K-ASSAY® Cystatin C

For the Quantitative Determination of Human Cystatin C in Serum or Plasma

INTENDED USE
For the quantitative determination of human cystatin C in serum, EDTA plasma, or lithium heparin plasma by immunoturbidimetric assay. Cystatin C measurements are used as an aid in the diagnosis and treatment of renal diseases. FOR IN VITRO DIAGNOSTIC USE.

INTRODUCTION AND SUMMARY
Cystatin C is a small, 13.4 kDa, non-glycosylated basic protein belonging to the cystatin super-family of cysteine protease inhibitors. Cystatin C is produced by virtually all nucleated cells, and is present in all investigated body fluids. The production rate is constant and is unaffected by inflammatory processes, gender, age, and muscle mass. In normal kidneys, cystatin C is almost freely filtered through the glomerular membrane and then nearly completely reabsorbed and degraded by proximal tubular cells. Therefore, the plasma concentration of cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making cystatin C an excellent indicator of GFR function. Numerous studies and a meta-analysis incorporating 4,492 subject samples have shown that serum cystatin C is superior to serum creatinine as a marker for GFR function.1

PRINCIPLE OF TEST
The K-ASSAY® Cystatin C quantifies the cystatin C in the patient’s serum or plasma based on immunoturbidimetric assay.

The cystatin C reagent contains a suspension of latex particles coated with purified goat anti-human cystatin C polyclonal antibodies. A sample is mixed with this suspension. The resulting immune complexes are measured by turbidimetry. The signal generated is correlated with the concentration of cystatin C in the sample. By interpolation on a standard curve, the concentration of cystatin C in the sample is calculated.

The K-ASSAY® Cystatin C assay can be run using a two-reagent clinical chemistry analyzer. Six calibrators are provided in the K-ASSAY® Cystatin C Calibrator. These calibrators are used to prepare a calibration curve for quantifying the levels of cystatin C present in the patient’s serum or plasma sample.

KIT COMPOSITION
Reagents (Liquid Stable)

R1: Buffer Reagent, pH 7.5
HEPES (100 mM)
< 0.1% Sodium Azide

R2: Latex Suspension, pH 6.0
Latex particles coated with goat anti-human cystatin C antibodies (0.20% w/v) MES (25 mM)

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

Not to 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
Reagents are ready to use and do not require reconstitution.

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 package and bottle labels.

REAGENT STABILITY
Opened reagents can be used for one month if stored at 2-8°C. Discard reagents if they become contaminated.

Evidence of obvious precipitation in reagent 2 (R-2) solution is cause to discard.

INSTRUMENT
Measurement of absorbance is to be made with an instrument able to accurately read absorbance at 570 nm. Refer to the instrument manual from the manufacturer regarding the following:

a) Use or function
b) Installation procedures and requirements
c) Principles of operation
d) Performance characteristics, operating instructions
e) Calibration procedures including materials and equipment to be used
f) Operational precautions, limitations, and hazards
g) Service and maintenance information

SPECIMEN COLLECTION AND PREPARATION
It is recommended that specimen collection be carried out in accordance with CLSI document M29-A3. No method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood samples should be considered potentially infectious.

Serum or plasma (EDTA or lithium heparin) test samples must be collected in the manner routinely used for clinical laboratory tests. Freshly drawn serum is preferred and should be used within the day of collection. Samples may also be stored refrigerated (2-8°C) for five days or at -30°C for up to 1 year. Avoid excessive freeze/thaw of specimens.1,4

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

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

PROCEDURE
Materials Supplied

KAI-073, Cystatin C

KAI-074, Cystatin C (L)

Materials Required But Not Supplied
Calibrators: K-ASSAY® Cystatin C Calibrator, Cat. No. KAI-0996 (6 calibrators containing known amounts of human cystatin C). Two-Reagent Clinical Chemistry Analyzer: Capable of accurate absorbance readings at 570 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 917):

Sample 3.0 µL
• R1 (Buffer Reagent) 120 µL
37 °C, 5 min.
• R2 (Latex Suspension) 120 µL
2-point endpoint, 570 /800 nm

