

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k112624

B. Purpose for Submission:

New Device

C. Measurand:

Human Epididymis Protein 4 (HE4)

D. Type of Test:

Quantitative, Automated Chemiluminescence Immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys HE4 Assay

Elecsys HE4 CalSet

Elecsys PreciControl HE4

Elecsys HE4 CalCheck 5

G. Regulatory Information:

1. Regulation section:

21 CFR §866.6010 – Tumor-Associated Antigen Immunological Test System

21 CFR §862.1150 – Calibrator

21 CFR §862.1660 – Quality Control Material (Assayed and Unassayed)

2. Classification:

Class II (Elecsys HE4 Assay and Elecsys HE4 CalSet)

Class I (Elecsys PreciControl HE4 and Elecsys HE4 CalCheck 5)

3. Product code:

OIU – Test, Epithelial Ovarian Tumor Associated Antigen (HE4)

JIT – Calibrator, Secondary

JJX – Single (Specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

Immunology (82)

Chemistry (75)

H. Intended Use:

1. Intended use(s):

Elecsys HE4 Assay:

The Elecsys HE4 assay is an immunoassay for the quantitative determination of HE4 in human serum and plasma. The assay is used as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. Serial testing for patient HE4 assay values should be used in conjunction with other clinical findings used for monitoring ovarian cancer.

The electrochemiluminescence immunoassay “ECLIA” is intended for use on Elecsys and cobas e immunoassay analyzers.

Elecsys HE4 CalSet:

Elecsys HE4 CalSet is used for calibrating the quantitative Elecsys HE4 assay on the Elecsys and cobas e immunoassay analyzers.

Elecsys PreciControl HE4:

Elecsys PreciControl HE4 is used for quality control of the Elecsys HE4 immunoassay on Elecsys and cobas e immunoassay analyzers.

Elecsys HE4 CalCheck 5:

The Elecsys HE4 CalCheck 5 is an assayed control for use in calibration verification and for use in the verification of the assay range established by the Elecsys HE4 reagent on the indicated Elecsys and cobas e immunoassay analyzers. For In vitro diagnostic use only.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

Prescription use only

4. Special instrument requirements:

Elecsys and cobas e immunoassay analyzers

I. Device Description:

The Elecsys HE4 assay is a two-step sandwich immunoassay for quantitative determination of HE4 antigen in human serum and plasma. The kit contains the following reagents:

- M: Streptavidin-coated microparticles, 1 bottle (6.5 mL)
- R1: Biotinylated monoclonal anti-HE4 antibody (mouse), 1 bottle (10 mL)
- R2: Monoclonal anti-HE4 antibody (mouse) labeled with ruthenium, 1 bottle (10 mL)

The Elecsys HE4 CalSet consists of two levels of lyophilized HE4 from the cell line OvCar-3:

- HE4 Cal1: ~5 pmol/L HE4 in an equine serum matrix with preservative, 2 bottles (1.0 mL/bottle)
- HE4 Cal2: ~200 pmol/L HE4 in an equine serum matrix with preservative, 2 bottles (1.0 mL/bottle)

The Elecsys PreciControl HE4 (PC HE4) consists of two lyophilized human sera.

- PC HE4 1: ~ 50 pmol/L HE4 in human serum, 2 bottles (1.0mL/bottle)
- PC HE4 2: ~400 pmol/L HE4 in human serum, 2 bottles (1.0 mL/bottle)

The Elecsys HE4 CalCheck 5 set contains 5 vials of lyophilized human HE4 from OvCar-3 cell culture in equine serum with one for each level of HE4 (Check 1 – 5). Target values and approximate target ranges of the reconstituted calibrators are listed below:

Level	Target Value [pmol/mL]	Target Range [pmol/mL]
Check 1	≤3000 counts	-
Check 2	100	80 – 120
Check 3	750	675 – 825
Check 4	1200	1080 – 1320
Check 5	1500	1400 – 1600

J. Substantial Equivalence Information:

1. Predicate device name(s) and predicate 510(k) number(s):
ARCHITECT HE4 assay, k093957
Elecsys hGh CalSet, k103221
Elecsys PreciControl Multimarker, k102157
Elecsys DHEA-S CalCheck 5, k103402
2. Comparison with predicate:

Similarities		
Item	Device Elecsys HE4 Assay	Predicate ARCHITECT HE4
Intended Use	Quantitative determination of HE4 antigen in human serum. The assay is used as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.	Same
Antigen Detection	HE4	Same

Similarities		
Item	Device Elecsys HE4 Assay	Predicate ARCHITECT HE4
Assay Format	Quantitative, automated	Same

Differences		
Item	Device Elecsys HE4 Assay	Predicate ARCHITECT HE4
Detection Protocol	Electrochemiluminescent	Chemiluminescent
Instrument Platform	Elecsys 2010 and MODULAR ANALYTICS E170; Cobas e 411, cobas e 601 and cobas e 602	ARCHITECT <i>i</i> System
Sample Type	Human serum and plasma treated with K ₂ -EDTA, K ₃ -EDTA or lithium heparin	Human serum only
Capture Antibody	Biotinylated mouse monoclonal antibody (12A2)	Mouse monoclonal antibody (2H5)
Detection Antibody	Monoclonal anti-HE4 antibody (mouse) labeled with ruthenium (2H5)	Monoclonal anti-HE4 antibody (mouse) labeled with acridinium (3D8)
Calibrators	Elecsys HE4 CalSet: 2 levels (5 and 200 pmol/L)	ARCHITECT HE4 Calibrator Kit: 6 levels (0, 30, 100, 250, 750 and 1500 pmol/L)
Controls	Elecsys HE4 PreciControl: 2 levels (50, and 400 pmol/L)	ARCHITECT HE4 Control Kit: 3 levels (50, 175, and 700 pmol/L)
Sample Size	10 µL	25 µL
Measuring Range	15 – 1500 pmol/L	20 – 1500 pmol/L

Similarities and Differences		
Item	Device Elecsys HE4 CalSet	Predicate Elecsys hGh CalSet
Intended Use	For calibrating the quantitative Elecsys HE4 assay on the Elecsys and cobas e immunoassay analyzers	For calibrating the quantitative Elecsys hGH assay on the Elecsys and cobas e immunoassay analyzers.
Levels	Two	Same
Matrix	Equine serum	Human serum
Format	Lyophilized	Same
Handling	Add exactly 1.0 mL of distilled	Same

Similarities and Differences		
Item	Device Elecsys HE4 CalSet	Predicate Elecsys hGh CalSet
	water and allow standing closed for 15 minutes to reconstitute.	
Stability – Unopened	2 – 8°C: up to stated expiration date	Same
Stability – Reconstituted	2 – 8°C: 7 days -15 – -25°C: 8 weeks (freeze only once)	Same
Stability – On-board	On Elecsys 210/cobas e 411: 20 – 25°C: 5 hours On MODULAR ANALYTICS E170, cobas e 601 and cobas e 602: 20 – 25°C: Use only once	Same

