510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K220176

B Applicant

Roche Diagnostics

C Proprietary and Established Names

Elecsys AFP

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LOJ	Class II	21 CFR 866.6010 - Tumor-Associated Antigen Immunological Test System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Modification of a previously cleared assay to decrease interference to biotin.

B Measurand:

α1-fetoprotein (AFP)

C Type of Test:

Quantitative electrochemiluminescent immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Immunoassay for the in vitro quantitative determination of α1-fetoprotein in human serum and plasma to aid in the management of patients with non-seminomatous germ cell tumors.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on the cobas e immunoassay analyzers.

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only
For in vitro diagnostic use.

D Special Instrument Requirements:

Roche cobas e 601 immunoassay analyzer

IV Device/System Characteristics:

A Device Description:

The Elecsys AFP reagent rackpack (M, R1, R2) consists of:

- M: Streptavidin-coated microparticles (transparent cap), one bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1: Anti-AFP-Ab~biotin (gray cap), one bottle, 17 mL: Biotinylated monoclonal anti-AFP antibodies (mouse) 4.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.
- R2: Anti-AFP-Ab~Ru(bpy) (black cap), one bottle, 17 mL: Monoclonal anti-AFP antibodies (mouse) labeled with ruthenium complex 12.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

B Principle of Operation:

The Elecsys AFP is a one-step sandwich immunoassay. AFP present in the patient sample, ruthenium-labeled AFP specific antibody, and biotin-labeled AFP specific antibody to form a sandwich complex. The complexes are immobilized onto the surface of magnetic microparticles via biotin-streptavidin binding. The reaction mixture is aspirated into the measuring cell where

the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Current is applied to the electrode to stimulate chemiluminescent emission that 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 bar code; the measured electrochemiluminescence signal is proportional to the amount of AFP in the sample.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Elecsys AFP

B Predicate 510(k) Number(s):

K981282

C Comparison with Predicate(s):

Device & Predicate Device(s):		
Device Trade Name	Elecsys AFP	Same
General Device Characteristic Similarities		
Intended Use/ Indications For Use	Immunoassay for the in vitro quantitative determination of α1-fetoprotein in human serum and plasma to aid in the management of patients with non-seminomatous germ cell tumors. The electrochemiluminescence immunoassay "ECLIA" is intended for use on the cobas e immunoassay analyzers.	Immunoassay for the in vitro quantitative determination of α1-fetoprotein in human serum and plasma to aid in the management of patients with non-seminomatous germ cell tumors. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and cobas e immunoassay analyzers.
Detection method	ECLIA	Same
Test type and format	Quantitative sandwich	Same
Assay Reaction Time	18 minutes	Same
Traceability	1st IRP WHO Reference Standard 72/225	Same
Sample Matrices	Human serum, lithium heparin plasma, K2-EDTA heparin plasma, and K3-EDTA heparin plasma	Same

Device & Predicate Device(s):	K220176	K981282
Calibrators and Control Materials	AFP Calset II, PreciControl Tumor Marker, PreciControl Universal	Same
General Device Characteristic Differences		
Catalog Number	09015086160	04491742160
Biotin Tolerance	No interference up to 1200 ng/mL	No interference up to 60 ng/mL
Measuring Range	All platforms: 1.5 – 1000 IU/mL	cobas e 411, cobas e 601, and cobas e 602 analyzers: 0.500-1000 IU/mL cobas e 801:1.5–1000 IU/mL

VI Standards/Guidance Documents Referenced:

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

CLSI EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

   Precision of the Elecsys AFP was conducted following the CLSI EP05-A3 guideline. Aliquots of six human serum samples and two quality control (QC) samples were measured in quadruplicate (separated into two parts) in one run per day for 21 days using one reagent lot on one cobas e 601, for a total of 84 measurements. The within-laboratory precision is shown in the table below:

Sample ID	N	Mean (IU/mL)	Repeatability		Between-Part		Between-Day		Within-Lab	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	84	1.7	0.05	3.0	0.04	2.6	0.03	1.6	0.07	4.2
2	84	5.3	0.15	2.7	0.12	2.2	0	0	0.19	3.5
3	84	16.0	0.50	3.1	0.35	2.2	0.08	0.5	0.62	3.9
4	84	157.0	4.58	2.9	3.24	2.1	0	0	5.61	3.6
5	84	515.0	15.40	3.0	12.00	2.3	0	0	19.60	3.8
6	84	923.0	29.10	3.2	18.50	2.0	11.00	1.2	36.20	3.9
QC 1	84	7.5	0.16	2.2	0.20	2.6	0	0	0.26	3.4
QC 2	84	70.9	1.31	1.9	2.06	2.9	0	0	2.45	3.5

Lot-to-lot precision was evaluated on one cobas e 601 analyzer by testing six serum samples and two QC samples in five replicates, one run per day for five days, using three reagent lots. The results are summarized in the table below:

Sample ID	N	Mean (IU/mL)	Repeatability		Between-Day		Between-Lot		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	75	1.9	0.1	4.69	0	0	0.1	7.76	0.2	9.07
2	75	5.5	0.2	3.42	0	0	0.1	2.40	0.2	4.18
3	75	16.3	0.6	3.40	0.1	0.44	0.1	0.63	0.6	3.49
4	75	158.0	5.2	3.30	0	0	1.8	1.14	5.5	3.49
5	75	515.0	19.2	3.73	0	0	5.9	1.15	20.1	3.90
6	75	922.0	42.0	4.55	0	0	4.5	0.49	42.2	4.58
QC 1	75	7.7	0.2	3.10	0	0	0.1	1.69	0.3	3.53
QC 2	75	71.1	2.32	3.26	0	0	1.1	1.56	2.6	3.61

2. Linearity:

Linearity of the Elecsys AFP was evaluated using one human serum sample with high analyte content above the measuring range that was diluted to the lower end of the measuring range with human serum sample without AFP for a total of 15 concentration levels across the measuring range. Samples were assayed in triplicate within a single run on one cobas e 601 analyzer using one reagent lot. The linear regression analysis was performed and the results of the study were as follows:

Slope (95% CI)	Y-Intercept (95% CI) (IU/mL)	R^2
0.965 (0.909; 1.021)	0.433 (0.397; 0.462)	0.958

The results support the claimed measuring range 1.5 – 1000.0 IU/mL.

High Dose Hook Effect:

Two extremely high AFP serum samples were serially diluted to determine the hook effect. The results indicated that there was no hook effect up to 1,000,000 IU/mL.

Dilution of over-range samples:

A dilution study was performed to compare cobas e 601 analyzers' ability to correctly dilute and then measure very high analyte samples. Four samples with very high native concentration samples were diluted in Diluent Universal at 1:50 manually and by automated dilution using two cobas e 601 analyzers. The diluted samples were tested in triplicate and the recovery of instrument vs manual was calculated. The percent recovery for all four samples was within 91 – 106%, supporting the recommended 1:50 dilution in the instructions for use for the Elecsys AFP.

3. Analytical Specificity/Interference:

Endogenous Interference:

Potential interference from some endogenous substances was assessed using three serum samples with AFP concentrations of low-range (~3 IU/mL), mid-range (~420 IU/mL), and high-range (~700 IU/mL). Each of the three samples was further divided into two aliquots for a control sample (with no added interferent) and test sample (with added interferent) then combined in different ratios into a series of 11 samples of varying substance concentration by inter-mixing the control and test samples with known volumes. Each sample of the dilution series was assayed in five replicates using one lot and one instrument. From measurement of the dilution series, the highest interferent concentration within the allowable error was found and identified as the highest concentration at which no significant interference is observed. For each interferent concentration level, the recovery (absolute difference or % recovery) was calculated by comparing the mean of the replicates of each sample containing interference to the control. No significant interference (an absolute difference of < 0.4 IU/mL for samples between 1.5 to 4 IU/mL and a recovery of 100% ± 10% for samples > 4.0 IU/mL) was observed for the samples containing each interference substance at the concentrations shown in the table below:

Endogenous Interferent	**Highest concentration tested without interference**
Albumin	7 g/dL
Bilirubin	6 mg/dL
Hemoglobin	2200 mg/dL
Intralipid	1500 mg/dL
IgG	7 g/dL
Rheumatoid Factor	1500 IU/mL

Biotin Interference:

One aliquot of each AFP sample was spiked with biotin up to 3600 ng/mL and used as "interference pool". A series of 11 dilution steps were prepared by mixing the interference pools and the related dilution pools in 10% increments. Each sample of the dilution series

was assayed in five replicates using one reagent lot on one cobas e 601 analyzer. Recovery (absolute deviation or % recovery) was calculated based on the mean of the replicates compared to the expected value. For all samples with biotin concentrations ≤ 2400 ng/mL, an absolute deviation of < 0.4 IU/mL was observed for samples with AFP concentration between 1.5 to 4 IU/mL, and a recovery of 100% ± 10% was observed for samples with AFP concentration > 4.0 IU/mL. The results support that the device has no significant biotin interference up to 1200 ng/mL.

Exogenous Interference:

Interference from 17 common pharmaceutical drugs (e.g., antibiotics), 10 pharmaceutical drugs that might be used in the clinical context of the assay's intended use were assessed using two serum pools, one with a low concentration of AFP (~3.1 IU/mL) and one with a high concentration of AFP (~700 IU/mL) samples. The two samples were divided into aliquots and spiked with the potential interferents or with solvent only. No significant interference (an absolute difference of < 0.4 IU/mL for samples between 1.5 to 4 IU/mL and a recovery of 100% ± 10% for samples > 4.0 IU/mL) was observed for the samples containing each interference substance at the concentrations shown in the table below:

Common Drugs	Concentration Tested (mg/L)	Pharmaceutical Drugs	Concentration Tested (mg/L)
Acetylcysteine	150	Doxorubicin	15
Acetylsalicylic acid	30	Cyclophosphamide	200
Ampicillin-Na	75	Cisplatin	45
Ascorbic acid	52.5	5-Fluorouracil	100
Cefoxitin	750	Methotrexate	200
Doxycycline	18	Tamoxifen	10
Levodopa	7.5	Mitomycin	5
Methyldopa	22.5	Carboplatin	200
Metronidazole	123	Etoposide	80
Rifampicin	48	Taxol	5.5
Acetaminophen	156		
Cyclosporine	1.8		
Ibuprofen	219		
Theophylline	60		
Phenylbutazone	321		
Itraconazole	30		
Heparin	3300 IU/L		

4. Assay Reportable Range:

The claimed reportable range for the Elecsys AFP is 1.5 – 1000.0 IU/mL.

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

Traceability:

The assay is traceable to the 1st IRP WHO Reference Standard 72/225.

Stability:

An on-board stability study of the modified assay reagents was performed using six samples across the measuring range to verify the claim established in K981282. The data supported the package insert claim of 4 weeks on-board stability.

A real-time study was conducted using six samples across the measuring range and tested at various time points after storage at 2–8 °C to verify a shelf-life stability of 21 months.

6. Detection Limit:

The limit of blank (LoB), limit of detection (LoD), and limit of quantitation (LoQ) were determined in accordance with CLSI EP17-A2.

Limit of Blank (LoB):
For determination of LoB, five analyte-free serum samples were measured in duplicate in six runs, distributed over six days, with three different reagent lots on one cobas e 601 analyzer. In total, 60 measured values of analyte free samples were obtained per lot. The LoB corresponds to the concentration below which analyte-free samples are found with a probability of 95%. The LoB determined in all three lots were less than the LoB claim in the labeling, 0.75 IU/mL.

Limit of Detection (LoD):
For determination of LoD, five low-level native human samples were measured in duplicate in six runs, distributed over six days, with three different lots on one cobas e 601 analyzer. In total, 60 measured values of samples with low analyte concentrations were obtained per lot. The LoD corresponds to the concentration determined by LoD = LoB + 1.653 x SD total (of low analyte samples). The LoD determined in all three lots were less than the LoD claim in the labeling, 1.5 IU/mL.

Limit of Quantitation (LoQ):
For the determination of LoQ, six low-level native human samples were measured five-fold in one run a day for five days, with three different lots on one cobas e 601 analyzer. The LoQ was set as the lowest concentration of analyte that can be reproducibly measured with a total error of ≤ 20 %. The LoQ determined in all three lots support the claim of LoQ equal to LoD in the labeling, 1.5 IU/mL.

7. Assay Cut-Off:

Not applicable.

B Comparison Studies:

1. Method Comparison with Predicate Device:

A method comparison study was performed to show comparability between the performance of the current Elecsys AFP assay and the biotin-updated Elecsys AFP assay. At least 180 native human samples spanning the assay range (1.58 – 996 IU/mL by the predicate) were tested using one reagent lot of the predicate assay on one cobas e 411 analyzer and three reagents lots of the updated assay on three different cobas e 601 analyzers. The results of Passing-Bablok regression analysis of each lot are shown below:

Lot	N	Slope (95% CI)	Intercept (95% CI)	Pearson r
1	181	0.965 (0.956 – 0.973)	0.101 (0.072 – 0.144)	0.999
2	181	0.968 (0.964 – 0.975)	0.157 (0.110 – 0.178)	1.000
3	180	0.971 (0.965 – 0.977)	0.251 (0.189 – 0.306)	0.999

2. Matrix Comparison:

A matrix equivalency study to serum was conducted to support use of the Elecsys AFP assay on the cobas e 601 analyzer with sample matrices: lithium (Li) heparin plasma, dipotassium (K2) EDTA plasma, and tripotassium (K3) EDTA plasma. In the study, at least 48 donor matched venous specimens were collected. Each specimen was tested in singlicate. The results were analyzed by Passing-Bablok linear regression with serum results as the reference (x-axis). The slope and intercept of the regression line were calculated, and summarized as follows:

Serum vs.	N	Serum Sample Range (IU/mL)	Slope (95% CI)	Intercept (95% CI)	Pearson r value
Lithium Heparin	52	1.63 – 901	0.985 (0.962 – 1.013)	0.095 (-0.076 – 0.338)	0.999
K2-EDTA Heparin	48	1.63 – 901	0.983 (0.957 – 1.005)	0.028 (-0.208 – 0.171)	0.998
K3-EDTA Heparin	53	1.70 – 927	0.976 (0.963 – 0.984)	-0.013 (-0.147 – 0.178)	0.999

C Clinical Studies:

Refer to K981282

D Clinical Cut-Off:

Refer to K981282

E Expected Values/Reference Range:

Refer to K981282

The reference range of AFP values were determined in a normal cohort of 140 test subjects in K981282. In these samples, 97% of the cohort had an AFP value ≤ 6.90 IU/mL (≤ 8.7 ng/mL).

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.