510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K160090

B. Purpose for Submission:

New Device

C. Measurand:

ROMA (Risk of Ovarian Malignancy Algorithm) – Ovarian adnexal mass assessment score based on two analytes

D. Type of Test:

Software algorithm and two immunoassays

E. Applicant:

Fujirebio Diagnostics, Inc

F. Proprietary and Established Names:

Lumipulse® ***G*** Risk of Ovarian Malignancy Algorithm (ROMA®)

G. Regulatory Information:

1. Regulation section:

   21 CFR§866.6050 – Ovarian adnexal mass assessment score test system

2. Classification:

   Class II

3. Product code:

   ONX; Ovarian adnexal mass assessment score test system

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   Lumipulse® ***G*** Risk of Ovarian Malignancy Algorithm (ROMA®) is a qualitative serum and plasma (lithium heparin or dipotassium EDTA) test that combines the results of Lumipulse ***G*** HE4, Lumipulse ***G*** CA125II and menopausal status into a numerical score.

   Lumipulse ***G*** ROMA is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery. Lumipulse ***G*** ROMA is indicated for women who meet the following criteria: over age 18; ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. Lumipulse ***G*** ROMA must be interpreted in conjunction with an independent clinical and radiological assessment. The test is not intended as a screening or stand-alone diagnostic assay.

PRECAUTION: Lumipulse ***G*** ROMA should not be used without an independent clinical /radiological evaluation and is not intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use of Lumipulse ***G*** ROMA carries the risk of unnecessary testing, surgery, and/or delayed diagnosis.

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:

Lumipulse ***G*** ROMA is a qualitative serum and plasma test that combines the results of two analytes, HE4 (Lumipulse ***G*** HE4) and CA125 (Lumipulse ***G*** CA 125 II) and menopausal status into a numerical score between 0.00 and 10.00. The premenopausal or postmenopausal status must be based on ovarian function determined with information available from clinical evaluation and medical history. The test system consists of Lumipulse ***G*** HE4, Lumipulse ***G*** CA 125 II, the Lumipulse ***G*** ROMA Calculator Tool and the LUMIPULSE *G*1200 System. The LUMIPULSE *G*1200 System is not capable of calculating the ROMA score. The immunoassays are performed according to the directions detailed in each product insert.

Both Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II are previously 510(k) cleared Class II devices (K151378 and K142895 respectively). The Lumipulse ***G*** HE4 assay is a chemiluminescent enzyme immunoassay (CLEIA) for the quantitative determination of HE4 antigen in human serum and plasma (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 epithelial ovarian cancer. Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer. Lumipulse ***G*** CA 125 II assay 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.

Lumipulse ***G*** ROMA scores (numerical score from 0.00–10.00) for both premenopausal and postmenopausal women are calculated using the Lumipulse ***G*** ROMA Calculator Tool to indicate a low likelihood or high likelihood for finding malignancy on surgery using the value of the two immunoassays (Lumipulse ***G*** HE4 and Lumipulse ***G*** CA125II).

J. Substantial Equivalence Information:

1. Predicate device name:

   Fujirebio Diagnostics, Inc., ROMA™ (HE4 EIA + ARCHITECT CA 125 II)

2. Predicate 510(k) number:

   K103358

3. Comparison with predicate:

Similarities		
Item	Device Lumipulse ***G*** ROMA	Predicate ROMA (HE4 EIA + ARCHITECT CA 125 II) K103358
Intended Use/Indication for Use	Lumipulse® ***G*** Risk of Ovarian Malignancy Algorithm (ROMA®) is a qualitative serum and plasma (lithium heparin or dipotassium EDTA) test that combines the results of Lumipulse ***G*** HE4, Lumipulse ***G*** CA125II and menopausal status into a numerical score.	The Risk of Ovarian Malignancy Algorithm (ROMA™) is a qualitative serum test that combines the results of HE4 EIA, ARCHITECT CA 125 IITM and menopausal status into a numerical score.

Similarities		
Item	Device Lumipulse ***G*** ROMA	Predicate ROMA (HE4 EIA + ARCHITECT CA 125 II) K103358
	Lumipulse ***G*** ROMA is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery. Lumipulse ***G*** ROMA is indicated for women who meet the following criteria: over age 18; ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. Lumipulse ***G*** ROMA must be interpreted in conjunction with an independent clinical and radiological assessment. The test is not intended as a screening or stand-alone diagnostic assay.	ROMA is intended to aid in assessing whether a premenopausal or postmenopausal woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery. ROMA is indicated for women who meet the following criteria: over age 18; ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist. ROMA must be interpreted in conjunction with an independent clinical and radiological assessment. The test is not intended as a screening or stand-alone diagnostic assay.
Black box warning (PRECAUTION)	PRECAUTION: Lumipulse ***G*** ROMA should not be used without an independent clinical /radiological evaluation and is not intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use of Lumipulse ***G*** ROMA carries the risk of unnecessary testing, surgery, and/or delayed diagnosis.	Same
Type of test	Algorithm	Same
Measurand	Score based on two analytes and menopausal status	Same
Clinical Cut-off	<u>Premenopausal</u> Lumipulse ***G*** ROMA score ≥ 1.31: High likelihood of finding malignancy	Same

Similarities		
Item	Device Lumipulse ***G*** ROMA	Predicate ROMA (HE4 EIA + ARCHITECT CA 125 II) K103358
	Lumipulse ***G*** ROMA score < 1.31: Low likelihood of finding malignancy <u>Postmenopausal</u> Lumipulse ***G*** ROMA score ≥ 2.77: High likelihood of finding malignancy Lumipulse ***G*** ROMA score < 2.77: Low likelihood of finding malignancy	
Software	Provided as separate CD-ROM for manual entry of assay values to obtain ROMA score	Same

Differences		
Item	Device Lumipulse ***G*** ROMA	Predicate ROMA (HE4 EIA + ARCHITECT CA 125 II) K103358
Analyte	Fujirebio Lumipulse ***G*** HE4 and Lumipulse ***G*** CA125 II	Fujirebio manual HE4 EIA and ARCHITECT CA125 II
Sample matrix	Serum, K_2-EDTA, Li-Heparin EDTA	Serum
Instrument platform	LUMIPULSE *G*1200 system	Manual ELISA for HE4 and ARCHITECT i2000SR for CA125
Assay Format	Same immunoassay platform for the detection of HE4 and CA125 in a single sample	Separate immunoassay platforms for the detection of HE4 and CA125 in a single sample

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

CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Third Edition

CLSI guideline EP07-A2, Interference Testing in Clinical Chemistry; Approved

Guideline-Second Edition

CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; approved Guideline – Third Edition

CLSI guideline C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition

Guidance document entitled Class II Special Controls Guidance Document: Ovarian Adnexal Mass Assessment Score Test System

L. Test Principle:

Lumipulse ***G*** HE4 is an assay system, including a set of immunoassay reagents, for the quantitative measurement of HE4 in specimens based on CLEA technology by a two-step sandwich immunoassay method on the LUMIPULSE *G*1200 System. HE4 in specimens specifically binds to anti-HE4 monoclonal antibody (mouse) 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-HE4 monoclonal antibody (mouse) specifically binds to HE4 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. 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 HE4.

Lumipulse ***G*** CA125II is an assay system, including a set of immunoassay reagents, for the quantitative measurement of CA125 in specimens based on CLEA technology by a two-step sandwich immunoassay method on the LUMIPULSE ***G*** System. CA125 in specimens specifically binds to anti-CA125 monoclonal antibody (mouse) 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-CA125 monoclonal antibody (mouse) specifically binds to CA125 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. 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 CA125.

The Lumipulse ***G*** ROMA Calculator Tool will be used for calculating the ROMA score. Using the value of the two analytes, Lumipulse ***G*** ROMA scores (numerical score from 0.00–10.00) for both premenopausal and postmenopausal will be calculated and will indicate whether a woman is at low likelihood or high likelihood for finding malignancy on surgery. Both premenopausal and postmenopausal Lumipulse ***G*** ROMA results will be reported to the ordering physician who will decide which result to use based on patient's menopausal status.

M. Performance Characteristics:

1. Analytical performance:

Both Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II are previously cleared devices. Analytical performance for Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II were validated in K151378 and K142895, respectively. There has been no modification of assay methods for Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II since the original clearance for each assay. Thus, a limited study was done to evaluate the analytical performance of the Lumipulse ***G*** ROMA.

All studies were performed on the Lumipulse *G*1200 instrument.

a. Precision/Reproducibility:

Total Imprecision:

A panel of five serum samples were tested using one lot each of Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II reagents and calibrator kits according to each assay's package insert. The panel consisted of four pooled human serum samples and one sample that contained 55% native HE4 and 45% spiked HE antigen (panel 5).

Total imprecision was calculated at one site by testing each sample in two runs with two replicates per run for 20 non-consecutive days (n = 80 replicates per sample). The overall study was performed based on CLSI guideline EP05-A3.

The following table displays the results for the repeatability and with-in laboratory reproducibility. All data met the manufacturer's predetermined acceptance criteria.

Sample	Mean ROMA Value	Within-Run		Between-Runs		Between-Days		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Premenopausal Lumipulse ***G*** ROMA score									
1	1.08	0.03	2.6%	0.01	0.9%	0.03	3.1%	0.05	4.2%
2	1.96	0.04	1.9%	0.03	1.6%	0.05	2.7%	0.07	3.6%
3	4.17	0.08	1.9%	0.03	0.6%	0.04	1.1%	0.09	2.2%
4	8.82	0.03	0.3%	0.05	0.5%	0.00	0.0%	0.05	0.6%
5	9.94	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%
Postmenopausal Lumipulse ***G*** ROMA score									
1	1.57	0.03	1.7%	0.00	0.2%	0.03	2.2%	0.04	2.8%

Sample	Mean ROMA Value	Within-Run		Between-Runs		Between-Days		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
2	3.21	0.04	1.1%	0.02	0.5%	0.05	1.6%	0.06	2.0%
3	5.74	0.04	0.7%	0.03	0.5%	0.05	0.9%	0.07	1.2%
4	8.82	0.01	0.2%	0.02	0.3%	0.01	0.1%	0.03	0.3%
5	9.80	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.01	0.1%

Lot-to-Lot Reproducibility Study:

A panel of five serum samples were tested using three lots each of Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II reagents and calibrator kits according to each assay's package insert. The panel consisted of four pooled human serum samples and one sample that contained 55% native HE4 and 45% spiked HE antigen (panel 5).

Imprecision was calculated at one site by testing each sample in two runs with two replicates per run for 10 non-consecutive days (n = 40 replicates per sample for each lot). The overall study was performed based on CLSI guideline EP5-A3.

The following tables display the results for the lot-to-lot precision. All data met the manufacturer's predetermined acceptance criteria.

Sample	Mean ROMA Value	Within-Run		Between-Run		Between-Day		Between-Lot		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Premenopausal Lumipulse ***G*** ROMA score											
1	1.02	0.02	2.1%	0.02	1.8%	0.02	2.2%	0.03	3.0%	0.05	4.6%
2	1.87	0.04	1.9%	0.04	2.2%	0.03	1.8%	0.05	2.9%	0.08	4.5%
3	4.04	0.07	1.7%	0.04	1.0%	0.05	1.2%	0.09	2.3%	0.13	3.2%
4	8.75	0.03	0.4%	0.04	0.4%	0.01	0.1%	0.06	0.7%	0.08	0.9%
5	9.94	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%
Postmenopausal Lumipulse ***G*** ROMA score											
1	1.55	0.02	1.4%	0.01	0.9%	0.03	1.7%	0.01	0.4%	0.04	2.5%
2	3.16	0.04	1.1%	0.03	1.0%	0.04	1.2%	0.02	0.5%	0.06	2.0%
3	5.71	0.04	0.7%	0.04	0.7%	0.03	0.6%	0.03	0.5%	0.07	1.2%
4	8.81	0.02	0.2%	0.02	0.2%	0.01	0.1%	0.02	0.2%	0.03	0.4%
5	9.80	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.01	0.1%

Site-to-Site Reproducibility study:

A panel of five serum samples were tested at three different sites using one lot each of Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125 II reagents and calibrator kits according to each assay's package insert. The panel consisted of four pooled human serum samples and one sample that contained 55% native HE4 and 45% spiked HE antigen (panel 5).

Imprecision was calculated by testing each sample in two runs with two replicates per run for 10 non-consecutive days at each site (n = 40 replicates per sample at each site). The overall study was performed based on CLSI guideline EP05-A3.

The following tables display the results for the site-to-site precision. All data met the manufacturer's predetermined acceptance criteria.

