

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

A. 510(k) Number:

k072939

B. Purpose for Submission:

New device

C. Measurand:

HE4 protein (human epididymis protein 4)

D. Type of Test:

Quantitative, Enzymatic Immunoassay (EIA)

E. Applicant:

Fujirebio Diagnostics, Inc

F. Proprietary and Established Names:

HE4 EIA Kit

G. Regulatory Information:

1. Regulation section:
21 CFR § 866.6010, Tumor-associated Antigen Immunological Test System
2. Classification:
Class II HE4 assay
3. Product code:
OIU, epithelial ovarian tumor associated antigen test (HE4)
4. Panel:
Immunology 82 (HE4)

H. Intended Use:

1. Intended use(s):
The HE4 EIA is an enzyme immunometric assay for the quantitative determination of HE4 in human serum. The assay is to be used as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. Serial testing for patient HE4 assay values should be used in conjunction with other clinical methods used for monitoring ovarian cancer.
2. Indication(s) for use:
Same as intended use.
3. Special conditions for use statement(s):
Prescription use only.
4. Special instrument requirements:
Microplate reader capable of measuring optical density (OD) at 620 nm or 405 nm.

I. Device Description:

Each device contains the following:
microplate strips with breakaway microwells (12 strips x 8 wells/strip) coated with streptavidin; biotin-labeled anti-HE4 monoclonal antibody from mouse; 2 levels of HE4 controls; 6 levels of calibrators; stock solution of horseradish peroxidase-labeled anti-HE4 monoclonal antibody from mouse; wash buffer 25X concentrate; diluent; peroxide substrate; 0.1M Hydrochloric acid stop solution.

A component required but not supplied with the device is a microplate spectrophotometer capable of reading light at 620 or 450 nm in an absorbance range of 0 to 3.0. No specific spectrophotometer is listed

J. Substantial Equivalence Information:

1. Predicate device name(s):
Architect CA 125 II
2. Predicate K number(s):
k042731
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
	HE4 kit	Architect CA 125 II
Technology	Sandwich Immunoassay	Same
Calibrators	6 levels	Same
Assay format	Quantitative	Same
Platform	96 well microtiter plates	Same

Differences		
Item	Device	Predicate
Intended use	Aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer	Aid in monitoring the response to therapy in patients with epithelial ovarian cancer
Type of specimen	Human serum only	Human serum or plasma (EDTA, Li heparin, Na heparin)
Antigen detected	HE4	CA 125
Capture antibody	2H5 mouse monoclonal	OC 125 mouse monoclonal
Detection antibody	3D8 mouse monoclonal	M11 mouse monoclonal
Controls	2 levels (50 and 400 pM) supplied with kit	3 levels (supplied as separate kit)
Assay Signal	Visible color	Chemiluminescence
OD measurement	Within 2-10 minutes	Within one hour
Results Interpretation	picoMolar Units based on molecular weight derived from gene sequence	Arbitrary Mass Units

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

NCCLS (CLSI) EP5-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline-Second Edition (2004)

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

L. Test Principle:

The HE4 EIA is a solid-phase, non-competitive immunoassay based upon the direct sandwich technique using two mouse monoclonal antibodies, 2H5 and 3D8, directed against two epitopes on the HE4 molecule. Calibrators, controls and patient samples are incubated together with biotinylated Anti-HE4 monoclonal antibody (MAb) 2H5 in streptavidin coated microstrips. HE4 present in calibrators or samples is captured by the biotinylated Anti-HE4 MAb to the streptavidin coated microstrips during the incubation. The strips are then washed and incubated with enzyme-labeled Anti-HE4 MAb 3D8. After washing, buffered substrate/chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetra-methyl-benzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue color will develop if antigen is present. The intensity of the color is proportional to the amount of HE4 present in the samples. The color intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution). Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The HE4 concentrations of patient samples are then read from the calibration curve. The HE4 EIA Kit measures concentrations between 15 and 900 pM.

M. Performance Characteristics (if/when applicable):1. Analytical performance:a. *Precision/Reproducibility:*

Three studies at three different sites were performed to assess the precision of the HE4 EIA Kit. These studies were modeled after NCCLS guideline EP5-A2 "Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition (2004)". Each site performed the assay non-consecutively for 20 total days performing 2 runs per day with 4 samples tested as unknowns. The 2 runs per day are separated by a minimum of 2 hours. The 4 samples and 2 kit controls were tested in 2 replicates using 2 lots of HE4 Kits. One trained technician participated in the study. Samples 2 through 4 are comprised of native HE4 antigen supplemented into normal human sera. Panel 1 is normal sera only. The total number of replicates was 160 per sample. The individual replicates were evaluated using valid calibration curves. The kit controls were tested and evaluated for each assay to determine assay validity. A summary of these results are as follows:

Lot 1

Panel	Mean (pM) n=80	Total		Between Day		Between Run		Within Run	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	50.27	2.34	4.7	0.81	1.6	2.19	4.4	0.00	0.0
2	75.26	2.96	3.9	1.81	2.4	2.34	3.1	0.0	0.0
3	255.29	11.97	4.7	5.68	2.2	10.54	4.1	0.00	0.0
4	406.77	14.51	3.6	6.22	1.5	11.84	2.9	5.63	1.4
Control 1	48.9	2.81	5.7	0.89	1.8	2.31	4.7	1.33	2.7
Control 2	401.9	14.77	3.7	7.68	1.9	10.67	2.7	6.73	1.7