Similarities and Differences		
Item	Device Elecsys PreciControl HE4	Predicate Elecsys PreciControl Multimarker
Intended Use	For quality control of Elecsys HE4 immunoassay on the indicated Elecsys and cobas e immunoassay analyzers.	For quality control of specified Elecsys immunoassays on the Elecsys and cobas e immunoassay analyzers.
Levels	Two	Same
Matrix	Human serum	Same
Format	Lyophilized	Same
Handling	Add exactly 1.0 mL distilled or deionized water to one bottle. Allow to stand closed for 15 minute to reconstitute.	Add exactly 2.0 mL of distilled or deionized water to one bottle. Allow to stand closed for 30 minutes to reconstitute.
Stability– Unopened	2 – 8°C: 12 months	Same
Stability – Reconstituted	-20°C: 4 weeks (freeze only once) 2 – 8°C: 14 days 20 – 25°C: 24 hours	-20°C: 31 days (freeze only once) 2 – 8°C: 72 hours
Stability – On-board	20 – 25°C: 5 hours	Same

Similarities and Differences		
Item	Device Elecsys HE4 CalCheck 5	Predicate Elecsys DHEA-S CalCheck 5
Intended Use	For use in calibration verification and for use in the verification of the assay range established by the Elecsys HE4 reagent on the indicated Elecsys and cobas e immunoassay analyzers.	For use in calibration verification and for use in the verification of the assay range established by the Elecsys DHEA-S reagent on the indicated Elecsys and cobas e immunoassay analyzers.
Analyte	HE4	DHEA-S
Levels	Five	Same
Matrix	Equine serum	Human serum
Format	Lyophilized	Same
Handling	Reconstitute Check 1, Check 2, Check 3, Check 4 and Check 5 with exactly 1.0 mL distilled water or ionized water. Allow to stand closed for 15 minutes, then mix gently by inversion	Same
Stability– Unopened	2 – 8°C: up to expiration date	Same
Stability – Reconstituted	20 – 25°C: 5 hours	Same

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

CLSI EP5-A2: Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline – Second Edition

CLSI EP6-A: Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline

CLSI EP17-A: Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline

L. Test Principle:

The Elecsys HE4 assay is a two-step sandwich immunoassay. First, sample is incubated with a biotinylated monoclonal HE4-specific antibody and a monoclonal HE4-specific antibody labeled with a ruthenium to form a sandwich complex. After addition of streptavidin-coated microparticles, the complex binds to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. A voltage is applied to the electrode to induce chemiluminescent emission which is measured by a photomultiplier. The results are

determined via a calibration curve that is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

The precision of Elecsys HE4 assay was evaluated using five pooled human serum samples and two control samples (PeciControl Elecsys HE4). The pooled serum samples were prepared at the following levels: 25.3 pmol/L, 53.7 pmol/L, 142 pmol/L, 779 pmol/L, and 1437 pmol/L and concentrations of the controls were 45.7 pmol/L and 345 pmol/L. Samples were tested in 4 replicates (2 replicates/run), 2 runs a day for 21 days on Roche cobas e 411 immunoassay analyzer. A total 84 observations were generated for each sample. The results are summarized in the following table.

Samples	Mean HE4 Level (pmol/L)	Repeatability (Within-Run)		Total Precision (Between-Day)	
		SD (pmol/L)	% CV (95% CI)	SD (pmol/L)	% CV (95% CI)
Human serum 1	25.3	0.45	1.8 (1.5 – 2.2)	0.95	3.7 (3.1 – 4.8)
Human serum 2	53.7	0.99	1.8 (1.6 – 2.2)	2.28	4.2 (3.5 – 5.5)
Human serum 3	142	2.33	1.6 (1.4 – 2.0)	6.11	4.3 (3.5 – 5.5)
Human serum 4	779	11.30	1.5 (1.2 – 1.8)	32.60	4.2 (3.4 – 5.4)
Human serum 5	1437	18.90	1.3 (1.1 – 1.6)	39.40	2.7 (2.3 – 3.5)
PC Elecsys HE4 1	45.7	0.66	1.4 (1.2 – 1.8)	1.92	4.2 (3.4 – 5.5)
PC Elecsys HE4 2	345	5.67	1.6 (1.4 – 2.0)	11.80	3.4 (2.9 – 4.3)

Lot-to-lot reproducibility was evaluated by testing a total of 126 samples with HE4 values from 29.4 to 1387 pmol/L with different lots of reagent. The regression analysis (Passing-Bablok) by comparing the data generated by different lots of reagent shows the following parameters: slope=0.958 (95% CI: 0.929 – 0.975), intercept = 1.71 pmol/L (95% CI; 0.458 – 3.76 pmol/L), correlation tau=0.999.

Site-to-site precision was evaluated on two cobas e 411 immunoassay analyzers at two external sites according to CLSI EP5-A2 using four human serum samples (within difference concentration ranges covering the measuring range) and two

PreciControl HE4 samples. Samples were tested in 4 replicates per run for 21 days at each site. The %CV for site-to-site precision is <5.2% for all tested samples.

b. Linearity/assay reportable range:

i) Linearity:

To determine the linearity of the Elecsys HE4 assay, the dilution series were prepared using three high concentration levels of HE4 human serum samples. For each sample, 17 concentrations (15 dilutions) throughout the measuring range were prepared. Each dilution was tested in triplicate. The linearity data were analyzed with regards to linear, quadratic and cubic polynomials according to CLSI EP6-A. Regression analyses of the observed results (y) and the expected results (x) for each sample and pooled data are presented below:

Sample	Range (pmol/L)	Regression equation	Slope 95% CI	Intercept 95% CI
1	0 – 1780	$y=1.016x + 0.309$	1.005 – 1.027	0.296 – 0.322
2	0 – 1827	$y=0.947x + 0.393$	0.932 – 0.963	0.156 – 0.629
3	0 – 1887	$y=0.945x + 0.225$	0.934 – 0.956	0.051 – 0.398
Pooled	0 – 1887	$y=0.973x + 0.103$	0.916 – 1.029	0.079 – 0.126

Linearity was confirmed in the range from 12.6 to 1510 pmol/L. The claimed measuring range for Elecsys HE4 assay is 20 – 1500 pmol/L.

ii) High Dose Hook-effect:

The high dose hook effect of the Elecsys HE4 assay was assessed on the cobas e 411. Two samples were spiked with HE4 to concentrations of 48,864 pmol/L and 48,795 pmol/L, respectively. For each sample, a dilution series was made to have 5 to 6 samples above the measuring range. Each dilution was tested in triplicate. The hook concentration reported corresponds to the analyte concentration with a signal corresponding to at least 10% above the highest master calibrator. No hook effect is detected with HE4 concentrations up to 40,000 pmol/L.

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

i) Traceability:

The Elecsys HE4 assay was standardized using the Fujirebio HE4 EIA method from Fujirebio Diagnostics, Inc as no international standard is available for HE4.

ii) Value Assignment:

(a) Elecsys HE4 CalCheck5: Elecsys HE4 CalCheck5 set contains 5 lyophilized levels of human HE4 from OvCar-3 culture in equine serum and has been standardized against the HE4 EIA method from Fujirebio Diagnostics, Inc.

For each lot of Elecsys HE4 CalCheck 5 manufactured, the CalCheck are run in duplicate on at least three Modular Analytics E170 analyzers. The E170

master calibrator curve is used to calibrate pmol/L from counts. The assigned target value of each CalCheck is defined as the median value obtained over at least 6 determinations (duplicate runs on at least 3 analyzers) of respective CalCheck. The representative summary data for the value assignment process for Elecsys HE4 CalCheck 5 are shown below:

Level	Target Value [pmol/L]	Target Range [pmol/L]	Assigned Value [pmol/L]	Assigned Range [pmol/L]
Check 1	≤3000 counts	-	<15	-
Check 2	100	80 – 120	101	70.7 – 131
Check 3	750	675 – 825	726	508 – 944
Check 4	1200	1080 – 1320	1160	812 – 1508
Check 5	1500	1400 – 1600	1570	1099 – 2041

For additional analyzers, the same value assignment procedure was performed. The assigned values obtained on the additional analyzers are compared to those obtained on the Modular Analytics E170 analyzer. The acceptance criteria for analyzer-to-analyzer variability are within 10% for each level of CalCheck.

- (b) Elecsys HE4 CalSet: Elecsys HE4 CalSet set contains lyophilized equine serum with added HE4 protein (human, cell line OvCar-3) in two concentration ranges (approx. 5 pmol/L and approx. 200 pmol/L).

For each lot of Elecsys HE4 CalSet manufactured, the calibrators are run in duplicate on at least 3 Elecsys 2010 analyzers and at least 3 Modular Analytics E170 analyzers with all Elecsys HE4 reagent lots available. The master calibrator curve is generated from 5 master calibrators at 1, 11.59, 28.49 144.4 and 1903 pmol/L. The assigned target value of each calibrator is the median value obtained over at least 6 determinations (duplicate runs on at least 3 analyzers) of respective calibrator.

- (c) Elecsys PreciControl HE4: Elecsys PreciControl HE4 contains lyophilized human serum with HE4 (human, from cell line OvCar-3) in two concentration ranges (approx. 50 pmol/L and approx. 400 pmol/L).

Each level of the Elecsys PreciControl HE4 is assigned an analyte-specific value with the Elecsys HE4 assay. The controls are run in duplicate on at least three Modular Analytics E170 analyzers. The assigned value of each control level is the median value obtained over at least 6 determinations of the respective control level. The acceptance criteria for value assignment include: precision of the control determination (10%CV for control 1 and 9%CV for control 2), acceptance range of assigned value (25 – 90 pmol/L for control 1 and 200 – 650 pmol/L for control 2). For additional analyzer platforms, the

same value assignment procedure is performed. The assigned values obtained on the additional analyzers are compared to those obtained on the Modular Analytics E170. The acceptance criterion for analyzer-to-analyzer variability is 9%.

iii) *Stability:*

- (a) Sample stability: Sample stability was evaluated for the following storage conditions: 22°C for 6 hours, 2-8°C for three days, or -20°C for 13 weeks. Ten samples for each sample type (Serum, K₂-EDTA-plasma, K₃-EDTA-plasma, Li-Heparin-plasma) were aliquoted and evaluated directly after collection (reference) and after the tested storage condition, respectively. In addition, ten samples for each sample type were evaluated directly after collection (reference) and after three/thaw cycles. Measurements were performed with three-fold determination on a cobas e 411 immunoassay analyzer and recovery was calculated as percent of the reference value. The resulting data support the claim that serum, Li-Heparin-, K₂-EDTA- and K₃-EDTA-plasma specimens are stable at the following conditions: 5 hours at 15-25°C, 2 days at 2-8°C, 12 weeks at -20°C with up to two freeze/thaw cycles.
- (b) Calibration curve stability: The calibration curve stability was performed using two Elecsys HE4 reagent lots on a cobas e 411. Five human serum samples and two control samples were tested. The data support that the calibrator curve is stable up to 28 days. During that time period, fresh reagent kits of the same lot can be used without calibration using the calibration curve of the Day 0 reagent kit.
- (c) Assay kit stability:

Closed-vial stability: The real-time and accelerated stability studies were performed on cobas e 411 analyzer. Five human serum samples and two controls were tested. For real-time stability, data were collected at point 0, 6, 13, 16, 22 and 30 months at 2-8°C. For accelerated stability study, data were collected for 3 weeks after 35°C. The data support that Elecsys HE4 reagent kits have a shelf life up to 12 months when stored at 2-8°C.

Open-vial stability: Stability study was done to evaluate the reagent stability after first opening. A fresh kit was placed on the analyzer and calibrated. Reference values for the samples tested were determined. After the measurements, the kit was removed from the analyzer and kept at 2-8 °C for 15 weeks. After 15 weeks the kit was placed on the analyzer again, calibrated and the samples were run again. Five human serum samples and two controls were tested. The results support that Elecsys HE4 reagent kits are stable for up to 12 weeks when stored at 2-8°C after first opening.

Open-vial/On-board stability: Reagent on-board stability and calibration stability were evaluated on one cobas e 411 and one cobas e 601 analyzers. A fresh Reagent Rack-Pack was placed on the analyzer and calibrated. All samples were measured on Day 1. On Day 8 and Day 36, the same samples were measured with the same reagent kit (kept at $20^{\circ}\text{C} \pm 3^{\circ}\text{C}$, simulating on-board conditions) using the calibration curves established on Day 1 and Day 29, respectively. The results support that Elecsys HE4 reagent kits can be stored on board of the analyzers for up to 28 days. A new calibration of the kit kept on-board is recommended every 7 days.

(d) Elecsys HE4 CalCheck 5 stability:

Open-vial stability: One lot of Elecsys HE4 CalCheck 5 was evaluated in duplicate on the cobas e 411. The test material was reconstituted and stored at 25°C for 6 hours (in an open vial). The reference material was a freshly reconstituted set of CalCheck. The recovery of test material was calculated as a percent of the reference value. The acceptance criterion is 90 – 110% recovery of the reference material value. The data support the claimed stability – Elecsys HE4 CalCheck 5 is stable up to 5 hours at $20\text{-}25^{\circ}\text{C}$.

Closed-vial stability: The real-time and accelerated stability study was done to evaluate the shelf life of the Elecsys HE4 CalCheck 5. For accelerated stability study, one lot of Elecsys HE4 CalCheck 5 was evaluated in duplicate on the cobas e 411. The test material was stored at 35°C for 3 weeks. The reference material was a freshly reconstituted set of CalCheck (stored at $2\text{-}8^{\circ}\text{C}$). After 3 weeks, the test and reference materials were tested in duplicate. The recovery of test material was calculated as a percent of the reference value. The acceptance criterion is 90 – 110% recovery of the reference materials. For real-time stability study, the test material is stored at $2\text{-}8^{\circ}\text{C}$. Samples at 0, 6, 13, 19, 25, 31, and 37 months will be tested in duplicate. Recovery values are calculated by comparing to the unstressed reference value (stored at -20°C). The acceptance criterion is $<15\text{ pmol/L}$ for CalCheck Level 1 and recovery of 90-110% for CalCheck Level 2-5.

The results from the accelerated stability and on-going real-time stability support an initial shelf-life claim of 18 months at $2\text{-}8^{\circ}\text{C}$ for the Elecsys HE4 CalCheck 5.

(e) Elecsys HE4 CalSet stability:

Stability after reconstitution: The test and reference materials were tested in duplicate. The test material was reconstituted and stored in closed vials for 29 days at $2\text{--}8^{\circ}\text{C}$, 13 weeks at $-15\text{ to }-25^{\circ}\text{C}$. In addition, stability for 5 freeze/thaw cycles was evaluated. The reference material was a freshly reconstituted CalSet. The recovery of test material was calculated as percentage of the reference value. The acceptance criterion of recovery is 95

– 105%. The data support the following stability claim for the reconstituted Elecsys HE4 CalSet: 7 days at 2 – 8°C, 8 weeks at -15 to -25°C with 1 freeze/thaw cycle.

Open vial/On-board stability: Two lots of Elecsys were evaluated in duplicate on the cobas e 411 and the cobas e 601. The test material was reconstituted and stored for at 25°C for 6 hours (in an open vial). The reference material was a freshly reconstituted set of CalSet. The recovery of test material was calculated as a percent of the reference value. The acceptance criterion for recovery is 95 – 105%. The data support the claimed stability – Elecsys HE4 CalSet is stable up to 5 hours on board (at 20-25°C) of the Elecsys and cobas e analyzers. According to the sponsor, the Elecsys HE CalSet should only be used once on MODULAR ANALYTICS E170/cobas e 601.

Closed vial stability: The stability of Elecsys HE4 CalSet was evaluated with the real-time and accelerated stability study. For accelerated stability study, one Elecsys HE4 CalSet lot was evaluated in duplicate on the cobas e 411. The test material was stored lyophilized at 35°C for 3 weeks. The reference material was a freshly reconstituted set of CalCheck (stored at 4°C). After 3 weeks, the test and reference materials were tested in duplicate. The recovery of test material was calculated as a percent of the reference value. The acceptance criterion is 95 – 105% recovery of the reference materials. For real-time stability, the test material is stored at 2-8°C. Sample at time-points at 0, 6, 13, 19, 25, 31, and 37 months will be tested in duplicate. Calibration curves are generated using calibrators stored at 2-8°C and calibrators stored at -20°C (reference). Measurement values for HE4 PreciControl 1 and PreciControl 2 are read from the calibration curve generated using calibrators stored at 2 – 8°C and compared to measurement values read from a calibration curve using calibrators stored at -20°C (reference). The acceptance criterion for HE4 PreciControl 1 and PreciControl 2 is recovery of 90-110% of the reference value.

The results from the accelerated stability and on-going real-time stability support an initial shelf-life claim of 12 months at 2 – 8°C for the Elecsys CalSet.

(f) Elecsys PreciControl HE4 stability:

Stability after reconstitution: The test and reference materials were tested in duplicate. The test material was reconstituted and stored in closed vials for 29 days at 2 – 8°C, 13 weeks at -15 to -25°C or 25 hours at 25°C. The reference material was a freshly reconstituted PreciControl HE4. The recovery of test material was calculated as percentage of the reference value. The acceptance criterion of recovery is 90 – 110%. The data support the following stability

claim for the reconstituted Elecsys PreciControl HE4: 14 days at 2 – 8°C, 4 weeks at -20°C, and 24 hours at 20-25°C.

Open-vial stability after reconstitution: Two lots of Elecsys PreciControl HE4 were evaluated in duplicate on the cobas e 411 and the cobas e 601 analyzer. The test material was reconstituted and stored for 6 hours at 25°C in open vial on the instrument. The reference material was a freshly reconstituted set of PreciControl HE4. The recovery of test material was calculated as a percent of the reference value. The acceptance criterion was 90 – 110%. The data support the claimed stability – Elecsys PreciControl HE4 is stable up to 5 hours at 20-25°C on the instrument.

Closed-vial stability: The real-time and accelerated stability study was done to evaluate the shelf-life of the Elecsys PreciControl. For accelerated stability study, one Elecsys PreciControl HE4 lot was evaluated in duplicate on the cobas e 411. The test material was stored lyophilized at 35°C for 3 weeks. The reference material was a freshly reconstituted set of PreciControl (stored at 2-8°C). After 3 weeks, the test and reference materials were tested in duplicate. The recovery of test material was calculated as a percent of the reference value. The acceptance criterion is 90 – 110% recovery of the reference materials. For real-time stability, the test material is stored at 2-8°C. Samples at 0, 6, 13, 19, 25, 31, and 37 months are tested in duplicate. The recovery of test material is calculated as percent recovery compared to the reference value (stored at -20°C). The acceptance criterion for PreciControl HE4 is 90-110% of the reference value.

The results from the accelerated stability and on-going real-time stability support the shelf-life claim of 9 months at 2 – 8°C.

d. Detection limit:

The limit of blank (LoB), limit of detection (LoD) and limit of quantitation (LoQ) of the Elecsys HE4 assay were determined on cobas e 411 and cobas e 601 in accordance with the CLSI EP17-A requirements.

For LoB, five analyte-free samples were analyzed on two cobas e 411 and two cobas e 601 over 3 days, 2 runs per day with a single replicate per run, for a total of 60 determinations. LoB was determined as the 95th percentile of measurements of blank samples. The LoB was 0.358 pmol/L on cobas e 411 and 0.230 pmol/L on cobas e 601.

For LoD, five human serum samples with low analyte concentration were analyzed on two cobas e 411 and two cobas e 601 over 3 days, 2 runs per day with a single replicate per run. A pooled estimate of the precision (SD_{total}) for the 5 low level samples was calculated, $LoD = LoB + 1.653 \times SD_{total}$. The LoD calculated was 0.661 pmol/L on cobas e 411 and 0.497 pmol/L on cobas e 601.

For LoQ, a low concentration level sample set of 6 samples was prepared by diluting 3 human serum samples with an analyte-free calibrator matrix. The sample set was tested in singlicate for three days, two runs per day on two cobas e 411 and two cobas e 601 analyzers. Each run was calibrated separately using a two-point calibration in combination with the master curve stored on the reagent barcode. The Elecsys PreciControl HE4 was tested and evaluated for each run to determine assay validity. The Total Error (TE) was calculated based on the average bias and SDs for each member of the sample set: $TE = | \text{bias} | + 1.96 *SD$. LoQ is derived from a plot of the allowable error versus the expected HE4 value at a Total Error of 30%. The calculated LoQ was 2.15 pmol/L on cobas e 411 and 4.42 pmol/L on cobas e 601.

According to the sponsor, all other instruments meet the same specification as that for cobas e 411 and cobas e 601. The results support a claim for LoB of 5 pmol/L, LoD of 15.0 pmol/L, and LoQ of 20.0 pmol/L in the labeling.

e. Analytical specificity:

i) Endogenous Substance Interference:

Effect on quantitation of analyte in the presence of endogenous interfering substances using the Elecsys HE4 assay was determined on cobas e 411 analyzer using pooled native human serum samples with 3 HE4 levels (54.2–60.6 pmol/L, 141–161 pmol/L, and 416–485 pmol/L) spiked with varying levels of interferent. The interfering substances tested included hemoglobin, biotin, lipemia, bilirubin, rheumatoid factor and IgG.

