Elecsys IL-6

cobas®

REF		Σ	SYSTEM
			cobas e 411
09015604190	09015604500	100	**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

Elecsys IL-6 is an immunoassay for the in vitro quantitative determination of Interleukin-6 (IL-6) in human serum and plasma. It is intended for use in conjunction with clinical evaluation to aid in:

- management of critically ill patients as an early indicator for acute inflammation
- diagnosis of sepsis in neonates with suspected or confirmed bacterial infection

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

Summary

Interleukin-6 (IL-6) is a pleiotropic cytokine and key mediator of the acute phase response induced by infection, inflammation, or trauma.[1,2,3] Numerous cell types can produce IL-6, but the most significant sources are macrophages and monocytes at the affected lesion.[4] Following initial synthesis, IL-6 is transported via the bloodstream to the liver, where it stimulates synthesis of the inflammatory acute phase proteins, such as C-reactive protein (CRP).

IL-6 levels in blood rise within the first hour post inflammatory insult and peak after 2-3 hours.[5] The rapid increase in IL-6 levels caused by inflammation and infection makes IL-6 a useful clinical aid to guide diagnosis of acute inflammatory diseases, including sepsis.[6] Elevations of IL-6 in neonates are indicative of infection and can guide diagnosis of neonatal sepsis.[7,8]

Monitoring IL-6 levels in critically ill patients provides prognostic information, as the magnitude of IL-6 elevation correlates with the severity of inflammatory insult or disease.[9,10,11] Post-operative IL-6 levels can also be indicative of infection before presentations of clinical signs.[12,13,14] Furthermore, elevated IL-6 levels can be predictive of impending respiratory failure and need for mechanical ventilation in patients with COVID-19.[15] IL-6 levels are also elevated in patients with chronic inflammatory disorders, such as rheumatoid arthritis, and can provide clinically useful information to guide patient management.[4,16]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL 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 ($Ru(bpy)_3^{2+}$)

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 µg/mL; phosphate buffer 95 mmol/L, pH 7.3; preservative.

R2 Anti-IL-6~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 9 mL:
Monoclonal anti-IL-6 antibody (mouse) labeled with ruthenium complex 1.5 µg/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.

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: 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.

Elecsys IL-6

cobas®

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, K_2-EDTA and K_3-EDTA plasma.

Plasma tubes containing separating gel can be used.

Criterion: Slope 0.9-1.1 + intercept within ≤ ± 3 pg/mL + coefficient of correlation ≥ 0.95.

Stable for 6 hours at 20-25 °C, 2 days at 2-8 °C, 24 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

Use Elecsys PreciControl Multimarker or other suitable controls for routine quality control procedures.

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 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	≤ 684 µmol/L or ≤ 40 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1500 mg/dL
Biotin	≤ 4912 nmol/L or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL

Criterion: Recovery within ± 15 % of initial value.

There is no high-dose hook effect at IL-6 concentrations up to 200000 pg/mL.

Elecsys IL-6

cobas®

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special therapeutic drugs were tested. No interference with the assay was found.

Special therapeutic drugs

Drug	Concentration tested mg/mL
Imipenem	1.18
Cefotaxime	0.9
Vancomycin	3.5
Dopamine	0.13
Noradrenaline	0.002
Dobutamine	0.0112
Furosemide	0.02
Fentanyl	0.01

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 Limit of Detection and the maximum of the master curve). Values below the Limit of Detection 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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 1.0 pg/mL

Limit of Detection = 1.5 pg/mL

Limit of Quantitation = 3.5 pg/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 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 ≥ 450 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 adults a reference range up to 7 pg/mL (95th percentile) was determined. For 134 neonates who did not have positive microbiological findings, a reference range of 66.4 pg/mL (95th percentile) was determined within the first 24 hours after birth.

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.[17] 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	122
Septic shock	659	8835	8.55	171257	62	

Median of mean IL-6 values (pg/mL) per day of life (DOL) period with minimum and maximum measured values are presented for 1695 neonates. Controls were defined as surviving neonates for whom measured laboratory parameters of infection did not increase. Sepsis and laboratory parameters of infection were defined according to Neo-KISS criteria.[7,18]

DOL 1		DOL 2-7		DOL > 7	
Control pg/mL	Sepsis pg/mL	Control pg/mL	Sepsis pg/mL	Control pg/mL	Sepsis pg/mL
23 1.5-3765	465 55-19907	18 2-1452	153 7-1149	10 2-1183	95 5-10980

IL-6 is a very dynamic indicator of inflammation and changes to its concentration in blood can be influenced by numerous non-infectious sources. IL-6 titres from non-infected and septic neonates can overlap significantly. IL-6 can aid in diagnosing neonatal sepsis, but must only be considered within the context of clinical assessment and history of the patient.

