

BIOLATEX®**Cystatin C**

For the quantitative determination of Cystatin C in human serum by immunoturbidimetry.

Diagnostic Relevance

Cystatin C is a nonglycosylated 13-kDa basic protein belonging to the cystatin super-family of cysteine proteinase inhibitors. Cystatin C is produced by virtually all nucleated cells, and is present in all investigated body fluids. The production rate is constant and unaffected by inflammatory processes, sex, age, diet and nutritional status. In the normal kidney, cystatin C is freely filtered through the glomerular membrane of the nephron 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 GFR (glomerular filtration rate), making cystatin C an excellent indicator of GFR. At the same time cystatin C is becoming acknowledged as a marker of elevated risk of death from cardiovascular complications – myocardial infarction and stroke.

Principle of the Method

This Cystatin C test is based upon 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 between 0 and 10 mg/L. The measuring temperature is 37°C. The assay can be performed on all instruments allowing turbidimetric measurements at 500 to 600 nm.

Reagents

Each Cystatin C kit contains:

A.- Buffer - 30 mL of TRIS buffer, pH: 7,2, containing detergents, polyethyleneglycol and 0,09 % sodium azide as preservative..

B.- Latex reagent – 5.1 ml of Polystyrene particles (0.5%) coated with antibodies anti-human-Cystatin C serum in a glycine buffer (0.1 M, pH: 8.2), containing NaCl (0.15 M) and bovine serum albumin (0.5%). Preservative: Sodium azide 0.075%

Precautions

For in vitro diagnostic use only. Do not pipette by mouth. Reagents containing sodium azide must be handled with precaution. Sodium azide can form explosive azides with lead and copper plumbing. Since absence of infectious agents cannot be proven, all specimens and reagents obtained from human blood should always be handled with precaution using established good laboratory practices.

Disposal of all waste material should be in accordance with local guidelines.

As with other diagnostic tests, results should be interpreted considering all other test results and the clinical situation of the patient.

Materials required but not provided

Automatic analyzer.

Calibrator.

Controls.

Storage and Stability

The Cystatin C reagents should be stored tightly capped at +2...+8°C when not in use. **Do not freeze.** Reagents in the original vials are stable to the expiration date on the vial label when capped and stored at +2...+8°C. Immediately following the completion of an assay run, the reagent vials should be capped until next use in order to maximize curve stability. Once opened the reagent can be used within 1 month if stored tightly closed at +2...+8°C after use.

The Cystatin C buffer reagent should be clear and colourless. Any turbidity may be sign of deterioration and reagent should be discarded.

The Cystatin C latex reagent should have a white, turbid appearance free of granular particulate. Visible agglutination or precipitation may be a sign of deterioration, and the reagent should be discarded.

Specimens

Fresh or deep frozen serum. Cystatin C remain stable for 12 days at +2 to +8°C. If the test should be performed later, it is recommended to freeze the serum. Avoid successive freezing and thawing. Discard haemolysed or contaminated samples.

Procedure

The reagents are ready to use as supplied. Latex reagent should be gently shaken before each use.

Follow the instructions of the operator's manual to load the cartridge, technique programation, calibration, sample measurement and control.

Calibration. Quality control

Use Biolatex Calibrator. The Cystatin C concentration of the Standards is given on the label.

This curve is stored in memory by the analyzer and recalled for later use. Calibration curves are stable for up to 14 days, after which a new curve must be generated. Additionally, recalibration must be performed whenever reagent lots are changed.

For quality control use BioLatex Control or other suitable control material. The control intervals and limits must be adapted to the individual laboratory requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits. Control must be assayed and evaluated as for patient samples.

Calculation

The turbidimetric analysers automatically calculate the Cystatin C concentration of each sample.

Conversion: mg/L = µg/ml.

Reference Values

Values 0.59 – 1.03 mg/L are considered within the normal range. Each laboratory should establish an expected range for the geographical area in which it is located.

Assay range and Sensitivity

The assay range is established from 0 to 10 mg/L. Manually dilute samples having higher concentration with 0,9 % NaCl. Multiply the result by the appropriate factor.

Calculating the mean plus 3SD of twenty replicates of zero standard resulted in a lower limit of detection of 0,05 mg/L

Specificity

The assay is specific for Cystatin C determination.

Due to the assay measuring principle (wavelength 550 nm), no endogenous interference by bilirubin (up to 18 mg/dL), haemoglobin (up to 5 g/L).

Other substances can interfere. For a comprehensive review of interfering substances, refer to the publication by Young.

Precision

Intra-assay coefficients of variation (CV) for four samples (Cystatin C values ranging from 0.86 to 5 mg/L) were between 0.70 and 1.22 %. Inter-assay CVs were between 1.54 and 3.37 %.

Assay 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 8 mg/L. Linear regression values of Cystatin C mg/L vs concentration yielded correlation coefficients, $r > 0.999$, for all samples. Within the assay's 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 16 mg/L.

Method comparison

43 samples were correlated with a commercial procedure. When comparing the results by lineal regression the result was: $y=0.97x-0.05$ and $r=0,9886$.

Analytical characteristics have been obtained in a single experiment in a Cobas-Mira plus analyser. As is well known the analytical characteristics of a clinical chemistry reagent depend on both the reagents and the 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

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Michael G. Shlipak and al. Cystatin C and the risk of Death and cardiovascular events among elderly persons. NEJM 2005 volume 352:2049-2060.

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Young DS. Effects of Drugs on Clinical Laboratory Test. 5th Edition, AACCPress, 2000.

Significados de los símbolos indicados en las etiquetas. Explanation of symbols used on labelling. Explication des symboles figurant sur les étiquettes. Spiegung der Symbole utilized auf den Etiketten. Significado dos símbolos indicados nas etiquetas. Erläuterung der Symbole auf den Etiketten.

	LOT	REF	CE	REAG	CAL	Buffer	LYOPH	Conc.	Control H / Control L		
Fecha de Caducidad Expiry Date Date de Périemption Data di Scadenza Data Expiração Verwendbar bis	Temperatura de almacen Storage Temperature Temperatura de Conservación Temperatura di Conservazione Temperatura de Conservação Lagertemperatur	Número de Lote Lot Number Número de Lot Número di Lotto Número de Lote Charact-Nr	Para Diagnóstico In Vitro For In Vitro Diagnostic Usage In Vitro Per Uso Diagnostico In Vitro Utilizar em Diagnostico In Vitro In Vitro Diagnosticum	Número de catálogo Catalog Number Número de catalogue Número di catalogo Número de catálogo Katalognummer	Conformidad Europea European Conformity Conformité aux normes européennes Conformità europea Conformidade com as normas europeias CE-Konformitätskennzeichnung	Fabricado por Manufactured by Fabrique par Fabbriato da Fabricado por Reagente Reagenz	Calibrador Calibrator Calibreur Calibratore Calibratore Calibrator Kalibrator	Tampón Buffer Tampon Tampone Buffer Puffer	Liofilizado Lyophilised Lyophilisé Liofilo Liofilizado Lyophilisiert	Concentración Concentration Concentration Concentrazione Concentração Konzentration	Control Alto / Control Bajo Control High / Control Low Contrôle élevé / Contrôle Bas Controllo Alto / Controllo Basso Controlo Alto / Controllo Basso Kontrolle Hoch / Kontrolle Niedrig

Cystatin C / Cistatina C Procedure for / Procedimiento para COBAS MIRA PLUS

<p>GENERAL</p> <p>Measurement mode: Absorb</p> <p>Reaction mode: R-S-SR1</p> <p>Calibration mode: LOGIT/LOG5</p> <p>Reagent blank: REAG/DIL</p> <p>Cleaner: NO</p> <p>Wavelength: 550 nm</p> <p>Decimal position: 2</p> <p>Units: mg/L</p> <p>ANALYSIS</p> <p>POST DIL. FACTOR: NO</p> <p>CONC. FACTOR: NO</p> <p>SAMPLE Cycle:3 Volume: 3.0 µL</p> <p>DILUENT Name: H₂O Volume: 10.0 µL</p> <p>REAGENT Cycle: 1 Volume: 250 µL</p> <p>START R1 Cycle: 1 Volume: 50.0 µL</p> <p>DILUENT Name: H₂O Volume: 10.0 µL</p> <p>CALCULATION</p> <p>Sample limit: NO</p> <p>Reaction Direction: INCREASE</p> <p>Check: ON</p> <p>Antigen excess: NO</p>	<p>CALCULATION (continued)</p> <p>Conversion factor: 1.00000</p> <p>Offset: 0.00000</p> <p>Normal range low: NO</p> <p>Normal range high: NO</p> <p>Number of steps: 1</p> <p>Calc. Step A: ENDPOINT</p> <p>Reading first: 3</p> <p>Reading last: 15</p> <p>CALIBRATION</p> <p>Calibr. Interval: ON REQUEST</p> <p>Reagent blank:</p> <p>Reag. Range low: NO High: NO</p> <p>Blank range low: NO High: NO</p> <p>STANDARDS POS:**</p> <p>1: * 2: *</p> <p>3: * 4: *</p> <p>5: * 6: *</p> <p>7: NO 8: NO</p> <p>REPLICATE: SINGLE</p> <p>Deviation: NO</p> <p>Correction std: NO</p>
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(*) Standard value on the label / insert
(**) Select one position on the standard rack