08057427500V8.0

CA2

Calcium Gen.2

Order information

REF	🛈	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057427190*	08057427500	Calcium Gen.2 (1500 tests)	System-ID 2034 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057427214*	08057427500	Calcium Gen.2 (1500 tests)	System-ID 2034 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 × 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

* Some kits shown may not be available in all countries.

English

System information

CA2: ACN 20340 (Serum/plasma)

CA2U: ACN 20341 (Urine)

Intended use

In vitro test for the quantitative determination of calcium in human serum, plasma and urine on **cobas c** systems.
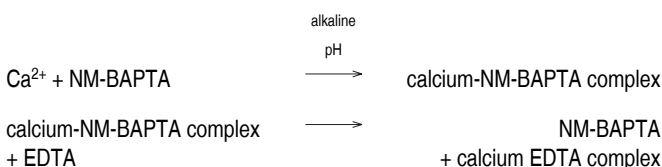
Summary

Measurements of calcium, measured with this device, in human serum, plasma and urine, are used in the diagnosis of hypercalcemia/hypercalciuria (such as observed in hyperparathyroidism and cancer, endocrine disorders, inherited hypercalcemia, excessive vitamin D intake, chronic kidney disease) and of hypocalcemia/hypocalciuria (such as observed in hypoparathyroidism, vitamin D or magnesium deficiency, calcium homeostasis bone disease).[1]

Calcium is the most abundant mineral element in the body with about 99 % in the bones primarily as hydroxyapatite. The remaining calcium is distributed between the various tissues and the extracellular fluids where it performs a vital role for many life sustaining processes. Among the extra skeletal functions of calcium are involvement in blood coagulation, neuromuscular conduction, excitability of skeletal and cardiac muscle, enzyme activation, and the preservation of cell membrane integrity and permeability. Urinary calcium results from glomerular filtration of albumin-free plasma calcium and intense calcium reabsorption along the different tubular segments.[2]

Serum calcium levels and hence the body content are controlled by parathyroid hormone (PTH), calcitonin, and vitamin D. An imbalance in any of these modulators leads to alterations of the body and serum calcium levels. Increases in serum PTH or vitamin D are usually associated with hypercalcemia. Increased serum and urine calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcemia may be observed e.g. in hypoparathyroidism, nephrosis, and pancreatitis.[1]

Test principle

Calcium ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA.

$$Ca^{2+} + NM\text{-}BAPTA \xrightarrow[\text{pH}]{\text{alkaline}} calcium\text{-}NM\text{-}BAPTA\ complex$$

$$calcium\text{-}NM\text{-}BAPTA\ complex + EDTA \longrightarrow NM\text{-}BAPTA + calcium\ EDTA\ complex$$

The change in absorbance is directly proportional to the calcium concentration and is measured photometrically.

Reagents - working solutions

R1 CAPSO:[a] 557 mmol/L; NM-BAPTA: 2 mmol/L; pH 10.0; non-reactive surfactant; preservative

R3 EDTA: 7.5 mmol/L; pH 7.3; non-reactive surfactant, preservative

a) 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid
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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H318	Causes serious eye damage.

Prevention:

P280	Wear eye protection/ face protection.

Response:

P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling
Ready for use

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:	26 weeks

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cobas®

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: Fresh serum collected in the fasting state is the preferred specimen. Plasma: Li-heparin plasma.

Serum or plasma should be separated from blood cells as soon as possible, because prolonged contact with the clot may cause lower calcium values.[3] Sera from patients receiving EDTA (treatment of hypercalcemia) are unsuitable for analysis, since EDTA will chelate the calcium and render it unavailable for reaction with NM-BAPTA. Co-precipitation of calcium with fibrin (i.e. heparin plasma), lipids, or denatured protein has been reported with storage or freezing.[1,4]

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.

Urine

Urine specimens should be collected in acid-washed bottles. 24-hour specimens should be collected in containers containing 20-30 mL of 6 mol/L HCl to prevent calcium salt precipitation. Precipitated calcium salts may not be completely dissolved by the addition of HCl following urine collection.[5]

If stabilizers are added to the sample, the sample index feature must not be used.

