Complement C4
For the Quantitative Determination of Human Complement C4 in Serum

INTENDED USE
For the quantitative determination of human complement C4 (4th complement component) in serum by immunoturbidimetric assay. Complement is a group of serum proteins that destroy infectious agents. Measurement of these proteins aids in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY
C4 is the fourth complement component. It is one of a group of serum proteins that are active in the body's immune response to destroy infectious agents. Measurement of the level of the 4th complement component (C4) in serum can aid in the diagnosis of immunological disorders, especially those associated with deficiencies of complement components. The K-ASSAY® Complement C4 assay is intended for the quantitative determination of human complement C4 by immunoturbidimetric assay. The antiserum used in the kit was produced against purified human complement C4. The complement C4 antibody interacts with the complement C4 in the serum forming immune complexes. The immune complexes cause an increase in light scattering that correlates with the concentration of serum complement C4.

The K-ASSAY® Complement C4 assay has been measured using a variety of methods, including radioimmunoassay (RIA), radial diffusion, nephelometric, and enzyme-linked immunosorbent assay. The K-ASSAY® Complement C4 assay uses an immunoturbidimetric assay format.

PRINCIPLE OF TEST
The K-ASSAY® Complement C4 assay quantifies the 4th complement component in the patient’s serum based on immunoturbidimetric assay. Calibrators, controls, and patient samples are pipetted into sample cups. Microliter volumes of samples and antibody diluent are automatically pipetted into individual cuvettes. Following an initial incubation and measurement of sample blank, antiserum is added to the cuvettes. The samples (antigen) and antiserum are then mixed in the reaction cuvettes. Insoluble antigen-antibody (immune) complexes form. The immune complexes cause an increase in light scattering that correlates with the concentration of serum complement C4. Following an incubation period lasting approximately 5 min., the absorbance of the solution is measured at 600 nm.

A calibration curve is generated by assaying a series of calibrators with known concentrations of proteins and using the instrument's data reduction capability or manually plotting the change in absorbance versus concentration. Concentration of the control and patient samples are interpolated from the calibration curve. The antiserum used in the kit is a goat polyclonal antibody specific for human complement C4.

The K-ASSAY® Complement C4 assay can be run using a two-reagent clinical chemistry analyzer. Six calibrators are provided in the K-ASSAY® Multi-Analyte Calibrator. These calibrators are used to prepare a calibration curve for quantifying the levels of complement C4 present in the patient's serum sample.

KIT COMPOSITION
Reagents (Liquid Stable)
R1: Buffer Reagent 4 x 20 mL
Tris(hydroxymethyl)aminomethane
R2: Antiserum Reagent 2 x 10 mL
Anti-human complement C4 goat antiserum (40%)

WARNINGS AND PRECAUTIONS
FOR IN VITRO DIAGNOSTIC USE. Rx only.

To not be used internally in humans or animals. Normal precautions exercised in handling laboratory reagents should be followed.

Do not mix or use reagents from one test kit with those from a different lot number.

Do not use reagents past their expiration date stated on each reagent container label.

Do not pipette by mouth. Avoid ingestion and contact with skin.

Reagents in this kit contain sodium azide as a preservative. Sodium azide may form explosive compounds in metal drain lines. When disposing of reagents through plumbing fixtures, flush with copious amounts of water. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control, Atlanta, Georgia.

REAGENT PREPARATION
The reagents are ready to use. They do not need to be reconstituted.

STORAGE AND HANDLING
All reagents should be stored refrigerated (2-8°C). Return all reagents to 2-8°C promptly after use. Unopened reagents can be used for up to 18 months from the date of manufacture, as indicated by the expiration date on the package and bottle labels.

REAGENT STABILITY
Opened reagents can be used for 1 month if stored at 2-8°C. Discard reagents if they become contaminated. Evidence of cloudiness or particulate matter in solution is cause to discard.

INSTRUMENT
Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 600 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements

PRINCIPLES OF operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and/or equipment to be used
f) Operational precautions, limitations, and hazards

Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION
It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum is required for this assay. Soon after the blood is drawn, it should be allowed to clot, centrifuged, and the serum separated from the clot to a plastic tube (not glass) within 2 hours. Serum should be stored refrigerated (2-8°C) and can be used within 1 week or should be stored frozen for up to 2 months.

Use plastic tubes for storing the sample, do not use glass.

AUTOMATED ANALYZER APPLICATION
Suitable for two-reagent automated analyzers that use a multi-point calibration method.

PROCEDURE
Materials Supplied
Reagent 1 (R-1) Buffer Reagent 4 x 20 mL
Reagent 2 (R-2) Antiserum Reagent 2 x 10 mL

Materials Required But Not Supplied
Calibrators: K-ASSAY® Multi-Analyte Calibrator, Cat. No. KAI-016C. (6 calibrators containing human serum with known levels of complement C4).

