Calcium OCP Fluid 1+1
IN VITRO TEST FOR THE QUANTITATIVE DETERMINATION OF CALCIUM IN HUMAN SERUM, PLASMA AND URINE BY O-CRESOLPHTHALEIN METHOD.

CALIBRATION
Calcium standard (10 mg/dl) 5ml 88 71 97
QUANTITY 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 extracellular 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. Hypercalcemia 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 (pHT) 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 hypercalcuria and hypocalcuria 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 → 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
Criteron: Recovery within ±10% of initial value.

Lactera: 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/l/24 h (100–320 mg/dl), corresponding to 1.7–5.3 mmol/l (6.7–21.3 mg/dl) (#)

Reference range acc. to Tietz:

Serum/plasma

Children (0–10 years): 7.6–10.4 mg/dl (1.90–2.60 mmol/l)

Children (10–12 years): 9.0–11.0 mg/dl (2.25–2.75 mmol/l)

Children (12–14 years): 8.8–10.8 mg/dl (2.20–2.70 mmol/l)

Adults (12–60 years): 8.4–10.2 mg/dl (2.12–2.55 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 evaluate 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/dl</th>
<th>SD mg/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.25</td>
<td>0.03</td>
<td>1.40</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.85</td>
<td>0.05</td>
<td>1.41</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.69</td>
<td>0.04</td>
<td>1.40</td>
</tr>
</tbody>
</table>

Within run

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean mg/dl</th>
<th>SD mg/dl</th>
<th>CV %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>2.21</td>
<td>0.03</td>
<td>1.42</td>
</tr>
<tr>
<td>Sample 2</td>
<td>3.80</td>
<td>0.05</td>
<td>1.50</td>
</tr>
<tr>
<td>Sample 3</td>
<td>2.64</td>
<td>0.04</td>
<td>1.40</td>
</tr>
</tbody>
</table>

METHOD COMPARISON

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

\[ y = 0.901 \times +0.275; \quad r = 0.940 \]
**REAGENT CONCENTRATION**

R1: Ethanolamine, pH 10.7 3.5 mol/l

R2: o-Cresolphthalein complexone 0.16 mmol/l

8-hydroxychinolone 6.9 mmol/l

Hydrochloric acid 160 mmol/l

**PREPARATION and stabilizer**

Preservation and stabilizer

**PREPARATION 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
- 0.9% NaCl solution

**Additional materials required:**

- Calibrators and controls as indicated below

**Manual procedure:**

- Wavelength
- Temperature +25°C
- Couvette 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.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Standard</th>
<th>Calibrator</th>
</tr>
</thead>
<tbody>
<tr>
<td>blank</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>R1-R2-mixture</td>
<td>1000 µl</td>
<td>1000 µl</td>
</tr>
<tr>
<td>Standard</td>
<td>20 µl</td>
<td>---</td>
</tr>
<tr>
<td>Sample</td>
<td>---</td>
<td>20 µl</td>
</tr>
</tbody>
</table>

Mix and incubate 5 minutes at +25°C - +37°C, then read absorbance of sample (A sample) and standard (A standard) against the blank.

**Calculation:**

\[ \text{A standard} \times \text{standard conc.} = \text{Calcium conc. (mg/dI)} \]

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**AQUIM Product range Clinical Chemistry**

**Enzymes**

- Acid Phosphatase
- Alkaline Phosphatase
- α-Amylase direct
- CK-NAC activated
- CK-MB (NAC-activated)
- γ-GT fluid
- LDH fluid
- Cholinesterase
- GOT/ASAT fluid
- GPT/ALAT fluid
- Lipase UV fluid
- Lactate PAP fluid
- α-HBDH

**Ions**

- Ammonium fluid
- Copper fluid
- Calcium fluid
- Chloride fluid
- Glucose G6PAP fluid
- Glucose Hexokinase fluid
- Inorganic Phosphorus fluid
- Iron fluid
- Urea Enzymatic fluid
- Urea UV fluid
- TIBC
- Uric Acid PAP fluid
- Magnesium fluid
- Potassium fluid
- Sodium fluid
- Control Serum N
- Control Serum P

**Other Metabolites**

- Creatinine fluid
- Bilirubin T/D
- Glucose G6PAP fluid
- Urea Enzymatic fluid
- Urea UV fluid
- Control Serum N
- Control Serum P

**Lipids**

- Albumin
- Cholesterol fluid
- HDL Cholesterol
- LDL Cholesterol
- Protein Total fluid

**Proteins**

- Albumin
- CSF-Protein fluid
- Microprotein fluid
- Hemoglobin
- Protein Total fluid

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**REFERENCES**