

Cystatin C



Application Notes for Cystatin C Immunoassay on Beckman Coulter® IMMAGE® 800 SYSTEMS

Using Cystatin C Reagent Kit A52761 and IMMAGE User-Defined Reagent cartridges

Intended Use

The cystatin C immunoassay on Beckman Coulter® IMMAGE® 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 [5]: The equation is valid for persons above 14 years.

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

Assay Principle

Serum or plasma sample from human is mixed with cystatin C immunoparticles. Cystatin C from the sample and anti cystatin C from the immunoparticles aggregates. 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 IMMAGE® platforms, will automatically calculate the results.

Reagents Provided in Reagent Kit

Reaction Buffer 1 (R1)

Cystatin C Reaction buffer, 1 vial of 100mL. 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. 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.

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| Items included in CYSX IMMAGE Sales Group REF B69746: | |
|---|------------|
| Gentian Cystatin C Reagent Kit | REF A52761 |
| User-Defined Reagent Cartridge (pkg. of 10) | REF 447250 |
| Evaporation Caps (pkg. of 20) | REF 447170 |
| Items required (not included): | |
| Gentian Cystatin C Calibrator Kit (6 x 1 ml) | REF A52763 |
| Gentian Cystatin C Control Kit, low & high (2 x 1 ml) | REF A52765 |

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 characterized 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

Shelf life of unopened reagents at 2-8°C: See expiry date on the label.
Stability after opening: Until expiry date when using hard caps at 2 - 8°C.
In-use stability: 28 days when using evaporation caps.

Specimen Collection and Handling

Required sample material is human serum or EDTA/heparinized plasma. It is recommended to analyze the samples as fresh as possible. However, sample stability testing showed that cystatin C in serum and plasma samples are stable for 26 days at room temperature (8 - 25°C) or 26 days if stored at 2 - 8°C. Additionally, it has been published that samples can be stored below -70°C for up to 5 years [6]. Mix samples well before analyzing.

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.com. Instrument set up, maintenance, operation and precautions must be handled in accordance with the Beckman Coulter® IMMAGE® instrument manuals.

Reagent Preparation

Reagent is supplied ready for transfer into IMMAGE Reagent Cartridge. Transfer the contents of reagent 1 and reagent 2 into appropriate compartments of the User Defined Cartridge as shown in the table below and apply evaporation caps when in use. Use care to avoid contamination. Reagents should be stored with original hard caps at 2-8°C when not in use.

| Cystatin C Kit | Compartment A | Compartment B |
|---------------------|---------------|---------------|
| R1, Reagent Buffer | - | 8.5 mL |
| R2, Immunoparticles | 1.5 mL | - |

Establishing the Calibration Curve

Use standards 1 to 6 to establish a 6-point standard curve as defined in the Beckman Coulter® IMMAGE® 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. New calibration should be performed once every 4 weeks.

QC Controls

The controls low and high must be assayed each day before any samples are assayed in order to validate 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. 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® IMMAGE® systems Instrument Manuals.

Results

The results are calculated automatically by the Beckman Coulter® IMMAGE® systems. The results are presented in mg/L.

Limitations

The materials should not be used past expiration date.

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 136 self-declared healthy subjects 20-80 years of age. The samples were analyzed 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.

Performance Characteristics

All studies were performed using one lot of reagents in one laboratory unless otherwise stated.

Precision

The Gentian Cystatin C Immunoassay was used in a 3 day precision study designed in accordance with CLSI protocol EP5-A. Four serum pools and 2 control levels were measured on the Beckman Coulter® IMMAGE 800* system.

| ID | Mean value (mg/L) | Within run CV(%) | Total CV (%) | N |
|----|-------------------|------------------|--------------|----|
| P1 | 0.72 | 1.71 | 3.86 | 10 |
| P2 | 0.62 | 2.35 | 5.59 | 10 |
| P3 | 0.67 | 3.74 | 3.76 | 10 |
| P4 | 0.96 | 1.63 | 3.10 | 10 |
| P5 | 0.82 | 2.14 | 6.78 | 10 |
| P6 | 2.54 | 0.67 | 3.84 | 10 |

Linearity

The Gentian Cystatin C Immunoassay was used in a linearity study on the IMMAGE 800 system, a linear range of 0.35 – 6.79 mg/L was observed. Concentrations outside this range were not tested.