The resulting samples series (ten levels of interferent per sample) were tested and the %of recovery was calculated. Bias exceeding 10% for recovery was considered interference. No significant interference was noted for samples containing hemoglobin (up to 1,000 mg/dL), biotin (up to 50 ng/mL), lipemia (up to 2,000 mg/dL), bilirubin (up to 66 mg/dL), rheumatoid factors (up to 1,500 IU/mL), and IgG (up to 70 g/L).

ii) Human Anti-Mouse Antibody (HAMA) Interference:

The effect of the presence of human anti-mouse antibodies (HAMA) on the Elecsys HE4 assay was assessed on the cobas e 411 analyzer. A high HAMA serum pool (805 ng/mL) was divided into five aliquots which were further spiked with analyte to yield five different HE4 concentrations: 49.9 (unspiked), 176, 347, 535, and 1187 pmol/L. The control samples were five aliquots containing no HAMA and had concentrations corresponding to the test samples. Each high HAMA serum pool was diluted in 11 steps with the corresponding control sample. All dilutions of samples and controls were analyzed. All samples recovered within $\pm 10\%$ of their controls. The data support that HAMA at levels up to 805 ng/mL had no significant effect on the performance of the Elecsys HE4 assay.

iii) *Exogenous Substance Interference:*

Eighteen (18) pharmaceutical compounds and fourteen (14) cancer drugs were spiked into human serum samples with two HE4 levels (~50–63 pmol/L and ~707–813 pmol/L) and examined for potential interference on HE4 determination using Elecsys HE4 assay on the cobas e 411 analyzer. Significant interference was defined as $\pm 3SD$ of the reference value (unspiked sample) or $\pm 10\%$ deviation from the reference value. No significant interference was found for each compound and drug at the concentration listed below.

Commonly used pharmaceuticals:

Name of Agent	Concentration ($\mu\text{g/mL}$)	Name of Agent	Concentration ($\mu\text{g/mL}$)
Acetylcysteine	150	Methyldopa	20
Ampicillin-Na	1000	Metronidazole	200
Ascorbic acid	300	Phenylbutazone	400
Ca- Dobesilate	200	Doxycycline	50
Cyclosporine	5	Acetylsalicylic Acid	1000
Cefoxitin	2500	Rifampicin	60
Heparin	5000 U	Acetaminophen	200
Intralipid	10000	Ibuprofen	500
Levodopa	20	Theophylline	100

Cancer Drugs:

Name of Agent	Concentration ($\mu\text{g/mL}$)	Name of Agent	Concentration ($\mu\text{g/mL}$)
Carboplatin	600	Methotrexate-Dinatrium	150
Cisplatin	180	Paclitaxel	265
Cyclophosphamide	500	Fluorouracil	900
Dexamethasone	20	Bevacimab (Avastin)	750
Doxorubicin	120	Erlotinib (Tarceva)	150
Leucovorin	750	Rituximab (Mab Thera)	750
Melphalan	15	Trastuzumab (Herceptin)	600

iv) *Related Proteins:*

The specificity of the Elecsys HE4 assay was determined using native human serum samples (single donors) spiked with potential cross-reacting compounds (related proteins): Secretory Leucocyte Protease Inhibitor (SLPI) and Elastase-specific inhibitor (Elafin)/Skin-derived antileukoproteinase (SKALP). The spiked and non-spiked samples were tested in duplicates on cobas e 411. No cross-reactivity was observed for SLPI at 20,833 pmol/L and Elafin/SKALP at 54,500 pmol/L.

f. *Assay cut-off:*

There is no assay cut-off for monitoring the progression of epithelial ovarian cancer using this marker.

2. Comparison studies:

a. *Method comparison with predicate device:*

See clinical study.

b. *Matrix comparison:*

To validate different sample matrices, matched samples were collected in serum, Li-heparin, K₂-EDTA, and K₃-EDTA plasma tubes. The sample pairs were tested in duplicate using one reagent lot on cobas e 411 analyzer. Comparability between matrices was evaluated. Regression analyses of the plasma results (y) and the serum results (x) for each plasma sample type are presented below:

Matrix compared to serum	N=	Range (pmol/L)	Comparison (Passing/Bablok)
Li-Heparin	40	17.1 – 1458	$y = 0.999x + 1.50$ (r = 1.000) Slope (95% CI): 0.986 to 1.010 Intercept (95%CI): 0.654 to -2.51
K ₂ -EDTA	40	17.1 – 1458	$y = 0.980x - 0.016$ (r = 1.000) Slope (95% CI): 0.963 to 1.000 Intercept (95%CI): -0.928 to 0.702
K ₃ -EDTA	40	19.3 – 1450	$y = 1.010x - 2.310$ (r = 0.999) Slope (95% CI): 0.979 to 1.020 Intercept (95% CI): -2.980 to -1.040

3. Clinical studies:

a. *Clinical Sensitivity/Clinical Specificity:*

The effectiveness of the HE4 assays as an aid in monitoring of disease status in ovarian cancer subjects was determined by assessing changes in HE4 levels in serial serum samples from 80 female subjects diagnosed with epithelial ovarian cancer

(EOC) compared to changes in their disease status. The sample inclusion and exclusion criteria are as follows:

Inclusion criteria:

- confirmed diagnosis of ovarian cancer;
- appropriate clinical data/information;
- normal appearance of sample;
- minimum 0.5 ml volume available;
- informed Consent available;
- ≥ 3 draws available.

Exclusion criteria:

- no clinical observation per visit;
- < 3 draws per longitudinal series;
- < 18 years of age;
- insufficient volume;
- insufficient clinical information;
- ≥ 3 freeze/thaw cycles of samples;
- stored or shipped at $> 0^{\circ}\text{C}$;
- Icteric, lipemic, hemolytic, substantial particulates;
- no Informed Consent.

Serum samples were obtained from 80 women with EOC undergoing serial surveillance monitoring of cancer progression. Changes in clinical status were determined by physicians based on the clinical information (medical imaging, physical examination, and other clinical investigations). The following definition was used to categorize the patient's disease status:

1. No Evidence of Disease (NED) – a complete lack of clinical evidence of disease as determined by the treating physician.
2. Stable Disease – clinical evidence that the disease had not changed since the last assessment as determined by the treating physician.
3. Responding Disease – Clinical evidence that the primary tumor had shrunk and there was no evidence of new tumors as determined by the treating physician.
4. Progressive Disease – Clinical evidence of growth in the primary tumor or the appearance of new tumors since the last assessment as determined by the treating physician.

Among 80 subjects (age from 20 to 85 years old), 9 (11.25%) subjects were premenopausal and 71 (88.75%) were postmenopausal. Majority of subjects ($n = 69$) were Caucasian, the remaining subjects included 3 African American and 1 Asian, and 7 others with unknown races. Of 80 subjects, 51 subjects had staging information. Of 51 staged subjects, 18 (22.5%) were stage I, II while 43 (53.75%)

were stage III, IV. A total of 493 values consisting of 80 baseline values and 413 monitoring observations were obtained with the mean number of sample draws of 5.16 per subject.

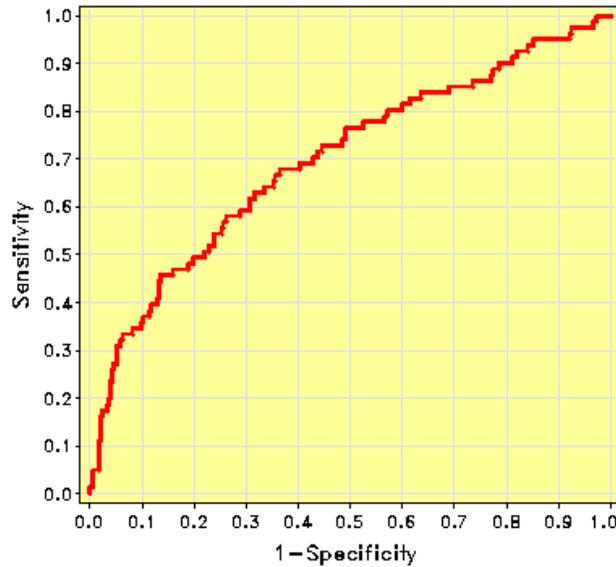
In this study, HE4 serum levels for all serum samples were measured using the Elecsys HE4 assay on cobas e 411. The performance of the Elecsys HE4 assay as an aid in monitoring of disease status in ovarian cancer subjects was determined by assessing percentage changes in HE4 levels correlated to the clinical assessment at the time of assay.

The following table shows the median (and 1st and 3rd quartile values) %change in HE4 in each clinical disease states:

Clinical Condition	N	Min	1st quartile	Median	3rd quartile	Max
NED	176	-55.4%	-9.7%	-1.9%	9.8%	252.0%
Stable Disease	85	-81.4%	-13.4%	-0.7%	20.8%	566.8%
Responding Disease	71	-93.3%	-43.1%	-15.8%	2.5%	258.1%
Progressive Disease	81	-76.0%	-2.6%	14.8%	48.9%	2642.8%

The results indicated that median %change for samples with NED and stable disease are below 0%. The median %change for samples with responding disease was -15.8% which was significantly below 0%change. Samples with progression had a median %change significantly above 0% change. The results indicated that the median %change for samples with progression and no-progression categories are related to the disease state of the subject.

For evaluation of clinical sensitivity and specificity of Elecsys HE4 assay, the clinical disease status was condensed into 2 categories: Progression and No-progression. Subjects with progression contained those monitoring events defined as progressive disease. Subjects with no-progression contained those monitoring events defined as “NED”, “Stable disease” and “Responding disease”. Sponsor shows a receiver-operator characteristics (ROC) curve analysis of the percentage change in HE4. The ROC plot gives the sensitivity/specificity for all possible cutoffs.



The area under the ROC curve is 0.699 (95% CI: 0.630 – 0.767) and is significantly better than the non-association area of 0.5. Note the observed area under the ROC curve is statistically equivalent to the area under the ROC curve reported by predicate in the k093957 (area=0.684; 95% CI: 0.618 – 0.750).

Currently, there is no clinically accepted cut-off for use in monitoring cancer progression in epithelial ovarian cancer subjects with this assay. Sponsor summarized the analysis and suggested a series of %change in HE4 cutoff values with associated sensitivity and specificity performance characteristics. The following table provides sensitivities and specificities for HE4 using a series of %change in HE4 values as determined in the clinical study.

Change in HE4	Sensitivity (n/N)	95% CI for Sensitivity	Specificity (n/N)	95% CI for Specificity
0%	69.14% (56/81)	57.61 – 80.88%	57.83% (192/332)	52.35 – 63.44%
5%	62.96% (51/81)	50.63 – 75.61%	68.07% (226/332)	62.71 – 73.08%
10%	54.32% (44/81)	42.13 – 66.67%	74.70% (248/332)	69.47 – 79.32%
15%	49.38% (40/81)	38.28 – 60.48%	78.31% (260/332)	73.68 – 82.51%
20%	46.91% (38/81)	35.44 – 58.44%	84.04% (279/332)	80.12 – 87.76%
25%	39.51% (32/81)	28.84 – 51.23%	87.05% (289/332)	83.56 – 90.34%
50%	24.69% (20/81)	16.00 – 34.52%	95.78% (318/332)	93.65 – 97.64%
75%	17.28% (14/81)	9.20 – 26.75%	97.59% (324/332)	95.92 – 99.02%
100%	14.81% (12/81)	7.50 – 23.94%	97.89% (325/332)	96.29 – 99.19%

The results indicated that for increasing % change in HE4 cutoff values, the sensitivity decreases and the specificity increases. The labeling presents a similar table of differing sensitivity and specificity values at different % change in HE4 so clinicians can choose a value that reflects their own preferences in sensitivity or specificity.

The clinical performance of the Elecsys HE4 assay at different %change in HE4 was evaluated against that of the predicate ARCHITECT HE4. The table below compared the clinical sensitivity and specificities between the Elecsys HE4 and ARCHITECT HE4 using a series of %change in HE4 values as determined in two separate clinical study.

Elecsys HE4			ARCHITECT HE4 (k093975)		
Change in HE4 (%)	Sensitivity (%) (n/N)	Specificity (%)	Change in HE4 (%)	Sensitivity (%) (n/N)	Specificity (%)
0	69.1 (56/81)	57.83	0	69.7(69/99)	58
5	62.96 (51/81)	68.07	5	62.6 (62/99)	69
10	54.32 (44/81)	74.70	10	56.6 (56/99)	75
14	50.62 (41/81)	77.41	14	53.5 (53/99)	78
15	49.38 (40/81)	78.31	15	NA	NA
20	46.91 (38/81)	84.04	20	47.5 (47/99)	84
25	39.51 (32/81)	87.05	25	39.4 (39/99)	87
50	24.69 (20/81)	95.78	50	28.3 (28/99)	94
75	17.28 (14/81)	97.59	75	18.2 (18/99)	97
100	14.81 (12/81)	97.89	100	16.2 (16/99)	98

Similar clinical sensitivity and specificity at each %change of HE4 are provided for two assays. Note neither Elecsys HE4 assay nor ARCHITECT HE4 assay should be used interchangeably with other manufactures' methods for HE4 determinations for the same patient during the monitoring of disease status.

b. Other clinical supportive data:

In this study, HE4 was determined for 80 female subjects diagnosed with epithelial ovarian cancer (EOC) compared to changes in their disease status to evaluate the performance of Elecsys HE4 assay. The percentage change in assay value was chosen to ensure that the change in the test device would not be attributed to assay variation. To evaluate the performance characteristics at a fixed %change threshold, sponsor chose a cut-off which represented 2 times the total %CV of the assay.

