



## Calcium OCP Fluid 1+1

IN VITRO TEST FOR THE QUANTITATIVE DETERMINATION OF CALCIUM IN HUMAN SERUM, PLASMA AND URINE BY O-CRESOLPHTALEIN METHOD.

REF	88 59 36	2x100ml
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### CALIBRATION

Calcium standard (10 mg/dl)	5ml	88 71 97
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### QUALITY CONTROL

Control Serum N	6x5ml	88 41 48B
Control Serum P	6x5ml	88 46 85B

The control intervals and limits must be adapted to the individual laboratory and country-specific requirements. Values obtained should fall within established limits. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

### SUMMARY

The calcium content of an adult is somewhat over 1 kg (25000 mmol), i.e. about 2% of the body weight. Of this, 99% is present as calcium hydroxyapatite in bones and less than 1 % is present in the extra-osseous ICS (intracellular space) or ECS (extracellular space). The calcium level in the ECS (approx. 100 mmol) is in dynamic equilibrium with the rapidly exchangeable fraction of bone calcium. Calcium ions affect the contractility of the heart and the skeletal musculature and are essential for the function of the nervous system. In addition, calcium ions play an important role in blood clotting and bone mineralization. In plasma, calcium is bound to a considerable extent to proteins (approx. 40%), 10% is in the form of inorganic complexes and 50% is present as free (ionized) calcium. The body's calcium balance is regulated by the parathyroid hormone (PTH), calcitriol (CT) and calcitonin.

The test is used for the diagnosis and monitoring of hypocalcemia (calcium deficiency) and hypercalcemia (excess calcium) in serum. The characteristic symptom of hypocalcemia is latent or manifest tetany and osteomalacia. Hypocalcemia is due to the absence or impaired function of the parathyroid or impaired vitamin D-synthesis. Hypercalcemia is brought about by increased mobilization of calcium from the skeletal system (osteoporosis) or increased intestinal absorption. The majority of cases are due to primary hyperparathyroidism (pHPT) or bone metastasis of carcinoma of the breast, prostate or thyroid and bronchial carcinoma.

The main significance of determining urinary calcium lies in the differentiation between hypercalciuria and hypocalciuria and the differential diagnosis of nephrolithiasis.

Complexometric methods are used in addition to atomic absorption spectrometry (AAS) for determining calcium. The following calcium determination is based on the reaction of calcium with o-cresolphthalein complexone in alkaline solution. Magnesium is masked with 8-hydroxy-quinoline.

### TEST PRINCIPLE

Colorimetric assay with endpoint determination and sample blank

- Sample and addition of R1 (buffer)
- Addition of R2 (chromogen) and start of reaction:

Calcium + o-cresolphthaleincomplexone  $\xrightarrow{\text{alkaline solution}}$  calcium-o-cresolphthalein-complex

The colour intensity of the purple complex formed is directly proportional to the calcium concentration and is measured photometrically.

### NOTES

For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents.

### LIMITATIONS - INTERFERENCE

Criterion: Recovery within  $\pm 10\%$  of initial value.

Icterus: No significant interference up to an I index of 100 (approximate at bilirubin concentration: 100 mg/dl).

Hemolysis: No significant interference up to an index H of 200 (approximate hemoglobin conc.: 200 mg/dl).

Lipemia (Intralipid): No significant interference up to an index L of 100 (approximate triglycerides concentration: 200 mg/dl). There is poor correlation between turbidity and triglycerides concentration.

### MEASURING/REPORTABLE RANGE

Serum/plasma, urine

Measuring range: 0.05-4.00 mmol/l (0.2-16 mg/dl)

Determine samples having higher concentrations manually dilute with 0.9% NaCl or distilled/deionized water (e.g. 1 + 1). Multiply the result by the appropriate dilution factor (e.g. 2).

### EXPECTED VALUES

Serum/plasma

2.15-2.55 mmol/l (8.60-10.2 mg/dl)

24-hour urine

2.5-8.0 mmol/24 h (100-320 mg/24 h), corresponding to 1.7-5.3 mmol/l\* (6.7-21.3 mg/dl\*)

\*Assuming a urine volume of 1.5 l/24 h

Reference range acc. to Tietz:

Serum/plasma

Children (0-10 days): 7.6-10.4 mg/dl (1.90-2.60 mmol/l)

Children (10 days-2 years): 9.0-11.0 mg/dl (2.25-2.75 mmol/l)

Children (2-12 years): 8.8-10.8 mg/dl (2.20-2.70 mmol/l)

Adults (12-60 years): 8.4-10.2 mg/dl (2.10-2.55 mmol/l)

Adults (60-90 years): 8.8-10.2 mg/dl (2.20-2.55 mmol/l)

Adults (> 90 years): 8.2-9.6 mg/dl (2.05-2.40 mmol/l)

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 range. For diagnostic purposes the calcium results should always be assayed in conjunction with the patient's medical history, clinical examinations and other findings.

### ANALYTICAL SENSITIVITY (LOWER DETECTION LIMIT)

Detection limit: 0.05 mmol/l (0.2 mg/dl)

The detection limit represents the lowest measurable calcium concentration that can be distinguished from zero.

### IMPRECISION

Serum

Reproducibility was determined using controls. The following results were obtained.

