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 |

Intended Use

IMTEC-C3d-CIC is an enzyme immunoassay (ELISA) for the qualitative 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., 1978), two independent methods should be used for measurement of circulating immune complexes.

The determination of C3d-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 | Calibrators IgG | aggregated IgG, inked according to concentration, ready for use |
| 1.5 ml | 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 | 20A | 50 ml | Washing Buffer (black cap) |
| Concentrate (20x) for 1 l |
| TRIS buffer | pH 6.9 ± 0.2 |
| DIL | 100 ml | Dilution Buffer (blue cap) | ready for use |
| Phosphate buffer | pH 7.0 ± 0.2 |
| DB04 | 15 ml | Conjugate Solution (white cap) | anti-human-IgG HRP conjugate, ready for use |
| SUB | 15 ml | TMB solution (black cap) | ready for use |
| pH 3.7 ± 0.2 |
| Hydrogen peroxide | 3 mmol/l |
| STOP | 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.

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]20A 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] and 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] and 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:

- $[\text{PC}]$ is within the indicated range (see label).
- $[\text{CAL}]_4$ does not fall below an absorbance value of 0.6.
- The absorbances of $[\text{CAL}]_1$ - $[\text{CAL}]_4$ keep raising.

In order to improve accuracy of the test results we recommend to run $[\text{CAL}]_1$ - $[\text{CAL}]_4$, $[\text{PC}]$ and patient samples in duplicate.

Interpretation of Results
Plot measured absorbances against concentrations of $[\text{CAL}]_1$ - $[\text{CAL}]_4$ ($0 \mu g/ml$ (blank), $25 \mu g/ml$, $50 \mu g/ml$, $100 \mu g/ml$, $200 \mu g/ml$) 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 $40 \mu g/ml$ (cut-off value) are considered as elevated. Values exceeding $[\text{CAL}]_4$ are to be reported as $>200 \mu g/ml$. 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:

References