Sample	Mean ROMA Value	Within-Run		Between-Run		Between-Day		Between-Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Premenopausal Lumipulse ***G*** ROMA score											
1	1.01	0.03	2.6%	0.03	3.0%	0.02	2.4%	0.07	6.7%	0.08	8.1%
2	1.86	0.03	1.8%	0.06	3.4%	0.04	2.3%	0.11	5.8%	0.14	7.3%
3	4.01	0.08	1.9%	0.12	3.0%	0.10	2.5%	0.18	4.4%	0.25	6.1%
4	8.75	0.03	0.3%	0.09	1.0%	0.02	0.3%	0.09	1.1%	0.13	1.5%
5	9.94	0.00	0.0%	0.00	0.0%	0.00	0.0%	0.01	0.1%	0.01	0.1%
Postmenopausal Lumipulse ***G*** ROMA score											
1	1.51	0.03	1.8%	0.02	1.6%	0.03	1.9%	0.06	4.2%	0.08	5.2%
2	3.11	0.03	1.1%	0.05	1.7%	0.05	1.7%	0.11	3.5%	0.14	4.4%
3	5.63	0.04	0.8%	0.08	1.5%	0.07	1.2%	0.12	2.1%	0.16	2.9%
4	8.77	0.02	0.2%	0.06	0.6%	0.02	0.2%	0.06	0.7%	0.09	1.0%
5	9.79	0.00	0.0%	0.01	0.1%	0.01	0.1%	0.01	0.1%	0.02	0.2%

Simulation precision:

In order to demonstrate the effect on precision of all possible combinations of precision of the two analytes, a simulation precision study for Lumipulse ***G*** ROMA score was conducted based on the precision profiles of HE4 and CA 125 with different combinations of values of these two analytes. The statistical analysis of simulation of Lumipulse ***G*** ROMA score precision showed acceptable precision covering the range of Lumipulse ***G*** ROMA score from 0–10.

b. Linearity/assay reportable range:

Linearity studies for HE4 and CA 125 assay kits were presented in K151378 and K142895, respectively. No new linearity data were presented in this submission.

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

Traceability and stability studies for the HE4 and CA 125 assay kits were presented in K151378 and K142895, respectively. No new traceability and stability data were presented in this submission.

Calibrators and Controls:

Each assay uses its own calibrator and controls.

*Lumipulse **G** CA125II Calibrators*

The Lumipulse ***G*** CA125II Calibrators are for the use in the calibration of the LUMIPULSE ***G*** System for the quantitative measurement of CA125 in serum or plasma (sodium heparin, lithium heparin, and dipotassium EDTA). The OC125 defined antigen is used in the LUMIPULSE ***G*** CA125II Calibrators. This material is obtained from Fujirebio Diagnostics, Inc. proprietary human ovarian carcinoma cell line.

*Lumipulse **G** HE4 Calibrators*

The Lumipulse ***G*** HE4 Calibrators are for the use in the calibration of the LUMIPULSE ***G*** System for the quantitative measurement of HE4 in serum or plasma (lithium heparin and dipotassium EDTA). The HE4 antigen is used in the Lumipulse ***G*** HE4 Calibrators. This material is provided by Fujirebio Diagnostics, AB. The Lumipulse ***G*** HE4 assay is standardized against the Fujirebio Diagnostics HE4 EIA.

Stability:

Sample Stability: Lumipulse ***G*** ROMA is intended for use with serum and plasma (lithium heparin and dipotassium EDTA). The specimen stability and storage claims are limited to the Lumipulse ***G*** HE4 assay. Samples can be stored at 2–8°C for 3 days or at −10°C or colder for up to 1 month before being tested.

Calibration Curve: For Lumipulse ***G*** HE4 and Lumipulse ***G*** CA125II, the calibration curve is stable up to 30 days.

Reagent Stability: Users are instructed to refer to the individual stability information in the package insert of each assay.

Lumipulse ***G*** HE4 is stable when stored at 2–10°C until the expiration date

stated on the label outside of the kit box. The current shelf life of Lumipulse ***G*** HE4 is 8 months.

Lumipulse ***G*** CA125II is table when stored at 2–10°C until the expiration date stated on the label outside of the kit box. The current shelf life of Lumipulse ***G*** CA125II is 10 months.

d. Detection limit:

The limits of detection and limits of quantitation reported in each assay's package insert are incorporated into the algorithm such that results outside of the measuring interval are not imported into the algorithm and do not yield a Lumipulse ***G*** ROMA score.

e. Analytical specificity:

Interference: Studies were conducted to evaluate the interference of Lumipulse ***G*** ROMA score by endogenous substances. CLSI guideline, EP07-A2 "Interference Testing in Clinical Chemistry, Approved Guideline – Second Edition", was used to design the interference experiments.

Five patient samples with Lumipulse ***G*** ROMA scores across the measuring range (0.55–9.86) were tested in this study. These samples were then supplemented with each interfering substance. The control samples were prepared without corresponding interfering substance. The control samples and test samples were tested in replicates of three using Lumipulse ***G*** ROMA. The Lumipulse ***G*** ROMA score was calculated for each sample and its control sample using a mean of three replicates of Lumipulse ***G*** HE4 and the mean of three replicates of Lumipulse ***G*** CA 125II. The effect of each interfering substance on the Lumipulse ***G*** ROMA score was assessed by comparing the measurement of each test sample to the control. The summary of the results are shown in the following table, and all data met the manufacturer's predetermined acceptance criteria.

Interferent	Substance Concentration	% Difference From Control					
		Mean ROMA Score					
		Sample 1		Sample 2		Sample 3	
		0.55	2.10	5.10	6.43	9.72	9.57
		Pre[1]	Post[2]	Pre	Post	Pre	Post
Bilirubin (Unconjugated)	60 mg/dL	2	0	−1	−1	0	0
Bilirubin (Conjugated)	60 mg/dL	2	0	0	0	0	0
Lipid	3 g/dL	4	−1	1	0	0	0
Hemoglobin	500 mg/dL	−3	−3	−2	0	0	0
Protein	12 g/dL	−1	−3	−1	−2	0	0
Immunoglobulin (IgG)	5 g/dL	−8	−5	1	0	0	0
Biotin	19.8 mg/dL	0	0	0	0	0	0
HAMA	1000 ng/mL	1	−1	1	0	0	0
Rheumatoid Factor	1000 IU/mL	−4	−1	−1	0	0	0

Interferent	Substance Concentration	% Difference From Control			
		Mean ROMA Score			
		Sample 4		Sample 5	
		9.95	9.86	3.60	5.96
		Pre[1]	Post[2]	Pre	Post
Bilirubin (Unconjugated)	60 mg/dL	0	0	−1	0
Bilirubin (Conjugated)	60 mg/dL	0	0	1	1
Lipid	3 g/dL	0	0	−1	−1
Hemoglobin	500 mg/dL	0	0	4	2
Protein	12 g/dL	0	0	−1	−1
Immunoglobulin (IgG)	5 g/dL	0	0	−1	0
Biotin	19.8 mg/dL	0	0	1	0
HAMA	1000 ng/mL	0	0	1	1
Rheumatoid Factor	1000 IU/mL	0	0	1	0

[1]ROMA score used the equation for premenopausal status
[2]ROMA score used the equation for postmenopausal status

f. Assay cut-off:

See clinical cut-off

2. Comparison studies:

a. Method comparison with predicate device:

A total of 168 samples were used for the method comparison study. The enrolled patients consist of 150 diseased patients and 38 apparently healthy women. 53 of the samples were from premenopausal women, and 115 of the samples were from postmenopausal women. The premenopausal ROMA range for the samples was 0.3–10.0 and for the postmenopausal samples was 0.5–10.0. No samples were excluded from the data analyses. Data analysis was performed using Deming and Passing-Bablok regression analysis and all data met the manufacturer's predetermined acceptance criteria. The results are summarized in the following table:

Menopausal Status	Regression	Regression Equation	Slope (95% CI)	Intercept (95% CI)	r
Premenopausal	Deming	y = 1.00x−0.004	0.99–1.01	−0.09–0.08	0.99
	Passing-Bablok	y = 1.00x−0.02	0.99–1.01	−0.10–0.04	0.99
Postmenopausal	Deming	y = 1.00x−0.10	0.99–1.02	−0.25–0.05	0.99
	Passing-Bablok	y = 1.02x−0.20	1.00–1.03	−0.30–−0.13	0.99

b. Matrix comparison:

Matrix Comparison Study:

A matrix comparison study was performed to compare the results of Lumipulse ***G*** ROMA in serum and K_2-EDTA plasma. 86 matched serum and K_2-EDTA plasma samples were spiked with recombinant HE4 and CA 125 to cover the analytical measuring range of the device. The premenopausal ROMA range for the samples was 0.33–9.93 and for the postmenopausal samples was 0.37–9.78. Data analysis was performed using Weighted Deming regression analysis and all data met the manufacturer's predetermined acceptance criteria. The results are summarized in the following table:

Menopausal Status	Regression	Regression Equation	Slope (95% CI)	Intercept (95% CI)	r
Premenopausal	Weighted Deming	y = 1.00x−0.07	0.99–1.01	−0.11–0.03	0.99
Postmenopausal	Weighted Deming	y = 1.00x−0.06	1.00–1.01	−0.06–0.01	0.99

Simulated Matrix Comparison Study:

A simulation study was performed to evaluate the worst-case conditions where both the Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125II results showed the maximal matrix effects. The maximal matrix effects were calculated using the data for the K_2-EDTA plasma and Li-Heparin plasma matrix comparisons that were completed for Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125II in K151378 and K142895, respectively. The results of the simulation study were acceptable.

3. Clinical studies:

a. Clinical Sensitivity/Clinical Specificity:

A clinical study was done to validate Lumipulse ***G*** ROMA in pre- and postmenopausal women presenting to a generalist with an adnexal mass, for whom a decision to undergo surgery has been made. The study enrolled 512 patients at the 13 study sites. The patients were female patients over 18, presenting to a generalist at a general or specialty hospital with an ovarian cyst or an adnexal mass (defined as a simple, complex or a solid ovarian/pelvic mass) who were scheduled to undergo surgery. Blood samples were collected from all patients and tested with Lumipulse ***G*** HE4 and Lumipulse ***G*** CA 125II at Fujirebio Diagnostics, Inc.

The Initial Cancer Risk Assessment (ICRA) and all clinical information relating to the surgical procedures, including imaging reports and final pathology reports, were collected. All patients underwent surgery and tissues were examined by local pathologists. An independent pathologist reviewed

all imaging reports, case report forms and histopathology reports from each patient's institution pathologist, checking for discrepancies in the data. The performance of standalone use of ICRA, standalone use of Lumipulse ***G*** ROMA and adjunctive use of ICRA and Lumipulse ***G*** ROMA were evaluated by comparing to histopathology results for detecting the presence of ovarian malignancy.

Of the 512 patients, 53 patients were excluded from analysis. The most common reason for exclusion was no surgery was performed to remove an adnexal mass. 10 additional patients were excluded because there was not enough sample available for testing, and one patient was excluded because the Lumipulse ***G*** HE4 value was outside of the measuring range of the device. In the final total of 450 evaluable patients, 244 (54%) were premenopausal and 206 (46%) were postmenopausal. All of the major racial groups were represented with 84% White, 7% of Black, 3% Hispanic, 3% Asian, and 3% of other ethnicity. The age range of the patients was 18–89 with a median age of 49.

The statistics for the 450 enrolled subjects with pathology classification are summarized in the following table:

Classification	All N = 450		Pre-menopausal N = 244		Post-menopausal N = 206	
	N	%	N	%	N	%
Histopathology Benign	366	81.3%	223	91.4%	143	69.4%
Borderline/LMP[1]	18	4.0%	7	2.9%	11	5.3%
EOC[2]	47	10.4%	9	3.7%	38	18.4%
Non-EOC	2	0.4%	0	0.0%	2	1.0%
Other Gynecological Cancer	9	2.0%	3	1.2%	6	2.9%
Other Cancer	7	1.6%	1	0.4%	6	2.9%
Metastatic Cancer	1	0.2%	1	0.4%	0	0.0%

[1]Low malignant potential
[2]Epethelilian ovarian cancer

The Lumipulse ***G*** ROMA test used the following cut points to evaluate the performance of the test in pre- and postmenopausal women presenting to a generalist with an adnexal mass, for whom a decision to undergo surgery has been made. The cut-offs are the same for serum, K_2-EDTA plasma, and Li-Heparin plasma samples.

<u>Premenopausal:</u>

Lumipulse ***G*** ROMA score $\geq$ 1.31: High likelihood of finding malignancy
Lumipulse ***G*** ROMA score $<$ 1.31: Low likelihood of finding malignancy

Postmenopausal:

Lumipulse ***G*** ROMA score $\geq$ 2.77: High likelihood of finding malignancy
Lumipulse ***G*** ROMA score $<$ 2.77: Low likelihood of finding malignancy

The information provided by the Lumipulse ***G*** ROMA test should be used only as an adjunctive test to complement, not replace, other diagnostic and clinical procedures. The ability of Lumipulse ***G*** ROMA to contribute to the ICRA was evaluated by comparing the sensitivity, specificity, positive predictive values (PPV) and negative predictive values (NPV) for standalone use of Lumipulse ***G*** ROMA, and adjunctive use of ICRA and Lumipulse ***G*** ROMA. The performance of Lumipulse ***G*** ROMA evaluated for diagnosis of EOC including LMP are presented below.

Performance of Lumipulse G ROMA for Diagnosis of EOC including LMP (431 patients):

Combined pre- and postmenopausal subjects:

For diagnosis of EOC including LMP, the counts for all pre- and postmenopausal subjects with malignancy by pathology and with no malignancy by pathology are summarized in separate tables below.

Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	48	9	57
	Negative	2	6	8
	Total	50	15	65

No Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	27	61	52
	Negative	31	247	322
	Total	58	308	366

To examine whether the Lumipulse ***G*** ROMA test provides additional information when used in combination with ICRA, the ability of Lumipulse ***G*** ROMA to contribute to the ICRA was analyzed.

The following table presents the observed frequencies of malignancy tabulated according to ICRA and Lumipulse ***G*** ROMA test results from 431 patients.

	Frequency of Malignancy	**95% CI**
Prevalence of malignancy among patients with adnexal mass assessed: 15.1% (65/431)		
ICRA alone "Positive"	46.3% (50/108)	37.2%–55.7%
ICRA alone "Negative"	4.6% (15/323)	2.8%–7.5%
Lumipulse ***G*** ROMA alone "Positive"	39.3% (57/145)	31.7%–47.4%
Lumipulse ***G*** ROMA alone "Negative"	2.8% (8/286)	1.4%–5.4%
ICRA "Positive" and Lumipulse ***G*** ROMA "Positive"	64.0% (48/75)	52.7%–73.9%
ICRA "Positive" and Lumipulse ***G*** ROMA "Negative"	6.1% (2/33)	1.7%–19.6%
ICRA "Negative" and Lumipulse ***G*** ROMA "Positive"	12.9% (9/70)	6.9%–22.7%
ICRA "Negative" and Lumipulse ***G*** ROMA "Negative"	2.4% (6/253)	1.1%–5.1%

The same information about the frequencies of malignancy is presented by the likelihood ratios: Likelihood ratio (Result) = Pr(Result|Malignancy) / Pr(Result|No Malignancy). Likelihood ratio is a way of quantifying how much a given test result changes the pre-test probability of malignancy in a patient.

	Likelihood Ratio	**95% CI**
ICRA alone "Positive"	4.85	3.33–5.22
ICRA alone "Negative"	0.27	0.16–0.31
Lumipulse ***G*** ROMA alone "Positive"	3.65	2.61–3.86
Lumipulse ***G*** ROMA alone "Negative"	0.16	0.08–0.21
ICRA "Positive" and Lumipulse ***G*** ROMA "Positive"	10.01	6.25–11.21
ICRA "Positive" and Lumipulse ***G*** ROMA "Negative"	0.36	0.09–1.03
ICRA "Negative" and Lumipulse ***G*** ROMA "Positive"	0.83	0.41–1.07
ICRA "Negative" and Lumipulse ***G*** ROMA "Negative"	0.14	0.06–0.19

The likelihood ratio for identifying malignancy by adjunctive use of Lumipulse ***G*** ROMA and ICRA is 10.01, 2.1 times higher than the likelihood ratio by ICRA alone (4.85).

The performance of adjunctive use of Lumipulse ***G*** ROMA and ICRA for diagnosis of EOC including LMP was further evaluated by calculating sensitivity, specificity, PPV, and NPV compared to standalone use of ICRA.

Performance of the Test for Diagnosis of EOC including LMP for both Pre- and Postmenopausal Subjects			
	ICRA	Lumipulse ***G*** ROMA	ICRA and Lumipulse ***G*** ROMA
Sensitivity (95% CI)	76.9% (50/65) (65.4%–85.4%)	87.7% (57/65) (77.5%–93.6%)	90.8% (59/65) (81.3%–95.6%)
Specificity (95% CI)	84.2% (308/366) (80.1%–87.5%)	76.0% (278/366) (71.3%–80.0%)	67.5% (247/366) (62.5%–72.1%)
PPV (95% CI)	46.3% (50/108) (37.2%–55.6%)	39.3% (57/145) (31.7%–47.4%)	33.1% (59/178) (26.7%–40.3%)
NPV (95% CI)	95.4% (308/323) (92.5%–97.2%)	97.2% (278/286) (94.6%–98.6%)	97.6% (247/253) (94.9%–98.9%)
Prevalence	15.1% (65/431)		

With adjunctive use of ICRA and Lumipulse ***G*** ROMA for diagnosis of EOC including LMP, sensitivity for malignancy increased from 76.9% to 90.8%. Specificity for malignancy decreased from 84.2% to 67.5%. PPV for the adjunctive use of ICRA and Lumipulse ***G*** ROMA decreased from 46.3% to 33.1% due to an increase in the number of false positive tests added by the addition of Lumipulse ***G*** ROMA to ICRA. NPV of the adjunctive use of ICRA and Lumipulse ***G*** ROMA increased from 95.4% to 97.6%. The increase of NPV was 2.2% (95% CI 1.0%–4.0%) and was statistically significant.

Premenopausal subjects:

To evaluate the Lumipulse ***G*** ROMA for diagnosis of EOC including LMP in premenopausal subjects, data and statistical analysis were performed and summarized below.

The counts for premenopausal subjects with malignancy by pathology and with no malignancy by pathology are shown in the following tables.

Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	7	6	13
	Negative	0	3	3
	Total	7	9	16

No Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	9	47	56
	Negative	14	153	167
	Total	23	200	223

The performance of adjunctive use of Lumipulse ***G*** ROMA and ICRA for diagnosis of EOC including LMP was further evaluated by calculating sensitivity, specificity, PPV, and NPV compared to standalone use of ICRA.

Performance of the Test for Diagnosis of EOC including LMP for Premenopausal Subjects			
	ICRA	Lumipulse ***G*** ROMA	ICRA and Lumipulse ***G*** ROMA
Sensitivity (95% CI)	43.8% (7/16) (23.1%–66.6%)	81.3% (13/16) (57.0%–93.1%)	81.3% (13/16) (57.0%–93.1%)
Specificity (95% CI)	89.7% (200/223) (85.0%–93.0%)	74.9% (167/223) (68.8%–80.1%)	68.6% (153/223) (62.2%–74.3%)
PPV (95% CI)	23.3% (7/30) (11.8%–40.8%)	18.8% (13/69) (11.4%–29.6%)	15.7% (13/83) (9.4%–24.9%)
NPV (95% CI)	95.7% (200/209) (92.0%–97.7%)	98.2% (167/170) (94.9%–99.4%)	98.1% (153/156) (94.5%–99.3%)
Prevalence	6.7% (16/239)		

With adjunctive use of ICRA and Lumipulse ***G*** ROMA for diagnosis of EOC including LMP, sensitivity for malignancy increased from 43.8% to 81.3%. Specificity for malignancy decreased from 89.7% to 68.6%. PPV for the adjunctive use of ICRA and Lumipulse ***G*** ROMA decreased from 23.3% to 15.7% due to an increase in the number of false positive tests added by the addition of Lumipulse ***G*** ROMA to ICRA. NPV of the adjunctive use of ICRA and Lumipulse ***G*** ROMA increased from 95.7% to 98.1%. The increase of NPV was 2.4% (95% CI 0.6%–4.4%) and was statistically significant.

Postmenopausal subjects:

To evaluate the Lumipulse ***G*** ROMA for diagnosis of EOC including LMP in postmenopausal subjects, data and statistical analysis were performed and summarized below.

The counts for premenopausal subjects with malignancy by pathology and with no malignancy by pathology are shown in the following tables.

Malignancy by Pathology				
		ICRA		
		Positive	Negative	Total
Lumipulse *G* ROMA	Positive	41	3	44
	Negative	2	3	5
	Total	43	6	49

No Malignancy by Pathology				
		ICRA		
		Positive	Negative	Total
Lumipulse *G* ROMA	Positive	18	14	32
	Negative	17	94	111
	Total	35	108	143

The performance of adjunctive use of Lumipulse ***G*** ROMA and ICRA for diagnosis of EOC including LMP was further evaluated by calculating sensitivity, specificity, PPV, and NPV compared to standalone use of ICRA.

Performance of the Test for Diagnosis of EOC including LMP for Postmenopausal Subjects			
	ICRA	Lumipulse ***G*** ROMA	ICRA and Lumipulse ***G*** ROMA
Sensitivity (95% CI)	87.8% (43/49) (75.8%–94.2%)	89.8% (44/49) (78.2%–95.5%)	93.9% (46/49) (83.5%–97.8%)
Specificity (95% CI)	75.5% (108/143) (67.9%–81.8%)	77.6% (111/143) (70.1%–83.7%)	65.7% (94/143) (57.6%–73.0%)
PPV (95% CI)	55.1% (43/78) (44.1%–65.6%)	57.9% (44/76) (46.7%–68.3%)	48.4% (46/95) (38.6%–58.3%)
NPV (95% CI)	94.7% (108/114) (89.0%–97.5%)	95.7% (111/116) (90.3%–98.1%)	96.9% (94/97) (91.3%–98.9%)
Prevalence	25.5% (49/192)		

With adjunctive use of ICRA and Lumipulse ***G*** ROMA for diagnosis of EOC including LMP, sensitivity for malignancy increased from 87.8% to 93.9%. Specificity for malignancy decreased from 75.5% to 65.7%. PPV for the adjunctive use of ICRA and Lumipulse ***G*** ROMA decreased from 55.1% to 48.4% due to an increase in the number of false positive tests added by the addition of Lumipulse ***G*** ROMA to ICRA. NPV of the adjunctive use of ICRA and Lumipulse ***G*** ROMA increased from 94.7% to 96.9%. The increase of NPV was 2.2% (95% CI 0.2%–5.2%) and was statistically significant.

Performance of Lumipulse G ROMA for Diagnosis of All Cancers including LMP (450 patients):

Combined pre- and postmenopausal subjects:

For diagnosis of EOC including LMP, the counts for all pre- and postmenopausal subjects with malignancy by pathology and with no malignancy by pathology are summarized in separate tables below.

Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	56	13	69
	Negative	5	10	15
	Total	61	23	84

No Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	27	61	88
	Negative	31	247	278
	Total	58	308	366

To examine whether the Lumipulse ***G*** ROMA test provides additional information when used in combination with ICRA, the ability of Lumipulse ***G*** ROMA to contribute to the ICRA was analyzed.

The following table presents the observed frequencies of malignancy tabulated according to ICRA and Lumipulse ***G*** ROMA test results from 450 patients.

	Frequency of Malignancy	95% CI
Prevalence of malignancy among patients with adnexal mass assessed: 18.7% (84/450)		
ICRA alone "Positive"	51.3% (61/119)	42.4%–60.1%
ICRA alone "Negative"	6.9% (23/331)	4.7%–10.2%
Lumipulse ***G*** ROMA alone "Positive"	43.9% (69/157)	36.4%–51.8%
Lumipulse ***G*** ROMA alone "Negative"	5.1% (15/293)	3.1%–8.3%
ICRA "Positive" and Lumipulse ***G*** ROMA "Positive"	67.5% (56/83)	56.8%–76.6%
ICRA "Positive" and Lumipulse ***G*** ROMA "Negative"	13.9% (5/36)	6.1%–28.7%
ICRA "Negative" and Lumipulse ***G*** ROMA "Positive"	17.6% (13/74)	10.6%–27.8%
ICRA "Negative" and Lumipulse ***G*** ROMA "Negative"	2.1% (10/257)	2.1%–7.0%

The same information about the frequencies of malignancy is presented by the likelihood ratios: Likelihood ratio (Result) = Pr(Result|Malignancy) / Pr(Result|No Malignancy). Likelihood ratio is a way of quantifying how much a given test result changes the pre-test probability of malignancy in a patient.

	Likelihood Ratio	95% CI
ICRA alone "Positive"	4.58	3.20–4.89
ICRA alone "Negative"	0.33	0.21–0.36
Lumipulse ***G*** ROMA alone "Positive"	3.42	2.49–3.59
Lumipulse ***G*** ROMA alone "Negative"	0.24	0.14–0.27
ICRA "Positive" and Lumipulse ***G*** ROMA "Positive"	9.04	5.71–10.06
ICRA "Positive" and Lumipulse ***G*** ROMA "Negative"	0.70	0.27–1.11
ICRA "Negative" and Lumipulse ***G*** ROMA "Positive"	0.93	0.51–1.11
ICRA "Negative" and Lumipulse ***G*** ROMA "Negative"	0.18	0.09–0.22

The likelihood ratio for identifying malignancy by adjunctive use of Lumipulse ***G*** ROMA and ICRA is 9.04, 2.0 times higher than the likelihood ratio by ICRA alone (4.58).

The performance of adjunctive use of Lumipulse ***G*** ROMA and ICRA for diagnosis of EOC including LMP was further evaluated by calculating sensitivity, specificity, PPV, and NPV compared to standalone use of ICRA.

Performance of the Test for Diagnosis of All Cancers Including LMP for both Pre- and Postmenopausal Subjects			
	ICRA	Lumipulse ***G*** ROMA	ICRA and Lumipulse ***G*** ROMA
Sensitivity (95% CI)	72.6% (61/84) (62.3%–81.0%)	82.1% (69/84) (72.6%–88.8%)	88.1% (74/84) (79.5%–93.4%)
Specificity (95% CI)	84.2% (308/366) (80.1%–87.5%)	76.0% (278/366) (71.3%–80.0%)	67.5% (247/366) (62.5%–72.1%)
PPV (95% CI)	51.3% (61/119) (42.4%–60.0%)	43.9% (69/157) (36.4%–51.8%)	38.3% (74/193) (31.8%–45.4%)
NPV (95% CI)	93.1% (308/331) (89.8%–95.3%)	94.9% (278/293) (91.7%–96.9%)	96.1% (247/257) (93.0%–97.9%)
Prevalence	18.7% (84/450)		

With adjunctive use of ICRA and Lumipulse ***G*** ROMA for diagnosis of EOC including LMP, sensitivity for malignancy increased from 72.6% to 88.1%. Specificity for malignancy decreased from 84.2% to 67.5%. PPV for the adjunctive use of ICRA and Lumipulse ***G*** ROMA decreased from 51.3% to 38.3% due to an increase in the number of false positive tests added by the addition of Lumipulse ***G*** ROMA to ICRA. NPV of the adjunctive use of ICRA and Lumipulse ***G*** ROMA increased from 93.1% to 96.1%. The increase of NPV was 3.0% (95% CI 1.4%–5.0%) and was statistically significant.

Premenopausal subjects:

To evaluate the Lumipulse ***G*** ROMA for diagnosis of EOC including LMP in premenopausal subjects, data and statistical analysis were performed and summarized below.

The counts for premenopausal subjects with malignancy by pathology and with no malignancy by pathology are shown in the following tables.

Malignancy by Pathology				
		ICRA		
		Positive	**Negative**	**Total**
Lumipulse *G* ROMA	**Positive**	7	8	15
	Negative	1	5	6
	Total	8	13	21

No Malignancy by Pathology				
		ICRA		
		Positive	Negative	Total
Lumipulse *G* ROMA	Positive	9	47	56
	Negative	14	153	167
	Total	23	200	223

The performance of adjunctive use of Lumipulse ***G*** ROMA and ICRA for diagnosis of EOC including LMP was further evaluated by calculating sensitivity, specificity, PPV, and NPV compared to standalone use of ICRA.

Performance of the Test for Diagnosis of All Cancers Including LMP for Premenopausal Subjects			
	ICRA	Lumipulse ***G*** ROMA	ICRA and Lumipulse ***G*** ROMA
Sensitivity (95% CI)	38.1% (8/21) (20.7%–59.0%)	71.4% (15/21) (50.0%–86.0%)	76.2% (16/21) (54.9%–89.2%)
Specificity (95% CI)	89.7% (200/223) (85.0%–93.0%)	74.9% (167/223) (68.8%–80.1%)	68.6% (153/223) (62.2%–74.3%)
PPV (95% CI)	25.8% (8/31) (13.7%–43.1%)	21.1% (15/71) (13.2%–31.9%)	18.6% (16/86) (11.8%–28.1%)
NPV (95% CI)	93.9% (200/213) (89.8%–96.4%)	96.5% (167/173) (92.6%–98.4%)	96.8% (153/158) (92.8%–98.6%)
Prevalence	8.6% (21/244)		

With adjunctive use of ICRA and Lumipulse ***G*** ROMA for diagnosis of EOC including LMP, sensitivity for malignancy increased from 38.1% to 76.2%. Specificity for malignancy decreased from 89.7% to 68.6%. PPV for the adjunctive use of ICRA and Lumipulse ***G*** ROMA decreased from 25.8% to 18.6% due to an increase in the number of false positive tests added by the addition of Lumipulse ***G*** ROMA to ICRA. NPV of the adjunctive use of ICRA and Lumipulse ***G*** ROMA increased from 93.9% to 96.8%. The increase of NPV was 2.9% (95% CI 0.8%–5.4%) and was statistically significant.

Postmenopausal subjects:

To evaluate the Lumipulse ***G*** ROMA for diagnosis of EOC including LMP in postmenopausal subjects, data and statistical analysis were performed and summarized below.

The counts for premenopausal subjects with malignancy by pathology and with no malignancy by pathology are shown in the following tables.

Malignancy by Pathology				
		ICRA		
		Positive	Negative	Total
Lumipulse *G* ROMA	Positive	49	5	54
	Negative	4	5	9
	Total	53	10	63

No Malignancy by Pathology				
		ICRA		
		Positive	Negative	Total
Lumipulse *G* ROMA	Positive	18	14	32
	Negative	17	94	111
	Total	35	108	143

The performance of adjunctive use of Lumipulse ***G*** ROMA and ICRA for diagnosis of EOC including LMP was further evaluated by calculating sensitivity, specificity, PPV, and NPV compared to standalone use of ICRA.

Performance of the Test for Diagnosis of All Cancers Including LMP for Postmenopausal Subjects			
	ICRA	Lumipulse ***G*** ROMA	ICRA and Lumipulse ***G*** ROMA
Sensitivity (95% CI)	84.1% (53/63) (73.2%–91.1%)	85.7% (54/63) (75.0%–92.2%)	92.1% (58/63) (82.7%–96.5%)
Specificity (95% CI)	75.5% (108/143) (67.9%–81.8%)	77.6% (111/143) (70.1%–83.7%)	65.7% (94/143) (57.6%–73.0%)
PPV (95% CI)	60.2% (53/88) (49.8%–69.8%)	62.8% (54/86) (52.2%–72.2%)	54.2% (58/107) (44.8%–63.3%)
NPV (95% CI)	91.5% (108/118) (85.1%–95.3%)	92.5% (111/120) (86.4%–96.0%)	94.9% (94/99) (88.7%–97.8%)
Prevalence	30.6% (63/206)		

With adjunctive use of ICRA and Lumipulse ***G*** ROMA for diagnosis of EOC including LMP, sensitivity for malignancy increased from 84.1% to 92.1%. Specificity for malignancy decreased from 75.5% to 65.7%. PPV for the adjunctive use of ICRA and Lumipulse ***G*** ROMA decreased from 60.2% to 54.2% due to an increase in the number of false positive tests added by the addition of Lumipulse ***G*** ROMA to ICRA. NPV of the adjunctive use of ICRA and Lumipulse ***G*** ROMA increased from 91.5% to 94.9%. The increase of NPV was 3.4% (95% CI 0.8%–6.8%) and was statistically significant.

Association between the Lumipulse ***G*** ROMA Score and Likelihood of Malignancy:

Summary statistics for the Lumipulse ***G*** ROMA scores, for subjects who had a primary ovarian malignancy (EOC + LMP) are given by cancer stage in the table below.

Number of Patients and Average ROMA Score for Patients with EOC + LMP						
		Unstaged	**Stage I**	**Stage II**	**Stage III**	**Stage IV**
Premenopausal	**N** **Mean**	3 3.44	5 4.89	1 9.47	7 8.25	0 N/A
Postmenopausal	**N** **Mean**	4 7.19	13 4.49	3 4.70	27 9.01	2 9.70

Summary statistics for the Lumipulse ***G*** ROMA scores, for subjects with all cancers + LMP are given by cancer stage in the table below.

Number of Patients and Average ROMA Score for Patients with all cancers + LMP						
		Unstaged	**Stage I**	**Stage II**	**Stage III**	**Stage IV**
Premenopausal	**N** **Mean**	3 3.44	7 4.00	1 9.47	9 7.62	1 0.95
Postmenopausal	**N** **Mean**	6 6.26	19 4.35	4 5.57	31 8.56	3 7.65

To demonstrate whether higher Lumipulse ***G*** ROMA is associated with an increased likelihood of cancer, additional analysis was conducted by splitting the patients at the cut-off point and finding the median Lumipulse ***G*** ROMA score within each split giving two balanced groups below the cutoff and additional groups above. The results are summarized below.

