

CALCIUM OCC

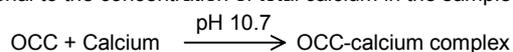
CONTENTS			
REF	1115000	Calcium OCC	2 x 50 mL
	1115010	Calcium OCC	4 x 100 mL
For <i>in vitro</i> diagnostic use only			

CALCIUM OCC

TOTAL
Colorimetric method
ENDPOINT

PRINCIPLE

The method¹ is based on the specific binding of cresolphthalein complexone (OCC), a metallochromic indicator², and calcium at alkaline pH with the resulting shift in the absorption wavelength of the complex. The intensity of the chromophore formed is proportional to the concentration of total calcium in the sample.



REAGENT COMPOSITION

- R1** **OCC indicator.** o-Cresolphthalein complexone 0.16 mmol/L, HCl 60 mmol/L, 8-quinolinol 7 mmol/L.
- R2** **OCC buffer.** AMP 0.35 mol/L, pH 10.7.
- CAL** **Calcium standard.** Calcium 10 mg/dL (2.5 mmol/L). Organic matrix based primary standard.

STORAGE AND STABILITY

 Store at 15-30°C.
The reagents are stable until the expiry date stated on the label.

REAGENT PREPARATION

Working reagent. Mix 1 volume of **R1** + 1 volume of **R2**. Stable 8 days at 2-8°C. Recap reagents immediately after use. Discard the mixture if the blank presents an absorbance above 0.600 at 570 nm against distilled water or if it fails to recover the declared values of control sera (see Notes).

SAMPLES

Serum or heparinized plasma, and urine (see Notes). Other anticoagulants (EDTA, oxalate and citrate) must not be used. Calcium in serum or plasma is stable for 1 week at 20-25°C and up to 3 weeks at 2-8°C. Freeze for longer storage.

Calcium in acidified samples of urine (see Notes) is stable for 2 days at 20-25°C and up to 4 days at 2-8°C. Freeze for longer storage.

INTERFERENCES³

- Effects of bilirubin (>20 mg/dL), hemoglobin (>500 mg/dL), albumin (>10 g/L) and phosphates (>1.0 g/L) are negligible. Magnesium (>20 mg/dL) does not interfere.
- Lipemic samples (triglycerides up to >1.25 g/L) can cause spurious results.
- Many detergents and water supplies are a major source of contamination of the glassware used for this test.

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 570 ± 10 nm.
- Pipettes with disposable plastic tips to measure reagents and samples.
- Disposable plastic tubes for the tests.

PROCEDURE

- Bring reagents and samples to room temperature.
- Pipette into labelled test tubes:

TUBES	Blank	Sample	Standard
Working reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	10 µL	–
Standard	–	–	10 µL

- Mix and let stand the tubes 2 minutes at room temperature.
- Read the absorbance (A) of the samples and the standard at 570 nm against the reagent blank.

The color is stable for at least 1 hour.

CALCULATIONS

Serum, plasma

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL total calcium}$$

Samples with concentrations higher than 15 mg/dL (3.75 mmol/L) should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

Urine

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times 200 = \text{mg/24-hours total calcium}$$

If results are to be expressed as SI units apply:
mg/dL x 0.25 = mmol/L



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REFERENCE VALUES⁴

Serum, plasma

Newborns (< 10 days)	7.6-10.4 mg/dL (1.9-2.6 mmol/L)
Children (2-12 years)	8.8-10.4 mg/dL (2.2-2.6 mmol/L)
Adults (12-60 years)	8.4-10.2 mg/dL (2.1-2.5 mmol/L)

Urine

Adults (normal diet)	100-300 mg/24-h (25-75 mmol/24-h)
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It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.

To ensure adequate quality control (QC), each run should include a set of controls (normal and abnormal) with assayed values handled as unknowns.

REF BC600 HUMAN MULTISERA NORMAL
Borderline level of calcium. Assayed.

REF BC650 HUMAN MULTISERA ABNORMAL
Elevated level of calcium. Assayed.

CLINICAL SIGNIFICANCE

Calcium exists in the blood in three forms: ionized (13%), complexed (47%) and bound to protein, mainly albumin (40%). When calcium determinations are performed, the total calcium concentration is determined regardless of the amount of calcium present in each form. A depressed concentration of total calcium can be due to hypoproteinemia, but the concentration of physiologically active (ionized) calcium in such case may be normal. For this reason, a protein determination should accompany each calcium analysis so that the calcium value can be interpreted properly.

Depressed serum calcium levels usually accompany hypoparathyroidism, some bone diseases, certain kidney diseases, and low protein levels.

Elevated serum calcium levels occur in hyperparathyroidism, vitamin-D poisoning, and sarcoidosis.

The plasma level in calcium is greatly affected by the plasma level of inorganic phosphate. In most cases, there is an inverse relationship between calcium and inorganic phosphate.

Conditions associated with *hypercalcemia*, such as primary hyperparathyroidism are usually associated with *hypophosphatemia*; the opposite is true as well.

Urine calcium excretion parallels the serum calcium level. Large amounts of calcium are excreted in the urine in hyperparathyroidism, metabolic acidosis, renal tubular insufficiency, multiple myeloma and bone malignancies.

NOTES

- Most of the detergents and water softening products used in the labs contain quelating agents. A defective rinsing will invalidate the procedure. Keep the glassware acid washed and thoroughly rinsed at all times.
- Collect a 24-hour urine specimen into a plastic bottle containing 20 mL of 50% (v/v) HCl. Bring to 2 L with distilled water. Mix completely and test as described for serum.

ANALYTICAL PERFORMANCE

- **Linearity.** Up to 15 mg/dL

- **Precision**

mg/dL	Intraserial			Interserial		
Media	6.5	9.8	17.6	6.7	9.9	17.3
DE	0.024	0.009	0.088	0.16	0.1	0.18
CV%	0.37	0.1	0.5	2.4	1.01	1.04
N	10	10	10	5	5	5

Replicates: 10 for each level.

Replicates: 5 for each level

Instrument: CECIL CE 2021

for 8 days.

- **Sensitivity.** Using a 1:100 sample/reagent at 570 nm, 1mg of calcium will produce a net absorbance of approximately 0.055.

- **Correlation.** This assay (y) was compared with a similar commercial method (x). The results were:

$$N = 30 \quad r = 0.995 \quad y = 0.96x + 0.378$$

REFERENCES

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3. Morin, L.G. Am. J. Clin. Path. 61 : 114 (1974).
4. Tietz, N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).

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