



Food and Drug Administration
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Silver Spring, MD 20993-0002

Fujirebio Diagnostics, Inc.
c/o Stacey Dolan, RAC-US
Manager, Regulatory Affairs
201 Great Valley Parkway
Malvern, PA 19355

Re: K151378

Trade/Device Name: Lumipulse **G** HE4 Immunoreaction Cartridges, Lumipulse **G** HE4
Calibrators

Regulation Number: 21 CFR 866.6010

Regulation Name: Tumor-associated antigen immunological test system

Regulatory Class: II

Product Code: OIU, JIT

Dated: October 14, 2015

Received: October 15, 2015

Dear Ms. Dolan:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>. Also, please note the regulation entitled, “Misbranding by reference to premarket notification” (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to <http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm> for the CDRH’s Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address <http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm>.

Sincerely yours,

Kelly Oliner -S
2015.11.23 16:36:14 -05'00'

FOR
Leonthena R. Carrington, MS, MBA, MT(ASCP)
Director
Division of Immunology and Hematology Devices
Office of In Vitro Diagnostics
and Radiological Health
Center for Devices and Radiological Health

Enclosure

Indications for Use

510(k) Number (if known)
k151378

Device Name

Lumipulse G HE4 Immunoreaction Cartridges
Lumipulse G HE4 Calibrators

Indications for Use (Describe)

Lumipulse G HE4 Immunoreaction Cartridges:

For in vitro diagnostic use.

Lumipulse G HE4 is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of HE4 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 values should be used in conjunction with other clinical methods used for monitoring epithelial ovarian cancer.

Lumipulse G HE4 Calibrators:

Lumipulse G HE4 Calibrators are for use in the calibration of the Lumipulse G System for the quantitative measurement of HE4 in human serum and plasma (lithium heparin or dipotassium EDTA).

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

Prescription Use (Part 21 CFR 801 Subpart D)

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

CONTINUE ON A SEPARATE PAGE IF NEEDED.

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-
4. Panel:
82, Immunology (Assay)
75, Chemistry (Calibrators)

H. Intended Use:

1. Intended use(s):
See indications for use below.

2. Indication(s) for use:

Lumipulse **G** HE4 Immunoreaction Cartridges

For in vitro diagnostic use.

The Lumipulse **G** HE4 is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of HE4 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 epithelial ovarian cancer.

Lumipulse **G** HE4 Calibrators

The Lumipulse **G** HE4 Calibrators are for use in the calibration of the LUMIPULSE **G** System for the quantitative measurement of HE4 in human serum and plasma (lithium heparin or dipotassium EDTA).

3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
LUMIPULSE **G**1200 System

I. Device Description:

The Lumipulse **G** HE4 is an assay system, including a set of immunoassay reagents, for the quantitative measurement of HE4 in specimens based on CLEIA technology by a two-step sandwich immunoassay method on the LUMIPULSE **G** System.

Lumipulse **G** HE4 Immunoreaction Cartridges REF 234174

The Lumipulse **G** HE4 Immunoreaction Cartridges consists of 3 × 14 tests. Each kit contains the following:

Antibody-Coated Particle Solution
(Liquid when used, 250 µL/Immunoreaction Cartridge)

Contains 150 µg/mL anti-HE4 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: Proclin 300.

Enzyme-Labeled Antibody Solution
(Liquid, 350 µL/Immunoreaction Cartridge)

Contains 0.25 µg/mL alkaline phosphatase (ALP: calf) labeled anti-HE4 monoclonal antibody (mouse), protein stabilizers (bovine, calf and mouse) and chemical stabilizers in 0.1 M sodium chloride/MES buffer. Preservative: Proclin 300.

LUMIPULSE **G HE4 Calibrators **CAL** 234181**

Each calibrator kit contains one bottle each of Calibrators 1 and 2. The calibrator kit is packaged separately.

CAL Liquid, 1 × 2 Concentrations

CAL 1 0 pM HE4 calibrator (1 × 1.5 mL)

CAL 2 1500 pM HE4 calibrator (1 × 1.5 mL)

Contains sodium chloride and potassium chloride in phosphate buffer with protein stabilizer (bovine). Preservative: Proclin 950.

