

## Calcium Arsenazo III Colorimetric Method

**IVD** For in vitro-diagnostic use only.

2°C ↕ 8°C  
Store at 2-8°C

### INTENDED USE

For the measurement of calcium concentration in human serum, plasma or urine.

### INTRODUCTION

Calcium the most abundant mineral in the body, is found in some foods, added to others, available as a dietary supplement, and is present in some medicines (such as antacids). Calcium is required for vascular contraction and vasodilation, muscle function, nerve transmission, intracellular signaling and hormonal secretion, though less than 1% of total body calcium is needed to support these critical metabolic functions. The remaining 99% of the body's calcium supply is stored in the bones and teeth where it supports their structure and function.

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 based on a single test result; it should integrate clinical and other laboratory data.

### PRINCIPLE

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

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

### MATERIAL

#### MATERIAL PROVIDED

R	Imidazol buffer ph 6.5	100mmol/L
(Arsenazo III)	Arsenazo III	120mmol/L
Calcium STD	Calcium aqueous primary standard	10mg/dl

#### MATERIALS REQUIRED BUT NOT PROVIDED

- Spectrophotometer or colorimeter measuring at 650 nm.
- Matched cuvette 1.0 cm light path.
- General laboratory equipment.

#### PRECAUTIONS

R: May damage fertility or the unborn child.

STD: May be corrosive to metals.

#### STORAGE AND STABILITY

- All 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 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.

#### PREPARATION

Reagents are ready to use.

#### 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 result by 2 (dilution factor).

Stability of the samples: calcium is stable for 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 Blank. The color is stable for at least 1 hour.

#### CALCULATIONS

##### Serum, plasma

$$(A)_{\text{Sample}} - (A)_{\text{Blank}} \times 10 (\text{STD conc.}) = \text{mg/dl total calcium}$$

$$\frac{\text{mg/dl total calcium}}{(A)_{\text{Standard}} - (A)_{\text{Blank}}}$$

##### Urine

$$(A)_{\text{Sample}} - (A)_{\text{Blank}} \times 10 \times \text{vol. (dl) urine} / 24 \text{ h} = \text{mg/24-hours total calcium}$$

$$\frac{\text{mg/24-hours total calcium}}{(A)_{\text{Standard}} - (A)_{\text{Blank}}}$$

**Conversion factor:** mg/dl X 0.25 = mmol/l

#### REFERENCE VALUES

##### Serum, plasma

Newborns	8-13 mg/dl (2-3.25 mmol/L)
Children	10-12 mg/dl (2.5-3 mmol/L)
Adults	8.5-10.5 mg/dl (2.1-2.6 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 purposes, each laboratory should establish its own reference range.

#### QUALITY CONTROL

- If control values are found outside the defined range, check the instrument, reagents and calibration material.
- Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

#### PERFORMANCE CHARACTERISTICS

##### 1. 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.

## 2. Precision

	INTRA -ASSAY (N=20)		INTER -ASSAY (N=20)	
	Mean (mg/dl)	8.35	14.28	8.58
SD	0.08	0.08	0.19	0.34
CV (%)	0.96	0.56	2.21	2.33

## 3. Sensitivity.

1mg/dl=0.0316 A.

## 4. 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 as follows:

Correlation coefficient(r):0.9506.

Regression equation=0.8944x+1.3421.

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

## INTERFERENCES

No interference was observed with triglyceride up to 1.25g/l.

A list of drugs and other interfering substances with calcium determination has been reported by Young.

## NOTES

1. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
2. Use clean disposable pipette tips for its dispensation.
3. CALCIUM CAL: proceed carefully with this product as, due its nature, it can get contaminated easily.
4. Most of the detergents and water softening products used in the laboratories contain chelating agents. Defective rinsing will invalidate the procedure.

## REFERENCES

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3. Young, D.S., Pestaner, L.D. y Gibberman, V. Clin. Chem. 21, Vol. 5, 10-432D (1975).
4. Tietz. N.W. Clinical Guide to Laboratory Tests, 3<sup>rd</sup> Edition. W.B. Saunders Co. Philadelphia, PA. (1995).

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	Catalogue Number		Temperature limit
	In Vitro diagnostic medical device		Caution
	Contains sufficient for <n> tests and Relative size		Consult instructions for use (IFU)
	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