Calcium O-CPC

Diagnostic reagent for the in-vitro quantitative determination of Calcium in human serum, plasma and urine on manual systems.

REF: V/CA02.025	50 test	REF: V/CA01.050	100 test
REF: V/CA04.025	100 test	REF: V/CA02.100	200 test

CLINICAL SIGNIFICANCE

Calcium is the fifth most common element in the body, most of which (98 %) is present in the skeleton. One half of the remaining calcium is found in extracellular fluid and the rest in tissues. Calcium has a crucial role in bone mineralization and is also vital for basic physio- logical processes such as blood coagulation, neuromuscular conduction, and normal muscle tone. Calcium is constantly lost from the body through excretion in feces, urine and to a small extent in sweat. The determination of serum calcium is useful for monitoring myeloma, renal failure, acid base balance, and cirrhosis. Both serum and tissue calcium in the body are controlled by parathyroid hormone, calcitonin and vitamin D. Hypocalcemia may be observed in hypoparathyrodism, steatorrhea, pancreatitis and nephrosis. Increased levels may be associated with multiple myeloma and other neoplastic diseases.

METHOD PRINCIPLE

Calcium ions react with O-cresolphthalein complexone (O-CPC) under alkaline conditions to form a violet colored complex. Magnesium and iron.

*Ca*2+ + O-CPC $\xrightarrow{\text{Alkaline pH}}$ 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 578 nm.

REAGENT COMPOSITION

R1: Standard	10 mg/dl (2.5 mmol/l)
R2: Buffer 2-Amino-2-methyl-1-propanol PH 10.5	0.3 mmol/L
R3: Chromogen O-cresolphthalein complexone 8-hydroxyquinoline	 0.16 mmol/L 7.0 mmol/L

PRECAUTIONS AND WARNINGS

Reagent to be handled by entitled and professionally educated person.

Good Laboratories practices using appropriate precautions should be followed in:

- Wearing personnel protective equipment (overall, gloves, glasses,).
- Do not pipette by mouth.
- In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
- Respect country requirement for waste disposal.
  S56: dispose of this material and its container at hazardous or special waste collection point.
  S57: use appropriate container to avoid environmental contamination.
  S61: avoid release in environment.

For further information, refer to the **Lab.Vie** Calcium o-cpc reagent material safety data sheet.

REAGENT PREPARATION, STORAGE AND STABILITY

Lab.Vie.Calcium o-cpc reagents are supplied ready-to-use. All reagents are stable until expiration date stated on label when stored at 2-8°C.

Deterioration
The **Lab.Vie**. Calcium o-cpc reagent is normally clear, do not use reagent if it is turbid.

SPECIMEN COLLECTION AND PRESERVATION

Serum and Plasma
Use nonhemolyzed serum. Heparin is the only acceptable antico-agulant. No other anticoagulant can be used. Fresh serum collected in the fasting state is the preferred specimen. Serum or plasma should be separated from cells as soon as possible, because prolonged contact with the clot may cause lower calcium values. 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. The biological half-life of calcium in blood is few hours.

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 < 3 with 6 mol/L HCl. Dilute urine specimen with distilled water (1volume urine + 1volume distilled water) before assay.

Stability (serum): 7 days at 15 – 25 °C; 3 weeks at 4 – 8 °C; 8 months at -20 °C.
Stability (urine): 2 days at 15 – 25 °C; 4 days at 4 – 8 °C; 3 weeks at -20 °C.
Stored serum or urine specimens must be mixed well prior to analysis.

