09014985500V2.0

Elecsys PIVKA-II

REF	(Instructions for use)	Σ	SYSTEM
09014985190	09014985500	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

System information

For **cobas e** 411 analyzer: test number 1580
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 470

Please note

The measured PIVKA-II 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 PIVKA-II assay method used. PIVKA-II 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.

Intended use

Immunoassay for the quantitative measurement of protein induced by vitamin K absence or antagonist-II (PIVKA-II) in human serum and plasma. The assay is used as an aid in the diagnosis of hepatocellular carcinoma (HCC). The results must be interpreted in conjunction with other methods in accordance with standard clinical management guidelines.

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

Summary

Hepatocellular carcinoma (HCC) is the 6th most common cancer worldwide and accounts for more than 90 % of primary liver cancer.[1,2] It is the 2nd most common cause of death from cancer in males and the 6th in females worldwide. Major risk factors of developing HCC are chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) as indicated by the strong correlation between the prevalence of HCC and chronic hepatitis B and C.[3] Diagnosis of HCC depends on typical findings on cross-sectional imaging such as arterial hypervascularity as well as washout of contrast agents in the portal and late phase. If there is no typical cross-sectional imaging, liver biopsy is recommended.[4]

As most HCCs develop in cirrhotic livers,[5] ultrasound surveillance of patients with advanced chronic liver disease is recommended.[6,7,8,9] However, as ultrasound performance is operator-dependent, degrades in overweight and obese patients and is sub-optimal for early detection of HCC,[10,11] addition of biomarkers is recommended.[12] α1-fetoprotein (AFP) is the most commonly used marker for primary liver tumors worldwide. While AFP is elevated during hepato-carcinogenesis, it can also be found in other tumors such as testicular, embryonic[13] or gastric cancer.[14] AFP has reported sensitivities ranging from 39 to 65 %, and specificities from 76 to 94 % in HCC patients.[15] The divergence in sensitivity and specificity of AFP in these studies is probably due to a variety of factors including different etiologies, variable study designs, and different cutoff values. Protein induced by vitamin K absence or antagonist-II (PIVKA-II, also known as des-γ-carboxy prothrombin [DCP]), as well as AFP-L3% (Lens culinaris agglutinin-reactive fraction of α-fetoprotein [AFP-L3] expressed as a percentage of AFP) have been identified as promising biomarkers, which may have utility in the surveillance, diagnosis, and management of HCC.[16,17]

PIVKA-II is an abnormal form of prothrombin secreted into the bloodstream when the activity of vitamin K-dependent carboxylase in the liver is inhibited as a result of the absence of vitamin K or the presence of vitamin K antagonists.[16,18] Serum PIVKA-II was found to have sensitivities of 48-62 %, specificities of 81-98 %, and an accuracy of 59-84 % in diagnosing HCC in several studies, mostly from Asian cohorts.[19,20,21,22] According to recent data, PIVKA-II has better diagnostic effectiveness than AFP in differentiating HCC from non-HCC hepatic diseases. In addition, the combination of the two markers could significantly improve the diagnostic performance.[23] In another study which compared PIVKA-II, AFP and AFP-L3%, PIVKA-II was found to be significantly superior to the others in differentiating primary liver cancer from cirrhosis (sensitivity 86 % and specificity 93 %).[24] PIVKA-II is an independent predictor of HCC presence and a better diagnostic biomarker than AFP in discriminating between neoplastic and non-neoplastic lesions in cirrhotic patients with initial ultrasound evidence of suspicious liver nodules.[25]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 40 µL of sample are automatically prediluted 1:5 with Diluent Universal 2. The antigen (in 20 µL of prediluted sample), a biotinylated monoclonal PIVKA-II-specific antibody, and a monoclonal PIVKA-II-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 PIVKA.

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

R1 Anti-PIVKA-II-Ab~biotin (gray cap), 1 bottle, 10.0 mL:
Biotinylated monoclonal anti-PIVKA-II antibody (rabbit) 1.2 mg/L; phosphate buffer 40 mmol/L, pH 6.5; preservative.

