Elecsys AFP

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REF		Σ	SYSTEM
09015060160	09015060501	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

For use in the USA only

System Information

For **cobas e** 411 analyzer: Test Number 1810
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 134

Please note

The measured AFP value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the AFP assay method used. AFP values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations. If there is a change in the AFP assay procedure used while monitoring therapy, then the AFP values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

CAUTION: US federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and its use is restricted to, by or on the order of a physician.

Intended use

Immunoassay for the in vitro quantitative determination of α_1-fetoprotein in human serum and plasma to aid in the management of patients with non-seminomatous germ cell tumors.

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

Summary

Alpha 1-fetoprotein, an albumin-like glycoprotein with a molecular weight of 70000 daltons, is formed in the yolk sac, non-differentiated liver cells, and the fetal gastro-intestinal tract.[1,2]
70-95 % of patients with primary hepatocellular carcinoma have elevated AFP values.[3]
The later the stage of non-seminomatous germ cell tumors, the higher the AFP values. Human chorionic gonadotropin (hCG) and AFP are important parameters for estimating the survival rate of patients with advanced, non-seminomatous germ cell tumors.[4,5,6]
No correlation between the AFP concentration and tumor size, tumor growth, stage or degree of malignancy has so far been demonstrated.
Greatly elevated AFP values generally indicate primary liver cell carcinoma. When liver metastasis exists, the AFP values are generally below 350-400 IU/mL.[7] As the AFP values rise during regeneration of the liver, moderately elevated values are found in alcohol-mediated liver cirrhosis and acute viral hepatitis as well as in carriers of HBsAg.[7,8]
The determination of AFP to screen the general population for cancer is, however, not to be recommended.
The Elecsys AFP assay is indicated for serial measurements of AFP to aid in the management of patients with germ cell tumors.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal AFP-specific antibody, and a monoclonal AFP-specific antibody labeled with a ruthenium complex[a)] react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as AFP.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-AFP-Ab~biotin (gray cap), 1 bottle, 10 mL: Biotinylated monoclonal anti-AFP antibodies (mouse) 4.5 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

R2 Anti-AFP-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-AFP antibodies (mouse) labeled with ruthenium complex 12.0 mg/L; phosphate buffer 100 mmol/L, pH 6.0; preservative.

Precautions and warnings

For in vitro diagnostic use for healthcare professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: 1-800-428-2336

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

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Reagent handling

The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated.

All information required for correct operation is read in from the respective reagent barcodes.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on **cobas e** 411 and **cobas e** 601 analyzers	8 weeks
on **cobas e** 602 analyzer	4 weeks

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin, K_2-EDTA and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + coefficient of correlation ≥ 0.95.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 6 months at -20 °C (± 5 °C). The samples may be frozen 3 times.

For information on the stability of serum obtained with tubes containing separating gel, note the data provided by the tube manufacturer.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

- REF 09227261190, AFP CalSet II, for 4 x 1.0 mL
- REF 11776452160, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 11731416160, PreciControl Universal, for 4 x 3.0 mL
- REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Additional materials for **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup , 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Additional materials for all analyzers:

- REF 11298500160, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator’s manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers.

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Calibration

Traceability: This method has been standardized against the 1st IRP WHO Reference Standard 72/225.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 28 days when using the same reagent lot
- after 7 days when using the same reagent kit on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

Use PreciControl Tumor Marker or PreciControl Universal or other suitable controls for routine quality control procedures.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory’s individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the analyte concentration of each sample (either in IU/mL, ng/mL, KIU/L or additionally in IU/L for the **cobas e** 601 and **cobas e** 602 analyzers).

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Conversion factors: IU/mL x 1.21 = ng/mL
ng/mL x 0.83 = IU/mL

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1112 µmol/L or ≤ 65 mg/dL
Hemoglobin	≤ 1.37 mmol/L or ≤ 2200 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factor	≤ 1500 IU/mL
Serum albumin	≤ 7 g/dL
IgG	≤ 7 g/dL

Criterion: ± 0.4 IU/mL of initial value for samples ≤ 4.0 IU/mL and within ± 10 % of initial value for samples > 4.0 IU/mL.

Specimens containing biotin up to a concentration of 1200 ng/mL demonstrated ≤ 10 % change in Elecsys AFP assay results. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day[9] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[10]

There is no high-dose hook effect at AFP concentrations up to 1 million IU/mL (1.21 million ng/mL).

Pharmaceutical substances

In vitro tests were performed on 17 commonly used drugs. No interference was observed at the levels tested.

Drug	Concentration tested mg/L
Acetylcysteine	150
Acetylsalicylic acid	30
Ampicillin-Na	75
Ascorbic acid	52.5
Cefoxitin	750
Doxycycline	18
Heparin	3300 IU/L
Levodopa	7.5
Methyldopa	22.5
Metronidazole	123
Rifampicin	48
Acetaminophen	156
Cyclosporine	1.8
Ibuprofen	219
Theophylline	60
Phenylbutazone	321
Itraconazole	30

In addition, the following 10 special drugs were tested. No interference with the assay was found.

Drug	Concentration tested mg/L
Doxorubicin	75
Cyclophosphamide	1000
Cisplatin	225
5-Fluorouracil	500
Methotrexate	1000
Tamoxifen	50
Mitomycin	25
Carboplatin	1000
Etoposid	400
Taxol	5.5

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Limits and ranges

Measuring range

1.50-1000 IU/mL or 1.82-1210 ng/mL (defined by the Limit of Quantitation and the maximum of the master curve). Values below the Limit of Quantitation are reported as < 1.50 IU/mL or < 1.82 ng/mL. Values above the measuring range are reported as > 1000 IU/mL or > 1210 ng/mL (or up to 50000 IU/mL or 60500 ng/mL for 50-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.75 IU/mL (or 0.91 ng/mL)

Limit of Detection = 1.50 IU/mL (or 1.82 ng/mL)

Limit of Quantitation = 1.50 IU/mL (or 1.82 ng/mL)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

Dilution

Samples with AFP concentrations above the measuring range can be diluted with Elecsys Diluent Universal 2. The recommended dilution is 1:50 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 18 IU/mL (> 21.8 ng/mL).

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the software automatically takes the dilution into account when calculating the sample concentration.

Expected values

The following AFP values were found in samples from 140 healthy test subjects: ≤ 6.90 IU/mL or ≤ 8.30 ng/mL for 95 % of the results.

The following table includes AFP values found in samples from 140 healthy test subjects, 76 subjects with non-malignant disease, and 96 subjects with malignant disease.

Population	Number of subjects	0.0-8.7 ng/mL (%)	8.8-15.0 ng/mL (%)	15.1-20.0 ng/mL (%)	20.1-100 ng/mL (%)	>100 ng/mL (%)
Normal	**140**	**136(97)**	**4(3)**	**0**	**0**	**0**

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Population	Number of subjects	0.0-8.7 ng/mL (%)	8.8-15.0 ng/mL (%)	15.1-20.0 ng/mL (%)	20.1-100 ng/mL (%)	>100 ng/mL (%)
Female (not pregnant)	70	68(97)	2(3)	0	0	0
Male	70	68(97)	2(3)	0	0	0
Benign Diseases (Tot.)	**76**	**74(97)**	**1(1)**	**0**	**1(1)**	**0**
Liver (cirrhosis)	16	16(100)	0	0	0	0
Liver (hepatitis)	17	15(88)	1(6)	0	1(6)	0
Pancreatitis	5	5(100)	0	0	0	0
Gastointestinal and pelvic	10	10(100)	0	0	0	0
Inflammatory disease						
Benign prostatic hypertrophy(BPH)	21	21(100)	0	0	0	0
Urogenital	7	7(100)	0	0	0	0
Malignant Diseases (Tot.)	**165**	**112(68)**	**6(4)**	**4(2)**	**15(9)**	**28(17)**
Colorectal	13	12(92)	1(8)	0	0	0
Breast	5	5(100)	0	0	0	0
Stomach	2	1(50)	0	0	1(50)	0
Genitourinary	36	35(97)	1(3)	0	0	0
Cervical/Ovarian	8	6(75)	0	0	0	2(25)
Pancreatic	8	8(100)	0	0	0	0
Liver	12	8(67)	0	0	1(8)	3(25)
Testicular						
Non-seminoma	62	22(36)	3(4)	4(6)	11(18)	22(36)
Seminoma	9	6(67)	1(11)	0	2(22)	0
Others	10	9(90)	0	0	0	1(10)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzer are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in accordance with a modified protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%	IU/mL	ng/mL	%
HS[b] 1	1.61	1.95	0.097	0.117	6.0	0.131	0.159	8.1
HS 2	5.29	6.40	0.161	0.195	3.0	0.287	0.347	5.4
HS 3	16.1	19.5	0.441	0.534	2.7	0.864	1.05	5.4
HS 4	160	194	4.18	5.06	2.6	7.83	9.47	4.9
HS 5	460	557	12.2	14.8	2.7	22.3	27.0	4.8
HS 6	943	1141	20.9	25.3	2.2	48.4	58.6	5.1

cobas e 411 analyzer								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%	IU/mL	ng/mL	%
PC TM[c]1	7.45	9.01	0.162	0.196	2.2	0.391	0.473	5.2
PC TM2	71.2	86.2	1.98	2.40	2.8	3.86	4.67	5.4

b) HS = human serum

c) PC TM = PreciControl Tumor Marker

cobas e 601 and **cobas e** 602 analyzers								
			Repeatability			**Intermediate precision**		
Sample	Mean		SD		CV	SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%	IU/mL	ng/mL	%
HS 1	1.72	2.08	0.051	0.062	3.0	0.073	0.088	4.2
HS 2	5.31	6.43	0.145	0.175	2.7	0.185	0.224	3.5
HS 3	16.0	19.4	0.500	0.605	3.1	0.618	0.748	3.9
HS 4	157	190	4.58	5.54	2.9	5.61	6.79	3.6
HS 5	515	623	15.4	18.6	3.0	19.6	23.7	3.8
HS 6	923	1117	29.1	35.2	3.2	36.2	43.8	3.9
PC TM1	7.50	9.08	0.164	0.198	2.2	0.255	0.309	3.4
PC TM2	70.9	85.8	1.31	1.59	1.9	2.45	2.96	3.5

Method comparison

A comparison of the Elecsys AFP assay, REF 04481798190 (x) with the Elecsys AFP assay, REF 09015060160 (y) on a **cobas e** 601 analyzer gave the following correlations (IU/mL):

Number of samples measured: 181

Passing/Bablok[11]	Linear regression
y = 0.101 + 0.965x	y = -0.148 + 0.969x
tau = 0.985	r = 0.999

The sample concentrations were between 1.58 and 966 IU/mL (1.91 and 1169 ng/mL).

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For further information, please refer to the appropriate user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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