Lot 2

Panel	Mean (pM) n=80	Total		Between Day		Between Run		Within Run	
		SD	CV%	SD	CV%	SD	CV%	SD	CV%
1	48.02	2.17	4.5	0.69	1.4	2.05	4.3	0.0	0.0
2	72.35	4.70	6.5	1.73	2.4	2.33	3.2	3.69	5.1
3	241.84	12.85	5.3	5.21	2.2	9.02	3.7	7.52	3.1
4	384.77	21.61	5.6	8.71	2.3	12.89	3.4	15.00	3.9
Control 1	48.9	2.42	5.0	1.82	3.7	1.48	3.0	0.63	1.3
Control 2	389.4	12.66	3.3	8.26	2.1	9.59	2.5	0.0	0.0

The average total imprecision for the 4 panel members and the 2 kit controls ranged from 3.3% and 6.5% CV. The HE4 EIA Kit meets the predetermined acceptance criteria of $\leq 20\%$ total CV and supports the performance claims of the assay of $< 15\%$ CV.

b. *Linearity/assay reportable range:*

The assay measures concentrations between 15 and 900 pM. Dilution linearity within the assay range was evaluated using CLSI guideline EP6-A “Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline,” as a guide. Six serum samples with elevated HE4 values were diluted with 6 serum samples with low HE4 values or with HE4 Calibrator A for 12 combinations. The samples were tested as unknowns with the assay in 2 replicates for all 12 combinations. Two kit lots were used in the study. The individual sample replicates were evaluated using valid calibration curves. The kit controls were tested and evaluated for each assay to determine assay validity. The mean concentration obtained from the replicates of each sample for each dilution was compared to the mean of the expected values. The percent recovery was calculated according to the CLSI guideline. Similar percent recovery results were observed using serum as well as diluent buffer as sample diluent. The recoveries of each sample at the various dilutions (n=60) range from 84.8 to 102.1%, - average recovery 96.7%.

Analysis of the linearity data for the 6 samples using the method described in the EP6-A guideline indicates linearity for the 6 samples. Comparison of the slopes of the best fit linear line for each sample across the diluent type (serum or buffer diluent) indicates no difference in slopes. Similarity of slopes indicates no significant difference in diluent type.

Accuracy

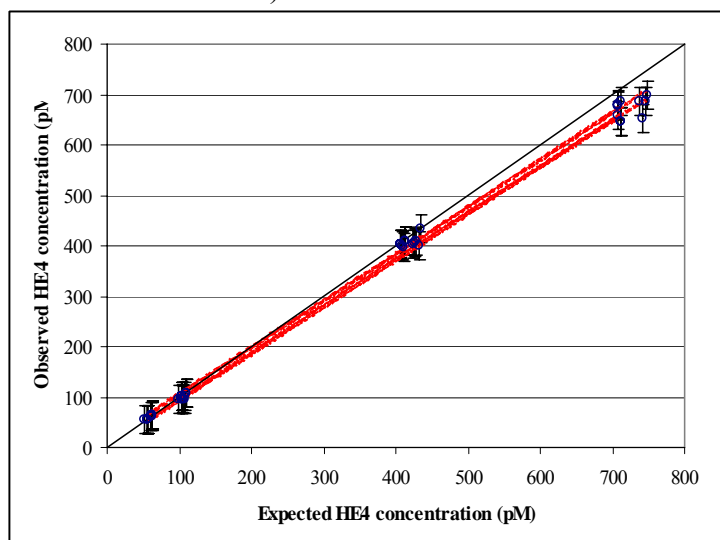
To assess assay accuracy, HE4 was added to human serum and recovery was calculated from the measured HE4 concentration in 2 separate determinations. Five (5) serum samples from apparently normal subjects were supplemented with 4 different HE4 concentrations (approximately 15 pM, 75 pM, 350 pM, and 650 pM) and tested along with unspiked samples in duplicate in the assay. The individual sample replicates were evaluated using a valid calibration

curve. The kit controls were tested and evaluated for each assay to determine assay validity. The mean concentration obtained from the 2 replicates of each sample with the added HE4 antigen was compared to the mean value of the corresponding unspiked sample without added HE4 antigen. The results for the 5 samples in both studies are as follows:

Normal sample 1 - 5

HE4 Kit Lot 1	HE4 (pM)		CV%	% Recovery
	Observed	Expected		
Unspiked	43.21		2.3	
Spiked level 1 (~15 pM)	58.17	57.57	1.1	101.0
Spiked level 2 (~75 pM)	98.74	104.79	2.4	94.26
Spiked level 3 (~350 pM)	406.63	418.65	1.9	97.16
Spiked level 4 (~650 pM)	674.23	724.13	2.5	93.13
Mean %Recovery \pm SEM				96.2 \pm 0.7

The mean recovery from both data sets meets the acceptance criteria of 100 + 15%. The percent recovery is slightly lower at concentrations above 650 pM, as noted in the lowered mean % recovery in spiked level 4. The difference is additionally noted in the following graph of observed and expected HE4 concentrations (The line of 100% recovery, or identity of observed and expected, is the solid black line).



