Calcium

o-Cresolphtalein v/v. Colorimetric

Quantitative determination of calcium

CE

CE

IVD

PACKAGING

Ref: 101-0338	Cont.: 2 x 100 mL
Ref: 101-0450	Cont.: 8 x 100 mL

Store at 2-8º C

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. Among causes of hypercalcemia are cancers, large intake of vitamin D, enhaced renal retention, osteoporosis, sarcosidosis, thyrotoxicosis, hyperparathyroidism. Low levels of calcium are found in hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, malnutrition and intestinal malabsortion[1,6,7].

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

PRINCIPLE OF THE METHOD

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

$$Ca^{++} + o\text{-Cresolphtalein} \xrightarrow{OH^+} \text{Colored complex}$$

The intensity of the colour formed is proportional to the calcium concentration in the sample[1,2,3].

REAGENTS

R 1 Buffer	Ethanolamine	500 mmol/L
R 2 Chromogen	*o*-Cresolphtalein 8-Hidroxyquinolein	0.62 mmol/L 69 mmol/L
CALCIUM CAL	Calcium aqueous primary standard	10 mg/dL

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PRECAUTIONS

R1/R2: Corrosive (C):R35:Causes severe burns.

PREPARATION

All the reagents are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

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

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 570 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment (Note 1, 2).

SAMPLES

- Serum or plasma[1]: Separated from cells as rapidly as possible. Blood anticoagulants with oxalate or EDTA are not acceptable since these chemicals will strongly chelate calcium.
- Urine[1]: 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 2-8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

CALCIUM CAL: Proceed carefully with this product because due its nature it can get contamined easily.

It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with diluted 1/1 HNO_3 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.

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

	Blank	Calibrator	Sample
R 1 (mL)	1.0	1.0	1.0
R 2 (mL)	1.0	1.0	1.0
Calibrator(Note 3, 4) (µL)	--	20	--
Sample (µL)	--	--	20

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

CALCULATIONS

Serum and plasma

$\frac{(A)\,Sample - (A)\,Blank}{(A)\,Standard - (A)Blank}$ x 10 (Calibrator conc.) = mg/dL Ca in the sample

Urine 24 h

$\frac{(A)\,Sample - (A)\,Blank}{(A)\,Standard - (A)Blank}$ x 10 (Calibrator conc.) x vol. (dL) urine/24 h =mg/24 h Ca

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

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

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REFERENCE VALUES[1]

Serum or plasma:

Adults	8.5 - 10.5 mg /dL	≅ 2.1 - 2.6 mmol/L
Children	10 - 12 mg/dL	≅ 2.5 - 3 mmol/L
Newborns	8 -13 mg/dL	≅ 2 - 3.25 mmol/L

Urine:

Adults	50 - 300 mg/24h	≅ 1.25 – 7.5 mmol/24h
Children	80 -160 mg/24h	≅ 2 - 4 mmol/24h

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From detection limit of 0.071 mg/dL to linearity limit of 35 mg/dL.

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

Precision:

	Intra-assay (n=20)		Inter-assay (n=20)	
Mean (mg/dL)	9.14	16.0	9.34	16.3
SD	0.07	0.11	0.20	0.37
CV (%)	0.74	0.68	2.16	2.27

Sensitivity: 1 mg/dL = 0.044 A.

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient (r): 0.981.

Regression equation: y=0.8234x + 1.5484.

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

INTERFERENCES

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[4,5].

BIBLIOGRAPHY

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