



CALCIUM

Quantitative Determination of Calcium Colorimetric Arsenazo III Method

For professional in vitro diagnostic use only.

INTENDED USE

Quantitative determination of Calcium in Serum or Plasma urine. Arsenazo III Colorimetric method.

GENERALITIES

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, 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, and multiple myeloma and bone malignancies.

TEST PRINCIPLE

With Arsenazo III and at neutral pH, Calcium produces a blue complex, whose optical absorbance, proportional to the Calcium concentration in the sample, is photometrically measurable at 600 nm.

REAGENT COMPOSITION

Reagent	
Imidazole buffer, pH 6.5	70 mmol/L
Arsenazo III	80 µmol/L
Detergents	2 %
Standard	
Calcium	value on label

STORAGE, PREPARATION AND SHELF LIFE

Liquid and ready to use reagents, stable until the expiry date shown, if stored as indicated on the label and avoid contamination, evaporation and prolonged exposure to direct light. Do not freeze the reagents. Discard the reagent if signs of deterioration appear, such as the presence of particles and turbidity or failure to recover the values of certified control sera. After opening the bottles, it is advisable to withdraw the necessary volume, to immediately close the bottles and store them in the fridge to avoid contamination, degradation from direct light and evaporation.

SAMPLE COLLECTION AND PREPARATION

Serum, heparinized plasma, urine. Do not use EDTA plasma. The 24h Urine must be acidified with 10 mL of concentrated hydrochloric acid. The morning urine should be acidified with a few drops of concentrated hydrochloric acid.

The urine sample should be diluted 1: 2 (1 + 1) with distilled water.

Stability in serum/plasma: 7 days at 15° - 25 °C, 3 weeks at 2° - 8 °C, 3 months at -20 °C.

Stability in urine: 2 days at 15-25 °C, 4 days at 2-8°C, 3 weeks at -20 °C.

Discard contaminated samples.

TEST PROCEDURE

Wavelength	: 600 nm (578 – 630)
Light path	: 1 cm
Temperature	: 37 °C
Measurement:	: Against Reagent Blank
Assay type	: ENDPOINT

Assay:

	Blank	Assay
Reagent	1000 µL	1000 µL
Sample/ Standard/ Cal	/	10 µL
-Mix and incubate for 5 min at 37 °C.		
-Read the absorbance (A) of the samples and the standard against the reagent blank.		

CALCULATION

Serum/ Plasma:

$$\text{Calcium Concentration} = \frac{\text{Abs Sample} - \text{Abs blank}}{\Delta \text{Abs Std/Cal} - \text{Abs blank}} \times \text{Std/ Cal.}$$

Urine:

Calculate as for the serum and multiply the result by 2 (initial sample dilution).

Conversion Factor: [mg/dL] x 0.25 = [mmol/L]

QUALITY CONTROL

Normal and abnormal control sera of known concentration should be analyzed routinely with each group of unknown samples.

EXPECTED VALUES

Serum/plasma:

- Newborn: 8.0 - 13.0 mg/dL.
- Children: 8.5 - 12.0 mg/dL.
- Adults: 8.5 - 10.5 mg/dL.

Urine :

- Women: < 250 mg/24 h.
- Men: < 300 mg/24 h
-

Each laboratory should establish a range of expected values based on its patient population and, if necessary, determine its own reference interval. For diagnostic purposes, results should always be assessed together with the patient's medical history, clinical examination and other results.

PERFORMANCE

PRECISION:

Low Level: Samples= 20; Average = 8.79; S.D. = 0.09; CV = 1.02%.

High Level: Samples = 20; Average = 14.0; S.D. = 0.24; CV = 1.71%.

ACCURACY:

A comparison between this method (x) and a certified method of trade (y) gave the following correlation:

$$y = 1.02x - 0.2 \quad r = 0.999$$

SENSITIVITY: 0.1 mg/dL.
LINEARITY: 20 mg/dL.

PRECAUTIONS

R: Harmful if swallowed, in contact with skin or inhaled.
in case of contact of reagents with the operator, you must apply the following first aid: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. After contact with skin, wash immediately with plenty of water. Wear suitable protective clothing, gloves and eye/face protection.
Note: Most of the detergents and water softening products used in the labs contain chelating agents. A defective rinsing will invalidate the procedure. Keep the glassware acid washed and thoroughly always rinsed.

INTERFERENCES

No interference was observed by ascorbic acid up to 30 mg/dL, bilirubin up to 40 mg/dL, hemoglobin up to 500 mg/dL, lipemia up to 2.000 mg/dl triglycerides and magnesium up to 15 mg/dL.

LITERATURE

- Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. Anal Biochem 1981;110:61-72.
- Endres DB, Rude RK. Mineral and bone metabolism. In: Burtis CA, Ashwood ER, editors. Tietz Textbook of Clinical Chemistry. 3rd ed. Philadelphia: W.B Saunders Company; 1999. p. 1395-1457.
- Michaylova V, Ilkova P. Photometric determination of micro amounts of calcium with arsenazo III. Anal ChimActa 1971;53:194-8.
- Thomas L. Clinical Laboratory Diagnostics. 1st ed. Frankfurt: TH Books Verlag gesellschaft; 1998. p. 192-202.

USED SYMBOLS

	In Vitro Diagnostic Medical Device
	Manufacturer
	Date of Manufacture
	Catalogue Number
	Batch Code
	Use by YYYY-MM (MM = end of month)
	Operator's Manual; Operating Instructions
	Keep away from Sunlight
	Keep away from Rain
	Temperature Limit
	Caution
	Do not use if Package is Damaged
	Do Not Re-Use
	Contains Sufficient for <N> Tests