510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K160915

B. Purpose for Submission:

New Device

C. Measurand:

CYFRA 21-1 (cytokeratin 19 fragments)

D. Type of Test:

Quantitative, electrochemiluminescence immunoassay

E. Applicant:

Roche Diagnostics

F. Proprietary and Established Names:

Elecsys CYFRA 21-1
Elecsys CYFRA 21-1 CalSet
Elecsys PreciControl Tumor Marker

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 – Assay and Calibrators
   Class I – Controls

3. Product code:

   OVK – Cytokeratin fragments 21-1 Eia kit

JIT – Calibrator, secondary
JJX – Single (specified) analyte controls (assayed and unassayed)

4. Panel:

   Immunology (82) (Assay)
   Clinical Chemistry (75) (Calibrators and Controls)

H. Intended Use:

1. Intended uses:

   Elecsys CYFRA 21-1:
   Immunoassay for the in vitro quantitative determination of fragments of cytokeratin 19 in human serum and plasma (Li-Heparin, K2-EDTA and K3-EDTA). The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients. Serial testing for patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.

   The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

   Elecsys CYFRA 21-1 CalSet:
   CYFRA 21-1 CalSet is used for calibrating the quantitative Elecsys CYFRA 21-1 assay on the Elecsys and cobas e immunoassay analyzers.

   Elecsys PreciControl Tumor Marker:
   PreciControl Tumor Marker is used for quality control of Elecsys immunoassays on Elecsys and **cobas e** immunoassay analyzers.

2. Indications for use:

   Same as Intended Use

3. Special conditions for use statement:

   Prescription use only

4. Special instrument requirements:

   Elecsys and **cobas e** immunoassay analyzers

I. Device Description:

Each Elecsys CYFRA 21-1 kit contains the following materials:

- M (1 bottle, 6.5 mL): 0.72 mg/mL of streptavidin-coated microparticles with preservative
- R1 (1 bottle, 10 mL): 1.5 mg/L of biotinylated monoclonal anti-cytokeratin 19 antibody in phosphate buffer with preservative
- R2 (1 bottle, 10 mL): 2 mg/L of monoclonal anti-cytokeratin 19 antibody labeled with ruthenium complex in phosphate buffer with preservative

The Elecsys CYFRA 21-1 CalSet is a lyophilized product consisting of cytokeratin from cell culture of the cell line MCF-7 in two concentrations ranges in a cytokeratin free human serum matrix with preservative. The CalSet includes:

- CYFRA 21-1 Cal1: 2 bottles, each containing 1.0 mL of calibrator 1 (Target value: 0 ng/mL)
- CYFRA 21-1 Cal2: 2 bottles, each containing 1.0 mL of calibrator 2 (Target value: 50 ng/mL)

PreciControl Tumor Marker is a lyophilized human serum in two clinically relevant ranges and includes:

- PC TM1: 2 bottles, each containing 3.0 mL of control serum (Target value: 3.29 ng/mL)
- PC TM2: 2 bottles, each containing 3.0 mL of control serum (Target value: 27.2 ng/mL)

J. Substantial Equivalence Information:

1. Predicate device names and 510(k) numbers:

   Fujirebio CYFRA 21-1 EIA, K100831
   Elecsys HE4 CalSet, K112624
   Elecsys PreciControl Tumor Marker, K050387

2. Comparison with predicate:

Similarities		
Item	Device Elecsys CYFRA 21-1	Predicate CYFRA 21-1 EIA
Intended Use / Indication for Use	Immunoassay for the in vitro quantitative determination of fragments of cytokeratin 19 in human serum and plasma (Li-Heparin, K2-EDTA and K3-EDTA). The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer	The CYFRA 21-1 EIA kit is intended for the quantitative determination of soluble cytokeratin 19 fragments in human serum. The assay is to be used as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients. Serial testing for

Similarities		
Item	Device Elecsys CYFRA 21-1	Predicate CYFRA 21-1 EIA
	patients. Serial testing for patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.	patient CYFRA 21-1 assay values should be used in conjunction with other clinical methods used for monitoring lung cancer.
Analyte	cytokeratin 19 fragments	Same
Assay format	Quantitative	Same
Results interpretation	A positive change in CYFRA 21-1 is defined as an increase in the value that is at least 50 % greater than the previous value of the test.	Same

Differences		
Item	Device Elecsys CYFRA 21-1	Predicate CYFRA 21-1 EIA Kit
Sample type	human serum and plasma (Li-heparin, K2-EDTA, K3-EDTA)	human serum
Assay operation	Automatic	Manual
Detection Antibody	Ruthenium complex labeled mouse anti-CYFRA 21-1 monoclonal antibody (mAb)	Horse radish peroxidase (HRP) labeled mouse anti-CYFRA 21-1 mAb
Substrate	not applicable	TMB
Detection method	Electrochemiluminescence	Spectrophotometry
Instrument	Elecsys and **cobas e** analyzer	ELISA reader
Sample volume	20 µL	50 µL
Assay range	0.5–100 ng/mL	0.5–50 ng/mL
Traceability / Standardization	The assay has been standardized against the Enzymun-Test CYFRA 21-1 method.	The concentration of the assay primary calibrators has been assigned by using Roche CK19 antigen.
Calibrators	Elecsys CYFRA 21-1 CalSet: two levels (sold separately)	CYFRA 21-1 Calibrator set: six levels (included in the kit)
Controls	PreciControl Tumor Marker: two levels (sold separately)	CYFRA 21-1 controls two levels (included in the kit)

Elecsys CYFRA 21-1 CalSet

Similarities and Differences		
Item	Device Elecsys CYFRA 21-1 CalSet	Predicate Elecsys HE4 CalSet
Intended Use	CYFRA 21-1 CalSet is used for calibrating the quantitative Elecsys CYFRA 21-1 assay on the Elecsys and **cobas e** immunoassay analyzers	Elecsys HE4 CalSet is for calibrating the quantitative Elecsys HE4 assay on the Elecsys and cobas e immunoassay analyzers
Levels	Two	Same
Matrix	Human serum	Same
Format	Lyophilized	Same
Handling	Add exactly 1.0 mL of distilled water and allow to stand closed for 15 minutes to reconstitute.	Same
Stability–Unopened	2–8°C: up to stated expiration date	Same
Stability–Reconstituted	2–8°C: seven days −15 – −25°C: eight weeks (freeze only once)	2–8°C: seven days -20°C: eight weeks (freeze only once)
Stability–On-board	20–25°C: up to five hours	Same

Elecsys PreciControl Tumor Marker

Similarities and Differences		
Item	Device PreciControl Tumor Marker	Predicate PreciControl Tumor Marker (K050387)
Intended Use	PreciControl Tumor Marker is used for quality control of Elecsys immunoassays on Elecsys and **cobas e** immunoassay analyzers	PreciControl Tumor Marker is used for quality control of the Elecsys immunoassays on Elecsys immunoassay analyzers
Analyte	AFP, CEA, CA 15-3 II, CA 125 II, Ferritin, tPSA, fPSA, CA 19-9, CYFRA 21-1	AFP, CEA, CA 15-3 II, CA 125 II, Ferritin, tPSA, fPSA, CA 19-9
Levels	Two levels	Same
Matrix	Human serum	Same
Format	Lyophilized	Same

Similarities and Differences		
Item	Device PreciControl Tumor Marker	Predicate PreciControl Tumor Marker (K050387)
Handling	Add exactly 1.0mL of distilled water and allow to stand closed for 30 minutes to reconstitute.	Same
Stability–unopened	2–8°C: until expiration date	Same
Stability–reconstituted	2–8°C: 14 days −15 – −25°C: four weeks 20–25°C: 24 hours	Same
Stability–on-board	20–25°C: five hours	Same

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

- CLSI EP05-A3, Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline—Third Edition
- CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach; Approved Guideline
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline—Second Edition

L. Test Principle:

The Elecsys CYFRA 21-1 assay is a two-step sandwich immunoassay with streptavidin microparticles and an electrochemiluminescence detection system. Cytokeratin 19 fragments in the sample react with labeled antibodies to form a sandwich complex. This complex binds to streptavidin coated magnetic microparticles, which are magnetically captured onto an electrode. Application of voltage to the electrode induces chemiluminescence which is measured by a photomultiplier tube. Results are determined via a calibration curve which is instrument-specific, generated by 2-point calibration and a master curve provided via the reagent barcode.

M. Performance Characteristics:

1. Analytical performance: All results presented below were within the Manufacturer's pre-determined acceptance criteria for each study.

   a. Precision/Reproducibility:

   Precision: Precision of the Elecsys CYFRA 21-1 assay was evaluated according to CLSI guideline EP05-A3. A seven-member panel consisting of five pooled serum samples and two controls (PreciControl TM Level 1 and 2) were measured. Each sample was tested in single determinations in four separate

aliquots (divided in two runs per day) for 21 days using one reagent lot on the **cobas e** 411 (total of 84 measurements per sample) The results are summarized in the table below.

Sample	Mean (ng/mL)	Within-Run		Between-Run		Between-Day		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	0.77	0.02	2.7	0.03	3.7	0.01	0.9	0.04	4.7
2	2.99	0.04	1.4	0.05	1.8	0.01	0.4	0.07	2.3
3	3.11	0.04	1.4	0.07	2.1	0.00	0.0	0.08	2.5
4	22.30	0.29	1.3	0.27	1.2	0.22	1.0	0.46	2.1
5	71.50	1.18	1.7	0.99	1.4	0.32	0.4	1.57	2.2
PC TM1	2.69	0.05	1.8	0.06	2.1	0.02	0.9	0.08	2.9
PC TM2	25.60	0.38	1.5	0.53	2.1	0.00	0.0	0.65	2.6

Reproducibility:

To evaluate lot-to-lot reproducibility, five pooled serum samples two controls (PreciControl TM Level 1 and 2) were tested using three reagent lots. Each sample was tested in two replicates per run, two runs per day for 21 days to generate 84 data points for each sample for each lot, or a total of 252 measurements. The results are summarized in the table below:

Sample	Mean (ng/mL)	Within-Run		Between-Day		Between-Lots		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	0.79	0.02	2.7	0.00	0.0	0.03	3.6	0.05	6.0
2	3.01	0.04	1.4	0.00	0.0	0.04	1.3	0.08	2.6
3	3.14	0.05	1.5	0.00	0.0	0.05	1.7	0.09	2.9
4	22.27	0.32	1.4	0.19	0.8	0.00	0.0	0.44	2.0
5	71.15	1.07	1.5	0.28	0.4	0.19	0.3	1.56	2.2
PC TM1	2.72	0.05	1.7	0.00	0.0	0.04	1.6	0.08	3.0
PC TM2	26.01	0.38	1.5	0.00	0.0	0.42	1.6	0.74	2.8

To evaluate site-to-site reproducibility, an eight-member panel consisting of six pooled serum samples and two controls (PreciControl TM Level 1 and 2) were tested using one reagent lot at three sites. The study was done on one **cobas e** 411 analyzer at each site. Each sample was tested in two replicates per run, two runs per day for 20 days, to generate 80 data points for each sample at each site, or a total of 240 measurements for three sites combined. Data were analyzed for within run, between run, within site, between site and total reproducibility. The results are summarized in the table below.

Sample	Mean (ng/mL)	Within-Run		Between-Day		Between-Sites		Total	
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
1	0.53	0.03	4.8	0.04	6.7	0.01	2.3	0.05	9.6
2	1.31	0.03	2.1	0.03	2.4	0.02	1.6	0.05	4.0
3	1.37	0.03	2.5	0.03	2.1	0.04	2.6	0.06	4.6
4	2.80	0.05	1.9	0.04	1.4	0.03	0.9	0.07	2.6
5	18.98	0.25	1.3	0.21	1.1	0.23	1.2	0.41	2.2
6	79.37	1.07	1.3	0.94	1.2	1.21	1.5	1.89	2.4
PC TM1	2.66	0.03	1.2	0.07	2.6	0.07	2.8	0.11	4.3
PC TM2	21.73	0.16	0.7	0.59	2.7	0.50	2.3	0.87	4.0

b. Linearity/assay reportable range:

Linearity: Linearity was evaluated according to CLSI guideline EP6-A on the **cobas e** 411 immunoassay analyzer. Three serum samples containing high levels of CYFRA 21-1 were mixed with analyte striped serum to make three dilution series to cover the measuring range of the assay. Each dilution series contained at least 11 dilution samples and each sample was tested with one reagent lot. The results are summarized as follows:

	Test Range (ng/mL)	Slope (95% CI)	Intercept (95% CI)	R^2
1	0.27–133.00	1.00 (0.99–1.02)	-0.13 (-0.31–0.05)	1.00
2	0.45–116.00	1.00 (0.99–1.02)	-0.11 (-0.27–0.05)	1.00
3	0.65–127.00	1.00 (0.99–1.02)	-0.11 (-0.29–0.08)	1.00

The results support the linearity of the claimed measuring range (0.5–100 ng/mL) for Elecsys CYFRA 21-1

Dilution: A dilution study for the CYFRA 21-1 assay was performed on the **cobas e** 411 using five human samples spiked to analyte concentrations above the measuring range and diluted automatically on the instrument with the Elecsys Diluent Universal at a 1:5 dilution. The same set of samples prepared manually was used as reference. The % recovery values from individual results obtained with the instrument dilution compared to results obtained with the manual dilution were between 93.3% and 101.9%.

High dose hook effect: The high dose hook effect of the CYFRA 21-1 assay was assessed on the **cobas e** 411 analyzer. Two high positive samples were spiked to analyte concentration to 2470 ng/mL. Each sample was diluted to make five dilutions samples which had analyte concentrations above the upper limit of the measuring range. Each dilution sample was tested by single determination. The results showed no hook effect up to 2470 ng/mL.

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

Traceability: There is no recognized reference standard for CYFRA 21-1. The Elecsys CYFRA 21-1 has been standardized against the Enzymun test CYFRA 21-1 method.

Value assignment:

For each lot of Elecsys CYFRA 21-1 CalSet manufactured, the value assignment was performed by testing each calibrator in duplicate on at least three cobas e 411 analyzers and at least three Modular Analytics E170 analyzers/cobas e 601/cobas e 601 analyzers using three CYFRA 21-1 reagent lots. The test value was determined based on the master calibrator curve generated from five master calibrators (0.1, 2.08, 4.27, 8.93, 41.78 and 205 ng/mL). The assigned value of each calibrator is the median value obtained over at least six determinations on at least three analyzers.

For each lot of PreciControl Tumor Marker manufactured, the values are assigned using the **cobas e** 411 Immunoassay Analyzer. The value assigned was performed by testing each PreciControl in duplicate on six to eight analyzers. The concentration of each control is determined using the corresponding calibration curve. The assigned value of each PreciControl is the median value obtained.

