Cystatin C

Gentian Cystatin C Reagent Kit

REF 1101

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

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 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

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

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

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 NaN_3 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 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.

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 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), and 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. The samples can be shipped without special cooling and must then be analyzed within 14 days after shipment.

Procedure

Methods for the Gentian Cystatin C Immunoassay are established on multiple clinical chemistry analyzers. Detailed, validated Application Notes describing the procedures for installation and analyzing on specific instruments are available upon request to marketing@gentian.com. Instrument set up, maintenance, operation and precautions must be handled in accordance with the specific instrument manuals.

Reagent preparation

For instrument setup information, consult the Gentian Cystatin C Immunoassay Application Note specific to the instrument as well as the instrument manual. Reagents are supplied ready to use, mix gently before loading into instrument. The reagent bottles may fit directly into the instrument unless otherwise stated on the application notes.

[1]Architect ci8000 is a registered trademark of Abbott

Assay kit components

Materials provided	
Gentian Cystatin C Reagent Kit • R1 Assay Buffer (58 mL) • R2 Immunoparticles (10 mL)	REF 1101
Materials required but not provided	
Gentian Cystatin C Calibrator (1 mL), or	REF 1012
Gentian Cystatin C Calibrator Kit (6 levels x 1 mL)	REF 1051
Gentian Cystatin C Control Kit (2 levels x, 1 mL), or	REF 1019
Gentian Cystatin C Control kit (2 levels x 5 mL) or	REF 1026
Gentian Cystatin C Single Use Control Low (100 x 150 µL)	REF 1029
Gentian Cystatin C Single Use Control High (100 x 150 µL)	REF 1030

All materials are ready for use.

Stability

Stability after opening: Until expiry date at 2-8 °C. On-board stability: 9 weeks at 2-8 °C.

Calibrator standardization

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 1051) or the Gentian Cystatin C Calibrator (REF 1012).

QC controls

Please refer to the package insert of the Gentian Cystatin C Controls (REF 1019, REF 1026, REF 1029, or REF 1030).

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 Application Note and the instrument manual.

Results

The results are calculated automatically by the analyzer 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

CLSI Guideline, C28 [7] was used to determine the transferability of the reference interval. The reference interval was determined from a population of ostensibly healthy subjects with no history of renal disease. A total of 136 samples from individuals (57 males, 79 females) ranging in age from 20 to 84 years were tested on the Architect ci8200 instrument. 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.

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 in the analytical value sheet available on www.gentian.com.

Specific performance characteristics

The performance characteristics of Gentian Cystatin C Immunoassay are related to type of instrument. Performance characteristics included in this package insert are for Architect ci8000[1] system. See Application Notes for characteristics on specific instruments.

Limit of quantification

Limit of quantification 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. Limit of quantification was measured as 0.40 mg/L in a study on the Architect ci8000[1] system.

Limit of detection

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 Detection was measured as of 0.03 mg/L in the same study as for Limit of Quantification.

Linearity

Gentian Cystatin C Immunoassay on Architect ci8000[1] is linear in the range of 0.27-8.8 mg/L. Concentrations outside this range has not been tested.

Security zone

For Gentian Cystatin C Immunoassay there is no hook effect (antigen excess) in samples less than 16 mg/L.

Precision

The Gentian Cystatin C Immunoassay was used in a 5-days precision study designed in accordance with CLSI protocol EP05 [8]. 4 serum pools and 2 controls were measured on Architect ci8000[1] system.

Sample ID	Mean value (mg/L)	Within Run CV (%)	Between Day CV (%)	Between Run CV (%)	Total CV (%)
P1	0.69	1.51	1.07	2.11	2.81
P2	5.71	1.12	2.23	3.35	4.18
P3	3.38	1.93	1.62	2.77	3.75
P4	1.35	0.97	1.30	2.12	2.67
CL	0.88	1.42	1.51	3.09	3.72
CH	3.58	0.71	0.10	1.21	1.41

Recovery

Recovery was analyzed by spiking low analyte sample with high analyte sample. Gentian Cystatin C Immunoassay has a recovery of 103-108 %.

[1]Architect ci8000 is a registered trademark of Abbott

Interference

No interference was detected with Triglycerides (15 mmol/L), Hemoglobin (8 g/L), Intralipid (11 g/L) or Bilirubin (420 mg/L) on Architect ci8000[1] in a study based on protocol EP07 from CLSI [9]. Previously, no interference has been detected when using this assay with the drugs tested on recommendation from Sonntag and Scholer [10]. There is no rheumatoid factor interference present in the Gentian Cystatin C Immunoassay due to the use of antibodies of avian origin [11]. The potential interferents listed in the table below were spiked into two human plasma samples; one low analyte serum sample and one high analyte serum sample. No interference is defined as no statistically significant difference (< 5 %) between spiked and unspiked sample.

Potential Interferents	Interferent Conc.
Triglycerides	15 mmol/L
Hemoglobin	8 g/L
Intralipid	11 g/L
Bilirubin	420 mg/L

Methods comparison

Results obtained with the Gentian Cystatin C Immunoassay were compared with a commercially available nephelometric assay:

Passing Bablok regression	N	Range specimen (mg/L)	Term	Coeffi-cient	95% CI of Coefficient
Modular P vs BN ProSpec	79	0.59-7.47	Intercept	-0.030	-0.058 to 0.003
			Slope	1.010	0.984 to 1.043
Architect ci8000[1] vs BN ProSpec	87	0.52-7.91	Intercept	-0.062	-0.099 to 0.023
			Slope	0.983	0.959 to 1.005

Bibliography

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Gentian AS

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

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Shipping damage

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

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.

[1]Architect ci8000 is a registered trademark of Abbott