IMTEC-COMPLEMENT ACTIVITY

Complement ELISA for the Quantitative Determination of Complement Activity

Package Size

Package Code: ITC59035

Reagent Kit: 96 Tests

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 defense 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 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 changes 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

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Microliter Strips (in 1 strip holder)</td>
<td>12</td>
</tr>
<tr>
<td>CAL</td>
<td>Calibrator 4</td>
<td>3 x for 1.5 ml</td>
</tr>
<tr>
<td>PC</td>
<td>Control Sample, lyophilised</td>
<td>3 x for 2.5 ml</td>
</tr>
<tr>
<td>WASH</td>
<td>Washing Buffer</td>
<td>50 ml</td>
</tr>
<tr>
<td>WB03</td>
<td>TRIS buffer</td>
<td>20x</td>
</tr>
<tr>
<td>OIL</td>
<td>Dilution Buffer</td>
<td>100 ml</td>
</tr>
<tr>
<td>DB15</td>
<td>ready for use</td>
<td>20x</td>
</tr>
<tr>
<td>Anti-C9</td>
<td>anti-C9 Solution (red cap)</td>
<td>12 ml</td>
</tr>
<tr>
<td>CON</td>
<td>Conjugate Solution (white cap)</td>
<td>15 ml</td>
</tr>
</tbody>
</table>

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 HIV and HCV. Do not re-use any test kit lot.

Procedure

1. Prepare calibrators and standards.
2. Allow all other components to reach room temperature before use! Used bottles should be closed tightly and stored at 2-8°C.

Perform reconstitution, pipetting, handling and storage of S4 (CAL4) and PC on ice prior to use.

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

Prepare calibrators S1 to S4 on ice by diluting S4 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

Any crystallised salt inside the bottle must be resolved before use. Dilute 1 part with 19 parts distilled water. 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 just before starting the test (mix 10 µl serum with 0.5 ml dil:)

Procedure

1. Pipette 100 µl of diluted serum, (CAL) and (PC) into (MTP) for blank use instead of serum dilution, seal (MTP) with adhesive strip.

2. Incubate for 1 hour at 37°C.

3. Discard the solution from (MTP) and wash (MTP) 3 times using 300 µl per well.

4. 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

References