



## Calcium

### Arsenazo III. Colorimetric Quantitative determination of calcium

For in vitro diagnostic use only

Store at 2-8°C

#### PRINCIPLE OF THE METHOD

Calcium with Arsenazo III (1,8-Dihydroxy-3,6-disulpho-2,7-naphthalene-bis (azo)-dibenzeneazonic acid), at neutral pH, yields a blue colored complex.

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

#### 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.

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

#### REAGENTS

<b>R</b>	Imidazol Buffer pH 6.5	100 mmol/L
Arsenazo III	Arsenazo III	120 mmol/L
<b>CALCIUM STD</b>	Calcium aqueous primary standard 10 mg/dL	

#### 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.

**CALCIUM STD** Once open is stable up to 1 month when stored tightly closed at 2-8°C, protected from light and contaminations prevented during their use.

#### Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 650 nm  $\geq$  0.50.

#### ADDITIONAL EQUIPMENT

1. Spectrophotometer or colorimeter measuring at 650 nm.
2. Matched cuvettes 1.0 cm light path
3. General laboratory equipment

#### SAMPLES

- Serum or plasma<sup>1</sup>: Separated from cells as rapidly as possible. Blood anticoagulants with oxalate or EDTA are not acceptable since these chemicals will strongly chelate calcium.
- Urine<sup>1</sup>: 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..... 650 nm  
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 (mL)	1.0	1.0	1.0
Standard(μL)	--	10	--
Sample (μL)	--	--	10

4. Mix and incubate for 2 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 1 hour

#### CALCULATIONS

Serum and plasma (A)  $\frac{\text{Sample}}{\text{Standard}} \times 10$  (Standard Conc.) = mg/dL calcium

Urine 24 h (A)  $\frac{\text{Sample}}{\text{Standard}} \times 10 \times \text{vol. (dL) urine/24 h}$  =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<sup>1</sup>

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 0.22 mg/dL to linearity limit of 30 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)	9.11	13.7	9.31	14.1
SD	0.10	0.15	0.20	0.28
CV (%)	1.10	1.07	2.16	1.98

Sensitivity: 1 mg/dL = 0.017 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): 0.99

Regression equation:  $y = 0.9741x + 0.2595$ .

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 by Young et. al<sup>1</sup>.

#### NOTES

1. It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with diluted 1/1 HNO<sub>3</sub> in water and then thoroughly rinsed it with distilled water.
2. Most of the detergents and water softening products used in the laboratories contains chelating agents. A defective rinsing will invalidate the procedure.
3. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
4. Use clean disposable pipette tips for its dispensation.

#### BIBLIOGRAPHY

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