ms_04491742190V5.0

AFP

cobas®

AFP α1-fetoprotein

REF	Σ	SYSTEM
		MODULAR ANALYTICS E170
04491742 190	200	**cobas e** 601
		cobas e 602

English

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.

Intended use

Immunoassay for the in vitro quantitative determination of α_1-fetoprotein in human serum and plasma.

This assay is intended for the use as:

- An aid in the management of patients with non-seminomatous germ cell tumors.
- As one component in combination with other parameters to evaluate the risk of trisomy 21 (Down syndrome). Further testing is required for diagnosis of chromosomal aberrations.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 immunoassay analyzers.

Summary

Alpha1-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. 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]

The determination of AFP to screen the general population for cancer is, however, not to be recommended.

Elevated AFP concentrations in maternal serum or amniotic fluid during pregnancy can indicate spina bifida, anencephalia, atresia of the oesophagus or multiple pregnancy.[8,9,10,11]

Measurement of AFP makes a contribution to the risk assessment for trisomy 21 (Down syndrome) in the second trimester of pregnancy together with hCG+β and other parameters, such as exact gestational age and maternal weight. In a trisomy 21 affected pregnancy the maternal serum concentration of AFP is decreased whereas the maternal serum hCG+β concentration is approximately twice the normal median.[12] The risk for a trisomy 21 affected pregnancy in the second trimester can be calculated by a suitable software (see "Materials required, but not provided" section) using the algorithm as described by Wald[13] and the respective assay-specific parameters.[12,13,14,15,16,17,18]

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.

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, 12 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-AFP-Ab~biotin (gray cap), 1 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~$Ru(bpy)_3^{2+}$ (black cap), 1 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.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

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

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 the analyzers	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, sodium heparin, K_3-EDTA and sodium citrate plasma. When sodium citrate is used, the results must be corrected by + 10 %.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8 °C, 3 months at -20 °C.[19]

The suitability of plasma samples for estimating the risk of trisomy 21 has not been evaluated.

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.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 04487761190, AFP CalSet II, for 4 x 1 mL
- REF 11776452122, PreciControl Tumor Marker, for 2 x 3 mL each of PreciControl Tumor Marker 1 and 2 or
  REF 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or
  REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment

MODULAR ANALYTICS E170, **cobas e** 601 or **cobas e** 602 analyzer

Accessories for MODULAR ANALYTICS E170, **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 Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M
- REF 11298500316, Elecsys SysClean, 5 x 100 mL system cleaning solution

For risk calculation of trisomy 21:

- A suitable software, e.g.
  REF 05126193, SsdwLab (V5.0 or later), single user licence
  REF 05195047, SsdwLab (V5.0 or later), multi user licence
- REF 03271749190, HCG+β, 100 tests
- REF 03302652190, HCG+β CalSet, for 4 x 1 mL

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 approx. 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). Renewed calibration is recommended as follows:

- after 1 month (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

For quality control, use PreciControl Tumor Marker or PreciControl Universal.

In addition, other suitable control material can be used.

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.

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.

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

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), hemolysis (Hb < 1.4 mmol/L or < 2.2 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 246 nmol/L or < 60 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL.

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

In vitro tests were performed on 26 commonly used pharmaceuticals. No interference with the assay was found.

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

0.500-1000 IU/mL or 0.605-1210 ng/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 IU/mL or < 0.605 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

Lower detection limit of the test

Lower detection limit: 0.50 IU/mL (0.61 ng/mL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two

standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with AFP concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:50 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 20 IU/mL (> 24 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

Results of following studies using the Elecsys AFP assay see below:

a) Multicenter study "Elecsys 2010 analyzer" status September 1997 and reference range study in Germany and France, data evaluated in September 1998.

Following AFP values were found in serum samples from 646 healthy test subjects:

≤ 5.8 IU/mL or ≤ 7.0 ng/mL for 95 % of the results.

AFP median values for completed weeks of pregnancy (defined as completed weeks of pregnancy beginning with the start of the last menstruation phase):

Weeks	14	15	16	17	18	19
N	382	1782	2386	975	353	146
IU/mL	23.2	25.6	30.0	33.5	40.1	45.5
ng/mL	27.9	30.9	36.1	40.4	48.3	54.8

b) Multicenter study to determine reference values for evaluating the risk of trisomy 21 in maternal serum (study No. BO1P019, status March 2003).

Values from serum samples of 1753 pregnant women in total (relevant gestational weeks 14 to 18) were evaluated.

Measurements with the Elecsys HCG+β assay and the Elecsys AFP assay were conducted in 5 clinical centers in Belgium, France, and Germany.

The gestational age in days determined by ultrasound was given for each sample. From a log-linear regression analysis of all 1753 AFP values versus gestational age the following median values were calculated for the middle of the respective weeks (e.g. week 14 + 3 days):

Weeks	14	15	16	17	18
IU/mL	20.9	24.0	27.6	31.7	36.4
ng/mL	25.3	29.0	33.3	38.3	44.0

Note: For prenatal testing it is recommended that the median values be re-evaluated periodically (1 to 3 years) and whenever methodology changes.

The transferability of the reference values to plasma samples has not been verified.

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 analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, pooled human sera and controls in accordance with a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60); repeatability on MODULAR ANALYTICS E170 analyzer, n = 21. The following results were obtained:

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Repeatability				
Sample	Mean		SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%
Human serum 1	14.8	17.8	0.27	0.33	1.8
Human serum 2	46.7	56.5	0.65	0.79	1.4
Human serum 3	745	901	11.7	14.2	1.6
PreciControl TM[b)]1	9.35	11.3	0.21	0.25	2.2
PreciControl TM2	104	126	2.49	3.01	2.4

b) TM = Tumor Marker

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
	Intermediate precision				
Sample	Mean		SD		CV
	IU/mL	ng/mL	IU/mL	ng/mL	%
Human serum 1	14.1	17.0	0.53	0.64	3.8
Human serum 2	44.6	53.9	1.14	1.38	2.6
Human serum 3	711	860	23.4	28.3	3.3
PreciControl TM1	9.1	11.0	0.26	0.31	2.8
PreciControl TM2	103	125	2.54	3.07	2.5

Method comparison

A comparison of the Elecsys AFP assay (y) with the Enzymun-Test AFP method (x) using clinical samples gave the following correlations (IU/mL):

Number of samples measured: 77

Passing/Bablok[20]	Linear regression
y = 0.92x - 1.51	y = 0.90x + 0.35
τ = 0.975	r = 0.998

The sample concentrations were between approximately 2 and 500 IU/mL (2.4 and 600 ng/mL).

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

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

CE 0123

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