Cystatin C

Gentian Cystatin C Immunoassay on Beckman Coulter Synchron UniCel System

REF A52761

Intended Use

The Gentian Cystatin C Immunoassay 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 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

Human serum or plasma sample is mixed with cystatin C immunoparticles. Cystatin C from the sample aggregates with anti cystatin C antibodies from the immunoparticles solution. 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.

Assay Reagents

Materials required but not provided	
Gentian Cystatin C Calibrator Kit, level 1-6, vials of 1 mL	REF A52763
Gentian Cystatin C Control Kit, Low & High, vials of 1 mL	REF A52765
User-Defined Reagent Cartridge (pkg. of 12)	REF 442835

Composition

Reaction Buffer 1 (R1): 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): 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.

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. The immunoparticles, calibrators and controls contain potentially infectious substances of animal and human origin and should be handled with due caution. Disposal of any discarded materials should be in accordance to local requirements.
4. Use only instrument applications validated and approved by Gentian AS.
5. Reagents containing sodium azide 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 NaN_3 in lead and copper pipes may cause generation of explosive metal azides. To prevent this, rinse thoroughly if discarded into the drain.
6. Reagents containing MOPS can be irritating to eye and skin. Handle with due caution.
7. R2 contains polystyrene nanoparticles.
8. Avoid using highly lipemic, icteric or hemolytic samples.
9. Do not mix reagents of different reagent lots or switch caps between reagents.

Reagent Storage and Stability

Shelf life of unopened reagents at 2 - 8°C: See expiry date on the label. Stability after opening: 9 weeks at 2 - 8°C. On-board stability: 4 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. 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 [6]. Mix samples well before analysing. The samples can be shipped without special cooling and must then be analysed within 14 days after shipment.

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 lot specific calibrator value as indicated on the Analytical Value sheet.

Assay Procedure

Application Notes

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 Synchron UniCel system’s instrument manual.

Reagent Preparation

Reagent is supplied ready to 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. Reagents should be stored capped at 2-8°C when not in use.

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

Establishing the Calibration Curve

Use standards 1 to 6 to establish a 6-point standard curve as defined in the Beckman Coulter Synchron UniCel system 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. The Beckman Coulter Synchron UniCel system require recalibration every 2 weeks.

QC Controls

The Gentian Cystatin C 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 Synchron UniCel system Instrument Manual.

Results

The results are calculated automatically by the Beckman Coulter Synchron UniCel system. The results are presented in mg/L.

Reference Intervals

CLSI Guideline, C28-A2 [7] was used to determine the transferability of the reference interval. The reference interval is based on a reference interval study performed at Karolinska University Hospital, Stockholm, Sweden, including serum samples from 138 self-declared healthy subjects 20-80 years of age. The samples were analysed for cystatin C on the Synchron LX20. Equivalency between the Synchron LX and UniCel DxC systems has been established. The reference interval was calculated non parametrically and was determined to be 0.62-1.15 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 down to 5 years of age [8].

Performance Characteristics

Studies based on measurements on Synchron UniCel DxC 800PRO at one instrument site using one lot of reagents unless otherwise stated.

Detection Limit

Limit of Detection is defined as the smallest amount of an analyte that the method can reliably detect to determine presence or absence of the analyte. 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.

The Gentian Cystatin C Immunoassay on the Synchron UniCel DxC has a Limit of Quantification of 0.46 mg/L.

Precision

The Gentian Cystatin C Immunoassay was used in a 5-days precision study on the UniCel DxC, designed in accordance with CLSI protocol EP5-A. Three serum pools and 2 control levels were measured on the DxC instrument with recalibration between days.

Sample	Mean (mg/L)	Within run (CV %)	Between day (CV %)	Between run (CV %)	Total CV %
P1	1.12	1.72	2.13	5.08	5.77
P2	2.83	1.04	0.84	2.73	3.03
P3	5.79	3.33	2.40	2.38	4.75
P4	0.97	2.62	0.97	4.29	5.12
L (Low)	1.00	2.88	1.50	2.95	4.38
H (High)	3.90	1.67	0.58	2.36	2.95

Linearity

Gentian Cystatin C Immunoassay on is linear in the range of 0.34-7.95 mg/L for UniCel DxC. Concentrations outside this range have not been tested.

Analytical Recovery

For the Gentian Cystatin C Immunoassay on Synchron UniCel DxC a recovery of 98-107% can be expected.

Hook Effect

A study was performed on the Synchron LX20 instrument, for which equivalency has been established. No hook effect was detected below 25 mg/L. However, the Synchron UniCel DxC is programmed to not report any results above the highest calibrator level.

Interference

No interference is detected with Triglycerides (15 mmol/L), Haemoglobin (4 g/L), Intra Lipid (12 g/L) or Bilirubin (200 mg/L). There is no interference detected with the drugs tested on recommendation from Sonntag and Scholer [9]. The interference study was designed in accordance with the protocol EP7-A from CLSI [11]. There is no RF interference present in the Gentian Cystatin C Immunoassay because the antibodies are of avian origin [10].

Instrument Variation and Method Comparison

The table below shows instrument variation between Gentian Cystatin C on Synchron LX20, and 1) Another Gentian Cystatin C turbidimetric assay application, and 2) UniCel DxC by Passing-Bablok regression analysis. In addition the table shows a comparison between Gentian Cystatin C on Synchron LX20, and 3) a commercially available nephelometric assay by Passing-Bablok regression analysis.

Method	N	Range specimen (mg/L)	Term	Coefficient	95% CI of Coefficient
LX20 vs DxC	53	0.68-7.65	Intercept	0.03	-0.01-0.07
			Slope	0.97	0.95-1.00
LX20 vs BN ProSpec	51	0.87-7.46	Intercept	0.30	0.21-0.34
			Slope	1.04	1.01-1.08

Additional Information

For more detailed information on the Synchron UniCel system, 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.

For other languages visit:

http://gentian.no/products/beckman-coulter-customers/

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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Shipping Damage

Please notify your local distributor if the product received is damaged.

References

1. Abrahamson M et al: Biochem J 1990;268:287-94.
2. Laterza OF et al: Clin Chem 2002;48:63-99.
3. Grubb AO. Adv Clin Chem 2000;35:63-99.
4. Filler G et al: Clin Biochem 2005 ;38 :1-8.
5. Flodin M et al: Scand J Clin Lab Invest 2007;67:560-567
6. Shlipak M.G, et al: Clinical Chemistry 57: 737-745, 2011
7. CLSI; Document C28-A2; Reference Intervals in the Clinical Lab; Approved Guideline.
8. Nitsch D. et al: The Uppsala Family Stud. Am J Kidney Dis. 2011;57(6):863-872
9. Sonntag O, Scholer A: Ann Clin Biochem 2001 ;38 :376-85.
10. Larsson A et al: Poultry Science 1993 ;72 :1807-1
11. CLSI; Document EP7-A ; Interference testing in Clinical Chem ; Approved Guideline.

Instrument Parameters

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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]