

Elecsys Anti-CCP

cobas®

REF		Σ	SYSTEM
05031656160	05031656501	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 810

For **cobas e 601** and **cobas e 602** analyzers: Application Code Number 202

Please note

The measured anti-CCP value of a patient's sample can vary depending on the testing procedure used. The laboratory finding must therefore always contain a statement on the anti-CCP assay method used.

Anti-CCP values determined on patient samples by different testing procedures cannot be directly compared with one another and could be the cause of erroneous medical interpretations.

Therefore, the results reported by the laboratory to the physician should include: "The following results were obtained with the Elecsys Anti-CCP assay. Results from assays of other manufacturers cannot be used interchangeably."

The performance characteristics for this assay have not been established for pediatric specimens. The diagnostic value of anti-CCP antibodies has not been determined for juvenile arthritis.

Intended use

Immunoassay for the in vitro semi-quantitative determination of human IgG autoantibodies to cyclic citrullinated peptides in human serum. The results of the assay are intended to be used as an aid in the diagnosis of rheumatoid arthritis in combination with other clinical and laboratory findings.

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

Summary

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases, affecting 0.5-1 % of the world population. This systemic disease is characterized by chronic inflammation of the synovial joints and progressive joint degeneration eventually leading to disability of affected individuals.¹

The diagnosis of RA often relies on clinical manifestations and laboratory tests such as rheumatoid factor (RF) and C-reactive protein (CRP). However, RF is non-specific for RA and may be present in healthy elderly persons or in patients with other autoimmune and infectious diseases and CRP is a general inflammation marker.

Recently, the identification of citrulline as a target of a whole set of autoantibodies like anti-perinuclear factor (APF), anti-keratin antibodies (AKA), anti-filaggrin antibodies (AFA) etc. detected in the sera of RA patients has led to the development of anti-CCP assays that possess a high specificity for RA. The clinical performance of anti-CCP assays has been further improved by the use of multiple citrullinated peptides, resulting in a second generation of anti-CCP assays.^{2,3,4,5,6,7,8,9,10,11}

The Elecsys Anti-CCP assay uses a set of cyclic citrullinated peptides and is therefore a so-called second generation assay.

Test principle

IgG-capture test principle. Total duration of assay: 18 minutes.

- 1st incubation: 15 µL of sample are incubated with biotinylated cyclic citrullinated peptides and ruthenylated^{a)} monoclonal antibody against human IgG, forming a complex when CCP-specific antibodies are present in the sample.
- 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)₃²⁺)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as A-CCP.

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 CCP-biotin (gray cap), 1 bottle, 9 mL: Biotinylated cyclic citrullinated peptides (synthetic) approximately 1.1 µg/mL, phosphate buffer 100 mmol/L, pH 5.0; preservative.
- R2 Anti-human aggregated IgG-Ru(bpy)₃²⁺ (black cap), 1 bottle, 10 mL: Ruthenylated monoclonal anti-human IgG antibody (mouse) 0.75 µg/mL; phosphate buffer 100 mmol/L, pH 6.0; preservative.

A-CCP Cal1 Anti-CCP calibrator 1 (white cap), 2 bottles (lyophilized) for 1.0 mL each: Anti-CCP antibodies (human) approximately 20 U/mL in a human serum matrix.

A-CCP Cal2 Anti-CCP calibrator 2 (black cap), 2 bottles (lyophilized) for 1.0 mL each: Anti-CCP antibodies (human) approximately 200 U/mL in a human serum matrix.

Calibrators: The exact lot-specific calibrator values are encoded in the barcoded labels of the test-specific reagent.

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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

2-methyl-2H-isothiazol-3-one hydrochloride

EUH 208 May produce an allergic reaction.

Product safety labeling follows EU GHS guidance.

All human material should be considered potentially infectious. The calibrators (A-CCP Cal1, A-CCP Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

However, as no testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.^{12,13}

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

Reagent rackpack

The reagent rackpack (M, R1, R2) in the kit is ready for use and is supplied in bottles compatible with the system.

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Calibrators

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer aliquots of the reconstituted calibrators into empty labeled snap-cap bottles (CalSet Vials). Attach the supplied labels to the additional bottles. Store the aliquots immediately at -20 °C (± 5 °C).

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note for **cobas e 602** analyzers: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. Please turn the vial cap 180° into the correct position so that the barcode between the yellow markers can be read by the system. Place the vial on the analyzer as usual.

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 of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
on all analyzers	1 week or maximum of 40 hours on the analyzer; up to 4 weeks when stored alternately in the refrigerator and on the analyzer

Stability of the calibrators	
lyophilized calibrators	up to the stated expiration date
reconstituted calibrators at -20 °C (± 5 °C)	4 weeks (freeze only once)
on the analyzers at 20-25 °C	up to 2 hours
after thawing	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

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.

Stable for 3 days at 20-25 °C, 8 days at 2-8 °C, 12 months at -20 °C (± 5 °C).

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 and frozen samples 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.

- 2 x 6 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- [REF](#) 05031664160, PreciControl Anti-CCP, for 4 x 2.0 mL
- [REF](#) 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- cobas e** analyzer

- Distilled or deionized water

Additional materials for **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](#) 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.

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.

Place the reconstituted calibrators (in the system-compatible bottles with barcoded labels) in the sample zone.

Perform **only one** calibration procedure per aliquot.

Calibration

Traceability: This method has been standardized against a commercially available second-generation anti-CCP assay.

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.

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Calibration frequency: Calibration must be performed once per reagent lot using A-CCP Cal1, A-CCP Cal2 and 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 Anti-CCP.

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 U/mL.

Note: Results are reported in arbitrary units as there is no generally recognized international standard for anti-CCP autoantibodies.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 427 µmol/L or < 25 mg/dL), hemolysis (Hb < 0.311 mmol/L or < 0.5 g/dL), lipemia (Intralipid < 1500 mg/dL), and biotin (< 123 nmol/L or < 30 ng/mL).

Criteria of recovery: maximum deviation of < 5 U/mL for samples < 25 U/mL and ± 15 % for samples ≥ 25 U/mL.

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.

Interference was observed from rheumatoid factor above a concentrations of 150 U/mL.

Autoantibodies are heterogeneous and this gives rise to non-linear dilution phenomena for certain individual samples.

There is no high-dose hook effect at anti-CCP concentrations up to 7000 U/mL.

IgG (hypergammaglobulinaemia)

Interference with pathologic levels of unspecific IgG cannot be excluded. However, the coincidence of RA and gammopathy in one patient has been reported to be very low.¹⁴

The anti-CCP test results can be false negative in patients with hypergammaglobulinaemia. Results from patients suffering from this disorder should not be used for diagnostic purposes.

In vitro tests were performed on 18 commonly used pharmaceuticals and in addition on methotrexate and prednisolone. 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

8.00-500 U/mL (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 8.00 U/mL. Values above the measuring range are reported as > 500 U/mL.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank = 7.00 U/mL

Limit of Detection = 8.00 U/mL

Limit of Quantitation = 8.00 U/mL

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements. The Limit of Quantitation was determined using the result of functional sensitivity testing.

The Limit of Blank is the 95th percentile value from $n \geq 60$ 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 (functional sensitivity) is the lowest analyte concentration that can be reproducibly measured with an intermediate precision CV of ≤ 20 %.

It has been determined using low concentration anti-CCP samples.

Dilution

Samples with anti-CCP concentrations above the measuring range can be diluted manually using an anti-CCP negative serum pool. The recommended dilution is 1:2 to 1:5.

After manual dilution, multiply the result by the dilution factor.

Note: Autoantibodies are heterogeneous and this gives rise to non-linear dilution phenomena for certain individual samples.