Automated Method (Example)

Chemistry Parameters for Automatic Analyzer

<table>
<thead>
<tr>
<th>INSTRUMENT</th>
<th>Roche / Hitachi 917</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEMPERATURE</td>
<td>37°C</td>
</tr>
<tr>
<td>TEST (CysC)</td>
<td></td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT END) (10)</td>
</tr>
<tr>
<td>(18)</td>
<td>(28)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(800)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(120)</td>
</tr>
<tr>
<td>REAGENT VOL (R1)</td>
<td>(120)</td>
</tr>
<tr>
<td>REAGENT VOL (R2)</td>
<td>(0)</td>
</tr>
<tr>
<td>REAGENT VOL (R3)</td>
<td>(120)</td>
</tr>
<tr>
<td>REAGENT VOL (R4)</td>
<td>(0)</td>
</tr>
<tr>
<td>ABS. LIMIT (SLOPE)</td>
<td>(32000)</td>
</tr>
<tr>
<td>PROZONE LIMIT</td>
<td>(-32000)</td>
</tr>
<tr>
<td>CALIB. TYPE</td>
<td>(SP LINE)</td>
</tr>
<tr>
<td>POINT</td>
<td>(6)</td>
</tr>
<tr>
<td>SPAN POINT</td>
<td>(6)</td>
</tr>
<tr>
<td>SD LIMIT</td>
<td>(999)</td>
</tr>
<tr>
<td>DUPLICATE LIMIT</td>
<td>(10000)</td>
</tr>
<tr>
<td>SENSITIVITY LIMIT</td>
<td>(0)</td>
</tr>
<tr>
<td>STABS RANGE</td>
<td>(-32000)</td>
</tr>
<tr>
<td>INSTRUMENT FACTOR</td>
<td>a=1.0)</td>
</tr>
<tr>
<td>UNIT</td>
<td>(mg/L)</td>
</tr>
<tr>
<td>STD.(1) Conc.-POS.</td>
<td>(*1) - (1)</td>
</tr>
<tr>
<td>STD.(2) Conc.-POS.</td>
<td>(*2) - (2)</td>
</tr>
<tr>
<td>STD.(3) Conc.-POS.</td>
<td>(*3) - (3)</td>
</tr>
<tr>
<td>STD.(4) Conc.-POS.</td>
<td>(*4) - (4)</td>
</tr>
<tr>
<td>STD.(5) Conc.-POS.</td>
<td>(*5) - (5)</td>
</tr>
<tr>
<td>STD.(6) Conc.-POS.</td>
<td>(*6) - (6)</td>
</tr>
</tbody>
</table>

1-6: Input concentration of calibrators.

Parameters for other automated analyzers are available.

CALIBRATION
It is recommended that cystatin C levels be determined using a multi-point calibration curve prepared using the K-ASSAY® Cystatin C Calibrator. On the Roche / Hitachi 917, calibration curves were found to be stable for up to
one month. However, it is recommended that the user determine calibration curve frequency as this depends on the instrument and type/number of other assays being performed.

QUALITY CONTROL

Normal and abnormal controls of known concentration should be included in each assay performed. These controls should fall within the ranges established by each lab for the particular lot of 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 established recovery range.

RESULTS / CALCULATIONS

Cystatin C levels are determined using the prepared calibration curve.

LIMITATIONS OF PROCEDURE

The measuring range for cystatin C is between 0.40 and 8.00 mg/L (0.34 - 6.80 mg/L ERM-DA471/IFCC Standardized). Grossly lipemic samples and samples with very high triglyceride concentrations should be diluted 1 part sample with 4 part isotonic saline or filtered to decrease nonspecific light scattering. Multiply results by 5 to compensate for the dilution. If the cystatin C concentration of a patient sample is greater than 8.00 mg/L (6.80 mg/L ERM-DA471/IFCC Standardized), dilute 1 part sample with 3 parts isotonic saline or filtered to standardize. Multiply results by 4 to compensate for the dilution.