J. Substantial Equivalence Information:

1. Predicate device name(s):
Fujirebio Diagnostics, Inc. HE4 EIA
2. Predicate 510(k) number(s):
k072939

3. Comparison with predicate:

Similarities		
	Lumipulse G HE4 Assay (Proposed Device)	Fujirebio Diagnostics, Inc. HE4 EIA Assay (Predicate Device) K072939
Device Type	<i>In vitro</i> diagnostic	<i>In vitro</i> diagnostic
Classification	Class II	Class II
CFR section	866.6010	866.6010
Product Code	OIU	OIU
Product Usage	Clinical and Hospital laboratories	Clinical and Hospital laboratories
Intended Use	<p>For <i>in vitro</i> diagnostic use.</p> <p>The Lumipulse G HE4 is a Chemiluminescent Enzyme Immunoassay (CLEIA) for the quantitative determination of HE4 in human serum and plasma (lithium heparin or dipotassium EDTA) on the LUMIPULSE G System.</p> <p>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 epithelial ovarian cancer.</p>	<p>For <i>in vitro</i> diagnostic use.</p> <p>The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum.</p> <p>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 epithelial ovarian cancer</p>
Specimen Collection Method	Routine Phlebotomy Techniques	Routine Phlebotomy Techniques
Calibrator Antigen	Ig-HE4	Ig-HE4
Analyte	Human HE4	Human HE4

Differences		
	Lumipulse G HE4 Assay (Proposed Device)	Fujirebio Diagnostics, Inc. HE4 EIA Assay (Predicate Device) K072939
Instrument System	LUMIPULSE G 1200 System	None
Principle of Operation	Chemiluminescent Enzyme Immunoassay (CLEIA)	Enzyme Immunometric Assay (EIA)
Type of Specimen	Human Serum or Plasma (, lithium heparin, or dipotassium EDTA)	Human Serum
Assay Range	20 – 1500 pM	15 – 900 pM
Interpretation of Results	Calibrator Curve	Standard Curve
Calibrators	2 Levels (0 and 1500 pM) Ready to Use -Liquid -Supplied as separate kit	5 Levels (Lot specific) -Lyophilized
Antibodies	12A2 and 3D8 (mouse monoclonal)	2H5 and 3D8 (mouse monoclonal)

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

- ISO 17511:2003 Measurement of Quantities in Biological Samples - Metrological Traceability of Values Assigned to Calibrator and Control Materials
- CLSI EP5-A3 - Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline - Third Edition
- CLSI EP7-A2 - Interference Testing in Clinical Chemistry; Approved Guideline-Second Edition
- CLSI C28-A3c - Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition
- CLSI EP17-A2 - Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline Second Edition
- CLSI EP6-A - Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP9-A3 – Measurement Procedure Comparison and Bias Estimation Using Patient Samples; approved Guideline – Third Edition
- FDA Guidance Document for the Submission of Tumor Associated Antigen Premarket Notifications, [510(k)], to FDA
- Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff

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.

M. Performance Characteristics (if/when applicable):

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

1. Analytical performance:

a. *Precision/Reproducibility:*

Lumipulse **G** HE4 is $\leq 10\%$ total (within-laboratory) CV (coefficient of variation). Lumipulse **G** HE4 demonstrated precision $\leq 3.5\%$ (total %CV) in a study run according to the Clinical and Laboratory Standards Institute (CLSI) guideline EP5-A3. Eight human serum-based samples (specimen pools), and two commercially available serum-based controls were assayed in replicates of two at two separate times of the day for 20 days (n=80 for each sample) using one LUMIPULSE **G**1200 System. Data from this study are presented below.

