Intended Use

The Cystatin C Immunoassay on the Beckman Coulter* AU* Systems is an in vitro diagnostic test for quantitative determination of cystatin C in human serum and plasma. The measurement of cystatin C is used in the diagnosis and treatment of renal diseases.

Summary and Explanation of Test

The non-glycosylated basic protein, cystatin C (molecular weight 13.2 kD), is produced at a constant rate in nearly every nucleated cell in the human body [1]. It is freely filtered through a normal Glomerular membrane, and is then reabsorbed and almost entirely catabolized in the proximal tubules. Hence, the cystatin C concentration in human blood is closely related to glomerular filtration rate (GFR) [2]. A reduction in the GFR causes a rise in the concentration of cystatin C. The cystatin C concentration has not been shown to be significantly influenced by other factors such as muscular mass, inflammatory diseases, sex, age or diet [2, 3, 4].

Calibrator Standardisation

Gentian Cystatin C Calibrator is standardised against the international calibrator standard ERM-DA471/IFCC.

GFR Prediction Calculation

Several cystatin C based prediction equations for calculation of GFR for adults and children have been published. It should be noted that these formulas were evaluated with different cystatin C assays (particle-enhanced nephelometric immunoassay PENIA or particle enhanced turbidimetric immunoassay PETIA) and may reveal inaccurate GFR results if an inappropriate combination of formula and assay is used. For calculation of GFR from cystatin C values measured with the Gentian assay the following prediction equation is recommended using mg/L as the unit factor [9]: The equation is valid for persons above 14 years.

\[
GFR \left[ \frac{\text{mL/min}}{1.73 \text{ m}^2} \right] = \frac{79.901}{\text{Cystatin C (mg/L)}}^{1.4389}
\]

Assay Principle

Serum or plasma sample from human, cystatin C from the sample and anti cystatin C from the immunoparticles agglomerates. The complex particles created absorb light, and by turbidimetry the absorption is related to cystatin C concentration via interpolation on an established standard calibration curve. The AU* platforms, will automatically calculate the results.

Reagents Provided in Reagent Kit

Reaction Buffer 1 (R1):

Cystatin C Reaction buffer, 1 vials of 100mL, REF A52761. R1 is a MOPS [3-(N-Morpholino)-propane sulfonic acid] buffered saline, preserved with sodium azides (0.09 % (w/v)). The buffer is ready for use.

Reaction Buffer 2 (R2):

Cystatin C Immunoparticles, 2 vials of 10 mL, REF A52761. R2 contains immunoparticles, which is a purified immunoglobulin fraction that is directed against cystatin C, which is covalently attached to uniform polystyrene particles. Human cystatin C was used as immunogen in the process of generating the immunoparticles. It is provided as a ready to use suspension, preserved with 0.09 % (w/v) sodium azide and antibiotics.

Items required but not provided:

Gentian Cystatin C Reagent Kit
Gentian Cystatin C Control Kit, Low & High, vials of 1 mL

Warnings and Precautions

1. This test is for in vitro use only, and must be handled by qualified personnel.
2. Reagents contain antibiotics and must be handled with due caution.
3. Reagents contain sodium azide preservative and must be handled with due caution: Do not ingest or allow contact to skin or mucous membranes. The sodium azide concentration of this product is not characterised as dangerous. Although, accumulated NaN₃ in lead and copper pipes may cause generation of explosive metal azides. To prevent this, rinse thoroughly if discarded into the drain.
4. The immunoparticles contain substances of animal origin. Disposal of any discarded materials should be in accordance to local requirements.
5. Serum used in the manufacture of calibrators and controls was tested for hepatitis HBsAG, anti-HCV, anti-HIV1 and anti-HIV2 and found to be negative. Nevertheless, the materials contain substances of human and animal origin and must be handled with due care. Disposal of any discarded materials should be in accordance to local requirements.

