Elecsys CA 125 II

**System information**

For cobas e 411 analyzer: test number 341
For MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers: Application Code Number 053

**Intended use**

Immunossay 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 electrochemiluminescence immunossay “ECLIA” is intended for use on Elecsys and cobas e immunossay 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

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.6 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 gynaecological 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 complex16 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 backpack 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/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.
R2 Anti-CA 125-Ab-Ru(bpy)3²⁺ (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).

**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:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Expiration Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unopened at 2-8 °C</td>
<td>Up to the stated expiration date</td>
</tr>
<tr>
<td>After opening at 2-8 °C</td>
<td>12 weeks</td>
</tr>
<tr>
<td>On the analyzers</td>
<td>6 weeks</td>
</tr>
</tbody>
</table>

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.

- Lip-heparin, K₂-EDTA and K₃-EDTA plasma as well as plasma tubes containing separating gel.

**Criteria:** 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). 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)

- **07030207190**, CA125 II CalSet II, for 4 x 1 mL
- **11776452122**, PreciControl Tumor Marker, for 4 x 3 mL
- **11732277122**, Diluent Universal, 2 x 16 mL sample diluent or 
  **03183971122**, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- MODULAR ANALYTICS E170 or cobas e analyzer

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

- **05950929190**, Elecsys HE4, 100 tests
- **05950945190**, HE4 CalSet, for 4 x 1 mL
- **05950953190**, PreciControl HE4, for 2 x 1 mL each of PreciControl HE4 1 and 2
- **03609878190**, Diluent MultiAssay, 2 x 16 mL sample diluent

**Accessories for cobas e 411 analyzer:**

- **11662988122**, ProCell, 6 x 380 mL system buffer
- **11662970122**, CleanCell, 6 x 380 mL measuring cell cleaning solution
- **11930346122**, Elecsys SysWash, 1 x 500 mL washer additive
- **11933159001**, Adapter for SysClean
- **11706802001**, AssayCup, 60 x 60 reaction cups
- **11706799001**, AssayTip, 30 x 120 pipette tips
- **11800507001**, Clean-Liner

**Accessories for MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers:**

- **04880340190**, ProCell M, 2 x 2 L system buffer
- **04880293190**, CleanCell M, 2 x 2 L measuring cell cleaning solution

- **03023141001**, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- **03005712190**, ProbeWash M, 12 x 70 mL cleaning solution for run initialization and rinsing during reagent change
- **12102137001**, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- **03023150001**, WasteLiner, waste bags
- **030227651001**, SysClean Adapter M

Accessories for all analyzers:

- **11298500316**, IS/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. In exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e 602** analyzer).

Bring the cooled reagents to approximately 20 °C and place on the reagent deck (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

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 bilirubin (< 287 µmol/L or < 70 mg/dL).

**Citation:** Recovery within ± 10 % of initial value.

Samples should not be taken from patients receiving therapy with high bilirubin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.
Elecsys CA 125 II

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 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 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 %.

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

<table>
<thead>
<tr>
<th>cobas e 411 analyzer</th>
<th>MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limit of Blank (U/mL)</td>
<td>0.600 0.449</td>
</tr>
<tr>
<td>Limit of Detection (U/mL)</td>
<td>0.697 0.548</td>
</tr>
<tr>
<td>Limit of Quantitation (U/mL)</td>
<td>1.05 1.29</td>
</tr>
</tbody>
</table>

**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 (EPS-5-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:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean U/mL</td>
<td>SD U/mL</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>14.7</td>
<td>0.423</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>3.08</td>
<td>0.090</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>2400</td>
<td>60.1</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>4950</td>
<td>93.2</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>35.2</td>
<td>0.686</td>
</tr>
<tr>
<td>PreciControl TM1a</td>
<td>31.1</td>
<td>0.327</td>
</tr>
<tr>
<td>PreciControl TM2</td>
<td>97.9</td>
<td>0.864</td>
</tr>
</tbody>
</table>

a) TM = Tumor Marker

**MODULAR ANALYTICS E170, cobas e 601 and cobas e 602 analyzers**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean U/mL</td>
<td>SD U/mL</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>15.1</td>
<td>0.121</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>3.21</td>
<td>0.099</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>2480</td>
<td>16.2</td>
</tr>
<tr>
<td>Human serum 4</td>
<td>4790</td>
<td>98.4</td>
</tr>
<tr>
<td>Human serum 5</td>
<td>35.5</td>
<td>0.301</td>
</tr>
<tr>
<td>PreciControl TM1</td>
<td>30.0</td>
<td>0.201</td>
</tr>
<tr>
<td>PreciControl TM2</td>
<td>95.8</td>
<td>0.762</td>
</tr>
</tbody>
</table>

**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**
  - Linear regression
  - $y = 0.93x + 5.57$
  - $y = 0.96x + 5.82$
  - $r = 0.81$
  - $r = 0.981$

The sample concentrations were between approximately 4 and 500 U/mL.
Elecsys CA 125 II

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

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.