Premenopausal (cut-off 1.31)					
Lumipulse *G* ROMA Score		**0–0.74**	**0.74–1.31**	**1.31–2.31**	**2.31–10**
Benign	**Observed**	85	82	36	20
	Expected	79.5	78.6	32.9	32.0
Cancer	**Observed**	2	4	0	15
	Expected	7.5	7.4	3.1	3.0
Total		87	86	36	35
Cancer %		2.3% (2/87)	4.7% (4/86)	0.0% (0/36)	42.9% (15/35)
Postmenopausal (cut-off 2.77)					
Lumipulse *G* ROMA Score		**0–0.1.39**	**0.1.39–2.77**	**2.77–5.83**	**5.83–10**
Benign	**Observed**	58	54	27	4
	Expected	42.3	41.7	29.8	29.2
Cancer	**Observed**	3	6	16	38
	Expected	18.7	18.3	13.2	12.8
Total		61	60	43	42
Cancer %		4.9% (3/61)	10.0% (6/60)	37.2% (16/43)	90.5% (38/42)

4. Clinical cut-off**:**
   The following cut-offs are used to interpret the result. The Lumipulse ***G*** ROMA score is between 0.0 and 10.0.

   Premenopausal:

   Lumipulse ***G*** ROMA score $\geq$ 1.31: High likelihood of finding malignancy
   Lumipulse ***G*** ROMA score $<$ 1.31: Low likelihood of finding malignancy

   Postmenopausal:

   Lumipulse ***G*** ROMA score $\geq$ 2.77: High likelihood of finding malignancy
   Lumipulse ***G*** ROMA score $<$ 2.77: Low likelihood of finding malignancy

5. Expected values/Reference range:

   Expected values in Healthy Subjects:

   In order to determine the normal reference ranges of the Lumipulse ***G*** ROMA score in healthy women, 120 premenopausal samples and 118 postmenopausal samples (total = 238 samples) were tested. Samples covered age ranging from 18 to 79 and represented whites (74.8%), African American (4.6%), Hispanic (17.2%) and Asian (3.3%) subjects. The results for Lumipulse ***G*** ROMA score obtained from the pre- and post-menopausal populations are presented below:

	All Tested Subjects (N = 238)	**Premenopausal Healthy Subjects (N = 120)**	**Postmenopausal Healthy Subjects (N = 118)**
	Lumipulse ***G*** ROMA score		
Mean (SD[1])	0.97 (0.79)	0.85 (1.00)	1.10 (0.49)
Median	0.83	0.65	0.99
Range (min, max)	0.23–9.92	0.23–9.92	0.38–2.94
Reference Interval (5^{th}, 95^{th} percentile)	0.33, 1.98	0.29, 1.73	0.52, 2.03
	ROMA Likelihood of finding malignancy (N, %)		
High Likelihood	17 (7.1%)	15 (12.5%)	2 (1.7%)
Low Likelihood	221 (92.9%)	105 (87.5%)	116 (98.3%)

Overall, 95% of the premenopausal health female subjects had a Lumipulse ***G*** ROMA score equal to or below 1.73. Ninety five percent of the postmenopausal healthy female subjects had a Lumipulse ***G*** ROMA score equal to or below 2.03. These values were chosen based on the 95th percentile of the population tested. It is recommended that each laboratory established its own reference value for the population of interest.

Expected values in Non-Ovarian Malignancy Conditions:

To evaluate the performance of Lumipulse ***G*** ROMA in subjects with other benign and other malignant conditions, Lumipulse ***G*** ROMA was evaluated in women with benign conditions (benign gynecological disease, congestive heart failure (CHF), hypertension, pregnant, and other benign disease) and in women with other malignant conditions (bladder cancer, breast cancer, endometrial cancer, gastrointestinal cancer, and lung cancer). A total of 880 subjects were analyzed. The tables below summarize the results analyzed for premenopausal and postmenopausal samples.

	Bladder Cancer (N=40)		Breast Cancer (N=40)		Endometrial Cancer (N=40)		GI Cancer (N=40)		Lung Cancer (N=40)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
N	2	38	4	36	8	32	7	33	8	32
Lumipulse ***G*** ROMA Score										
Mean (SD)	5.56 (6.23)	3.10 (1.99)	0.96 (0.54)	3.11 (2.51)	4.53 (3.20)	2.86 (1.88)	2.64 (2.81)	2.60 (1.50)	2.66 (3.49)	4.20 (2.04)
Median	5.56	2.46	0.89	1.84	4.50	2.01	0.87	1.92	1.03	4.03
Range (min-max)	1.15–9.96	0.64–9.25	0.49–1.51	0.50–9.81	1.11–9.54	1.20–8.58	0.60–8.04	0.86–6.61	0.51–9.80	0.81–9.33
5^{th}, 95^{th} percentile	1.59, 9.52	0.91, 6.90	0.49, 1.51	0.87, 8.87	1.13, 8.82	1.33, 7.02	0.62, 6.90	1.10, 5.64	0.51, 8.60	1.34, 7.62
ROMA Likelihood of finding malignancy (N, %)										
High Likelihood	1 (50%)	16 (42%)	1 (25%)	15 (42%)	6 (75%)	12 (38%)	3 (42.9%)	13 (39%)	3 (37.5%)	24 (75%)
Low Likelihood	1 (50%)	22 (58%)	3 (75%)	21 (58%)	2 (25%)	20 (62%)	4 (57.1%)	20 (61%)	5 (62.5%)	8 (25%)

	Benign Gynecological Disease (N=366)		Other Benign Disease (N=40)		CHF (N=40)		Hypertension (N=40)		Pregnant (N=40)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
N	223	143	3	37	3	37	20	20	40	-
Lumipulse ***G*** ROMA Score										
Mean (SD)	1.12 (0.81)	2.07 (1.45)	6.85 (5.43)	1.85 (1.10)	0.97 (1.02)	3.94 (1.97)	3.52 (3.30)	3.52 (2.89)	0.64 (0.26)	-
Median	0.86	1.64	9.99	1.57	0.56	3.74	2.03	1.99	0.58	-
Range (min-max)	0.17–4.86	0.33–8.47	0.59–9.99	0.62–4.57	0.22–2.14	0.72–7.78	0.43–10.0	0.94–9.85	0.28–1.57	-
5^{th}, 95^{th} percentile	0.37, 2.83	0.63, 4.68	1.52, 9.99	0.69, 4.04	0.25, 1.98	1.40, 7.20	0.46, 9.56	1.14, 9.35	0.31, 1.02	-
ROMA Likelihood of finding malignancy (N, %)										
High Likelihood	57 (25.6%)	32 (22.4%)	2 (66.7%)	7 (18.9%)	1 (33%)	23 (62.2%)	13 (65.0%)	7 (35%)	1 (2.5%)	-
Low Likelihood	166 (74.4%)	111 (77.3%)	1 (33.3%)	30 (81.1%)	2 (67%)	14 (37.8%)	7 (35.0%)	13 (65%)	39 (97%)	-

N. Proposed Labeling:

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

O. Conclusion:

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