Stability in *serum/plasma*:[6]	7 days at 15-25 °C
	3 weeks at 2-8 °C
	8 months at -20 °C (± 5 °C)

Freeze only once.

Stability in *urine*:[6]	2 days at 15-25 °C
	4 days at 2-8 °C
	3 weeks at -20 °C (± 5 °C)

Freeze only once.

Stored serum or urine specimens must be mixed well prior to analysis.

Centrifuge samples containing precipitates before performing the assay.

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

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.

Application for serum and plasma

Test definition

Reporting time	10 min
Wavelength (sub/main)	376/340 nm
Reagent pipetting	Diluent (H$_2$O)
R1	15 µL	120 µL
R3	15 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	–	–
Decreased	2.3 µL	–	–
Increased	2.3 µL	–	–

Application for urine

Test definition

Reporting time	10 min
Wavelength (sub/main)	376/340 nm
Reagent pipetting	Diluent (H$_2$O)
R1	15 µL	120 µL
R3	15 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	15 µL	60 µL
Increased	1.5 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 serum/plasma (ACN 20340)

Calibrators	S1: H$_2$O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - every 8 weeks - as required following quality control procedures

Application for urine (ACN 20341)
Transfer of calibration from serum/plasma application (ACN 20340)

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

Traceability: This method has been standardized against the SRM 956 c Level 2 reference material.

Quality control

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

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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 26 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 mmol/L (mg/dL, mg/L).

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Conversion factors:

mmol/L × 4.01 = mg/dL

mmol/L × 40.1 = mg/L

In studies with 24-hour urine, multiply the value obtained by the 24-hour volume in order to obtain a measurement in mg/24 h or mmol/24 h.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a calcium concentration of 2.2 mmol/L.

Serum/plasma

Icterus:[7] 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:[7] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 1000. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Magnesium: No significant interference from magnesium up to a concentration of 15 mmol/L (36.5 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

The interference of intravenously administered gadolinium containing MRI (magnetic resonance imaging) contrast media was tested (Omniscan®, Optimark®) but no interference was found at the therapeutic concentration. Interferences at higher concentrations were observed.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[10]

Urine

Icterus: No significant interference up to a conjugated bilirubin concentration of 1026 µmol/L or 60 mg/dL.

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

Magnesium: No significant interference from magnesium up to a concentration of 60 mmol/L (145.8 mg/dL).

Urea: No significant interference from urea up to a concentration of 1600 mmol/L (9610 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9]

The interference of intravenously administered gadolinium containing MRI (magnetic resonance imaging) contrast media was tested (Omniscan®, Optimark®). For Omniscan® no interference was observed at the therapeutic concentration, but there was interference at higher concentrations. For Optimark® interference was observed at therapeutic and higher concentrations.

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

Serum/plasma

0.20-5.0 mmol/L (0.8-20.1 mg/dL)

Urine

0.20-7.5 mmol/L (0.8-30.1 mg/dL)

Determine urine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma and urine

Limit of Blank	= 0.10 mmol/L (0.4 mg/dL)
Limit of Detection	= 0.20 mmol/L (0.8 mg/dL)
Limit of Quantitation	= 0.20 mmol/L (0.8 mg/dL)

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 calcium samples.

Expected values[11]

mmol/L

Serum/plasma

Children (0-10 days):	1.90-2.60 mmol/L
Children (10 days-2 years):	2.25-2.75 mmol/L
Children (2-12 years):	2.20-2.70 mmol/L
Children (12-18 years):	2.10-2.55 mmol/L
Adults (18-60 years):	2.15-2.50 mmol/L
Adults (60-90 years):	2.20-2.55 mmol/L
Adults (> 90 years):	2.05-2.40 mmol/L

Urine

2.5-7.5 mmol/24 h with normal food intake.

mg/dL

Serum/plasma

Children (0-10 days):	7.6-10.4 mg/dL
Children (10 days-2 years):	9.0-11.0 mg/dL
Children (2-12 years):	8.8-10.8 mg/dL
Children (12-18 years):	8.4-10.2 mg/dL
Adults (18-60 years):	8.6-10.0 mg/dL
Adults (60-90 years):	8.8-10.2 mg/dL
Adults (> 90 years):	8.2-9.6 mg/dL

Urine

100-300 mg/24 h with normal food intake.

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

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(2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Serum/plasma

Repeatability	Mean	SD	CV
	mmol/L	mmol/L	%
PCCC1[b]	2.22	0.0103	0.5
PCCC2[c]	3.41	0.0136	0.4
Human serum 1	0.398	0.00739	1.9
Human serum 2	1.43	0.00982	0.7
Human serum 3	2.12	0.0112	0.5
Human serum 4	2.42	0.0152	0.6
Human serum 5	4.13	0.0155	0.4

Intermediate precision	Mean	SD	CV
	mmol/L	mmol/L	%
PCCC1[b]	2.21	0.0140	0.6
PCCC2[c]	3.41	0.0272	0.8
Human serum 1	0.398	0.00924	2.3
Human serum 2	1.43	0.0109	0.8
Human serum 3	2.13	0.0134	0.6
Human serum 4	2.42	0.0212	0.9
Human serum 5	4.13	0.0185	0.4

b) PreciControl ClinChem Multi 1
c) PreciControl ClinChem Multi 2

Urine

Repeatability	Mean	SD	CV
	mmol/L	mmol/L	%
Control 1[d]	1.73	0.0138	0.8
Control 2[d]	2.39	0.0150	0.6
Human urine 1	0.374	0.0104	2.8
Human urine 2	1.44	0.0127	0.9
Human urine 3	2.25	0.0161	0.7
Human urine 4	3.56	0.0217	0.6
Human urine 5	6.25	0.0294	0.5

Intermediate precision	Mean	SD	CV
	mmol/L	mmol/L	%
Control 1[d]	1.73	0.0150	0.9
Control 2[d]	2.39	0.0180	0.8
Human urine 1	0.374	0.0170	4.5
Human urine 2	1.43	0.0159	1.1
Human urine 3	2.25	0.0236	1.0
Human urine 4	3.56	0.0282	0.8
Human urine 5	6.22	0.0425	0.7

d) commercially available control material

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Calcium values for human serum, plasma and 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).

Serum/plasma

Sample size (n) = 71

Passing/Bablok[12]	Linear regression
y = 0.988x + 0.0216 mmol/L	y = 0.985x + 0.0295 mmol/L
τ = 0.964	r = 0.999

The sample concentrations were between 0.260 and 4.84 mmol/L.

Urine

Sample size (n) = 68

Passing/Bablok[12]	Linear regression
y = 0.964x + 0.0148 mmol/L	y = 0.967x + 0.0055 mmol/L
τ = 0.987	r = 1.000

The sample concentrations were between 0.270 and 7.12 mmol/L.

Calcium values for human serum, plasma and 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).

Serum/plasma

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 1.024x - 0.0379 mmol/L	y = 1.024x - 0.0304 mmol/L
τ = 0.977	r = 0.999

The sample concentrations were between 0.250 and 4.63 mmol/L.

Urine

Sample size (n) = 71

Passing/Bablok[12]	Linear regression
y = 0.996x - 0.00631 mmol/L	y = 0.990x + 0.00625 mmol/L
τ = 0.989	r = 0.999

The sample concentrations were between 0.300 and 7.42 mmol/L.

Calcium values for human serum, plasma and urine samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Serum/plasma

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 1.021x - 0.0334 mmol/L	y = 1.011x - 0.0110 mmol/L
τ = 0.955	r = 0.999

The sample concentrations were between 0.464 and 4.85 mmol/L.

Urine

Sample size (n) = 72

Passing/Bablok[12]	Linear regression
y = 1.017x + 0.00977 mmol/L	y = 1.016x + 0.0110 mmol/L
τ = 0.992	r = 1.000

The sample concentrations were between 0.250 and 7.13 mmol/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 navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
⟶	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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⟨Roche⟩