Two Reagent Clinical Chemistry Analyzer:
Capable of accurate absorbance readings at 600 nm
Capable of accurately dispensing the required volumes
Capable of maintaining 37°C

ASSAY PROCEDURE
Note: Allow all reagents and specimens to warm to room temperature. Mix all reagents gently before using.

An example of automated application (Hitachi 717):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>250</td>
</tr>
<tr>
<td>R2</td>
<td>70</td>
</tr>
<tr>
<td>Wash</td>
<td>37 °C, 5 min.</td>
</tr>
</tbody>
</table>

2-point endpoint, 600 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Parameter</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hitachi 717</td>
<td>Temperature</td>
<td>37°C</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Test</td>
<td>(C4)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Sample Volume</td>
<td>(24) (50)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>R-1 Volume</td>
<td>(250) (1)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>R-2 Volume</td>
<td>(70) (1)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Wavelength</td>
<td>(600)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Calibration Method</td>
<td>Nonlinear (1) (6)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>STD.1 Conc.-POS.</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>STD.2 Conc.-POS.</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>STD.3 Conc.-POS.</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>STD.4 Conc.-POS.</td>
<td>4 (4)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>STD.5 Conc.-POS.</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>STD.6 Conc.-POS.</td>
<td>6 (6)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>SD Limit</td>
<td>999</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Duplicate Limit</td>
<td>10000</td>
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<tr>
<td>Hitachi 717</td>
<td>Sensitivity Limit</td>
<td>0</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Abs. Limit (Slope)</td>
<td>32000</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Prozone Limit</td>
<td>(32000)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Expected Value</td>
<td>(99999)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Panic Value</td>
<td>(99999)</td>
</tr>
<tr>
<td>Hitachi 717</td>
<td>Instrument Factor</td>
<td>1.00</td>
</tr>
</tbody>
</table>

1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION
It is recommended that complement C4 levels be determined using a multi-point calibration curve prepared using the K-ASSAY® Multi-Analyte Calibrator. It is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed. Initially, calibration should be performed each day.
QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the stated values assigned to the controls. The validity of the assay is in question if the value for the controls generated by the assay's calibration curve does not fall within the stated range. Recalibrate if the value determined for the controls falls outside the stated recovery range.

RESULTS / CALCULATIONS

Complement C4 levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measurable range for complement C4 is between 8 - 80 mg/dL. Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

If the complement C4 concentration of a patient sample is greater than the highest calibrator value, dilute 1 part sample with 1 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 2 to compensate for the dilution.

PERFORMANCE

Precision

The precision for the K-ASSAY® Complement C4 assay was determined using packaged reagents, pooled human serum, and a Hitachi 717 analyzer.

Accuracy / Correlation

A comparison of the K-ASSAY® Complement C4 assay and an Incstar Complement C4 Test Kit was performed using a Hitachi 717. The test results provided the following data:

\[ y = 0.739x + 2.46 \]
\[ r = 0.988 \]
\[ n = 50 \]
\[ x = INCSTAR Complement C4 Test Kit \]
\[ y = K-ASSAY® Complement C4 assay \]

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample I</td>
<td>20</td>
<td>5.7</td>
<td>0.29</td>
<td>5.17</td>
</tr>
<tr>
<td>Sample II</td>
<td>20</td>
<td>36.3</td>
<td>0.76</td>
<td>2.10</td>
</tr>
<tr>
<td>Sample III</td>
<td>20</td>
<td>58.6</td>
<td>1.98</td>
<td>3.38</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample I</td>
<td>10</td>
<td>6.51</td>
<td>0.24</td>
<td>3.72</td>
</tr>
<tr>
<td>Sample II</td>
<td>10</td>
<td>30.6</td>
<td>1.18</td>
<td>3.87</td>
</tr>
<tr>
<td>Sample III</td>
<td>10</td>
<td>53.2</td>
<td>1.12</td>
<td>2.10</td>
</tr>
</tbody>
</table>

Assay Range

8 - 80 mg/dL

INTERFERENCE

- Bilirubin C: No interference up to 20 mg/dL
- Bilirubin F: No interference up to 20 mg/dL
- Hemoglobin: No interference up to 500 mg/dL
- Intralipid: No interference up to 500 mg/dL

EXPECTED VALUE

The expected value as reported is between 14 - 39 mg/dL. Each laboratory should establish its own expected values using this kit.

REFERENCES


LABELING SYMBOLS

G / TECHINCAL INFORMATION

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive
Seattle, WA 98168 USA
TEL: (206) 575-8068 / (800) 526-4925
FAX: (206) 575-8094

ORDERING / PRICING / TECHNICAL INFORMATION

Advena Ltd.
Tower Business Centre, 2nd Flr.,
Tower Street, Swatar, BKR 4013 Malta