

REF	CONTENT	Analyzer(s) on which cobas c pack(s) can be used
04975723 190	Tina-quant Cystatin C (225 tests)	System-ID 07 6988 6 COBAS INTEGRA 400 plus COBAS INTEGRA 800
04975901 190	Calibrator f.a.s. Cystatin C (4 × 1 mL)	System-ID 07 6989 4
04975936 190	Cystatin C Control Set Control I (low) (4 × 1 mL) Control II (high) (4 × 1 mL)	System-ID 07 6990 8 System-ID 07 6991 6
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0

English

System information

Test CYSC, test-ID 0-195

Intended use

In vitro test for the quantitative immunological determination of cystatin C in human serum and plasma on COBAS INTEGRA systems.

Summary^{1,2,3,4,5,6,7,8,9,10,11,12,13,14}

Chronic kidney disease is a worldwide health problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m² for 3 months or more, regardless of cause. GFR is the most frequently used criteria in the assessment of renal function.

Serum creatinine is the most commonly used marker for estimation of GFR. However, it has become evident that the creatinine concentration is far from ideal because it is significantly changed by other factors such as muscle mass, diet, gender, age and tubular secretion. To compensate for these drawbacks several prediction equations have been developed, with the Modification of Diet in Renal Disease (MDRD), the Schwartz, and Counahan-Baratt equations being the ones most widely accepted.

Creatinine clearance measurements using serum and urine creatinine levels suffer from a similar problem, since the creatinine is endogenous and the factors mentioned above also complicate the interpretation.

Cystatin C is produced by all nucleated cells at a constant rate and the production rate in humans is remarkably constant over the entire lifetime. Elimination from the circulation is almost entirely via glomerular filtration. For this reason the serum concentration of cystatin C is independent from muscle mass and gender in the age range 1 to 50 years. Therefore cystatin C in plasma and serum has been proposed as a more sensitive marker for GFR, and several studies, as well as one meta analysis, have suggested that cystatin C is superior to serum creatinine for estimation of GFR. Patient groups which benefit most are those with mild to moderate kidney disease and also those in acute renal failure, where toxic drugs have to be administered which are excreted by glomerular filtration, especially elder people (> 50 years), children, pregnant women with suspicion of pre-eclampsia, diabetics, people with diseases of skeletal muscle and renal transplant recipients. Additionally cystatin C has been discussed in recent literature as a prognostic marker for acute heart failure.

As with creatinine 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 Roche assay the following prediction equation is recommended using only concentration in mg/L and a prepubertal factor:

$$\text{GFR [mL/min/1.73 m}^2\text{]} = \frac{84.69}{\text{cystatin C (mg/L)}^{1.680}} \times 1.384^*$$

*for children < 14 y

Test principle⁵

Particle enhanced immunoturbidimetric assay

Human cystatin C agglutinates with latex particles coated with anti-cystatin C antibodies. The aggregate is determined turbidimetrically at 552 nm.

Reagents - working solutions

R1 Solution of polymers in MOPS-buffered saline; preservative, stabilizers

R2 = SR Latex particles in glycine buffer coated with anti-cystatin C antibodies (rabbit); preservative, stabilizers

R1 is in position B and SR is in position C.

Precautions and warnings

Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.

For USA: For prescription use only.

Reagent handling

Ready for use

COBAS INTEGRA 400 plus analyzers:

CYSC **cobas c** packs must be mixed for 1 minute using the Cassette Mixer before loading on the analyzer.

COBAS INTEGRA 800 analyzer:

CYSC **cobas c** packs must be mixed well before placing on-board the instrument.

Storage and stability

Shelf life at 2-8 °C See expiration date on **cobas c** pack label

COBAS INTEGRA 400 plus system

On-board in use at 10-15 °C 8 weeks

COBAS INTEGRA 800 system

On-board in use at 8 °C 8 weeks

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.

Serum

Plasma: Li-heparin plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

Stability:¹⁵ 7 days at 2-8 °C

6 months at (-15)-(-25) °C

Frozen samples should carefully be thawed and mixed well before analysis.

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

NaCl Diluent 9 %, Cat. No. 20756350322, system-ID 07 5635 0 for automatic sample dilution and standard serial dilutions.

NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board COBAS INTEGRA 400 plus/800 analyzers.