SYSTEM PARAMETERS

Wavelength	578 nm
Optical path	1 cm
Assay type	End-point
Direction	Increase
Sample Reagent Ratio e.g.: Reagent volume Sample volume	1:100 1 ml 10 µl
Temperature	15-25°C
Incubation time	5 min at 15-25°C
Zero adjustment	Reagent blank
Sensitivity	2.5 mg/dl
Linearity	18 mg/dl

EQUIPMENT REQUIRED NOT PROVIDED

- Sterile Syringe
- Analytical tubes, automatic pipet
- Centrifuge and spectrophotometer

ASSAY PROCEDURE

	Blank	Standard	Specimen
Standard		10 µl	
Specimen			10 µl
Reagent 2	0.5 ml	0.5 ml	0.5 ml
Reagent 3	0.5 ml	0.5 ml	0.5 ml

Mix, incubate for 5 minutes at room temp. Measure absorbance of Specimen and standard against reagent blank. The color is stable for at least 1 hour.

CALCULATION

Calcium concentration (mg/dl) = $\frac{\text{Abs. specimen}}{\text{Abs. standard}} \times 10$

Urine Calcium (mg/24 hrs.) = $\frac{\text{Abs. specimen}}{\text{Abs. standard}} \times 10 \times 10^{*} \times 2^{**} \times V^{***}$

* The factor "10" converts mg/dl to mg/liter
** The factor "2" represents the dilution factor
*** "v" represents the 24-hour urine volume in liters

QUALITY CONTROL

To ensure adequate quality control, it is recommended that normal and abnormal commercial control serum of known concentrations included in each run. If control values are found outside the defined range, check the instrument calibration, and reagent for problems. If control still out of range please contact **Lab.Vie**. technical support.

PERFORMANCE CHARACTERISTICS

Precision	Within run (Repeatability)		Run to run (Reproducibility)	
	Normal level	High level	Normal level	High level
n	20	20	20	20
Mean mg/dl	9.58	13.97	9.6	14.15
SD.	0.12	0.207	0.23	0.221
CV. %	1.33	1.48	1.42	1.53

The results of the performance characteristics depend on the analyzer used.

Accuracy (Methods Comparison)
Result obtained from **Lab.Vie**.Calcium o-cpc reagent compared with commercial reagent of the same methodology performed on 20 human sera give a correlation of 0.979.

Sensitivity
When run as recommended, the minimum detection limit of the assay is 2.5 mg/dl.

Linearity
The reaction is linear up to concentration of 18 mg/dl. Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result×2).

INTERFERING SUBSTANCES

Haemoglobin
Avoid haemolysis.
Icterus
No significant interference.
lipemia
No significant interference.
Anticoagulants
Complexing anticoagulants such as citrate, oxalate and EDTA must be avoided.

EXPECTED VALUES

	mg/dl	mmol/l
Serum / Plasma		
Adults 20-50 years	8.8 – 10.2	2.20 – 2.55
Adults > 50 years	8.4 – 9.7	2.09 – 2.42
Children 4-18 years	9.2 – 11.0	2.30 – 2.75
Children >4 years	7.2 – 11.2	1.80 – 2.8
Urine(24 h)	<250 mg/day	<6.25 mmol/day
Females	<300 mg/day	<7.5 mmol/day
Males	<6 mg/Kg/day	<0.15 mmol/day
Children		

DYNAMIC RANGE

2.5– 18 mg/dl.

REFERENCES

1. Barnett RN: A scheme for the comparison of quantitative methods. AM J Clin Pathol 43: 562, 1965.
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3. Kessler G, wolfman M: □An automated procedure for the simultaneous determination of calcium and phosphorus.Clin Chem 10:686, 1964.
4. Peters JP, Van Slyke, DD: Quantitative clinical chemistry, vol 2, williams and wilkins, Baltimor (MD),1932, p 760.
5. Tietz NW: Blood gases and electrolytes. In:Fundamentals of clinical chemistry, NW tietz, editor,Saunders, Philadelphia, 176,pp 903, 908.
6. Young DS, Effects of drugs on clinical laboratory tests. AACC press, Washington, D.C. 1990.

SYMBOLS IN PRODUCT LABELLING			
IVD	For in-vitro diagnostic use	Σ	Number of <n> test in the pack
LOT	Batch Code/Lot number		Caution
REF	Catalogue Number		Do not use if package is damaged
	Temperature Limitation		Consult Instruction for use
	Expiration Date		
	Manufactured by		