Elecsys CA 125 II

cobas®

REF		Σ	SYSTEM
			cobas e 411
11776223190	11776223500	100	**cobas e** 601
			cobas e 602

English

System information

For **cobas e** 411 analyzer: test number 341
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 053

Please note

The measured CA 125 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 125 assay method used. CA 125 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 125 assay procedure used while monitoring therapy, then the CA 125 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 OC 125 reactive determinants in human serum and plasma.

These determinants are associated with a high molecular weight glycoprotein in serum and plasma of women with primary epithelial invasive ovarian cancer (excluding those with cancer of low malignant potential).

This assay is indicated for use as an aid in the detection of residual or recurrent ovarian carcinoma in patients who have undergone first-line therapy and would be considered for second-look procedures. This assay is further indicated for serial measurement of CA 125 to aid in the management of cancer patients.

This assay is also intended to be used in conjunction with the Elecsys HE4 assay as part of ROMA (Risk Of Ovarian Malignancy Algorithm) for the risk assessment of ovarian cancer in premenopausal and postmenopausal women presenting with pelvic mass.

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

Summary

CA 125 is a repeating peptide epitope of the mucin MUC16,[1,2] which promotes cancer cell proliferation and inhibits anti-cancer immune responses.[3,4,5,6]

MAb OC 125 was an antibody obtained from mice that had been immunized with OVCA (ovarian carcinoma cell line) 433, an adenocarcinoma cell line from the ovary.[7] Subsequently, the MAb M11 antibody was developed against CA 125.[8] In the Elecsys test, OC 125 is used as a detection antibody. MAb M 11 is used as the capture antibody (solid-phase antibody); this has been employed in second-generation CA 125 assays since 1992.

CA 125 has been found in the amniotic fluid and in the coelomic epithelium; both of these tissues are of fetal origin. In tissues of adult origin, the presence of CA 125 has been demonstrated in the epithelium of the oviduct, in the endometrium and in the endocervix.[9]

CA 125 is found in a high percentage of ovarian tumors of epithelial origin and can be detected in serum.[10,11] Elevated values are sometimes found in various benign gynecological diseases such as ovarian cysts and endometriosis.[12] Slight elevations of this marker may also occur in early pregnancy and in various benign diseases (e.g. pancreatitis, cirrhosis, hepatitis, benign gastrointestinal diseases, renal insufficiency, and others).[13] Although the highest CA 125 values occur in patients suffering from ovarian carcinoma, elevated values are also observed in malignancies of the endometrium, breast, gastrointestinal tract, and various other malignancies.

Recent findings show that combination of CA 125 and HE4 can help to determine whether a pelvic mass is benign or malignant in pre- and postmenopausal women. The dual marker combination CA 125 and HE4 is a more accurate predictor of malignancy than either alone.[14] Huhtinen et al. reported a 78.6 % sensitivity at 95 % specificity in ovarian carcinoma vs. endometriotic cysts.[15] Moore et al. reported 94 % accuracy in identifying malignant vs benign pelvic masses when combining CA 125 and HE4 in the ROMA algorithm.[16]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 20 µL of sample, a biotinylated monoclonal CA 125-specific antibody, and a monoclonal CA 125-specific antibody labeled with a ruthenium complex[a)] 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 CA125 II.

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

R1 Anti-CA 125-Ab~biotin (gray cap), 1 bottle, 9 mL:
Biotinylated monoclonal anti-CA 125 antibody (M 11; mouse) 1 mg/L; phosphate buffer 100 mmol/L, pH 7.4; preservative.

R2 Anti-CA 125-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-CA 125 antibody (OC 125; mouse) labeled with ruthenium complex 1 mg/L; phosphate buffer 100 mmol/L, pH 7.4; 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	6 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 as well as plasma tubes containing separating gel.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x Limit of Blank + coefficient of correlation ≥ 0.95.

Stable for 8 hours at 20-25 °C, 5 days at 2-8 °C, 24 weeks 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 07030207190, CA 125 II CalSet II, for 4 x 1.0 mL
- REF 11776452122, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

For epithelial ovarian cancer risk assessment with ROMA (Risk of Ovarian Malignancy Algorithm):

- REF 05950929190, Elecsys HE4, 100 tests
- REF 05950945190, HE4 CalSet, for 4 x 1 mL
- REF 05950953190, PreciControl HE4, for 2 x 1 mL each of PreciControl HE4 1 and 2
- REF 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent

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 125 II method. This in turn has been standardized against the CA 125 II RIA from Fujirebio Diagnostics.

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 8 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

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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, U/L or kU/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1129 µmol/L or < 66 mg/dL), hemolysis (Hb < 2.0 mmol/L or < 3.2 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 287 nmol/L or < 70 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 1200 IU/mL.

There is no high-dose hook effect at CA 125 concentrations up to 50000 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

0.6-5000 U/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 0.6 U/mL. Values above the measuring range are reported as > 5000 U/mL (or up to 25000 U/mL for 5-fold diluted samples).

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.6 U/mL

Limit of Detection = 1.2 U/mL

Limit of Quantitation = 2.0 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 defined as the lowest amount of analyte in a sample that can be accurately quantitated with a total allowable relative error of ≤ 20 %.

A study was performed based on guidance from the CLSI, protocol EP17-A2 using 5 diluted human serum samples each for Limit of Blank and Limit of Detection respectively. The samples were tested in 6 runs over 3 days on 2 analyzers resulting in n = 60 values. For Limit of Quantitation 3 human serum samples were diluted and measured in 6 runs over ≥ 3 days on 2 analyzers with a total allowable relative error of ≤ 20 %.

Limit of Blank, Limit of Detection and Limit of Quantitation were calculated to be the following:

	cobas e 411 analyzer	**cobas e** 601 and **cobas e** 602 analyzers
Limit of Blank (U/mL)	0.600	0.449
Limit of Detection (U/mL)	0.697	0.548
Limit of Quantitation (U/mL)	1.05	1.29

Dilution

Samples with CA 125 concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:5 (either automatically by analyzers, or manually). The concentration of the diluted sample must be > 1000 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.

Note: In rare cases, sample-dependent non-linearity upon dilution is seen with samples having analyte levels beyond the measuring range.

Expected values

Studies using the Elecsys CA 125 II assay in 593 samples from healthy females (pre- and postmenopausal) yielded a value of 35 U/mL (95th percentile). Values > 35 U/mL indicate an increased probability for residual or recurrent ovarian carcinoma in patients treated for primary epithelial invasive ovarian cancer.

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

Risk estimation in patients with pelvic mass

For risk estimation with ROMA see package insert of the Elecsys HE4 assay.

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 (EP5-A2) 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 U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	14.7	0.423	2.9	0.591	4.0
Human serum 2	3.08	0.090	2.9	0.148	4.8
Human serum 3	2400	60.1	2.5	82.0	3.4
Human serum 4	4950	93.2	1.9	193	3.9
Human serum 5	35.2	0.686	1.9	1.56	4.4
PreciControl TM[b)]1	31.1	0.327	1.0	0.790	2.5
PreciControl TM2	97.9	0.864	0.9	3.98	2.1

b) TM = Tumor Marker

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cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	15.1	0.121	0.8	0.326	2.2
Human serum 2	3.21	0.099	3.1	0.208	6.5
Human serum 3	2480	16.2	0.7	44.1	1.8
Human serum 4	4790	98.4	2.1	169	3.5
Human serum 5	35.5	0.301	0.8	0.710	2.0
PreciControl TM1	30.0	0.201	0.7	1.02	3.4
PreciControl TM2	95.8	0.762	0.8	2.70	2.8

Method comparison

A comparison of the Elecsys CA 125 II assay (y) with Fujirebio Diagnostics CA 125 II RIA (x) using clinical samples gave the following correlations.

Number of samples measured: 139

Passing/Bablok[17]	Linear regression
y = 0.93x + 5.57	y = 0.96x + 5.82
τ = 0.81	r = 0.981

The sample concentrations were between 4 and 500 U/mL.

Analytical specificity

The Elecsys CA 125 II tumor marker assay is based on the monoclonal M 11 and OC 125 antibodies which are only available from Fujirebio Diagnostics, its licensees and its representatives. The performance characteristics of test procedures using these antibodies cannot be assumed for test methods using other antibodies.

References

1 O'Brien TJ, Beard JB, Underwood LJ, et al.The CA 125 gene: an extracellular superstructure dominated by repeat sequences. Tumor Biol 2001;22(6):348-366.

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16 Moore, RG, Miller MC, Skates SJ, et al. Evaluation of the Diagnostic Accuracy of the Risk of OvarianMalignancy Algorithm in Women With a Pelvic Mass. Obstet Gynecol. 2011;118 (2, Part 1):280-288.

17 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.

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CA 125 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 after reconstitution or mixing
GTIN	Global Trade Item Number

CE 0123

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