08890595500V5.0

Elecsys HCG STAT

cobas®

REF		Σ	SYSTEM
08890595190	08890595500	100	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
HCGST	10203

Intended use

Immunoassay for the in vitro quantitative determination of human chorionic gonadotropin in human serum and plasma. This assay is intended for use especially in the diagnosis and monitoring of pregnancy.

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

Summary

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 four of these glycoprotein hormones are virtually identical, whereas the β-chains have greatly differing structures and are responsible for the respective specific hormonal functions.

Human chorionic gonadotropin consists of a number of isohormones[1] 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 one week after conception. The determination of hCG in the 1st trimester of pregnancy is of particular importance.[2] Elevated values here serve as an indication of hydatidiform mole or multiple pregnancy.[3,4,5] Depressed values indicate threatening or missed abortion, ectopic pregnancy[6,7] or intra-uterine death. Elevated values in the absence of a pregnancy are indicative of a tumor.[8]

The specific monoclonal antibodies used recognize the holo-hormone.[9] The Elecsys HCG STAT assay should therefore be used in particular in the diagnosis and monitoring of pregnancy. The ruthenium-labeled and biotinylated antibodies used are directed against different epitopes of the hCG molecule.

Test principle

Sandwich principle. Total duration of assay: 9 minutes.

- During a 9 minute incubation, antigen in the sample (6 µL), a biotinylated monoclonal hCG-specific antibody, a monoclonal hCG-specific antibody labeled with a ruthenium complex [a)] and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.
- 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 II 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 **cobas** link.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The **cobas e** pack is labeled as HCGST.

M Streptavidin-coated microparticles, 1 bottle, 6.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-hCG-Ab~biotin, 1 bottle, 9.9 mL:
Biotinylated monoclonal anti-hCG antibody (mouse) 2.3 mg/L; phosphate buffer 40 mmol/L, pH 7.5; preservative.

R2 Anti-hCG-Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 10.3 mL:
Monoclonal anti-hCG antibody (mouse) labeled with ruthenium complex 6.0 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 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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **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
on the analyzers	16 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.

Plasma tubes containing separating gel can be used.

Stable for 5 days at 20-25 °C, 14 days at 2-8 °C, 12 months 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 and calibrators are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators 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 09013296190, HCG STAT CalSet, for 4 x 1.0 mL
- REF 11731416190, PreciControl Universal, for 4 x 3.0 mL
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

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.

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 **cobas e** pack 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 12 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl Universal.

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 **cobas e** pack, 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 mIU/mL or IU/L).

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 496 µmol/L or ≤ 29 mg/dL
Hemoglobin	≤ 0.932 mmol/L or ≤ 1500 mg/dL
Intralipid	≤ 2400 mg/dL
Biotin	≤ 14326 nmol/L or ≤ 3500 ng/mL
Rheumatoid factors	≤ 667 IU/mL

Criterion: For concentrations of 1.0-10 mIU/mL the deviation is ≤ ± 1.0 mIU/mL. For concentrations > 10 mIU/mL the deviation is ≤ ± 10 %.

There is no high-dose hook effect at hCG concentrations up to 500000 mIU/mL.

Pharmaceutical substances
In vitro tests were performed on 16 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

1.0-10000 mIU/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 1.0 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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 0.5 mIU/mL

Limit of Detection = 1.0 mIU/mL

Limit of Quantitation = 1.0 mIU/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 the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

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 Belgium, Netherlands, and Germany with the Elecsys HCG STAT assay (REF 03300811) in specimen from healthy individuals are listed below (Study No. B01P023):

- ≤ 1 mIU/mL hCG for 97.5 % of the values obtained from 182 healthy, non-pregnant premenopausal women. The corresponding upper 95 % confidence limit ranges up to 4.9 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 8.1 mIU/mL.

The following hCG values were determined during pregnancy (completed weeks of pregnancy from first day of the last menstruation cycle):

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	5th-95th percentile
3	25	18.7	5.44-72.0
4	43	135	10.2-708
5	23	1420	217-8245
6	19	3475	152-32177
7	13	35873	4059-153767
8	23	83603	31366-149094
9	23	104475	59109-135901
10	20	85304	44186-170409
12	17	61730	27107-201615
14	20	37082	24302-93646
15	546	28696	12540-69747
16	766	24346	8904-55332
17	190	22064	8240-51793
18	64	22464	9649-55271

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 protocol (EP05-A3) 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 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	3.28	0.122	3.7	0.164	5.0
Human serum 2	4.22	0.0969	2.3	0.153	3.6
Human serum 3	3125	46.5	1.5	113	3.6
Human serum 4	5148	97.6	1.9	181	3.5
Human serum 5	8742	130	1.5	297	3.4
PC[b)] Universal1	4.78	0.139	2.9	0.213	4.5
PC Universal2	41.5	1.08	2.6	1.67	4.0

b) PC = PreciControl

Method comparison

A comparison of the Elecsys HCG STAT assay, REF 08890595190 (**cobas e** 801 analyzer; y) with the Elecsys HCG STAT assay, REF 07229569190 (**cobas e** 801 analyzer; x) gave the following correlations (mIU/mL):

Number of samples measured: 178

Passing/Bablok[10]	Linear regression
y = 1.00x + 1.86	y = 0.994x + 19.6
τ = 0.994	r = 1.000

The sample concentrations were between 2.32 and 9887 mIU/mL.

A comparison of the Elecsys HCG STAT assay, REF 08890595190 (**cobas e** 402 analyzer; y) with the Elecsys HCG STAT assay, REF 08890595190 (**cobas e** 801 analyzer; x) gave the following correlations (mIU/mL):

Number of samples measured: 132

Passing/Bablok[10]	Linear regression
y = 0.980x - 1.53	y = 0.981x - 7.49
τ = 0.985	r = 1.000

The sample concentrations were between 5.14 and 9911 mIU/mL.

Analytical specificity

For the monoclonal antibodies used, the following cross-reactions were found:

LH: 0.2 %, FSH: n. d.[c)], TSH: n. d.

c) n. d. = not detectable

References

1 Choi J, Schmitz J. Luteinizing hormone and human chorionic gonadotropin: Origins of difference. Mol Cell Endocrinology. 2014;383:203-213.

2 Seeber BE. What serial hCG can tell you, and cannot tell you, about an early pregnancy. Fertil Steril 2012;98(5):1074-1077.

3 Guth B, Hudelson J, Hifbie J, et al. Predictive Value of hCG 14 Days After Embryo Transfer. J of Assist Reprod Genet 1995;12(1):13-14.

4 Chung K, Sammel MD, Coutifaris C, et al. Defining the rise of serum HCG in viable pregnancies achieved through use of IVF. Hum Reprod 2006;21(3):823-828.

5 Cavailere A, Ermito S, Dinatale A, et al. Management of molar pregnancy. J Prenat Med 2009;3(1):15-17.

6 Dillon KE, Sioulas VD, Sammel MD, et al. How and when human chorionic gonadotropin curves in women with an ectopic pregnancy mimic other outcomes: differences by race and ethnicity. Fertil Steril 2012;98(4):911-916.

7 Ustunyurt E, Duran M, Coskun E, et al. Role of initial and day 4 human chorionic gonadotropin levels in predicting the outcome of single-dose methotrexate treatment in women with tubal ectopic pregnancy. Arch Gynecol Obstet 2013;288(5):1149-1152.

8 Iles RK, Delves PJ, Butler SA. Does hCG or hCGHβ play a role in cancer cell biology. Mol Cell Endocrinol 2010;329(1-2):62-70.

9 Whittington J, Fantz CR, Gronowski AM, et al. The analytical specificity of human chorionic gonadotropin assays determined using WHO International Reference Reagents. Clin Chim Acta 2010;411(1-2):81-85.

10 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.

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 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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