R2 Anti-PIVKA-II-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10.0 mL:
Monoclonal anti-PIVKA-II antibody (rabbit) labeled with ruthenium complex 2.0 mg/L; phosphate buffer 40 mmol/L, pH 6.5; preservative.

Precautions and warnings

For in vitro diagnostic use for health care 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 dust/fume/gas/mist/vapours/spray.
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: all countries: +49-621-7590

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	8 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.

Li-heparin plasma tubes containing separating gel can be used.

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, 12 weeks at -20 °C (± 5 °C). The samples may be frozen up to 3 times.

(Acceptance criteria: For serum and plasma: ≤ 30 ng/mL ± 4.5 ng/mL; > 30 ng/mL ± 15 %.)

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 08333637190, CalSet PIVKA-II, for 4 x 1.0 mL
- REF 08333645190, PreciControl HCC, for 4 x 1.0 mL or
- REF 08754551190, PreciControl HCC V2, for 4 x 1.0 mL
- REF 05192943190, Diluent Universal 2, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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 11298500316, 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.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

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 purified recombinant des-γ-carboxy prothrombin from cell culture.

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

For quality control, use PreciControl HCC or PreciControl HCC V2.

In addition, other suitable control material can be used.

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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 in ng/mL.

Limitations - interference

The effect of the following endogenous substances and pharmaceuticals 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	≤ 66 mg/dL or ≤ 1129 µmol/L
Hemoglobin	≤ 1000 mg/dL or ≤ 0.621 mmol/L
Intralipid	≤ 2000 mg/dL
Biotin	≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL
Albumin	≤ 7.0 g/dL

Criterion: Recovery ± 3.0 ng/mL of initial value ≤ 30 ng/mL and within ± 10 % of initial value > 30 ng/mL.

There is no high-dose hook effect at PIVKA-II concentrations up to 145000 ng/mL.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

Commonly used pharmaceuticals

Pharmaceutical	Concentration tested
Acetylcysteine	553 µg/mL
Ampicillin-Na	1000 µg/mL
Ascorbic acid	300 µg/mL
Cyclosporine	5.00 µg/mL
Cefoxitin	2500 µg/mL
Heparin	5000 IU/L
Levodopa	20.0 µg/mL
Methyldopa + 1.5	20.0 µg/mL
Metronidazole	200 µg/mL
Phenylbutazone	400 µg/mL
Doxycyclin	50.0 µg/mL
Acetylsalicylic acid	1000 µg/mL
Rifampicin	60.0 µg/mL
Acetaminophen	200 µg/mL
Ibuprofen	500 µg/mL
Theophylline	100 µg/mL
Itraconazole	50.0 µg/mL

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

Special drugs

Drug	Concentration tested
5-FU (Fluorouracil)	900 µg/mL
Doxorubicin	165 µg/mL
Cisplatin	180 µg/mL
Mitomycin	25.0 µg/mL
Epoetin	25 mU/L
Metoclopramide	7.50 µg/mL
Neupogen	0.9 µg/mL
Dexamethasone	20.0 µg/mL
Sorafenib	800 µg/mL
SN-38	525 µg/mL
Pegylated Interferon-α	0.026 µg/mL
Vitamin K	0.09 µg/mL
Ribavirin	1200 µg/mL
Uridine-analog-triphosphate of sofosbuvir	80.0 µg/mL
Entecavir	1.00 µg/mL
Tenofovir	245 µg/mL
Ledipasvir	18.0 µg/mL
Daclatasvir	60.0 µg/mL

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

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

3.5-12000 ng/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 3.5 ng/mL. Values above the measuring range are reported as > 12000 ng/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank ≤ 3.0 ng/mL

Limit of Detection ≤ 3.5 ng/mL

Limit of Quantitation ≤ 4.5 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 95^{th} 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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with an intermediate precision CV of ≤ 20 %.

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Linearity

The Elecsys PIVKA-II assay is linear across the measuring range from 3.5-12000 ng/mL. Samples were prepared according to CLSI EP6-A by diluting 3 serum and 3 plasma samples each with Diluent Universal 2 in multiple steps ranging from > 12000 ng/mL downwards to Limit of Detection.

