

# IMTEC-CCP-ANTIBODIES

## CCP

### ELISA for the Quantitative Determination of Antibodies against Cyclic Citrullinated Peptides

#### Package Size

<b>[REF]</b>	ITC60021	96 Tests	Complete Testkit
<b>[VD]</b>			

Please read the instructions carefully before testing.

#### Procedural precautions:

Do not use the reagents beyond the date of expiry.

**[DIL] DB18, [WASH]20x WB04, [SUB] TMB CCP and [STOP] STOP ELISA may be interchanged between lots and test kits that share the same reagent designation.**

**All other reagents are specific for the individual test kit lot and must not be interchanged with other lots and test kits**

Store reagents at 2...8°C.

#### Intended Use

IMTEC-CCP-Antibodies is an indirect solid-phase enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against cyclic citrullinated peptide (CCP) in human serum or plasma. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of rheumatoid arthritis.

Rheumatoid arthritis (RA) is one of the most common systemic autoimmune diseases and affects up to 1-2 % of the world population. Classification criteria for RA include the serological marker rheumatoid factor (RF). RF has been shown to be associated with unfavourable outcome for joint destruction and disability, particularly when present in high titre.

However, RF is often negative or present at a low titre in the early disease stages. Anti-cyclic citrullinated peptide antibodies (anti-CCP) have been shown to occur in a significant number of well defined RA patient sera with an excellent specificity against disease controls. Anti-CCP antibodies can be detected years before onset of clinical manifestations and can therefore be used as a predictive marker for the development of RA.

The IMTEC-CCP-Antibodies ELISA contains improved synthetic peptides selected on the basis of superior performance and serves as a highly specific and sensitive marker to add to the diagnosis of RA.

#### Method

The test is based on the immobilisation of cyclic citrullinated peptides (CCP) to the surface of a microtiter plate and subsequent binding of anti-CCP antibodies from human serum or plasma. The bound anti-CCP antibodies are detected with a horseradish peroxidase (HRP)-labelled antibody that is directed against human IgG. After the addition of substrate solution, a colour stain develops and its intensity is proportional to the concentration of the anti-CCP antibodies. Following the addition of stop solution, the colour changes from blue to yellow.

#### Contents

<b>[MTP]</b>	12	<b>Microtiter Strips</b> (in 1 strip holder) 8-well snap-off strips, ready for use coated with cyclic citrullinated peptides
<b>[CAL]</b>	1 – 5 5 x 1.5 ml	<b>Anti-CCP Calibrators IgG</b> (white cap), human serum, inked according to concentration, ready for use anti-CCP level: 25 U/ml (1), 50 U/ml (2), 200 U/ml (3), 800 U/ml (4), 3200 U/ml (5)
<b>[NC]</b>	1.5 ml	<b>Anti-CCP Negative Control</b> (green cap), human, ready for use
<b>[PC]</b>	1.5 ml	<b>Anti-CCP Positive Control</b> (red cap), human, ready for use
<b>[CC]</b>	1.5 ml	<b>Anti-CCP Reference Control</b> (yellow cap), human, ready for use
<b>[WASH]20x WB04</b>	50 ml	<b>Washing Buffer</b> (black cap) Concentrate (20x) for 1 l Phosphate buffer pH 7.4 ± 0.2
<b>[DIL] DB18</b>	100 ml	<b>Dilution Buffer</b> (blue cap) ready for use Phosphate buffer pH 7.4 ± 0.2

++++ Change of **[I]** ++++ Please read **marked text carefully!** ++++

<b>[CON]</b>	15 ml	<b>Conjugate Solution</b> (white cap) anti-human-IgG HRP conjugate, ready for use
<b>[SUB]</b> TMB CCP	15 ml	<b>TMB solution</b> (black cap) ready for use, 3,3', 5,5'-tetramethylbenzidin Hydrogen peroxide pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
<b>[STOP]</b> STOP ELISA	15 ml	<b>Stop Solution</b> (red cap) Sulphuric acid, ready for use 0.5 mol/l
	1	<b>Adhesive Strip</b>

#### Safety Notes

Do not swallow the reagents. Avoid contact with eyes, skin and mucous membranes. All patient specimens and controls should be handled as potentially infectious. The controls have been checked on donor level for HCV and HIV-1/2 antibodies and HBsAg and found negative. Wear protective clothing and disposable gloves according to Good Laboratory Practices.

All materials contaminated with patient specimens or controls should be inactivated by validated procedures (autoclaving or chemical treatment) in accordance with applicable regulations.

#### Stability

The reagents are stable up to the stated expiry dates on the individual labels when stored at 2...8 °C.

#### Reagent Preparation

**Allow the test kit and all its components to reach room temperature before use!** Used bottles should be closed carefully and stored at 2...8 °C. Store **[SUB]** protected from light.

Do not use polystyrene vessels for handling of **[CON]**.

To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.

#### Washing Buffer Solution **[WASH]**

Any crystallised salt inside the bottle must be dissolved before use. Dilute 1 part **[WASH]20x** with 19 parts deionised water. **[WASH]** is stable for 6 weeks stored at 2...8 °C.

#### Specimen

Patient Sera and Plasma

Use samples freshly collected or freeze samples at -20 °C. Freeze and thaw once only. Do not use serum samples inactivated by heat treatment at 56 °C.

Allow the samples to reach room temperature (30 min.).

Dilute samples 1:50 with **[DIL]** (add 10 µl sample to 490 µl **[DIL]**).

#### Procedure

- Quantitative:  
Pipette 100 µl diluted sample, **[CAL]**, **[PC]** and **[NC]** into **[MTP]**, for blank use **[DIL]** instead of sample dilution, seal **[MTP]** with adhesive strip.  
Qualitative:  
Pipette 100 µl diluted sample, **[CC]**, **[PC]** and **[NC]** into **[MTP]**, for blank use **[DIL]** instead of sample dilution, seal **[MTP]** with adhesive strip.
- Incubate for 1 hour at RT.
- Discard the solution from **[MTP]**. Wash **[MTP]** 3 times using 300 µl **[WASH]** per well.
- Discard **[WASH]** and knock out residues on an absorbent paper or cloth.
- Pipette 100 µl **[CON]** and seal **[MTP]** with adhesive strip.
- Incubate for 30 min. at RT.
- Discard the solution from **[MTP]**. Wash **[MTP]** 3 times using 300 µl **[WASH]** per well.
- Discard buffer and knock out residues on an absorbent paper or cloth.
- Pipette 100 µl **[SUB]** and incubate for 30 min. At room temperatures above 25°C the substrate incubation can be shortened, but should never be less than 25 min..
- Add 100 µl **[STOP]** per well.
- Read absorbance values at 450 nm within the next 10 min. after stopping. Bi-chromatic measurement with a reference wavelength at 620 – 690 nm is recommended.

## Automation

The IMTEC-CCP-Antibodies ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated in prior to diagnostic use.

## Validation of the test

The test results are valid provided the following criteria are met for the obtained results:

### Quantitative

- $[PC]$  is within the indicated range (see label).
- $[NC]$  is lower than the cut-off value of the test.
- $[CAL]5$  does not fall below an absorbance value of 0.9.
- The absorbances of  $[CAL]1$  –  $[CAL]5$  keep raising.

### Qualitative

- The absorbance ratio of  $[PC] / [CC]$  is within the indicated range (see label).
- The absorbance ratio of  $[NC] / [CC]$  is  $< 1.0$ .

In order to improve the accuracy of the test results we recommend to run  $[CAL]1$  –  $[CAL]5$ ,  $[CC]$ ,  $[PC]$ ,  $[NC]$  and patient samples in duplicate.

## Interpretation of Results

### Quantitative

Plot measured absorbances against concentrations/units of  $[CAL]1$ - $[5]$  in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of CCP-specific antibodies in the patient samples can be determined.

The calibration curve cannot be used for absorbance values below  $[CAL]1$  (25 U/ml). Values should be reported as  $< 25$  U/ml.

Results above 25 U/ml (cut-off value) are considered positive.

### Qualitative

Qualitative interpretation of results is possible by correlating the absorbances of the  $[CC]$  and of the samples:

- Absorbances  $> 1.0 \times [CC]$  have to be considered as positive.
- Absorbances  $< 0.95 \times [CC]$  have to be considered as negative.
- Absorbances  $\geq 0.95 \times [CC]$  and  $\leq 1.0 \times [CC]$  have to be considered as equivocal (recommend repeat testing).

## Limitations

A positive result must be used in association with clinical evaluation and diagnostic procedures. The values obtained from this assay are intended to be an aid for diagnosis only.

Elevated anti-CCP antibodies may occur in individuals with no evidence of clinical disease.

Anti-CCP antibody concentration does not necessarily correlate to disease activity.

The performance characteristics for this assay have not been established for paediatric specimens.

## Performance Characteristics

Typical performance data can be found in the Verification Report, accessible via:

[www.human.de/data/gb/vr/el-60021.pdf](http://www.human.de/data/gb/vr/el-60021.pdf) or

[www.human-de.com/data/gb/vr/el-60021.pdf](http://www.human-de.com/data/gb/vr/el-60021.pdf)

If the performance data are not accessible via internet, they can be obtained free of charge from your local distributor.

## Safety Notes

### **[STOP]** Warning

#### • Hazard statements

H315 Causes skin irritation.

H319 Causes serious eye irritation.

#### • Precautionary statements

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P332+P313 If skin irritation occurs: Get medical advice/attention.

P337+P313 If eye irritation persists: Get medical advice/attention.

## References

1. Coenen D. *et al.*, Clin Chem. **53**, 498-504 (2007)
2. Bizzaro N. *et al.*, Clin Chem. **53**, 1527-33 (2007)
3. Van Boekel *et al.*, Arthritis Res. **4**, 87-93 (2002)
4. Rantapää-Dahlqvist S. *et al.*, Arthritis Rheum. **48**, 2741-2749 (2003)
5. Kastborn A. *et al.*, Ann. Rheum. Dis. **63**, 1085-1089 (2004)
6. Nell V.P.K. *et al.*, Ann Rheum Dis. **64**, 1731-1736 (2005)

EL-60021

INF ITC60021 GB

08-2018-14M



**Human**