



Store at: +2+8°C.

Presentation:

Cod. SU009 CONT: R 2 x 125 mL. + CAL 1 x 5 mL.

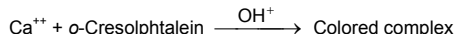
Procedure

Quantitative determination of calcium.

Only for *in vitro* use in clinical laboratory (IVD)

TEST SUMMARY

The measurement of calcium in the sample is based on formation of color complex between calcium and o-cresolphthalein in alkaline medium:



The intensity of the colour formed is proportional to the calcium concentration in the sample^{1,2,3}.

REAGENTS COMPOSITION

R.1 Buffer	Ethanolamine	500 mmol/L
R.2 Chromogen	o-Cresolphthalein 8-Hydroxyquinolein	0.62 mmol/L 69 mmol/L
Calcium Cal	Calcium aqueous primary calibrator	10 mg/dL

REAGENT PREPARATION AND STABILITY

All the reagents (R.1) (R.2) are ready to use. All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use. Do not use reagents over the expiration date

Calcium Cal: Once open is stable up to 1 month when stored tightly closed at 2-8°C, protected from light and contamination prevented during their use.

Signs of Reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 570 nm \geq 0.2.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8°C. Do not use reagents over the expiration date.

SPECIMEN

Serum or plasma¹: Separated from cells as rapidly as possible. Blood anticoagulants with oxalate or EDTA are not acceptable since these chemicals will strongly chelate calcium.

Urine¹: Collect 24 hour urine specimen in calcium free containers. The collecting bottles should contain 10 ml of diluted Nitric acid (50% v/v). Record the volume. Dilute a sample 1/2 in distilled water. Mix. Multiply results by 2 (dilution factor).

Stability of the samples: Calcium is stable 10 days at 2-8°C..

MATERIAL REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 570 nm.
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment^(note 1 and 2).

TEST PROCEDURE

- Assay Conditions
 - Wavelength : 570 nm. (550-590).
 - Cuvette: 1 cm light path.
 - Temperature 37/ 15-25°C.
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

	Blank	Standard	Sample
R 1 (mL)	1	1	1
R 2 (mL)	1	1	1
Standard ^(Note 3-4) (µL)	--	20	--
Sample (µL)	--	--	20

- Mix and incubate for 5 minutes at 37/ 15-25°C.
- Read the absorbance (A) of the samples and calibrator, against the Blank. The color is stable for at least 40 minutes.

CALCULATIONS

Serum or plasma:

$$\text{Calcium(mg/dL.)} = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times 10 \text{ (Calibrator conc.)}$$

Urine 24:

$$\text{Calcium(mg/24 h.)} = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times 10 \text{ (Cal. conc.)} \times \text{vol(dL) urine/24 h}$$

Conversion factor: mg/dL x 0.25 = mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of the procedure, Control Normal Ref. QC001 and Control Pathological Ref. QC002. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES¹

Serum or plasma:	
Adults	8.5-10.5 mg /dL \cong 2.1-2.6 mmol/L
Children	10 -12 mg/dL \cong 2.5 - 3 mmol/L
Newborns	8 -13 mg/dL \cong 2 - 3.25 mmol/L
Urine:	
Adults	50 - 300 mg/24h \cong 1.25 - 7.5 mmol/24h
Children	80 -160 mg/24h \cong 2 - 4 mmol/24h

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE

Calcium is the most abundant and one of the most important minerals in the human body. Approximately 99% of body calcium is found in bones.

A decrease in albumin level causes a decrease in serum calcium. Low levels of calcium are found in hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsorption.

Among causes of hypercalcemia are cancers, large intake of vitamin D, enhanced renal retention, osteoporosis, sarcoidosis, thyrotoxicosis, hyperparathyroidism^{1,6,7}.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE

- **Measuring Range:**

From detection limit of 0.10 mg/dL. to linearity limit of 15 mg/dL., under the described assay conditions.

If results obtained were greater than linearity limit, dilute the sample 1/2 with NaCl 9 g/L. and multiply result by 2.

- **Precision:**

	Intra-assay n= 20		Inter-assay n= 20	
Mean (mg/dL)	9.08	15.7	9.03	14.28
SD	0.17	0.24	0.17	0.23
CV %	1.97	1.53	1.99	1.62

- **Sensitivity:** 1 mg/dL. = 0.034 A.

- **Accuracy:** Results obtained GPL reagents did not show systematic differences when compared with other commercial reagents.

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.91.

Regression Equation: y= 0.9069x + 0.9114

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

INTERFERING SUBSTANCES

- No interferences were observed with triglycerides up to 1.25 g/L^{1,2,3}.
- A list of drugs and other interfering substances with calcium determination has been reported by Young et. al^{2,3}.

NOTES

- It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with diluted 1/1 HNO₃ in water and then thoroughly rinsed it with distilled water.
- Most of the detergents and water softening products used in the laboratories contains chelating agents. A defective rinsing will invalidate the procedure.
- Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
- Use clean disposable pipette tips for its dispensation.

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