Because the acceptance criterion for total imprecision is no more than 7%, a 20% change was selected. The following table represents the number of all clinical visits for all 80 subjects as which a clinical evolution of progression/no-progression occurred and the percentage change of the subjects at these clinical evaluations:

		Disease Status		Total
		Progression	No-Progression	
Change in HE4	≥20%	38	53	91
	<20%	43	279	322
Total		81	332	413

Sensitivity: 46.9% (95% CI: 37.3 – 56.3%)

Specificity: 84.0% (95% CI: 81.7 – 86.3%)

At this cut-off, the assay is informative with respect to progression/no-progression. The true positive rate (0.469) minus the false positive rate (0.16) is greater than zero (difference 0.309, 95% confidence interval of difference 0.190 to 0.427).

The table below shows the distribution of results when compared to disease status:

		Disease Status				Total
		NED	Responding Disease	Stable Disease	Progressive Disease	
Change in HE4	≥20%	22	7	24	38	91
	<20%	154	64	61	43	322
Total		176	71	85	81	413

Note for the subjects with NED, this change in HE4 is not informative as 75% of subjects with NED has the %change in HE4 less than ± 20% (a %change in HE4 between -9.7% in the first quartile and 9.8% in the third quartile was observed). However, a different type of cutoff may be informative in subjects with NED vs. subjects without NED. According to the sponsor's study, 99.7% of the apparently healthy women had a HE4 assay value at or below 140 pmol/L (see the section 5, Expected values/Reference range). Since 175 out of 176 (99.4%) subjects with NED had HE4 levels below 140 pmol/L, the performance of the assay was assessed by comparing a patient's HE4 value for elevation above the normal range to detect a change in status from NED. The results are summarized in the following table:

	NED	not NED	Total
HE4 ≤140 pmol/L	175	156	331
HE4 >140 pmol/L	1	81	82
Total	176	237	413

The sensitivity of HE4 assay for NED at 140 pmol/L value was 99.4%. This indicates that 99% of subjects with NED will have HE4 concentrations less than 140 pmol/L. The specificity of the HE4 assay for NED at 140 pmol/L value was 34.2%.

For subjects with responding disease, stable disease and progressive disease, assay performance was examined in a single table to correlate these 3 different clinical states with three categories of %change in HE4 value, e.g., a %change less than 20%, %change between -20% and 20%, and %change greater than 20%. The table below represents subject counts for all subjects and all visits based upon data in the clinical study:

Change in HE4	Disease Status			
	Responding Disease	Stable Disease	Progressive Disease	Total
<-20%	32	16	8	56
-20% ≤ x ≤ 20%	32	45	35	112
> 20%	7	24	38	69
Total	71	85	81	237

The performance parameters for each clinical disease state are as follows:

<i>Responding Disease</i>			
Sensitivity	45.07% (95% CI: 33.33 – 56.52%)	Sensitivity - (1-Specificity)	30.61% (95% CI: 18.04 – 43.01%)
Specificity	85.54% (95% CI: 80.00 – 90.38%)		
<i>Stable Disease</i>			
Sensitivity	52.94% (95% CI: 42.36 – 63.25%)	Sensitivity - (1-Specificity)	8.86% (95% CI: -3.03 – 21.14%)
Specificity	55.92% (95% CI: 46.62 – 65.06%)		
<i>Progressive Disease</i>			
Sensitivity	46.91% (95% CI: 35.44 – 58.44%)	Sensitivity - (1-Specificity)	27.04% (95% CI: 15.70 – 39.13%)
Specificity	80.13% (95% CI: 74.41 – 85.81%)		

For the three clinical disease states, responding disease, stable disease and progressive disease, the HE4 assay is informative since the difference in sensitivity (true positive rate) minus 1-specificity (the false positive rate) is greater than 0.

Among 80 subjects, 71 (88.75%) subjects were postmenopausal women and 9 (11.25%) were premenopausal women. The clinical performance characteristics of Elecsys HE4 assay was analyzed based on menopausal status: The results are summarized in the below:

Change in HE4	Clinical Status				Total
	NED	Responding Disease	Stable Disease	Progressive Disease	
Postmenopausal subject					
>20% (n, %)	18 (11.5%)	4 (6.7%)	22 (28.6%)	32 (44.4%)	76
≤20% (n, %)	139 (88.5%)	56 (93.3%)	55 (71.4%)	40 (55.6%)	290
Total	157	60	77	72	366
Premenopausal subjects					
>20% (n, %)	4 (21.1%)	3 (27.3%)	2 (25.0%)	6 (66.7%)	15
≤20% (n, %)	15 (78.9%)	8 (72.7%)	6 (75.0%)	3 (33.3%)	32
Total	19	11	8	9	47

The clinical sensitivity of the 20% elevation in HE4 values for postmenopausal subjects is 44.4% (95% CI: 34.4 – 54.4%) with clinical specificity of 85% (95% CI: 82.6 – 87.5%). The clinical sensitivity for premenopausal subjects is 66.7 % (95% CI: 33.5 – 90.3%) with clinical specificity of 76.3% (95% CI: 68.5 – 81.9%).

In addition, sponsor provided the summarized results based on disease stages.

Change in HE4	NED	Responding Disease	Stable Disease	Progressive Disease	Total
Stage I and II					
≥20%	3 (5.8%)	3 (23.1%)	2 (28.6%)	3 (37.5%)	11
<20%	49 (94.2%)	10 (76.9%)	5 (71.4%)	5 (62.5%)	69
Total	52	13	7	8	80
Stage III					
≥20%	11 (12.9%)	2 (5.7%)	16 (31.4%)	23 (47.9%)	52
<20%	74 (87.1%)	33 (94.3%)	35 (68.6%)	25 (52.1%)	167
Total	85	35	51	48	219
Stage IV					
≥20%	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0
<20%	8 (100.0%)	1 (100.0%)	4 (100.0%)	3 (100.0%)	16
Total	8	1	4	3	16
Stage Unknown					
≥20%	8 (25.8%)	2 (9.1%)	6 (26.1%)	12 (54.6%)	28
<20%	23 (74.2%)	20 (90.9%)	17 (73.9%)	10 (45.4%)	70
Total	31	22	23	22	98

With the cut-off of 20% change in HE4 as determined by Elecsys HE4 assay, clinical sensitivity and specificity for patient with stage III ovarian cancer is 47.9% (95%CI: 35.4 – 60.5%) and 83.0% (95% CI: 79.5 – 86.4%), respectively. While 37.5% (95% CI: 10.7 – 71.0%) clinical sensitivity with 88.9% (95% CI: 85.9 – 92.6%) specificity is observed for patient with stage I or II ovarian cancer.

4. Clinical cut-off:

Currently, there is no clinically accepted cut-off for use in monitoring cancer progression in epithelial ovarian cancer subjects with this assay. A cut-off could be a percentage change from a previously determined value and would be expected to correlate with the clinical state at the time of assay and clinical evaluation. The labeling contains various percentage changes in HE4 from a previous value as determined in the clinical study. The assay sensitivity and specificity at each cut-off are indicated for use in a serial surveillance monitoring situation where clinical outcome is categorized as cancer progression/non-progression.

