IMTEC-C3d-CIC

C3d-CIC
ELISA for the Quantitative Determination of Circulating C3d-binding Immune Complexes (IgG)

Package Size

REF	ITC59032	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 **DB04,** BUF WASH 10x **WB03,** SUBS TMB **TMB ELISA and** SOLN 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

The formation of immune complexes is a physiological defence mechanism for the rapid elimination of endogenous or exogenous antigens.

In autoimmune diseases, the detection of circulating immune complexes is an important criterion for the evaluation of the disease activity and the organic manifestation as well as for indications of new therapy approaches.

In accordance with the recommendations of a WHO study (Lambert P.H. *et al*., 1978), two independent methods should be used for measurement of circulating immune complexes.

The determination of C1q-binding immune complexes registers the classical path of complement activation. The detection of C3d-bound immune complexes may be caused by activation of the classical pathway or by alternative pathways.

The use of both test systems thus makes it possible to differentiate between the classical pathway and the alternative pathway of complement system activation.

Principle

The test is based on the immobilisation of an anti-C3d monoclonal antibody to the solid phase of microtiter strips and subsequent binding of C3d-containing circulating immune complexes from patient serum.

The bound immune complexes are detected with a peroxidase-labelled secondary antibody that is directed against human IgG. After addition of substrate solution, a colour appears which intensity is proportional to the concentration of C3d-containing circulating immune complexes. 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 anti-C3d-antibodies
CAL 1 – CAL 4	**4 x 1 ml Calibrators IgG** (white cap), aggregated IgG, inked according to concentration, ready for use **1**: 25 µg/ml **2**: 50 µg/ml **3**: 100 µg/ml **4**: 200 µg/ml
CONTROL +	**1 ml Positive Control Serum** (red cap), human, ready for use Concentrations are stated on the labels.
BUF WASH 10x WB03	**50 ml Washing Buffer** (black cap) Concentrate (10x) for about 0.5 l Phosphate buffer pH 6.7 ± 0.2
DIL DB04	**100 ml Dilution Buffer** (blue cap) ready for use, Phosphate buffer pH 7.0 ± 0.2
CONJ a(hum IgG):HRP	**12 ml Conjugate Solution** (white cap) anti-human-IgG HRP conjugate, ready for use
SUBS TMB TMB ELISA	**12 ml TMB Solution** (black cap) ready for use, pH 3.7 ± 0.2 colourless to bluish 3,3',5,5'-tetramethylbenzidine 1.2 mmol/l Hydrogen peroxide 3 mmol/l
SOLN STOP STOP ELISA	**12 ml Stop Solution** (red cap) Sulphuric acid, ready for use 0.5 mol/l
	1 Pc Adhesive Strip

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.

SOLN STOP, SUBS TMB can irritate eyes, skin and mucous membranes. Upon contact, rinse thoroughly with copious amounts of water and consult a doctor.

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 SUBS TMB protected from light.

Do not use polystyrene vessels for handling of CONJ a(hum IgG):HRP.

If the test is run on an automated system, use fresh conjugate each time. Remove traces of old conjugate completely.

Washing Buffer Solution WASH

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part BUF WASH 10x with 9 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. **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 sera 1:11 with DIL (add 20 µl serum to 0.2 ml DIL).

Procedure

- **Pipette 100 µl** diluted sample, CAL and CONTROL + into MTP, for blank use DIL instead of sample dilution, seal MTP with adhesive strip.
- Incubate for **1 hour** at RT.
- **Wash** MTP using 250 µl WASH per well. **Repeat** procedure **3 times.**
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µl** CONJ a(hum IgG):HRP and seal MTP with adhesive strip.
- Incubate for **30 min.** at RT.
- **Wash** MTP using 250 µl WASH per well. **Repeat** procedure **3 times.**
- **Discard buffer and knock out residues** on an absorbent paper or cloth.
- **Pipette 100 µl** SUBS TMB 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** SOLN STOP per well.
- **Measure at 450 nm** within the next 30 min. after stopping.

Validation of the test

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

- CONTROL + is within the indicated range (see label).
- CAL 4 does not fall below an absorbance value of 0.6.
- The absorbances of CAL 1-4 keep raising.

In order to improve accuracy of the test results we recommend to run CAL 1-4, CONTROL + and patient samples in duplicate.

Interpretation of Results

Plot measured absorbances against concentrations of CAL 1-4 (**0 µg/ml (blank)**, 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml) in semi log. By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of C3d-containing immune complexes as equivalents of aggregated IgG can be determined.

Results above 40 µg/ml (cut-off value) are considered as elevated. Values exceeding the fourth standard are to be reported as >200 µg/ml. Retesting at higher dilutions is not recommended, because the dilution behaviour of C3d-containing immune complexes is non-linear.

Performance Characteristics

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

www.human.de/data/gb/vr/el-59032.pdf or

www.human-de.com/data/gb/vr/el-59032.pdf

Literature

1. Ritzmann, S.E., Daniels J.C., Clin. Chem. **26**, 1259-1271 (1982)
2. Lambert P.H. *et al.*, J. Clin. Lab. Immunol. **1**, 1-15 (1978)
3. Theofilopoulos, A.N., Progress in Clin. Immunol. **4**, 63-106 (1980)

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