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CA 125 II

cobas®

Cancer Antigen 125

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
11776223 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

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 belongs to the family of hybridoma-defined tumor markers. The values measured are defined by the use of the monoclonal antibody (MAb) OC 125.

The antigenic determinant CA 125 is located on a high-molecular weight glycoprotein (200-1000 kD) isolated from cell culture or serum. The antigenic determinant CA 125 has a protein structure with associated carbohydrate side-chains.[1]

MAb OC 125 was obtained from lymphocytes from mice that had been immunized with OVCA (ovarian carcinoma cell line) 433, an adenocarcinoma cell line from the ovary.[2] 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 is found in a high percentage of non-mucinous ovarian tumors of epithelial origin[3] and can be detected in serum.[4,5] It does not occur on the surface epithelium of normal ovaries (adult and fetal). Ovarian carcinoma accounts for about 20 % of gynecological tumors; the incidence is 15/100000.[6]

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.[7]

Elevated values are sometimes found in various benign gynecological diseases such as ovarian cysts, ovarian metaplasia, endometriosis, uterus myomatosus or cervicitis. Slight elevations of this marker may also occur in early pregnancy and in various benign diseases (e.g. acute and chronic pancreatitis, benign gastrointestinal diseases, renal insufficiency, autoimmune diseases and others). Markedly elevated levels have been found in benign liver diseases such as cirrhosis and hepatitis. Extreme elevations can occur in any kind of ascites due to malignant and benign diseases. Although the highest CA 125 values occur in patients suffering from ovarian carcinoma, clearly elevated values are also observed in malignancies of the endometrium, breast, gastrointestinal tract, and various other malignancies.

Although CA 125 is a relatively unspecific marker,[8,9,10,11,12] it is today the most important tumor marker for monitoring the therapy and progress of patients with serous ovarian carcinoma. At primary diagnosis the sensitivity of CA 125 depends on the FIGO stage (FIGO = Federation of Gynecology and Obstetrics); higher tumor stages are associated with higher CA 125 levels.[13]

The diagnostic sensitivity and specificity of the Elecsys CA 125 II test was calculated by comparing ovarian carcinoma patients at primary diagnosis (FIGO stage I to IV) with patients suffering from benign gynecological diseases. At a cutoff value of 65 U/mL, the sensitivity is 79 % (at a low specificity of 82 %). The cutoff level has to be raised if higher specificity is desired. The optimal clinical value is reached at 150 U/mL (sensitivity 69 %, specificity 93 %). If the specificity is 95 %, in accordance with the recommendations of van Dalen, et al.,[14] a sensitivity of 63 % is obtained (cutoff 190 U/mL).

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.

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.
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).

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Reagent handling

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

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

Storage and stability

Store at 2-8 °C.

Do not freeze.

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

Stability:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on 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 (LoB) + coefficient of correlation ≥ 0.95.

Stable for 5 days at 2-8 °C, 3 months at -20 °C.[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 07030207190, CA 125 II 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
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

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

- REF 05950929190, 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

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- 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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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

Accessories 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). 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

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.

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).

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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 (< 143 nmol/L or < 35 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 (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 0.6 U/mL

Limit of Detection = 1.2 U/mL

Limit of Quantitation = 2.0 U/mL with a total allowable error of ≤ 20 %

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 LoB and LoD respectively. The samples were tested in 6 runs over 3 days on 2 analyzers resulting in n = 60 values. For LoQ 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 %.

LoB, LoD and LoQ were calculated to be the following:

	Elecsys 2010 and **cobas e** 411 analyzers	MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers
LoB (U/mL)	0.600	0.449
LoD (U/mL)	0.697	0.548
LoQ (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 the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** 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 MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** 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 duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		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

MODULAR ANALYTICS E170, **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.

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Number of samples measured: 139

Passing/Bablok[16]	Linear regression
y = 0.93x + 5.57	y = 0.96x + 5.82
τ = 0.81	r = 0.981

The sample concentrations were between approximately 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

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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.

FUJIREBIO Diagnostics, Inc.

CA 125 is a registered trademark of Fujirebio Diagnostics, Inc.

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
GTIN	Global Trade Item Number

CE

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