Reagent Storage and stability

Shell life of unopened reagents at 2-8°C: See expiry date on the label. Stability after opening: Until expiry date at 2-8°C
On-board stability: 9 weeks at correct temperature (2-8°C)

Specimen Collection and Handling

Required sample material is human serum or EDTA/Heparinized plasma. It is recommended to analyse the samples as fresh as possible. However sample stability testing showed that cystatin C in serum and plasma samples are stable for 14 days at room temperature (8-25°C), 21 days if stored at 2-8°C, and if stored below -20°C for at least 10 years [10]. Mix samples well before analysing. The samples can be shipped without special cooling and must then be analysed within 14 days after shipment.

Assay Procedure

Application Notes/Assay Installation

A detailed Instrument Parameter list is available in the section “Instrument Parameters” below. The Application Note is also available at: www.gentian.no, Instrument set up, maintenance, operation and precautions must be handled in accordance with the Beckman Coulter* AU* instrument manuals.
Reagent Preparation
Gentian Cystatin C reagents are supplied ready to use. Mix gently before loading into instrument. Reagents should be stored capped at 2-8°C when not in use.

Establishment of the Calibration Curve
Use standards 1 to 6 to establish a 6-point standard curve as defined in the Beckman Coulter® AU® Systems Instrument Manuals. Calibrator values are lot dependent and a new calibration must be performed whenever a new calibration lot is used. The calibrator’s assigned values are given on the analytical value sheet provided with the calibrator.

QC Controls
The controls low and high must be assayed each day before any samples are assayed in order to calibrate the calibration curve. The controls have an assigned value range that must be met before measuring samples. The assigned values are given in the Analytical Value sheet included with the Gentian Cystatin C Control Kit (REF 1019). If the control values are not valid, repeat the control measurements. If the calibration cannot be performed without error, or valid control values cannot be reproduced, contact Beckman Coulter* for support.

Measuring Patient Samples
When a valid calibration has been performed and the control values are within the valid range, serum or plasma samples may be measured. Check that minimum volume of sample is present and assay the samples according to the instructions given in the Beckman Coulter® AU® Systems Instrument Manuals.

Results
The results are calculated automatically by the Beckman Coulter* AU* Systems. The results are presented in mg/L.

Measuring range
The measuring range of cystatin C for the assay is approximately 0.4 - 8.0 mg/L. The exact range is dependent on the calibrator set points of the Gentian Cystatin C Calibrator Kit lot number.

Reference intervals
Gentian follows the CLSI Guideline, C28-A2; How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline, Second edition to determine the transferability of the reference interval. The reference interval is based on a reference interval study performed at Växjö Hospital, Sweden, including serum samples from 138 self-declared healthy subjects 20-80 years of age. The samples were analysed for cystatin C on the AU* 2700 platform. The reference interval was calculated non-parametrically and was determined to be 0.53-1.01 mg/L. This represents the central 95% of the whole population tested. It is recommended that every laboratory should determine a local reference interval since values may vary depending on the population tested.
In a separate study involving 850 healthy children (46% boys, 54 % girls) in the age from 5 to 15 years, the reference range 0.51 - 1.05 mg/L was confirmed in all ages down to 5 years of age [11].

Limitations
The materials shall not be used past expiration date.

Additional Information
For more detailed information on AU* Systems, refer to the appropriate system manual.
Since Beckman Coulter* does not manufacture the reagent or perform quality control or other tests on individual lots, Beckman Coulter* cannot be responsible for the quality of the data obtained which is caused by performance of the reagent, any variation between lots of reagent, or protocol changes by the manufacturer.

Shipping Damage
Please notify your distributor if this product is received damaged.
For technical assistance please contact your local Beckman Coulter* Representative
For other languages visit: www.gentian.no/BCI_Applications

Bibliography
7. CLSI; Document EP7-A ; Interference testing in Clinical Chem ; Approved Guideline.

Symbols Key

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<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<tr>
<td>LOT</td>
<td>Lot number</td>
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<tr>
<td>ATP</td>
<td>Temperature limitations</td>
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<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
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<tr>
<td>E</td>
<td>Expiration date</td>
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<td>i</td>
<td>Consult Instructions for Use</td>
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<td>M</td>
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<td>REF</td>
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