Stability:

Sample stability: Sample stability was evaluated for the following storage conditions: 15–25°C for five days, 2–8°C for 14 days, −20°C for 12 weeks, stored at −80°C with freeze/thaw cycles. Four samples from each sample type (Serum, K_2-EDTA plasma, K_3-EDTA plasma, Li-heparin plasma) were aliquoted and tested directly after collection (as reference value) and after each tested storage condition. The recovery was calculated as percent of the reference value. In addition, the sample stability was evaluated at −80°C by testing ten samples from each sample type. The resulting data support the claim that serum, Li-heparin, K_2-EDTA and K_3-EDTA plasma specimens are stable at the following conditions: five days at 15–25°C, 14 days at 2–8°C, 12 weeks at −20°C, and three years at −80°C with one freeze/thaw cycle.

Elecsys CYFRA 21-2 assay kit stability:

Closed-vial stability: The real-time stability was performed on the **cobas e** 411 analyzer. Three reagent kits were stored at 2–8°C. The stored assay reagents were tested using two PreciControls (Level 1 and Level 2) at Day 0 and at specified intervals over the shelf-life of the device up to the planned shelf-life plus one month. The real-time stability study data support that the Elecsys CYFRA 21-1 reagent kits have a shelf-life up to 21 months when stored at 2–

8°C.

Open-vial stability: The reagent stability after first opening was determined on one **cobas e** 411 analyzer by testing five serum samples. Three reagent kits were opened on Day 0, one kit was placed on the analyzer, calibrated and reference values for the testing samples were determined. The other two kits were stored at 2–8°C. At Day 50 and Day 92, one of the stored kits was placed on the analyzer, calibrated, and the test samples were measured. The results were compared to the reference values at Day 0. The results support that the Elecsys CYFRA 21-1 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 analyzers by testing four serum samples. A fresh kit was placed on the analyzer, calibrated and reference values for the samples were determined. After measurement, the kit was closed and kept at 20°C ± 3°C for 64 days to simulate on-board conditions. Measurements were repeated at Day 36, Day 50, and Day 64. The recovery was compared to the reference values from Day 0. The results support that Elecsys CYFRA 21-1 kit can be stored on-board on the analyzer for up to eight weeks.

Calibration stability:

Lot calibration stability: The study was performed to determine the stability of the calibration curve made with the same lot. In this study, three kits from the same lot were used. On Day 0, the first reagent kit was opened and used to generate the calibration curve on one **cobas e** 411. Five serum samples were tested. On Day 36 and Day 64, the same samples were measured with a newly-opened kit using the calibration generated by the first kit on Day 0. The % recovery was calculated and the results support that the lot calibration stability is up to 8 weeks.

On-board calibration stability: To determine on-board calibration stability, one reagent kit was opened and calibration was generated on Day 0. Four serum samples were measured on Day 0. On Day 8, the same samples were retested using the calibration from Day 0 and a new opened reagent kept at on-board condition. Recovery was calculated and the data support the on-board calibration stability up to seven days on **cobas e** 411.

Elecys CYFRA 21-1 CalSet Stability:

Stability after reconstitution: The test material was reconstituted and stored in the closed vials at 2–8°C or −15 to −25°C. The reference material was a freshly reconstituted CalSet. The recovery of test material was calculated as percentage of the reference value. The data support the following stability claim for the reconstituted Elecsys CYFRA 21-1 CalSet: seven days at 2–8°C, eight weeks at

−15 to −25°C.

Open vial/On-board stability: The test material was reconstituted and stored in the closed vials at 20–25°C (on-board conditions). The reference material was a freshly reconstituted CalSet. The recovery of test material was calculated as percentage of the reference value. The data support that the Elecsys CYFRA 21-1 CalSet is stable up to five hours at 20–25°C.

Closed vial stability: Real-time stability was tested using three production lots of the Elecsys CYFRA 21-1 CalSet. The test materials were stored at 2–8°C and tested at Day 0 and at specified intervals over the shelf-life of the device up to the planned shelf life plus one month. The recovery was calculated compared to the reference value at Day 0. The data support that the Elecsys CYFRA 21-1 is stable up to 29 months stored at 2–8°C.

Transportation (stress) stability: The test material was reconstituted and stored in closed vials at 35°C. The reference material was a freshly reconstituted CalSet. The recovery of test material was calculated as percentage of the reference value. The data support that the Elecsys CYFRA 21-1 CalSet is stable up to one week when kept at 35°C.

Elecsys PreciControl Tumor Marker Stability

Stability after reconstitution: The test material was reconstituted and stored in closed vials at 2–8°C or −15 to −25°C. The recovery of test material was calculated as the percentage of the assigned value. The data support the following stability claim for the reconstituted Elecsys PreciControl Tumer Markers: 14 days at 2–8°C, four weeks at −15 to −25°C.

Open vial/On-board stability: The test material was reconstituted and stored in the closed vials under 20–25°C (on-board conditions). The recovery of test material was calculated as percentage of the assigned value. The data support that the PreciControl Tumor Marker is stable up to five hours at 20–25°C.

Closed vial stability: Real-time stability was tested using three lots of the PreciControl Tumor Marker. The test materials were stored at 2–8°C and tested at Day 0 and at specified intervals over the shelf-life of the device up to the planned shelf life plus one month. The recovery was calculated as percent recovery compared to the reference value. The data support that the PreciControl Tumor Marker is stable up to 24 months stored at 2–8°C.

d. Detection limit: The limit of blank (LoB), limit of detection (LoD) and limit of Quantitation (LoQ) was determined according to CLSI EP17-A2.