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 (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 411 analyzer					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	2.92	0.768	26.3	0.916	31.4
Human serum 2	5.95	0.649	10.9	0.922	15.5
Human serum 3	23.3	0.565	2.4	1.22	5.2
Human serum 4	2593	33.9	1.3	85.5	3.3
Human serum 5	4763	50.9	1.1	145	3.0
PC MM[b)] 1	42.3	0.804	1.9	1.76	4.2
PC MM 2	265	2.35	0.9	7.93	3.0

b) PC MM = PreciControl Multimarker

Elecsys IL-6

cobas®

cobas e 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean pg/mL	SD pg/mL	CV %	SD pg/mL	CV %
Human serum 1	3.12	0.269	8.6	0.472	15.1
Human serum 2	6.13	0.277	4.5	0.490	8.0
Human serum 3	23.3	0.384	1.7	0.979	4.2
Human serum 4	2570	43.0	1.7	91.8	3.6
Human serum 5	4759	59.6	1.3	143	3.0
PC MM 1	42.2	0.588	1.4	1.37	3.3
PC MM 2	261	2.20	0.8	6.42	2.5

Method comparison

A comparison of the Elecsys IL-6 assay, REF 09015604190 (**cobas e** 601 analyzer; y), with the Elecsys IL-6 assay, REF 05109442190 (**cobas e** 601 analyzer; x), gave the following correlations (pg/mL):

Number of samples measured: 123

Passing/Bablok[19]	Linear regression
y = 0.990x - 0.154	y = 0.991x + 2.61
τ = 0.987	r = 1.00

The sample concentrations were between 1.76 and 4929 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-1α	50
Interleukin-1β	50
Interleukin-2	50
Interleukin-3	50
Interleukin-4	50
Interleukin-8	50
Interferon-γ	50
TNF-α	50

References

1 Jawa RS, Anillo S, Huntoon K, et al. Interleukin-6 in Surgery, Trauma, and Critical Care: Part I: Basic Science. J Intensive Care Med 2011;26(1):3-12

2 Heinrich PC, Castell JV, Andus T. Interleukin-6 and the acute phase response. Biochem J 1990;265(3):621-636.

3 Tanaka T, Narazaki M, Kishimoto T. IL-6 in Inflammation, Immunity, and Disease. Cold Spring Harb Perspect Biol 2014;6(10):a016295.

4 Gabay C. Interleukin-6 and chronic inflammation. Arthritis Res Ther 2006;8 Suppl 2(Suppl 2):S3.

5 Thorsted A, Nielsen EI, Friberg LE. Pharmacodynamics of immune response biomarkers of interest for evaluation of treatment effects in bacterial infections. International Journal of Antimicrobial Agents 2020;56(3):106059.

6 Song M, Kellum JA. Interleukin-6. Critical Care Medicine 2005;33(12):S463.

7 Küng E, Unterasinger L, Waldhör T, et al. Cut-off values of serum interleukin-6 for culture-confirmed sepsis in neonates. Pediatr Res Published online October 10, 2022:1-6.

8 Ebenebe CU, Hesse F, Blohm ME, et al. Diagnostic accuracy of interleukin-6 for early-onset sepsis in preterm neonates. J Matern Fetal Neonatal Med 2021;34(2):253-258.

9 Watt DG, Horgan PG, McMillan DC. Routine clinical markers of the magnitude of the systemic inflammatory response after elective operation: a systematic review. Surgery 2015;157(2):362-380.

10 Feng M, Sun T, Zhao Y, et al. Detection of Serum Interleukin-6/10/18 Levels in Sepsis and Its Clinical Significance. J Clin Lab Anal 2016;30(6):1037-1043.

11 Song J, Park DW, Moon S, et al. Diagnostic and prognostic value of interleukin-6, pentraxin 3, and procalcitonin levels among sepsis and septic shock patients: a prospective controlled study according to the Sepsis-3 definitions. BMC Infect Dis 2019;19(1):968.

12 Fink-Neuboeck N, Lindenmann J, Bajric S, et al. Clinical impact of interleukin 6 as a predictive biomarker in the early diagnosis of postoperative systemic inflammatory response syndrome after major thoracic surgery: A prospective clinical trial. Surgery 2016;160(2):443-453.

13 Rettig TCD, Verwijmeren L, Dijkstra IM, et al. Postoperative Interleukin-6 Level and Early Detection of Complications After Elective Major Abdominal Surgery. Ann Surg 2016;263(6):1207-1212.

14 Sander M, von Heymann C, von Dossow V, et al. Increased interleukin-6 after cardiac surgery predicts infection. Anesth Analg 2006;102(6):1623-1629.

15 Herold T, Jurinovic V, Arnreich C, et al. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. Journal of Allergy and Clinical Immunology 2020;146(1):128-136.e4.

16 Pandolfi F, Franza L, Carusi V, et al. Interleukin-6 in Rheumatoid Arthritis. International Journal of Molecular Sciences 2020;21(15):5238.

17 American College of Chest Physicians/Society of Critical Care Medicine Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20:864-874.

18 Gastmeier, P, Geffers C, Schwab F, et al. Development of a surveillance system for nosocomial infections: the component for neonatal intensive care units in Germany. J. Hosp. Infect 2004;57:126-131.

19 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 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):

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

Elecsys IL-6

cobas®

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche