

HCG STAT

Human chorionic gonadotropin, STAT (Short Turn Around Time)

cobas®

REF	Σ	SYSTEM
03300811 190	100	Elecsys 2010 cobas e 411 cobas e 601 cobas e 602

English

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 electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

Summary

References^{1,2,3,4}

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 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. 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.

The specific monoclonal antibodies used recognize the holo-hormone. 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.

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

- 1st incubation: 10 μ L 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.

cobas e 601 and **cobas e 602** analyzers:

- During a 9 minute incubation, antigen in the sample (10 μ L), a biotinylated monoclonal hCG-specific antibody, a monoclonal hCG-specific antibody labeled with a ruthenium complex and streptavidin-coated microparticles react to form a sandwich complex, which is bound to the solid phase.

All analyzers:

- 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 ($\text{Ru}(\text{bpy})_3^{2+}$)

Reagents - working solutions

The reagent rackpack is labeled as HCGSTAT.

- 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 antibody (mouse) 2.3 mg/L; phosphate buffer 40 mmol/L, pH 7.5; preservative.
- R2 Anti-hCG-Ab~ $\text{Ru}(\text{bpy})_3^{2+}$ (black cap), 1 bottle, 10 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.

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 automatically 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, $\text{K}_3\text{-EDTA}$, sodium citrate and sodium fluoride/potassium oxalate plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within $\pm 2x$ analytical sensitivity (LDL) + coefficient of correlation > 0.95 .

Stable for 3 days at 2-8 °C, 12 months at -20 °C. Freeze only once.⁵

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.

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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] 03303071190, HCG STAT CalSet, for 4 x 1 mL
- [REF] 11731416190, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2
- [REF] 11731416160, PreciControl Universal, for 2 x 3 mL each of PreciControl Universal 1 and 2 (for USA)
- [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 or **cobas e** analyzer

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 **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
- [REF] 11298500160, Elecsys SysClean, 5 x 100 mL system cleaning solution (for USA)

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 prior to use and the reading in of the test-specific parameters via the reagent barcode take place automatically. No manual input is necessary. 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 approx. 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). 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.

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 mIU/mL or IU/L).

Limitations - interference

The assay is unaffected by icterus (bilirubin < 496 µmol/L or < 29 mg/dL), hemolysis (Hb < 0.932 mmol/L or < 1.5 g/dL), lipemia (Intralipid < 2400 mg/dL) and biotin (< 164 nmol/L or < 40 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 667 IU/mL and samples from dialysis patients.

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

In vitro tests were performed on 26 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

0.500-10000 mIU/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 0.500 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: 0.500 mIU/mL

The lower detection limit represents the lowest analyte level that can be distinguished from zero. It is calculated as the value lying two 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 Elecsys 2010 or **cobas e** 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 Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

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Expected values

Results from a multicenter study in 5 clinical centers in Belgium, France, and Germany with the HCG STAT assay (REF 03300811) in specimen from healthy individuals are listed below (Study No. BO1P019, status March 2003):

- ≤ 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 (weeks of pregnancy completed following the start 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	5 th -95 th percentile
3	25	18.7	5.40-72.0
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 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:

Elecsys 2010 and cobas e 411 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	42.1	0.64	1.5	0.91	2.2
Human serum 2	618	9.43	1.5	14.1	2.3
Human serum 3	4634	67.7	1.5	96.5	2.1
PreciControl U ^b 1	10.6	0.23	2.2	0.32	3.1
PreciControl U2	27.9	0.35	1.3	0.54	1.9

b) U = Universal

Precision was determined using Elecsys reagents, pooled human sera and controls in a separate study according to 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:

cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean mIU/mL	SD mIU/mL	CV %	SD mIU/mL	CV %
Human serum 1	3.56	0.12	3.4	0.13	3.8
Human serum 2	1.83	0.10	5.2	0.10	5.5
Human serum 3	7.04	0.17	2.3	0.21	2.9
Human serum 4	93.5	1.77	1.9	2.31	2.5
Human serum 5	744	15.3	2.1	19.0	2.6
Human serum 6	7091	132	1.9	178	2.5
PreciControl U1	8.27	0.12	1.4	0.18	2.2
PreciControl U2	30.8	0.32	1.0	0.52	1.7

Method comparison

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

Number of samples measured: 81

Passing/Bablok⁶ Linear regression

$$y = 1.0x - 7.38 \quad y = 1.05x - 52.6$$

$$r = 0.986 \quad r = 0.999$$

The sample concentrations were between 6.00 and 7675 mIU/mL.

Analytical specificity

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

LH 0.29 %, FSH 0.09 %, TSH: no cross-reactivity

References

- 1 Thomas CMG, Reijnders FJL, Segers MFG, et al. Human Choriongonadotropin (HCG): Comparisons between Determinations of Intact HCG, Free HCG β -Subunit, and "Total" HCG + β in Serum during the First Half of High-Risk Pregnancy. Clin Chem 1990;36(4):651-655.
- 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.
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- 5 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 1996:16-19. ISBN 3-928865-22-6.
- 6 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.

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

HCG STAT

Human chorionic gonadotropin, STAT (Short Turn Around Time)

cobas®

REAGENT

Reagent

CALIBRATOR

Calibrator



Volume after reconstitution or mixing

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