CA2

cobas®

Calcium Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05061482 190	Calcium Gen.2 300 tests	System-ID 07 7476 6	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 × 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 × 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 × 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 × 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 × 3 mL)	Code 301	
12149443 160	Precipath U plus (10 × 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 × 5 mL)	Code 300	
10171735 122	Precinorm U (4 × 5 mL)	Code 300	
10171778 122	Precipath U (20 × 5 mL)	Code 301	
10171760 122	Precipath U (4 × 5 mL)	Code 301	
05117003 190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 × 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 × 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

CA2: ACN 698

S-CA2: ACN 699 (STAT, reaction time: 3)

For **cobas c** 502 analyzer:

CA2: ACN 8698

S-CA2: ACN 8699 (STAT, reaction time: 3)

Intended use

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

Summary[1]

Calcium is the most abundant mineral element in the body with about 99 percent 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.

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 calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcemia may be observed e.g. in hypoparathyroidism, nephrosis, and pancreatitis.

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.

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

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

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 and stabilizer

R2 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 R2 is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

CA2

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	6 weeks

Diluent NaCl 9 %

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.
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.[2] 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.[1,3]

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.[4]

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

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

Centrifuge samples containing precipitates before performing the assay.

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

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 6-8 (STAT 3 / 6-8)	
Wavelength (sub/main)	376/340 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	20 µL	160 µL
R2	20 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	3 µL	–	–
Increased	3 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 10-13 (STAT 3 / 10-13)	
Wavelength (sub/main)	376/340 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	20 µL	160 µL
R2	20 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3 µL	–	–
Decreased	3 µL	–	–
Increased	3 µL	–	–

Application for urine

cobas c 311 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 6-8 (STAT 3 / 6-8)	
Wavelength (sub/main)	376/340 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	20 µL	160 µL
R2	20 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15 µL	135 µL
Increased	2 µL	–	–

cobas c 501/502 **test definition**

Assay type	2-Point End	
Reaction time / Assay points	10 / 10-13 (STAT 3 / 10-13)	
Wavelength (sub/main)	376/340 nm	
Reaction direction	Decrease	
Units	mmol/L (mg/dL)	
Reagent pipetting		Diluent (H_2O)
R1	20 µL	160 µL
R2	20 µL	-

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2 µL	–	–
Decreased	4 µL	15	135
Increased	2 µL	–	–

Calibration

Calibrators	S1: H_2O
	S2: C.f.a.s.

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Calcium Gen.2

cobas®

Calibration mode	Linear
Calibration frequency	2-point calibration • after reagent lot change • as required following quality control procedures

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

Quality control

Serum/plasma

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

Urine

Quantitative urine controls are recommended for routine quality control.

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

Roche/Hitachi **cobas c** systems automatically calculate the analyte concentration of each sample.

Conversion factors:	mmol/L x 4.01 = mg/dL

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 ± 0.22 mmol/L (0.9 mg/dL) of initial value of samples ≤ 2.2 mmol/L (8.8 mg/dL) and within ± 10 % for samples > 2.2 mmol/L.

Serum/plasma

Icterus:[6] 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:[6] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[6] 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 up to a concentration of 15 mmol/L.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[7,8]

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.[9]

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 a hemoglobin concentration of 621 µmol/L or 1000 mg/dL.

Magnesium: No significant interference up to a concentration of 60 mmol/L.

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

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 Roche/Hitachi **cobas c** systems. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/Multiclean/SCCS or the NaOHD/SMS/SmpCln1+2/SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is not required.

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

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 (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ)

Serum/plasma and urine

Serum/plasma

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[10]

Serum/plasma

Children (0-10 days):	1.90-2.60 mmol/L (7.6-10.4 mg/dL)
Children (10 days-2 years):	2.25-2.75 mmol/L (9.0-11.0 mg/dL)
Children (2-12 years):	2.20-2.70 mmol/L (8.8-10.8 mg/dL)
Children (12-18 years):	2.10-2.55 mmol/L (8.4-10.2 mg/dL)
Adults (18-60 years):	2.15-2.50 mmol/L (8.6-10.0 mg/dL)
Adults (60-90 years):	2.20-2.55 mmol/L (8.8-10.2 mg/dL)
Adults (> 90 years):	2.05-2.40 mmol/L (8.2-9.6 mg/dL)

Urine

2.5-7.5 mmol/24 h (100-300 mg/24 h) with normal food intake.

