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-cyslosylated 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.

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

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

Open 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/or 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.

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
Reagent 1 (R-1) Buffer Reagent 1 x 10 mL
Reagent 2 (R-2) Latex Suspension 1 x 10 mL
KAI-074, Cystatin C (L)
Reagent 1 (R-1) Buffer Reagent 2 x 10 mL
Reagent 2 (R-2) Latex Suspension 2 x 10 mL

Materials Required But Not Supplied

Calibrators: K-ASSAY® Cystatin C Calibrator, Cat. No. KAI-099C (6 calibrators containing known amounts of human cystatin C).

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

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

↓

- 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</td>
<td>(CysC)</td>
</tr>
<tr>
<td>ASSAY CODE</td>
<td>(2 POINT END) (10)</td>
</tr>
<tr>
<td>WAVELENGTH</td>
<td>(800) (570)</td>
</tr>
<tr>
<td>SAMPLE VOLUME</td>
<td>(30) (2) (0) (0)</td>
</tr>
<tr>
<td>REAGENT VOL(R1)</td>
<td>(2) (0)</td>
</tr>
<tr>
<td>REAGENT VOL(R2)</td>
<td>(0) (0)</td>
</tr>
<tr>
<td>REAGENT VOL(R3)</td>
<td>(120) (0)</td>
</tr>
<tr>
<td>REAGENT VOL(R4)</td>
<td>(0) (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 (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
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 3 parts isotonic saline and 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 and reassay. 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>N</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>Within Run C. %</td>
<td>1.094</td>
<td>0.712</td>
<td>0.640</td>
<td>0.690</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. %</td>
<td>1.066</td>
<td>2.496</td>
<td>0.960</td>
<td>1.811</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. %</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:

\[
y = 1.0093x + 0.411 \\
r = 0.9983 \\
n = 50 \\
x = \text{another company's Cystatin C assay} \\
y = \text{K-ASSAY® Cystatin C Assay}
\]

\[
x \text{ min} = 0.41 \\
x \text{ max} = 7.43 \\
x \text{ mean} = 2.650
\]

\[
y \text{ min} = 0.40 \\
y \text{ max} = 7.68 \\
y \text{ mean} = 2.633
\]

Linearity

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP8-A guideline on diluted samples and the CLSI EP17-A guideline with the following results.

Linearity: 0.06 - 8.00 mg/L (0.05 - 6.80 mg/L*)

Limit of Blank (LoB) = 0.012 mg/L

Limit of Detection (LoD) = 0.024 mg/L (0.020 mg/L*)

(* ERM-DA471/IFCC Standardized)

INTERFERENCE

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP7-A2 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.\textsuperscript{5} Due to population differences, each laboratory should establish its own expected values using this kit.

REFERENCES


LABELING SYMBOLS

- **LOT**: Lot Number
- **REF**: Catalog Number
- **IVD**: For In Vitro Diagnostics Use
- **EC/REP**: Authorized Representative in the European Community
- **EC/REP**: Potential Human Biohazard
- **EC/REP**: Manufacturer
- **EC/REP**: Consult Package Insert for Instructions for Use

EU AUTHORIZED REPRESENTATIVE

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

ORDERING / PRICING / TECHNICAL INFORMATION

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