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Application for serum and plasma

COBAS INTEGRA 400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A	552 nm
Calc. first/last	35/69
Typical prozone effect	> 20 mg/L
Antigen excess check	No
Predilution factor	No
Unit	mg/L

Pipetting parameters

		Diluent (H ₂ O)
R1	154 µL	
Sample	2 µL	5 µL
SR	34 µL	13 µL
Total volume	208 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A	552 nm
Calc. first/last	46/98
Typical prozone effect	> 20 mg/L
Antigen excess check	No
Predilution factor	No
Unit	mg/L

Pipetting parameters

		Diluent (H ₂ O)
R1	154 µL	
Sample	2 µL	5 µL
SR	34 µL	13 µL
Total volume	208 µL	

Calibration

Calibrator	Calibrator f.a.s. Cystatin C
Calibration dilution ratio	1:1, 1:1.63, 1:2.31, 1:3.88, 1:9.33, and 1:18.22, performed automatically by the instrument
Calibration mode	Logit/log 4
Calibration replicate	Duplicate recommended
Calibration interval	Each lot and after 90 days on board and as required following quality control procedures

Enter the assigned lot specific cystatin C value of the undiluted calibrator, indicated in the package insert of the calibrator C.f.a.s. Cystatin C.

Traceability: This method has been standardized against an in-house reference preparation of pure recombinant human cystatin C. The cystatin C concentration of this reference preparation was established by dry mass determination as described in reference Bilrup-Jensen.¹⁶

Quality control

Quality control	Cystatin C Control Set
Control interval	24 hours and using a new cobas c pack recommended
Control sequence	User defined
Control after calibration	Recommended

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400 plus/800 analyzers).

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum, plasma

Icterus:¹⁷ No significant interference up to an I index of 60 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:¹⁷ No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):¹⁷ No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference up to a rheumatoid factors level of 1200 IU/mL.

High-dose hook effect: Does not occur at cystatin C concentrations below 20 mg/L.

Drugs: No interference was found at therapeutic concentrations using common drug panels.^{18,19}

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.²⁰

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on COBAS INTEGRA analyzers. Refer to the CLEAN Method Sheet for further instructions and for the latest version of the Extra wash cycle list.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.4-8.0 mg/L (typical measuring range)

The upper and lower limits of the measuring range depend on the actual calibrator value.

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement*Limit of Blank and Limit of Detection*

Limit of Blank = 0.3 mg/L

Limit of Detection = 0.4 mg/L

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from $n \geq 60$ measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Values below the Limit of Detection (≤ 0.4 mg/L) will not be flagged by the instrument.

Expected values¹⁵

For individuals 20-70 years ($n = 500$)^{a)}: 0.47-1.09 mg/L

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

a) measured in a well characterized reference population of healthy donors on a Roche/Hitachi system

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability ($n = 21$) and intermediate precision (1 aliquot per run, 1 run per day, 21 days). The following results were obtained:

Repeatability	Mean mg/L	CV %
Level 1	1.11	1.2
Level 2	4.47	1.5

Intermediate precision	Mean mg/L	CV %
Level 1	1.04	2.7
Level 2	4.37	1.1

Method comparison

Cystatin C values for human serum samples obtained on a COBAS INTEGRA 400 analyzer with the COBAS INTEGRA Tina-quant Cystatin C test (CYSC) (y) were compared to those obtained with the Cystatin C test on a Roche/Hitachi 917 analyzer (x).

Roche/Hitachi 917 analyzerSample size (n) = 104

Passing/Bablok Lin. regression

 $y = 0.987x - 0.009$ mg/L $y = 0.991x - 0.044$ mg/L $r = 0.952$ $r = 0.995$

SD (md 95) = 0.240 SPC (SD) = 0.109

The sample concentrations were between 0.75 and 6.51 mg/L.

Cystatin C values for human serum samples obtained on a COBAS INTEGRA 400 analyzer with the COBAS INTEGRA Tina-quant Cystatin C test (CYSC) (y) were compared to those determined with a nephelometric Cystatin C test (x).

Nephelometric methodSample size (n) = 70Passing/Bablok²¹ Lin. regression $y = 0.931x + 0.249$ mg/L $y = 0.921x + 0.3$ mg/L $r = 0.911$ $r = 0.984$

SD (md 95) = 0.327 SPC (SD) = 0.204

The sample concentrations were between 0.84 and 7.49 mg/L.

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

	Contents of kit
	Volume after reconstitution or mixing
	Global Trade Item Number

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