B2MG

cobas®

Tina-quant β2-Microglobulin (urine application)

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08105529190	Tina-quant β2-Microglobulin (150 tests)	System-ID 2025 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

08047545190	Calibrator β2-Microglobulin (2 × 1 mL)	Code 20474	
08362785190	Control Set β2-Microglobulin - Level I (2 × 1 mL) - Level II (2 × 1 mL)	 Code 20144 Code 20145	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

B2MGU: ACN 20251

Intended use

Immunoturbidimetric assay for the quantitative in vitro determination of β2-microglobulin (B2MG) in human urine on **cobas c** systems.

Summary

B2MG is a low-molecular-weight protein with approximately 12 kDa. It is identical to the light chain of the major histocompatibility complex (MHC) antigens (HLA, A, B, C). Thus B2MG is expressed on the cell membrane of nearly all nucleated cells (exception: trophoblasts).[1,2] Lymphocytes are the major location of synthesis. B2MG is constantly released into the blood in small quantities. Due to its low molecular weight, it is rapidly filtered through the renal glomeruli. Thereafter, up to 99.9 % is reabsorbed by the proximal tubules.[3]

Drug nephrotoxicity causing severe, acute changes in tubular reabsorption and progressive renal diseases causing irreversible structural tubular defects impair tubular reabsorption of numerous smaller proteins including B2MG.[4] Thus, urinary B2MG is discussed as a marker for the diagnosis and monitoring of tubulointerstitial renal damage.[2] Excretion of B2MG is increased in Fanconi syndrome, a generalized dysfunction of the proximal tubules. Causes for acquired Fanconi syndrome include exposure to toxins and drugs.[5]

Elevated B2MG values may identify patients at higher risk of glomerular filtration rate (GFR) decline in other kidney diseases such as membranous nephropathy.[6] Furthermore, there is evidence that B2MG excretion is associated with acute allograft rejection in renal transplant recipients.[7]

Various assay methods are available for B2MG determination, such as radioimmunoassays (RIA), enzyme-linked immunosorbent assays (ELISA), nephelometric immunoassays, and turbidimetric methods.[2] The Roche B2MG assay is based on the principle of immunological agglutination with latex reaction enhancement.

Test principle

Immunoturbidimetric assay.

Latex-bound anti-β2-microglobulin antibodies react with antigen from the sample to form antigen/antibody complexes which are determined turbidimetrically after agglutination.[8]

Reagents - working solutions

R1 TRIS/HCl buffer: 23 g/L, pH 8.2; NaCl: 19 g/L; EDTA: 1.3 g/L; preservative

R3 Latex particles coated with polyclonal anti-human β2-microglobulin antibody (rabbit); preservative

R1 is in position B and R3 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.

Reagent handling

Ready for use

Carefully invert reagent container several times prior to use to ensure that the reagent components are mixed.

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 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.
Urine

Each urine sample must be centrifuged (10 minutes at approximately 3000 × g) prior to testing.[9]
B2MG is unstable in acidic conditions and degradation occurs at pH < 6 within 2 hours.[2,10] Thus, pre-analytical conditions are highly important. Since the degradation also takes place in the bladder, collection of a spot urine sample should not be performed in the morning due to a lower urine pH.[2]
A strict control of the urine pH after collection is required: urine samples must be adjusted to pH 7-9 by the addition of 1 N NaOH as soon as possible after receipt.[10]

Centrifuge samples containing precipitates before performing the assay.

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

Stability in urine:	5 days at 15-25 °C
	14 days at 2-8 °C
	12 weeks at –20 ± 5 °C
	Freezing and thawing up to 2 times is allowed.

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.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

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.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

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Application for urine

Test definition

Reporting time	10 min		
Wavelength (sub/main)	-/700 nm		
Reagent pipetting		Diluent (H_2O)	
R1	63 µL	–	
R3	63 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1 µL	–	–
Decreased	1 µL	10 µL	100 µL
Increased	1 µL	–	–

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Application for urine (ACN 20251)
Transfer of calibration from serum/plasma application (ACN 20250)

Application for serum/plasma (ACN 20250)

Calibrators	S1: H_2O
	S2: Calibrator β2-Microglobulin
Calibration mode	Linear
Calibration frequency	Full calibration - after reagent lot change - 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 the WHO standard.

Quality control

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. It is recommended to perform quality control always after lot calibration and subsequently at least every 12 weeks. 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 c systems automatically calculate the analyte concentration of each sample in the unit mg/L (nmol/L, µg/L).

Conversion factors:	mg/L × 84.7 = nmol/L
	mg/L × 1000 = µg/L

Limitations - interference

Criterion: Recovery within ± 0.1 mg/L of initial values at a β2-microglobulin concentration of ≤ 1.0 mg/L and within ± 10 % for samples > 1.0 mg/L.

Hemolysis: No significant interference up to an H index of 1100 (approximate hemoglobin concentration: 1100 mg/dL).[11]

High-dose hook effect: No false result occurs up to a B2MG concentration of 240 mg/L (20328 nmol/L).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[12]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

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 c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

0.2-5.8 mg/L (16.9-491 nmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:11 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 11.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.1 mg/L (8.5 nmol/L)
Limit of Detection	= 0.15 mg/L (12.7 nmol/L)
Limit of Quantitation	= 0.2 mg/L (16.9 nmol/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 20 %. It has been determined using low concentration human β2-microglobulin samples.

Expected values

mg/L*

B2MG (urine), male: ≤ 0.300 mg/L[14]

B2MG (urine), female: ≤ 0.183 mg/L[14]

B2MG/creatinine (urine): ≤ 0.029 mg/mmol[14]

24 h urine: 0.033-0.363 mg[10]

nmol/L*

B2MG (urine), male: ≤ 25.4 nmol/L[14]

B2MG (urine), female: ≤ 15.5 nmol/L[14]

B2MG/creatinine (urine): ≤ 2.46 nmol/mmol[14]

24 h urine: 2.80-30.7 nmol[10]

*calculated by unit conversion factor

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

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Repeatability	*Mean mg/L*	*SD mg/L*	*CV %*
CS B2MG1[a)]	2.54	0.0295	1.2
CS B2MG2[b)]	4.57	0.0355	0.8
Human urine 1	0.274	0.0373	13.6
Human urine 2	0.395	0.0385	9.8
Human urine 3	0.807	0.0457	5.7
Human urine 4	2.53	0.0425	1.7
Human urine 5	5.07	0.0389	0.8

Intermediate precision	*Mean mg/L*	*SD mg/L*	*CV %*
CS B2MG1[a)]	2.54	0.0385	1.5
CS B2MG2[b)]	4.57	0.0692	1.5
Human urine 1	0.274	0.0385	14.0
Human urine 2	0.395	0.0424	10.7
Human urine 3	0.807	0.0473	5.9
Human urine 4	2.53	0.0449	1.8
Human urine 5	5.06	0.0458	0.9

a) Control Set β2-Microglobulin Level I

b) Control Set β2-Microglobulin Level II

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s).

Method comparison

β2-Microglobulin values for human urine samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 68

Passing/Bablok[15]	Linear regression
y = 1.000x + 0.00300 mg/L	y = 0.998x + 0.00986 mg/L
τ = 0.983	r = 1.000

The sample concentrations were between 0.260 and 5.52 mg/L.

β2-Microglobulin values for human urine samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 76

Passing/Bablok[15]	Linear regression
y = 1.021x - 0.00731 mg/L	y = 1.023x - 0.0105 mg/L
τ = 0.968	r = 0.999

The sample concentrations were between 0.200 and 5.39 mg/L.

References

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12 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

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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 for reconstitution
GTIN	Global Trade Item Number

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche