Application Sheet

cobas®

Laboratory Name

Test Name: Calcium Gen.2

System information

For **cobas c** 311/501 analyzers:
CA2: ACN 698
S-CA2: ACN 699 (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.

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

Stored serum 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
Other suitable control material can be used in addition.

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 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	–	–

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

At least once daily run solutions at two levels of a quality control material with known concentrations.

Refer to Brown Clinic Quality Control Requirements, Rules and Reviews Policy
Refer to Brown Clinic Quatliy Control Specialty and Subspecialty Policy

Calculation

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

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

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]

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)

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

Limit of Blank:	= 0.10 mmol/L (0.4 mg/dL)
Limit of Detection:	= 0.20 mmol/L (0.8 mg/dL)
Limit of Quantitaion:	= 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 95^{th} 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 %).

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)

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

For Known Interfering Substances section refer to package insert.
For Known Non-Interfering Substance refer to package insert.
For Additional Technical Information refer to package insert.

References

1. Endres DB, Rude RK. Mineral and Bone Metabolism. In: Burtis CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. St. Louis (MO): Saunders Elsevier 2006:1891-1965.
2. Heins M, Heil W, Withold W. Storage of Serum or Whole Blood Samples? Effects of Time and Temperature on 22 Serum Analytes. Eur J Clin Chem Clin Biochem 1995;33:231-238.
3. Wilding P, Zilva JF, Wilde CE. Transport of specimens for clinical chemistry analysis. Ann Clin Biochem 1977;14:301-306.
4. Burtis CA, Ashwood ER, Bruns DE, ed. Tietz Fundamentals of Clinical Chemistry, 6th ed. St. Louis (MO): Saunders Elsevier 2008:715.
5. Use of Anticoagulants in Diagnostic Laboratory Investigations. WHO Publication WHO/DIL/LAB 99.1 Rev. 2. Jan. 2002.
6. Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.
7. Breuer J. Report on the Symposium "Drug Effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.
8. Sonntag O, Scholer A. Drug interferences in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.
9. Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.
10. Wu AHB, ed. Tietz Clinical Guide to Laboratory Tests, 4th ed. St. Louis (MO): Saunders Elsevier 2006:202-207.
11. Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

Alternative method

Refer to Brown Clinic Back-up Testing Policy

Source document

Reagent Name: CA2
Package Insert Version: 2013-10, V 3.0 English

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 x 5 mL)	Code 300	
10171778 122	Precipath U (20 × 5 mL)	Code 301	
10171760 122	Precipath U (4 x 5 mL)	Code 301	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

Effective date

Effective date for this procedure: _____

Author

Source documentation compiled by Roche Diagnostics

Revised by: Heather J Hall, MBA, MT(ASCP), CG(ASCP)^{cm} Date: 4/9/2018

Approved by: Aaron Shives MD (Signature on file Date: 4/11/2018

REVIEW – REVISION SUMMARY DOCUMENTATION

Date: By: Revision Summary: