Calcium

Order information

COBAS INTEGRA Calcium	300 Tests	Cat. No. **20763128** 322 System-ID 07 6312 8
Calibrator f.a.s.	12 × 3 mL	Cat. No. **10759350** 190
Calibrator f.a.s. (for USA)	12 × 3 mL	Cat. No. **10759350** 360 System-ID 07 3718 6
Precinorm U	20 × 5 mL	Cat. No. **10171743** 122 System-ID 07 7997 0
Precipath U	20 × 5 mL	Cat. No. **10171778** 122 System-ID 07 7998 9
Precinorm U plus	10 × 3 mL	Cat. No. **12149435** 122
Precinorm U plus (for USA)	10 × 3 mL	Cat. No. **12149435** 160 System-ID 07 7999 7
Precipath U plus	10 × 3 mL	Cat. No. **12149443** 122
Precipath U plus (for USA)	10 × 3 mL	Cat. No. **12149443** 160 System-ID 07 8000 6
PreciControl ClinChem Multi 1	20 × 5 mL	Cat. No. **05117003** 190
PreciControl ClinChem Multi 1 (for USA)	4 × 5 mL	Cat. No. **05947626** 160 System-ID 07 7469 3
PreciControl ClinChem Multi 2	20 × 5 mL	Cat. No. **05117216** 190
PreciControl ClinChem Multi 2 (for USA)	4 × 5 mL	Cat. No. **05947774** 160 System-ID 07 7470 7

● Indicates analyzer(s) on which **cobas c** pack can be used

COBAS INTEGRA 400/400 plus	COBAS INTEGRA 800
●	●

System information
COBAS INTEGRA Calcium (CA)
Test CA, test ID 0-012 for serum and plasma
Test CAU, test ID 0-112 for urine

Intended use
In vitro test for the quantitative determination of calcium in human serum, plasma, and urine on COBAS INTEGRA systems.

Summary[1,2]
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 believed to be 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 in hypoparathyroidism, steatorrhea, nephrosis, and pancreatitis.

Test principle
Method according to Schwarzenbach with o-cresolphthalein complexone.[3] Calcium ions react with o-cresolphthalein complexone (o-CPC) under alkaline conditions to form a violet colored complex. The addition of 8-hydroxyquinoline prevents interference by magnesium and iron.

$$Ca^{2+} + \text{o-CPC} \xrightarrow{\textit{Alkaline pH}} \text{calcium-o-CPC complex}$$

The color intensity of the complex formed is directly proportional to the calcium concentration. It is determined by measuring the increase in absorbance at 552 nm.

Reagents - working solutions
R1 CAPS (3-[cyclohexylamino]-1-propanesulfonic acid): 525 mmol/L; NaOH: 400 mmol/L, pH 11.5; nonreactive surfactant

R2 = SR o-cresolphthalein complexone: 0.5 mmol/L; 8-hydroxyquinoline: 30 mmol/L; pH 1.1; stabilizer

Precautions and warnings
Pay attention to all precautions and warnings listed in Section 1 / Introduction of this Method Manual.
This kit contains components classified as follows according to the European directive 99/45/EC:


C R1 contains sodium hydroxide 1.7 % w/w.
Corrosive

R 35 Causes severe burns.

S 26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S 36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

Contact phone: all countries: +49-621-7590,
USA: +1-800-428-2336

Reagent handling
Ready for use.

Storage and stability

Shelf life at 15 to 25 °C	See expiration date on **cobas c** pack label
COBAS INTEGRA 400/400 plus systems	
On-board in use at 10 to 15 °C	12 weeks
COBAS INTEGRA 800 systems	
On-board in use at 8 °C	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.[4] 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 o-cresolphthalein complexone. Co-precipitation of calcium with fibrin (i.e. heparin plasma), lipids, or denatured protein has been reported with storage or freezing.[5,6]

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 5 mL of 6 mol/L HCl. If the specimen is collected without acid, the pH should be adjusted to 3-4 with 6 mol/L HCl.[1]

Stability in *serum/plasma:*[7]	7 days at 15-25 °C 3 weeks at 2-8 °C 8 months at (-15)-(-20) °C
Stability in *urine:*[7]	2 days at 15-25 °C 4 days at 2-8 °C 3 weeks at (-15)-(-20) °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.

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, and urine

COBAS INTEGRA 400/400 plus test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	33/36
Unit	mmol/L

Pipetting parameters

Serum, plasma		Diluent (H_2O)
R1	20 µL	95 µL
Sample	3 µL	35 µL
SR	20 µL	50 µL
Total volume	223 µL	
Urine		Diluent (H_2O)
R1	20 µL	95 µL
Sample	2 µL	35 µL
SR	20 µL	50 µL
Total volume	222 µL	

COBAS INTEGRA 800 test definition

Measuring mode	Absorbance
Abs. calculation mode	Endpoint
Reaction mode	R1-S-SR
Reaction direction	Increase
Wavelength A/B	552/629 nm
Calc. first/last	43/46
Unit	mmol/L

Pipetting parameters

Serum, plasma		Diluent (H_2O)
R1	20 µL	95 µL
Sample	3 µL	35 µL
SR	20 µL	50 µL
Total volume	223 µL	
Urine		Diluent (H_2O)
R1	20 µL	95 µL
Sample	2 µL	35 µL
SR	20 µL	50 µL
Total volume	222 µL	

Calibration

Calibrator	Calibrator f.a.s. Use deionized water as zero calibrator.
Calibration mode	Linear regression
Calibration replicate	Duplicate recommended
Calibration interval	**COBAS INTEGRA 400/400 plus systems:** Each **cobas c** pack, every 3 days, and as required following quality control procedures **COBAS INTEGRA 800 systems:** Each **cobas c** pack, every 2 weeks, and as required following quality control procedures

Traceability: This method has been standardized against the SRM 909 b reference material.

Quality control

Quality control: serum, plasma	Precinorm U, Precinorm U plus or PreciControl ClinChem Multi 1 Precipath U, Precipath U plus or PreciControl ClinChem Multi 2
Quality control: urine	Quantitative urine controls are recommended for routine quality control.
Control interval	24 hours 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

COBAS INTEGRA analyzers automatically calculate the analyte concentration of each sample. For more details, please refer to Data Analysis in the Online Help (COBAS INTEGRA 400/400 plus/800 analyzers).

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

Limitations - interference

Criterion: Recovery within ± 10 % of initial value.

Serum, plasma

Icterus[8]	No significant interference.
Hemolysis[8]	No significant interference.
Lipemia[8]	No significant interference.
Anticoagulants	Complexing anticoagulants such as citrate, oxalate, and EDTA must be avoided.
Drugs	Therapeutic drug interference was tested according to the recommendations of the VDGH[a]. No interference was found. *Exception:* Drugs containing strontium salts may lead to significantly increased calcium results.
Other	Intravenously administered contrast media for MRI (magnetic resonance imaging) contain chelating complexes which may interfere with the determination of calcium.
	A sharp decrease in calcium values was observed when gadodiamide (GdDTPA-BMA) was administered. Follow the instructions of the manufacturer with regard to the retention time of the contrast medium.
	Results flagged with HIGH ACT ("high activity") indicate potentially elevated results due to gradient formation within the sample. Such samples should be re-run after transfer to a secondary tube. The HIGH ACT flag can also occur in extremly low or zero aqueous samples (e.g. when "0" calibrator is run as a sample). This is because in these samples the typical slight decrease in the absorbance after addition of SR (which is the measuring principle of the High activity flag) is not observed. Therefore mixing of the aqueous zero sample will not prevent a flagged result because in a zero sample the formation of a gradient is not possible.
	In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.

a) Verband der Diagnostica und Diagnostica Geräte Hersteller
Refer to section 1 / Introduction of this Method Manual, for a list of tested drugs and their concentrations.

Urine

Drugs	Drugs containing strontium salts may lead to significantly increased calcium results.

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 Method Manual, Introduction, Extra Wash Cycles for further instructions.
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
0.10-5.0 mmol/L (0.4-20 mg/dL)

Urine
0.15-7.0 mmol/L (0.6-28 mg/dL)

Determine 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

Serum, plasma
Lower detection limit of the test:
0.10 mmol/L (0.4 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD, repeatability, n = 30).

Urine
Lower detection limit of the test:
0.15 mmol/L (0.6 mg/dL)
The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of a zero sample (zero sample + 3 SD,repeatability, n = 30).

Expected values

Serum/plasma[9]
2.15-2.55 mmol/L (8.6-10.2 mg/dL)

24-hour urine[10]
2.5-8.0 mmol/24 h (100-321 mg/24 h), corresponding to 1.7-5.3 mmol/L (6.8-21.3 mg/dL)*
*Assuming a urine volume of 1.5 L/24 h

Reference range acc. to Tietz[11]
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.

Urine
2.5-7.5 mmol/24 h (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 for serum and plasma

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

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability[b] and intermediate precision[c] (2 aliquots per run, 2 runs per day, 20 days).
The following results were obtained:

	Level 1	Level 2
Mean	2.33 mmol/L (9.34 mg/dL)	3.37 mmol/L (13.5 mg/dL)
CV repeatability[b]	1.0 %	0.8 %
CV intermediate precision[c]	3.5 %	3.1 %

b) repeatability = within-run precision
c) intermediate precision = total precision / between-run precision / between-day precision

Method comparison

Calcium values for human serum and plasma samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Calcium reagent (*y*) were compared to those determined using a commercially available reagent for calcium on an alternative clinical chemistry system (*x*). Samples were measured in duplicate. Sample size (n) represents all replicates.

		Alternative system
Sample size	(n)	196
Corr. coefficient	(r)	0.987
	(r_s)	0.970
Lin. regression		$y = 1.057x - 0.13$ mmol/L
Passing/Bablok[12]		$y = 1.060x - 0.13$ mmol/L

The sample concentrations were between 1.74 and 3.50 mmol/L (6.98 and 14.0 mg/dL).

Specific performance data for urine

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

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability[d] and intermediate precision[e] (2 aliquots per run, 2 runs per day, 20 days).
The following results were obtained:

	Level 1	Level 2
Mean	3.92 mmol/L (15.7 mg/dL)	1.45 mmol/L (5.81 mg/dL)
CV repeatability[d]	1.4 %	2.2 %
CV intermediate precision[e]	3.0 %	3.8 %

d) repeatability = within-run precision
e) intermediate precision = total precision / between-run precision / between-day precision

Method comparison

Calcium values for human urine samples obtained on a COBAS INTEGRA 700 analyzer using the COBAS INTEGRA Calcium reagent (*y*) were compared to those determined using a commercially available reagent for calcium on an alternative clinical chemistry system (*x*). Samples were measured in duplicate. Sample size (n) represents all replicates.

		Alternative system
Sample size	(n)	115
Corr. coefficient	(r)	0.997
	(r_s)	0.995
Lin. regression		$y = 1.086x - 0.054$ mmol/L
Passing/Bablok[12]		$y = 1.065x - 0.028$ mmol/L

The sample concentrations were between 0.03 and 4.04 mmol/L (0.12 and 16.2 mg/dL).

References

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12. Passing H, Bablok W, Bender R, et al. A general regression procedure for method transformation. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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.

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