



LIQUID STABLE IMMUNOTURBIDIMETRIC
ASSAY FOR THE MEASUREMENT OF
CYSTATIN C ON RX SERIES ANALYSERS

P. Armstrong, E. Donnelly, P. McGivern, J. Campbell, S.P. Fitzgerald.
Randox Laboratories Ltd., Crumlin, United Kingdom

INTRODUCTION

What is Cystatin C?

Cystatin C is a small cysteine proteinase inhibitor that is produced at a constant rate by all nucleated cells. The small molecular weight of cystatin C allows it to be completely removed and broken down by the kidneys, levels therefore remain steady if the kidneys are working efficiently and the glomerular filtration rate (GFR) is normal. Cystatin C is broken down in renal tubular cells and is not secreted nor excreted by the kidneys. Therefore, levels of cystatin C in serum/plasma are almost entirely dependent on GFR.

What is Cystatin C used for?

The use of methods enabling accurate determination of this compound is advantageous for the estimation of renal function in clinical and therapeutic applications. Cystatin C is a particularly useful marker of renal function in patients where creatinine measurements are not reliable. E.g. individuals who are obese, malnourished, have liver cirrhosis or reduced muscle mass. Furthermore, unlike creatinine, cystatin C does not have a 'blind area' - up to 50% of renal function can be lost before significant creatinine levels occurs. Cystatin C is extremely sensitive to very small changes in GFR and is therefore capable of detecting early stage kidney dysfunction. Due to the strong link between kidney dysfunction and later cardiovascular problems, cystatin C has also been proposed for the early detection of coronary heart disease, stroke and death in the elderly.

Clinical Significance of Cystatin C

The small molecular weight of cystatin C allows it to be freely filtered by the glomerular membrane and therefore cystatin C levels in the blood are indicative of a normal or impaired Glomerular Filtration Rate (GFR). The GFR gives a good indication of renal health, and is also used to calculate the dosage of antibiotics and other therapeutics.

Expected Values

In serum the cystatin C normal range is between 0.57 – 1.05 mg/l. Cystatin C concentration measurements have shown utility in the calculation of estimated glomerular filtration rate (eGFR).

The formula recommended for the Randox cystatin C method is as follows:

$$\text{eGFR (ml/min/1.73m}^2\text{)} = -4.32 + 80.35 / \text{Cystatin C value}$$

It is recommended that each lab establishes its own reference range to reflect the age, sex, diet and geographical location of the population.

Scientific Study

This study carried out by Randox Laboratories reports the development of a liquid stable immunoturbidimetric assay for the measurement of cystatin C with an analytical range that allows determinations in human serum/plasma samples without additional dilutions. The assay is applied to the fully automated RX series analysers and is also suitable for use on many mainstream clinical chemistry platforms. This is of value as an accurate, stable and convenient tool for determination of cystatin C using these automated systems.

Methodology

The principle of the assay is immunoturbidimetric. A latex agglutination complex (read at 570nm) is found between cystatin C and latex particles. The assay (CYS 4004) is applicable to the fully automated RX Series Analysers, RX daytona and RX imola. These systems require 2.1µl of neat sample, generate the first result after 14 minutes and include dedicated software for data management. The assay was performed following manufacturer's instructions. Cystatin C Calibrator (CYS2699) and Cystatin C Controls Level 2 (CYS5019) and Level 3 (CYS5020) were used.

Analytical parameters:

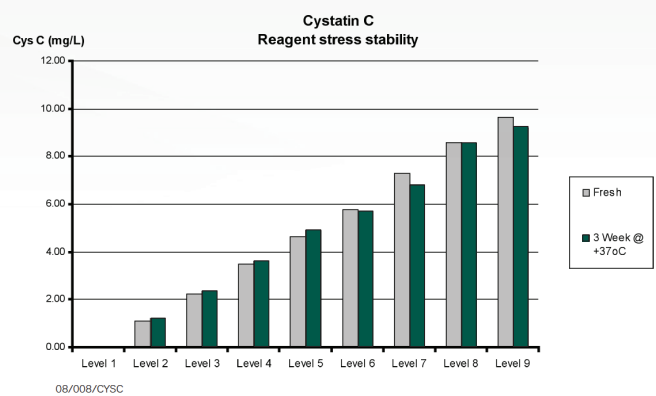
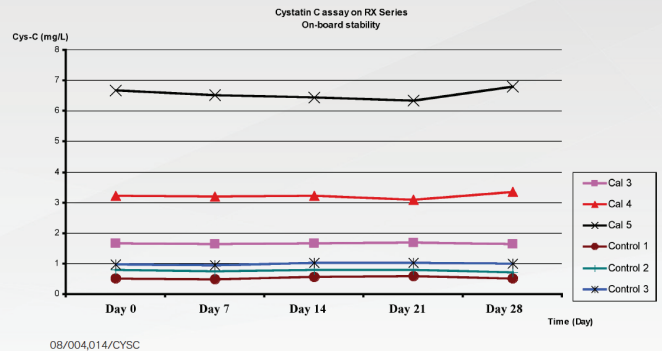
Sensitivity: sensitivity was assessed by analysis of replicates of serial dilutions of control material and was determined as the lowest concentration with imprecision <20% and within 20% from target for 10 replicates.

Linearity: linearity was determined by assessment of replicates of serial dilutions of control material with an upper limit level of 10 mg/L. This may vary depending on the lot specific values of the calibrators.

Stability: On-board and calibration stabilities were tested by storing two lots of reagent uncapped on the RX series analysers for a period of 28 days. Stressing studies were also carried out for the reagents, calibrators and controls. All were stored at 37°C for 3 weeks and then performance was compared to fresh material.

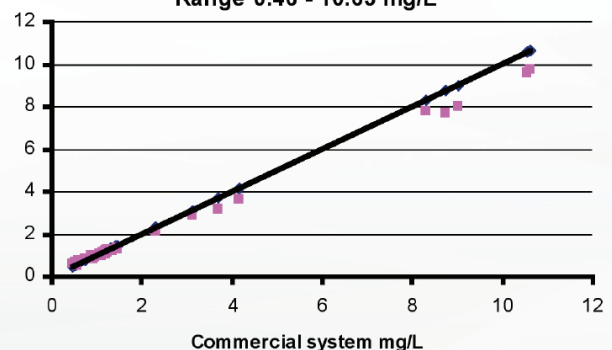
Precision: within-run and total precision were assessed by testing serum samples at defined medical decision levels, 2 replicates twice a day for 11 days. Results are expressed as %CV.

Correlation: a correlation was conducted with 40 serum samples using another commercially available cystatin C assay. Correlation coefficients were determined by linear regression analysis.



Cystatin C		Within run precision	Total precision
		%CV	%CV
Level 1 n=44	0.78mg/L	4.2	6.2
Level 2 n=44	3.37mg/L	2.6	4.3
Level 3 n=44	5.35mg/L	2.6	4.4

Cystatin C assay on RX Series vs Commercial Assay
 n=40, r=1.0
 Range 0.46 - 10.63 mg/L



RESULTS

Cystatin C assay on RX series	
Sensitivity (mg/L)	Linearity* (mg/L)
0.4	10

09/015, 016/CYSC
 *This may vary slightly with batch of calibrators

Sample type: serum, plasma

Correlation with another commercially available method

In correlation studies the following linear regression equation was obtained

$$Y = 0.90x + 0.07$$

And a correlation coefficient of $r = 1.0$

Forty patient samples were analysed spanning the range 0.46 to 10.63 mg/l.

Interference

The analytes below were tested up to the following levels and were found not to interfere:

Total Bilirubin	60 mg/dl
Direct Bilirubin	60 mg/dl
Haemoglobin	750 mg/dl
Triglycerides	1200 mg/dl
Intralipid®	1200 mg/dl

Findings

Data shows optimal analytical performance of the assay for the measurement of cystatin C on the fully automated RX series Analysers.

- Accurate and precise determination of cystatin C concentrations across the assay range of 0.4-10 mg/L.
- Liquid stable reagent, calibrators and controls.
- Stable on-board the RX series instruments for 28 days with a calibration frequency of 7 days.
- It can be used with serum and plasma.
- Excellent correlation with other commercially available systems.

- This is of value in the accurate determination of this analyte in human serum/plasma for clinical and therapeutic applications.

Further information on the assay and RX analysers used within this study

Cystatin C features:

- Sample type – Suitable for use with serum and plasma
- Latex enhanced Immunoturbidimetric method- Making it simple and quick to perform
- Liquid ready to use reagents – For ease of use and convenience
- Excellent stability – All reagents are stable to expiry date when stored at +2-8°C or 28 days on board the analyser at approximately 10°C.

Advantages of the RX series for direct testing:

- Low water consumption
- User friendly software
- 450- 560 tests per hour including ISE
- Low sample volume required
- STAT sample functionality
- Dual 5 speed stirrers optimized for reach chemistry reaction
- Reagent micropipette with liquid level sensor and crash detection
- Liquid level sensor, crash, bubble and clot detection

References

1. Laterza, O.F., et al. Cystatin C: An Improved Estimator of Glomerular Filtration Rate? Clin. Chem. 2002; 48: 699-707.
2. Finney H., Newman D.J., Gruber W., Merle P., Price C.P. Initial evaluation of cystatin C measurement by particle-enhanced immunonephelometry on the Behring nephelometer systems.
3. Finney H., Newman D.J., Price C.P. Adult reference ranges for cystatin C, creatinine and predicted creatinine clearance. Ann. Clin. Biochem 2000; 37: 49-59.
4. Flodin M., Hansson L-O. and Larsson A. Evaluation of Dade Behring N latex Cystatin C reagent on Abbott Ci8200. Upsala Journal of Medical Science 2006; 111:209-214.
5. Laterza, O.F., Price C.P. and Scott M.G. Cystatin C: An Improved Estimator of Glomerular Filtration Rate? Clinical Chemistry 2002; 48:699-707.
6. Paskalev E., Lambreva L., Simeonov P., Koicheva N., Beleva B., Genova M., Marcovska R. and Nashkov A. serum Cystatin C in renal transplant patients. Clinica Chimica Acta 2001; 310:53-56.
7. Christensson A., Ekberg J., Grubb A., Ekberg H., Lindström V., and Lilja H. Serum Cystatin C is a more sensitive and more accurate marker of glomerular filtration rate than enzymatic measurements of creatinine in renal transplantation. Nephron Physiol 2003; 94:19-27.
8. Pucci L. Triscornia S., Lucchesi D., Fotito C., Pellegrini G., Pardini E., Miccoli R., Del Prato S. and Penno G.
9. Cystatin C and estimates of renal function: searching for a better measure of kidney function in diabetic patients Clinical Chemistry 2007; 53:480-488.
10. Stabuc B., Vrhovec L., Stabuc-Silih M. and Cizej T.E. Improved prediction of decreased creatinine clearance by serum Cystatin C: use in cancer patients before and during chemotherapy. Clinical Chemistry 2000; 46:193-197.
11. Demirtas S., Bozbas A., Akbay A., Yavuz Y. and Karaca L. Diagnostic value of serum cystatin C for evaluation of hepatorenal syndrome. Clinica Chimica Acta.2001; 311:81-89.
12. Villa P., Jiménez M., Soriano M-C., Manzanares J. and Casasnovas P. Serum cystatin C concentration as a marker of acute renal dysfunction in critically ill patients. Critical Care 2005; 9:R139-R143.
13. Herro-Morin J.D., Málaga S., Fernández N., Rey C., Diéguez M.Á., Solis G., Concha A. and Medina A. Cystatin C and beta2-microglobulin: markers of glomerular filtration in critically ill children. Critical Care 2007; 11:R59.
14. Stowe H., Lawrence D., Newman D.J. and Lamb E.J. Analytical Performance of a particle-enhanced Nephelometric immunoassay for Serum Cystatin C using rate analysis. Clinical Chemistry 2001; 8:1482-1485.
15. Hoek F.J., Kemperman F.A.W., Krediet R.T. A Comparison between Cystatin C, Plasma Creatinine and the Cockcroft and Gault Formula for the Estimation of Glomerular Filtration Rate. Nephrol Dial Transplant 2003; 18: 2024-2031.

Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom
+44 (0) 28 9442 2413 • marketing@randox.com • randox.com/clinical-chemistry-analysers/