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.

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.

PRINCIPLE OF TEST

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

KIT COMPOSITION

Reagents (Liquid Stable)

R-1: Buffer Reagent, pH 7.5
50 mM HEPES
1700 mM Sodium Chloride
≤ 0.1% Sodium Azide

R-2: Latex Suspension, pH 6.0
0.11% (w/v) solution of latex particles coated with goat anti-human cystatin C antibodies
25 mM MES

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.
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 parts 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 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>%</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>0.008</td>
<td>0.001</td>
<td>0.015</td>
<td>0.033</td>
</tr>
<tr>
<td>Within Run C.V. %</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.V. %</td>
<td>1.068</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>0.016</td>
<td>0.016</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>0.067</td>
<td>0.024</td>
<td>0.027</td>
<td>0.068</td>
</tr>
<tr>
<td>Total C.V. %</td>
<td>1.421</td>
<td>2.462</td>
<td>1.353</td>
<td>2.006</td>
</tr>
</tbody>
</table>

Accuracy / Correlation

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP6-A guideline. A comparison of the K-ASSAY® Cystatin C and another company’s cystatin C assay was performed with the following results:

\[
y = 1.0093x - 0.0411 \\
B = 0.9983 \\
N = 50 \\
x = another company’s cystatin C assay \\
y = K-ASSAY® Cystatin C Assay
\]

LINEARITY

Testing was performed on a Roche / Hitachi 917 analyzer according to the CLSI EP7-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 (0.010 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 EP8-A2 guideline on diluted samples and the CLSI EP17-A guideline with the following results.

<table>
<thead>
<tr>
<th>Interference</th>
<th>Concentration</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin, Conjugated</td>
<td>No interference up to 60 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Bilirubin, Unconjugated</td>
<td>No interference up to 60 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>No interference up to 900 mg/dL</td>
<td></td>
</tr>
<tr>
<td>Intrapluid</td>
<td>No interference up to 11 g/L</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid Factor</td>
<td>No interference up to 1,000 IU/L</td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>No interference up to 1,500 mg/dL</td>
<td></td>
</tr>
</tbody>
</table>

EXPECTED VALUES

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

REFERENCES