prompts and inputs the appropriate sample, rack, and assay information. The LUMIPULSE ***G*** System automatically performs various processes including dispensing, agitation, washing, photometric measurement, and result calculation.
Two methods of designating the assay to be performed are: Rack ID assay, in which the entire rack of samples is tested by one assay (sample position in the rack is manually entered) and Patient ID assay, in which each sample is individually designated for its own assay. The Rack ID assay is conducted according to the Rack ID and rack position where samples are registered during the sample registration process. Samples are manually set in the positions in the rack where they are registered. Patient ID assay is conducted according to the Patient ID barcode on the sample containers. Sample containers can be set in any position of any rack.

5. Calibration

Calibrators are provided in a cartridge that bears a bar code containing lot-specific calibration information and standard curve. The CA 125 concentration of a specimen is automatically calculated from the calibration curve.

Recalibration is required after a pre-determined calibration interval, when 30 days have elapsed since the previous calibration, quality control results fall out of range, or when a new Lumipulse G CA125II Immunoreaction Cartridge lot is loaded.

6. Quality Control

Quality control materials, Tumor Markers 1 and 2, are not provided, but should be tested

at least two levels: low and high. Quality control materials must pass acceptance criteria for the assay to proceed. If the controls fail to pass the acceptance criterion the instrument calls out the failure by error code errors, e.g., aspiration, for operator review and re-test if identified as such.

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The "Performance Characteristics" Section above:

Not applicable

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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