Dilution

Samples with PIVKA-II concentrations above the measuring range can be diluted manually with Diluent Universal 2. The recommended dilution is 1:10. The concentration of the diluted sample must be > 1200 ng/mL.

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

Expected values

The following PIVKA-II concentration values (ng/mL) were found in serum samples from 811 apparently healthy adults (431 males, 380 females) aged between 20 and 79 (mean 47.05) years (thereof 803 Caucasian):

	Min/Max	Mean (SD)	Median (95 % CI[b])	95th percentile (95 % CI)
All (N = 811)	8.40/131	19.7 (6.38)	18.7 (18.4; 19.0)	28.4 (26.9; 29.4)
Female (N = 380)	8.40/54.4	19.2 (5.32)	18.1 (17.7; 18.7)	27.8 (26.7; 31.1)
Male (N = 431)	11.2/131	20.3 (7.15)	19.0 (18.7; 19.6)	28.6 (26.7; 30.0)

b) CI = confidence interval

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

Study cohort for the evaluation of PIVKA-II clinical performance

A study was performed with 376 patients with liver disease. Of these 376 patients, 168 had HCC and 208 had liver disease but no diagnosis of HCC (control).

	Median age	Gender (% male)	Race				
			Asian (%)	Caucasian (%)	Black (%)	Other (%)	Missing (%)
Control (N = 208)	53	60.6	47.6	48.6	1.4	0	2.4
HCC (N = 168)	64	83.9	42.3	56.5	0	0.6	0.6

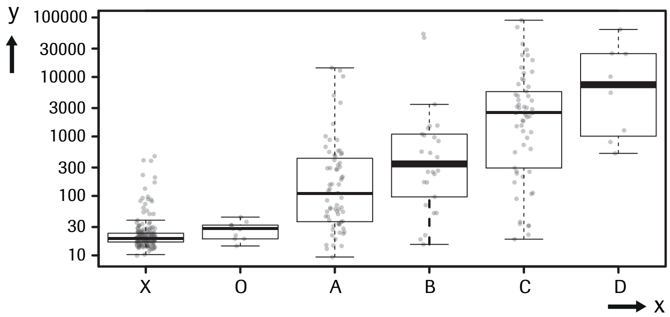
Range of PIVKA-II concentration in HCC cases compared to controls

The following table and graph show the range of PIVKA-II concentration in samples from HCC patients staged according to Barcelona clinic liver cancer classification (BCLC)[26] compared to controls. For the 168 patients with a diagnosis of HCC, the PIVKA-II concentration increased with disease progression. All concentrations are in ng/mL. The thick line in the box plots represents the median value.

Disease stage	N	Min/Max	Mean (SD)	Median	25th-75th perc.[c]
Control[d]	208	9.92/465	31.7 (53.9)	19.4	16.8-23.6
Early (Stage 0 + A)	77	9.39/14233	783 (2493)	63	32.3-329
BCLC Stage 0	10	14.4/44	27.5 (9.14)	28.1	-
BCLC Stage A	67	9.39/14233	895 (2657)	111	36.5-460
Late (Stages B, C and D)	91	15.3/89918	7468 (15840)	1486	252-5056
BCLC Stage B	26	15.3/53067	4378 (13319)	357	96.4-1094
BCLC Stage C	57	18.7/89918	7636 (15858)	2508	295-5672
BCLC Stage D	8	520/62941	16309 (21339)	7785	-

c) not calculated if sample size is 20 or below

d) In the graphical representation below, this group is designated with an “X”.



x ---> X: Control; O: Stage 0; A: Stage A; B: Stage B; C: Stage C; D: Stage D
y ---> PIVKA-II (ng/mL)

