CYSC2

Tina-quant Cystatin C Gen.2

cobas®

Specific proteins

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
06600239 190	Tina-quant Cystatin C Gen.2 (225 tests)	System-ID 07 7550 9	COBAS INTEGRA 400 plus

Materials required (but not provided):

04975901 191	C.f.a.s. Cystatin C (4 × 1 mL)	System-ID 07 7566 5	
06729371 190	Cystatin C Control Set Gen.2 Control 1 (3 × 1 mL) Control 2 (3 × 1 mL) Control 3 (3 × 1 mL)	 System-ID 07 7561 4 System-ID 07 7562 2 System-ID 07 7563 0	
20756350 322	NaCl Diluent 9 % (6 × 22 mL)	System-ID 07 5635 0	

English

System information

Test CYSC2, test ID 0-139

Intended use

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

Summary[1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20]

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 m^2 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.

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. There is a small dependency of cystatin C concentration from age in the age range 1 to 50 years whereas the cystatin C concentration of healthy individuals > 50 years increases with age. Therefore, cystatin C in plasma and serum has been proposed as a more sensitive marker for GFR in children and adults, 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.

CKD-EPI cystatin C equation for estimating GFR:[21]

Serum cystatin C ≤ 0.8 mg/L:

Male $133 \times (Scys/0.8)^{-0.499} \times 0.996^{Age}$

Female $133 \times (Scys/0.8)^{-0.499} \times 0.996^{Age} \times 0.932$

Serum cystatin C > 0.8 mg/L:

Male $133 \times (Scys/0.8)^{-1.328} \times 0.996^{Age}$

Female $133 \times (Scys/0.8)^{-1.328} \times 0.996^{Age} \times 0.932$

Cystatin C equation for estimating GFR acc. to Horio M et al.:[22]

Male $96 \times SCysC^{-1.324} \times 0.996^{Age}$

Female $96 \times SCysC^{-1.324} \times 0.996^{Age} \times 0.894$

Cystatin C equation for estimating GFR acc. to Grubb A et al.:[23]

$eGFR = 130 \times Cystatin\ C^{-1.069} \times Age^{-0.117} - 7$

Test principle[5]

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

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

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Mix all brand new (non-punctured) **cobas c** packs for 1 minute on a cassette mixer before loading on the analyzer.

Storage and stability

Shelf life at 2-8 °C	See expiration date on **cobas c** pack label
On-board in use at 10-15 °C	8 weeks

Specimen collection and preparation[7]

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

Only the specimens listed below were tested and found acceptable.
Serum, collected using serum separating tubes
Plasma: Li-heparin plasma, K_2-, K_3-EDTA 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.

See the limitations and interferences section for details about possible sample interferences.

Blood collected in capillary blood collection tubes is unsuitable for use in this assay.[24]

Stability in serum:	7 days at 15-25 °C
	7 days at 2-8 °C
	24 months at –25 °C[25]
Stability in Li-heparin, K_2-, K_3-EDTA plasma:	7 days at 15-25 °C
	7 days at 2-8 °C
	6 months at –20 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Frozen samples should be thawed carefully 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 postdilution and standard serial dilutions. NaCl Diluent 9 % is placed in its predefined rack position and is stable for 4 weeks on-board the COBAS INTEGRA 400 plus analyzer.

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/plasma

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_2O)
R1	154 µL	
Sample	2 µL	
SR	34 µL	20 µL
Total volume	210 µL	

Calibration

Calibrator	C.f.a.s. Cystatin C
	1:1, 1:1.5, 1:2.58, 1:5.2, 1:9.35, and 0, performed automatically by the instrument
Calibration mode	Spline
Calibration replicate	Duplicate recommended
Calibration frequency	Each lot and after 90 days on-board and as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against ERM-DA471/IFCC reference material.

Quality control

Quality control	Cystatin C Control Set Gen.2
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

The COBAS INTEGRA 400 plus analyzer automatically calculates the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help.

Limitations - interference

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.[26]

Levels of cystatin C are sensitive to changes in thyroid function and should not be used without knowledge of the patient's thyroid status.[27]

Criterion: Recovery within ± 0.100 mg/L of initial values of samples ≤ 1.00 mg/L and within ± 10 % for samples > 1.00 mg/L.

Icterus:[28] 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:[28] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[28] 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 < 1200 IU/mL do not interfere.

High dose hook-effect: No false result occurs up to a cystatin C concentration of 12 mg/L.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[29,30]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[31]

In very rare cases falsely elevated results for cystatin C will be obtained from samples taken from patients who have been treated with rabbit antibodies or have developed anti-rabbit antibodies.[32]

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

0.40-6.80 mg/L

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 using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Limit of Blank , Limit of Detection and Limit of Quantitation

Limit of Blank = 0.30 mg/L

Limit of Detection = 0.40 mg/L

Limit of Quantitation = 0.40 mg/L

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 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 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 30 %. It has been determined using low concentration cystatin C samples.

Expected values

Aliquots of samples from a reference panel containing healthy subjects were analyzed. Study participants with an eGFR > 80 (mL/min/1.73 m^2) were included in this study (273 samples). The age of the study population ranged from 21 to 77 years.

The analysis of the data with the 2.5 % and the 97.5 % percentile gave a cystatin C range from 0.61 mg/L to 0.95 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.

Specific performance data

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

Precision

Repeatability and intermediate precision were determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP5 requirements (2 aliquots per run, 2 runs per day, 21 days). The following results were obtained:

Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
Control 1	0.915	0.015	1.7
Control 2	1.75	0.02	1.0
Control 3	4.10	0.04	1.1
Human serum 1	0.519	0.016	3.1
Human serum 2	2.78	0.03	1.1
Human serum 3	6.39	0.09	1.5

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
Control 1	0.915	0.019	2.0
Control 2	1.75	0.03	1.7
Control 3	4.10	0.07	1.7
Human serum 1	0.519	0.016	3.1

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
Human serum 2	2.78	0.05	1.8
Human serum 3	6.39	0.11	1.7

Method comparison

Cystatin C values for human serum samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 132

Passing/Bablok[33]	Linear regression
y = 1.015x - 0.051 mg/L	y = 1.017x - 0.059 mg/L
τ = 0.980	r = 0.999

The sample concentrations were between 0.430 and 6.67 mg/L.

Cystatin C values for human serum samples obtained on a COBAS INTEGRA 800 analyzer (y) were compared with those determined using the corresponding reagent on a COBAS INTEGRA 400 analyzer (x).

Sample size (n) = 130

Passing/Bablok[33]	Linear regression
y = 1.032x - 0.035 mg/L	y = 1.035x - 0.047 mg/L
τ = 0.975	r = 0.999

The sample concentrations were between 0.410 and 6.65 mg/L.

References

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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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.roche.com for definition of symbols used):

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

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