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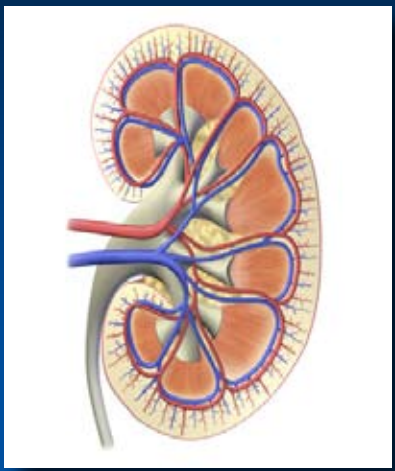
Health Canada Approved

CYSTATIN C

Estimated Glomerular Filtration Rate (GFR)



An Introduction to Cystatin C in the Diagnosis and Management of Kidney Disease

"Cystatin C is emerging as a biomarker superior to serum creatinine for estimating GFR and predicting the risk of death and cardiovascular events"

(DIABETES, VOL 56, NOVEMBER 2007)

INNOVATIONS IN CLINICAL DIAGNOSTICS

DIAZYME

CYSTATIN C

Estimated Glomerular Filtration Rate (GFR)

The Epidemic of Chronic Kidney Disease (CKD)

Recent epidemiological studies in the United States report that there has been a 40% increase in the prevalence of CKD in recent years with a corresponding doubling of the incidence of end-stage renal disease and a tripling of Americans on dialysis. The prevalence of chronic kidney disease has reached epidemic proportions now affecting 13.8 to 15.8 percent of the general population.[1] The expense of CKD is staggering claiming an estimated one-third of Medicare and Medicaid budgets.[2]

The Importance of Early Detection and Treatment of CKD

The National Kidney Disease Education Program (NKDEP) was initiated by the National Institutes of Health in an effort to address this major public health issue. The NKDEP's objective is "to raise awareness of the seriousness of kidney disease, the importance of testing those at high risk (those with diabetes, high blood pressure, or a family history of kidney failure), and the availability of treatment to prevent or slow kidney failure."[3] There is a growing body of evidence that indicates that some of the negative outcomes of chronic kidney disease can be averted with early diagnosis and treatment. Unfortunately, it has also been reported that chronic kidney disease is significantly underdiagnosed and undertreated.[3,4] In an effort to improve early diagnosis, the National Kidney Foundation has issued standardized clinical practice guidelines according to the Kidney Disease Quality Initiative (K/DOQI). In these guidelines and recommendations the primary measure of renal function is the glomerular filtration rate (GFR).

Stage of Kidney Disease NKDEP Classification	
Normal	Healthy kidneys GFR > 90 mL/min per 1.73 m^2
Stage 1	Kidney damage with normal or elevated GFR GFR > 90 mL/min per 1.73 m^2
Stage 2	Kidney damage and mild decrease in GFR GFR of 60 -89 mL/min per 1.73 m^2
Stage 3	Moderate decrease in GFR GFR of 30 – 59 mL/min per 1.73 m^2
Stage 4	Severe decrease in GFR GFR <16 – 29 mL/min per 1.73 m^2
Stage 5	Kidney failure - End Stage Renal Disease (ESRD) GFR of <15 mL/min per 1.73 m^2

Measurement of GFR

The GFR is a measure of the rate at which water and dissolved substances (low molecular weight, ultrafiltrateable compounds) are filtered out of the blood per unit time. Normal GFR's for males are about of 150 mL/min per 1.73 m^2 and 130 mL/min per 1.73 m^2 for females. Procedures for determining GFR with high accuracy require the injection of exogenous substances which are known to be only filtered at the glomerulus and not absorbed or secreted by the renal tubules. These gold standard procedures include Cr-EDTA, radiological contrast media (Iohexol) and inulin. Procedures determining GFR using exogenous substances are invasive and carry some risk to the patient which usually are considered too expensive and time consuming for routine clinical use. Historically, creatinine has been considered the renal marker of choice because it is a naturally occurring endogenous compound that is freely filtered at the glomerulus and has relatively minor absorption and secretion by the renal tubules. Even though serum creatinine determination remains the most commonly used

