Elecsys CA 72-4

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REF		Σ	SYSTEM
			cobas e 411
09005692190	09005692500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 2510
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 307

Please note

The measured CA 72-4 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 CA 72-4 assay method used. CA 72-4 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 CA 72-4 assay procedure used while monitoring therapy, then the CA 72-4 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 CA 72-4 in human serum and plasma. The assay in particular serves as an aid in the therapeutic monitoring of carcinomas of the stomach and ovaries.

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

Summary

The tumor associated glycoprotein (TAG) 72, also known as CA 72-4 is a mucin protein of high molecular weight (approximately 200-400 kD) and found on the surface of many cancer cells, including stomach, ovary, breast, colon and pancreatic cells.[1] An antibody construct directed against TAG 72 has been proposed as an anti-tumor agent against ovarian and prostate cancer.[2]

Elevated serum levels are primarily found in gastric cancer patients,[3,4] but can also be found in certain non-malignant diseases like pneumonia, pancreatitis, liver cirrhosis and ovarian cysts.[5] The most important advantage of CA 72-4 is its ability to discriminate between malignant and non-malignant gastric and ovarian diseases.[3,6]

Gastric and Ovarian Cancer:

For gastric cancer, a diagnostic sensitivity of 33 % was reported for CA 72-4.[7] Monitoring treatment and disease course in patients with gastric and ovarian cancer is the main indication for CA 72-4. After surgical intervention, CA 72-4 levels return to normal and remain within the normal range in cases where tumor tissue is no longer present.[8]

A diagnostic sensitivity of 47-76 % has been reported in ovarian carcinoma.[9] Especially for mucinous ovarian cancer, the diagnostic sensitivity of CA 72-4 is greater than that of CA 125.

The Elecsys CA 72-4 assay utilizes the following two monoclonal antibodies to detect the mucin, TAG 72:[10]

- B72.3 monoclonal antibody, which has been raised against a membrane-enriched extract of mammary carcinoma metastases[11] and
- CC49 monoclonal antibody, specific to highly-purified TAG 72.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a biotinylated monoclonal CA 72-4-specific antibody (CC49), and a monoclonal CA 72-4-specific antibody (B72.3) 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 CA72-4.

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

R1 Anti-CA 72-4-Ab~biotin (gray cap), 1 bottle, 8 mL:
Biotinylated monoclonal anti-CA 72-4 antibody (CC49; mouse) 1 mg/L; phosphate buffer 100 mmol/L, pH 6.8; preservative.

R2 Anti-CA 72-4-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
Monoclonal anti-CA 72-4 antibody (B72.3; mouse) labeled with ruthenium complex 6 mg/L; phosphate buffer 100 mmol/L, pH 6.8; 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.

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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.

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

Stable for 24 hours at 20-25 °C, 30 days at 2-8 °C, 90 days at -20 °C (± 5 °C). Freeze only once.

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 09175130190, CA 72-4 CalSet, for 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, 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 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 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.

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 Enzymun-Test CA 72-4 method.

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 12 weeks 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.

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.

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 U/mL or kU/L).

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.

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Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 1130 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: Recovery ± 0.4 U/mL of intial value for samples ≤ 4 U/mL, within ± 10 % of initial value for samples > 4 to 100 U/mL, and within ± 12 % of initial value for samples > 100 U/mL.

There is no high-dose hook effect at CA 72-4 concentrations up to 15000 U/mL.

In vitro tests were performed on 27 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

0.500-250 U/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.500 U/mL. Values above the measuring range are reported as > 250 U/mL (or up to 500 U/mL for 2-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.5 U/mL

Limit of Detection = 1.0 U/mL

Limit of Quantitation = 1.5 U/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 CA 72-4 concentrations above the measuring range can be diluted with Diluent Universal 2. The recommended dilution is 1:2 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 110 U/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.

Please note: A sample dependent non-linearity of dilutions has been observed for samples which are outside the measuring range.

Expected values

Extended studies with the Elecsys CA 72-4 assay in clinical centers in Belgium, Germany, and Roche-internal studies gave the following results for a total of 635 healthy individuals:

6.9 U/mL (95 % percentile)

5.6-8.2 U/mL (95 % confidence range of the percentile)[12]

Status: Elecsys CA 72-4 multicenter evaluation; study No. B99P026, 7/2001

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 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 mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	3.74	0.081	2.2	0.157	4.2
Human serum 2	5.18	0.080	1.5	0.246	4.8
Human serum 3	5.75	0.091	1.6	0.264	4.6
Human serum 4	137	2.57	1.9	7.00	5.1
Human serum 5	243	3.98	1.6	14.2	5.8
PreciControl TM[b)]1	4.45	0.074	1.7	0.190	4.3
PreciControl TM2	53.3	1.14	2.1	2.99	5.6

b) TM = Tumor Marker

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	3.51	0.044	1.3	0.116	3.3
Human serum 2	4.89	0.058	1.2	0.154	3.2
Human serum 3	5.44	0.064	1.2	0.183	3.4
Human serum 4	133	1.72	1.3	4.89	3.7
Human serum 5	236	2.80	1.2	8.34	3.5
PreciControl TM1	4.32	0.051	1.2	0.134	3.1
PreciControl TM2	51.0	0.797	1.6	1.70	3.3

Method comparison

A comparison of the Elecsys CA 72-4 assay REF 11776258122 (y) with the Enzymun-Test CA 72-4 method (x) using clinical samples gave the following correlations:

Number of samples measured: 144

Passing/Bablok[13]	Linear regression
y = 0.93x - 1.59	y = 0.95x - 1.43
τ = 0.877	r = 0.954

The sample concentrations were between 0.3 and 87 U/mL.

A comparison of the Elecsys CA 72-4 assay, REF 09005692190 (**cobas e** 601 analyzer; y) with the Elecsys CA 72-4 assay, REF 11776258122 (**cobas e** 601 analyzer; x) gave the following correlations (U/mL):

Number of serum samples measured: 147

Passing/Bablok[13]	Linear regression
y = 0.998x - 0.218	y = 1.006x - 0.430
τ = 0.985	r = 1.000

The sample concentrations were between 0.767 and 241 U/mL.

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Analytical specificity

The Elecsys CA 72-4 tumor marker assay is based on the monoclonal B72.3 and CC49 antibodies which are only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of testing procedures using these antibodies cannot be assumed for test methods using other antibodies.

References

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7 Yang A-P, Liu J, Lei H-Y, et al. CA 72-4 combined with CEA, CA-125 and CA 19-9 improves the sensitivity for the early diagnosis of gastric cancer. Clin Chim Acta 2014;437:183-86.

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9 Hasholzner U, Baumgartner L, Stieber P, et al. Clinical significance of the tumor markers CA 125 II and CA 72-4 in ovarian carcinoma. Int J Cancer (Pred Oncol) 1996;69(4):329-334.

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12 Hahn GJ, Meeker WQ. Statistical Intervals: A Guide for Practitioners. John Wiley & Sons, Inc. New York 1991.

13 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, 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.

FUJIREBIO Diagnostics, Inc. CA 72-4 is a registered trademark of Fujirebio Diagnostics, Inc.

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

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche