DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Food and Drug Administration
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VERMILLION, INC. May 26, 2016
BENJAMIN A. KIMBALL
SENIOR DIRECTOR OF REGULATORY AND
QUALITY AFFAIRS, COMPLIANCE OFFICER
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AUSTIN, TEXAS 78738

Re: K150588
Trade/Device Name: OVA1 Next Generation
Regulation Number: 21 CFR §866.6050
Regulation Name: Ovarian adnexal mass assessment score test system
Regulatory Class: Class II
Product Code: ONX
Dated: February 22, 2016
Received: March 1, 2016

Dear Mr. Kimball:

This letter corrects our substantially equivalent letter of March 18, 2016.

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 regulation (21 CFR Part 801), 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" (21CFR 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

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

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement below.

510(k) Number *(if known)*
K150588

Device Name
OVA1 Next Generation

Indications for Use *(Describe)*

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.

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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510(k) Summary
OVA1 Next Generation

510(k) Number: K150588

Manufacturer Identification

Submitted by: Vermillion, Inc.
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Date Prepared:	03.06.2015
Proprietary Name	OVA1 Next Generation
Common Name	Ovarian adnexal mass assessment score test system
Device Classification	21 CFR 866.6050
Proposed Regulatory Class	Class II
Device Product Code	ONX

Purpose of this Special 510(k)

This Traditional 510(k) seeks clearance for a new test.

Device Description

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

The assays used to generate the numeric result (OVA1 NG test result) are APO, CA 125 II, FSH, HE4 and TRF.

Biomarker values are determined using assays on the Roche cobas® 6000 system, which is a fully automated, software-controlled system for clinical chemistry and immunoassay analysis. The biomarker assays are run according to the manufacturer's instructions as detailed in the package insert for each reagent.

The OVA1 NG software (OvaCalc v4.0.0) contains a proprietary algorithm that utilizes the results (values) from the five biomarker assays, (APO, CA 125 II, FSH, HE4 and TRF). 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 NG test result between 0.0 and 10.0. A low- or high-risk result is then determined by comparing the software-generated risk score to a single cutoff (low-risk result <5, high-risk result ≥5).

The analytes and corresponding analytes and calibrators are as follows:

Analyte	Reagent and Calibrator	Instrument
Apolipoprotein A-1	cobas APO A1, C.F.A.S. Lipids	Roche cobas 6000: Roche cobas® c501
CA 125	cobas CA 125 Gen 2, 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
Transferrin	cobas Transferrin, C.F.A.S. Proteins	Roche cobas 6000: Roche cobas® c501

Substantial Equivalence Information:

Predicate Device and K number:
OVA1: K081754

See Tables 1 and 2 below for a comparison with predicate technological characteristics.

Table 1 - Similarities

Item	Subject Device	Predicate OVA1
Intended 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	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

Item	Subject Device	Predicate OVA1
	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.	and radiological evaluation does not indicate malignancy. The test is not intended as a screening or stand-alone diagnostic assay.
Indications for Use	The OVA1 Next Generation test is a qualitative serum test that combines the results of five immunoassays, Apolipoprotein A-1 (APO), cancer antigen 125 (CA 125 II), Follicle Stimulating Hormone (FSH), Human epididymis protein 4 (HE4), and Transferrin (TRF) 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.	Substantially the same as Subject device
Boxed Warning	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 as Subject device
Sample Matrix	Serum	Same as Subject device
Type of Test	Algorithm	Same as Subject device
Analytes	APO, CA 125, FSH, HE4, TRF	APO, CA 125, TRF

Table 2 - Differences

Item	Subject Next Generation	Predicate OVA1
Analytes	FSH	Beta-2 microglobulin, Prealbumin
Measurement	Score based on 5 analytes	Score based on 5 analytes
Clinical Cutoff	5.0	5.0 Premenopausal, 4.4 Postmenopausal
Platform	Roche *cobas* e601, c501	BNII, Roche Elecsys 2010

Intended Use of the Device

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 intended to be part of the preoperative evaluation to aid in assessing whether a woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy at surgery. The OVA1 Next Generation test must be interpreted in conjunction with an independent clinical and imaging evaluation. 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.

Indications for Use

The OVA1 Next Generation test is a qualitative serum test that combines the results of five immunoassays, Apolipoprotein A-1 (APO), cancer antigen 125 (CA 125 II), Follicle Stimulating Hormone (FSH), Human epididymis protein 4 (HE4), and Transferrin (TRF) into a single numeric result. The OVA1 Next Generation test is intended to aid in assessing whether a woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy as part of the preoperative evaluation. 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 must be interpreted in conjunction with an independent clinical and imaging evaluation. The test is not intended as a screening or stand-alone diagnostic assay.

Clinical Performance Evaluation:

Clinical and Analytical testing included:

- Clinical Performance on OVA500 cohort
- Clinical Specificity - Healthy Pre- and Postmenopausal women

- Clinical Specificity - Other Cancers and Diseases
- Precision
- Reproducibility
- Sample Stability
- Interference

Clinical Performance Evaluation

Description of Subjects:

The clinical study used a banked sample set from a prospective, multi-site pivotal study of OVA1 – the OVA500 Study – which was published in a leading peer-reviewed specialty journal in Feb 2013. Four hundred ninety-three of the banked samples were used to conduct a side-by-side clinical validation for Substantial Equivalence purposes.

The external sites received the samples for blinded validation testing and did not have access to patient or physician information. The validation sites sent the OvaCalc reports to an independent 3rd party statistician for analysis.

Clinicians were required to document the results of physical examination, family history, imaging, laboratory tests (including CA 125, when available, but not OVA1), and to make a formal pre-surgical prediction of malignancy. In cases where the formal prediction was done by a clinician other than the enrolling physician, the referral history and the specialty of the clinician who made the prediction were recorded, as was the specialty of the surgeon who ultimately operated on each patient. In order to reflect their routine clinical judgment and referral behavior, physicians were not asked to either follow any specific prediction algorithm or justify their prediction. Postoperative pathology diagnosis was recorded at each enrolling site and independently reviewed.