LoB: One analyte-free serum sample was tested in 10 replicates per run, two runs per day for three days with three reagent lots on one **cobas e** 411 analyzer.

For each lot, the LoB was determined as the 95th percentile of the measurement of blank samples based on a total of 60 measurements and found to be 0.09, 0.07 and 0.09 ng/mL for three lots. The claimed LoB is 0.15 ng/mL.

LoD: Five serum samples with low analyte concentration were tested. Each sample was run in two replicates per run, two runs per day for three days with three reagent lots on one **cobas e** 411 analyzer. For each lot, the LoD value was calculated based on the LoB + 1.645 x SD of the replicates for the samples and found to be 0.18, 0.13 and 0.15 ng/mL for three lots. The claimed LoD is 0.3 ng/mL.

LoQ: Eight serum samples with low analyte concentration were tested in five replicates per run, one run per day for five days with three reagent lots on one **cobas e** 411 analyzer. For each lot, the LoQ is defined as the mean value of the sample which fulfills the specification for the intermediate precision (<16.6% CV) and was determined to be 0.29, 0.21 and 0.21 ng/mL for three lots. The results support the specification of LoQ: ≤ 20% CV at 0.5 ng/mL. The claimed LoQ is 0.5 ng/mL.

e. Analytical specificity:

i) *Endogenous Substance Interference*:

The effect on quantitation of analyte in the presence of endogenous interfering substances using the Elecsys CYFRA 21-1 assay was evaluated on the **cobas e** 411 analyzer using three serum samples with concentrations at 3 ng/mL, 20 ng/mL, and 91 ng/mL 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 1,500 mg/dL, biotin up to 50 ng/mL, lipemia up to 1,500 mg/dL, bilirubin (a mixture of 10% conjugated and 90% unconjugated) up to 66 mg/dL, rheumatoid factors up to 1,500 IU/mL, and human serum albumin up to 7 g/dL.

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

The effect on quantitation of analyte in the presence of human anti-mouse antibodies (HAMA) using the Elecsys CYFRA 21-1 assay was assessed on the **cobas e** 411 analyzer. Two serum samples with analyte concentrations of 3.2 ng/mL and 96.3 ng/mL were tested by spiking with HAMA serum and preparing a dilution series each containing 11 different levels of HAMA interferent. The control samples were spiked analogously with a serum without interferent. The data support that no significant effect on the performance of the Elecsys CYFRA 21-1 assay with HAMA up to 805 µg/L.

iii) *Exogenous Substance Interference*:

The effect on quantitation of analyte in the presence of drugs using the Elecsys CYFRA 21-1 assay was evaluated on the **cobas e** 411 analyzer using two serum samples with concentration at 3.0 ng/mL and 95.0 ng/mL spiked with interferent. A total of 31pharmaceutical compounds including 16 common drugs and 15 additional cancer drugs were tested for the potential interference of the assay. No significant interference was found for each compound and drug at the concentration listed below.

Commonly used pharmaceuticals:

Name of Agent	Concentration (mg/L)	Name of Agent	Concentration (mg/L)
Acetylcysteine	553	Methyldopa	20
Ampicillin-Na	1000	Metronidazole	200
Ascorbic acid	300	Phenylbutazone	400
Cyclosporine	5	Acetylsalicylic Acid	1000
Cefoxitin	2500	Rifampicin	60
Heparin	5000 U/L	Acetaminophen	200
Doxycycline	50	Ibuprofen	500
Levodopa	20	Theophylline	100

Cancer Drugs*:*

Name of Agent	Concentration (mg/L)	Name of Agent	Concentration (mg/L)
Carboplatin	1000	Methotrexate	1000
Cisplatin	45	Paclitaxel	265
Clotrimazole	0.3	Tarceva	30
Cyclophosphamide	1000	5-Fluorouracil	500
Dexamethasone	20	Tamoxifen	50
Doxorubicin	120	Mitomycin	25
Leucovorin	750	Etoposid	400
Melphalan	15	Rituximab	750
Bevacizumab	750	Nivolumab	225
Pembrolizumab	150		

f. Assay cut-off:

See clinical cut-off

2. Comparison studies:

a. Method comparison with predicate device:

The method comparison study was performed on 153 serum samples obtained

from three commercial vendors. A total of 121 out of 153 tested samples had the results within the range of 0.5–100 ng/mL and were used for the analysis. Because the measuring range for the predicate CYFRA 21-1 EIA is 0.5–50 ng/mL, the samples above 50 ng/mL were diluted according to the instruction for use, and the value of the samples were calculated by actual reading x Dilution Factor. Deming regression analysis was performed to evaluate the analytical equivalence of the Elecsys CYFRA 21-1 to the predicate. The results are summarized as follows:

N	Range (ng/mL)	Slope (95% CI)	Intercept (95% CI)	R2
121	0.56–96.39	0.91 (0.86–0.96)	-1.40 (-2.86–0.06)	0.96

b. Matrix comparison:

The effect on quantitation of analyte in the presence of anticoagulants with the Elecsys CYFRA 21-1 assay was determined by comparing values obtained from samples drawn into serum, Li-heparin Plasma, K2- and K3-EDTA plasma primary tubes. At least 44 serum/plasma pairs per sample type were tested on one **cobas e** 411 analyzer. Passing/Bablok regression analyses of the plasma results (y) and the serum results (x) for each plasma sample type are presented below:

	N	Range (ng/mL)	Slope (95% CI)	Intercept (95% CI)	Correlation coefficient
Li-heparin vs. Serum	44	0.7–95.4	0.99 (0.98–1.01)	0.12 (0.08–0.16)	0.97
K_2-EDTA vs. Serum	45	0.7–95.4	1.00 (0.98–1.03)	−0.02 (−0.05– −0.08)	0.97
K_3-EDTA vs. Serum	45	0.7–95.4	1.01 (0.98–1.03)	−0.01 (−0.05–0.05)	0.96

3. Clinical studies:

a. Clinical Sensitivity and specificity

The effectiveness of the CYFRA 21-1 assay as an aid in monitoring of disease status in lung cancer subjects was determined from a retrospective clinical study by assessing the changes of CYFRA 21-1 levels in serial serum samples from subjects diagnosed with lung cancer compared to the changes in their disease status.

In this study, subject draws were performed at various stages of disease, including pre-treatment, during treatment, or standard of care follow-up. Subjects that had samples drawn at any of these time points with corresponding clinical data were included. The sample inclusion and exclusion criteria are as

follows:

Inclusion criteria:

- Eighteen years of age or older
- Diagnosed with lung cancer;
- Minimum of 3 serial draws available
- Appropriate clinical data
- Minimum 0.4 mL volume of serum available
- Normal appearance of sample

Exclusion criteria:

- No diagnosis of lung cancer
- Less than 3 serial draws available
- Insufficient sample volume
- Multiple freeze-thaw cycles
- Icteric lipemic, hemolytic substantial particulates

The clinical data collected for each subject included the following: subject ID, date of birth, the date of blood draw, gender, race/ethnicity, smoking status, including smoking history and date of smoking cessation, date of lung cancer diagnosis, histology, grade, and stage. For each subject sample draw, the following information was collected: chemotherapeutic treatment information (onset date, end date, regimen), imaging information (date, type, findings, disease status), physical exam date and findings, sample draw date, procedure information (type, date and findings), clinical disease status, date of recurrence, and date of death (if subject expired).

A total of 421 samples from 86 subjects were obtained and tested for CYFRA 21-1 levels. For data analysis, three subjects (with nine samples) and additional 14 samples from seven subjects were excluded for reasons including: (i) the subject had undergone monitoring for only two weeks, (ii) samples which were serially collected less than 7 days apart from the same subject, (iii) duplicate samples, or (iv) results outside the measuring range. Therefore, a total of 398 samples from 83 subjects were used in the study analysis.

Of the 83 subjects, 58% were male. The mean age was 65 years ranging from 34 to 88 years. The majority of subjects (n=72, 87%) were Caucasian, the remaining subjects included five (6%) African American, four (5%) Asian, and two (2%) Hispanic.

A total of 398 draws were obtained from 83 subjects. The mean number of draws per subject was 4.8 ranging from three to 17 draws. The length of time over which the subjects were monitored ranged from 41 days to 2174 days with a median of 175. The median interval between successive visits was 35 days ranging from seven days to 1878 days.

For the 83 lung cancer cases, 80 were classified as non-small cell lung cancer (NSCLC) and three were classified as small cell lung cancer (SCLC). The histopathology distribution of the subjects is summarized below:

Histologic Subtype	**N**
Non-Small Cell	
Adenocarcinoma	54
Squamous Cell	13
Large Cell	2
Mixed: Adnocarcinoma and Mucinous	1
Mixed: Adenosquamous Carcinoma	1
Mixed: Squamous Cell and Adenocarcinoma	1
Bronchoalveolar Carcinoma	4
No Further Histology	4
Small Cell	3
Total	83

Of 83 subjects, 69 subjects had staging information: nine (13%) were stage I or II, while 60 (87%) were stage III or IV. As a consequence, the performance of the Elecsys CYFRA 21-1 has not been adequately assessed in the patients with Stage I or II diseases.

In addition, of 83 subjects, only three patients were diagnosed with small-cell lung cancer; therefore, the performance of the Elecsys CYFRA 21-1 has not been adequately assessed in the patients with small cell lung cancer.

Results:

A total of 398 samples from 83 subjects consisting of 83 baseline values and 315 monitoring observations were measured for the CYFRA 21-1 values using the Elecsys CYFRA 21-1 assay on **cobas e** 411. At each follow-up visit, the percentage change of the CYFRA 21-1 was calculated by comparing the test result to the result obtained from the previous visit. The performance of the Elecsys CYFRA 21-1 assay as an aid in monitoring disease progression during the course of disease and treatment in lung cancer patients was determined by assessing percentage changes of CYFRA 21-1 levels correlated to the clinical assessment at the time of each follow-up visit. The changes in clinical status were determined by the physicians based on the clinical information (medical imaging, physical examination, physician transcription notes, and/or other laboratory data). The following definitions were used to categorize the patient's disease status:

- No Evidence of Disease (NED): A complete lack of clinical evidence of disease as determined by the treating physician.
- Stable Disease: Clinical evidence that the disease has not changed since last assessment as determined by the treating physician.

