ms_05031656190V5.0

Anti-CCP

cobas®

Antibody to cyclic citrullinated peptide (anti-CCP)

REF	Σ	SYSTEM
05031656 190	100	Elecsys 2010 MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

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."

Intended use

Immunoassay for the in vitro semi-quantitative determination of human IgG autoantibodies to cyclic citrullinated peptides in human serum and plasma. 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 **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "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.

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

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) approx. 1.1 µg/mL, phosphate buffer 100 mmol/L, pH 5.0; preservative.

R2 Anti-human aggregated IgG~$Ru(bpy)_3^{2+}$ (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) approx. 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) approx. 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.

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 applied were FDA-approved 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

Anti-CCP

cobas®

Antibody to cyclic citrullinated peptide (anti-CCP)

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

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

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 of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
on all analyzers	1 week or up to 4 weeks with max. 5 x 8 hours at 15-25 °C if 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	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 in a sufficient number and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-heparin and K_3-EDTA plasma.

Criterion: Slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LoB), coefficient of correlation > 0.95.

Stable for 3 days at 2-8 °C, 1 month at -20 °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.

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 05031664190, PreciControl Anti-CCP, for 2 x 2 mL each of PreciControl Anti-CCP 1 and 2
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer
- Distilled or deionized water

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 MODULAR ANALYTICS E170, **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 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

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 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.

Place the reconstituted calibrators 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 Anti-CCP 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 A-CCP Cal1 and A-CCP Cal2.

Anti-CCP

cobas®

Antibody to cyclic citrullinated peptide (anti-CCP)

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

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.

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.

No interference was observed from rheumatoid factors up to a concentration of 150 IU/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 (hypergammaglobulinemia)

Interference with pathologic levels of unspecific IgG can not 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 hypergammaglobulinemia. 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

7-500 U/mL (defined by the Limit of Blank and the maximum of the master curve). Values below the Limit of Blank are reported as < 7 U/mL. Values above the measuring range are reported as > 500 U/mL.

Lower limits of measurement

Limit of Blank (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ):

Limit of Blank = 7 U/mL

Limit of Detection = 8 U/mL

Limit of Quantitation = 8 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 ≥ 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.[15,16]

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 cut-off of 17 U/mL was determined; samples with a concentration ≥ 17 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 duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		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

Anti-CCP

Antibody to cyclic citrullinated peptide (anti-CCP)

b) PC = PreciControl

MODULAR ANALYTICS E170, **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
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 cut-off of 17 U/mL was determined. At this cut-off 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 defined by normal results for a standard clinical chemistry and hematology profile and a brief medical examination. 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 positive with the Elecsys Anti-CCP assay	Sensitivity %
Non-RA samples in total	1327	40	97.0
Healthy	420	4	99.0
Non-RA disease samples in total	907	36	96.0
Non-RA disease subsets:			
Connective tissue diseases	166	9	94.6
Vasculitides	47	4	91.5
Spondyloarthropathies	146	8	94.5
Other rheumatic diseases	108	2	98.1
Inflammatory bowel diseases	52	0	100
Non-rheumatic autoimmune diseases	31	2	93.5
Renal failure	31	1	96.8
Liver cirrhosis	26	2	92.3
Infectious diseases	300	8	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 cut-off of ≥ 17 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
Concordance in clinical subgroups				
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.

The obtained results were also used to perform a ROC (receiver operating characteristic) analysis. The area under the curve (AUC) for the Elecsys Anti-CCP assay was 0.86 (95 % confidence interval: 0.84-0.88) and 0.81 (95 % confidence interval: 0.79-0.84) for the commercially available, second-generation anti-CCP assay used in this method comparison, thus indicating that both assays are comparable with respect to their clinical differentiation.

References

1 Feldmann M, Brennan FM, Maini RN. Rheumatoid arthritis. Cell 1996;85:307-310.

2 Raptopoulou A, Sidiropoulos P, Katsouraki M. Anti-citrulline antibodies in the diagnosis and prognosis of rheumatoid arthritis: Evolving concepts. Crit Rev Clin Lab Sci 2007;44:339-363.

3 Nishimura K, Sugiyama D, Kogata Y, et al. Diagnostic Accuracy of Anti-Cyclic Citrullinated Peptide Antibody and Rheumatoid Factor for Rheumatoid Arthritis. American College of Physicians 2007;146:797-808.

4 Schellekens GA, De Jong B, van den Hoogen F, et al. Citrulline is an Essential Constituent of Antigenic Determinants Recognized by Rheumatoid Arthritis-specific Autoantibodies. J Clin Invest 1998;101:273-281.

Anti-CCP

Antibody to cyclic citrullinated peptide (anti-CCP)

5 Vossenaar ER, Despres N, Lapointe E, et al. Rheumatoid arthritis specific anti-SA antibodies target citrullinated vimentin. Arthritis Res Ther 2004;6:R142-150.

6 Vossenaar ER, Radstake TRD, van der Heijden A, et al.. Expression and activity of citrullinating peptidylarginine deiminase enzymes in monocytes and macrophages. Ann Rheum Dis 2004;63:373-381.

7 Vossenaar ER, Zendman AJW, van Venrooij WJ, et al. PAD, a growing family of citrullinating enzymes: genes, features and involvement in disease. BioEssays 2003;25:1106-1118.

8 Zendman AJW, van Venrooij WJ, Pruijin GJM. Use and significance of anti-CCP autoantibodies in rheumatoid arthritis. Rheumatology 2006;45:20-25.

9 Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systemic literature review. Ann Rheum Dis 2006;65:845-851.

10 Rantapää-Dahlqvist S, de Jong BAW, Berglin E, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis & Rheumatism 2003;48:2741-2749.

11 Nielen MMJ, van Schaardenburg D, Reesink HW, et al. Specific Autoantibodies Precede the Symptoms of Rheumatoid Arthritis (A Study of Serial Measurements in Blood Donors). Arthritis & Rheumatism 2004;50:380-386.

12 Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

13 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

14 Kelly C, Baird G, Foster H, et al. Prognostic significance of paraproteinaemia in rheumatoid arthritis. Ann Rheum Dis 1991;50:290-294.

15 Klee GG. Interferences in hormone immunoassays. Clin Lab Med 2004;24:1-18.

16 Ismail AAA. On detecting interference from endogenous antibodies in immunoassays by doubling dilution test. Clin Chem Lab Med 2007;45(7):851-854.

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
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com