A total of 519 subjects were reported on for this study, of which 493 were evaluable for OVA1 and Physician Assessment (PA). The demographics are detailed in Table 3 below:

Table 3 - Specimen and subject disposition – Demography

		Evaluable Subjects		
	All Enrolled Subjects (N= 519)	**All Evaluable Subjects (N= 493)**	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
Age, years				
N	519	493	276	217
Mean (SD)	48.4 (14.32)	48.6 (14.16)	39.5 (8.96)	60.2 (10.74)
Median	47	48	41	60
Range (min, max)	18, 87	18, 87	18, 60	33, 87
Ethnicity/race, n(%)				
Asian	13 (2.5)	13 (2.6)	8 (2.9)	5 (2.3)
Black or African American	86 (16.6)	81 (16.4)	54 (19.6)	27 (12.4)

		Evaluable Subjects		
	All Enrolled Subjects (N= 519)	**All Evaluable Subjects (N= 493)**	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
Native Hawaiian/Pacific islander	1 (0.2)	1 (0.2)	1 (0.4)	0 (0.0)
White	365 (70.3)	347 (70.4)	173 (62.7)	174 (80.2)
Other	5 (1.0)	5 (1.0)	4 (1.4)	1 (0.5)
Hispanic or Latino	49 (9.4)	46 (9.3)	36 (13.0)	10 (4.6)
No. of pregnancies, n(%)				
None	87 (16.8)	80 (16.2)	56 (20.3)	24 (11.1)
1	87 (16.8)	86 (17.4)	52 (18.8)	34 (15.7)
2	141 (27.2)	131 (26.6)	70 (25.4)	61 (28.1)
3	97 (18.7)	94 (19.1)	50 (18.1)	44 (20.3)
4 or more	107 (20.6)	102 (20.7)	48 (17.4)	54 (24.9)
No. of live births, n(%)				
None	123 (23.7)	116 (23.5)	84 (30.4)	32 (14.7)
1	94 (18.1)	91 (18.5)	54 (19.6)	37 (17.1)
2	163 (31.4)	152 (30.8)	82 (29.7)	70 (32.3)
3	87 (16.8)	84 (17.0)	39 (14.1)	45 (20.7)
4 or more	52 (10.0)	50 (10.1)	17 (6.2)	33 (15.2)

Sensitivity and Specificity:
Specificity and sensitivity are primary clinical measures of the performance of diagnostic tests. When comparing specificities and sensitivities across multiple diagnostic tests (Subject compared to the Predicate), the different tests are applied to groups of subjects with the same disease status for a disease or medical condition under consideration, in this case ovarian cancer. Therefore, direct comparisons to clinical performance can be drawn from one device to another, in light of the "gold standard," pathology, as discussed above. This is especially informative when the different methods are compared in matched subjects; i.e. head to head, such that direct inferences can be made about concordance or differences on a per-subject basis, rather than a similar population.

In the case of ovarian cancer triage, specificity measures the percent of benign masses (a mass is diagnosed as benign after surgery and pathology review) that are correctly predicted as low risk.

Sensitivity measures the percent of all subjects with a malignant mass (adnexal malignancy is diagnosed after surgery and pathology review) that are correctly identified as such (i.e., the percentage of subjects who are correctly predicted as high risk).

Specificity – with PA:
Overall specificity for the Subject device improved by ~14% as compared to the Predicate OVA1 device. Postmenopausal specificity improved ~23% and premenopausal

specificity improved ~8% as compared to the Predicate OVA1 device. Please see Table 4 below for the Specificity with PA.

Table 4 – Specificity with PA

	All Evaluable Subjects (N= 493)	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
All benign			
PA OR Subject OVA1 Next Generation			
Specificity, %	64.8	67.3	60.9
n/N	260/ 401	165/ 245	95/ 156
95% CI	60.0 to 69.4	61.2 to 72.9	53.1 to 68.2
PA OR Predicate OVA1			
Specificity, %	50.9	59.2	37.8
n/N	204/ 401	145/ 245	59/ 156
95% CI	46.0 to 55.7	52.9 to 65.2	30.6 to 45.6
PA OR Subject OVA1 Next Generation vs PA OR Predicate OVA1			
Difference in specificity, %	13.97	8.16	23.08
95% CI for difference	8.52 to 19.41	1.75 to 14.58	13.53 to 32.62
Ratio of specificity	1.275	1.138	1.610
95% CI for ratio	1.158 to 1.410	1.027 to 1.267	1.309 to 2.010

Note: In this table, a positive combined test result is where the women has a high risk index score OR the pre-surgical PA was malignant. When the women has both a low risk index score and the pre-surgical PA was benign the combined test result is negative.

Sensitivity – with PA

Differences between the sensitivity of the Subject device and Predicate OVA1 device were clinically small. Clinically small is defined as the difference in any characteristic as a difference where; the 95% CI of the difference between the subject OVA1 Next Generation and the predicate OVA1 values bounds or contains the value 0, or; where the 95% CI of the ratio between the Subject OVA1 Next Generation and the predicate OVA1 values bounds or contains the value 1. Values where differences do not bound or contain 0, or where ratios do not bound or contain 1 will be described as "clinically significant."

For all subjects, premenopausal women, and postmenopausal women, the differences were ~2%, ~3%, and ~2%, respectively. Please see Table 5 below for the Sensitivity with PA.

Table 5 – Sensitivity with PA

	All Evaluable Subjects (N= 493)	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
All malignancies			
PA OR Subject OVA1 Next Generation			
Sensitivity, %	93.5	90.3	95.1
n/N	86/ 92	28/ 31	58/ 61
95% CI	86.5 to 97.0	75.1 to 96.7	86.5 to 98.3
PA OR Predicate OVA1			
Sensitivity, %	95.7	93.5	96.7
n/N	88/ 92	29/ 31	59/ 61
95% CI	89.3 to 98.3	79.3 to 98.2	88.8 to 99.1
PA OR Subject OVA1 Next Generation vs PA OR Predicate OVA1			
Difference in sensitivity, %	-2.17	-3.23	-1.64
95% CI for difference	-7.37 to 3.03	-14.12 to 7.67	-7.19 to 3.91
Ratio of sensitivity	0.977	0.966	0.983
95% CI for ratio	0.908 to 1.043	0.808 to 1.136	0.899 to 1.066

Note: In this table, a positive combined test result is where the women has a high risk index score OR the pre-surgical PA was malignant. When the women has both a low risk index score and the pre-surgical PA was benign the combined test result is negative.

Specificity – Standalone:

The overall standalone specificity for the Subject device improved ~16% as compared to the Predicate OVA1 device. For premenopausal women the improvement was ~10% and for postmenopausal women the improvement was ~24%. Please see Table 6 below for a summary of the specificity data.

Table 6 – Standalone Specificity

	All Evaluable Subjects (N= 493)	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
Specificity			
Subject OVA1 Next Generation			
Specificity, %	69.1	71.4	65.4
n/N	277/ 401	175/ 245	102/ 156
95% CI	64.4 to 73.4	65.5 to 76.7	57.6 to 72.4
Predicate OVA1			
Specificity, %	53.6	61.6	41.0
n/N	215/ 401	151/ 245	64/ 156
95% CI	48.7 to 58.4	55.4 to 67.5	33.6 to 48.9
Subject OVA1 Next Generation vs Predicate OVA1			
Difference in specificity, %	15.46	9.80	24.36
95% CI for difference	9.79 to 21.13	3.12 to 16.47	14.41 to 34.31

	All Evaluable Subjects (N= 493)	Pre-menopausal Women (N= 276)	Post-menopausal Women (N= 217)
Ratio of specificity	1.288	1.159	1.594
95% CI for ratio	1.172 to 1.423	1.047 to 1.290	1.304 to 1.974

Sensitivity – Standalone:
Standalone sensitivity was substantially equivalent for the Subject and Predicate OVA1 device. For all subjects the difference was clinically small at ~1%. For post-menopausal women both the Subject and Predicate had 91.8% performance and for pre-menopausal women there was a ~3% difference. Please see Table 7 below for a summary of the sensitivity data.

Table 7 – Standalone Sensitivity

	All Evaluable Subjects (N= 493)	Pre-menopausal Women (N= 276)	Post-menopausal Women (N= 217)
All malignancies			
Subject OVA1 Next Generation			
Sensitivity, %	91.3	90.3	91.8
n/N	84/ 92	28/ 31	56/ 61
95% CI	83.8 to 95.5	75.1 to 96.7	82.2 to 96.4
Predicate OVA1			
Sensitivity, %	92.4	93.5	91.8
n/N	85/ 92	29/ 31	56/ 61
95% CI	85.1 to 96.3	79.3 to 98.2	82.2 to 96.4
Subject OVA1 Next Generation vs Predicate OVA1			
Difference in sensitivity, %	-1.09	-3.23	0.00
95% CI for difference	-7.47 to 5.30	-14.12 to 7.67	-7.87 to 7.87
Ratio of sensitivity	0.988	0.966	1.000
95% CI for ratio	0.909 to 1.071	0.808 to 1.136	0.898 to 1.113

Sensitivity – Subtype of Ovarian Malignancy
All malignancies were evaluated and stratified by five subtypes;

1. Epithelial ovarian cancer (EOC)
2. Non-EOC malignancies
3. Low malignant potential (LMP)
4. Malignancies metastatic to the ovaries, and
5. Other, non-ovarian malignancies

The sensitivity of detection across these five ovarian cancer subtypes was retained in the Subject OVA1 Next Generation device as compared to the Predicate OVA1 device. The Subject OVA1 Next Generation device identified one less non-ovarian malignancy than the Predicate device. However, guidelines for GO referral focus on primary ovarian

cancer, since the specialized GO procedures are effective for primary ovarian cancer but clinically irrelevant for non-ovarian pelvic malignancies. In any case, when one considers the balance between specificity and sensitivity; and the improvement in the specificity of the Subject device, performance differences between the Subject and Predicate OVA1 device are de minims and clinically small. Please see Table 8 below for the Sensitivity based on Subtype of Ovarian Malignancy.

Table 8 – Sensitivity – Subtype of Ovarian Malignancy

	Epithelial ovarian cancer	**Non-EOC malignancies**	**Low malignant potential**	**Malignancies metastatic to the ovaries**	**Other non-ovarian malignancies**
All evaluable subjects					
Subject OVA1 Next Generation					
Sensitivity, %	95.0	80.0	82.4	100.0	75.0
n/N	57/ 60	4/ 5	14/ 17	6/ 6	3/ 4
95% CI	86.3 to 98.3	37.6 to 96.4	59.0 to 93.8	61.0 to 100.0	30.1 to 95.4
Predicate OVA1					
Sensitivity, %	95.0	80.0	82.4	100.0	100.0
n/N	57/ 60	4/ 5	14/ 17	6/ 6	4/ 4
95% CI	86.3 to 98.3	37.6 to 96.4	59.0 to 93.8	61.0 to 100.0	51.0 to 100.0
Premenopausal women					
Subject OVA1 Next Generation					
Sensitivity, %	100.0	80.0	80.0	100.0	0.0
n/N	18/ 18	4/ 5	4/ 5	2/ 2	0/ 1
95% CI	82.4 to 100.0	37.6 to 96.4	37.6 to 96.4	34.2 to 100.0	0.0 to 79.3
Predicate OVA1					
Sensitivity, %	100.0	80.0	80.0	100.0	100.0
n/N	18/ 18	4/ 5	4/ 5	2/ 2	1/ 1
95% CI	82.4 to 100.0	37.6 to 96.4	37.6 to 96.4	34.2 to 100.0	20.7 to 100.0
Postmenopausal women					
Subject OVA1 Next Generation					
Sensitivity, %	92.9	--	83.3	100.0	100.0
n/N	39/ 42	0/0	10/ 12	4/ 4	3/ 3
95% CI	81.0 to 97.5	--	55.2 to 95.3	51.0 to 100.0	43.9 to 100.0
Predicate OVA1					
Sensitivity, %	92.9	--	83.3	100.0	100.0
n/N	39/ 42	0/0	10/ 12	4/ 4	3/ 3
95% CI	81.0 to 97.5	--	55.2 to 95.3	51.0 to 100.0	43.9 to 100.0

Sensitivity – Stage of Primary Ovarian Malignancy:
The Subject OVA1 Next Generation device performed substantially equivalent to the Predicate OVA1 device for sensitivity across all early stage malignancies. For all evaluable subjects with early stage malignancies (stage I or II) the Subject OVA1 Next Generation device had a sensitivity of ~89% as compared to ~91% for the Predicate OVA1 device. For all evaluable subjects with late stage malignancies (stage III or IV) the sensitivity of the Subject device was 100% as compared to the Predicate OVA1 device at ~97%. Please see Table 9 below for a summary of results.

Table 9 – Summary of Results

	Stage I	Stage II	All Early Stage (I or II)	Stage III	Stage IV	All Late Stage (III or IV)
All evaluable subjects						
Subject OVA1 Next Generation						
Sensitivity, %	85.7	100.0	88.6	100.0	100.0	100.0
n/N	24/ 28	7/ 7	31/ 35	25/ 25	5/ 5	30/ 30
95% CI	68.5 to 94.3	64.6 to 100.0	74.0 to 95.5	86.7 to 100.0	56.6 to 100.0	88.6 to 100.0
Predicate OVA1						
Sensitivity, %	89.3	100.0	91.4	96.0	100.0	96.7
n/N	25/ 28	7/ 7	32/ 35	24/ 25	5/ 5	29/ 30
95% CI	72.8 to 96.3	64.6 to 100.0	77.6 to 97.0	80.5 to 99.3	56.6 to 100.0	83.3 to 99.4
Premenopausal women						
Subject OVA1 Next Generation						
Sensitivity, %	88.9	100.0	90.9	100.0	100.0	100.0
n/N	8/ 9	2/ 2	10/ 11	10/ 10	2/ 2	12/ 12
95% CI	56.5 to 98.0	34.2 to 100.0	62.3 to 98.4	72.2 to 100.0	34.2 to 100.0	75.8 to 100.0
Predicate OVA1						
Sensitivity, %	88.9	100.0	90.9	100.0	100.0	100.0
n/N	8/ 9	2/ 2	10/ 11	10/ 10	2/ 2	12/ 12
95% CI	56.5 to 98.0	34.2 to 100.0	62.3 to 98.4	72.2 to 100.0	34.2 to 100.0	75.8 to 100.0
Postmenopausal women						
Subject OVA1 Next Generation						
Sensitivity, %	84.2	100.0	87.5	100.0	100.0	100.0
n/N	16/ 19	5/ 5	21/ 24	15/ 15	3/ 3	18/ 18
95% CI	62.4 to 94.5	56.6 to 100.0	69.0 to 95.7	79.6 to 100.0	43.9 to 100.0	82.4 to 100.0
Predicate OVA1						
Sensitivity, %	89.5	100.0	91.7	93.3	100.0	94.4
n/N	17/ 19	5/ 5	22/ 24	14/ 15	3/ 3	17/ 18
95% CI	68.6 to 97.1	56.6 to 100.0	74.2 to 97.7	70.2 to 98.8	43.9 to 100.0	74.2 to 99.0

[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.

Table 10 shows a comparison of clinical performance of OVA1 Next Generation and OVA1®, with samples collected from selected larger prospective studies. These larger prospective studies recruited premenopausal and postmenopausal women presenting with an adnexal mass requiring surgical intervention. The purpose of the comparison was to demonstrate that for samples archived less than one year prior to testing, on the OVA1 Next Generation showed equivalent clinical performance when compared to OVA1. This blinded study included twenty eight patients confirmed by pathology to have primary ovarian malignancy, along with 105 block-randomized patients with benign conditions,

selected to balance the malignancy rate within each menopausal subgroup as well as to approximate the prevalence of primary ovarian malignancies found in OVA1 pivotal clinical trials. All serum samples had been archived at -65 °C to -85 °C and tested for OVA1 Next Generation and OVA1 tests no more than one year after collection. Tables 13 and 14 show comparisons of OVA1 Next Generation and OVA1 in this set of samples.

Table 10. Comparison of OVA1 Next Generation and OVA1 performance for a selected set of serum samples from patients confirmed by pathology to have primary ovarian malignancies (N=28) or benign ovarian conditions (N=105). Samples were tested within one year of collection

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

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

Table 11. OVA1 Next Generation and OVA1 test sensitivity by stage of primary ovarian malignancy for a selected set of samples tested within one year of collection

Stage	**N**	**OVA1 Next Generation % Sensitivity (n/N)**	**OVA1 % Sensitivity (n/N)**
I	10	90 (9/10)	90 (9/10)
II	1	100.0 (1/1)	100.0 (1/1)
III	9	88.9 (8/9)	88.9 (8/9)
IV	3	66.7 (2/3)	100.0 (3/3)
Not Staged	5	40.0 (2/5)	40.0 (2/5)

Positive and Negative Predictive Value:

Positive and negative predictive values are proportions of positive and negative results representing true positive and true negative diagnostic (pathology) findings. Positive predictive value (PPV) is the percent of subjects with a positive test result who truly have the disease. Negative predictive value (NPV) is the percent of subjects with a negative test result who truly do not have a malignancy.

It should be noted that the PPV is not intrinsic to the test as it critically depends also on the prevalence of the disease state. However, PPV of the Subject OVA1 Next Generation and the Predicate OVA1 device can be compared here because both results were generated on the same set of patient serum samples.

Positive Predictive Value (PPV):
As with the improved standalone specificity discussed above, the standalone PPV for the Subject device improved significantly as well, with a 9% increase overall in PPV as compared to the Predicate OVA1 device. Please see Table 12 for a summary of PPV results.

Table 12 – Positive Predictive Values

	All Evaluable Subjects (N= 493)	**Premenopausal Women (N= 276)**	**Postmenopausal Women (N= 217)**
Positive Predictive Value , %			
Subject OVA1 Next Generation	40.4	28.6	50.9
n/N	84/ 208	28/ 98	56/ 110
95% CI	33.9 to 47.2	20.6 to 38.2	41.7 to 60.1
Predicate OVA1	31.4	23.6	37.8
n/N	85/ 271	29/ 123	56/ 148
95% CI	26.1 to 37.1	16.9 to 31.8	30.4 to 45.9
Subject OVA1 Next Generation vs Predicate OVA1			
Difference in PPV, %	9.02	4.99	13.07
95% CI for difference	5.02 to 13.02	0.24 to 9.75	6.88 to 19.27
Ratio of PPV	1.288	1.212	1.345
95% CI for ratio	1.155 to 1.435	1.018 to 1.443	1.171 to 1.546
95% CI	93.6 to 98.5	95.4 to 99.6	84.1 to 96.9

Negative Predictive Value (NPV):
The NPV for the Subject device was substantially equivalent to the Predicate OVA1 device at ~97% for both. Please see Table 13 for a summary of NPV results.

Table 13 – Negative Predictive Values

	All Evaluable Subjects (N= 493)	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
Negative Predictive Value, %			
Subject OVA1 Next Generation	97.2	98.3	95.3
n/N	277/ 285	175/ 178	102/ 107
95% CI	94.6 to 98.6	95.2 to 99.4	89.5 to 98.0
Predicate OVA1	96.8	98.7	92.8
n/N	215/ 222	151/ 153	64/ 69
95% CI	93.6 to 98.5	95.4 to 99.6	84.1 to 96.9
Subject OVA1 Next Generation vs Predicate OVA1			
Difference in NPV, %	0.35	-0.38	2.57
95% CI for difference	-1.97 to 2.66	-2.38 to 1.62	-3.20 to 8.35
Ratio of NPV	1.004	0.996	1.028
95% CI for ratio	0.980 to 1.028	0.976 to 1.017	0.966 to 1.093

Please see Table 14 below for the Specificity, Sensitivity, PPV, NPV – standalone values. Please see Table 15 below for values with PA.

Table 14 – Sensitivity, Specificity, PPV, NPV – Standalone

Comparison of the standalone performance of the subject OVA1 Next Generation **to the Predicate OVA1**												
	Sensitivity			**Specificity**			**Positive Predictive Value**			**Negative Predictive Value**		
	All	Pre	Post	All	Pre	Post	All	Pre	Post	All	Pre	Post
OVA1 Next Generation (95% CI)	**91.3%** 83.8 to 95.5	**90.3%** 75.1 to 96.7	**91.8%** 82.2 to 96.4	**69.1%** 64.4 to 73.4	**71.4%** 65.5 to 76.7	**65.4%** 57.6 to 72.4	**40.4%** 39.9 to 47.2	**28.6%** 20.6 to 38.2	**50.9%** 41.7 to 60.1	**97.2%** 94.6 to 98.6	**98.3%** 95.2 to 99.4	**95.3%** 89.5 to 98.0
OVA1 (95% CI)	**92.4%** 85.1 to 96.3	**93.5%** 79.3 to 98.2	**91.8%** 82.2 to 96.4	**53.6%** 47.8 to 58.4	**61.6%** 55.4 to 67.5	**41.0%** 33.6 to 48.9	**31.4%** 26.1 to 37.1	**23.6%** 16.9 to 31.8	**37.8%** 30.4 to 45.9	**96.8%** 93.6 to 98.5	**98.7%** 95.4 to 99.6	**92.8%** 84.1 to 96.9

Table 15 – Sensitivity, Specificity, PPV, NPV, with PA

	Sensitivity			**Specificity**			**Positive Predictive Value**			**Negative Predictive Value**		
	All	**Pre**	**Post**	**All**	**Pre**	**Post**	**All**	**Pre**	**Post**	**All**	**Pre**	**Post**
OVA1 Next Generation (95% CI)	**93.5%** 86.5 to 97.0	**90.3%** 75.1 to 96.7	**95.1%** 86.5 to 98.3	**64.8%** 60.0 to 69.4	**67.3%** 61.2 to 72.9	**60.9%** 53.1 to 68.2	**37.9%** 31.8 to 44.3	**25.9%** 18.6 to 34.9	**48.7%** 39.9 to 57.6	**97.7%** 95.2 to 99.0	**98.2%** 94.9 to 99.4	**96.9%** 91.4 to 99.0
OVA1 (95% CI)	**95.7%** 89.3 to 98.3	**93.5%** 79.3 to 98.2	**96.7%** 88.8 to 99.1	**50.9%** 46.0 to 55.7	**59.2%** 52.9 to 65.2	**37.8%** 30.6 to 45.6	**30.9%** 25.8 to 36.5	**22.5%** 16.1 to 30.4	**37.8%** 30.6 to 45.6	**98.1%** 95.2 to 99.2	**98.6%** 95.2 to 99.6	**96.7%** 88.8 to 99.1

Likelihood ratios (LR):
There are two likelihood ratios; The LR+ represents the probability of a person who has a malignancy testing positive divided by the probability of a person who does not have the disease testing positive.

Conversely, the LR- represents the probability of a person who has the disease testing negative divided by the probability of a person who does not have the disease testing negative.

A likelihood ratio of greater than 1 indicates the test result is associated with the disease. A likelihood ratio less than 1 indicates that the result is associated with absence of the disease.

The Subject device demonstrated an improved LR+ as compared to the Predicate device, as well as an improved LR-. Please see Table 16 for a summary of the Likelihood ratios.