Analytical Recovery

Using the Gentian Cystatin C Immunoassay on a Beckman Coulter® IMMAGE 800 instrument, a recovery of 100-110% was observed.

Limit of Quantification

Limit of Quantification is defined as the lowest actual amount of an analyte that can be reliably detected and at which the total error meets the requirements for accuracy.

Using the Gentian Cystatin C assay on an IMMAGE 800 instrument, a lower limit of quantification was measured as 0.24mg/L.

Security Zone

In a study on IMMAGE 800, the security zone for antigen excess extended up to 43 mg/L using the Gentian Cystatin C assay.

Interference

In a study, no significant interference was detected with Hemoglobin (8 g/L), Intralipid (16 g/L) or Bilirubin (800 mg/L) in cystatin C samples. The interference study was designed in accordance with the protocol EP7-A from CLSI [7]. Previously, no significant interference was detected with the drugs tested as recommended in a publication by Sonntag and Scholer [8]. There is no RF interference present in the Gentian Cystatin C Immunoassay because the antibodies are made using avian antibodies (chicken) [9].

Instrument Variation

Instrument variation between Gentian Cystatin C on IMMAGE and Hitachi 917 instruments was measured and the results analyzed using Passing-Bablok regression analysis:

| Methods | N | Range specimen (mg/L) | Term | Coefficient | 95% CI of Coefficient |
|----------------------------|----|-----------------------|-----------|-------------|-----------------------|
| IMMAGE 800 vs. Hitachi 917 | 24 | 0.58 - 6.97 | Intercept | 0.08 | 0.05 - 0.11 |
| | | | Slope | 1.01 | 0.99 - 1.03 |

Additional Information

For more detailed information on IMMAGE® 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 held 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:

<https://www.gentian.com/clinical-diagnostic-products/beckman-coulter-customers-cystatin-c>

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Symbols Key



Lot number



Temperature limit



Use by date



Consult instructions for use



Manufacturer



Catalogue number



In vitro diagnostic medical device



Caution



Biological risks



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Instrument Settings for Cystatin C Immunoassay on Beckman Coulter® IMMAGE Systems*(IMMAGE 800)

Page 1

| | |
|----------------------------------|--|
| Test name: cysx | Unit: mg/L |
| Reagent lot number: * | Protocol: Non-competitive NIPIA |
| Cartridge number: ** | Reagent expiry date: * |
| Reagent series number: ** | Tests per lot: 40 |
| AGXS Limit: | AGXS Enabled: |

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| | |
|-------------------------------------|---------------------------------|
| Buffer: BUF10 | Diluent: DIL10 |
| Sample volume: 5 µl | Calibrator dilution: 1:1 |
| Reaction buffer volume: 0 µl | Sample dilution: 1:1 |
| Reagent A volume: 30 µl | Reaction time: 5 minutes |
| Reagent B volume: 195 µl | |

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| | |
|--|-------------------------|
| Levels: 6 | Calibrator Level |
| Replicates: 2 | Level 1: * |
| Level update: 3 | Level 2: * |
| Replicates: 2 | Level 3: * |
| Calibration Type: 3.order polynom | Level 4: * |
| | Level 5: * |
| | Level 6: * |

Slope and Offset (under 'Reagent and Calibration')

Factor a (a·x + b) : 1.10

Transfer Gentian Cystatin C Assay Buffer into compartment B and Gentian Cystatin C immunoparticles in compartment A. The maximum volume of compartment "A" and "B" of the UDR cartridge is 8.5 mL. Hence the maximum number of tests loaded into the cartridge is approximately 40 tests.

* Lot dependent

** User defined