Sample	Mean Conc. (pM) n=80	Within-run SD (pM)	Within-run %CV	Total SD (pM)	Total %CV
Serum 1	58.9	0.70	1.2	1.15	1.9
Serum 2	77.4	0.78	1.0	1.45	1.9
Serum 4	119.0	1.58	1.3	1.87	1.6
Serum 5	310.7	3.18	1.0	7.12	2.3
Serum 6	1125.1	14.03	1.2	24.19	2.1
Serum 7	28.6	0.35	1.2	0.99	3.5
Serum 8	627.8	7.06	1.1	8.49	1.4
Serum 9	1329.9	21.89	1.6	29.88	2.2
Control 1	95.1	1.99	2.1	2.27	2.4
Control 2	751.8	10.27	1.4	13.88	1.8

Lumipulse **G** HE4 demonstrated precision $\leq 6.1\%$ total %CV when tested at 3 external laboratory sites. The human serum-based samples (specimen pools) and two commercially available serum-based controls were assayed in replicates of two at two separate times of the day at each of the sites for 10 days (n=40 for each sample) using one LUMIPULSE **G**1200 System. Data from this study are presented below.

Sample	Mean Conc. (pM) n=120	Within-run SD (pM)	Within-run %CV	Total SD (pM)	Total %CV
Serum 1	57.1	0.70	1.2	2.14	3.8
Serum 2	75.4	0.89	1.2	2.78	3.7
Serum 4	115.9	1.47	1.3	4.85	4.2
Serum 5	303.0	2.85	0.9	15.44	5.1
Serum 6	1110.2	14.99	1.4	62.98	5.7
Serum 7	27.5	0.35	1.3	1.68	6.1
Serum 8	609.3	8.17	1.3	32.89	5.4
Serum 9	1323.2	25.92	2.0	68.74	5.2
Control 1	95.4	1.90	2.0	5.57	5.8
Control 2	733.4	12.27	1.7	29.16	4.0

Lumipulse **G** HE4 demonstrated precision $\leq 3.2\%$ total %CV when tested using 3 lots of Lumipulse **G** HE4 immunoreaction cartridges and calibrators. The human serum-based samples (specimen pools) and two commercially available serum-based controls were assayed in replicates of two at two separate times of the day for each of the lots for 10 days (n=40 for each sample) using one LUMIPULSE **G**1200 System. Data from this study are presented below.

Sample	Mean Conc. (pM) n=120	Within-run SD (pM)	Within-run %CV	Total SD (pM)	Total %CV
Serum 1	57.5	0.56	1.0	1.24	2.2
Serum 2	75.3	0.76	1.0	1.75	2.3
Serum 4	116.1	1.42	1.2	2.71	2.3
Serum 5	302.7	3.81	1.3	9.76	3.2
Serum 6	1119.3	15.36	1.4	24.91	2.2
Serum 7	27.6	0.35	1.2	0.75	2.7
Serum 8	617.5	9.66	1.6	13.83	2.2
Serum 9	1334.0	25.33	1.9	33.60	2.5
Control 1	94.9	1.66	1.8	2.20	2.3
Control 2	746.5	8.35	1.1	11.82	1.6

b. Linearity/assay reportable range:

Lumipulse **G** HE4 demonstrated linearity in a study consistent with the guidelines in the CLSI Protocol EP6-A. One human serum specimen pool and one dipotassium EDTA plasma specimen pool with high HE4 levels were diluted with one human serum specimen pool and one dipotassium EDTA plasma specimen pool with low HE4 levels throughout the range of the assay. The linearity was found in the range of 20.0 to 1500.0 pM. Lumipulse **G** HE4 correlated with expected concentrations according to the linear regression formulas:

Serum: $y = 0.9909 (x) - 10.3161$; R-squared: 1.0000

Plasma: $y = 1.0104 (x) + 12.4869$; R-squared: 1.0000

Lumipulse **G** HE4 recovery is $100 \pm 10\%$, ranging from 91% to 107%. A study was performed where known concentrations of HE4 antigen were added to human serum and dipotassium EDTA plasma samples with low endogenous HE4 levels. The concentration of HE4 was determined using the Lumipulse **G** HE4 and the resulting percent recovery was calculated.

Sample	Target HE4 Levels (pM)	Measured Conc. (pM) (n=3)	Expected Conc. (pM)	% Recovery
Serum 1	80	132.8	125.2	106
	150	188.2	186.7	101
	450	484.4	474.2	102
	750	768.0	761.0	101
	1050	1056.5	1064.4	99
	1350	1350.4	1390.5	97
Serum 2	80	148.3	140.8	105
	150	196.0	191.5	102
	450	488.7	479.0	102
	750	767.2	765.8	100
	1050	1046.9	1069.2	98
	1350	1316.9	1395.3	94
Serum 3	80	116.8	109.3	107
	150	202.4	197.4	103
	450	491.7	484.9	101
	750	763.8	771.7	99
	1050	1081.1	1075.1	101
	1350	1324.1	1401.2	94
Plasma 1	80	127.5	122.2	104
	150	242.4	240.4	101
	450	518.1	527.9	98
	750	796.3	814.7	98
	1050	1069.3	1118.1	96
	1350	1366.4	1444.2	95
Plasma 2	80	138.5	134.5	103
	150	206.5	203.4	102
	450	492.7	490.9	100
	750	780.6	777.7	100
	1050	1035.8	1081.1	96
	1350	1274.7	1407.2	91
Plasma 3	80	137.9	130.9	105
	150	204.8	201.1	102
	450	494.0	488.6	101
	750	784.3	775.4	101
	1050	1057.1	1078.8	98
	1350	1344.9	1404.9	96

High dose hook is a phenomenon whereby very high level specimens may read within the dynamic range of the assay. For Lumipulse **G** HE4, no high dose hook effect was observed for samples containing up to 300,000 pM of HE4 antigen. However values above 30,000 pM should be interpreted with caution because reading may be inaccurate above this concentration.

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

The Lumipulse **G** HE4 Calibrators are for use in the calibration of the LUMIPULSE **G** System when used for the quantitative measurement of HE4 in human serum and plasma (lithium heparin or dipotassium EDTA). 2 Bottles (1.5 mL each) are supplied for the LUMIPULSE **G** HE4 Calibrators. Preservatives: Proclin 950. The calibrators are at the following concentrations:

Calibrator	Concentration (pM)
CAL 1	0
CAL 2	1500

There is currently no known internationally recognized consensus reference method or reference material for standardization. Lumipulse **G** HE4 values are expressed as pM (or pmol/L). This value is related to a Fujirebio Diagnostics maintained reference preparation. Calibration of the Lumipulse **G** HE4 is traceable to in-house reference calibrators, whose values have been assigned to correlate to Fujirebio Diagnostics' HE4 EIA.

The real time stability (including open use) has been demonstrated for 10 months. The instrument cartridge and calibrators were found to be stable under simulated transport conditions. Shelf life assignment for the Lumipulse **G** HE4 Immunoreaction Cartridges and Calibrators will be 8 months at 2–10°C.

d. Detection limit:

The Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) of Lumipulse **G** HE4 is ≤ 20.0 pM.

1) LoB and LoD

The LoB for Lumipulse **G** HE4 was 0.1 pM.

The LoD for Lumipulse **G** HE4 was 3.5 pM, determined consistent with the guidelines in the CLSI Protocol EP17-A2. Seven low-level specimens were tested over 3 days using three LUMIPULSE **G** Systems and three Lumipulse **G** HE4 lots giving 180 determinations for each panel.

2) LoQ

The LOQ for Lumipulse **G** HE4 was 3.5 pM, determined consistent with the guidelines in the CLSI guideline EP17-A2. Since the percent total error estimate is $\leq 30\%$, LoQ equals LoD.

e. *Analytical specificity:*

The Lumipulse **G** HE4 demonstrated an average interference of $\leq 10\%$ (for each compound) in a study consistent with the guidelines in the CLSI guideline EP7-A2. Human serum specimens were supplemented with potentially interfering compounds. The following compounds were tested and found not to interfere with the test.

Endogenous Interferences	Test Concentration
Free Bilirubin (unconjugated)	60 mg/dL
Conjugated Bilirubin	60 mg/dL
Triglycerides (Intralipid 20% Emulsion)	3000 mg/dL
Hemoglobin	500 mg/dL
Human Serum Albumin	12 g/dL
Immunoglobulin G (IgG)	5 g/dL
Biotin	19.8 mg/dL
Human Anti-Mouse Antibodies (HAMA)	1,000 ng/mL
Rheumatoid Factor (RF)	1,000 IU/mL

Therapeutic drug Interferences	Test Concentration
Bevacizumab	750 μ g/mL
Carboplatin	600 μ g/mL
Cisplatin	180 μ g/mL
Clotrimazole	0.3 μ g/mL
Cyclophosphamide	800 μ g/mL
Dexamethasone	20 μ g/mL
Docetaxel	10.5 mg/dL
Doxil [®] (Pegylated Liposomal Doxorubicin)	5.2 mg/dL
Doxorubicin	120 μ g/mL
Erlotinib	150 μ g/mL
Etoposide	10 μ g/mL
5-Fluorouracil	900 μ g/mL
Gemcitabine	100 μ g/mL
Leucovorin	750 μ g/mL
Magestrol Acetate	10 μ g/mL
Melphalan	15 μ g/mL
Methotrexate	450 μ g/mL
Olaparib	28.1 mg/dL
Paclitaxel	3.5 ng/mL
Rituximab	750 μ g/mL
Tamoxifen	60 μ g/mL
Topotecan	0.155 mg/dL
Trastuzumab	600 μ g/mL

f. *Assay cut-off:*

See Clinical Cutoff in M (4) below

2. Comparison studies:

a. *Method Comparison*

Lumipulse **G** HE4 method comparison study was performed using specimens consistent with CLSI guideline EP9-A3. The weighted Deming regression method was used to compare Lumipulse **G** HE4 to HE4 EIA. The HE4 EIA has a different measuring range than Lumipulse **G** HE4, therefore the results were limited to the measuring range of both devices (143 samples). The data are summarized in the following table.

Range of samples:

35.2 – 969.5 pM (Lumipulse **G** HE4)

33.4 – 899.0 pM (HE4 EIA)

Lumipulse **G HE4 vs. HE4 EIA**

n	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)	Average Bias (pM)
143	0.9891	-3.2350 -5.3628 – -1.1071	1.0349 1.0074 – 1.0624	8.8

The data summarized in the following table include results from a study with specimens above the measurement range of both devices requiring dilution (168 samples).

Range of samples:

35.2 – 4602.0 pM (Lumipulse **G** HE4)

33.4 – 4067.0 pM (HE4 EIA)

Lumipulse **G HE4 vs. HE4 EIA**

n	Correlation Coefficient (r)	Intercept (95% CI)	Slope (95% CI)	Average Bias (pM)
168	0.9917	-2.8518 -4.8632 – -0.8404	1.0289 1.0045 – 1.0533	13.8

b. *Matrix Comparison*

The Lumipulse **G** HE4 matrix comparison study was performed to evaluate the difference across tube types (SST, K2EDTA, and Lithium Heparin,) versus the means of the control samples (Red top serum) analyzed per CLSI guideline EP9-A3. The slope for each tube type when compared to the control had 95% confidence intervals that lay entirely within the range 0.9 to 1.1 and the correlation coefficients were ≥ 0.9 .

3. Clinical studies:

a. *Clinical sensitivity:*

See 3(c) below

b. *Clinical specificity:*

See 3(c) below

c. *Other clinical supportive data (when a. and b. are not applicable):*

Monitoring of Disease status in Patients Diagnosed with Epithelial Ovarian Cancer

The effectiveness of Lumipulse **G** HE4 as an aid in monitoring of disease status in EOC patients was determined by assessing changes in HE4 levels in serial serum samples from 72 patients compared to changes in disease status. A study involving a total of 330 pairs of observations was undertaken with an average number of 5.6 observations per patient. A positive change in HE4 was defined as an increase in the value that was at least 18% greater than the previous value of the test. This level of change takes into account the variability of the assay. Forty-nine percent (49%) or 30/61 of the patient samples with a positive change correlated with the disease progression while eighty percent (80%) or 214/269 of the patient serial samples with no significant change in HE4 value correlated with no progression. The total concordance was seventy-four percent (74% or 245/330).

Lumipulse **G** HE4 Performance Measurements (%):

Performance Measurement	SE	Lower 95% CI	Upper 95% CI
Sensitivity	49.2	10.74	70.6
Specificity	79.6	6.87	93.2
Total Concordance	73.9	5.69	85.3
PPV	35.3	7.71	50.7
NPV	87.4	7.54	102.4

SE = Standard Error

CI = Confidence Interval

The following table presents the data in a 2 x 2 format.

Change in Disease State per Sequential Pair			
Change in HE4 Concentration	No progression	Progression	Total
≤ 18%	214	31	245
> 18%	55	30	85
Total	269	61	330

4. Clinical cut-off:

HE4 is significantly elevated if it is at least 18% higher than the previous assay.

5. Expected values/Reference range:

The distribution of HE4 levels determined in healthy premenopausal and postmenopausal women is shown in the table below:

	All Healthy Subjects	Premenopausal Healthy Subjects	Postmenopausal Healthy Subjects
N	240	120	120
Mean	59.5	58.0	61.0
(SD)	(68.8)	(94.8)	(22.5)
Median	51.3	47.0	55.9
Range (min, max)	29.4, 1076.6	29.9, 1076.6	29.4, 176.7
Reference Interval (2.5th percentile, 97.5th percentile)	32.5, 108.4	31.9, 87.1	32.5, 112.2
Proportion below 90.0 pM	96%	98%	93%
Proportion below 135.0 pM	99%	99%	98%

All Lumipulse **G** HE4 concentrations are presented as pM

The distribution of HE4 levels determined in benign conditions is shown in the table below:

	Benign Gynecological Disease	Other Benign Disease	Congestive Heart Failure	Hypertension	Pregnant
N	366	40	40	40	40
Mean (SD)	70.7 (44.0)	232.6 (548.8)	185.3 (99.7)	397.0 (999.9)	45.4 (8.3)
Median	57.8	81.4	173.9	108.8	44.5
Range (min, max)	26.3, 386.8	42.7, 2762.0	30.6, 417.7	39.0, 5773.0	31.9, 71.0
Reference Interval (2.5th percentile , 97.5th percentile)	35.0, 202.0	44.2, 2374.9	43.5, 407.5	40.9, 2874.3	32.2, 58.3
Proportion below 90.0 pmol/L	82%	55%	18%	43%	100%
Proportion below 135.0 pmol/L	94%	70%	38%	65%	100%

*All Lumipulse **G** HE4 concentrations are presented as pM

The distribution of HE4 levels determined in malignancy conditions is shown in the table below:

	Epithelial Ovarian Cancer	Bladder Cancer	Breast Cancer	Endometrial Cancer	GI Cancer	Lung Cancer
N	155	40	40	40	40	40
Mean (SD)	1188.7 (2447.4)	188.3 (230.0)	173.0 (398.0)	145.9 (119.5)	103.4 (51.8)	157.6 (127.5)
Median	341.0	127.5	95.3	99.5	91.2	115.8
Range (min, max)	24.6, >15000.0	44.9, 1381.9	32.4, 2591.0	50.0, 646.9	42.2, 258.9	41.1, 658.5
Reference Interval (2.5th percentile , 97.5th percentile)	48.6, 7602.3	50.3, 569.9	38.3, 419.8	60.5, 500.0	44.3, 247.7	42.3, 562.9
Proportion below 90.0 pmol/L	14%	30%	48%	35%	48%	33%
Proportion below 135.0 pmol/L	25%	58%	78%	68%	83%	63%

All Lumipulse **G** HE4 concentrations are presented as pM

It is recommended that each laboratory establish its own reference value for the population of interest for Lumipulse **G** HE4 on the LUMIPULSE **G**1200.

6. Conclusion

The results of these analytical (nonclinical) and clinical studies demonstrate that the Lumipulse **G** HE4 assay is substantially equivalent to the performance of the Fujirebio Diagnostics, Inc. HE4 EIA assay.

N. Proposed Labeling:

The labeling satisfies the requirements of 21 CFR Part 809.10.