Expected values

In an external study using the Elecsys Anti-CCP assay on samples from 420 asymptomatic healthy individuals, 792 confirmed RA patients and 907 patients with other rheumatic and non-rheumatic disorders, an optimal cutoff of 17.0 U/mL was determined; samples with a concentration ≥ 17.0 U/mL being considered positive for anti-CCP (for details see section "Clinical sensitivity and specificity").

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 (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					
		Repeatability		Intermediate precision	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	16.9	0.106	0.6	0.527	3.1
Human serum 2	356	8.36	2.3	16.0	4.5
PC ^{b)} A-CCP1	24.6	0.242	1.0	0.740	3.0
PC A-CCP2	137	1.90	1.4	3.48	2.5

b) PC = PreciControl

cobas e 601 and cobas e 602 analyzers					
		Repeatability		Intermediate precision	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 1	17.8	0.2	1.0	0.3	1.9

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	Repeatability			Intermediate precision	
Sample	Mean U/mL	SD U/mL	CV %	SD U/mL	CV %
Human serum 2	77.9	0.6	0.8	1.1	1.4
PC A-CCP1	20.0	0.2	0.8	0.3	1.5
PC A-CCP2	94.1	0.7	0.8	1.2	1.3

Clinical sensitivity and specificity

In cohorts of 792 confirmed RA patients, 420 asymptomatic healthy individuals and 907 patients with other rheumatic and non-rheumatic disorders an optimal cutoff of 17.0 U/mL was determined. At this cutoff the sensitivity was calculated to be 67.4 % with a specificity of 97.0 %. The area under the receiver operating characteristic (ROC) curve was 0.85. The health status of 420 asymptomatic healthy volunteers (213 men, 207 women) was confirmed by a standard clinical chemistry and hematology profile and a brief medical examination. In particular, elevated CRP levels, elevated white blood cell counts and elevated uric acid levels were excluded for this group. A family history of rheumatic / autoimmune disorders was excluded through a medical questionnaire. The cohort of established RA patients consisted of patients with unknown disease duration as well as patients with a known disease duration of more than 2 years or less than 2 years. Disease duration was measured from the time point of RA diagnosis by an experienced rheumatologist.

	N	Number of samples found positive with the Elecsys Anti-CCP assay	Sensitivity %
RA samples in total	792	534	67.4
RA > 2 years	378	273	72.2

Clinical specificity

	N	Number of samples found negative with the Elecsys Anti-CCP assay	Specificity %
Non-RA samples in total	1327	1287	97.0
Healthy	420	416	99.0
Non-RA disease samples in total	907	871	96.0
Non-RA disease subsets:			
Connective tissue diseases	166	157	94.6
Vasculitides	47	43	91.5
Spondyloarthropathies	146	138	94.5
Other rheumatic diseases	108	106	98.1
Inflammatory bowel diseases	52	52	100
Non-rheumatic autoimmune diseases	31	29	93.5
Renal failure	31	30	96.8
Liver cirrhosis	26	24	92.3
Infectious diseases	300	292	97.3

Method comparison

A subset of the sample collectives described to determine the clinical performance of the Elecsys Anti-CCP assay was also used to compare the Elecsys Anti-CCP assay to a commercially available, second-generation

anti-CCP microtiter plate ELISA assay. The respective assay was used according to the manufacturer's instructions given in the package insert.

Using a cutoff of ≥ 17.0 U/mL for the Elecsys Anti-CCP assay the following results were obtained:*

N = 1606		Commercially available, second-generation anti-CCP assay	
		positive	negative
Elecsys Anti-CCP assay	positive	428	18
	negative	26	1134

	Total	Samples concordant in both assays	Concordance %	95 % confidence interval
Positive concordance	454	428	94.3	91.7-96.2
Negative concordance	1152	1134	98.4	97.5-99.1
<i>Concordance in clinical subgroups</i>				
Non-RA group	992	968	97.6	96.4-98.4
RA group	614	594	96.7	95.0-98.0
Concordance over all samples	1606	1562	97.3	96.3-98.0

* Representative data, results from individual laboratories might differ.

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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).

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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