Table 16 – Positive and Negative Likelihood Ratios

	All Evaluable Subjects (N= 493)	**Pre-menopausal Women (N= 276)**	**Post-menopausal Women (N= 217)**
Positive Likelihood Ratio			
Subject OVA1 Next Generation	2.953	3.161	2.652
95% CI	2.518 to 3.463	2.514 to 3.975	2.111 to 3.332
Predicate OVA1	1.992	2.438	1.557
95% CI	1.766 to 2.247	2.029 to 2.930	1.339 to 1.810
Subject OVA1 Next Generation vs Predicate OVA1			
Ratio of positive LR	1.482	1.297	1.704
95% CI for ratio	1.242 to 1.769	1.012 to 1.661	1.311 to 2.214
Negative Likelihood Ratio			
Subject OVA1 Next Generation	0.126	0.135	0.125
95% CI	0.065 to 0.245	0.046 to 0.398	0.054 to 0.293
Predicate OVA1	0.142	0.105	0.200
95% CI	0.069 to 0.291	0.027 to 0.401	0.084 to 0.472
Subject OVA1 Next Generation vs Predicate OVA1			
Ratio of negative LR	0.887	1.294	0.627
95% CI for ratio	0.374 to 2.106	0.382 to 4.383	0.188 to 2.097

Clinical Specificity –Healthy Women Study:

A Clinical Specificity Study on healthy women was conducted to determine test result reference intervals of the Subject device on healthy women. Healthy women are not within the definition of the intended use population and would therefore not be considered for testing according to labeling. Nevertheless, the characterization study data will help physicians understand the representative range and distribution of OVA1 Next Generation test results in the intended use population, and to answer questions on how they might differ or compare with healthy women.

Study Design:

Study subjects were healthy women ages 18 to 92 years old. "Healthy" was defined as: no viral or bacterial infection, no substance abuse, no chronic disease state (for example, diabetes, lupus, or hepatitis), and no diagnosis of malignancy in the last 10 years, with the exception of non-melanoma skin cancer.

A total of 152 results were obtained from 68 premenopausal and 84 postmenopausal subjects (there were no unevaluable results). Single samples were run using the same calibrator reference curve, the same kit reagents lots, and the same control lots for the entire study duration. Serum concentrations of each protein biomarker were determined using the *cobas* 6000 instrument. Each operator generated the test result for the test he or she ran using the Subject OvaCalc software.

Study Results:
The Subject OVA1 Next Generation test demonstrated approximately 50% reduction in the test-positive subjects, as compared to the Predicate device. For example, ~13% of premenopausal subjects were test-positive with the Subject OVA1 Next Generation compared with 29% for the Predicate OVA1; and ~17% of postmenopausal subjects were test-positive for Subject OVA1 Next Generation compared with ~33% for the Predicate OVA1. These improvements are consistent with the design intent of lowering the percent of test-positive non-malignant subjects. However, no claim is made since this study was designed to inform physicians on the representative range and distribution of OVA1 Next Generation test results in healthy women, and to answer questions on how they might differ or compare with the intended use population. Therefore, we conclude the results to be substantially equivalent.

Please see Table 17 below for a summary of the clinical specificity of the Subject and Predicate OVA1 device. The Study demonstrated that the performance of the Subject device is substantially equivalent to the Predicate device.

Table 17 – Clinical Specificity –Healthy Women

	All Healthy Subjects	**Pre-menopausal Women**	**Post-menopausal Women**
N	152	68	84
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
Percentile (5% to 95%)	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%)
Predicate OVA1			
N	147	69	78
Positive	46 (31.3%)	20 (29.0%)	26 (33.3%)

Clinical Specificity - Other Cancers and Disease States:
A Clinical Specificity Study was conducted to determine the representative range of the Subject OVA1 Next Generation test results from women with other (non-ovarian) cancers and benign conditions including the following:

- Bladder cancer
- Breast cancer
- Cervical cancer
- Colon cancer
- Endometrial cancer
- Leukemia
- Lung cancer
- Lymphoma
- Autoimmune disease
- Cardiac disease
- Diabetes
- Endometriosis
- Hepatitis
- Kidney disease
- Pregnancy

Similar to the Clinical Specificity Study – Healthy Women, this characterization study was conducted to help physicians better understand and compare the representative range and distribution of OVA1 Next Generation test results in the intended use population to various benign and malignant diseases they may encounter when managing patients.

Study Design:
Four hundred and one single samples were run using the Subject OVA1 Next Generation device, using the same calibrator reference curve, the same kit reagent lots, and the same control lots for the entire study duration. Serum concentrations of each protein biomarker were determined using the *cobas* 6000 instrument. Each operator generated the test result for the test he or she ran using the Subject OvaCalc software.

Study Results:
As previously discussed; the nature of the test is to assign a high risk or low risk of malignancy. It is therefore not unexpected that the Subject device might detect certain other (non-ovarian cancer) cancers, disease states and conditions – as previously shown for the Predicate OVA1 device. Indeed the results of the Clinical Specificity for Other Cancers and Diseases indicate that other cancers and disease conditions can yield positive OVA1 Next Generation test result in some cases. However, the clinical study was not designed and Sponsor is not making any claims regarding other disease states. This study was designed simply to characterize and report the expected range and distribution of Subject OVA1 Next Generation test results for various benign and malignant diseases they may encounter when managing patients, relative to the Predicate OVA1 device.

Nevertheless the risk of detecting other non-ovarian cancers is mitigated by including physician assessment as part of the intended use for the Subject device. Lastly, both the Predicate OVA1 device has been on the market for several years without any reported events related to misdiagnosis versus other cancers, evidencing that the PA, as required for proper use of the Subject device, is adequate to mitigate risk related to other cancers.

The characterization study results and comparison to the Predicate OVA1 characterization results support a conclusion that the performance of the Subject OVA1 Next Generation device is substantially equivalent to the Predicate device. Please see Table 18 for a summary of results.

Table 18 - Clinical Specificity - Other Cancers and Diseases States

	All Evaluable Subjects	**Bladder Cancer**	**Breast Cancer**	**Cervical Cancer**	**Colon Cancer**	**Endometrial Cancer**	**Leukemia**	**Lung Cancer**	**Lymphoma**
N	221	20	40	20	40	40	11	40	10
OVA1 Next Generation Result, n (%)									
Positive	103 (46.6)	10 (50.0)	6 (15.0)	13 (65.0)	18 (45.0)	20 (50.0)	10 (90.9)	18 (45.0)	8 (80.0)
Negative	118 (53.4)	10 (50.0)	34 (85.0)	7 (35.0)	22 (55.0)	20 (50.0)	1 (9.1)	22 (55.0)	2 (20.0)
Specificity, %	53.4	50.0	85.0	35.0	55.0	50.0	9.1	55.0	20.0
	All Evaluable Subjects	**Bladder Cancer**	**Breast Cancer**	**Cervical Cancer**	**Colon Cancer**	**Endometrial Cancer**	**Leukemia**	**Lung Cancer**	**Lymphoma**
N	NA	16	45	12	40	44	10	13	13
Predicate OVA1									
Positive	NA	6	11	8	18	15	9	3	6
Negative	NA	10	34	4	22	29	1	10	7
Specificity %	NA	62.5	75.6	33.3	55.0	65.9	10	76.9	53.8
	All Evaluable Subjects	**Autoimmune Disease**		**Cardiac Disease**	**Diabetes**	**Endo-metriosis**	**Hepatitis**	**Kidney Disease**	**Pregnant Women**
N	180	20		20	40	40	20	20	20
OVA1 Next Generation Result, n (%)									
Positive	99 (55.0)	11 (55.0)		15 (75.0)	14 (35.0)	11 (27.5)	11 (55.0)	18 (90.0)	19 (95.0)
Negative	81 (45.0)	9 (45.0)		5 (25.0)	26 (65.0)	29 (72.5)	9 (45.0)	2 (10.0)	1 (5.0)
Specificity, %	45.0	45.0		25.0	65.0	72.5	45.0	10.0	5.0
	All Evaluable Subjects	**Autoimmune Disease**		**Cardiac Disease**	**Diabetes**	**Endo-metriosis**	**Hepatitis**	**Kidney Disease**	**Pregnant Women**
N	NA	10		12	40	40	10	12	10
Predicate OVA1									
Positive	NA	5		7	10	17	3	12	3
Negative	NA	5		5	30	23	10	0	7
Specificity, %	NA	50.0		41.7	75.0	57.5	76.9	0	70.0

Analytical Performance Validation:

Precision Study:

The Precision Study established total precision for the risk score algorithm and individual analyte measurements in the subject device. End users were instructed to follow the package insert from the manufacturer of each respective immunoassay.

Study Design:

The sample set consisted of five pooled serum samples (samples were numbered 6 – 10 for Subject device and were 1 – 5 for Predicate OVA1 submission) spanning the Subject device test result range (low test result, high test result, and close to the cutoff at 5.0), as well as two control levels for each assay per run.

The five samples were tested using two separate aliquots on two runs on each day. Multiple operators were used to run the five samples. The samples were run over 20 separate days and used the same kit reagents lots and the same control lots for the entire study.

Serum concentrations of each protein biomarker were determined using the *cobas* 6000 instrument. Each operator generated the test result for the test they ran using the Subject OvaCalc software.

A total of 400 results were obtained – on each of the 20 days, the same five samples were analyzed in duplicate for two runs per day. There were no unevaluable results.

Study Results:

The overall coefficient of variation (%CV) was 1.54% across all days and pools, which demonstrates that the errors of measurement were well within the acceptable limits (< 10%) established in the Product Design Specification.

In addition, the precision for the Subject OVA1 Next Generation test was notably improved from the Predicate OVA1, as seen in comparison in Table 19 below. The %CVs for the Subject device are equivalent to or less than the %CVs for the Predicate OVA1 test. Pool 6 exhibited little variance. This was likely due to that fact that biomarker concentrations are at limits such that their individual variation does not affect the calculated OVA1 Next Generation test result.

Analysis of Results:

The overall %CV for the Subject device was 1.54% across all days and pools. The overall %CV for the Predicate OVA1 device in contrast was 4.09% (please refer to OVA1 precision data in K081754).

The Study demonstrated that the performance of the Subject device is substantially equivalent to the Predicate device.

Table 19 - Precision Study, subject device compared to predicate OVA1

	Serum Pool					
OVA1 Next Generation Test Value	**1**	**2**	**3**	**4**	**5**	**All Pools**
N	80	80	80	80	80	400
Mean	8.50	8.16	5.08	4.11	3.30	5.83
SD of Error	0.000	0.055	0.161	0.085	0.065	0.090
%CV of overall error	0.00	0.67	3.16	2.06	1.95	1.54

	Serum Pool					
OVA1 Test Value	**1**	**2**	**3**	**4**	**5**	**All Pools**
N	80	80	80	80	80	400
Mean	2.74	3.39	3.74	4.69	9.94	4.90
SD of Error	0.091	0.159	0.192	0.349	0.098	0.200
%CV of overall error	3.31	4.69	5.11	7.43	0.98	4.09

Reproducibility Study:

Study Design:
The study was run using the same five pooled serum samples used in the Precision Study. Pooled samples (low test result, high test result, and close to the cutoff at 5.0) were run over six, non-consecutive days at three sites and two operators as per site.

A total of 360 test results were obtained (one result was rejected due to operator error: operator used APO B, rather than APO A1) over the six days.

Study Results:
The Subject device showed very little variability over different sites, days, operators and runs. The overall %CV including all sites was 1.63% as compared to the Predicate OVA1 device with an overall %CV of 2.80% across all sites (please reference reproducibility study in K081754). Please see Table 20 below for a summary of reproducibility study results.

The Study demonstrated that the performance of the Subject device is substantially equivalent to the Predicate device.

Table 20 - Reproducibility Study, subject device compared to predicate device (OVA1)

OVA1 Next Generation Test Value	**Total – All Pools**
SD	0.10
%CV	1.6
Predicate OVA1	
SD	0.27
%CV	2.8

Stability Study:
A sample stability study was conducted to verify specimen sample stability for use with the Subject device. The study duration and temperatures represented instructions for use that include shipping and laboratory storage prior to running the assays and provide estimates of specimen stability for the Subject device test result and individual biomarker assays.

Study Design:
The sample set consisted of the seven pooled serum samples (Pools A-G) run for the other analytical studies, with two control levels for each assay per run.

Four independent aliquots of each pool's samples were removed from the freezer, thawed on the bench top, and then placed in the refrigerator (2-8°C) for time points from zero to eight days. Samples were run using the same calibrator reference curve, the same kit reagents lots, and the same control lots for the entire study duration.

Serum concentrations of each protein biomarker were determined using the *cobas* 6000 instrument. Each operator generated the test result for the test he or she ran using the Subject OvaCalc software.

Study Results:
Results from the Sample Stability study confirmed that the specimen sample provides stable OVA1 Next Generation test results over eight days of storage. For all pools, results at eight days of storage between 2°C and 8°C were within 10% of the initial (Day 0) value, which meets the acceptance criteria established in the Product Design Specification.

The Study demonstrated that the performance of the Subject device is substantially equivalent to the Predicate device. Please see Table 21 below for a summary of stability study results.

Table 21 - Sample Stability

	Analysis Metric	Day 0	Day 2	Day 6	Day 8	Day 9
Pool A	Mean	7.90	7.90	7.90	7.90	7.90
	SD	0.000	0.000	0.000	0.000	0.000
	Mean Change	-	0.00	0.00	0.00	0.00
	%Mean Change from Day 0	-	0.0%	0.0%	0.0%	0.0%
	95% CI of change	-	0.00 to 0.00	0.00 to 0.00	0.00 to 0.00	0.00 to 0.00
Pool B	Mean	8.30	8.35	8.35	8.30	8.30
	SD	0.000	0.071	0.071	0.000	0.000
	Mean Change	-	0.05	0.05	0.00	0.00
	%Mean Change from Day 0	-	0.6%	0.6%	0.0%	0.0%
	95% CI of change	-	-0.02 to 0.12	-0.02 to 0.12	-0.07 to 0.07	-0.07 to 0.07
Pool C	Mean	6.00	6.00	6.05	6.00	6.10
	SD	0.100	0.141	0.071	0.141	0.141
	Mean Change	-	0.00	0.05	0.00	0.10
	%Mean Change from Day 0	-	0%	0.8%	0%	1.7%
	95% CI of change	-	-0.22 to 0.22	-0.17 to 0.27	-0.22 to 0.22	-0.12 to 0.32
Pool D	Mean	7.96	7.85	7.90	7.95	8.00
	SD	0.055	0.071	0.000	0.071	0.000
	Mean Change	-	-0.11	-0.06	-0.01	0.04
	%Mean Change from Day 0	-	-1.4%	-0.8%	-0.1%	0.5%
	95% CI of change	-	-0.21 to -0.01	-0.16 to 0.04	-0.11 to 0.09	-0.06 to 0.14
Pool E	Mean	8.20	8.20	8.20	8.20	8.20
	SD	0.000	0.000	0.000	0.000	0.000
	Mean Change	-	0.0	0.0	0.0	0.0
	%Mean Change from Day 0	-	0.0%	0.0%	0.0%	0.0%
	95% CI of change	-	0.00 to 0.00	0.00 to 0.00	0.00 to 0.00	0.00 to 0.00
Pool F	Mean	4.00	4.00	4.05	4.05	4.05
	SD	0.071	0.000	0.071	0.354	0.071
	Mean Change	-	0.00	0.05	0.05	0.05
	%Mean Change from Day 0	-	0.0%	1.3%	1.3%	1.3%
	95% CI of change	-	-0.27 to 0.27	-0.22 to 0.32	-0.22 to 0.32	-0.22 to 0.32
Pool G	Mean	3.10	2.80	3.05	2.90	2.95
	SD	0.100	0.000	0.354	0.000	0.212
	Mean Change	-	-0.30	-0.05	-0.20	-0.15
	%Mean Change from Day 0	-	-9.7%	-1.6%	-6.5%	-4.8%
	95% CI of change	-	-0.61 to 0.01	-0.36 to 0.26	-0.51 to 0.11	-0.46 to 0.16

Interference Study:
An interference study was conducted to screen common interfering substances for potential effects to the Subject OVA1 Next Generation test. The study was adapted from and is consistent with the Clinical and Laboratory Standards Institute (CLSI) Interference Testing in Clinical Chemistry; Approved Guideline—Second Edition (EP07-A2). Additionally, the study was designed to be consistent with the Predicate OVA1 submission interference study in order to evaluate possible interfering substance bias for establishing substantial equivalence to the Predicate OVA1.

Study Design:
The study tested three serum sample pools (Pools 6, 8 and 10) spanning the OVA1 Next Generation test result range (low, close to the cutoff, and high), each spiked with various levels of five potential interfering substances. These were three of the five pools tested in the other analytical studies above. Vehicle control samples without potential interfering substances but with the same amount of solvent were also tested. Potential interfering substances and concentrations tested were as listed in Table 22 below.

Table 22 - Interfering Substances

Substance	Concentrations Tested
Hemoglobin	5.0 g/L 9.0 g/L
Bilirubin, conjugated	0.3 g/L 0.9 g/L
Bilirubin, unconjugated	0.3 g/L 0.9 g/L
Triglycerides	2.0 g/L 4.6 g/L 10.0 g/L
Rheumatoid factor	250 IU/mL 1000 IU/mL

Four replicates of each experimental group were prepared, each from an independent sample aliquot. Two control levels for each assay were run prior to each sample run. Samples were run using the same calibrator reference curve, the same kit reagents lots, and the same control lots for the entire study duration. Serum concentrations of each protein biomarker were determined using the *cobas* 6000 instrument. Each operator generated the test result for the test he or she ran using the Subject OvaCalc software.

Study Results:
All potential interfering substances tested were within acceptable limits established in the Product Design Specification and consistent with the assay manufacturer's instructions for use for interfering substances. For the purpose of this study, an interfering substance showed no effect if the 95% CI of the treated pooled sample was within the 10% margin of the untreated control.

Method Comparison:

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 Table 23. 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, or 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, or 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.

Table 23 - Dual assessment of PA with OVA1 Next Generation versus dual assessment of PA with OVA1 Test

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% (187/285) 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%				

Conclusions:
The subject OVA1 Next Generation Test device is substantially equivalent in indications for use, intended use, and functionality to the predicate device cleared in K081754.

- OVA1 510(k) K081754

All data indicates that the device will perform as intended.