Cystatin C

Gentian Cystatin C Immunoassay on Beckman Coulter® Synchron and UniCel System

REF A52761

This document describes the general use and the instrument specific settings of the product above and is applicable for USA only.

Intended use

The Cystatin C Immunoassay on the Beckman Coulter® Synchron and UniCel 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, gender, age or diet [2, 3, 4].

Principles of the procedure

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 Synchron and UniCel platforms will automatically calculate the results.

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

For *in vitro* diagnostic use by laboratory professionals.
Caution: Federal law restricts this device to sale by or on the order of a physician.

1. Contains substances from human or animal origin and should be considered as potentially infectious material. Serum used in Gentian Cystatin C Controls and calibrators is tested for hepatitis HBsAG, anti-HCV, anti-HIV1 and anti-HIV2 and found to be negative. Handle with caution and discard following local regulations.
2. Contains antibiotics and must be handled with due caution.
3. The sodium azide concentration of the assay is not characterized as hazardous. 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. Exposure may result in irritation of skin and eyes.
5. Avoid contact with incompatible materials.
6. Avoid exposure to heat and direct sunlight.

To obtain the SDS (Safety Data Sheet), 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.

Directions for reconstitution/dilution

The product is ready to use.

Storage instructions

Shelf life of unopened reagents at 2-8 °C: See expiry date on the label.

Specimen collection and preparation

Required sample material is human serum or EDTA/heparinized plasma. It is recommended to analyze 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), 21 days if stored at 2-8 °C. If stored below -70 °C the samples are stable for at least 5 years [5]. Mix samples well before analyzing.

Procedure

A detailed instrument parameter list is available in the section "Instrument settings for Cystatin C Immunoassay" 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

Gentian Cystatin C reagents are supplied ready for use. Transfer the content of Reagent 1 and Reagent 2 into appropriate compartments of the User Defined Cartridge 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

Assay kit components

Materials provided	
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)	REF A52761
Materials required but not provided	
Gentian Cystatin C Calibrator Kit (6 levels x 1 mL)	REF A52763
Gentian Cystatin C Control Kit (2 levels x 1 mL)	REF A52765
User-Defined Reagent Cartridge (pkg. of 12)	REF 442835

Stability
The in-use stability of the Gentian Cystatin C Reagent Kit was found to be at least 9 weeks on AU400 instrument performed as an on board study.

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

Establishment of the calibration curve
Please refer to the package insert of the Gentian Cystatin C Calibrator Kit REF A52763.

QC controls
Please refer to the package insert of the Gentian Cystatin C Control Kit REF A52765.

Measuring patient samples
When a valid calibration curve has been established and the control values are within the valid range, serum or plasma samples 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 Beckman Coulter® Synchron and UniCel Systems for all applications established for the Gentian Cystatin C Immunoassay. The results are presented in mg/L.

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 [6]. The equation is valid for persons above 14 years.

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

Limitations of the procedure
The materials should not be used past expiration date.

Expected values
Gentian follows the CLSI Guideline, C28 [10] 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 AU2700 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.

Measuring range
The measuring range of the Gentian Cystatin C Immunoassay was found to be 0.45-7.83 mg/L for Synchron and 0.46-7.95 mg/L for UniCel DxC. 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.

Interference
There is no interference detected with the drugs tested on the recommendations from Sonntag and Scholer [7]. As the antibodies in the Gentian Cystatin C Immunoassay are of avian origin, there is no interference due to Rheumatoid Factor in the samples [8]. Interference was tested in a study using a protocol based on the CLSI guideline EP07 [9]. No clinically relevant difference was detected at the tested interferent concentrations.

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

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

Precision
The Gentian Cystatin C Immunoassay was used in a 3 - day precision study on the Synchron LX20 and a 5-days precision study on the UniCel DxC, designed in accordance with CLSI protocol EP05 [11]. Three serum pools and 2 control levels were measured for Synchron LX20 (n=12), and 4 serum pools and 2 control levels were measured for UniCel DxC (n=20).

Synchron LX20:

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

UniCel DxC:

Sample ID	Mean [mg/L]	Within run CV [%]	Total CV [%]
P1	1.08	1.34	4.18
P2	1.37	1.17	3.07
P3	2.35	1.05	2.16
P4	3.94	0.62	2.18
CL	1.01	3.59	3.87
CH	3.37	0.99	2.19

Analytical sensitivity

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. Gentian Cystatin C Immunoassay has a limit of quantification of 0.42 mg/L on Synchron LX20, and on the UniCel DxC the limit of quantification is 0.46 mg/L.

Linearity

Gentian Cystatin C Immunoassay is linear in the range of 0.45-7.83 mg/L for Synchron LX20, and in the range 0.34-7.95 mg/L for UniCel DxC. Concentrations outside this range have not been tested.

Hook effect

For Gentian Cystatin C Immunoassay on Synchron LX20 and UniCel DxC there is estimated to be no hook effect in cystatin C samples below 25 mg/L. However, the Synchron LX20 and UniCel DxC is programmed to not report any results above the highest calibrator level.

Analytical recovery

For the Gentian Cystatin C Immunoassay on Synchron LX20 and UniCel DxC a recovery of 96-106 % and 98-107 % was observed respectively.

Instrument variation

Instrument variation between Gentian Cystatin C Immunoassay on Synchron LX20 and another Gentian Cystatin C turbidimetric assay application, and UniCel DxC by Passing-Bablok regression analysis.

Methods	N	Range of samples [mg/L]	Term	Coefficient	95 % CI
LX20 vs Architect	47	0.71–7.91	Intercept	0.17	0.13-0.22
			Slope	0.95	0.93-0.97
LX20 vs DxC	53	0.68-7.65	Intercept	0.03	-0.01-0.07
			Slope	0.97	0.95-1.00

Methods comparison

Comparison between Gentian Cystatin C Immunoassay on Synchron LX20 and a commercially available nephelometric assay, by Passing-Bablok regression analysis.

Methods	N	Range of samples [mg/L]	Term	Coefficient	95 % CI
LX20 vs BN ProSpec	51	0.87–7.46	Intercept	0.30	0.21-0.34
			Slope	1.04	1.01-1.08

Bibliography

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Gentian AS

Bjornasveien 5
N-1596 Moss
Norway
TEL: +47 99 33 99 05
www.gentian.com

CE 0123 UK CA RxOnly

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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.

Serious incidents

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

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
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
RxOnly	Caution: Federal law restricts this device to sale by or on the order of a physician.

Cystatin C

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		

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	[ ]

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]