Roche has not evaluated reference ranges in a pediatric population.

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:

The following results were obtained:

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Human serum 1	0.60 (2.4)	0.01 (0.0)	2.0
Human serum 2	2.55 (10.2)	0.02 (0.1)	0.8
Human serum 3	4.46 (17.9)	0.04 (0.2)	0.8
Precinorm U	2.25 (9.0)	0.02 (0.1)	0.8
Precipath U	3.51 (14.1)	0.03 (0.1)	0.8

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Human serum 1	0.60 (2.4)	0.02 (0.1)	2.5
Human serum 2	2.55 (10.2)	0.02 (0.1)	0.9
Human serum 3	4.46 (17.9)	0.04 (0.2)	0.9
Precinorm U	2.25 (9.0)	0.02 (0.1)	0.8
Precipath U	3.51 (14.1)	0.03 (0.1)	0.9

Urine

Repeatability	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Human urine 1	0.58 (2.3)	0.02 (0.1)	3.0
Human urine 2	3.92 (15.7)	0.04 (0.2)	1.1
Human urine 3	5.18 (20.8)	0.05 (0.2)	0.9
Human urine 4	6.09 (24.4)	0.08 (0.3)	1.3
Control Level 1	1.85 (7.4)	0.02 (0.1)	1.3
Control Level 2	2.72 (10.9)	0.03 (0.1)	1.1

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L (mg/dL)	*mmol/L (mg/dL)*	*%*
Human urine 1	0.58 (2.3)	0.02 (0.1)	3.1
Human urine 2	3.92 (15.7)	0.05 (0.2)	1.2
Human urine 3	5.18 (20.8)	0.06 (0.2)	1.1
Human urine 4	6.09 (24.4)	0.08 (0.3)	1.3
Control Level 1	1.85 (7.4)	0.03 (0.1)	1.5
Control Level 2	2.72 (10.9)	0.04 (0.2)	1.3

Method comparison

Calcium values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Roche Calcium Gen.2 reagent (x) were compared to those determined on a Roche/Hitachi MODULAR P analyzer using the same reagent (y).

Serum/plasma

Sample size (n) = 69

Passing/Bablok[11]	Linear regression
y = 0.982x + 0.061 mmol/L	y = 0.982x + 0.059 mmol/L
τ = 0.979	r = 1.00

The sample concentrations were between 0.33 and 4.76 mmol/L (1.3 and 19.1 mg/dL).

Urine

Sample size (n) = 65

Passing/Bablok[11]	Linear regression
y = 0.989x + 0.064 mmol/L	y = 0.983x + 0.079 mmol/L
τ = 0.989	r = 1.00

The sample concentrations were between 0.28 and 7.47 mmol/L (1.1 and 30.0 mg/dL).

Calcium values for human serum, plasma and urine samples obtained on a Roche/Hitachi **cobas c** 501 analyzer using the Roche Calcium Gen.2 reagent (y) were compared with those determined using the Roche Calcium reagent on a Roche/Hitachi MODULAR P analyzer (x).

Serum/plasma

Sample size (n) = 69

Passing/Bablok[11]	Linear regression
y = 1.018x - 0.027 mmol/L	y = 1.023x - 0.036 mmol/L
τ = 0.976	r = 1.00

The sample concentrations were between 0.28 and 4.65 mmol/L (1.1 and 18.6 mg/dL).

Urine

Sample size (n) = 65

Passing/Bablok[11]	Linear regression
y = 1.024x + 0.018 mmol/L	y = 1.020x + 0.029 mmol/L
τ = 0.988	r = 1.00

The sample concentrations were between 0.30 and 7.25 mmol/L (1.2 and 29.1 mg/dL).

References

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9 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

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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.

CONTENT	Contents of kit
→	Volume after reconstitution or mixing

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

Significant additions or changes are indicated by a change bar in the margin.

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