Limitations of Creatinine as a Marker for GFR		
Limitation	**Comments**	
Non-Renal Factors	Gender Ethnicity Diet Muscle mass Drugs which affect tubular secretion of creatinine	
Clinical Utility	Poor sensitivity for CKD "creatinine blind range"	Serum creatinine remains in the normal range until 50% of renal function is lost. Insensitive to loss of GFR in Stage 2 and Stage 3 of CKD.
Analytical Problems	Non-specific bias frequently reported with the commonly used Jaffé Assay Method (alkaline picrate)	Use of enzymatic assays for creatinine such as Diazyme's enzymatic creatinine method can significantly improve test performance by eliminating many sources of analytical error.

Measurement of GFR (continued)

renal marker for estimation of GFR, it is known to have a number of inherent difficulties which limit its clinical reliability.[5,6] These include the fact that measurement of GFR by creatinine is influenced by multiple non-renal factors including diet, gender, muscle mass and tubular secretion which can result in an overstatement of GFR up to 20%. In an attempt to improve the accuracy of serum creatinine measurements the NKDEP has advocated the use of GFR estimates calculated from serum creatinine levels. These include the Modification of Diet in Renal Disease (MDRD) and the Cockroft-Gault (CG). In addition, a quadratic formula has been introduced by the Mayo Clinic which has been referred to as the Mayo Clinic Quadratic (MCQ). Each

of these equations has strengths and weaknesses for use in specific patient sub-populations.

Even though creatinine based GFR equations such as the MDRD improve the accuracy of serum creatinine measurements, concentrations of creatinine can be within the normal range even with a GFR of around 40 mL/min/1.73 m^2 resulting in a so called "creatinine blind" range. This is due to the fact that MDRD understates normal and elevated GFR's and overstates decreases in GFR[5].

It is in this area of small to moderate decrease in kidney function that Cystatin C provides its greatest utility in the detection of both acute and chronic kidney disease.

Cystatin C as a GFR Marker

A substantial body of evidence has developed over the past several years which supports the use of Cystatin C as an alternative and more sensitive endogenous marker for the estimation of GFR than serum creatinine and serum creatinine based GFR estimations.[5,6,9-12]

Cystatin C is a small 13 –kDa protein that is a member of the cysteine proteinase inhibitor family that is produced at a constant rate by all nucleated cells. Due to its small size it is freely filtered by the glomerulus, and is not secreted but is fully reabsorbed and broken down by the renal tubules. This means the primary determinate of blood Cystatin C levels is the rate at which it is filtered at the glomerulus making it an excellent GFR marker. A recent meta-analysis demonstrated that serum Cystatin C is a better marker for GFR than serum creatinine.

Unlike creatinine, Cystatin C serum levels are virtually unaffected by age (>1 yr), muscle mass, gender and race. A number of very simple formulas have been introduced which can be used to obtain an estimated GFR using Cystatin C. Multiple studies have found Cystatin C to be more sensitive to actual changes in GFR in the early stages of CKD than creatinine based GFR estimates. A significant advantage of Cystatin C based formulas, unlike creatinine based equations, is that Cystatin C based estimated GFR formulas are not biased according to GFR[13-14] and there is no GFR blind area with Cystatin C.

Cystatin C Estimated GFR

Unlike creatinine, Cystatin C serum levels are virtually unaffected by age (>1 yr), muscle mass, gender and race. A number of very simple formulas have been introduced which can be used to obtain an estimated GFR using Cystatin C. Multiple studies have found Cystatin C to be more sensitive to actual changes in GFR in the early stages of CKD than creatinine based GFR estimates.

An example of a Cystatin C estimated GFR forumla is the one proposed by Larson and Grubb et al. In their study a cystatin C–based prediction equation using only concentration in mg/L and a factor: GFR (ml/min) = 99.43 x (cys C)$^{-1.5837}$ provided reliable and readily available GFR data based on single measurements of cystatinC concentrations. As with all new test methods the actual formula used for conversion should be evaluated by the laboratory prior to introduction.[18]

A normogram comparing serum Cystatin C concentration to GFR is depicted below.