PIVKA-II concentration and disease etiology

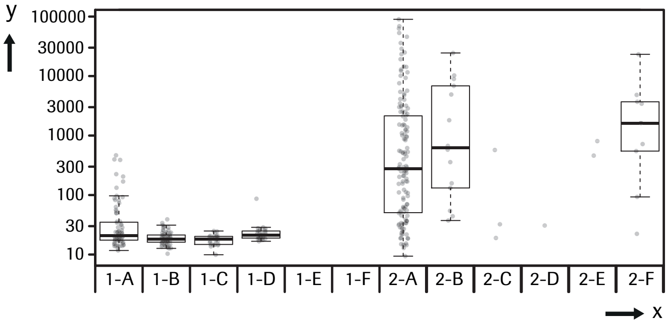
The PIVKA-II concentration as function of etiology for the two patient groups (Control, 1-A to 1-F and HCC, 2-A to 2F) is shown in the following table and graph.

Label	Etiology[e]	N	Min/Max	Mean (SD)	Median	25th-75th perc.[c]
1-A	Cirrhosis	79	11.7/465	50.6 (83.9)	20.7	17.4-35.7
1-B	Hepatitis B	72	10.3/39	19.4 (5.26)	18.2	16.1-21.3
1-C	Hepatitis C	27	9.92/24.9	18.0 (3.5)	18.2	14.8-20.4
1-D	NASH[f]	30	16.7/86.7	23.8 (12.3)	21.3	18.9-24.9
1-E	ALD[g]	0	-	-	-	-
1-F	Others	0	-	-	-	-
2-A	Cirrhosis	139	9.39/89918	4608 (13126)	277	49.7-2177
2-B	Hepatitis B	14	37.3/24432	4229 (6831)	625	-
2-C	Hepatitis C	3	19/571	208 (315)	32.3	-
2-D	NASH[f]	1	-	30.7 (-)	-	-
2-E	ALD[g]	2	460/807	633 (245)	633	-
2-F	Others	9	22.3/23205	4240 (7322)	1620	-

e) All etiologies except cirrhosis are non-cirrhotic

f) Non-alcoholic steatohepatitis

g) Alcoholic liver disease



y---> PIVKA-II (ng/mL)

Clinical performance of the Elecsys PIVKA-II assay in detecting HCC

The sensitivity and specificity of the Elecsys PIVKA-II assay in detecting HCC at a cut-off of 28.4 ng/mL (95^{th} percentile in the apparently healthy population) and the results of the Receiver Operating Characteristics (ROC) analysis are shown below.

	All HCC	Early Stage HCC[h]	Late Stage HCC[i]
Sensitivity (95 % CI)	86.9 % (80.8 %, 91.6 %)	77.9 % (67 %, 86.6 %)	94.5 % (87.6 %, 98.2 %)
Specificity (95 % CI)	83.7 % (77.9 %, 88.4 %)	83.7 % (77.9 %, 88.4 %)	83.7 % (77.9 %, 88.4 %)
ROC AUC[j]	90.8 %	84.7 %	95.9 %

h) BCLC stages 0, A

i) BCLC stages B,C,D

j) Area under the Curve

	Cirrhosis	Hep B	Hep C	NASH	ALD	Other
Sensitivity (95 % CI)[c]	85.6 % (78.7 %, 91 %)	-	-	-	-	-
Specificity (95 % CI)[c]	68.4 % (56.9 %, 78.4 %)	90.3 % (81 %, 96 %)	100 % (87.2 %, 100 %)	93.3 % (77.9 %, 99.2 %)	-	-
ROC AUC[j]	85.6 %	97.3 %		96.4 %		

Cutoffs of the Elecsys PIVKA-II assay at specified sensitivity or specificity

The following tables show the clinical performance of the Elecsys PIVKA-II assay at different cutoffs and specified sensitivity or specificity.

Specificity	PIVKA-II cutoff (ng/mL)	Sensitivity (95 % CI)
95 %	86.7	67.9 % (60.2 %, 74.8 %)
90 %	35.9	81 % (74.2 %, 86.6 %)
85 %	28.5	86.9 % (80.8 %, 91.6 %)
80 %	25.3	88.7 % (82.9 %, 93.1 %)
75 %	23.5	89.9 % (84.3 %, 94 %)
70 %	22.7	90.5 % (85 %, 94.5 %)

Sensitivity	PIVKA-II cutoff (ng/mL)	Specificity (95 % CI)
95 %	18.7	43.3 % (36.4 %, 50.3 %)
90 %	23.1	72.1 % (65.5 %, 78.1 %)
85 %	31.2	87.5 % (82.2 %, 91.7 %)
80 %	36.5	90.4 % (85.5 %, 94 %)
75 %	51.4	91.8 % (87.2 %, 95.2 %)
70 %	63.1	93.3 % (89 %, 96.3 %)

PIVKA-II values in different types of benign and malignant disorders

The following table and graph show the PIVKA-II concentration (ng/mL) in a panel of samples from patients with either a benign liver disease, an immune disorder, or a malignancy other than HCC (N total 397; median age 54 years, 58 % female, 39 % Asian and 61 % Caucasian).

Label	Etiology	N	Min/Max	Mean (SD)	Median	25^{th}-75^{th} perc.[c]
A	Benign liver diseases[k]	87	13.3/843	50.7 (134)	20.9	17.1-28.0
B	Rheumatoid arthritis	38	13.0/51.1	19.5 (6.19)	17.9	16.3-21.8
C	Crohn's disease	37	11.4/660	38.9 (105)	19.9	17.3-25.8
D	Ulcerative colitis	30	3.5/71.3	21.6 (10.9)	19.5	17.2-23.1
E	Other autoimmune diseases[l]	26	12.2/37.6	21.4 (7.38)	18.0	16.6-26.4
F	Lung cancer	24	11.3/176	28.5 (32.8)	19.7	16.3-26.1
G	Breast cancer	27	15.0/266	33.1 (47.8)	21.3	18.7-27.2
H	Renal cancer	10	13.9/4015	492 (1257)	24.1	-
I	Cholangio-carcinoma[m]	27	14.5/22463	2313 (5619)	143	42.6-834
J	Pancreatic cancer[m]	10	17.6/3034	674 (966)	211	-
K	Other gastrointestin-al cancers[n]	55	12.8/2342	72.1 (314)	19.9	17.5-29.4
L	Gynecological cancers[o]	26	14.7/3186	151 (619)	21.4	18.8-39.2

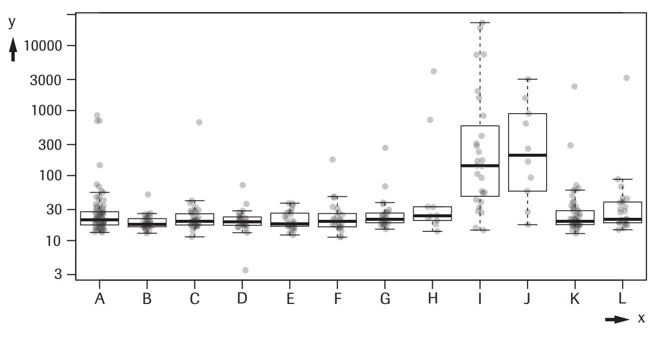
k) polycystic liver disease, simple cysts, focal nodular hyperplasia, hemangioma, hepatocellular adenoma, non-cirrhotic alcohol liver disease

l) systemic lupus erythematosus, autoimmune thyroiditis

m) of the 8 patients with cholangiocarcinoma or pancreatic cancer with a PIVKA-II concentration > 1000 ng/mL, 5 patients had evidence of cholestatic disease (e.g. cholestasis, cholangitis, jaundice, biliary obstruction) at the time point of blood draw. For the other 3 patients no detailed information could be obtained.

n) colorectal, gastric and esophageal cancer

o) ovarian, endometrial and cervical cancer



y---> PIVKA-II (ng/mL)

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Comparison of clinical performance

The clinical performance of the Elecsys PIVKA-II assay in terms of discriminating between HCC cases (N = 168) and disease controls (N = 208) was compared to that of the Fujirebio Lumipulse PIVKA-II test by ROC analysis: The Elecsys PIVKA-II assay has an AUC of 90.8 %, while the Fujirebio Lumipulse PIVKA-II test has an AUC of 89.4 %.

The following has to be taken into consideration

- For diagnostic purposes, results should be used in conjunction with other clinical data, e.g., symptoms, results of other tests, clinical impressions, etc.
- PIVKA-II levels, regardless of value, should not be interpreted as absolute evidence for the presence or absence of a malignant disease. In patients with suspected or known cancer, other tests and procedures must also be considered for diagnosis and good management.
- The concentration of PIVKA-II in a given specimen, determined with assays from different manufacturers, can vary due to differences in assay methods, calibration, and reagent specificity.

The following factors may influence the PIVKA-II level in an individual

- Medication containing vitamin K preparations may result in lower PIVKA-II values.
- Vitamin K antagonists and medical conditions causing Vitamin K deficiency (e.g., biliary obstruction or cholestasis) may result in higher PIVKA-II values. Samples from patients receiving vitamin K antagonists (warfarin, etc.) should not be measured with the Elecsys PIVKA-II assay.
- Increased concentrations of PIVKA-II have been observed in patients with renal dysfunction.[27] The evaluation of serum creatinine levels should be considered in cases of high PIVKA-II levels that are not consistent with diagnostic and clinical characteristics of the patient.

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, samples and controls in a 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 ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	7.15	0.161	2.20	0.398	5.60
Human serum 2	18.2	0.302	1.70	0.904	5.00
Human serum 3	25.3	0.644	2.50	1.45	5.70
Human serum 4	6659	175	2.60	410	6.20
Human serum 5	11800	291	2.50	696	5.90
Human serum 6	10838	269	2.50	652	6.00
PreciControl HCC 1	23.0	0.338	1.50	1.16	5.00
PreciControl HCC 2	348	10.2	2.90	19.1	5.50
PreciControl 1 HCC V2	20.9	0.410	2.0	0.768	3.7
PreciControl 2 HCC V2	325	7.08	2.2	12.7	3.9

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean ng/mL	SD ng/mL	CV %	SD ng/mL	CV %
Human serum 1	7.98	0.165	2.10	0.340	4.30
Human serum 2	19.2	0.309	1.60	0.707	3.70
Human serum 3	26.4	0.499	1.90	1.10	4.20
Human serum 4	6368	142	2.20	267	4.20
Human serum 5	10954	187	1.70	413	3.80
Human serum 6	10114	179	1.80	373	3.70
PreciControl HCC 1	23.6	0.333	1.40	0.816	3.50
PreciControl HCC 2	344	5.19	1.50	11.8	3.40
PreciControl 1 HCC V2	21.2	0.364	1.7	0.738	3.5
PreciControl 2 HCC V2	314	5.47	1.7	9.80	3.1

Method comparison

A comparison of the Elecsys PIVKA-II assay, REF 09014985190 (**cobas e** 601 analyzer; y) with the Elecsys PIVKA-II assay, REF 08333602190 (**cobas e** 601 analyzer; x) gave the following correlations (ng/mL):

Number of serum samples measured: 134

Passing/Bablok[28]	Linear regression
y = 1.09x + 0.087	y = 1.08x + 2.10
τ = 0.991	r = 1.00

The sample concentrations were between 3.74 and 11078 ng/mL.

References

1 Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2020, Cancer Incidence and Mortality Worldwide: IARC Lyon, France: International Agency for Research on Cancer; 2020. Available from: https://gco.iarc.fr/today/fact-sheets-cancers.

2 Akinyemiju T, Abera S, Ahmed M, et al. The burden of primary liver cancer and underlying etiologies from 1990 to 2015 at the global, regional, and national level: Results From the Global Burden of Disease Study 2015. JAMA Oncol 2017;3:1683-1691.

3 El-Seraq HB. Epidemiology of Viral Hepatitis and Hepatocellular Carcinoma. Gastroenterology 2012;142(6):1264-1273.

4 Gonzalez SA and Keeffe EB. Diagnosis of Hepatocellular Carcinoma: Role of Tumor Markers and Liver Biopsy. Clin Liver Dis 2011;15:297-306.

5 Llovet JM, Zucman-Rossi J, Pikarsky E, et al. Hepatocellular carcinoma. Nature Reviews Disease Primers. 2016;14:2:16018.

6 Heimbach JK, Kulik LM, Finn RS, et al. AASLD Guidelines for the Treatment of Hepatocellular Carcinoma. Hepatology 2018;67(1): 358-80.

7 EASL Clinical Practice Guidelines: Management of hepatocellular carcinoma. J Hepatol 2018;69(1):182-236.

8 Kokudo N, Hasegawa K, Akahane M, et al. Evidence-based Clinical Practice Guidelines for Hepatocellular Carcinoma: The Japan Society of Hepatology 2013 update (3rd JSH-HCC Guidelines). Hepatology Research 2015;45:123-127.

9 Omata M, Cheng AL, Kokudo N, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int 2017;11: 317-370.

10 Simmons O, Fetzer DT, Yokoo T, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. Aliment Pharmacol Ther 2017;45:169-177.

11 Tzartzeva K, Obi J, Rich NE, et al. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. Gastroenterology 2018; 154(6):1706-1718.e1.

12 Lok AS, Sterling RK, Everhart JE, et al. Des-gamma-Carboxy Prothrombin and alpha-Fetoprotein as Biomarkers for the Early Detection of Hepatocellular Carcinoma. Gastroenterology 2010;138:493-502.

13 Gupta S, Bent S, Kohlwes J. Test characteristics of alpha-fetoprotein for detecting hepatocellular carcinoma in patients with hepatitis C. A systematic review and critical analysis. Ann. Intern. Med. 2003;139(1):46-50.

14 Chen J, Röcken C, Treiber G, et al. Clinical implications of alpha-fetoprotein expression in gastric adenocarcinoma. Dig Dis 2003;21(4):357-362.

15 Daniele B, Bencivenga A, Megna AS, et al. Alpha-fetoprotein and ultrasonography screening for hepatocellular carcinoma. Gastroenterology 2004;127:108-112.

16 Liebmann HA, Furie BC, Tong MJ, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Eng J Med 1984;310:1427-1431.

17 Choi JY, Jung SW, Kim HY, et al. Diagnostic value of AFP-L3 and PIVKA-II in hepatocellular carcinoma according to total-AFP. World J. Gastroenterol 2013;19(3):339-346.

18 Bertino G, Ardiri AM, Boemi PM, et al. A study about mechanisms of des-gamma-carboxy prothrombin's production in hepatocellular carcinoma. Panminerva Med 2008;50(3):221-226.

19 Bertino G, Ardiri AM, Calvagno GS, et al. Prognostic and diagnostic value of des-γ-carboxy prothrombin in liver cancer. Drug News Perspect 2010;23(8):498-508.

20 Marrero JA, Su GL, Wei W, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in American patients. Hepatology 2003;3785):1114-1121.

21 Bertino G, Neri S, Bruno CM, et al. Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. Minerva Med 2011;102(5):363-371.

22 Kim MJ, Hyuck C, Kwon D, et al. Protein induced by vitamin K antagonist-II (PIVKA-II) is a reliable prognostic factor in small hepatocellular carcinoma. World J Surg 2013;37(6):1371-1378.

23 Yu R, Tan Z, Xiang X, et al. Effectiveness of PIVKA-II in the detection of hepatocellular carcinoma based on real-world clinical data. BMC Cancer 2017;17:608.

24 Volk ML, Hernandez JC, Su GL, et al. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers, a comparison of AFP, DCP, and AFP-L3. Cancer Biomarkers 2007;3(2):79-87.

25 Saitta C, Raffa G, Alibrandi A, et al. PIVKA-II is a useful tool for diagnostic characterization of ultrasound-detected livernodules in cirrhotic patients. Medicine, 2017;96:26(e7266).

26 Llovet JM, Brú C, Bruix J. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19(3):329-338.

27 McCabe KM, Adams MA, Holden RM. Vitamin K Status in Chronic Kidney Disease. Nutrients 2013;5(11):4390-4398.

28 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets 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.

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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.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

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