

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, 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-C3d-CIC is an enzyme immunoassay (ELISA) for the quantitative measurement of IgG containing C3d-binding circulating immune complexes in human serum. The assay is intended for in vitro diagnostic use only as an aid in the diagnosis and monitoring of immune dysfunctions.

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 – 4 4 x 1.5 ml	Calibrators IgG aggregated IgG, inked according to concentration, ready for use Concentration: 25 µg/ml (1), 50 µg/ml (2), 100 µg/ml (3), 200 µg/ml (4)	
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 DB04	100 ml	Dilution Buffer (blue cap) ready for use Phosphate buffer	pH 7.0 ± 0.2

++++ Change of  ++++ Please read **marked text carefully!** ++++

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	

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 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. **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 **PC** 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 buffer 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 **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 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-C3d-CIC ELISA may be processed with suitable automated ELISA analyzers. Applications have to be validated 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).
- [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, [PC] 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 [CAL]4 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.

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.

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-59032.pdf or

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

Human