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HE4

cobas®

Human epididymal protein 4

REF	Σ	SYSTEM
05950929 190	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Please note

The measured HE4 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 HE4 assay method used. HE4 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 HE4 assay procedure used while monitoring therapy, then the HE4 values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Intended use

Immunoassay for the quantitative determination of HE4 in human serum and plasma. The assay is used as an aid in monitoring recurrence or progressive disease in patients with epithelial ovarian cancer. Serial testing for patient HE4 values should be used in conjunction with other clinical findings used for monitoring ovarian cancer.

It is further intended to be used in conjunction with the Elecsys CA 125 II assay as an aid in estimating the risk of epithelial ovarian cancer in premenopausal and postmenopausal women presenting with pelvic mass. 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 Elecsys and **cobas e** immunoassay analyzers.

Summary

The human epididymal protein 4 (HE4, also known as WFDC2) belongs to the family of whey acidic four-disulfide core (WFDC) proteins with suspected trypsin inhibitor properties.[1,2] The corresponding gene codes for a 13 kD protein. In its mature glycosylated form the protein has a molecular weight of approximately 20-25 kD and consists of a single peptide chain containing two WFDC domains.[3]

HE4 was first determined in the epithelium of the distal epididymis.[4] It shows low expression in epithelia of respiratory and reproductive tissues including ovary, but high expression in ovarian cancer tissue.[5] High secreted levels can also be found in the serum of ovarian cancer patients.[6] HE4 is also expected to help in the risk assessment of epithelial ovarian cancer.

Ovarian cancer is the fourth most common cause of cancer-related death in women worldwide. It is the most lethal form of gynecological cancer, and potentially curable if diagnosed early[7,8] and treated by surgeons familiar with the management of ovarian cancer.[9,10] However, the symptoms of ovarian cancer are related to the presence of adnexal masses and are often vague and unspecific. Thus, 70-75 % of ovarian cancers are detected at a late stage. According to the International Agency for Research on Cancer, the 5 year survival rate of ovarian cancer patients is 46 %. However, if the disease is diagnosed early, the survival rate increases up to 94 %.

As a single tumor marker, HE4 had the highest sensitivity for detecting ovarian cancer, especially in stage I disease, the early non-symptomatic stage. Combined, CA 125 and HE4 yielded the highest sensitivity with 76.4 % at 95 % specificity. Additionally, HE4 is more sensitive in early-stage endometrial cancer compared to CA 125. Elevated serum HE4 with normal CA 125 would suggest the presence of either ovarian or other type of cancer, for example endometrial cancer.[11,12]

Combined with other markers such as CA 125, HE4 can help determining whether a pelvic mass is benign or malignant in pre- and post-menopausal women. The dual marker combination CA 125 and HE4 is a more accurate predictor of malignancy than either alone.[11] Huhtinen et al. reported a 78.6 % sensitivity at 95 % specificity in ovarian carcinoma vs. endometriotic cysts.[13] HE4 levels correlate with clinical response to therapy or recurrence status in women with diagnosis of ovarian carcinoma as determined by CT imaging. HE4 could thus be an important early indicator for disease recurrence.[14]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 10 µL of sample, a biotinylated monoclonal HE4-specific antibody, and a monoclonal HE4-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 HE4.

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

R1 Anti-HE4-Ab~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-HE4 antibody (mouse) 0.75 mg/L; phosphate buffer 100 mmol/L, pH 6.5; preservative.

R2 Anti-HE4-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL:
Monoclonal anti-HE4 antibody (mouse) labeled with ruthenium complex 1.5 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:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	12 weeks
on the analyzers	28 days

HE4

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Human epididymal protein 4

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- and K_3-EDTA plasma as well as Li-heparin plasma tubes containing separating gel.

Criterion: Slope 0.9-1.1 + intercept ≤ ± 10 pmol/L, coefficient of correlation ≥ 0.95.

Stable for 48 hours at 2-8 °C, 5 hours at 15-25 °C, 12 weeks at -20 °C. The samples may be frozen twice.

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 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
- 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 11776223322, CA 125 II, 100 tests
- REF 11776240322, CA 125 II CalSet, 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

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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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, 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.

MODULAR ANALYTICS E170, **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 the HE4 EIA method from Fujirebio Diagnostics, Inc.

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 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 HE4.

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 in pmol/L.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 1130 µmol/L or < 66 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 205 nmol/L or < 50 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 1500 IU/mL.

There is no high-dose hook effect at HE4 concentrations up to 40000 pmol/L.

In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.

Special cancer drugs were tested with concentrations shown in the table below. No interference with the assay was found.

Criterion: Recovery within ± 10 % of initial value.

Drug	Concentration (µg/mL)
Carboplatin	600
Cisplatin	180
Cyclophosphamide	500
Dexamethasone	20
Doxorubicin	120
Leucovorin	750
Melphalan	15
Methotrexat-disodium	150
Paclitaxel	265
Fluorouracil	900
Bevacizumab (Avastin)	750
Erlotinib (Tarceva)	150
Rituximab (MabThera)	750
Trastuzumab (Herceptin)	600

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

15.0-1500 pmol/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 15.0 pmol/L. Values above the measuring range are reported as > 1500 pmol/L (or up to 30000 pmol/L for 20-fold diluted samples).

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Limit of Blank = 5.00 pmol/L

Limit of Detection = 15.0 pmol/L

Limit of Quantitation = 20.0 pmol/L with a total allowable error of 30 %

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A 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 error of ≤ 30 %.

A study was performed using 5 equine serum samples and 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. LoB and LoD were calculated to be 0.358 pmol/L and 0.661 pmol/L respectively. For LoQ 3 human serum samples were diluted and measured in 6 runs over 3 days on 2 analyzers. At a total allowable error of ≤ 30 % the LoQ was 4.42 pmol/L.

Linearity

The Elecsys HE4 assay is linear across the measuring range from 15.0-1500 pmol/L. Samples were prepared according to CLSI EP6-A, by diluting 3 serum and 3 plasma samples each with Diluent MultiAssay in multiple steps ranging from > 1500 pmol/L down to LoB.

Dilution

Samples with HE4 concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:20 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 or **cobas e** analyzers or manually). The concentration of the diluted sample must be > 75 pmol/L.

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.

Expected values

A study in one clinical center in Germany with the Elecsys HE4 assay on sera from 358 apparently healthy women yielded the following results:

		HE4 (pmol/L)	
Age (years)	N	Median	95th percentile
< 40	127	42.0	60.5
40-49	65	44.3	76.2
50-59	60	47.9	74.3
60-69	60	55.0	82.9
≥ 70	46	62.1	104

The distribution in percentage (%) of HE4 assay values determined in two clinical centers in Spain and Germany with the Elecsys HE4 assay in 896 female specimens is summarized in the table below:

		Elecsys HE4 values (pmol/L)				
		0.0-70.0	70.1-140	140.1-500	500.1-1500	> 1500
	N (percentage distribution)					
Apparently healthy						
Premenopausal	90	76 (84.4 %)	13 (14.4 %)	1 (1.1 %)	0 (0.0 %)	0 (0.0 %)
Postmenopausal	106	63 (59.4 %)	40 (37.7 %)	3 (2.8 %)	0 (0.0 %)	0 (0.0 %)
Benign conditions						
Premenopausal	177	160 (90.4 %)	16 (9.0 %)	1 (0.6 %)	0 (0.0 %)	0 (0.0 %)
Postmenopausal	102	62 (60.8 %)	31 (30.4 %)	9 (8.8 %)	0 (0.0 %)	0 (0.0 %)
Pregnancy	50	50 (100 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)
Non-gynecological disease	35	16 (45.7 %)	6 (17.1 %)	6 (17.1 %)	7 (20.0 %)	0 (0.0 %)
CHF[b)]	23	9 (39.1 %)	11 (47.8 %)	3 (13.0 %)	0 (0.0 %)	0 (0.0 %)
Cancer						
Ovarian cancer Premenopausal	39	12 (30.8 %)	7 (17.9 %)	13 (33.3 %)	5 (12.8 %)	2 (5.1 %)
Ovarian cancer Postmenopausal	97	10 (10.3 %)	19 (19.6 %)	34 (35.1 %)	28 (28.9 %)	6 (6.2 %)
Endometrial cancer	49	18 (36.7 %)	20 (40.8 %)	9 (18.4 %)	1 (2.0 %)	1 (2.0 %)
Breast cancer	47	22 (46.8 %)	19 (40.4 %)	5 (10.6 %)	1 (2.1 %)	0 (0.0 %)
Gastrointestinal cancer	46	19 (41.3 %)	20 (43.5 %)	6 (13.0 %)	1 (2.2 %)	0 (0.0 %)
Lung cancer	23	5 (21.7 %)	7 (30.4 %)	10 (43.5 %)	1 (4.3 %)	0 (0.0 %)
Bladder cancer	12	3 (25.0 %)	4 (33.3 %)	4 (33.3 %)	1 (8.3 %)	0 (0.0 %)

b) CHF = congestive heart failure

In this study 84 % of the apparently healthy premenopausal women had an Elecsys HE4 assay value at or below 70 pmol/L and 97 % of the apparently healthy postmenopausal women had an Elecsys HE4 assay value at or below 140 pmol/L.

In this study the 95th percentiles for the apparently healthy pre- and postmenopausal women (all ages) were 92.1 pmol/L and 121 pmol/L, respectively.

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

Monitoring of disease status in patients diagnosed with ovarian cancer

The effectiveness of the Elecsys HE4 assay as an aid in monitoring of disease status in ovarian cancer patients was determined by assessing changes in HE4 levels in serial serum samples from 100 patients compared to changes in disease status. A study involving a total of 375 pairs of observations was undertaken with ≥ 3 blood withdrawals per patient. A positive change HE4 was defined as an increase in the value that was at least 20 % greater than the previous value of the test. 58.0 % (29/50) of the patient samples with a positive change correlated with the disease progression while 84.0 % (273/325) of the patient serial samples with no significant change in HE4 value correlated with no progression. The total concordance was 80.5 % (302/375). The following table presents the data in a 2 x 2 format.

Change in disease state per sequential pair			
Increase in HE4 concentration	Progression	No progression	Total
> 20 %	29	52	81
≤ 20 %	21	273	294
Total	50	325	375

Risk estimation in patients with pelvic mass

The effectiveness of the Elecsys HE4 assay in combination with the Elecsys CA 125 II assay for risk estimation of epithelial ovarian cancer of patients presenting with pelvic mass was determined in an international multi-center clinical trial using repository samples. An algorithm (ROMA = Risk of Ovarian Malignancy Algorithm) was developed for estimation of the risk of epithelial ovarian cancer. The algorithm takes into account the HE4 and CA 125 values as well as the menopausal status of the patient. The algorithm calculates a predictive probability of finding epithelial ovarian cancer on surgery.

Calculation of Predictive Index (PI)[15]

A Predictive Index is calculated for premenopausal and postmenopausal patients separately using equations (1) and (2) below. To calculate the PI, the assay values obtained from the Elecsys HE4 assay and the Elecsys CA 125 II assay are inserted into the equations below, depending on the menopausal status of the women.

(1) Premenopausal:
PI = -12.0 + 2.38*LN[HE4] + 0.0626*LN[CA125]

(2) Postmenopausal:
PI = -8.09 + 1.04*LN[HE4] + 0.732*LN[CA125]

where, LN = Natural Logarithm. Do not use LOG = Log_{10}.

Calculation of ROMA value[15]

To calculate the ROMA value (i.e. predictive probability), insert the calculated value for PI into equation (3):

(3) ROMA value (%) = exp(PI) / [1 + exp(PI)] * 100 where, exp(PI) = e^{PI}

NOTE: These equations were used for the calculation of ROMA values with the Elecsys HE4 assay from 28.8-3847 pmol/L and with the Elecsys CA 125 II assay from 6.42-5000 U/mL.

The examples below should be used in order to validate calculations of PI and ROMA before reporting patient results:

Menopausal status	Elecsys values		PI calculation	PI	ROMA %
	HE4 (pmol/L)	CA 125 II (U/mL)			
Premenopausal	37.5	74.9	-12.0 + (2.38*3.624) + (0.0626*4.316)	-3.10388	4.29
	387	21.8	-12.0 + (2.38*5.957) + (0.0626*3.082)	2.371517	91.5
Postmenopausal	66.7	11.3	-8.09 + (1.04*4.200) + (0.732*2.425)	-1.94683	12.5
	383	22.7	-8.09 + (1.04*5.948) + (0.732*3.122)	0.381799	59.4

Stratification into low risk and high risk groups

In a study a total of 384 repository patient samples were included and the predictive probability for ovarian cancer as well as the ability for separation into a low and a high risk group based on ROMA values were determined.

The risk of ovarian malignancy algorithm was used to stratify women into risk groups for finding epithelial ovarian cancer. The following cut-points were used in order to provide a specificity level of 75 % for the Elecsys HE4 and Elecsys CA 125 II assay combination:

Premenopausal women

ROMA value ≥ 11.4 % = high risk of finding epithelial ovarian cancer

ROMA value < 11.4 % = low risk of finding epithelial ovarian cancer

Postmenopausal women

ROMA value ≥ 29.9 % = high risk of finding epithelial ovarian cancer

ROMA value < 29.9 % = low risk of finding epithelial ovarian cancer

The risk stratification of all 384 patients (194 pre- and 190 postmenopausal) presenting with pelvic mass using the ROMA values for the Elecsys HE4 and Elecsys CA 125 II assay combination is shown in the following table:

Patient groups presenting with pelvic mass	Premenopausal patients			Postmenopausal patients		
	N	ROMA < 11.4 %	ROMA ≥ 11.4 %	N	ROMA < 29.9 %	ROMA ≥ 29.9 %
Stage I-II EOC[c)]	16	6 (37.5 %)	10 (62.5 %)	16	6 (37.5 %)	10 (62.5 %)
Stage I-IIIC EOC[d)]	21	7 (33.3 %)	14 (66.7 %)	34	9 (26.5 %)	25 (73.5 %)
Stage I-IV EOC	25	7 (28.0 %)	18 (72.0 %)	53	10 (18.9 %)	43 (81.1 %)
Stage III-IV EOC	9	1 (11.1 %)	8 (88.9 %)	37	4 (10.8 %)	33 (89.2 %)
Unstaged EOC	12	2 (16.7 %)	10 (83.3 %)	44	2 (4.5 %)	42 (95.5 %)
Benign	157	118 (75.2 %)	39 (24.8 %)	93	71 (76.3 %)	22 (23.7 %)

c) EOC = epithelial ovarian cancer

d) Stage I-IIIB and Stage I-IIIC (omentum negative, lymph node positive) EOC

The sensitivity for stratifying patients with stage I-IV epithelial ovarian cancer into the high risk group was 84.3 % at the set specificity of 75 %, such that 75.6 % of women with benign pelvic mass were classified into the low risk group. The positive and negative predictive values were 64.9 % and 90 % respectively.

AUC (95 % CI):

Premenopausal women = 0.858 (0.779-0.937)

Postmenopausal women = 0.923 (0.885-0.962)

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The following has to be taken into consideration

- The level of HE4 cannot be used as absolute evidence for the presence or absence of malignant disease and the Elecsys HE4 assay should not be used as a cancer screening test.
- Elecsys HE4 results should be used in conjunction with other clinical data; e.g. symptoms, medical history, etc.
- If the Elecsys HE4 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
- Elecsys HE4 results should not be used interchangeably with other manufacturers' methods for HE4 determinations.
- Elecsys CA 125 II results should not be used interchangeably with other manufacturers' methods for CA 125 determinations in the ROMA calculation.
- Patients with confirmed ovarian cancer may have Elecsys HE4 assay values in the same range as healthy women. Certain histological types of ovarian cancer (e.g. mucinous or germ cell tumors) rarely express HE4, therefore the use of the Elecsys HE4 assay is not recommended for monitoring of patients with known mucinous or germ cell ovarian cancer.[5] Conversely, elevated levels of HE4 antigen may be present in individuals with renal, liver and non-malignant diseases.
- The ROMA has not been validated for the following patient groups: patients previously treated for malignancy, patients currently being treated with chemotherapy, and patients less than 18 years of age. Failure of the Elecsys HE4 assay and/or the Elecsys CA 125 II assay to perform as indicated, or error in the calculation of results, could lead to inaccurate risk assessment and improper management of the patient. Specifically, a falsely low result of the assay(s) could result in a determination that the patient is at lower risk of having epithelial ovarian cancer, which could triage the patient to a less specialized level of care.

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 pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	25.3	0.450	1.8	0.945	3.7
Human serum 2	53.7	0.988	1.8	2.28	4.2
Human serum 3	142	2.33	1.6	6.11	4.3
Human serum 4	779	11.3	1.5	32.6	4.2
Human serum 5	1437	18.9	1.3	39.4	2.7
PreciControl HE4 1	45.7	0.661	1.4	1.92	4.2
PreciControl HE4 2	345	5.67	1.6	11.8	3.4

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
Human serum 1	27.4	0.481	1.8	0.798	2.9
Human serum 2	57.7	1.06	1.8	1.59	2.8
Human serum 3	155	2.28	1.5	3.95	2.6
Human serum 4	852	13.5	1.6	25.2	3.0
Human serum 5	1390	26.9	1.9	44.9	3.2

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pmol/L	SD pmol/L	CV %	SD pmol/L	CV %
PreciControl HE4 1	45.2	0.670	1.5	1.52	3.4
PreciControl HE4 2	345	6.06	1.8	11.2	3.2

Method comparison

A comparison of the Elecsys HE4 assay (y) with a manual HE4 method (x) using clinical samples gave the following correlations:

Number of samples measured: 1502

Passing/Bablok[16]	Linear regression
y = 1.018x + 5.52	y = 1.10x + 1.64
τ = 0.808	r = 0.978

The sample concentrations were between approximately 22 and approximately 1150 pmol/L.

A comparison of the Elecsys HE4 assay (y) with an automated HE4 method (x) using clinical samples gave the following correlations:

Number of samples measured: 703

Passing/Bablok[16]	Linear regression
y = 0.900x + 6.41	y = 0.875x + 11.0
τ = 0.836	r = 0.985

The sample concentrations were between approximately 20 and approximately 1400 pmol/L.

Analytical specificity

The following cross-reactivities were found, tested with HE4 concentrations of 65 and 155 pmol/L:

Proteins (WFDC family)	Concentration tested pmol/L	Cross-reactivity %
Elafin[e)]/SKALP[f)]	54500	0.025
SLPI[g)]	20833	0.088

e) Elafin = elastase-specific inhibitor

f) SKALP = skin-derived antileukoproteinase

g) SLPI = secretory leucocyte protease inhibitor

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