Sample	Between day		
	Mean mg/gl	SD mg/dl	CV %
Sample 1	2.25	0.03	1.40
Sample 2	3.85	0.05	1.17
Sample 3	2.69	0.04	1.40

Sample	Within run		
	Mean mg/gl	SD mg/dl	CV %
Sample 1	2.21	0.03	1.42
Sample 2	3.80	0.05	1.41
Sample 3	2.64	0.04	1.50

### METHOD COMPARISON

A comparison of the AXIOM Calcium OCP Fluid 1+1 (y) with a commercial obtainable assay (x) gave the following result:

$$y = 0.901x + 0.275; r = 0.940$$

**REAGENT CONCENTRATION**

<b>R1:</b>		
Ethanolamine, pH 10.7		3.5 mol/l
<b>R2:</b>		
o-Cresolphthalein complexone		0.16 mmol/l
8-hydroxyquinoline		6.9 mmol/l
Hydrochloric acid		160 mmol/l
Preservation and stabilizer		

**PREPARATION AND STABILITY***For assay series:*

The reaction mixture of R1 and R2 in ratio 1+1 is stable for 14 days at +2°C to 8°C or 5 days at +15°C to + 25°C.

*For single assays:*

All solutions are ready to use.

Stable up to the expiry date when stored at + 15°C to + 25°C. Avoid direct light! Keep bottles closed after use.

**SPECIMEN:**

Collect serum using standard sampling tubes.

*Heparin plasma.* Plasma must be assayed fresh. Do not use oxalate, EDTA, or citrate plasma.

Stability:	7 days	at +20°C to +25°C
	3 weeks	at + 4°C to + 8°C
	8 months	at - 20°C

*Urine:*

24-hour urine: Place 10 ml hydrochloric acid (6mol/l) in a collection bottle, or acidify (pH<2,0) after urine collection, in order to dissolve calcium salts.

Stability:	2 days	at +20°C to +25°C
	4 days	at + 4°C to + 8°C
	3 weeks	at - 20°C

Centrifuge samples containing precipitate before performing the assay.

**TESTING PROCEDURE***Materials provided*

- Working solutions as described above

*Additional materials required*

- Calibrators and controls as indicated below
- 0.9% NaCl solution

<b>Manual procedure:</b>			
Wavelength	Hg 579 nm, (550 – 590)		
Temperature	+25°C		
Cuvette	1cm light path		
Zero adjustment	against reagent blank, each series needs one reagent blank only		
Mix R1 and R2 in the ratio of 1+1.			
	<i>blank</i>	<i>Standard / Calibrator</i>	<i>Sample</i>
<i>R1-R2-mixture</i>	1000 µl	1000 µl	1000 µl
<i>Standard</i>	---	20 µl	---
<i>Sample</i>	---	---	20 µl
Mix and incubate 5 minutes at +25°C - +37°C, then read absorbance of sample (A sample) and standard (A standard) against the blank.			
<b>Calculation:</b>			
$\frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}} \times \text{standard conc.} = \text{Calcium conc. (mg/dl)}$			

**DISPOSAL**

Please note the legal regulations.

**LITERATURE**

1. Bablok W et al. A General Regression Procedure for Method Transformation. J Clin Chem Clin Biochem 1988;26:783-790.
2. Farrel E.C. Calcium. In: Pesce A.J., Kaplan L.A. (ed.). Methods in Clinical Chemistry. St. Louis/Washington/Toronto: CV Mosby, 1987:865-869.
3. Gindler E.M., King J.D. Am J Clin Pathol 1972;58:376.
4. Glick M.R., Ryder K.W., Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-474.
5. Gosling P. Analytical reviews in clinical biochemistry: Calcium measurement. Ann Clin Biochem 1986;23:146.
6. Guder W.G. Narayanan S., Wisser H., Zawta B. List of Analytes Preanalytical Variables. Broschüre in: Samples: From the Patient to the Laboratory. Darmstadt: GIT Verlag, 1996.
7. Külpmann W.R., Stummvoll H.K., Lehmann P. (ed.). Elektrolyte, Klinik und Labor, 2. Auflage. Wien/New York: Springer-Verlag, 1997.
8. Mathias D. Labordiagnostik bei Störungen des Knochenstoffwechsels. Clin Lab 1996;42:1069-1073.
9. Passing H., Bablok W., A New Biometrical Procedure for Testing the Equality of Measurements from Two Different Analytical Methods. J Clin Chem Clin Biochem 1983;21:709-720.
10. Schmidt-Gayk H., Blind E., Roth H.J. (ed.). Calcium Regulating Hormones and Markers of Bone Metabolism: Measurement and Interpretation, 2. Auflage. Heidelberg: Clin Lab Publications, 1997.
11. Tietz N.W., (ed.). Clinical Guide to Laboratory Tests, 3. Auflage. Philadelphia, Pa: WB Saunders Company, 1995:102.
12. Tietz N.W., (ed.). Fundamentals of Clinical Chemistry, 3. Auflage. Philadelphia, Pa: WB Saunders Company, 1987:718-719.

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**AXIOM Product range Clinical Chemistry**

Enzymes	Ions	Other Metabolites
Acid Phosphatase	Ammonium fluid	Bilirubin T/D
Alkaline Phosphatase	Copper fluid	Creatinine fluid
α-Amylase direct	Calcium fluid	Glucose GOD-PAP fluid
CK-NAC actived	Chloride fluid	Glucose Hexokinase fluid
CK-MB (NAC- actived)	Inorganic Phosphorus UV fluid	Urea Enzymatic fluid
γ-GT fluid	Iron fluid	Urea UV fluid
LDH fluid	TIBC	Uric Acid PAP fluid
Cholinesterase	Magnesium fluid	
GOT/ASAT fluid	Potassium fluid	
GPT/ALAT fluid	Sodium fluid	<b>Controls</b>
Lipase UV fluid		Control Serum N
Lactate PAP		Control Serum P
α-HBDH	<b>Proteins</b>	
	Albumin	
<b>Lipids</b>	CSF-Protein fluid	
Cholesterol fluid	Microprotein fluid	
HDL Cholesterol	Hemoglobin	
LDL Cholesterol	Protein Total fluid	
Triglycerides fluid		