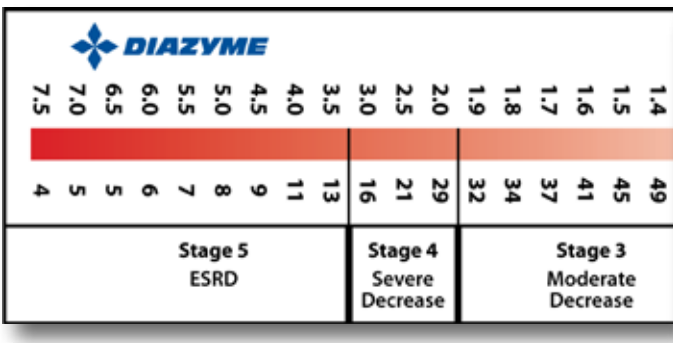
CONTRAINDICATIONS

Thyroid Function

Levels of Cystatin C are sensitive to changes in thyroid function and should not be used without knowledge of the patients thyroid status.[17]

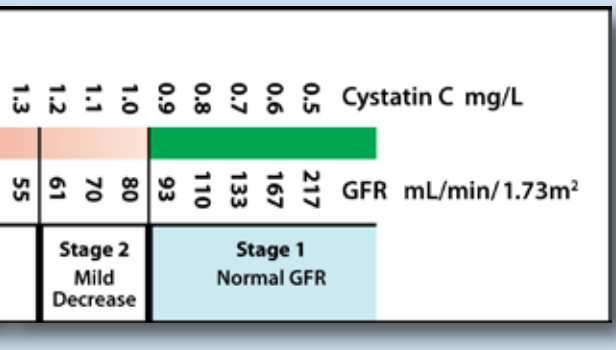
Corticosteroids

It has been reported that Cystatin C serum concentrations are not affected by standardized high-dose corticosteroid therapy but may be increased in patients with impaired renal function receiving corticosteroids.[14]



Advantages of Cystatin C as a GFR Marker

Advantage	Comments
Virtually unaffected by non-renal factors	Muscle Mass / Weight / Height Age (>1 year) – Cystatin C parallels age related decreases in GFR and may be used reliably with children Gender Diet Less inter individual variation than creatinine
Primary determinate of Cystatin C levels are renal factors	Cystatin C is not secreted but is fully absorbed and broken down by tubular cell. Since there is no tubular secretion of Cystatin C, it is extremely sensitive to small changes in GFR in the earliest stages of CKD.
Sensitive to changes in the so-called creatinine blind GFR range (40-70 ml/min/1.73 m^2)	Enables early detection and treatment of CKD.
Demonstrates higher diagnostic accuracy than MDRD, or C-G equations in patients with diabetes	Enables early detection and treatment of CKD in both Type 1 and Type 2 diabetes.
Can be used to detect and monitor kidney disease in patients with hepatic disease	Creatinine based GFR measurements are not reliable and are not recommended in hepatic disease. Cystatin C is reliable in cirrhotic patients.
Has been advocated as the preferred endogenous marker for dosing medication eliminated by the kidneys	May detect mild to moderate decreases in GFR that are not evident with serum creatinine based measurements, thus avoiding unnecessarily high drug doses which may pose an increased risk to the patient and the associated cost of possible resulting side effects.
Correlates to the appearance of microalbumin	Recent studies suggest that very early renal failure may be the first clinical indication of the progressive kidney damage associated with diabetes.