5. Expected values/Reference range:

The distribution of HE4 was determined in samples from healthy individuals and from patients with nonmalignant or malignant diseases.

For apparently healthy women, 198 premenopausal samples and 147 postmenopausal samples (total=345 samples) were tested. Samples covered age ranging from 20 to 79 and consisted of 289 White/Caucasians, 52 African American, and 2 American Indian/Alaskan Natives (the race of 2 subjects was unknown). The results are presented below:

Age	<40	40-49	50-59	60-69	>=70
N	129	63	67	59	27
Elecsys HE4 value (pmol/L)					
Mean (SD)	44.29 (11.84)	46.25 (9.67)	58.61 (28.17)	59.37 (16.15)	67.37 (16.94)
Median	41.87	44.78	50.34	55.54	63.97
Range (min, max)	27.61 – 107.40	30.78 – 73.61	28.80 – 222.50	35.11 – 114.30	44.11 – 106.00
Reference Interval (5 th , 95 th percentile)	30.36, 61.24	32.56, 63.55	37.27, 105.20	37.86, 96.46	45.77, 96.89

The results indicated that the Elecsys HE4 values among the apparently healthy women increase with increasing age.

Besides apparently healthy women population, the Elecsys HE4 value was determined in samples from 60 pregnant women, 325 patients with benign conditions and 425 patients with malignant conditions. The benign conditions included benign gynecological diseases (e.g., fibroids, cysts, pelvic inflammatory disease, HPV, endometriosis, Chlamydia, Candida), congestive heart failure (CHF), and other non-gynecological diseases (e.g., anemia, Sjögren’s syndrome, end stage renal disease, glaucoma, diabetes, diverticulitis, arthritis, hypothyroidism). The malignant conditions included epithelial ovarian cancer, endometrial cancer, breast cancer, gastrointestinal (GI) cancer, lung cancer and bladder cancer. The results of HE4 value in all tested cohort are summarized in the table below:

	N	Elecsys HE4 value				
		Mean (SD)	Median	Range (min, max)	Reference Interval	
					95 th	99 th
<i>Apparently Healthy Women</i>						
Premenopausal	198	46.92 (17.10)	43.96	27.61, 222.50	67.36	107.40
Postmenopausal	147	59.48 (18.37)	54.53	30.08, 126.40	96.89	122.10
Pregnant	60	40.22 (7.51)	39.26	26.15, 57.82	56.22	57.82
<i>Benign Diseases</i>						
Benign gynecologic diseases (Premenopausal)	145	48.65 (31.16)	43.15	19.64, 325.50	79.84	195.90
Benign gynecologic diseases (Postmenopausal)	61	95.91 (143.53)	67.54	19.66, 1139.00	201.10	1139.00
Non-gynecologic benign diseases	59	171.39 (383.94)	89.79	35.27, 2860.00	714.80	2860.00
CHF	60	181.00 (176.57)	130.90	43.59, 1172.00	481.85	1172.00
<i>Malignant Diseases</i>						
Epithelial ovarian cancer (Premenopausal)	17	318.99 (244.30)	293.70	46.90, 848.0	848.00	848.0
Epithelial ovarian cancer (Postmenopausal)	110	1118.61 (1857.32)	526.50	36.33, 12060.0	4274.50	10510.0
Endometrial cancer	57	156.29 (292.05)	72.99	33.95, 1813.0	399.80	1813.0
Breast cancer	61	251.19 (588.92)	81.18	34.43, 3510.0	890.80	3510.0
GI cancer	60	133.73 (150.15)	76.77	25.22, 933.5	337.55	933.5
Lung cancer	60	128.16 (71.32)	105.80	31.10, 308.5	269.05	308.5
Bladder cancer	60	469.07 (1017.61)	169.00	36.45, 6625.0	1858.50	6625.0

The distribution of HE4 value in healthy population, benign and malignant diseases is summarized in the table below:

	Total	Elecsys HE4 (pmol/L)				
		0.0-70.0	70.1-140	140.1- 500	500.1-1500	>1500
		N (Distribution %)				
<i>Apparently Healthy Women</i>						
Pre-menopausal	198	189 (95.5)	8 (4.0)	1 (0.5)	0 (0.0)	0 (0.0)
Post-menopausal	147	116 (78.9)	31 (21.1)	0 (0.0)	0 (0.0)	0 (0.0)
Pregnancy	60	60 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
<i>Benign Diseases</i>						
Benign gynecologic diseases (premenopausal)	145	132 (91.0)	11 (7.6)	2 (1.4)	0 (0.0)	0 (0.0)
Benign gynecologic diseases (postmenopausal)	61	32 (52.5)	22 (36.1)	6 (9.8)	1 (1.6)	0 (0.0)
Non-gynecological disease	59	17 (28.8)	31 (52.5)	8 (13.6)	2 (3.4)	1 (1.7)
CHF	60	14 (23.3)	18 (30.0)	25 (41.7)	3 (5.0)	0 (0.0)
<i>Malignant Diseases</i>						
Epithelial ovarian cancer (premenopausal)	17	2 (11.8)	3 (17.6)	8 (47.1)	4 (23.5)	0 (0.0)
Epithelial ovarian cancer (postmenopausal)	110	8 (7.3)	13 (11.8)	33 (30.0)	37 (33.6)	19 (17.3)
Endometrial cancer	57	23 (40.4)	20 (35.1)	12 (21.1)	1 (1.8)	1 (1.8)
Breast cancer	61	20 (32.8)	25 (41.0)	10 (16.4)	4 (6.6)	2 (3.3)
Gastrointestinal cancer	60	27 (45.0)	16 (26.7)	15 (25.0)	2 (3.3)	0 (0.0)
Lung cancer	60	10 (16.7)	32 (53.3)	18 (30.0)	0 (0.0)	0 (0.0)
Bladder cancer	60	9 (15.0)	19 (31.7)	22 (36.7)	7 (11.7)	3 (5.0)

In this study, 99.7% (344 of 345) of the apparently healthy women and 100% (60 of 60) pregnant women had a HE4 assay value at or below 140 pmol/L.

In patients with benign gynecologic disease, 98.6% of pre-menopausal women and 88.5% of the post-menopausal women displayed HE4 values below 140 pmol/L. Eleven women (18.6%) presenting with benign non-gynecologic diseases such as fibrocystic changes (n=6), Sjögren's syndrome (n=1), coronary artery disease (n=1), end stage renal disease (n=1), anemia (n=1) or hypothyroidism (n=1) showed HE4 values higher than 140 pmol/L. Approximately 46.7% of all subjects diagnosed with CHF presented with Elecsys HE4 values higher than 140 pmol/L.

Among patients with malignant conditions, those with EOC, bladder cancer and lung cancer showed the largest proportion of Elecsys HE4 values higher than 140 pmol/L. Four different cancer entities revealed values higher than 1500 pmol/L: Epithelial Ovarian (19), Bladder (3), Breast (2) and Endometrial (1). The post-menopausal EOC group represented the largest group with elevated HE4 values (17.3% at >1500 pmol/L).

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.