510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K150588

B. Purpose for Submission:

New device

C. Measurand:

Score based on 5 serum analytes

D. Type of Test:

Software algorithm that combines five immunoassays into a single score

E. Applicant:

Vermillion, Inc.

F. Proprietary and Established Names:

OVA1 Next Generation

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, Serum, algorithm, ovarian cancer assessment test

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   The OVA1 Next Generation test is a qualitative serum test that combines the results of five immunoassays into a single numeric result. It 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.

   The OVA1 Next Generation test is an aid to further assess the likelihood that malignancy is present when the physician's independent clinical and radiological evaluation does not indicate malignancy. The test is not intended as a screening or stand-alone diagnostic assay.

   PRECAUTION: The OVA1 Next Generation test should not be used without an independent clinical and imaging evaluation and is **not** intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use of the OVA1 Next Generation test 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:

   For use on Roche cobas® 6000 system

I. Device Description:

The OVA1 Next Generation test consists of software, instruments, assays and reagents. The software incorporates the results of five serum biomarker concentrations from immunoassays run separately to calculate a single, unitless numeric result indicating a low or high risk of ovarian malignancy.

The biomarkers and corresponding immunoassays and calibrators used to generate the numeric result (OVA1 Next Generation score) are:

Analyte	Reagent and Calibrator	Instrument
Apolipoprotein A-1 (APO)	cobas APO A1 C.f.a.s. Lipids	Roche cobas® 6000: Roche cobas® c501
CA 125 II	cobas CA 125 II CA 125 II Cal Set	Roche cobas® 6000: Roche cobas® e601
Follicle Stimulating Hormone (FSH)	cobas FSH FSH Cal Set II	Roche cobas® 6000: Roche cobas® e601
Human epididymis protein 4 (HE4)	cobas HE4 HE4 Cal Set	Roche cobas® 6000: Roche cobas® e601

Analyte	Reagent and Calibrator	Instrument
Transferrin (TRF)	cobas Transferrin C.f.a.s. Proteins	Roche cobas® 6000: Roche cobas® c501

The biomarker immunoassays and reagents are sold separately from the OVA1 Next Generation software (OvaCalc). All immunoassays are run on the Roche cobas® 6000 system according to the manufacturer's instructions as detailed in the product insert for each reagent. Users are instructed to use only qualified lot numbers for the immunoassays as posted on www.vermillion.com. Roche cobas® 6000 system is a fully automated, software-controlled system for clinical chemistry and immunoassay analysis.

The OvaCalc software (v4.0.0) contains a proprietary algorithm that utilizes the results (values) from the five biomarker immunoassays. The assay values from the cobas® 6000 system are either imported into OvaCalc through a .csv file or manually entered into the OvaCalc user interface to generate an OVA1 Next Generation score between 0.0 and 10.0.

OVA1 Next Generation score:
Low probability of malignancy Risk score < 5.0
High probability of malignancy Risk score ≥ 5.0

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s):

   Vermillion OVA1, K081754

2. Comparison with predicate:

Similarities		
Item	New Device OVA1 Next Generation	**Predicate** OVA1 Test
Intended Use/ Indication for Use	The OVA1 Next Generation test is a qualitative serum test that combines the results of five immunoassays into a single numeric result. It 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. The OVA1 Next Generation test is an aid to further assess the likelihood that malignancy is present when the physician's independent clinical and radiological evaluation does not indicate malignancy.	The OVA1 Test is a qualitative serum test that combines the results of five immunoassays into a single numerical score. It 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. The OVA1 Test is an aid to further assess the likelihood that malignancy is present when the physician's independent clinical and radiological evaluation does not indicate malignancy. The test is not intended as a screening or stand-alone diagnostic assay.

	The test is not intended as a screening or stand-alone diagnostic assay.	
	Should not be used without an independent clinical and imaging evaluation and is not intended to be a screening test or to determine whether a patient should proceed to surgery. Incorrect use carries the risk of unnecessary testing, surgery, and / or delayed diagnosis.	Same
Sample Matrix	Serum	Same
Type of Test	Algorithm	Same

Differences		
Item	**New Device** OVA1 Next Generation	**Predicate** OVA1 Test
Analytes	Roche Elecsys APO, CA 125, TRF, FSH, HE4	Roche Elecsys CA 125 and Siemens BN II APO, TRF, Prealbumin, β2 Microglobulin
Equation used for test	One equation with one cut-off	One equation with two cut-offs depending on menopausal status
Clinical cutoff	Pre-menopausal and Post-menopausal: • OVA1 risk score < 5.0 Low probability for malignancy • OVA1 risk score ≥ 5.0 High probability for malignancy	Pre-menopausal: • OVA1 risk score < 5.0 Low probability for malignancy • OVA1 risk score ≥ 5.0 High probability for malignancy Post-menopausal: • OVA1 risk score < 4.4 Low probability for malignancy • OVA1 risk score ≥ 4.4 High probability for malignancy
Platform	Roche cobas e601 (CA125, FSH and HE4) Roche cobas c501 (APO and TRF)	Roche Elecsys 2010 (CA 125) Siemens BNII (APO, TRF, Prealbumin, β2 Microglobulin)

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

- FDA Guidance "Class II Special Controls Guidance Document: Ovarian Adnexal Mass Assessment Score Test System."

- FDA Guidance "Guidance for the Content of Premarket Submissions for Software Contained in Medical Devices."

- ISO 14971:2012 Medical Devices-Application of Risk Management to Medical Devices, International Organization for Standardization.

- CLSI guideline EP05-A2, "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline."

- CLSI guideline EP07-A2, "Interference Testing in Clinical Chemistry; Approved Guideline – Second Edition."

L. Test Principle:

The individual assays for APO and TRF each contain a biomarker specific polyclonal antibody which forms an immune complex with the target when reacted with a serum specimen. The levels of immune complexes can be measured turbidimetrically and are proportional to the concentration of biomarker in the serum specimen for each specific assay. The individual assays for CA 125 II, FSH and HE4 each use two mouse monoclonal antibodies to their respective biomarkers. The quantity of each biomarker present is then measured by chemiluminescence emission.

The Cobas 6000 is an automated analyzer with electrochemiluminescence detection. The amount of analyte in each assay is determined against the calibration curve. Each assay uses its own specific calibrator and controls.

The user enters results of the five analytes manually into an Excel spreadsheet together with the headers needed by OvaCalc Software. There is no physical or electronic connection between the immunoassay devices and the OvaCalc Software. Using an algorithm and the values of these five analytes, the OvaCalc Software generates a single unit-less numerical score from 0.0 to 10.0.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance: All results met the manufacturer's pre-determined acceptance criteria.</u>

   a. Precision: Precision performance of the OVA1 Next Generation test was evaluated in accordance with CLSI guideline EP05-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline." Five pooled serum samples spanning the OVA1 Next Generation score range (three close to the cut-offs, one low and one high value) were tested over 20 days, two runs per day, and two replicates per run by multiple operators (equal to 80 replicate determinations per pool). There were no un-evaluable results. Total percent coefficient of variation (%CV) for all pools is 1.54%.

Specimen	n	OVA1 Next Generation (Mean score)	Within-run		Between-run		Total	
			SD	CV%	SD	CV%	SD	%CV
1	80	3.30	0.05	1.62	0.01	0.34	0.07	1.95
2	80	4.11	0.07	1.68	0.01	0.27	0.09	2.06
3	80	5.08	0.13	2.57	0.06	1.25	0.16	3.16
4	80	8.16	0.05	0.61	0.00	0.00	0.06	0.67
5	80	8.50	0.00	0.00	0.00	0.00	0.00	0.00

Reproducibility: Five pooled serum samples spanning the OVA1 Next Generation score range (three close to the cut-offs, one low and one high value) were tested in duplicate, two runs each day, over six non-consecutive days, by two operators at each of three sites. Each operator performed the test on three nonconsecutive days, i.e., operator 1 ran the test on days one, three, and five; operator 2 ran the test on days two, four, and six. At each site, the test was run with the same lots of calibrators, kit reagents, and controls for the duration of the study. Each operator performed the complete analysis of the OVA1Next Generation test for the day on the cobas® 6000 and imported the five biomarkers into Vermillion's OvaCalc Software for generation of the OVA1Next Generation score. For an assessment of the components of imprecision in the reproducibility study, the mean score, SD and %CV of each of the five pools were estimated and shown in the table below. The overall %CV including all sites was 1.63%.

Parameters		Specimen				
		1	2	3	4	5
OVA1 Next Generation test results						
Mean score		**3.28**	**4.08**	**5.09**	**8.16**	**8.50**
Repeatability (within-run)	SD	0.05	0.06	0.11	0.05	0.00
	%CV	1.44	1.38	2.25	0.56	0.00
Between-run	SD	0.02	0.07	0.10	0.03	0.00
	%CV	0.68	1.59	2.03	0.39	0.00
Between-day	SD	0.00	0.00	0.07	0.00	0.00
	%CV	0.00	0.00	1.43	0.00	0.00
Between-operator	SD	0.00	0.00	0.04	0.00	0.00
	%CV	0.00	0.00	0.69	0.00	0.00
Between-site	SD	0.00	0.02	0.03	0.04	0.00
	%CV	0.00	0.55	0.67	0.48	0.00
Reproducibility (total)	SD	0.05	0.09	0.18	0.06	0.00
	%CV	1.59	2.15	3.43	0.67	0.00
Individual biomarker immunoassay results						
APO, mg/dL						
Mean concentration		**157.96**	**153.09**	**166.68**	**149.69**	**133.07**
Repeatability (within-run)	SD	1.45	1.57	2.16	1.79	1.91
	%CV	0.92	1.03	1.30	1.19	1.43
Between-run	SD	2.30	2.19	2.67	1.87	2.30
	%CV	1.45	1.43	1.60	1.25	1.73

Between-day	SD	0.50	1.60	0.00	0.00	0.00
	%CV	0.32	1.04	0.00	0.00	0.00
Between-operator	SD	0.55	0.72	0.91	0.00	0.00
	%CV	0.35	0.47	0.55	0.00	0.00
Between-site	SD	0.87	0.37	1.64	1.08	0.00
	%CV	0.55	0.24	0.98	0.72	0.00
Reproducibility (total)	SD	2.88	3.19	3.77	2.73	2.98
	%CV	1.82	2.08	2.26	1.82	2.24
CA 125 II, IU/mL						
Mean concentration		**13.58**	**22.39**	**38.34**	**246.80**	**868.49**
Repeatability (within-run)	SD	0.30	0.43	0.55	3.16	11.16
	%CV	2.19	1.90	1.44	1.28	1.29
Between-run	SD	0.00	0.00	0.15	1.51	0.00
	%CV	0.00	0.00	0.38	0.61	0.00
Between-day	SD	0.13	0.18	0.14	0.00	5.13
	%CV	0.96	0.78	0.37	0.00	0.59
Between-operator	SD	0.00	0.00	0.08	0.00	3.72
	%CV	0.00	0.00	0.21	0.00	0.43
Between-site	SD	0.02	0.19	0.46	3.77	14.32
	%CV	0.15	0.85	1.19	1.53	1.65
Reproducibility (total)	SD	0.32	0.48	0.70	4.67	17.27
	%CV	2.38	2.16	1.83	1.89	1.99
FSH, mIU/mL						
Mean concentration		**25.74**	**29.08**	**32.48**	**31.84**	**34.66**
Repeatability (within-run)	SD	0.23	0.26	0.35	0.31	0.43
	%CV	0.90	0.89	1.08	0.97	1.24
Between-run	SD	0.28	0.35	0.42	0.30	0.37
	%CV	1.10	1.19	1.28	0.95	1.06
Between-day	SD	0.37	0.42	0.31	0.50	0.43
	%CV	1.42	1.45	0.96	1.57	1.23
Between-operator	SD	0.00	0.00	0.00	0.00	0.00
	%CV	0.00	0.00	0.00	0.00	0.00
Between-site	SD	0.33	0.28	0.37	0.36	0.46
	%CV	1.29	0.98	1.13	1.12	1.31
Reproducibility (total)	SD	0.58	0.64	0.69	0.71	0.80
	%CV	2.25	2.21	2.13	2.24	2.30
HE4, pmol/L						
Mean concentration		**60.33**	**55.96**	**81.10**	**213.18**	**532.00**
Repeatability (within-run)	SD	0.66	0.65	0.81	1.76	5.57
	%CV	1.09	1.15	1.00	0.82	1.05
Between-run	SD	0	0.13	0.52	0.51	1.96
	%CV	0	0.23	0.65	0.24	0.37
Between-day	SD	0.49	0.40	0.61	2.03	5.98
	%CV	0.81	0.72	0.75	0.95	1.12

Between-operator	SD	0.00	0.00	0.00	0.00	0.00
	%CV	0.00	0.00	0.00	0.00	0.00
Between-site	SD	1.07	1.05	1.45	3.27	8.95
	%CV	1.77	1.87	1.79	1.53	1.68
Reproducibility (total)	SD	1.19	1.153	1.65	3.81	11.12
	%CV	1.98	2.06	2.03	1.79	2.09
TRF, mg/dL						
Mean concentration		**288.69**	**276.82**	**276.75**	**255.80**	**225.31**
Repeatability (within-run)	SD	5.28	4.88	4.45	4.36	3.93
	%CV	1.83	1.76	1.61	1.70	1.75
Between-run	SD	5.49	3.47	3.78	4.10	2.34
	%CV	1.90	1.25	1.37	1.60	1.04
Between-day	SD	0.00	0.00	1.77	1.30	0.00
	%CV	0.00	0.00	0.64	0.51	0.00
Between-operator	SD	0.00	0.00	2.06	0.00	0.52
	%CV	0.00	0.00	0.74	0.00	0.23
Between-site	SD	1.56	3.25	1.08	3.87	1.49
	%CV	0.54	1.17	0.39	1.51	0.66
Reproducibility (total)	SD	7.69	6.54	6.43	6.88	4.76
	%CV	2.67	2.36	2.32	2.69	2.11

Lot-to-lot variability was evaluated and changes in the biomarkers ≤±3% due to different reagent lots resulted in difference of sensitivity of OVA1 Next Generation score ranging from -2.2% to 3.3% and specificity of OVA1 Next Generation score ranging from -5.5% to 4.5 %.

b. Linearity/assay reportable range:

For each analyte, measurement linearity (as claimed in the package inserts for the individual analytes and shown in the table below) was demonstrated for measurement intervals corresponding to those used in the OVA1 Next Generation test.

Analyte	**Measuring Range**
Apolipoprotein A-1 (APO)	20–400 mg/dL
CA 125 II	0.6–5000 IU/mL
Follicle Stimulating Hormone (FSH)	0.10–200 mIU/mL
Human epididymis protein 4 (HE4)	15.0–1500 pmol/L
Transferrin (TRF)	10–520 mg/dL

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

i. Traceability

Each biomarker immunoassay uses its own calibrator and controls. For APO, the method has been standardized against IFCC SP1‑01 (WHO IRP October 1992). For TRF, the method has been standardized against BCR470/CRM 470 (Reference Preparation for Proteins un Human Serum). For CA 125 II, the method is standardized against Enzymun-Test CA 125 II method which in turn has been standardized against the CA125 II RIA from Fujirebio Diagnostics. For FSH, the method is standardized against Enzymun-Test FSH method which in turn has been standardized against the 2nd IRP WHO reference standard 79/549. For HE4, the method is standardized against the HE4 EIA method from Fujirebio Diagnostics, Inc.

ii. Reagent Stability

Closed/open vial and on-board: For each biomarker immunoassay used in the OVA1 Next Generation test, users are instructed to refer to the storage and stability information in the package insert. This information is summarized in the table below.

Analyte	**Stability Data**
Apolipoprotein A-1 (APO)	Closed vial at 2–8° C: up to the expiration date On-board in use and refrigerated: 12 weeks
CA 125 II	Closed vial at 2–8° C: up to the expiration date Open vial at 2–8°C: 12 weeks On-board: 6 weeks
Follicle Stimulating Hormone (FSH)	Closed vial at 2–8°C: up to the expiration date Open vial at 2–8°C: 12 weeks On-board: 8 weeks
Human epididymis protein 4 (HE4)	Closed vial at 2–8°C: up to the expiration date Open vial at 2–8°C: 12 weeks On-board: 28 days
Transferrin (TRF)	Closed vial at 2–8°C: up to the expiration date On‑board in use and refrigerated: 12 weeks

iii. Specimen Stability

Seven serum pools spanning the OVA1 Next Generation risk score range (two close to the cut-off, one low and four high values) were tested to determine sample storage and freeze/thaw stability. The mean, SD, mean change, and %mean change from Day 0 (the day of specimen collection) were used to describe the OVA1 Next Generation risk score for each pool stored refrigerated up to nine days or four freeze/thaw cycles. The results support the following stability claims: fresh serum stored can be stored up to eight days between 2−8°C and may be subjected to four freeze/thaw cycles prior to testing.

d. Detection limit:

The limits of detection and limits of quantitation reported in each assay's package insert are summarized in the table below. They were confirmed and incorporated into the algorithm such

that results outside of the measuring interval are not imported and do not yield an OVA1 Next Generation score.

Analyte	Detection limit
Apolipoprotein A-1 (APO)	0.03 g/L (1.07 µmol/L)
CA 125 II	0.600 IU/mL
Follicle Stimulating Hormone (FSH)	0.100 mIU/mL
Human epididymis protein 4 (HE4)	LoB: 5.0 pmol/L LoD: 15.0 pmol/L LoQ: 20.0 pmol/L
Transferrin (TRF)	0.1 g/L (1.26 µmol/L)

e. Analytical specificity:

i. Endogenous Interference:

Three pooled serum samples with low (~3.28), medium (~5.15) and high (~8.50) OVA1 Next Generation scores were evaluated for interference by hemoglobin, bilirubin (conjugated and unconjugated), triglycerides and rheumatoid factor. The effect of each interfering substance on the OVA1 Next Generation score was assessed using a mean of four repeated measurements on each sample spiked with the potentially interfering substances and compared to control measurements of samples without the interfering substances but with the same amount of solvent. Based on the manufacturer's acceptance limits for non-interference, none of the interfering substances demonstrated significant interference on the OVA1 Next Generation score up to the concentrations evaluated.

		OVA1 Next Generation scores		
		Low	**Medium**	**High**
Interference substance	**Test concentration**	**Mean change (%) in score compared to control (95% CI of difference)**		
Bilirubin Unconjugated	0.3 g/L	0.92 (-0.10 to 0.15)	-0.96 (-0.32 to 0.22)	0.0 (0.0 to 0.0)
	0.9 g/L	2.94 (-0.02 to 0.22)	-1.23 (-0.34 to 0.19)	0.0 (0.0 to 0.0)
Bilirubin Conjugated	0.3 g/L	1.52 (-0.11 to 0.21)	1.87 (-0.25 to 0.45)	0.0 (0.0 to 0.0)
	0.9 g/L	4.02 (-0.01 to 0.31)	2.52 (-0.20 to 0.50)	0.0 (0.0 to 0.0)
Hemoglobin	5.0 g/L	1.52 (-0.06 to 0.16)	1.36 (-0.17 to 0.32)	0.0 (0.0 to 0.0)
	9.0 g/L	0.61 (-0.09 to 0.14)	1.26 (-0.17 to 0.32)	0.0 (0.0 to 0.0)
Triglycerides	2.0 g/L	- 0.61 (-0.12 to 0.07)	4.37 (0.01 to 0.44)	0.0 (0.0 to 0.0)
	4.6 g/L	-0.93	-0.95	0.0

		(-0.12 to 0.07)	(-0.27 to 0.17)	(0.0 to 0.0)
	10.0 g/L	0.0 (-0.10 to 0.10)	-0.37 (-0.24 to 0.19)	0.0 (0.0 to 0.0)
Rheumatoid Factor	250 IU/mL	-1.54 (-0.12 to 0.02)	-1.91 (-0.34 to 0.14)	0.0 (0.0 to 0.0)
	1000 IU/mL	-0.61 -0.10 to 0.05	-3.6 -0.44 to 0.04	0.0 (0.0 to 0.0)

f. Assay cut-off:

See clinical cut-off

2. Comparison studies:

a. Method comparison with predicate device:

For analysis of agreement with the predicate device, a total of 493 preoperatively collected serum specimens from pre-menopausal and post-menopausal women presenting with an adnexal mass requiring surgical intervention (refer to the section 'Clinical Studies' for a description of the clinical study and patient demographics) were assayed on both OVA1 Next Generation and OVA1 Test to generate OVA1 scores. All women had a physician's pre-surgical assessment (PA) by a non-gynecological oncologist and clinical prediction of malignant ovarian tumor (prior to surgery) using the combination of PA and OVA1 score (dual assessment) if either the PA was malignant or the OVA1 score indicated high risk for finding malignancy on surgery.

The comparison of performance for risk stratification between dual assessment of PA with OVA1 Next Generation (PA + OVA1 Next Generation) and dual assessment of PA with OVA1 Test (PA + OVA1 Test) for all evaluable subjects, and malignant and benign cases as determined by pathology is summarized in the table below. Results showed that PA+OVA1 Next Generation and PA+OVA1 Test agreed on 187 high risk cases and 168 low risk cases for a total percentage agreement of 355 of 493 cases (72%). For risk stratification agreement of malignant cases, PA+OVA1 Next Generation and PA+OVA1 Test agreed on 88 high risk cases and 2 low risk cases (misclassified) for a total percentage agreement of 86 of 92 cases (93.5%). For benign cases, PA+OVA1 Next Generation and PA+OVA1 Test agreed on the classification of 166 of 401 benign cases (41% of all benign cases) but incorrectly classified 103 benign cases as high risk (26%). PA+OVA1 Next Generation correctly classified 94 benign cases as low risk which PA+OVA1 Test classified as high risk (23% of benign cases correctly classified by PA+OVA1 Next Generation but not PA+OVA1 Test). PA+OVA1 Next Generation incorrectly classified 38 benign cases as high risk which PA+OVA1 Test classified as low risk (9% of benign cases correctly classified by PA+OVA1 Test but not PA+OVA1 Next Generation). Overall, PA+OVA1 Next Generation showed a net improvement of 14% in the classification of benign subjects.

All Evaluable Subjects				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	187	40	227
	Low risk	98	168	266
	Total	285	208	493
Positive Percent Agreement: 65.6% (87/285) 95% CI: 59.9% to 70.9% Negative Percent Agreement: 80.8% (168/208) 95% CI: 74.9% to 85.5% Total Percent Agreement: 72.0% (355/493) 95% CI: 67.9% to 75.8%				
Malignant Cases				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	84	2	86
	Low risk	4	2	6
	Total	88	4	92
Total Percent Agreement: 93.5% (86/92) 95% CI: 86.5% to 97.0%				
Benign Cases				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	103	38	141
	Low risk	94	166	260
	Total	197	204	401
Total Percent Agreement: 67.1% (269/401) 95% CI: 62.3% to 71.5%				

The proportions of high and low risk classifications between PA+OVA1 Next Generation and PA+OVA1 Test stratified by pre-menopausal subjects is summarized in the table below:

Pre-menopausal Subjects				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	84	24	108
	Low risk	45	123	168
	Total	129	147	276

Positive Percent Agreement: 65.1% (84/129) 95% CI: 56.6% to 72.8% Negative Percent Agreement : 83.7% (123/147) 95% CI: 76.9% to 88.8% Total Percent Agreement : 75.0% (207/276) 95% CI: 69.6% to 79.7%				
Malignant Cases				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	27	1	28
	Low risk	2	1	3
	Total	29	2	31
Total Percent Agreement: 90.3% (28/31) 95% CI: 75.1% to 96.7%				
Benign Cases				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	57	23	80
	Low risk	43	122	165
	Total	100	145	245
Total Percent Agreement: 73.1% (179/245) 95% CI: 67.2% to 78.2%				

The proportions of high and low risk classifications between PA+OVA1 Next Generation and PA+OVA1 Test stratified by post-menopausal subjects is summarized in the table below:

Post-menopausal Subjects				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	103	16	119
	Low risk	53	45	98
	Total	156	61	217
Positive Percent Agreement: 66.0% (103/156) 95% CI: 58.3% to 73.0% Negative Percent Agreement: 73.8% (45/61) 95% CI: 61.6% to 83.2% Total Percent Agreement: 68.2% (148/217) 95% CI: 61.7% to 74.0%				
Malignant Cases				
		Dual Assessment of PA with OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	57	1	58
	Low risk	2	1	3
	Total	59	2	61
Total Percent Agreement: 95.1% (58/61) 95% CI:86.5% to 98.3%				
Benign Cases				
		Dual Assessment of PA with		

		OVA1 Test		
		High risk	Low risk	Total
Dual Assessment of PA with OVA1 Next Generation	High risk	46	15	61
	Low risk	51	44	95
	Total	97	59	156
Total Percent Agreement: 57.7% (90/156) 95% CI: 49.8% to 65.2%				

b. Matrix comparison:

Serum is the only claimed matrix for each of the five analytes evaluated.

3. Clinical studies:

a. Clinical sensitivity and specificity:

The ability of OVA1 Next Generation to contribute to the physician's pre-surgical assessment (PA) was evaluated in two studies. The first study used archived serum specimens collected from a prospective, multi-site clinical study for pre-menopausal and post-menopausal women presenting with an adnexal mass requiring surgical intervention (Bristow et al. 2013; Ovarian malignancy risk stratification of the adnexal mass using a multivariate index assay. *Gynecologic Oncology* 128; 252-259). The clinical study subject enrollment centers are representative of institutions where ovarian tumor subjects potentially undergo a gynecologic examination. The specimens were collected at 27 non-gynecologic oncology practices.

A total of 493 women (age range 18–87) were evaluable in the study. For each patient, an initial PA was completed by a non-gynecological oncologist, providing the assessment of the patient's mass as benign (negative) or malignant (positive) based upon the information available to the non-gynecological oncologist during their work-up of the patient. The corresponding histopathology reports were collected after surgery.

Using a preoperatively collected serum sample, the OVA1 Next Generation test score was determined and the patient was stratified into a low or high risk group for finding malignancy on surgery. For each patient, the OVA1 Next Generation test result was compared to the pathology report from biopsied tissue. Malignancy included epithelial ovarian cancer (EOC), other primary ovarian malignancy, ovarian tumor with low malignant potential, malignancy extending to, or metastatic to ovaries, and malignancy that neither arose in nor involved the ovaries. A positive result, indicating high probability of malignancy for pre-menopausal and post-menopausal subjects was defined as an OVA1 Next Generation high risk result (risk score value of 5.0 or greater).

Subject enrollment demographics and pathology diagnoses are presented in the table below.

	All Evaluable	Pre-menopausal	Post-menopausal
N	493	276	217
Age, years			
Mean (SD)	48.6 (14.16)	39.5 (8.96)	60.2 (10.74)
Median	48	41	60
Range (min, max)	(18,87)	(18,60)	(33,87)
Ethnicity/race, n (%)			
White	347 (70.4)	173 (62.7)	174 (80.2)
Black or African American	81 (16.4)	54 (19.6)	27 (12.4)
Hispanic or Latino	46 (9.3)	36 (13.0)	10 (4.6)
Asian	13 (2.6)	8 (2.9)	5 (2.3)
Native Hawaiian/Pacific Islander	1 (0.2)	1 (0.4)	0 (0.0)
Other	5 (1.0)	4 (1.4)	1 (0.5)
No. of pregnancies, n (%)			
None	80 (16.2)	56 (20.3)	24 (11.1)
1	86 (17.4)	52 (18.8)	34 (15.7)
2	131 (26.6)	70 (25.4)	61 (28.1)
3	94 (19.1)	50 (18.1)	44 (20.3)
4 or more	102 (20.7)	48 (17.4)	54 (24.9)
Histopathological classification, n (%)			
Benign ovarian conditions	401 (81.3)	245 (88.8)	156 (71.9)
Epithelial ovarian cancer (EOC)	60 (12.2)	18 (6.5)	42 (19.4)
Other primary ovarian malignancies (non-EOC)	5 (1.0)	5 (1.8)	0 (0.0)
Ovarian tumors with low malignant potential (LMP)	17 (3.4)	5 (1.8)	12 (5.5)
Non-primary ovarian malignancies with involvement of the ovaries	6 (1.2)	2 (0.7)	4 (1.8)
Non-primary ovarian malignancies with no involvement of ovaries	4 (0.8)	1 (0.4)	3 (1.4)
Tumor stage, n (% of all primary malignant ovarian tumor)			
Stage 1	28 (43.1)	9 (39.1)	19 (45.2)
Stage 2	7 (10.8)	2 (8.7)	5 (11.9)
Stage 3	25 (38.5)	10 (43.5)	15 (35.7)
Stage 4	5 (7.7)	2 (8.7)	3 (7.1)

Performance for Pre-menopausal and Post-menopausal Subjects Combined

Among 493 subjects, there were 92 subjects with malignancy by pathology and 401 subjects with no malignancy by pathology. OVA 1 Next Generation performance was compared with clinical pathology:

Malignancy by Pathology				
		Physician Assessment (PA)		Total
OVA1 Next Generation	Positive	66	18	84
	Negative	2	6	8
Total		68	24	92
No Malignancy by Pathology				
		Physician Assessment (PA)		Total
OVA1 Next Generation	Positive	12	112	124
	Negative	17	260	277
Total		29	372	401

The analysis examined whether patient referral to gynecologic oncologist is supported when dual assessment is determined to be positive (either OVA1 Next Generation or PA is positive or both are positive). A dual assessment is negative when both OVA1 Next Generation and PA are negative.

Performance	Physician Assessment (PA)	Dual Assessment (PA and OVA1 Next Generation result used)
Sensitivity 95% CI	73.9% (68/92) 64.1% to 81.8%	93.5% (86/92) 86.5% to 97.0%
Specificity 95% CI	92.8 (372/401) 89.8% to 94.9%	64.8% (260/401) 60.0% to 69.4%
PPV 95% CI	70.1% (68/97) 61.9% to 77.3%	37.9% (86/227) 34.5% to 41.4%
NPV 95% CI	93.9% (372/396) 91.8% to 95.7%	97.7 (260/266) 95.4 to 98.9
Prevalence	18.7% (92/493)	

With dual assessment, sensitivity for malignancy increased from 73.9% to 93.5%. Specificity for malignant diagnoses decreased from 92.8% to 64.8% with dual assessment. PPV of the dual assessment decreased from 70% to 38%. However, NPV of the dual assessment significantly increased from 93.9% to 97.7%, supporting improved performance by dual assessment. The confidence interval for the observed 3.8% increase was 1.8% to 6.2%,

Performance characteristics of the OVA1 Next Generation test in combination with PA for the 493 patients classified according to their menopausal status are presented separately in the tables below:

Pre-menopausal subjects

Among 276 pre-menopausal subjects, there were 31 subjects with malignancy by pathology and 245 subjects with no malignancy by pathology. OVA 1 Next Generation was compared to the PA alone:

Malignancy by Pathology				
		Physician Assessment (PA)		Total
OVA1 Next Generation	Positive	23	5	28
	Negative	0	3	3
Total		23	8	31
No Malignancy by Pathology				
		Physician Assessment (PA)		Total
OVA1 Next Generation	Positive	5	65	70
	Negative	10	165	175
Total		15	230	245

With dual assessment, sensitivity for malignancy increased from 74.2% to 90.3%. PPV of the dual assessment decreased from 60.5% to 25.9% and NPV of the dual assessment increased from 96.6% to 98.2%. The confidence interval for the observed 1.6% increase was 0.1% to 3.7%.

Performance	**Physician Assessment (PA)**	**Dual Assessment (PA and OVA1 Next Generation result used)**
Sensitivity 95% CI	74.2% (23/31) 56.8% to 86.3%	90.3% (28/31) 75.1% to 96.7%
Specificity 95% CI	93.9% (230/245) 90.1% to 96.3%	67.3% (165/245) 61.2% to 72.9%
PPV 95% CI	60.5% (23/38) 47.0% to 72.3%	25.9% (28/108) 21.5% to 30.2%
NPV 95% CI	96.6% (230/238) 94.4% to 98.3%	98.2% (165/168) 95.9% to 99.5%
Prevalence	11.2% (31/276)	

Post-menopausal subjects

Among 217 post-menopausal subjects, there were 61 subjects with malignancy by pathology and 156 subjects with no malignancy by pathology. OVA 1 Next Generation was compared to the PA alone:

Malignancy by Pathology				
		Physician Assessment (PA)		Total
OVA1 Next Generation	Positive	43	13	56
	Negative	2	3	5
Total		45	16	61

No Malignancy by Pathology				
		Physician Assessment (PA)		Total
OVA1 Next Generation	Positive	7	47	54
	Negative	7	95	102
Total		14	142	156

With dual assessment, sensitivity for malignancy increased from 73.8% to 95.1%. PPV of the dual assessment decreased from 76.3% to 48.7% and NPV of the dual assessment significantly increased from 89.9% to 96.9%. The confidence interval for the observed 7.0% increase was 2.8% to 11.5%.

Performance	**Physician Assessment (PA)**	**Dual Assessment (PA and OVA1 Next Generation result used)**
Sensitivity 95% CI	73.8% (45/61) 61.6% to 83.2%	95.1% (58/61) 86.5% to 98.3%
Specificity 95% CI	91.0% (142/156) 85.5% to 94.6%	60.9% (95/156) 53.1% to 68.2%
PPV 95% CI	76.3% (45/59) 65.9% to 84.5%	48.7% (58/119) 43.8%% to 54.2%
NPV 95% CI	89.9% (142/158) 85.8% to 93.3%	96.9% (95/98) 92.0% to 99.0%
Prevalence	28.1% (61/217)	

Performance Characteristics for OVA1 Next Generation Alone

The OVA1 Next Generation test is not for use as a stand-alone test. Clinical/imaging evaluation is needed in order to identify patients in the intended use population (women over age 18, ovarian adnexal mass present for which surgery is planned, and not yet referred to an oncologist), for whom the performance characteristics of the test are established. These tables below compare the performance of the OVA1 Next Generation test to that of the predicate OVA1 Test as stand-alone tests, despite the fact that both are intended to be used in conjunction with PA.

Clinical Performance of OVA1 Next Generation and OVA1 Test Stand-Alone for All Evaluable Subjects and Stratified by Menopausal status						
	OVA1 Next Generation			**OVA1 Test**		
	All Evaluable subjects	Pre-menopausal women	Post-menopausal women	All Evaluable subjects	Pre-menopausal women	Post-menopausal women
All malignancies						
Sensitivity	**91.3%** (84/92)	**90.3%** (28/31)	**91.8%** (56/61)	**92.4%** (85/92)	**93.5%** (29/31)	**91.8%** (56/61)
95% CI	83.8% to 95.5%	75.1% to 96.7%	82.2% to 96.4%	85.1% to 96.3%	79.3% to 98.2%	82.2% to 96.4%
All benign						
Specificity	**69.1%** (277/401)	**71.4%** (175/245)	**65.4%** (102/156)	**53.6%** (215/401)	**61.6%** (151/245)	**41.0%** (64/156)
95% CI	64.4% to 73.4%	65.5% to 76.7%	57.6% to 72.4%	47.8% to 58.4%	55.4% to 67.5%	33.6% to 48.9%
PPV	**40.4%** (84/208)	**28.6%** (28/98)	**50.9%** (56/110)	**31.4%** (85/271)	**23.6%** (29/123)	**37.8%** (56/148)
95% CI	36.5% to 44.3%	23.7% to 33.5%	45.3% to 56.8%	28.7% to 34.0%	19.9% to 27.0%	34.2% to 41.7%
NPV	**97.2%** (277/285)	**98.3%** (175/178)	**95.3%** (102/107)	**96.8%** (215/222)	**98.7%** (151/153)	**92.8%** (64/69)
95% CI	94.9% to 98.6%	95.7% to 99.5%	90.3% to 97.9%	94.0% to 98.5%	95.8% to 99.7%	85.2% to 96.7%

[a]- Characterization evaluated stand-alone risk stratification versus cutoff, without regard to results of physician assessment. OVA1 Next Generation is not intended as a stand-alone diagnostic test.

Sensitivity of OVA1 Next Generation was 91.3%, sensitivity of OVA1 Test was 92.4% and difference was -1.1% with 95% CI: -7.5%–5.3%. Specificity of OVA1 Next Generation was 69.1%, specificity of OVA1™ Test was 53.6% and difference was 15.5% with 95% CI: 9.8%–21.1%.

The tables below provide further characterization of the OVA1 Next Generation stand-alone performance stratified by histological subtype of malignancy and stage of primary ovarian malignancy for all evaluable subjects, pre-menopausal and post-menopausal women.

Sensitivity of OVA1 Next Generation and OVA1 Test for Histological Subtype of Malignancy						
	OVA1 Next Generation			**OVA1 Test**		
	All Evaluable subjects	Pre-menopausal women	Post-menopausal women	All Evaluable subjects	Pre-menopausal women	Post-menopausal women
EOC						
Sensitivity	**95.0%** (57/60)	**100%** (18/18)	**92.9%** (39/42)	**95.0%** (57/60)	**100%** (18/18)	**92.9%** (39/42)
95% CI	86.3% to 98.3%	82.4% to 100%	81.0% to 97.5%	86.3% to 98.3%	82.4% to 100%	81.0% to 97.5%
Non-EOC Malignancies						
Sensitivity	**80.0%** (4/5)	**80.0%** (4/5)	**n/a**	**80.0%** (4/5)	**80.0%** (4/5)	**n/a**
95% CI	37.6% to 96.4%	37.6% to 96.4%		37.6% to 96.4%	37.6% to 96.4%	
Low Malignant Potential						
Sensitivity	**82.4%** (14/17)	**80.0%** (4/5)	**83.3%** (10/12)	**82.4%** (14/17)	**80.0%** (4/5)	**83.3%** (10/12)
95% CI	59.0% to 93.8%	37.6% to 96.4%	55.2% to 95.3%	59.0% to 93.8%	37.6% to 96.4%	55.2% to 95.3%
Malignancies Metastatic to the Ovaries						
Sensitivity	**100%** (6/6)	**100%** (2/2)	**100%** (4/4)	**100%** (6/ 6)	**100%** (2/2)	**100%** (4/4)
95% CI	61.0% to 100%	34.2% to 100%	51.0% to 100%	61.0% to 100%	34.2% to 100%	51.0% to 100%
Other non-Ovarian Malignancies						
Sensitivity	**75.0%** (3/4)	**0.0%** (0/1)	**100%** (3/3)	**100%** (4/4)	**100%** (1/1)	**100%** (3/3)
95% CI	30.1% to 95.4%	0.0% to 79.3%	43.9% to 100%	51.0% to 100%	20.7% to 100%	43.9% to 100%

[a]- Characterization evaluated stand-alone risk stratification versus cutoff, without regard to results of physician assessment. OVA1 Next Generation is not intended as a stand-alone diagnostic test.

There were no differences in the number of primary ovarian malignancies detected between OVA1 Next Generation and OVA1 Test (61/65 vs. 61/65). One fewer non-ovarian malignancy in a pre-menopausal woman was classified as high risk by OVA1 Next Generation (sensitivity of 75% or 3/4 for OVA1 Next Generation vs. 100% or 4/4 for OVA1 Test). One fewer Stage I primary ovarian malignancy (sensitivity of 85.7% or 24/28 for OVA1 Next Generation vs. 89.3% or 25/28 for OVA1 Test) and one more Stage III primary ovarian was detected (sensitivity of 100% or 25/25 for OVA1 Next Generation vs. 96% or 24/25 for OVA1 Test). The single stage I missed and the single stage III case picked up by OVA1 Next Generation were both in the post-menopausal subgroup.

Sensitivity of OVA1 Next Generation and OVA1 Test for Stage of Primary Ovarian Malignancy						
	OVA1 Next Generation			**OVA1 Test**		
	All Evaluable subjects	Pre-menopausal women	Post-menopausal women	All Evaluable subjects	Pre-menopausal women	Post-menopausal women
Stage I						
Sensitivity	**85.7%** (24/28)	**88.9%** (8/9)	**84.2%** (16/19)	**89.3%** (25/28)	**88.9%** (8/9)	**89.5%** (17/19)
95% CI	68.5% to 94.3%	56.5% to 98.0%	62.4% to 94.5%	72.8% to 96.3%	56.5% to 98.0%	68.6% to 97.1%
Stage II						
Sensitivity	**100%** (7/7)	**100%** (2/ 2)	**100%** (5/5)	**100%** (7/7)	**100%** (2/2)	**100%** (5/5)
95% CI	64.6% to 100%	34.2% to 100%	56.6% to 100%	64.6% to 100%	34.2% to 100%	56.6% to 100%
Stage III						
Sensitivity	**100%** (25/25)	**100%** (10/10)	**100%** (15/15)	**96.0%** (24/25)	**100%** (10/10)	**93.3%** (14/15)
95% CI	86.7% to 100%	72.2% to 100%	79.6% to 100%	80.5% to 99.3%	72.2% to 100%	70.2% to 98.8%
Stage IV						
Sensitivity	**100%** (5/5)	**100%** (2/2)	**100%** (3/3)	**100%** (5/5)	**100%** (2/2)	**100%** (3/3)
95% CI	56.6% to 100%	34.2% to 100%	43.9% to 100%	56.6% to 100%	34.2% to 100%	43.9% to 100%

[a]- Characterization evaluated stand-alone risk stratification versus cutoff, without regard to results of physician assessment. OVA1 Next Generation is not intended as a stand-alone diagnostic test.

One fewer Stage I primary ovarian malignancy (sensitivity of 85.7% or 24/28 for OVA1 Next Generation vs. 89.3% or 25/28 for OVA1 Test) and one more Stage III primary ovarian was detected (sensitivity of 100% or 25/25 for OVA1 Next Generation vs. 96% or 24/25 for OVA1 Test). The single stage I missed and the single stage III case picked up by OVA1 Next Generation were both in the post-menopausal subgroup.

In the second study, in addition to the long-term archived serum specimens collected from the multi-site clinical study described above, sensitivities and specificities of OVA1 Next Generation and OVA1 Test were compared using serum specimens that been stored at -65 °C to -85 °C and tested for OVA1 Next Generation and OVA1 Test no more than one year after collection. These samples were acquired from prospective studies that recruited premenopausal and postmenopausal women presenting with an ovarian adnexal mass requiring surgical intervention. The purpose of the comparison was to demonstrate that for samples archived less than one year prior to testing, the OVA1 Next Generation test showed similar sensitivity

and specificity when compared with the OVA1 Test. This blinded study included 28 patients confirmed by pathology to have primary ovarian malignancy, along with 105 patients with benign conditions. A comparison of OVA1 Next Generation and OVA1 Test standalone sensitivity in this set of samples is shown in the tables below.

	OVA1 Next Generation	**OVA1 Test**	**Difference OVA1 Next Generation - OVA1 Test**
All subjects			
Sensitivity	**78.6%** (22/28)	**82.1%** (23/28)	**-3.6%** (1/28)
95% CI	60.5% to 89.9%	64.4% to 92.1%	-19.2% to 12.0%
Specificity	**74.3%** (78/105)	**57.1%** (60/105)	**17.2%** (18/105)
95% CI	65.2% to 81.7%	47.6% to 66.2%	7.1% to 27.2%*

[a]- Characterization evaluated stand-alone risk stratification versus cutoff, without regard to results of physician assessment. OVA1 Next Generation is not intended as a stand-alone diagnostic test.

* - Performance was considered statistically different if the 95% CI of the difference did not bound or contain zero.

Stage	**N**	**OVA1 Next Generation Sensitivity%**	**OVA1 Test Sensitivity%**
I	10	90.0% (9/10)	90.0% (9/10)
II	1	100% (1/1)	100% (1/1)
III	9	88.9% (8/9)	88.9% (8/9)
IV	3	66.7% (2/3)	100% (3/3)
Not Staged	5	40.0% (2/5)	40.0% (2/5)

[a]- Characterization evaluated stand-alone risk stratification versus cutoff, without regard to results of physician assessment. OVA1 Next Generation is not intended as a stand-alone diagnostic test.

b. Other clinical supportive data (when a. is not applicable):

4. Clinical cut-off**:**

The results are interpreted as follows:

Low probability of malignancy OVA1 Next Generation risk score < 5.0
High probability of malignancy OVA1 Next Generation risk score ≥ 5.0

5. Expected values/Reference range:

The reference interval of OVA1 Next Generation test was determined in 68 pre-menopausal and 84 post-menopausal healthy women (total = 152 evaluable subjects). Ages ranged from 18 to 91 years and represented whites (84.9%), Hispanic/Latino (7.2%) and African American (5.3%) subjects. The mean, SD, median, range and 5^{th} to 95^{th} percentile of OVA1 Next Generation scores and the OVA1 Next Generation test results are shown for each group in the table below. It is recommended that each laboratory establish its own reference range for the population of interest.

OVA1 Next Generation Scores and Results in Healthy Subjects			
	All Evaluable Subjects	**Pre-menopausal Women**	**Post-menopausal Women**
n (%)	152 (100)	68 (44.7)	84 (55.3)
Mean age (SD)	51.0 (13.75)	39.2 (8.23)	60.5 (9.22)
Median age	51	41	59
OVA1 Next Generation score			
Mean (SD)	3.94 (0.984)	3.72 (0.938)	4.12 (0.989)
Median	3.90	3.60	4.05
Range (min, max)	(2.2, 7.1)	(2.2, 6.1)	(2.5, 7.1)
Reference interval (5^{th}, 95^{th} percentiles)	(2.5, 5.9)	(2.4, 5.3)	(2.9, 5.9)
OVA1 Next Generation result, n (%)			
Positive	23 (15.1%)	9 (13.2%)	14 (16.7%)
Negative	129 (84.9%)	59 (86.8%)	70 (83.3%)

Expected Values in Non-Ovarian Malignancy Condition: To evaluate the performance of the OVA1 Next Generation test in subjects with other disease conditions, patients with cancer conditions other than ovarian cancer (bladder cancer, breast cancer, cervical cancer, colon cancer, endometrial cancer, lung cancer, leukemia and lymphoma) and patients with non-cancer conditions (autoimmune disease, cardiac disease, diabetes, endometriosis, hepatitis and kidney diseases) were evaluated.

Evaluable Specimens from Subjects with non-Ovarian Cancers and Other Conditions	
Bladder cancer	20
Breast cancer	40
Cervical cancer	20
Colon cancer	40
Endometrial cancer	40
Leukemia	11
Lung cancer	40
Lymphoma	10
Autoimmune disease	20
Cardiac disease	20

Evaluable Specimens from Subjects with non-Ovarian Cancers and Other Conditions	
Diabetes	40
Endometriosis	40
Hepatitis	20
Kidney disease	20
Pregnant women	20
All specimens	401

The mean, median, standard deviation, 5th to 95th percentiles as observed in the data are shown for each condition group. Using the defined cut-off of 5.0 for OVA1 Next Generation scores, the number of positive (≥ 5.0) and negative (< 5.0) cases is presented below:

OVA1 Next Generation Scores and Results in Subjects with Non-Ovarian Cancers								
	Bladder Cancer	Breast Cancer	Cervical Cancer	Colon Cancer	Endometrial Cancer	Leukemia	Lung Cancer	Lymphoma
n	20	40	20	40	40	11	40	10
OVA1 Next Generation score, statistics								
Mean (SD)	5.32 (1.83)	4.03 (1.22)	6.56 (1.91)	5.11 (1.73)	5.45 (1.72)	6.66 (1.30)	5.02 (1.38)	6.10 (1.91)
Median	4.8	3.9	7.7	4.6	4.8	7.2	4.9	6.0
5th, 95th percentiles	2.9, 8.2	2.8, 6.6	3.5, 8.5	3.0, 7.7	3.1, 8.1	4.4, 8.1	3.1, 7.5	2.4, 8.5
Range min, max	2.8, 8.5	2.6, 8.4	2.6, 8.5	2.4, 8.1	3.0,8.1	4.4, 8.1	2.8, 7.8	2.4, 8.5
OVA1 Next Generation test result, n								
Positive	10	6	13	18	20	10	18	8
Negative	10	34	7	22	20	1	22	2
% negative results	50	85	35	55	50	9.1	55	20

OVA1 Next Generation Scores and Results in Subjects with Conditions Other than Cancers							
	Autoimmune Disease	Cardiac Disease	Diabetes	Endometriosis	Hepatitis	Kidney Disease	Pregnant Women
n	20	20	40	40	20	20	20
OVA1 Next Generation score, statistics							
Mean (SD)	5.52 (1.86)	6.12 (1.58)	4.72 (1.67)	4.35 (1.38)	5.19 (1.78)	6.65 (1.37)	5.31 (0.35)
Median	5.9	6.4	4.2	4.2	5.1	7.2	5.3
5th, 95th percentiles	2.8, 8.2	3.6, 8.1	2.5, 8.1	2.5, 7.2	3.0, 7.9	3.8, 8.3	4.8, 6.0
Range min, max	2.4, 8.3	3.3, 8.3	2.0, 8.1	2.2, 7.9	2.7, 7.9	3.3, 8.3	4.5, 6.2

OVA1 Next Generation test result, n							
Positive	11	15	14	11	11	18	19
Negative	9	5	26	29	9	2	1
% negative results	45	25	65	72.5	45	10	5

The number of cases is small within each of the examined diseases and conditions, but the results suggest that caution is warranted when interpreting OVA1 Next Generation results for pregnant women and patients with cervical cancer, leukemia, lymphoma, cardiac disease, kidney disease.

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.