Cystatin C

Gentian Cystatin C Reagent Kit on Beckman Coulter® Synchron and UniCel Systems

REF A52761

For *in vitro* diagnostic use by laboratory professionals.

This document describes the general use and the instrument specific settings of the product above. For instrument specific settings for IMMAGE, please refer to the application note 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	BCI REF	Gentian REF
Gentian Cystatin C Reagent Kit for Beckman Coulter® Synchron and UniCel systems • R1 Assay Buffer (1 x 100 mL) • R2 Immunoparticles (2 x 10 mL)	A52761	1100
Product required but not provided		
Gentian Cystatin C Calibrator Kit (6 levels x 1 mL)	A52763	1051
Gentian Cystatin C Control Kit (2 levels x 1 mL)	A52765	1019
Items required but not provided		
User-Defined Reagent Cartridge (pkg. of 12)	442835	Not applicable

All products are ready for use.

Composition

Reaction Buffer 1 (R1, 100 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 azides (0.09 % (w/v)).

Reaction Buffer 2 (R2, 2 x 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.

For additional safety information, please refer to the SDS (Safety Data Sheet) available on www.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. The in-use stability of the Gentian Cystatin C Reagent Kit was found to be at least 9 weeks on an AU400 instrument 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

All results refer to validation of the Gentian Cystatin C Immunoassay on a Synchron LX20 instrument at one site with one lot of reagents, unless otherwise stated.

Measuring range

The measuring range of the Gentian Cystatin C Immunoassay was found to be 0.45-7.83 mg/L. The exact measuring range is specific to the calibrator, please refer to the analytical value sheet for the lot specific calibrator values available on www.gentian.com.

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 found to be 0.42 mg/L.

Linearity

The linearity range of the Gentian Cystatin C Immunoassay was found to be 0.45-7.83 mg/L in a linearity study based on the CLSI guideline EP06 [8].

Security zone

No antigen excess effect in samples below 25 mg/L was observed for the Gentian Cystatin C Immunoassay in a study including 12 samples of spiked serum. Samples with a cystatin C concentration above the highest calibrator and up to 25 mg/L return a value above the highest calibrator and are flagged for rerun with automatic dilution.

Precision

Precision of the Gentian Cystatin C Immunoassay was tested in a study based on the CLSI guideline EP05 [9]. 3 serum pools and 2 controls were measured 2 times with 2 replicates (n=12).

Sample ID	Mean [mg/L]	Within run CV [%]	Between run CV [%]	Total CV [%]
P1	3.71	0.67	1.95	2.21
P2	0.88	3.25	2.80	5.35
P3	6.63	1.40	1.44	2.85
CL	1.04	2.40	0.00	4.83
CH	3.89	1.88	1.58	3.30

Recovery

Recovery was analysed by spiking a low analyte sample with a high analyte sample according to Westgard [10]. The Gentian Cystatin C Immunoassay had a recovery of 96-106 %.

Analytical specificity and limitations

There is no interference detected with the drugs tested on the recommendations from Sonntag and Scholer [11]. As the antibodies in the Gentian Cystatin C Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples [12]. Interference was tested in a study based on the CLSI guideline EP7 [13]. No clinically relevant difference was detected at the tested interferent concentrations.

Potential interferents	Concentration with no interference
Triglycerides	17 mmol/L
Haemoglobin	4 g/L
Intralipid	12 g/L
Bilirubin	0.2 g/L

Instrument variation

Results obtained with the Gentian Cystatin C Immunoassay on the Synchron LX20 instrument were compared using Passing-Bablok regression with results from the Architect ci8200 instrument (Abbott Laboratories) in a study based on the CLSI guideline EP09 [14].

n	Range of samples [mg/L]	Term	Co-efficient	95% CI
47	0.71-7.91	Intercept	0.17	[0.13, 0.22]
		Slope	0.95	[0.93, 0.97]

Assay procedure

A detailed instrument parameter list is available in the section "Instrument Settings" below. Instrument set up, maintenance, operation and precautions must be handled in accordance with the Beckman Coulter® Synchron and UniCel system's instrument manuals.

Reagent preparation

The reagents are supplied ready for use. Transfer the content of Reagent 1 and Reagent 2 into appropriate compartments of the user defined cartridges as shown in the table below. Use care to avoid contamination.

Gentian Cystatin C Reagent Kit	Compartment A	Compartment B
R1, Assay Buffer	25 mL	-
R2, Immunoparticles	-	5 mL

Establishment of the calibration curve
Please refer to the instruction for use of the Gentian Cystatin C Calibrator Kit REF A52763 available on www.gentian.com.

QC controls
Please refer to the instruction for use of the Gentian Cystatin C Control Kit REF A52765 available on 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) [15].

Reference intervals
The cystatin C reference intervals were determined in a study based on the CLSI guideline C28 [16] 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 [17].

Additional information
For more detailed information on Synchron and UniCel 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 local distributor if this product is received damaged. For technical assistance please contact your local distributor.

Symbols key

2°C 8°C	Temperature limit
	Use by date
	Consult instructions for use
	Manufacturer
CE 0123	CE mark with Notified Body number
UK CA	UKCA mark
CH REP	Swiss authorized representative
IVD	*In Vitro* Diagnostic medical device
LOT	Lot number
REF	Catalogue number
UDI	Unique Device Identifier
CONTENTS	Contents
R1	R1 Assay Buffer
R2	R2 Immunoparticles

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References

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Serious incidents

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

Modifications from previous version

- Included information about the SDS available on the Gentian website.

Date of issue

2023-12-07

For other languages visit:
www.gentian.com/products/ifu/cystatin-c/beckmancoulter

Instrument Settings for the Gentian Cystatin C Immunoassay on Beckman Coulter Synchron and UniCel Systems

Number [] **Chem [CYSX]**

Chemistry Parameters			**Page 1 of 3**	
Reaction Type		[Rate 1]		
Units		[mg/L]		
Precision		[X.XX]		
Reaction Direction		[Positive]		
Math Model		[8]		
Primary Wavelength		[410]		
Secondary Wavelength		[700]		
Calculation Factor		[1.000]		
No. of Calibrators		[6]		
Setpoints	1	[C]	4	[C]
	2	[C]	5	[C]
	3	[C]	6	[C]
Cal Time Limit		[336] hours		

Error Detection Limits			**Page 3 of 3**	
Blank	ABS Low/High Limits		[-1.500]/[2.200]	
	Rate Low/High Limits		[-1.500]/[2.200]	
	Mean Deviation		[2.200]	
Reaction 1	ABS Low/High Limits		[-1.500]/[2.200]	
	Rate Low/High Limits		[-1.500]/[2.200]	
	Mean Deviation		[2.200]	
Reaction 2	ABS Low/High Limits		[-1.500]/[2.200]	
	Rate Low/High Limits		[-1.500]/[2.200]	
	Mean Deviation		[2.200]	
Substrate Depletion				
	Initial Rate		[99.999]	
	Delta ABS		[2.200]	
Multipoint Span				
	1-2	[0.001]	4-5	[0.001]
	2-3	[0.001]	5-6	[0.001]
	3-4	[0.001]	6-1	[0.001]

Processing Parameters		**Page 2 of 3**
First Inject	Component	[A]
	Dispense Volume	[230] µL
Second Inject	Component	[None]
	Dispense Volume	[ ]
	Inject Time	[ ]
Third Inject	Component	[B]
	Dispense Volume	[45] µL
	Inject Time	[80] sec
Sample Volume	[5] µL	
ORDAC Volume	[None] µL	
Blank	Start Read	[56] sec
	End Read	[72] sec
Initial	Start Read	[81] sec
	End Read	[96] sec
Reaction 1	Start Read	[90] sec
	End Read	[154] sec
Reaction 2	Start Read	[ ] sec
	End Read	[ ] sec
Usable Result Range		
	Low Limit	[0.4]
	High Limit	[8.0]
ORDAC		
	Low Limit	[ ]
	High Limit	[ ]