PERFORMANCE

Precision

The precision for the K-ASSAY® Cystatin C assay was determined using packaged reagents, control material, and a Roche / Hitachi 917 analyzer according to the CLSI EP5-A2 guideline.

<table>
<thead>
<tr>
<th>Sample</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>80</td>
</tr>
<tr>
<td>Mean (mg/L)</td>
<td>0.511</td>
<td>0.968</td>
<td>1.999</td>
<td>4.389</td>
</tr>
<tr>
<td>Within Run S.D.</td>
<td>0.006</td>
<td>0.007</td>
<td>0.013</td>
<td>0.030</td>
</tr>
<tr>
<td>Between Run S.D.</td>
<td>0.005</td>
<td>0.024</td>
<td>0.019</td>
<td>0.079</td>
</tr>
<tr>
<td>Between Run C.V. %</td>
<td>1.094</td>
<td>0.712</td>
<td>0.640</td>
<td>0.690</td>
</tr>
<tr>
<td>Between Day S.D.</td>
<td>0.005</td>
<td>0.017</td>
<td>0.014</td>
<td>0.023</td>
</tr>
<tr>
<td>Between Day C.V. %</td>
<td>0.928</td>
<td>1.776</td>
<td>0.707</td>
<td>0.525</td>
</tr>
<tr>
<td>Total S.D.</td>
<td>0.007</td>
<td>0.024</td>
<td>0.027</td>
<td>0.088</td>
</tr>
<tr>
<td>Total C.V. %</td>
<td>1.421</td>
<td>2.462</td>
<td>1.353</td>
<td>2.008</td>
</tr>
</tbody>
</table>

Accuracy / Correlation

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP9-A2 guideline. A comparison of the K-ASSAY® Cystatin C and another company’s cystatin C assay was performed with the following results:

\[ y = 1.0093x + 0.411 \]

\[ r = 0.9983 \]

\[ n = 50 \]

\[ x = \text{another company’s Cystatin C assay} \]

\[ y = \text{K-ASSAY® Cystatin C Assay} \]

\[ x_{\text{min}} = 0.41 \quad y_{\text{min}} = 0.40 \]

\[ x_{\text{max}} = 7.43 \quad y_{\text{max}} = 7.88 \]

\[ \text{mean} = 2.650 \quad \text{mean} = 2.633 \]

Linearity

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP7-A2 guideline with the following results.

\[ \text{Linearity: 0.06 - 8.00 mg/L} \]

\[ (0.05 - 6.80 \text{ mg/L}^{*}) \]

\[ \text{Limit of Blank (LoB) = 0.012 mg/L} \]

\[ (0.010 \text{ mg/L}^{*}) \]

\[ \text{Limit of Detection (LoD) = 0.024 mg/L} \]

\[ (0.020 \text{ mg/L}^{*}) \]

(‘’ ERM-DA471/IFCC Standardized)

INTERFERENCE

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP17-A guideline with the following results.

- Bilirubin, Conjugated: No interference up to 60 mg/dL
- Bilirubin, Unconjugated: No interference up to 60 mg/dL
- Hemoglobin: No interference up to 900 mg/dL
- Lipemia: No interference up to 1,100 mg/dL
- Rheumatoid Factor: No interference up to 1,000 IU/L
- Triglycerides: No interference up to 1,500 mg/dL

MEASURING RANGE

Measuring Range: 0.40 - 8.00 mg/L (0.34 - 6.80 mg/L*)

(‘’ ERM-DA471/IFCC Standardized)

EXPECTED VALUE

The expected value as per the literature is between 0.5 and 1.0 mg/L.5 Due to population differences, each laboratory should establish its own expected values using this kit.

REFERENCES


LABELING SYMBOLS

LOT Lot Number

RT Reagent

REF Expiration or "Use By" Date

IVD For In Vitro Diagnostics Use

Potential Human Biohazard

Manufacturer

Consult Package Insert for Instructions for Use

Authorized Representative in the European Community

EU AUTHORIZED REPRESENTATIVE

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