Elecsys HCG + β

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REF		Σ	SYSTEM
03271749160	03271749501	100	**cobas e** 411 **cobas e** 601 **cobas e** 602

English

For use in the USA only

System Information

For **cobas e** 411 analyzer: test number 761
cobas e 601 and **cobas e** 602 analyzers: Application Code Number 148

Intended use

Immunoassay for the in vitro quantitative determination of the sum of human chorionic gonadotropin (hCG) plus the hCG β-subunit in human serum and plasma.

This assay is intended for the early detection of pregnancy.

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

Summary

References[1,2,3,4,5,6]

Similarly to LH, FSH and TSH, human chorionic gonadotropin (hCG) is a member of the glycoprotein family and consists of 2 subunits (α- and β-chains) which are associated to the intact hormone. The α-chains in all 4 of these glycoprotein hormones are virtually identical, whereas the β-chains have greatly differing structures and are responsible for the respective specific hormonal functions.

HCG is produced in the placenta during pregnancy. In non-pregnant women, it can also be produced by tumors of the trophoblast, germ cell tumors with trophoblastic components and some non-trophoblastic tumors. Human chorionic gonadotropin consists of a number of isohormones with differing molecular size. The biological action of hCG serves to maintain the corpus luteum during pregnancy. It also influences steroid production. The serum of pregnant women contains mainly intact hCG. Measurement of the hCG concentration permits the diagnosis of pregnancy just 1 week after conception. The determination of hCG in the 1st trimester of pregnancy is of particular importance. Elevated values here serve as an indication of chorionic carcinoma, hydatiform mole or multiple pregnancy. Depressed values indicate threatening or missed abortion, ectopic pregnancy, gestosis or intra-uterine death.

Elevated hCG concentrations not associated with pregnancy are found in patients with other diseases such as tumors of the germ cells, ovaries, bladder, pancreas, stomach, lungs, and liver.[5,6]

The combination of the specific monoclonal antibodies used in the Elecsys HCG + β assay recognize the holo-hormone, "nicked" forms of hCG, the β-core fragment and the free β-subunit. The ruthenium-labeled and biotinylated antibodies used are directed against different epitopes of the hCG molecule.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

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

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

R1 Anti-hCG-Ab~biotin (gray cap), 1 bottle, 9 mL: Biotinylated monoclonal anti-hCG antibodies (mouse) 2.6 mg/L; phosphate buffer 40 mmol/L, pH 7.5; preservative.

R2 Anti-hCG-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 10 mL: Monoclonal anti-hCG antibody (mouse) labeled with ruthenium complex 4.6 mg/L; phosphate buffer 40 mmol/L, pH 6.5; 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 mist or vapours.
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: 1-800-428-2336

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.

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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	4 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-, Na-, NH_4^+-heparin, Na_2-EDTA, K_3-EDTA, sodium citrate, and sodium fluoride/potassium oxalate plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + coefficient of correlation > 0.95 (Pearson).

Stable for 3 days at 2-8 °C, 12 months at -20 °C (± 5 °C). Freeze once only.[7]

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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- REF 03302652190, HCG+β CalSet, for 4 x 1.0 mL
- REF 11731416160, PreciControl Universal, for 4 x 3.0 mL
- REF 11776452160, PreciControl Tumor Marker, for 4 x 3.0 mL
- REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **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, 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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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 material for all analyzers:

- REF 11298500160, 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.

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 4th International Standard for Chorionic Gonadotropin from the National Institute for Biological Standards and Control (NIBSC) code 75/589.

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 1 month (28 days) 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 Universal or 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 in (mIU/mL or IU/L).

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Limitations - interference

The assay is unaffected by icterus (bilirubin < 24 mg/dL or < 410 µmol/L), hemolysis (Hb < 1.0 g/dL or < 0.621 mmol/L), lipemia (Intralipid < 1400 mg/dL), and biotin (< 327 nmol/L or < 80 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 3400 IU/mL and samples from dialysis patients.

There is no high-dose hook effect at hCG concentrations up to 750000 mIU/mL.

In vitro tests were performed on 15 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

0.100-10000 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.100 mIU/mL. Values above the measuring range are reported as > 10000 mIU/mL (or up to 1000000 mIU/mL for 100-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 0.100 mIU/mL

The Lower Detection Limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 2 standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with hCG concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:100 (either automatically by the analyzers or manually). The concentration of the diluted sample must be > 100 mIU/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.

Expected values

Results from a multicenter study in clinical centers in Belgium, France, and Germany using the Elecsys HCG+β assay (REF 03271749) are listed below (Study No. BO1P019, status March 2003).

Serum samples from healthy individuals:

- ≤ 1 mIU/mL hCG for 97.5 % of the values obtained from 181 healthy, non-pregnant premenopausal women. The corresponding upper 95 % confidence limit ranges up to 5.3 mIU/mL.
- ≤ 7 mIU/mL hCG for 97.5 % of the values obtained from 143 healthy, postmenopausal women. The corresponding upper 95 % confidence limit ranges up to 8.3 mIU/mL.
- < 2 mIU/mL hCG for 97.5 % of the values obtained from 290 men. The corresponding upper 95 % confidence limit ranges up to 2.6 mIU/mL.
- During pregnancy (weeks of pregnancy - defined as completed weeks of pregnancy beginning with the start of the last menstruation phase), the following values have been determined.

Data are given only for the weeks of gestation for which the case numbers (n) were greater than 10.

Weeks of gestation	N	HCG mIU/mL	
		Median	5-95 th percentile
3	25	17.5	5.8-71.2
4	43	141	9.5-750
5	23	1398	217-7138
6	19	3339	158-31795
7	13	39759	3697-163563
8	23	90084	32065-149571
9	23	106257	63803-151410
10	20	85172	46509-186977
12	17	66676	27832-210612
14	67	34440	13950-62530
15	666	28962	12039-70971
16	766	23930	9040-56451
17	190	20860	8175-55868
18	64	19817	8099-58176

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 modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60). 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	4.36	0.21	4.9	0.26	5.9
Human serum 2	822	13.0	1.6	15.6	1.9
Human serum 3	7040	133	1.9	189	2.7
PreciControl U[b)]1	8.17	0.16	1.9	0.24	2.9
PreciControl U2	21.5	0.71	3.3	0.78	3.6
PreciControl TM[c)]1	23.1	0.52	2.3	0.68	2.9
PreciControl TM2	2150	28.9	1.3	44.1	2.1

b) U = Universal

c) TM = Tumor Marker

cobas e 601 and **cobas e** 602 analyzers						
	Repeatability			Intermediate precision		
Sample	Mean mIU/mL	SD mIU/mL	CV %	Mean mIU/mL	SD mIU/mL	CV %
Human serum 1	8.52	0.24	2.8	4.73	0.35	7.4
Human serum 2	796	13.6	1.7	899	29.4	3.3
Human serum 3	7012	188	2.7	8082	344	4.3
PreciControl U1	7.20	0.18	2.5	8.49	0.29	3.4
PreciControl U2	19.6	0.55	2.8	22.5	1.05	4.6
PreciControl TM1	21.4	0.39	1.8	24.2	1.11	4.6
PreciControl TM2	2012	47.0	2.3	2316	84.2	3.6

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Method comparison

A comparison of the Elecsys HCG+β assay (y) with the Elecsys HCG STAT assay (x) using human sera gave the following correlations:

Number of samples measured: 81

Passing/Bablok[8]	Linear regression
y = 1.00x + 7.40	y = 0.95x + 53.4
τ = 0.986	r = 0.999

The sample concentrations were between 3 and 8550 mIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactivities were found:
TSH: not detectable, LH 0.12 %, FSH < 0.1 %.

Functional sensitivity

< 0.600 mIU/mL

The functional sensitivity is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of 20 %.

References

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2 Hoermann R, Berger P, Spoettl G, et al. Immunological Recognition and Clinical Significance of Nicked Human Chorionic Gonadotropin in Testicular Cancer. Clin Chem 1994;40(12):2306-2312.

3 Schwarz S, Berger P, Wick G. The Antigenic Surface of Human Chorionic Gonadotropin as Mapped by Murine Monoclonal Antibodies. Endocrinology 1986;118(1):189-197.

4 Runnebaum B, Rabe T. Gynäkologische Endokrinologie, Grundlagen, Physiologie, Pathologie, Prophylaxe, Diagnostik, Therapie. Berlin, Heidelberg, New York, London, Paris, Tokyo: Springer Verlag, 1987;8:43,489-541.

5 Sturgeon CM, McAllister EJ. Analysis of hCG: clinical applications and assay requirements. Ann Clin Biochem 1998;35:460-491.

6 Marcillac I, Troalen F, Bidart JM, et al. Free Human Chorionic Gonadotropin β Subunit in Gonadal and Nongonadal Neoplasms. Cancer Res 1992;52:3901-3907.

7 Guder WG, Narayanan S, Wisser H, et al. List of Analytes; Preanalytical Variables. Brochure in: Samples: From the Patient to the Laboratory. GIT-Verlag, Darmstadt 1996:16. ISBN 3-928865-22-6.

8 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 user guide or operator's manual for the analyzer concerned, the respective application sheets and the Method Sheets of all necessary components (if available in your country).

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.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.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
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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