IMTEC-Complement Activity

Complement
ELISA for the Quantitative Determination of Complement Activity

Package Size

REF	ITC59035	96 Tests	Testkit
IVD			

Please read the instructions carefully before testing.

Procedural precautions:

Do not use the reagents beyond the date of expiry.

DIL DB15, 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-Complement Activity is an enzyme immunoassay (ELISA) for the quantitative determination of the total classical complement activity in human serum. The assay is intended for in vitro diagnostic use only as an aid in the determination of a complement deficiency.

The complement system consists of at least 20 plasma proteins. It is responsible both for the elimination of circulating immune complexes over the classic pathway of the complement activation and for the defence against infectious pathogens over the alternative pathway of the complement activation. Both pathways of complement activation lead into the formation of the terminal lysis complexes (membrane attack complexes).

Decreased complement activity is of particular clinical significance. A hereditary defect of complement proteins and/or control proteins, can lead to recurrent infections, autoimmune diseases, and angioneurotic oedemas. Circulating immune complexes and bacterial infections can cause abnormally low levels of complement in the blood (hypocomplementemia). The complement activity in the serum reflects the functional condition of the complement system.

The IMTEC-Complement Activity test makes it possible to determine the total extent of activation of the complement system.

Principle

The assay procedure is based on the activation of complement in serum samples applied to a microtiter plate coated with a complement-activating substance. After completion of the entire complement cascade (from C1 to C9), the terminal activated C9 is labelled using a monoclonal antibody against a neoepitope of C9.

The label itself is detected with an anti-mouse IgG antibody conjugated with peroxidase. After addition of substrate solution, a colour appears. Following the addition of stop solution, the colour switches from blue to yellow which intensity is proportional to the concentration of the C9 neoepitope which itself, reflects the state of complement system activation. The test therefore correlates with a determination of hemolytic activity of the complement system (CH50 test).

Reagents and Contents

MTP	12	**Microtiter Strips** (in 1 strip holder) 8-well snap-off strips, ready for use coated with complement activator
CAL 4	3 x for 1.5 ml	**Calibrator 4** lyophilised human serum pool
PC	3 x for 2.5 ml	**Control Sample,** lyophilised, reduced complement activity Concentrations are stated on the labels.
WASH 20x WB03	50 ml	**Washing Buffer** (black cap) Concentrate (20x) for 0.5 l TRIS buffer pH 6.9 ± 0.2
DIL DB15	100 ml	**Dilution Buffer** (blue cap) ready for use TRIS buffer pH 7.3 ± 0.2
Anti-C9	12 ml	**anti-C9 Solution** (red cap), ready for use
CON	15 ml	**Conjugate Solution** (white cap) anti-mouse-IgG HRP conjugate, ready for use

SUB TMB ELISA	15 ml	**TMB Solution** (black cap) ready for use, pH 3.7 ± 0.2 colourless to bluish 3,3', 5,5'-tetramethylbenzidin 1.2 mmol/l Hydrogen peroxide 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 human Controls and calibrators were prepared from blood donations and 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

Equilibrate DIL on ice prior to the preparation of calibrators and control dilutions.

Equilibrate lyophilisates, as well as the dilution buffer (stored at 2...8°C) on ice prior to use.

Allow all other components to reach room temperature before use! Used bottles should be closed tightly 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.

Calibrators S1 - S4 (CAL 4) and PC

Perform reconstitution, pipetting, handling and storage of S4 (CAL 4) and PC on ice prior to use.

PC: Dissolve the lyophilisate of one bottle PC with exactly 2.5 ml ice chilled DIL. Swivel carefully from time to time for 20 min., avoid foaming. **DO NOT VORTEX.**

Calibrator S4 (200 U/ml): Dissolve the lyophilisate of one bottle CAL 4 with exactly 1.5 ml ice chilled DIL. Swivel gently from time to time for 20 min, avoid foaming. **DO NOT VORTEX.**

Prepare calibrators **S1** to **S3** on ice by diluting **S4** (200 U/ml) with ice chilled DIL:

0.5 ml **S4**	+	0.5 ml DIL	:	**S3** (100 U/ml),
0.5 ml **S3**	+	0.5 ml DIL	:	**S2** (50 U/ml),
0.5 ml **S2**	+	0.5 ml DIL	:	**S1** (25 U/ml).

Do not re-use Calibrators S1 to S4 and PC.

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

Use sera freshly collected or freeze samples **for extended storage** at -70°C. **In case sera were stored at –20°C, freeze and thaw only once**. Do not use serum samples inactivated by heat treatment at 56°C.

Store, dilute and handle sera on ice to prevent in vitro complement activation.

Dilute sera **1:51 on ice** with ice cold sample buffer DIL just before starting the test (mix 10 µl serum with 0.5 ml DIL).

Procedure

- **Pipette 100 µl** of **diluted serum,** CAL and PC into MTP, for blank use DIL instead of serum dilution, seal MTP with adhesive strip.
- Incubate for **1 hour** at 37°C.
- 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** of Anti-C9, seal MTP with adhesive strip.
- Incubate for **1 hour** at 37°C.
- 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 **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** 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..

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).
- S4 does not fall below an absorbance value of 0.6.
- The absorbances of **S1–S4** keep raising.

In order to improve accuracy of the test results we recommend to run **S1**–**S4**, PC and patient samples in duplicate.

Interpretation of Results

Plot the measured absorbances against concentrations **S1**-**S4** (25 (S1), 50 (S2), 100 (S3), 200 (S4) U/ml) in semi-log.

By interpolating the plotted measuring points, a calibration curve is obtained, from which the concentrations of activated C9 in the patient samples can be determined.

The normal range of complement activity (40-200 U/ml) was established using serum samples obtained from apparently healthy blood donors.

Performance Characteristics

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

www.human.de/data/gb/vr/el-59035.pdf or

www.human-de.com/data/gb/vr/el-59035.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**

H315 Causes skin irritation.

H319 Causes serious eye irritation.

SUB **Danger**

H360D May damage the unborn child.

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.

All donor units of human origin have been tested for HBsAg, HIV and HCV-antibodies and found to be negative using approved methods. However, the material should still be regarded as potentially infectious.

References

1. Zwirner J. *et al.*, J. Immunol. Methods **211**, 183–190 (1998)
2. Pickering M.C., Walport M.J., Rheumatology **39**, 133-141 (2000)

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Human Gesellschaft für Biochemica und Diagnostica mbH
Max-Planck-Ring 21 · 65205 Wiesbaden · Germany
Telefon +49 6122-9988-0 · Telefax +49 6122-9988-100 · e-Mail human@human.de