

Quantitative determination of calcium IVD

Store at 2-8°C

PRINCIPLE OF THE METHOD

Calcium with Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis (azo)-dibenzeneearsonic acid), at neutral pH, yields a blue colored complex. The intensity of the colour formed is proportional to the calcium concentration in the sample^{1,2,3}.

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.

REAGENTS

R	Imidazol Buffer pH 6,5	100 mmol/L
Arsenazo III	Arsenazo III	120 mmol/L
CALCIUM CAL	Calcium aqueous primary standard	10 mg/dL

PRECAUTIONS

R: H360- May damage fertility or the unborn child.

CAL: H290-May be corrosive to metals.

Follow the precautionary statements given in MSDS and label of the product.

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 2-8°C, protected from light and contaminations are 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 650 nm $\geq 0,50$.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 650 nm.
- Matched cuvettes 1,0 cm light path.
- General laboratory equipment (Note 2, 3).

SAMPLES

- 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.

PROCEDURE

- Assay conditions:
Wavelength: 650 nm
Cuvette: 1 cm. light path
Temperature: 37°C /15-25°C
- Adjust the instrument to zero with distilled water.
- Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1,0	1,0	1,0
Standard ^(Note 1,4,5) (µL)	--	10	--
Sample (µL)	--	--	10

- Mix and incubate for 2 min at 37°C / 15-25°C.
- Read the absorbance (A) of the samples and Standard, against the Blank. The color is stable for at least 1 hour.

CALCULATIONS

Serum and plasma $\frac{(A)_{\text{Sample}} - (A)_{\text{Blank}}}{(A)_{\text{Standard}} - (A)_{\text{Blank}}} \times 10$ (Standard conc.) = mg/dL

calcium

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

Conversion factor: mg/dL x 0,25= mmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures: CONTROL Normal and Pathologic .

If control values are found outside the defined range, check the instrument, reagent and calibration material.

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

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 - 3,0 mmol/L
Newborns	8 -13 mg/dL	\cong 2,00 - 3,25 mmol/L

Urine:

Adults	50 - 300 mg/24h	\cong 1,25 - 7,50 mmol/24h
Children	80 -160 mg/24h	\cong 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 0,026 mg/dL to linearity limit of 32 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)	SD	CV (%)	
Mean (mg/dL)	8,35	14,28	8,58	14,57
SD	0,08	0,08	0,19	0,34
CV (%)	0,95	0,59	2,24	2,31

Sensitivity: 1 mg/dL = 0,0316 A.

Accuracy: Results obtained using Audit Diagnostics 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): 0,9506

Regression equation: $y = 0,8944x + 1,3421$.

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 by Young et. al^{4,5}.

NOTES

- CALCIUM CAL: Proceed carefully with this product because due its nature it can get contaminated easily.
- It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with diluted (1/2) 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.
- Audit Diagnostics has instruction sheets for several automatic analyzers.**

BIBLIOGRAPHY

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