Cystatin C

Gentian Cystatin C Reagent Kit

REF 1101

For *in vitro* diagnostic use by laboratory professionals.

This document describes the general use of the product above. For instrument specific settings, please refer to the application notes available upon request to marketing@gentian.com.

Intended purpose

The Gentian Cystatin C Immunoassay is an immunoturbidimetric assay intended for the *in vitro* quantitative determination of cystatin C in human serum and plasma on automated clinical analysers by laboratory professional users. 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, gender, age, or diet [2, 3, 4].

Calibrator standardisation

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

Relevant calculations

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

The Gentian Cystatin C Immunoassay is a Particle-Enhanced Turbidimetric Immunoassay (PETIA). The plasma or serum sample is mixed with cystatin C immunoparticles. Cystatin C from the sample and the anti-cystatin C antibodies from the immunoparticle solution bind to form aggregates that increase the turbidity of the solution. The degree of turbidity is proportional to the concentration of cystatin C, which can be quantified via an established standard calibration curve.

Assay kit components

Products provided	
Gentian Cystatin C Reagent Kit • R1 Assay Buffer (58 mL) • R2 Immunoparticles (10 mL)	REF 1101
Products required but not provided	
Gentian Cystatin C Calibrator Kit (6 levels x 1 mL), or	REF 1051
Gentian Cystatin C Calibrator (1 mL)	REF 1012
Gentian Cystatin C Control Kit (2 levels x 1 mL), or	REF 1019
Gentian Cystatin C Control Kit (2 levels x 5 mL)	REF 1026

All products are ready for use.

Composition

Reaction Buffer 1 (R1, 58 mL inactive ingredient): Gentian Cystatin C Assay Buffer. R1 is a MOPS [3-(N-Morpholino)-propane sulfonic acid] buffered saline, containing avian proteins and preserved with sodium azide (0.09 % (w/v)).

Reaction Buffer 2 (R2, 10 mL active ingredient): Gentian Cystatin C Immunoparticles. R2 contains a purified immunoglobulin fraction directed against human cystatin C, which is covalently attached to polystyrene nanoparticles. The solution is preserved with 0.09 % (w/v) sodium azide and antibiotics.

Warnings and precautions

1. Contains substances from human or animal origin and should be considered as potentially infectious material. Handle with caution and discard following local regulations.
2. The sodium azide concentration of the assay is not characterised as hazardous. Although, accumulated NaN_3 in lead and copper pipes may cause generation of explosive metal azides. To prevent this, rinse thoroughly if discarded into the drain.
3. Contains a sensitizing substance below concentration limit. May produce an allergic reaction in some people and may cause respiratory irritation if inhaled.
4. Contains antibiotics and must be handled with due caution.
5. Exposure may result in irritation of skin and eyes.
6. Avoid contact with incompatible materials.
7. Avoid exposure to heat and direct sunlight.

To obtain the SDS (Safety Data Sheet), please contact Gentian at marketing@gentian.com.

Additional handling instructions

1. This test is for *in vitro* use only and must be handled by laboratory professionals.
2. Use only validated and approved instrument applications.
3. Do not use products after the expiration date has passed.
4. Do not mix reagents of different lots or interchange caps of reagents, controls, calibrators, and lots.
5. Tighten caps carefully back on after use of reagents, calibrators, and controls to avoid evaporation.

Reagent storage and stability

All products provided for the Gentian Cystatin C Immunoassay must be stored at 2-8 °C. The expiry date is printed on the labels. Using a Cobas c501 instrument (Roche), the in-use stability of the Gentian Cystatin C

Reagent Kit was found to be at least 5 weeks performed as an on board study.

Specimen collection and handling

Required sample material is human serum or plasma. It is recommended to analyse the samples as fresh as possible. Sample stability testing showed that cystatin C in serum and plasma samples are stable for 14 days at room temperature (8-25 °C) and 21 days if stored at 2-8 °C. If stored below -70 °C, the samples are stable for at least 5 years [6]. Mix samples well before analysing. The samples can be shipped without special cooling and must then be analysed within 14 days after shipment.

Performance characteristics

The results refer to the main validation of the Gentian Cystatin C Immunoassay on an Architect c4000 instrument (Abbott) at one instrument site with one lot of reagent, unless otherwise stated. For the instrument specific performance characteristics, please refer to the instrument specific application notes.

Measuring range

The measuring range of the Gentian Cystatin C Immunoassay is approximately 0.40–8.22 mg/L, with a security zone of up to 10 mg/L. The exact measuring range is calibrator- and instrument specific, please refer to the analytical value sheet for the lot specific calibrator values (available on www.gentian.com) and the instrument specific application notes.

Analytical sensitivity

The analytical sensitivity of the Gentian Cystatin C Immunoassay was tested in a study based on the CLSI guideline EP17 [7]. The Limit of Quantification (LoQ) is defined as the lowest concentration of an analyte that can be reliably detected and at which the total error meets the requirements for accuracy. The LoQ was measured to be 0.40 mg/L. For the instrument specific LoQ, please refer to the instrument specific application notes.

Precision

The total precision of the Gentian Cystatin C Immunoassay was measured with a CV of <5 % in a study based on the CLSI guideline EP05 [8]. For the instrument specific total precision, please refer to the instrument specific application notes.

Analytical specificity and limitations

No interference is detected for this product with triglycerides, haemoglobin, intralipid, or bilirubin at the tested concentrations in a study based on the CLSI guideline EP07 [9]. For the instrument specific interference, please refer to the instrument specific application notes. There is no interference detected with the drugs tested on the recommendations from Sonntag and Scholer [10]. As the antibodies in the Gentian Cystatin C Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples [11].

Assay procedure

Application notes

Applications of the Gentian Cystatin C Immunoassay have been established on several clinical chemistry analysers. Detailed, validated application notes describing the procedures for installation and analysis on specific instruments are available upon request to marketing@gentian.com. For instructions on how to install a new application, consult the instrument manual. Maintenance, operation and precautions must be handled in accordance with the specific instrument manual.

Reagent preparation

The reagents are ready for use. Mix the reagents gently before placing them into the assigned reagent positions. The reagent bottles may fit directly into the instrument unless otherwise stated in the application notes.

Establishment of the calibration curve

Please refer to the instruction for use of the Gentian Cystatin C Calibrator (REF 1012 and REF 1051) available at www.gentian.com.

QC controls

Please refer to the instruction for use of the Gentian Cystatin C Control Kit (REF 1019 and REF 1026) available at www.gentian.com.

Measuring patient samples

When a valid calibration curve has been established and the control values are within the valid range, the plasma or serum sample may be measured. Ensure that the minimum sample volume is present in the sample cups/tubes and assay the samples according to the instructions given in the instrument manual.

Results

The results are calculated automatically by the instrument for all applications established for the Gentian Cystatin C Immunoassay. The results are presented in mg/L.

Clinical performance

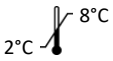
Sensitivity and specificity

With an eGFR cut off value of 60 ml/min/1.73 m^2 cystatin C has a sensitivity of 0.94 (95 % CI: 0.90-0.96) and specificity of 0.86 (95 % CI: 0.78-0.91) [12].

Reference intervals

The cystatin C reference intervals were determined in a study based on the CLSI guideline C28 [13] on an Architect ci8200 instrument (Abbott). The reference interval was determined from a population of ostensibly healthy subjects with no history of CKD. A total of 136 samples from individuals ranging in age from 20 to 84 years were measured. The samples used were serum samples. The reference interval was calculated non-parametrically and was determined to be 0.51-1.05 mg/L. This represents the central 95 % of the population. 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 [14].

Symbols key

	Temperature limit
	Use by date
	Consult instructions for use
	Manufacturer
	CE mark with Notified Body number
	UKCA mark
	Swiss authorized representative
	*In Vitro* Diagnostic medical device
	Lot number
	Catalogue number
	Unique Device Identifier
	Contents
	R1 Assay Buffer
	R2 Immunoparticles

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References

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Serious incidents

Please notify your manufacturer and competent authority if any serious incidents have occurred in relation to the device.

Modifications from previous version

- Added number of the Notified Body to CE mark
- Added UKCA mark
- Added chapter “Representatives”
- Minor editorial changes and corrections throughout the document

Date of issue

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