- Responding Disease: Clinical evidence that there is a shrinking of the primary tumor and no evidence of new tumors as determined by the treating physician.
- 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.

The summary statistics of the ratio of the Elecsys CYFRA 21-1 results and clinical disease status from the preceding draw for all follow-up draws is shown in the following table:

Clinical Disease Status	N	Ratio of the Elecsys CYFRA 21-1				
		Min	1st Quartile	Median	3rd Quartile	Max
NED	33	0.39	0.78	0.90	1.12	2.82
Stable	179	0.00	0.80	1.03	1.25	3.78
Responding	44	0.07	0.64	0.97	1.33	3.26
Progressive	59	0.04	0.94	1.38	2.31	22.26

Summary statistics showed the following:

- The NED, stable, and responding subjects have quite similar summary statistics
- Nearly three quarters of the subject visits showing disease progression had an increase in the Elecsys CYFRA 21-1 values
- A total of 26 out of 59 subject visits showing disease progression had an increase of the Elecsys CYFRA 21-1 values greater than 50%

For evaluation of clinical sensitivity and specificity of the Elecsys CYFRA 21-1 assay, the clinical disease status was condensed into two 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. The threshold for a statistically significant increase in CYFRA 21-1 values incorporated both assay and biological variability and was based on the published report of Trapé *et al* (Clinical Chemistry 51, pgs. 219-222, 2005). A positive change in CYFRA 21-1 was defined as measurable increase in the value that was at least 50% greater than the previous value of the test when the CYFRA 21-1 result was outside the normal range.

The following table represents the number of all clinical visits (n=315) for 83 subjects at which a clinical evaluation of progression/no-progression occurred and the percentage change of the subjects at these clinical evaluations using the cut-off value of 50% increase:

		Disease Status		Total
		Progression	No-Progression	
Change in CYFRA 21-1	**>50%**	26	23	49
	≤50%	33	233	266
	Total	59	256	315

The analysis of the performance measurements and 95% confidence interval (CI) was summarized in the following table:

Performance Measurement	Value	95% CI
Sensitivity	44.1%	32.2%–59.7%
Specificity	91.0%	86.9%–93.9%
Total Concordance	82.7%	78.3%–88.0%
Positive Predictive Value (PPV)	53.1%	39.4%–66.3%
Negative Predictive Value (NPV)	87.6%	83.1%–91.0%
Positive Likelihood Ratio	4.9	3.0–8.0
Negative Likelihood Ratio	0.6	0.5–0.8

With different cutoff values, there are tradeoffs between sensitivity and specificity as illustrated in the table below:

% Chance in Elecsys CYFRA 21-1	Sensitivity (%)	Specificity (%)	NPV (%)	PPV (%)
30	49.2	87.1	88.1	46.8
40	44.1	89.8	87.5	50.0
50	44.1	91.0	87.6	53.1
60	39.0	91.4	86.7	51.1
70	35.6	93.4	86.3	55.3

The summary statistics of the ratio of the Elecsys CYFRA 21-1 results from the preceding draw for all follow-up draws by disease stages is shown in the following table:

Stage	Subject	Pairs	Ratio of the Elecsys CYFRA 21-1				
			Min	1st Quartile	Median	3rd Quartile	Max
I	2	7	0.4	0.9	1.0	1.6	7.1
Ib	3	23	0.3	0.8	1.0	1.3	3.4
II	1	5	0.7	0.9	1.3	1.3	3.3
IIb	3	20	0.4	0.9	1.0	1.2	1.9
III	3	8	0.7	0.9	1.0	1.3	4.3
IIIa	17	71	0.4	0.8	1.1	1.6	3.9
IIIb	8	24	0.2	0.8	1.0	1.3	3.8
IV	32	117	0.0	0.8	1.0	1.4	22.3
Unknown	12	31	0.0	0.7	1.1	1.3	3.2
Unstaged	2	9	0.4	0.6	0.9	1.2	1.6
All	**83**	**315**	**0.0**	**0.8**	**1.0**	**1.4**	**22.3**

The following table summarized the diagnostic performance of CYFRA 21-1 values over successive visits by disease stage:

Stage	Subjects	Pairs	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
I	2	7	100.0	83.3	50.0	100.0
IB	3	23	0.0	95.7	0.0	100.0
II	1	5	0.0	50.0	0.0	25.0
IIB	3	20	0.0	95.0	0.0	100.0
III	3	8	100.0	100.0	100.0	100.0
IIIA	17	71	42.9	80.0	47.4	76.9
IIIB	8	24	33.3	90.5	33.3	90.5
IV	32	117	43.5	94.7	66.7	87.3
Unknown	12	31	57.1	95.8	80.0	88.5
Unstaged	2	9	0.0	100.0	0.0	100.0
Total	**83**	**315**	**44.1**	**91.4**	**54.2**	**87.6**

b. Other clinical supportive data:

The clinical performance of the Elecsys CYFRA 21-1 assay at different % change in CYFRA 21-1 was evaluated against that of the predicate CYFRA 21-1 EIA (K100831). The table below compared the clinical sensitivity and specificities between the Elecsys CYFRA 21-1 assay and the predicate using a series of % change in CYFRA 21-1 values as determined in two separate clinical studies.

	Elecsys CYFRA 21-1 (N=315/83 subjects)		CYFRA 21-1 EIA (k100831) (N=314/100 subjects)	
% Change in CYFRA 21-1	**Sensitivity (%)**	**Specificity (%)**	**Sensitivity (%)**	**Specificity (%)**
30	49.2	87.1	52.9	84.3
40	44.1	89.8	48.2	85.6
50	44.1	91.0	45.9	87.3
60	39.0	91.4	44.7	88.2
70	35.6	93.4	43.5	89.5

4. Clinical cut-off:

An absolute cutoff is not applicable for monitoring. CYFRA 21-1 value increases at least 50% higher than immediate previous sample are considered a significant change when the Elecsys CYFRA 21-1 result was outside the normal range.

5. Expected values/Reference range:

The expected value/reference range of CYFRA 21-1 was determined in samples from 240 healthy individuals and from 635 patients with nonmalignant or malignant

diseases.

i. *The upper limit of normal (ULN) for the Elecsys CYFRA assay:*

The Elecsys CYFRA 21-1 values were analyzed in a cohort of 240 apparently healthy individuals (125 females and 115 males, ages 25 to 87 years, with an average age of 60 years and median age of 61 years) including 120 smokers and 120 nonsmokers. The sample cohort included 127 Caucasian, 52 African American, 45 Hispanic, 13 Asian and three American Indian or Alaskan Native. The results are shown as follows:

	All	**Nonsmoker**	**Smoker**	**Female**	**Male**
N	**240**	**120**	**120**	**125**	**115**
Mean (ng/mL)	1.19	1.19	1.19	1.23	1.14
Median (ng/mL)	1.05	1.03	1.07	1.07	1.01
Min (ng/mL)	0.32	0.32	0.49	0.32	0.35
Max (ng/mL)	4.81	4.66	4.81	4.81	3.01
95^{th} percentile (ng/mL)	2.38	2.59	2.00	2.32	2.43
97.5^{th} percentile (ng/mL)	2.86	2.90	2.58	2.86	2.85

The results above were transformed using the Box-Cox transformation, the ULN for the Elecsys CYFRA 21-1 assay was calculated based on the 95^{th} percentile using the transformed data and determined as 2.37 ng/mL (95% CI: 2.21 ng/mL–2.90 ng/mL). It is recommended that each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

ii. *Distribution of the Elecsys CYFRA 21-1 values:*

The distribution of the Elecsys CYFRA 21-1 values in 875 of healthy subjects and patients with various benign and malignant conditions is summarized as follow:

	Number of Subjects (n/N)				
	Total	**Elecsys CYFRA 21-1 values (ng/mL)**			
		0.30–2.37	**2.38–5.00**	**5.01–20.00**	**>20.00**
Apparently Healthy					
All normals	240	228 (95.0%)	12 (5.0%)	0 (0.0%)	0 (0.0%)
Benign conditions					
Benign lung disease	75	70 (93.3%)	5 (6.7%)	0 (0.0%)	0 (0.0%)
CHF*	40	29 (72.5%)	11 (27.5%)	0 (0.0%)	0 (0.0%)
Benign kidney disease	40	8 (20.0%)	24 (60.0%)	8 (20.0%)	0 (0.0%)
Benign liver disease	40	35 (87.5%)	4 (10.0%)	1 (2.5%)	0 (0.0%)

	Number of Subjects (n/N)				
	Total	Elecsys CYFRA 21-1 values (ng/mL)			
		0.30–2.37	2.38–5.00	5.01–20.00	>20.00
Cancer					
Lung cancer	120	53 (44.2%)	33 (27.5%)	27 (22.5%)	7 (5.8%)
Bladder cancer	40	13 (32.5%)	9 (22.5%)	12 (30.0%)	6 (15.0%)
Breast cancer	40	32 (80.0%)	5 (12.5%)	3 (7.5%)	0 (0.0%)
Cervical cancer	40	28 (70.0%)	11 (27.5%)	1 (2.5%)	0 (0.0%)
ESCC**	40	21 (52.5%)	12 (30.0%)	6 (15.0%)	1 (2.5%)
GI tract cancer	40	23 (57.5%)	10 (25.0%)	6 (15.0%)	1 (2.5%)
Head and neck cancer	40	29 (72.5%)	11 (27.5%)	0 (0.0%)	0 (0.0%)
Prostate cancer	40	37 (92.5%)	1 (2.5%)	2 (5.0%)	0 (0.0%)
Ovarian cancer	40	25 (62.5%)	8 (20.0%)	5 (12.5%)	2 (5.0%)

* CHF: Congestive heart failure

** ESCC: Esophageal squamous cell carcinoma

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.