Cystatin C Assay

Early Detection of Kidney Disease in Type 1 and Type 2 Diabetes

Conclusions: **Our study provides convincing evidence that cystatin C may be more useful for detecting early renal impairment in both type 1 and type 2 diabetic patients than are creatinine and commonly employed creatinine-derived formulas.**

Cystatin C and Estimates of Renal Function:
Searching for a Better Measure of Kidney Function in Diabetic Patients
Clinical Chemistry 53:3 480–488 (2007)

Conclusion: **Cystatin C was more accurate in detecting decline in renal function than creatinine based methods in this population of subjects with Type 1 and a normal mean baseline GFR.**

Serial Measurements of Cystatin C Are More Accurate than Creatinine-based
Methods in Detecting Declining Renal Function in Type 1 Diabetes
Diabetes Care.2008; 0: dc07-1588v1-0

Conclusions: **Cystatin C may be considered as an alternative and more accurate serum marker than serum creatinine or the Cockcroft and Gault estimated GFR in discriminating type 2 diabetic patients with reduced GFR from those with normal GFR.**

Cystatin C Is a More Sensitive Marker Than Creatinine
for the Estimation of GFR in Type 2 Diabetic Patients
Kidney International, Vol. 61 (2002), pp. 1453–1461

Early Detection of Chronic Kidney Disease

Conclusions: **Among elderly persons without chronic kidney disease, cystatin C is a prognostic biomarker of risk for death, cardiovascular disease, and chronic kidney disease. In this setting, cystatin C seems to identify a "preclinical" state of kidney dysfunction that is not detected with serum creatinine or estimated GFR.**

Cystatin C and Prognosis for Cardiovascular and Kidney Outcomes
in Elderly Persons without Chronic Kidney Disease
Ann Intern Med. 2006;145:237-246

A recent meta-analysis demonstrated that serum cystatin C is a better marker for GFR than serum creatinine. In clinical practice, it has been suggested that serum cystatin C can optimize early detection for diabetic or hypertensive nephropathy. In addition, the use of serum cystatin C is possibly more appropriate for establishing an appropriate dose adjustment of drugs that are mainly eliminated by the kidney

A New Approach for Evaluating Renal Function and Its Practical Application
J Pharmacol Sci 105, 1 – 5 (2007)

Improved Marker of Drug Elimination

Cystatin C is a marker of drug elimination which is superior to serum creatinine for topotecan. It deserves to be further explored as a promising covariate for drug dosing as well as selection criteria for clinical studies of drugs eliminated mainly or partially by the kidney.

Serum Cystatin C is a Better Marker of
Topotecan Clearance than Serum Creatinine
Clin Cancer Res 2005;11(8) April 15, 2005

Early Detection of of Acute Renal Failure

Conclusions: **Serum cystatin C is a useful detection marker of acute renal failure (ARF), and may detect ARF one to two days earlier than creatinine.**

Early Detection of Acute Renal Failure by Serum Cystatin C
Kidney Int 2004; 66:1115-1122

Cardiovascular Risk

Conclusions: **High cystatin C concentrations predict substantial increased risks of all-cause mortality, cardiovascular events, and incident heart failure among ambulatory persons with CHD. This risk is not completely captured by measures of kidney function routinely used in clinical practice.**

Association of Cystatin C With Mortality, Cardiovascular Events,
and Incident Heart Failure Among Persons With Coronary Heart Disease
Circulation. 2007;115:173-179

Hepatic Disease

Conclusions: **Plasma cystatin C concentration is an accurate GFR marker in cirrhotic patients. Plasma creatinine concentration and calculated creatinine clearance are of no practical value, as their reference values vary with the severity of the liver disease.**

Diagnostic Value of Plasma Cystatin C as
A Glomerular Filtration Marker in Decompensated Liver Cirrhosis.
Clinical Chemistry 48:6 850–858 (2002)

Acute Kidney Injury (AKI)

Cystatin C has been shown to increase earlier than serum creatinine in patients developing AKI one to two days earlier than serum creatinine. Acute Kidney Injury also known as acute renal failure (ARF) is common in hospitalized patients, with a mortality rate between 30% and 90% The use of serum creatinine for estimating GFR in this group of critical ill patients is limited since serum creatinine does not accurately reflect the rapid changes in renal function seen in AKI.

Cystatin C and the MDRD

The creatinine based MDRD underestimates GFR in healthy subjects and shows decreased accuracy in older patients (with decreased muscle mass) and patients with body mass indexes (BMI) <21 and >30. While MDRD and serum creatinine show good diagnostic accuracy in severe renal failure (GFR <15 mL/min per 1.73 m^2) creatinine based measurements show a lack of sensitivity in stage 2 and stage 3 renal disease when early intervention may improve outcomes.[11] Cystatin C based estimates of GFR have been reported to be a more sensitive marker of decline in GFR especially in the earliest stages of CKD.

Cystatin C for Early Detection of CKD in Diabetes

Multiple reports indicate that Cystatin C is a reliable marker of GFR in patients with mild to moderate impairment of kidney function (stages 2-3 of CKD).[10] This high degree of sensitivity has been demonstrated in both Type 1 and Type 2 Diabetes.[13,14] In addition, several studies indicate that although clinical proteinuria was associated with both MDRD and Cystatin C estimates of GFR only Cystatin C was associated with microalbuminuria. This finding supports the enhanced sensitivity of Cystatin C based formulas for the early detection of kidney damage. Elevated serum Cystatin C levels have also recently been identified as a significant prognostic indicator for the development of cardiovascular disease in people with diabetes.

Cystatin C GFR as a Guide for Dose Adjustments of Medications

GFR is commonly determined in clinical practice to guide the dosage of potentially toxic drugs including digoxin, chemotherapy medications and aminoglycoside antibiotics. Serum creatinine often does not increase until the GFR has moderately decreased (about 40 ml/min/1.73 m^2). This insensitivity to small to moderate decreases in GFR in the so called creatinine blind GFR area (40–70 ml/min/1.73 m^2) may result in an unnecessarily high drug dose thus increasing the risk to the patient and the cost of possible resulting side effects. Some studies have advocated the preferential use of Cystatin C based GFR estimations for establishing the appropriate dose adjustment of drugs that are mainly eliminated by the kidneys.

DIAZYME

Diazyme Laboratories is a Division of General Atomics located in Poway, California.

Diazyme uses its proprietary enzyme technologies to develop diagnostic reagents which can be used on most automated chemistry analyzers in user-friendly formats.

Diazyme is a cGMP and ISO 13485 certified medical device manufacturer.

Diazyme's products include test kits for diagnosis of cardiovascular disease, liver disease, cancer markers, renal disease, and electrolytes.

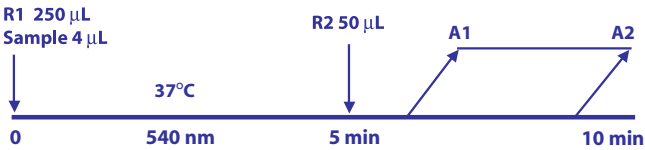
Diazyme Cystatin C

SUMMARY OF PERFORMANCE

Background

Cystatin C is a small, 13 kDa, protein that is produced by virtually all nucleated cells. Its production rate is constant and is unaffected by inflammatory process, gender, age and muscle mass. In the normal kidney, Cystatin C is freely filtered at the glomular membrane and then nearly completely reabsorbed and degraded by the 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, the Cockcroft-Gault estimation of creatinine clearance and is more reliable than the 24-h creatinine clearance.[i] 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, this may help facilitate prevention efforts in the elderly and those with diabetes, hypertension, or cardiovascular disease.[ii]

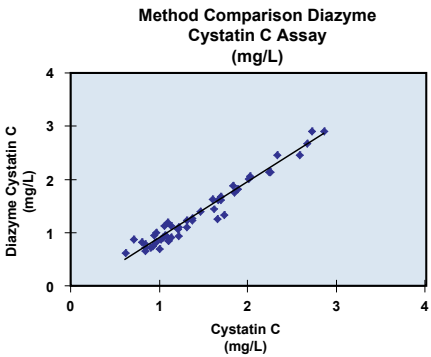
Assay Method



Diazyme Cystatin C assay is based on a latex enhanced immunoturbidimetric assay. Cystatin C in the sample binds to anti-Cystatin C antibody, which is coated on latex particles, and causes agglutination. The degree of the turbidity caused by agglutination can be measured optically and is proportional to the amount of Cystatin C in the sample. The instrument calculates the Cystatin C concentration of a patient specimen by interpolation of the obtained signal on a 6-point calibration curve.

Performance

Accuracy



The Diazyme Cystatin C assay method (y) results generated on a Roche Hitachi 717™ chemistry analyzer were compared with those obtained with a commercially available Cystatin C method (x). A total of forty five (45) patient samples with values ranging from 0.45 to 7.71 mg/L were analyzed over a period of 5 days. These studies yielded a correlation coefficient of 0.98 with a linear regression of $y = 0.930 + 0.1915$.

Precision

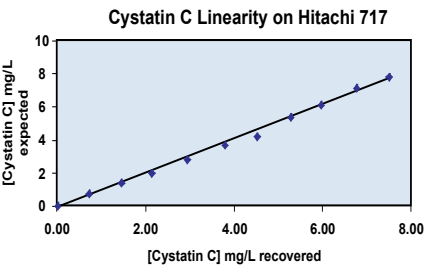
The precision of the Diazyme Cystatin C assay was evaluated according to Clinical Laboratory Standards Institute (formerly NCCLS) EP5-A guidelines on a Roche Hitachi 717 chemistry analyzer. In the study, three serum specimens were tested with 2 runs per day with duplicates over 20 working days.

Within-run Precision	Level 1	Level 2	Level 3
Mean mg/L	1.00	4.63	5.95
SD mg/L	0.04	0.10	0.16
CV%	3.9	2.1	3.9

Between-run Precision	Level 1	Level 2	Level 3
Mean mg/L	1.00	4.63	5.95
SD mg/L	0.05	0.17	0.22
CV%	4.8	3.7	3.7

Linearity

Eleven levels of a commercial linearity set were prepared by diluting a serum containing 7.8 mg/L Cystatin C with saline according to Clinical and Laboratory Standards Institute (formerly NCCLS) EP6-A.



Interference

The following substances normally present in serum produced less than 10% deviation at the listed concentrations: triglyceride at 2,400 mg/dL (formadine), RF at 450 IU/mL, Bilirubin at 18.2 mg/dL, Bilirubin Conjugated at 19.6 mg/dL, and Hemoglobin at 460 mg/dL.

[i]Diabetes Care 25:2004-2009, 2002
[ii]Annals of Internal Medicine 147:1, 21 2007

Available in a variety of convenient instrument specific packaging formats including Roche Hitachi, Olympus and Beckman.

Companion products available for this product include control and calibrator sets.

DIAZYME

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DIABETES, VOL 56, NOVEMBER 2007

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12) Use of serum Cystatin C to detect early decline of glomerular filtration rate in Type 2 diabetes A new approach for evaluating renal function and its practical application,
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Diabetes Care., 2007; 30:1988-1994

17) Serum Cystatin C is sensitive to small changes in thyroid function
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18) Larson A, Malm J, Grubb A, Hanson LO: Calculation of glomerular filtration rate express in ml/min from plasma cyctatin C values in mg/l.
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Cystatin C

Immunoturbidometric Assay

Highly Accurate

- Allows reliable estimation of GFR in the creatinine blind range of 50 and 90 ml/min/1.73 m^2
- Extended eGFR reportable range from 4 to 220 ml/min/1.73 m^2 with an assay range of 0.13 mg/L to 8.0 mg/L serum/plasma Cystatin C
- Enables earlier detection of Acute Kidney Damage (AKD) than serum creatinine or creatinine-based eGFR methods

Reproducible

- Excellent precision with CV's of less than 5%

Cost Effective

- Enables consolidation of multiple testing platforms
- Excellent performance at a reasonable price

Convenient

- Liquid Stable format and a choice of instrument specific packaging configurations for labs of all sizes and workflows

Flexibility

- Ability to test either serum or plasma
- Extensive list of instrument parameters

Instrument Specific Packaging

- Diazyme now offers our leading Cystatin C assay in specific packaging for open chemistry systems from world leading instrument manufacturers including Roche, Olympus, Beckman and Siemans (Dade). In addition to the added convenience these packaging options save operator time and improve laboratory efficiency by eliminating reagent transfer.

DIAZYME

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