High dose hook effect

No high dose hook effect was observed when samples containing up to approximately 300,000 pM HE4 antigen were assayed. This met the acceptance criteria of not less than 25,000 pM.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
 There is no standard or reference material for assay calibration. The HE4 Calibrator is a recombinant fusion protein consisting of the gene sequence for

human Fc antibody fragment and gene sequence for HE4. The protein is produced in a stably transfected cell line and purified from the cell line. The same recombinant HE4 antigen is used in the assay controls. Assigned values are arbitrary.

d. Detection limit:

A study was conducted to determine the lowest measurable HE4 concentration that can be distinguished from zero for the assay. NCCLS (CLSI) guideline, EP17-A “Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline,” was used as a guide to design the experiment. Two lots of HE4 calibrator B (30 pM concentration) were separately diluted with zero calibrator (0 pM concentration). Thirty-two (32) replicates of zero calibrator and the diluted calibrator of each lot were tested on 2 separate days in 4 assay runs by 2 technicians. The mean and standard deviation of assay signals were calculated for the samples with no HE4 and for samples with HE4. The Limit of Detection (LoD) of the assay was calculated to be in the range of 1.08 pM to 2.36 pM, meeting the predetermined acceptance criteria of < 15 pM. The 95% confidence interval for this determination (mean of 4 determination \pm 1.96*standard deviation) of the LoD was 1.1 to 2.2 pM.

A study was conducted to determine the lowest concentration of HE4 at which the sample CV’s was 20%. NCCLS (CLSI) guideline, EP5-A2 “Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition (2004)”, was used to design the experiment. A 5-member serum panel was made by serial 2-fold dilution of a normal serum sample with HE4 Calibrator A (0 pM). The limit of quantitation (LoQ) – described as functional sensitivity- of the HE4 EIA Kit was determined by testing each panel in 4 replicates using 2 lots of the assay at 2 separate times per day for 20 days. Two (2) technicians participated in the study. The order of the samples was changed for each run (n=160 per sample). The mean of each panel for each run, each day and an overall mean (total) for all runs was calculated. The LoQ was defined from the data as the concentration at which the CV% is less than or equal to 20%. The values determined for the HE4 EIA Kit were found to be < 5 pM, meeting the predetermined acceptance criteria of < 25 pM.

A more exact value found by modeling the trendline of %CV vs. HE4 concentration indicates that the LoQ is 3.85 pM with imprecision of 20%CV. The lowest calibrator target concentration in the assay is 30 pM. The LoQ is substantially below the lowest calibrator concentration.

e. Analytical specificity:

To evaluate the potential interference in the assay from lipids, bilirubin, hemoglobin, protein, human anti-mouse antibodies (HAMA), rheumatoid factor (RF), NCCLS (CLSI) guideline, EP7-A “Interference Testing in Clinical Chemistry, Approved Guideline,” was used to design the interference experiments. The percent recovery was calculated in each separate

experiment. The acceptance criterion was mean percent recovery of $100 \pm 15\%$.

The percent recoveries for samples with 30 mg/mL (3 g/dL) of supplemented lipid ranged from 88.8% to 93.6%, average recovery equaling 90.3%. Less than 15% average interference (i.e., mean recovery of $100 \pm 15\%$) was observed in the assay with samples containing an elevated level of lipids.

The percent recoveries for samples with 0.2 mg/mL (20 mg/dL) of supplemented bilirubin ranged from 98.3% to 101.0%, average recovery equaling 99.8%. Less than 15% average interference was observed in the assay with samples containing an elevated level of bilirubin.

The percent recoveries for samples with a hemoglobin concentration of 5 mg/mL (500 mg/dL) and 10 mg/mL (1000 mg/dL) ranged from 96.6% to 104.7%, average recovery equaling 99.6%. Less than 15% average interference was observed in the assay with samples containing an elevated level of hemoglobin.

The percent recoveries for samples with a total protein concentration of 120 mg/mL (12 g/dL) were 111.1% to 114.1%, average recovery equaling 113.1%. Less than 15% average interference was observed in the assay with samples containing an elevated level of protein.

To evaluate interference from HAMA, 5 specimens containing HAMA and 2 normal serum samples were each split into 3 equal aliquots. To two of the aliquots, 2 different concentrations of HE4 were added, one HE4 concentration in each aliquot. Samples were tested as unknowns with the assay, in 4 replicates. Two lots of kit were used in the study. The mean concentration obtained from the 4 replicates of each test sample was compared to the mean value of the corresponding control sample for each kit lot. The range of recoveries for HAMA samples supplemented with a low HE4 concentration (approximately 100 pM) was from 92% to 120%, average recovery equaling 103%. The range of recoveries for HAMA samples supplemented with a high HE4 concentration (approximately 500 pM) was from 93% to 105%, average recovery equaling 98%. The HAMA concentrations ranged from 80 to > 400 ng/mL. The overall average recovery was 101% for both HE4 concentrations. Less than 15% average interference was observed in the assay with samples up to 400 ng/mL of HAMA.

Rheumatoid factor interference was assessed in a manner similar with HAMA interference. Five RF specimens and 2 normal serum samples were each split into 3 equal aliquots and supplemented with two different concentrations of HE4. Samples were tested as unknowns with the assay, in 4 replicates. Two lots of kit were used in the study. The range of recoveries for rheumatoid factor samples supplemented with a low HE4 concentration (approximately 120 pM) was from 87% to 107%, average recovery equaling 98%. The range of recoveries for rheumatoid factor samples supplemented with a high HE4 concentration (approximately 525 pM) was from 90% to 106%, average recovery equaling 93%. The RF concentrations ranged from 21 to 568 IU/mL.

The overall average recovery was 95% for both HE4 concentrations. Less than 15% average interference was observed in the assay with samples containing elevated levels of rheumatoid factor up to 568 IU/mL.

To evaluate the potential interference from several chemotherapeutic agents in the assay, NCCLS (CLSI) guideline, EP7-A, was used to design the interference experiments. Two samples of human serum were supplemented with each chemotherapeutic as a potential interfering substance. The list of various chemotherapeutics prepared for spiking is described in the following table. Control samples were spiked with an equal volume of each respective solvent.

Chemotherapeutic Agent	Test Concentration
Carboplatin	500 µg/mL
Cisplatin	165 µg/mL
Clotrimazole	0.3 µg/mL
Cyclophosphamide	500 µg/mL
Dexamethasone	10 µg/mL
Doxorubicin	1.16 µg/mL
Leucovorin	2.68 µg/mL
Melphalan	2.8 µg/mL
Methotrexate	45 µg/mL
Paclitaxel	3.5 ng/mL

One sample of human serum was spiked with ovarian cancer patient serum to a concentration of about 90 pM HE4. This is used as a base pool and was split into 2 aliquots for each chemotherapeutic agent to be tested. One aliquot was spiked with the stock solutions of the chemotherapeutic agents (at a 1:20 dilution) to the test concentration. One aliquot was spiked with an equal volume of the respective solvent for use as a control sample.

A second sample of human serum was spiked with recombinant HE4 antigen rather than serum from an ovarian cancer patient to a concentration of ~90 pM. This is used as a base pool and was split into 2 aliquots for each chemotherapeutic to be tested. The control samples and the samples supplemented with chemotherapeutics were tested as unknowns with the HE4 EIA Kit, in 4 replicates, randomly pipetted. Two kit lots were used in the study. The individual sample replicates were evaluated using valid calibration curves and kit controls were tested and evaluated to determine assay validity. The mean concentration obtained from the 4 replicates of each sample with the added chemotherapeutics was compared to the mean value of the corresponding control sample with solvent only. The percentage recovery was calculated. The range of the percent recoveries for samples with added chemotherapeutics was 96.2% to 106.2%. Less than 15% interference was observed in the assay meeting the predetermined acceptance criteria of $100 \pm 15\%$ recovery for each chemotherapeutic tested.

f. Assay cut-off:

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

2. Comparison studies:

a. *Method comparison with predicate device:*

A predicate does not exist for this device so no comparison study was performed.

b. *Matrix comparison:*

Only serum is used as the sample matrix.

3. Clinical studies:

a. *Clinical Sensitivity Clinical specificity:*

The general study objective for the clinical study was to obtain sufficient clinical data to support the proposed intended use and to show non-inferiority of the HE4 EIA Kit to the Abbott ARCHITECT CA 125 11 immunoassay. Although the HE4 EIA Kit measures a different antigen than the ARCHITECT CA 125 11 assay, the intended uses are the same.

To determine the utility of the HE4 EIA Kit as an aid in monitoring ovarian cancer progression in women diagnosed with invasive epithelial ovarian cancer, serum sets were assessed from multiple serial samplings at various clinical evaluation times during surveillance monitoring. This study used only retrospective serum samples. The specimens used in the monitoring study were obtained from a large cancer center in the United States. No samples were specifically drawn for this study. The sample inclusion and exclusion criteria are as follows:

Inclusion criteria

- Ovarian cancer
- Appropriate clinical data
- Minimum 0.5 mL volume available
- Normal appearance
- Informed consent
- Appropriate information

Exclusion criteria

- No diagnosis of ovarian cancer
- Insufficient volume
- Multiple freeze-thaw stored or shipped @4°C
- Icteric, lipemic, hemolytic, substantial particulates
- No informed consent

Serum samples were obtained from 80 women with epithelial ovarian cancer undergoing serial surveillance monitoring of cancer progression. Changes in clinical status were determined using imaging data in the patient's record. This information used only tangible evidence of progression, or lack of progression, such as enlargement of lesions or other objective physical evidence. It should be noted that because the endpoint of this clinical trial was to demonstrate non-inferiority to a previous device, the primary endpoint was to compare changes in the HE4 EIA Kit concentrations over time with

changes in clinical status in comparison with changes in ARCHITECT CA 125 II concentrations over time and changes in clinical status. These 80 cases consisted of the following subgroups:

- 1) Women with no evidence of disease after therapy at last blood draw. No Evidence of Disease - NED: A complete lack of clinical evidence of disease as determined by the treating physician.
- 2) Women with evidence of residual but stable disease after therapy at last blood draw. Stable Disease - SD: Clinical evidence that the disease has not changed since last assessment as determined by the treating physician.
- 3) Women with progressive (recurring) disease after primary therapy at last blood draw. Progressive Disease - PD: Clinical evidence of growth in the primary tumor or the appearance of new tumors since the last assessment as determined by the treating physician.
- 4) Women with disease responsive to therapy at the last blood draw. Responding Disease - RD: Clinical evidence that there is a shrinking of the primary tumor and no evidence of new tumors as determined by the treating physician.

