IMTEC-Anti-C1q-Antibodies

ELISA for the Quantitative Determination of anti-C1q Antibodies (IgG)

Package Size

REF	ITC59033	96 Tests	Complete Testkit
IVD			

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

DIL DB11, WASH 20x WB03, SUB TMB ELISA 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-Anti-C1q-Antibodies is an enzyme immunoassay (ELISA) for the quantitative measurement of IgG class autoantibodies against C1q in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis of systemic lupus erythematosus and lupus nephritis.

Anti-C1q autoantibodies of the IgG type have been found in 17-46% of all patients with a systemic lupus erythematosus (SLE) and particularly in a large number of patients with lupus nephritis. It was also shown that an increased concentration of C1q autoantibodies has a sensitivity of 71%, a specificity of 92%, a positive predictive value of 50% and a negative predictive value of 97% for the development of renal involvement. Therefore, in patients with systemic LE, the detection of anti-C1q autoantibodies as well as detection of dsDNA autoantibodies are important criteria for determining potential renal involvement.

Principle

The test is based on the immobilisation of highly purified C1q to microtiter strips and subsequent binding of anti-C1q antibodies from patient serum. The bound antibodies are detected afterwards with a peroxidase-labelled secondary antibody that is directed against human IgG. After addition of substrate solution, a colour stain develops and its intensity is proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow.

Reagents and Contents

MTP	12	**Microtiter Strips** (in 1 strip holder) 8-well snap-off strips, ready for use coated with C1q	
CAL	1 – 5 5 x 1.5 ml	**Calibrators IgG** (white cap), human serum, inked according to concentration, ready for use anti-C1q level: 12.5 U/ml **(1)**, 25 U/ml **(2)**, 50 U/ml **(3)**, 100 U/ml **(4)**, 200 U/ml **(5)**	
NC	1.5 ml	**Negative Control Serum** (green cap), human, ready for use	
PC	1.5 ml	**Positive Control Serum** (red cap), human, ready for use Concentrations are stated on the labels.	
WASH 20x WB03	50 ml	**Washing Buffer** (black cap) Concentrate (20x) for 1 l TRIS buffer	pH 6.9 ± 0.2
DIL DB11	100 ml	**Dilution Buffer** (blue cap) ready for use Phosphate buffer	pH 6.8 ± 0.2
CON	15 ml	**Conjugate Solution** (white cap) anti-human-IgG HRP conjugate, ready for use	
SUB TMB ELISA	15 ml	**TMB solution** (black cap) ready for use, colourless to bluish 3,3', 5,5'-tetramethylbenzidin Hydrogen peroxide	pH 3.7 ± 0.2 1.2 mmol/l 3 mmol/l
STOP STOP ELISA	15 ml	**Stop Solution** (red cap) Sulphuric acid, ready for use	0.5 mol/l
	1	**Adhesive Strip**	

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Safety Notes

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 testkit 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 resolved before use. Dilute 1 part WASH 20x with 19 parts distilled water. WASH is stable for 6 weeks stored at 2...8°C.

Specimen

Patient sera

Use samples freshly collected or freeze samples at –20°C.

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

Dilute sera 1:101 with DIL (add 10 µl serum to 1 ml DIL).

Procedure

- **Pipette 100 µl** diluted sample, CAL, 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** WASH and knock out residues on an absorbent paper or cloth.
- **Pipette 100 µl** SUB and incubate for **10 min.**. At room temperatures above 25°C the substrate incubation could be shortened, but should never fall short of 5 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-Anti-C1q-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:

- 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 0.6.
- The absorbances of CAL 1-5 keep raising.

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

Interpretation of Results

Plot measured absorbances against U/ml of CAL 1-5 in semi-log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of anti-C1q autoantibodies in the patient samples can be determined.

Results above 20 U/ml are positive.

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-C1q antibodies may occur in individuals with no evidence of clinical disease.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

The performance characteristics for this assay have not been established for plasma samples.

Performance Characteristics

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

www.human.de/data/gb/vr/el-59033.pdf or

www.human-de.com/data/gb/vr/el-59033.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.

SUB **Danger**

· Hazard statements

H360D May damage the unborn child.

· Precautionary statements

CAL NC PC WASH 20x DIL CON SUB STOP

P234 Keep only in original container.

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P262 Do not get in eyes, on skin, or on clothing.

P281 Use personal protective equipment as required.

P303+P361+P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

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

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

P401 Store in accordance with local/regional/national/international regulations.

P501 Dispose of contents/container in accordance with local/regional/national/international regulations.

References

1. Mannik M., Wener M.H., Arthritis Rheum. **40**, 1504–1511 (1997)
2. Siegert C.E.H. *et al.*, Clin. Exp. Immunol. **116**, 4-8 (1999)
3. Marto N. *et al.*, Ann. Rheum. Dis. **64**, 444-448 (2005)

EL-59033 INF ITC59033 GB 08-2018-13M

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