510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K171605

B. Purpose for Submission:

Modification of Previously Cleared Device – Addition of **cobas e** 801 instrument for the use of the device

C. Measurand:

CA 15-3

D. Type of Test:

Quantitative, automated electrochemiluminescence immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys CA 15-3 II

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.6010 – Tumor-associated antigen immunological test system

2. Classification:

   Class II

3. Product code:

   MOI – System, test, immunological, antigen, tumor

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   Immunological in vitro assay for quantitative determination of CA 15-3 in human serum and Li-heparin plasma to aid in the management of breast cancer patients. In conjunction with other clinical and diagnostic procedures, serial testing with this assay is an aid

   - in the early detection of recurrence in previously treated stage II and III breast cancer patients
   - for monitoring response to therapy in metastatic breast cancer patients

   The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e 801** immunoassay analyzers.

2. Indications for use:

   Same as Intended Use above

3. Special conditions for use statement:

   For prescription use only

4. Special instrument requirements:

   cobas e 801 immunoassay analyzer (K162606)

I. Device Description:

The **cobas e** pack for Elecsys CA 15-3 II contains the following reagents:

M: Streptavidin-coated microparticles (0.72 mg/mL) in preservative, 1 bottle (12.4 mL)

R1: Biotinalyted monoclonal anti-CA 15-3 antibody (115D8; mouse) (1.75 mg/L) in phosphate buffer (pH 6.0) and preservative, 1 bottle (19.7 mL)

R2: Monoclonal anti-CA 15-3 antibody (DF3; mouse) (10 mg/L) labeled with ruthenium complex in phosphate buffer (pH 7.0) and preservative, 1 bottle (19.7 mL)

J. Substantial Equivalence Information:

1. Predicate device name:

   CA 15-3 II Assay

2. Predicate 510(k) number:

   K001468

3. Comparison with predicate:

Similarities		
Item	Device Elecsys CA 15-3 II on **cobas e** 801	Predicate CA 15-3 II
Intended Use	Immunological in vitro assay for quantitative determination of CA 15-3 in human serum to aid in the management of breast cancer patients. In conjunction with other clinical and diagnostic procedures, serial testing with this assay is an aid • in the early detection of recurrence in previously treated stage II and III breast cancer patients • for monitoring response to therapy in metastatic breast cancer patients The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on **cobas e 801** immunoassay analyzers.	Same
Capture Antibody	Biotinylated mouse monoclonal antibody (I1508) and DF3 antibodies	Same
Detection Antibody	Ruthenium labeled mouse monoclonal antibody (DF3)	Same
Calibrators	CA 15-3 II CalSet	Same
Controls	PreciControl Tumor Marker	Same
Traceability	The method has been standardized against the Enzymun-Test CA 15-3 method. This in turn has been standardized against the CA 15-3 RIA from Fujirebio Diagnostics.	Same
Sample Stability	2–8°C: 5 days −20°C: 90 days	Same

Differences		
Item	Device Elecsys CA 15-3 II on **cobas e** 801	Predicate CA 15-3 II
Instrument Platform	**cobas e** 801	Elecsys 2010 Modular Analytics E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

Differences		
Item	Device Elecsys CA 15-3 II on **cobas e** 801	Predicate CA 15-3 II
Sample type	Human serum and Li-heparin plasma	Human serum, (Li-, Na-, NH_4^+-) heparin plasma, and K3-EDTA plasma
Prewash	Added	None
Sample dilution	1:20	1:10
Sample volume	12 µL	20 µL
Measuring range	3.0–300 U/mL	1.0–300 U/mL
Calibration Frequency	After 28 days when using the same **cobas e** pack on the analyzer	After 7 days (when using the same reagent kit on the analyzer)
Reagent Stability	Unopened: 18 months Opened: N/A On-board: 16 weeks	Unopened: 18 months Opened: 12 weeks On-board: 5 weeks
Assay – Reagent Preparation	Place the cooled (stored at 2–8°C) **cobas e** pack on the reagent manager.	Bring the cooled reagents to approximately 20°C and place on the reagent disk (20°C) of the analyzer
Sample Stability	20–25 ^{o}C: 48 hours	Not tested

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

CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition

CLSI EP6-A, Evaluation of Linearity of Quantitative Measurement, Approved Guideline.

CLSI EP09-A3, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; Approved Guideline—Third Edition

CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

CLSI C28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

L. Test Principle:

The Elecsys CA 15-3 II Test System is a two-step sandwich immunoassay with streptavidin microparticles and electrochemiluminescence detection for the quantitative determination of CA 15-3 in human serum and plasma. In the first step, CA 15-3 in the sample binds to biotinylated monoclonal CA 15-3 II specific antibody and a monoclonal CA 15-3 II specific

antibody labeled with a ruthenium complex) to form a sandwich complex. In the second step, the complex binds to the streptavidin-coated microparticles. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode and unbound substances are then removed with ProCell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. Results are determined using a calibration curve that is generated specifically on each instrument by a 2-point calibration and a master curve provided with the reagent barcode.

M. Performance Characteristics (if/when applicable):

1. Analytical performance: The test results met the sponsor's pre-determined acceptance criteria for all analytical performance studies.

   a. Precision/Reproducibility:

   The within-laboratory precision of the Elecsys CA 15-3 II assay on **cobas e** 801 was evaluated according to CLSI guideline EP05-A3 by testing seven human serum pools samples and two control samples (PreciControl Tumor Marker: PC 1 and PC 2). Samples were tested in two replicates per run, two runs per day, for 21 days using one reagent lot on one **cobas e** 801 immunoassay analyzer (n=84 observations per sample). The results are summarized in the following table:

Sample	Mean (U/mL)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	3.33	0.08	2.3	0.00	0.0	0.22	6.6	0.23	7.0
1a	3.80	0.07	1.9	0.02	0.6	0.25	6.5	0.26	6.8
HS1	27.90	0.46	1.6	0.39	1.4	0.47	1.7	0.77	2.8
HS2	102.00	2.19	2.1	0.80	0.8	1.70	1.7	2.89	2.8
HS3	109.00	2.13	1.9	0.66	0.6	1.55	1.4	2.73	2.5
HS4	277.00	8.61	3.1	0.00	0.0	5.63	2.0	10.30	3.7
HS5	284.00	7.20	1.5	3.68	1.3	5.64	2.0	9.92	3.5
PC 1	23.20	0.30	1.3	0.34	1.5	0.42	1.8	0.62	2.7
PC 2	95.70	1.49	1.6	1.27	1.3	2.11	2.2	2.88	3.0

The lot-to-lot reproducibility was evaluated by testing five human sample pools and two controls (PC 1 and PC 2) using three lots of reagents. For each lot of reagent, each sample was tested in replicates of five per run, one run per day for five days on one **cobas e** 801 immunoassay analyzer (n=75 observations per sample). The results are summarized in the following table:

Sample	Mean (U/mL)	Within-Run		Between-Day		Between-Lot		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	3.71	0.10	2.8	0.10	2.6	0.12	3.2	0.18	4.9
2	25.66	0.67	2.6	0.10	0.4	0.21	0.8	0.71	2.8
3	123.57	3.37	2.7	2.59	2.1	0.00	0.0	4.25	3.4
4	268.40	5.66	2.1	5.99	2.2	2.88	1.1	8.75	3.3
5	294.48	7.63	2.6	8.05	2.7	0.00	0.0	11.1	3.8
PC 1	22.18	0.41	1.8	0.23	1.0	0.13	0.6	0.49	2.2
PC 2	90.96	1.71	1.9	0.69	0.8	0.65	0.7	1.95	2.1

The site-to-site reproducibility reproducibility was evaluated by testing four human sample pools and two controls (PC 1 and PC 2) at three sites using the same lot of reagent. Each sample was tested in replicates of five per run, one run per day for five days on one **cobas e** 801 immunoassay analyzer at each site (n=75 observations per sample). The results are summarized in the following table:

Sample	Mean (U/mL)	Within-Run		Between-Day		Between-Site/ Instrument		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	3.98	0.09	2.3	0.10	2.6	0.11	2.7	0.17	4.4
2	26.04	0.56	2.2	0.52	2.0	0.00	0.0	0.77	2.9
3	123.73	2.80	2.2	3.16	2.5	2.44	2.0	4.88	3.9
4	266.72	6.08	2.3	8.91	3.3	9.80	3.7	14.60	5.5
PC 1	22.63	0.36	1.6	0.52	2.3	0.13	0.6	0.64	2.8
PC 2	91.84	1.72	1.9	2.54	2.8	1.59	1.7	3.45	3.8

b. Linearity/assay reportable range:

Linearity: Linearity of the Elecsys CA 15-3 II assay was evaluated using the **cobas e** 801 immunoassay analyzer according to CLSI guideline EP6-A. Fifteen dilutions were prepared by diluting each of three high analyte serum samples with concentration above 300 U/mL down across the lower end of the measuring range. Each dilution was measured in replicates of three on **cobas e** 801 analyzer. The data summarized in the table below support linearity throughout the claimed measuring range from 3.0 U/mL to 300 U/mL.

Sample	Dilution Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
1	0.93–308.00	1.00 (0.99–1.01)	0.00 (−1.07–1.07)	0.99
2	1.33–313.00	1.00 (0.99–1.01)	0.00 (−1.12–1.12)	0.99
3	0.77–315.00	0.89 (0.99–0.01)	0.10 (−1.51–1.51)	0.99

Hook effect: The high-dose hook effect of the Elecsys CA 15-3 II assay was assessed using two positive serum samples spiked with CA 15-3 to achieve final analyte concentrations of 25,713 and 21,180 U/mL. Each sample was diluted to obtain five dilutions with CA 15-3 concentrations above the upper limit of the measuring range. Each dilution was tested in singulate using one lot of reagent on one **cobas e** 801 analyzer. No hook effect was observed up to 20,000 U/mL.

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

Traceability: There is no international standard available for CA 15-3. This method has been standardized against the Elecsys CA 15-3 assay. This in turn has been standardized against the Enzymun Test CA 15-3 method and CA 15-3 RIA by Fujirebio Diagnostics.

Value Assignment:

The predefined master curve is adapted to the analyzer using the relevant CalSet. The value assignment for the CalSet was described previously in K001468 and K010588.

Stability:

i) *Reagent stability*: Real-time stability studies for un-opened reagent stability (shelf life) and on-board stability studies were each performed. The results support that the reagents are stable when stored unopened at 2–8°C for 18 months or on-board (2–8°C) for 16 weeks on the **cobas e** 801 analyzer.

ii) *Calibraton stability*: The lot calibration and on-board calibration stability were performed on the **cobas e** 801 and support that the calibration for the Elecsys CA 15-3 II assay is stable for 12 weeks when using a same reagent lot and for 28 days when using the same cobas e pack on the **cobas e** 801 analyzer.

iii) *Sample Stability*: The studies were performed on the **cobas e** 801 for the following storage temperatures: 2–8°C, 20–25°C, and −20°C. The results support the claim that the specimens are stable up to five days at 2–8°C, up to two days at 20–25°C, and up to 90 days at −20°C.

d. *Detection limit:* The limit of blank (LoB), limit of detection (LoD), and limit of Quantitation (LoQ) of the Elecsys CA 15-3 II on the **cobas e** 801 analyzer were determined according to CLSI EP17-A2.

LoB was determined by testing analyte-free serum sample in 10 replicates per run, one run per day, for six days with three reagent lots on one **cobas e** 801 analyzer. The LoB was defined as the 95^{th} percentile of 60 measurements for each of tested lots and determined to be 0.58, 0.21 and 0.43 U/mL for three lots. The claimed LoB is 1.0 U/mL.

LoD was determined using five serum samples with low analyte concentration. Each

sample was tested in replicates of two per run, one run per day, for six days with three reagent lots on one **cobas e** 801 analyzer. The LoD value was calculated as the LoB + 1.653 x SD of the replicates for the samples and determined to be 1.10, 0.416, and 0.631 U/mL for each of three lots. The claimed LoD is 1.5 U/mL

LoQ was determined using a set of ten serum samples with low analyte concentration. Each sample was tested in replicates of five per run, one run per day, for five days with three reagent lots on one **cobas e** 801 analyzer to generate 25 data points per sample per reagent lot. The LoQ is defined as the mean value of the sample which fulfills the specification for the total within-laboratory imprecision (<20% CV) and was determined to be 1.6, 1.46 and 1.43 U/mL for each of three lots. The claimed LoQ is 3 U/mL.

e. Analytical specificity:

i). *Endogenous interferences:*

The effect of the presence of elevated level of hemoglobin, lipemia, bilirubin and rheumatoid factor (RF) on the performance of the Elecsys CA 15-3 II assay on the **cobas e** 801 analyzer was evaluated by testing three serum samples with CA 15-3 concentration of 26 U/mL (low), 125 U/mL (medium) and 230 U/mL (high) spiked with varying levels of interferent. The % recovery for each sample was calculated by comparing to the reference sample (without spiking the interference substance). No interference was noted for samples containing hemoglobin up to 1,000 mg/dL, lipemia up to 2,000 mg/dL, bilirubin up to 66 mg/dL, and rheumatoid factors up to 1,500 IU/mL.

The effect of the presence of HAMA on the performance of the Elecsys CA 15-3 II assay was evaluated using three serum samples with analyte concentrations of 14.9, 124, and 240 U/mL on one **cobas e** 801 analyzer. The samples were tested by spiking with HAMA serum with a concentration of 805 ng/mL. The control samples were spiked analogously with a serum without interferent. The data support that no significant effect with HAMA up to 805 ng/mL on the performance of the Elecsys CA15-3 II assay.

The biotin interference on the performance of the Elecsys CA 15-3 II assay on the **cobas e** 801 was evlauted by testing three serum samples with CA 15-3 concentration of 26.6 U/mL, 146 U/mL, and 238 U/mL spiked with varying level of biotin up to 800 ng/mL. The results (% bias) are summarized in the following table:

% Bias for Samples Containing Biotin									
Sample	**Biotin Concentration (ng/mL)**								
(U/mL)	**160**	**240**	**320**	**400**	**480**	**560**	**640**	**720**	**800**
26.6	−4.5%	−5.2%	−6.2%	−8.2%	−11.5%	−8.9%	−9.8%	−13.5%	−13.0%
146.0	−4.0%	−6.5%	−9.4%	−11.8%	−12.3%	−14.9%	−15.1%	−16.1%	−17.7%
238.0	−1.7%	−3.0%	−0.9%	−6.7%	−5.5%	−7.7%	−10.4%	−11.5%	−12.4%

The results indicated that sample with biotin concentrations up to 320 ng/mL demonstrated ≤10% bias in results. Biotin concentrations greater than 320 ng/mL can lead to falsely decreased CA15-3 results.

ii) *Exogenous interferences*:

The effect of the presence of drugs on the performance of the Elecsys CA 15-3 II assay on the **cobas e** 801 analyzer was evaluated by testing two serum samples with concentration of 30 U/mL and 230 U/mL spiked with interferent. A total of 27 pharmaceutical compounds including 16 common drugs and 11 additional specially used drugs were tested. No significant interference was found for each compound and drug at the concentrations listed below.

Commonly used pharmaceuticals:

Name of Agent	**Concentration**	**Name of Agent**	**Concentration**
Acetylcysteine	1660 mg/L	Methyldopa	20 mg/L
Ampicillin-Na	1000 mg/L	Metronidazole	120 mg/L
Ascorbic acid	300 mg/L	Phenylbutazone	400 mg/L
Cyclosporine	5 mg/L	Acetylsalicylic Acid	1000 mg/L
Cefoxitin	2500 mg/L	Rifampicin	60 mg/L
Heparin	5000 U	Acetaminophen	200 mg/L
Doxycycline	30 mg/L	Ibuprofen	500 mg/L
Levodopa	20 mg/L	Theophylline	100 mg/L

Special Cancer Drugs:

Name of Agent	**Concentration**	**Name of Agent**	**Concentration**
Carboplatin	1000 mg/L	Tamoxifen	50 mg/L
Cisplatin	225 mg/L	Mitomycin	25 mg/L
Cyclophosphamide	1000 mg/L	Etoposid	400 mg/L
Doxorubicin	75 mg/L	Flutamid	1000 mg/L
Methotrexate	200 mg/L	Taxol	5.5 mg/L
5-Fluoracil	500 mg/L		

f. Assay cut-off:

Not applicable

2. Comparison studies:

   a. *Method comparison with predicate device:*

   A total of 190 native human serum samples spanning the measuring range were tested with the Elecsys CA 15-3 II using the **cobas e** 801 (candidate) and the **cobas e** 601 (predicate) analyzers. Passing-Bablok regression analysis was performed and the data are summarized in the following table:

N	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
190	5.14–290.00	0.97 (0.95–0.98)	0.57 (0.06–1.31)	0.97

   b. *Matrix comparison:*

   A study was performed to demonstrate that Li-heparin plasma yield comparable values as serum by the Elecsys CA 15-3 II assay on the **cobas e** 801 analyzer. A total of 49 matrix-matched samples spread across the assay range were assayed using one lot of reagent on one instrument. Passing-Bablok regression analysis was performed and the data are summarized in the following table:

N	Range (U/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation Coefficient
49	8.37–248.00	1.01 (0.98–1.04)	−0.92(−1.36– -0.21)	0.97

3. Clinical studies:

   a. *Clinical Sensitivity and Clinical Specificity:*

   The clinical performance of CA 15-3 was demonstrated in K001468.

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

   Not applicable

4. Clinical cut-off**:**

   Not applicable

5. Expected values/Reference range:

   The reference range in the normal population was previously generated by testing 272 samples from apparently healthy subjects. 95% of the results were ≤25 U/mL (K010588). This reference range was verified per CLSI guidance C28-A3c by testing 20 apparently healthy women in age of 20 to 64 years on the **cobas e** 801 analyzer. The

results showed that all 20 samples had a CA 15-3 value below 25 U/mL.

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.