The outcome measure for this analysis was the determination of progression of disease from one time point to a succeeding time point. In this analysis, the total sample is the number of all clinical visits made by all patients after diagnosis of ovarian cancer and prior to death, loss to follow up or remission of disease. Disease progression from visit to visit was determined by the patient's physician based on either or both of the following:

1. Examination of the patient for clinical signs and symptoms, including the results of laboratory tests that are current standard of care for the assessment of ovarian cancer disease status. The determination of progression/non-progression included the use of a CA125 assay in some cases. In the instances where CA125 was used the ARCHITECT CA125II, was not used in determination of progression/non-progression.
2. Examination of radiographic findings (imaging) that can be used for the assessment of ovarian cancer disease status. Radiographic findings include results from CAT scans, PET scans, MRI and x-Ray images, as well as ultrasound. Second look surgery results, where available, will also be utilized.

Of 80 women in whom assay values and a progression/non-progression determination was made, 73 subjects had staging information. Of 73 staged subjects, 15% were stage I, II while 85% were stage III, IV. Of 71 subjects having menopausal status determinations, 66 were post-menopausal, 3 pre-menopausal, and 2 peri-menopausal.

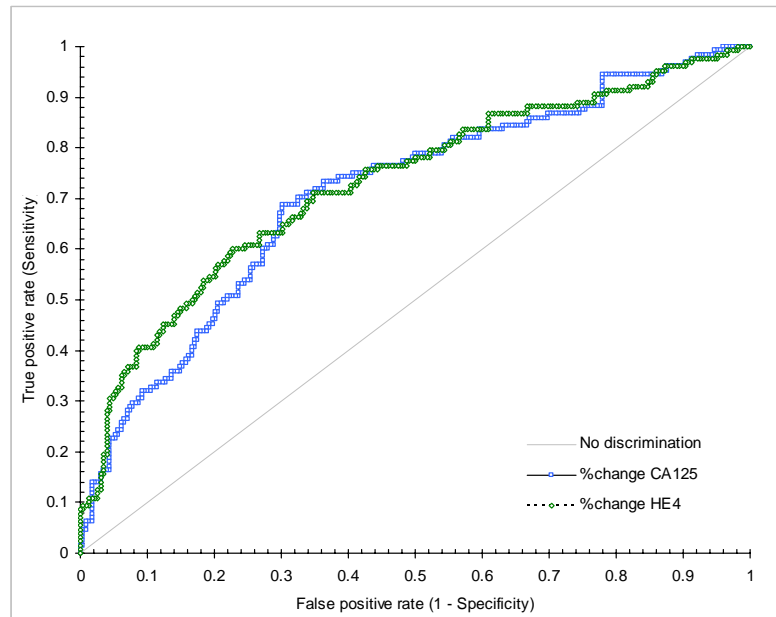
A definition of the percentage change in assay value was chosen to ensure that the change in the test device would not be attributed to assay variation. A positive change in the HE4 EIA Kit value was defined as an increase in the value that is at least 25% greater than the previous value of the test. The level

of change represented 2.5 times the total CV% of the assay. Because the maximum Total CV% for the HE4 EIA Kit was 9.3%, a 25% change was chosen. Based on this cutoff for HE4, the following table represents the number of all clinical visits for all 80 subjects at which a clinical evaluation of progression/non-progression occurred and the percentage change category of the subjects at these clinical evaluations:

%change HE4	Progression	No progression	Total
≥25%	76	57	133
<25%	50	171	221
Total	126	228	354

At this cut-off, the assay is informative with respect to progression/non-progression. The true positive rate (0.603) minus the false positive rate (0.250) is significantly greater than zero (difference 0.353, $p < 0.001$, 95% confidence interval of difference 0.251 to 0.455).

Receiver-Operator Characteristics (ROC) curves were drawn for both CA 125 and HE4. The two curves are similar in their shape, with AUCs of 0.725 (95% CI = 0.675 to 0.770) for HE4 and 0.709 (95% CI = 0.659 to 0.756) for CA 125. Given the overlap of the two 95% Confidence Intervals these two curves are not statistically significantly different. The ROC analysis indicates that the HE4 assay is not inferior to the CA125 assay for detecting cancer progression.



There is currently no clinically accepted cut-off for use in monitoring cancer progression in epithelial ovarian cancer subjects with this assay. The labeling contains various percentage changes in HE4 from a previous value as determined in the ROC analysis. The assay performance characteristics (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. The following table provides sensitivities and specificities for HE4 using several cut-offs of percent change in HE4 values as determined in the clinical study.

Cut-off HE4	Sensitivity HE4	Specificity HE4	Lower CI for Specificity	Upper CI for Specificity
-29.0%	94%	14%	9.8%	19.2%
-6.9%	84.1%	39%	32.7%	45.7%
6.8%	74%	57.5%	50.8%	64.0%
10.5%	70.6%	62.3%	55.6%	68.6%
15.4%	64.3%	69.3%	62.9%	75.2%
24.7%	60.3%	75%	68.9%	80.5%
41.2%	49.2%	83.3%	77.8%	87.9%
67.8%	39.7%	90.8%	86.3%	94.2%

A clinician could select any of several cut-off values but the estimated performance will be confined by the choice. Note further that at high sensitivity or specificity (for example > 85%) values, the cut-off is likely not practical. A decrease in HE4 of 29%, while giving approximately 95% sensitivity, is not practical to aid in excluding progression due to the very high false positive rate (0.86). Similarly, an increase in HE4 of 68%, while giving approximately 90% specificity, is also not practical to aid in detecting progression due to the very high false negative rate (~0.60). Intermediate cut-off values would lead to a trade-off in sensitivity and specificity and would reflect the clinician preference for a given patient with a set of clinical symptoms and/or signs.

Based on the 25% change cutoff for CA125, the following table represents the number of all clinical visits for all 80 subjects at which a clinical evaluation of progression/non-progression occurred and the percentage change category of the subjects at these clinical evaluations:

%change CA125	Progression	No progression	Total
≥ 25%	87	71	158
<25%	39	157	196
Total	126	228	354

At this cut-off, the CA125 assay is informative with respect to progression/non-progression. The true positive rate (0.691) minus the false positive rate (0.311) is significantly greater than zero (difference 0.379, $p < 0.001$, 95% confidence interval of difference 0.278 to 0.480).

Due to the slight difference in HE4 assay performance compared with CA125 assay performance at a given cutoff, the ROC analysis was utilized to fix the sensitivity percentage for cancer progression and estimate the cut-off value and specificity percentage for the HE4 assay and CA125 assay. The following table summarizes the cut-off and specificity values for the HE4 and CA125 assay at the same fixed sensitivity.

Cut-off HE4	Sensitivity HE4	Specificity HE4	Cut-off CA125	CA125 Specificity at matching Sensitivity
-29.0%	94%	14%	-59.7%	12.3%
-6.9%	84.1%	39%	-23.6%	32.9%
6.8%	74%	57.5%	6.9%	56.6%
10.5%	70.6%	62.3%	16.7%	63.6%
15.4%	64.3%	69.3%	32.8%	70.2%
24.7%	60.3%	75%	39.2%	71.1%
41.2%	49.2%	83.3%	71.9%	78.1%
67.8%	39.7%	90.8%	99.2%	82.9%

Note from the table that the cut-off and specificity for CA125 are different from the cut-off and specificity for HE4. The specificity values for CA125 and HE4 are not statistically different at the respective cut-offs and sensitivity values thus supporting non-inferiority of HE4 compared to CA 125. However, the cut-off values are different in value and reflect different points on the ROC curve compared with HE4 for a given sensitivity.

Of the subjects evaluated, there was no constant total follow-up time or interval between visits. The percentage change in biomarker values reflected the difference from the previous value regardless of the time interval between visits. In order to assess if the differing time intervals contributed to substantial percentage changes in biomarker and with marker performance, the percentage change was adjusted by the time in months between clinical evaluation visits. The adjustment was calculated as the percentage change per month between clinical visits. The percentage change per month was evaluated by ROC analysis against clinical progression/non-progression. The area under the ROC curve (0.73, 95% confidence interval 0.669 to 0.784) did not differ from the area without adjustment. Therefore, the time interval between clinical evaluation visits did not affect overall assay performance. The %change per month and specificity values at fixed sensitivity is summarized in the following table:

	HE4				CA125	
Sensitivity	cut-off	Specificity	lower CI	upper CI	cutoff	specificity
94.5%	-17.0%	12.3%	8.3%	17.3%	-33.2%	13.6%
80.5%	-1.4%	44.3%	37.7%	51.0%	-2.9%	44.7%
65.6%	5.9%	69.3%	62.9%	75.2%	14.0%	72.8%
50.0%	18.0%	83.8%	78.3%	88.3%	35.1%	86.0%
38.3%	24.9%	89%	84%	93%	48%	89%
25.0%	46.8%	96.9%	93.8%	98.8%	81.8%	95.6%

Note from the table that the percentage change per month values are dissimilar from the %change as described above. This analysis indicates substantial differences in actual percentage change but otherwise do not reflect differences in assay performance for HE4 or CA125. It is unlikely that a clinician would prefer or consistently evaluate marker values as a percent change per month compared with a percentage change from the previous

marker value. Since the performance is not altered and since special recommendations to clinicians would be confusing, no special suggestion need occur. It is unclear if the percentage change per month would become important with other biomarkers.

Assay performance for the HE4 assay was examined in a single table to correlate the 4 different clinical states (No evidence of disease, responding disease, stable disease, and progressive disease) with three categories of %change in HE4 value. The table below represents subject counts for all subjects and all visits based upon data in the clinical study:

%change HE4	NED	Responding	Stable	Progressive	Total
<-25%	6	12	22	10	50
-25% ≤ x ≤ 25%	50	10	71	40	171
>25%	18	4	35	78	135
Total	74	26	128	128	356

In the table the percentage change in HE4 is categorized as a %change less than -25%, %change between -25% and 25%, and %change greater than 25%. The performance parameters for each clinical disease state are as follows:

Performance parameter	Value	S.E.	Evaluation parameter	Value	S.E.
Sensitivity (responding)=	46.2%	± 0.098	TPR-FPR=	34.6%	± 9.9%
Specificity (responding)=	88.5%	± 0.018	95% CI	15.2%	54.1%
Sensitivity (stable)=	55.5%	± 0.044	TPR-FPR=	11.6%	± 5.5%
Specificity (stable)=	56.1%	± 0.033	95% CI	0.9%	22.4%
Sensitivity (progression)=	60.9%	± 0.043	TPR-FPR=	35.9%	± 5.2%
Specificity (progression)=	75.0%	± 0.029	95% CI	25.8%	46.1%

For the three clinical disease states responding disease, stable disease, and progressive disease the HE4 assay is informative since the difference in true positive rate and the false positive is higher than 0% difference ($p < 0.05$).

For the disease state no evidence of disease (NED), the sensitivity and specificity when HE4 is not increasing (i.e. $\leq 25\%$ change) was $75.7\% \pm \text{S.E. } 5.0\%$ and $41.5\% \pm \text{S.E. } 2.9\%$, respectively. The HE4 assay, when not increasing, is informative since the difference in true positive rate and false positive rate was $17.2\% \pm \text{S.E. } 5.8\%$ (95% confidence interval of difference 5.8% to 28.5%).

For the 4 clinical disease states evaluated, the HE4 assay is informative when categorized as $< -25\%$ change, between -25% and 25% change, and $> 25\%$ change.

In the 3 x 4 table above, of the 74 subjects with NED, a substantial number (50) have a %change in HE4 that is between -25% and 25%. This number would not be unexpected for subjects with no evidence of clinically active or

responding disease. It suggests that a %change in such subjects is small and not sufficiently robust at discriminating subjects with inactive or no evidence of disease compared with active or responding disease. One could assess performance by comparing a patient's HE4 value for elevation above the upper limit of the normal range to detect a change in status from no evidence or inactive disease.

	NED	not NED	Total
HE4 \leq 150 pM	66	102	168
HE4 > 150 pM	8	180	188
Total	74	282	356

When using the upper limit of normal subjects, 150 pM, as determined from normal healthy pre-menopausal or post-menopausal women, the sensitivity of the HE4 assay for no evidence or inactive disease was 89% (95% confidence interval 79.8% to 95.2%). The specificity of the HE4 assay for no evidence of disease at 150 pM value was 64% (95% confidence interval 57.9% to 69.4%). The sensitivity and specificity of the HE4 assay at a single HE4 value is higher than the sensitivity and specificity of the %change in HE4 of \leq 25% (75.7% sensitivity and 41.5% specificity).

b. Other clinical supportive data:

One thousand one hundred fifty (1150) serum patient specimens with various conditions were assessed using the HE4 assay to establish the ranges in various subject groups. The following patient cohorts were assembled to determine the distribution of the serum values in various benign and malignant conditions.

Cohort	Number
Apparently Healthy	
Normal healthy female: pre menopausal	76
Normal healthy female: post menopausal	103
Additional healthy female	3*
Total healthy females	182
Non Malignant Conditions	
Pregnant females	22
Benign gynecological disease	347
Other benign diseases	108
Hypertension/congestive hearth failure	96
Malignant Conditions	
Breast Cancer	46
GI Cancer	56
Endometrial Cancer	116
Lung Cancer	50
Ovarian Cancer	127
Total	1150

* Three subjects could not be categorized as pre- or post-menopausal based on their ages.

All of the serum samples that were used in this study were collected under an IRB approved protocol or when the local IRB determined that Informed Consent was not necessary. Samples were obtained from either commercial vendors of specimen banks or collected prospectively from clinical sites under the clinical study protocol. Samples were obtained from one source as “remnant samples” and the local IRB determined that Informed Consent was not necessary. All samples were tested in duplicate using the HE4 assay. The individual sample replicates were evaluated using valid calibration curves and the mean of the replicates was calculated. The HE4 assay controls were tested and evaluated for each assay to determine assay validity. This study was performed at three (3) sites.

Descriptive statistics for Healthy and Benign patient cohorts tested for Reference Ranges in the HE4 EIA Kit include the following:

	Healthy			Benign Diseases		
	Pre-menopausal	Post-menopausal	Pregnancy	Benign Gynecologic	Benign Other	Hypertension/ Congestive Heart Failure
N	76	103	22	347	108	96
Mean	64.0	71.1	63.7	82.5	233.0	165.0
SD	78.8	74.3	38.4	136.7	498.0	317.8
90 th Percentile	87.1	112.0	75.7	113.0	444.5	231.6
95 th Percentile	119.1	154.1	128.6	174.5	1133.4	297.5
Specificity (%)	94.7	94.2	95.5	93.4	75.9	78.1

Descriptive statistics for Cancer patient cohorts tested for Reference Ranges in the HE4 EIA Kit include the following:

	Cancers				
	Ovarian	Breast	Endometrial	Colon/GI	Lung
N	127.0	46	116	56	50
Mean	1197.2	96.8	205.2	163.8	169.3
SD	1965.2	75.0	399.9	527.7	102.0
90th Percentile	3342.7	185.9	442.7	165.5	310.5
95th Percentile	5339.0	260.9	755.3	194.2	393.2
Sensitivity (%)	78.7	13.0	25.9	16.1	42.0

The results shown in the above tables demonstrate that the HE4 assay showed reasonable specificity in healthy women, pregnant females, and in women

with benign gynecologic diseases. Twenty three (23) samples (6.6%) from the cohort of 347 women with benign gynecologic diseases had HE4 concentrations exceeding the Upper Limit of normal. These included: 3 subjects with cysts, 10 subjects with cystadenomas, 6 subjects with fibromas, and 4 subjects with other conditions (one normal sample, one hemorrhagic follicle, one ovarian torsion and one myoma).

The HE4 assay showed lower specificity relative to non-disease subjects in patients with congestive heart failure. This was an unexpected finding and is not associated with the known tissue expression of the HE4 (WFDC2) gene or protein. Similarly, the assay showed a lower specificity in patients with “Other Benign Diseases”. The patients with non-gynecologic benign disease would likely be clinically distinguishable from subjects with ovarian cancer or adnexal masses.

Sensitivity for breast, colon, and endometrial cancers was low. HE4 is elevated in a minority of these cancers. The sensitivity of HE4 in lung cancer was 42% and is consistent with the known up-regulation of the HE4 gene and protein in lung cancers, particularly adenocarcinoma of the lung.

4. Clinical cut-off:

There is currently no clinically accepted cut-off for use in monitoring cancer progression in epithelial ovarian cancer subjects with this assay. A cut-off for use in this situation 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 performance characteristics (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:

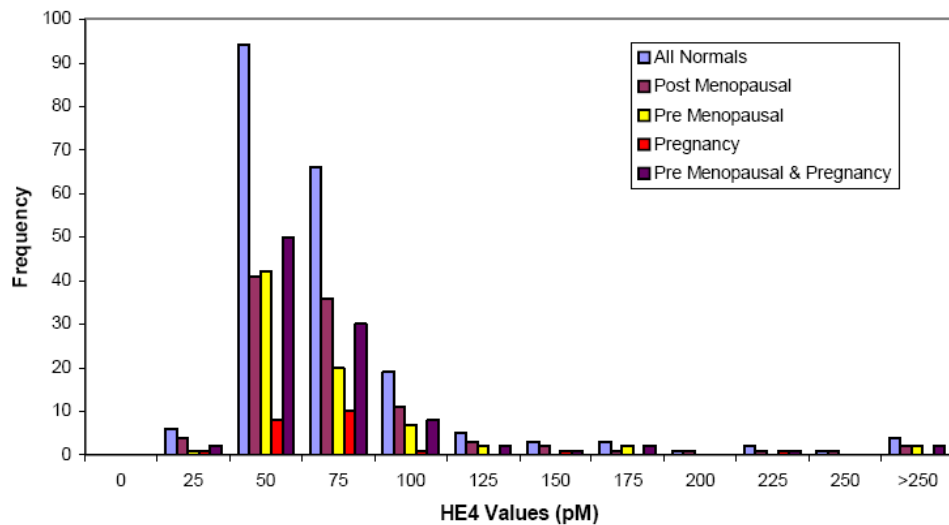
To determine the normal range of the HE4 EIA Kit using serum samples from an apparently healthy pre-menopausal and post-menopausal population, a sample of 204 females who were apparently disease free was assessed. The following table describes the included women:

Cohort	Number
Apparently Healthy	
Normal healthy female: pre menopausal	76
Normal healthy female: post menopausal	103
Additional Healthy Female	3*
Pregnant females	22
Total healthy female subjects	204

* Three subjects could not be categorized as pre- or post-menopausal based on their ages.

All samples were tested in duplicate. Two kit lots were used in the study. The individual sample replicates were evaluated using valid calibration curves and the mean of the two replicates was calculated. The kit controls were tested and evaluated for each assay to determine assay validity. This study was performed at two (2) sites.

Menopausal status was determined in one of two ways. For some patients, the menopausal status was known and recorded on data sheets. For other patients, only the age was known at the time of sample draw. For these patients, ages of <48y were considered pre-menopausal. Similarly, ages >52y were considered post-menopausal. This is based on the known median and mean age of menopause for US women (51 years of age) as measured by the American College of Obstetrics and Gynecology. The Figure below is a frequency plot of the assay concentrations grouped according to 25 pM HE4 concentrations ranges.



The figure shows that HE4 concentrations are not normally distributed for the subject populations. The sponsor determined 95% Upper Limits for each population using a non-parametric, 1-tailed analysis. The non-parametric upper 95%, one-tailed confidence interval was determined for the entire set, each subset and the subset containing pre-menopausal and pregnant females by percentile ranking. The results are presented in the following table.

Sample Class	N	Percentile Ranking Upper Limit (pM)	95% Confidence Limits	
			Lower	Upper
All Normal Samples	204	153.4	102.4	205.4
Post Menopausal	103	154.1	93.5	221
Pre Menopausal	76	119.1	84.5	293.5
Pre Menopausal + Pregnant	98	136.7	84.7	215.9

Using percentile ranking analysis, 95% of all healthy subjects and pregnant females had HE4 values below 153 pM. The 95% confidence intervals show a

wide range of values. This is affected, at least in part, by two samples with very high HE4 values (>600 pM). However, there is no information known about these healthy subjects that justifies removing them from the analysis. Because it is sometimes difficult to be certain of menopausal status, and because the 95% confidence interval for these samples ranges from 102 to 205 pM, the sponsor recommends an Upper Limit of 150 pM for the assay.

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.