Cystatin C - Liquid reagent Ref.:CYC-037B
Quantitative Determination of Cystatin C
Only for *in vitro* use in clinical laboratory
Store at 2-8ºC

Cystatin C

CE

CLINICAL SIGNIFICANCE

Cystatin C is a basic proteinase inhibitor which is produced at a constant rate in all nucleated cells and appears in human plasma and serum. Cystatin C is freely filtered through the glomerulus, is not secreted by the tubule or eliminated via any extra-renal route, and is almost completely absorbed and catabolized by proximal tubular cells. Therefore, the plasma concentration of Cystatin C is almost exclusively determined by the glomerular filtration rate (GFR), making Cystatin C an excellent indicator of GFR. Cystatin C has advantages over routine clinical measures of renal function. It is more accurate than plasma creatinine and the Cockcroft-Gault estimation of creatinine clearance and is more reliable than the 24-h creatinine clearance. There is a growing body of evidence that suggests that Cystatin C can be used to detect kidney disease at earlier stages than serum creatinine which may help facilitate prevention efforts in the elderly and those with diabetes, hypertension or cardiovascular disease.

PRINCIPLE OF THE TEST:

The assay is based on the reactions between Cystatin C and latex covalently bound antibodies against human Cystatin C. Cystatin C values are determined turbidimetrically using fixed- time measurement with sample blank correction. The relationship between absorbance and concentration permits a multipoint calibration with a measuring range of between 0 to 10 mg/L. The measuring temperature is 37ºC. The assay can be performed on all instruments allowing turbidimetric measurements at 500 to 600nm.

WARNINGS AND PRECAUTIONS:

When handling samples, take great care to prevent infection by HBV, HIV, and HCV, wear rubber gloves.
Be sure not to allow foreign substances including dust, fungi, bacteria, and detergent to get mixed into samples. In addition, take care to prevent contamination of reagents and cuvettes.
On testing, wear disposable gloves and avoid oral pipetting to prevent infection.
Dispose of the reagent under a large amount of running water, as each component reagent contains sodium azide. If they get into the eyes or mouth or adhere to skin, first aid measures such as thorough flushing with water should be taken. Consult a physician if necessary.

INSTRUMENTS:

Instrument applications are available upon request.

COMPONENT COMPOSITION:

Component	**Ingredients**	*Concentration in Tests*
Reagent 1	Tris Buffer pH7.2 with protein stabilisers	0.1M
	PRESERVATIVE	-
Reagent 2	Glycine Buffer pH8.2	0.1M
	NaCl	0.15M
	BSA	0.5%
	anti-human Cystatin C antibody	-
	PRESERVATIVE	-

REAGENT PREPARATION AND STABILITY:

Reagent 1 and 2 are ready for use.

Before use, mix reagent by gently inverting each bottle.
If stored and handled properly:

- Unopened components are stable until expiry date stated on the label.
- Once open, components are stable for 1 month at 2-8°C.

TYPE OF SPECIMEN:

Fresh or deep frozen serum can be used. Cystatin C remain stable for 12 days at +2 to +8ºC. If the test should be performed later, it is recommended to freeze the serum. Avoid successive freezing and thawing. Discard haemolysed or contaminated samples.
It is recommended to follow NCCLS procedures (or similar standardised conditions) regarding specimen handling. Specimen should be collected in an appropriate sampling container, with proper specimen identification. Serum/Plasma should be separated from cells within 2 hours after collection.

TEST PROCEDURE:

Materials required but not supplied:
Cystatin C controls: CYCT-03 (2x1ml)
Cystatin C calibrator: CYCB-03 (5x1ml)
General Laboratory Equipment

Assay procedure:

Wavelength: 550 nm
Temperature: 37°C
Optical path: 1 cm light path.

	Blank	**Calibrator**	**Sample**
Reagent 1	1ml	1ml	1ml
Sample	--	--	12 µl
Calibrator	--	12 µl	--
Gently mix and Incubate at 37°C for 5 minutes			
Reagent 2	250 µl	250 µl	250 µl
Gently mix and Incubate at 37°C, measure the Optical Density (OD1) after 30 sec. Measure the Optical Density (OD2) after further 5 minutes.			

Calibration:
Cystatin C calibrators are provided separately and ready for use. For automated analysers, use the recommended calibrator and calibrate the assay. The calibration curve is stable for up to 14 days after which a new curve must be generated. Recalibrate when:

- When using a new reagent kit or changing lot number.
- Following preventive maintenance or replacement of a critical part of the photometer used.
- When Quality Control results are out of range.

Calculation:

The Turbidimetric analysers automatically calculate the Cystatin C concentration of each sample.
Conversion mg/L = µg/ml

Quality Control

All clinical laboratories should establish an Internal Quality Control program. Verify instrument and reagent performance with recommended controls or similar. The values obtained for QC should fall within manufacturer's acceptable ranges or should be established according to the Laboratory's QC program:
Controls should be assayed:

- Prior to reporting patient results.
- Following any maintenance procedure.

At intervals established by the laboratory Q.C. programme.

EXPECTED VALUES:

The reference interval is 0.59 – 1.03 mg/L are considered within the normal range.

Each laboratory should establish its own reference range. Results should always be reviewed with the patient's medical examination and history.

PERFORMANCE CHARACTERISTICS:

Performance results can vary with the instrument used. Data obtained in each individual laboratory may differ from these values.

Linearity:
Linearity was evaluated using serial dilutions, prepared with saline solution, of three pooled samples, which contained values of Cystatin C in the range of analysis ranging from 0.05 to 8mg/L. Linear regression values of Cystatin C mg/L vs concentration yielded correlation coefficients , r>0.999, for all samples. Within the assays measuring range, the deviations of measurement from theoretical values did not exceed the 10% level. In addition, the system did not show prozone phenomenon at least up to 16mg/L.

Interfering substances: [5]
Results of study are as follows:

Bilirubin: Less than 10% interference up to 18 mg/dL
Haemoglobin: Less than 10% interference up to 5g/L

Sensitivity:
The Lower Detectable Level was estimated at 0.7µmol/L.

Precision:

Within Run N = 80	Mean *(mgl/L)*	*% CV*	*Between Run N = 80*	Mean *(mgl/L)*	*% CV*
Level 1	0.86	0.70	Level 1	0.86	1.54
Level 2	5	1.22	Level 2	5	3.37

Accuracy:
Various concentrations of Cystatin C (0.5 – 8.0mg/L) were added to 43 different serum samples. The linear regression gives correlation of r^2 value of 0.98, slope of 0.97 and Y intercept of 0.05.

Method Comparison:
Analytical characteristics have been obtained in a single experiment in a Cobras-Mira plus analyser. As is well known the analytical characteristics of a clinical chemistry reagent depend on both the reagents and instrument used. Multicenter studies indicate important differences in analytical characteristics among similar instruments. Therefore, the data expressed in the present document should be interpreted as a guide example.

BIBLIOGRAPHY:

1. A V Lewis, T J James, J B J Mc Guire,and R P Taylor. Improved immunoturbidimetric assay for Cystatin C . Ann Clin Biochem 2001;38: 111-114
2. Mutsumi Tanaka,kenje Matsuo, Masayasu Enomoto and Koji Mizuno. A Sol particle homogenous immunoassay for measuring serum Cystatin C. Cli. Biochem. 37 (2004) 27-35
3. David Massey. Commentary: Clinical diagnostic Use of Cystatin C. Journal of clinical Laboratory Analysis 18:50 – 60 (2004)
4. Michael G. Shilpak and al. Cystatin C and the risk of death and cardiovascular events among elderly persons. NEJM 2005 volume 352:2049-2060
5. David J Newman. Cystatin C. Ann Clin Biochem 2002;39:89 – 104
6. Li Hai, Xu Guo Bing and Xia Tie An. Serum Cystatin C for the detection of early renal impairment in diabetic patients. JCLA 2004;18:31-35
7. Young DS. Effects of Drugs on Clinical Laboratory Test. 5th Edition, AACC Press, 2000

V1-072018