Atlas Medical CE

CALCIUM O-Cresolphtalein Colorimetric test v/v

IVD For *in vitro* diagnostic use only

Store at 2-8°C

INTENDED USE

For the quantitative determination of calcium concentration in human serum, plasma and urine.

INTRODUCTION

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.

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

The intensity of the colour formed is proportional to the calcium concentration in the sample.

REAGENTS

R 1 Buffer	Ethanolamine Cloroformo Metanol	500 mmol/L 15 mmol/L 5700 mmol/L
R 2 Chromogen	o-Cresolphtalein 8-Hidroxyquinolein	0.62 mmol/L 69 mmol/L
CALCIUM STD	Calcium aqueous primary standard 10mg/dL	
Package Insert		

MATERIAL NEEDED BUT NOT PROVIDED

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

PREPARATION

- All the reagents are ready to use.

STORAGE AND STABILITY

- All the reagents are ready for use.
- Stored at 2-8ºC, it is stable up to the date of expiration as specified.
- Do not use reagents over the expiration date.
- Signs of reagent deterioration:
  - Presence of particles and turbidity.
  - Blank absorbance (A) at 570 nm >0.22.

SAMPLES

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

PROCEDURE

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

	Blank	Standard	Sample
R 1 (mL)	1.0	1.0	1.0
R 2 (mL)	1.0	1.0	1.0
Standard (µL)	--	20	--
Sample (A) (µL)	--	--	20

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

CALCULATIONS

Serum and plasma

$$\frac{(A)\text{Sample} - (A)\text{Blank}}{(A)\text{Standard} - (A)\text{Blank}} \times 10\ (\text{Standard conc.}) = \text{mg/dL (calcium in the sample)}$$

Urine 24 h

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

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.

REFERENCE VALUES Serum or plasma:

Serum or Plasma		
Adult	8.5-10.5 mg/dl	≈ 2.1-2.6 mmol/L
Children	10-12 mg/dl	≈ 2.5-3 mmol/L
Newborn	8-13 mg/dl	≈ 2-3.25 mmol/L
Urine		
Adults	50-300 mg/24h	≈1.25-7.5mmol/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 0.07 mg/dL to *linearity limit* of 35mg/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:

	Inter-assay (n=20)		Inter-assay (n=20)	
Mean(mg/dL)	9.14	16.02	9.34	16.27
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 Atlas 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 $(r)^2$: 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.
A list of drugs and other interfering substances with calcium determination has been reported.

NOTES

- 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.

REFERENCES

1. Farell E C. Calcium. Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton 1984; 1051-1255 and 418.
2. Kessler G. et al. Clin Chem Vol 10, No 8 1964; 686-706.
3. Connerty H. V. et al. Am J Clin Path Vol 45, No 3 1996; 200-296.
4. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
5. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed. AACC 2001.
6. Burtis A. et al. Tietz Textbook of Clinical Chemistry, 3rd ed. AACC 1999.
7. Tietz N W et al. Clinical Guide to Laboratory Tests, 3rd ed. AACC 19

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REF	Catalogue Number		Temperature limit
IVD	*In Vitro* diagnostic medical device		Caution
Σ	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
LOT	Batch code		Manufacturer
	Fragile, handle with care		Use-by date
	Manufacturer fax number		Do not use if package is damaged
	Manufacturer telephone number		Date of Manufacture
	Keep away from sunlight		Keep dry