

REF			SYSTEM
05109442190	05109442500	100	cobas e 411 cobas e 601 cobas e 602

English

System information

For **cobas e 411** analyzer: test number 590

For **cobas e 601** and **cobas e 602** analyzers: Application Code Number 203

Intended use

Immunoassay for the in vitro quantitative determination of Interleukin-6 (IL-6) in serum and plasma. This assay can be used to aid in the management of critically ill patients as early indicator for acute inflammation.

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

Summary

Interleukin-6 (IL-6) is a pleiotropic cytokine with a wide range of functions. It was first described as interferon- β 2, plasmacytoma growth factor, and hepatocyte stimulating factor. Later on it was described as human B-cell-stimulating factor 2 (BSF2). In 1988 it was proposed to name it IL-6 as further studies had demonstrated that the protein shows activities not only on B-cells but also on T-cells, hematopoietic stem cells, hepatocytes and brain cells.¹ IL-6 is produced from a single gene encoding a product of 212 amino acids, which is cleaved at the N-terminus to produce a 184 amino acid peptide with a molecular weight between 22-27 kDa.² In 1989 it was reported that also immunoreactive complexes in the range of 60-70 kDa were detected in human body fluids in patients with acute bacterial infections.³

IL-6 production is rapidly induced in the course of acute inflammatory reactions associated with injury, trauma, stress, infection, brain death, neoplasia, and other situations.²

IL-6 concentrations in trauma patients may predict later complications from additional surgical stress or indicate missed injuries or complications.^{4,5}

Sequential measurements of IL-6 in serum or plasma of patients admitted to the ICU (intensive care unit) showed to be useful in evaluating the severity of SIRS (systemic inflammatory response syndrome), sepsis and septic shock and to predict the outcome of these patients.^{6,7,8} IL-6 is also useful as an early alarm marker for the detection of neonatal sepsis.^{9,10,11,12} IL-6 also plays a role in chronic inflammation e.g. rheumatoid arthritis.^{13,14}

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 μ L of sample are incubated with a biotinylated monoclonal IL-6-specific antibody.
- 2nd incubation: After addition of a monoclonal IL-6-specific antibody labeled with a ruthenium complex^{a)} and streptavidin-coated microparticles, the antibodies form a sandwich complex with the antigen of the sample.
- 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)₃²⁺)

Reagents - working solutions

The reagent rackpack is labeled as IL6.

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

- R1 Anti-IL-6~biotin (gray cap), 1 bottle, 9 mL:

Biotinylated monoclonal anti-IL-6 antibody (mouse) 0.9 μ g/mL; phosphate buffer 95 mmol/L, pH 7.3; preservative.

- R2 Anti-IL-6~Ru(bpy)₃²⁺ (black cap), 1 bottle, 9 mL:

Monoclonal anti-IL-6 antibody (mouse) labeled with ruthenium complex 1.5 μ g/mL; phosphate buffer 95 mmol/L, pH 7.3; 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.

H412 Harmful to aquatic life with long lasting effects.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P273 Avoid release to the environment.

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 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-heparin, K₂-EDTA and K₃-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within $\pm 2 \times$ analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 5 hours at 20-25 °C, 1 day at 2-8 °C, 3 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, 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] 05109469190, IL-6 CalSet, for 4 x 2.0 mL
- [REF] 05341787190, PreciControl Multimarker, for 6 x 2.0 mL
- [REF] 03609987190, Diluent MultiAssay, 2 x 16 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for the **cobas e 411** analyzer:

- [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] 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 materials for all analyzers:

- [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. 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.

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 NIBSC (National Institute for Biological Standards and Control) 1st IS 89/548 Standard.

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:

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

- 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 Multimarker.

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 pg/mL.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 680 µmol/L or < 40 mg/dL), hemolysis (Hb < 0.621 mmol/L or < 1.0 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Recovery within ± 15 % 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 IL-6 concentrations up to 200000 pg/mL.

In vitro tests were performed on 18 commonly used and 13 special 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

1.5-5000 pg/mL (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 1.5 pg/mL. Values above the measuring range are reported as > 5000 pg/mL (or up to 50000 pg/mL for 10-fold diluted samples).

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: approximately 1.5 pg/mL

The Lower Detection Limit represents the lowest measurable 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 IL-6 concentrations above the measuring range can be diluted with Diluent MultiAssay. The recommended dilution is 1:10 (either automatically by the analyzers, or manually). The concentration of the diluted sample must be > 50 pg/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

In an external study using the Elecsys IL-6 assay on samples from 817 apparently healthy individuals a reference range up to 7 pg/mL (95th percentile) was determined.

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

Clinical performance

Measurements were performed on samples from 281 ICU patients with either a known or suspected infection. The patients were classified into categories based on the ACCP/SCCM (American College of Chest Physicians/Society of Critical Care Medicine) consensus criteria: SIRS, sepsis, severe sepsis and septic shock.¹⁵ The IL-6 values of the patients with SIRS (n = 94) or sepsis (n = 65), severe sepsis (n = 60) or septic shock (n = 62) were as follows (3 European centers):

	IL-6 (pg/mL)					
	Median	Mean	Minimum	Maximum	N = 281	N
SIRS	62.1	150	≤ 1.5	2062	94	159
Sepsis	131	294	6.47	3122	65	
Severe sepsis	346	1827	15.2	39121	60	
Septic shock	659	8835	8.55	171257	62	

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 duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 411 analyzer					
Sample	Mean pg/mL	Repeatability		Intermediate precision	
		SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	17.3	1.03	6.0	1.46	8.5
Human serum 2	117	2.90	2.5	3.71	3.2

cobas e 411 analyzer					
Sample	Mean pg/mL	Repeatability		Intermediate precision	
		SD pg/mL	CV %	SD pg/mL	CV %
Human serum 3	891	22.8	2.6	25.5	2.9
PC MM ^{b)} 1	38.3	0.540	1.4	1.04	2.7
PC MM 2	229	3.29	1.4	6.71	2.9

b) PC MM = PreciControl Multimarker

cobas e 601 and cobas e 602 analyzers					
Sample	Mean pg/mL	Repeatability		Intermediate precision	
		SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	12.1	0.346	2.9	0.371	3.1
Human serum 2	49.0	0.657	1.3	0.782	1.6
Human serum 3	1966	17.2	0.9	22.2	1.1
PC MM 1	37.7	1.27	3.4	1.60	4.2
PC MM 2	229	5.20	2.3	7.63	3.3

Method comparison

A comparison of the Elecsys IL-6 assay (y) with a commercially available IL-6 assay (x) using clinical samples gave the following correlations:

Number of samples measured: 113

Passing/Bablok¹⁶

$$y = 1.14x + 3.0$$

$$\tau = 0.908$$

Linear regression

$$y = 1.06x + 7.63$$

$$r = 0.980$$

The sample concentrations were between approximately 3 and 1000 pg/mL.

Analytical specificity

The Elecsys IL-6 assay does not show any significant cross-reactivity with the following substances, tested with IL-6 concentrations of approximately 10 pg/mL and 200 pg/mL (max. tested concentration):

Substances	Non-interfering concentrations (ng/mL)
Interleukin-1 α	50
Interleukin-1 β	50
Interleukin-2	50
Interleukin-3	50
Interleukin-4	50
Interleukin-8	50
Interferon- γ	50
TNF- α	50

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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.

The Summary of Safety & Performance Report can be found here:
<https://ec.europa.eu/tools/eudamed>

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):

	Contents of kit
	Analyzers/Instruments on which reagents can be used
	Reagent
	Calibrator
	Volume after reconstitution or mixing
	Global Trade Item Number

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