



# Ca-Color

## Arsenazo III AA

Direct colorimetric method for the determination of calcium in serum, plasma and urine

### SUMMARY

Calcium is an essential element in most blood clotting reactions and in the regulation of muscle fibers excitability. Calcium concentration in serum and urine is regulated by the action of factors such as parathormone levels, vitamin D and phosphorous. Physiological fluctuations are due to age, sex, pregnancy, physical activity, seasonal changes (sunlight incidence).

Hypercalcemia is related to different diseases: hyperparathyroidism, bone neoplasias, vitamin D poisoning. Hypocalcemia is associated to disorders such as hypoparathyroidism, vitamin D deficiency and malabsorption.

### PRINCIPLE

Calcium reacts with arsenazo III, yielding a blue colored complex, which is photocolorimetrically measured at 650 nm.

### PROVIDED REAGENTS

**A. Reagent A:** solution of 100 mg/l arsenazo III and 1.4 g/l 8-hydroxyquinoline sulphonate in 100 mM Tris buffer, pH 8.5.

**S. Standard\*:** 10 mg/dl calcium solution.

### NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus** when the automatic technique is used. It may also be employed in the calibration of manual techniques.
- Deionized or distilled water.

### INSTRUCTIONS FOR USE

**Provided Reagents:** ready to use.

**Standard:** whenever used, transfer an excess amount to a clean test tube and pipette the necessary volume, discarding the supernatant.

### WARNING

Reagents are for "in vitro" diagnostic use. Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

### STABILITY AND STORAGE INSTRUCTIONS

**Provided Reagents:** are stable in refrigerator (2-10°C) until the expiration date shown on the box. Avoid prolonged exposition of the Reagent A to direct light.

### INSTABILITY OR DETERIORATION OF REAGENTS

Turbidity or decoloration of Reagent A indicates deteriora-

tion, while blank absorbances > 1.200 O.D. at 650 nm (see PROCEDURE LIMITATIONS), indicate calcium contamination. Discard in both cases.

### SAMPLE

Serum, heparinized plasma or urine

#### a) Collection:

- Serum or plasma: obtain in the usual way.
- Urine: collect 24 hours urine over 20 ml 50% hydrochloric acid. Bring the sample volume to 2 liters with water. Homogenize.

**b) Additives:** if plasma is used as sample, heparin should be used as anticoagulant. If urine is used as sample, it should be acidified with 50% hydrochloric acid during collection.

**c) Known interfering substances:** anticoagulants other than heparin, bind to the calcium yielding a complex, thus giving erroneous results. No interferences are observed from bilirubin up to 200 mg/l, hemoglobin up to 350 mg/dl, magnesium up to 10 mg/dl nor from triglycerides up to 500 mg/dl. See Young, D.S. in References for effect of drugs on the present method.

**c) Stability and storage instructions:** sample should be preferably fresh. Sample may be kept for one week in refrigerator (2-10°C) or over 5 months in freezer without any preservatives.

### REQUIRED MATERIAL (non-provided)

- Spectrophotometer or photocolorimeter.
- Micropipettes or pipettes for measuring the stated volumes
- Test tubes or spectrophotometric cuvettes.
- Stopwatch.

### ASSAY CONDITIONS

- Wavelength: 650 nm in spectrophotometer or 620-650 nm in photocolorimeter.

- Reaction temperature: room temperature (15-25°C).

- Reaction time: 2 minutes

- Sample volume: 10 ul

- Final reaction volume: 1.01 ml

Reagent A and sample volumes may proportionally vary (e.g. 20 ul sample + 2 ml Reagent A or 5 ul sample + 0.5 ml Reagent A).

### PROCEDURE

In three test tubes labeled B (Blank), S (Standard or Calibrator) and U (Unknown) place:

\* Non-provided with all kit sizes

	B	S	U
<b>Sample</b>	-	-	10 ul
<b>Standard or Calibrator</b>	-	10 ul	-
<b>Distilled water</b>	10 ul	-	-
<b>Reagent A</b>	1 ml	1 ml	1 ml

Mix and incubate 2 minutes at room temperature (15-25°C). Read absorbance in spectrophotometer at 650 nm or in photocolormeter with red filter (620-650 nm) setting the instrument to zero O.D. with Blank.

### STABILITY OF FINAL REACTION

Final reaction color is stable for 1 hour. Therefore, absorbance should be read within that period.

### CALCULATIONS

$$1) \text{ Serum calcium (mg/dl)} = U \times f \quad f = \frac{10 \text{ mg/dl or C}}{S}$$

where:

C = calcium concentration in Calibrator A plus, if using this reagent

$$2) \text{ Urinary calcium (mg/24 hrs)} = \frac{U}{S} \times 200 \text{ mg/24 hrs}$$

For urine with diuresis volumes over 2 liters or that have not been brought to 2 liters with distilled water, use the following calculation:

$$\text{Urinary calcium (mg/24 hrs)} = \frac{U}{S} \times 100 \times V$$

where:

100 = Standard concentration in mg/l

V = diuresis volume in liters/24 hrs

### QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known calcium concentration. If running urine samples, a urine-based control should be used.

### REFERENCE VALUES

Serum: 8.5 - 10.5 mg/dl

Urine: up to 300 mg/24 hs (normal diet)

In a population including 120 healthy individuals from Rosario (Argentina) of both sexes (between 20-45 years old) with a calcium-free diet, was obtained the following result:

Urine: 60 - 200 mg/24 hs

It is recommended that each laboratory establishes its own reference values.

### CONVERSION UNITS

Ca (mg/dl) = Ca (mmol/l) x 4

Ca (mmol/l) = Ca (mg/dl) x 0.25

Ca (mg/dl) = Ca (mEq/l) x 2

Ca (mEq/l) = Ca (mg/dl) x 0.5

### PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

Blank readings higher than 1,200 O.D. may be obtained at wavelengths lower than 650 nm which do not affect the results.

Contamination: glassware should be thoroughly clean, free from calcium or any trace of anticoagulant. It is recommended to wash glassware with non-ionic detergents (Wiener lab's Noion) or diluted mineral acids, rinsing several times with distilled water.

It is advisable to keep pipettes and test tubes for exclusive use on this determination.

### PERFORMANCE

**a) Reproducibility:** assaying 20 replicates from the same samples on the same day, the following results were obtained:

	Level	S.D.	C.V.
<b>Serum</b>	9.2 mg/dl	± 0.18 mg/dl	1.93 %
	11.0 mg/dl	± 0.10 mg/dl	0.88 %
<b>Urine</b>	104 mg/24 hs	± 2.68 mg/24 hs	2.57 %
	372 mg/24 hs	± 6.64 mg/24 hs	1.79 %

Performing the same assay on different days, the following results were obtained:

<b>Serum</b>	9.1 mg/dl	± 0.16 mg/dl	1.74 %
	12.1 mg/dl	± 0.16 mg/dl	1.29 %
<b>Urine</b>	117 mg/24 hs	± 2.9 mg/24 hs	2.44 %
	266 mg/24 hs	± 7.0 mg/24 hs	2.62 %

**b) Linearity:** reaction is linear up to 20 mg/dl. For higher values, repeat testing using 1:2 or 1:4 diluted sample with saline solution, multiplying final result by 2 or 4 respectively.

**c) Quantification limit:** the minimum detectable calcium concentration will be of 2.5 mg/dl.

### PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user's manual of the autoanalyzer in use.

For calibration use Wiener lab's **Calibrador A plus**.

### WIENER LAB. PROVIDES

- 4 x 50 ml (Cat. Nº 1152004).

- 8 x 20 ml (Cat. Nº 1009307).

- 8 x 20 ml (Cat. Nº 1009248).

- 8 x 20 ml (Cat. Nº 1009606).

### REFERENCES

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- Tietz, N.W. - "Fundamentals of Clinical Chemistry", W.B. Saunders, Philadelphia, 2001.
- Burtis C., Ashwood, E. - Tietz Textbook of Clinical Chemistry, WB Saunders Co., 3<sup>o</sup> ed, 1999.


## SYMBOLS

The following symbols are used in the packaging for Wiener lab. diagnostic reagents kits.

 This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices

 Authorized representative in the European Community

 "In vitro" diagnostic medical device


 Contains sufficient for <n> tests

 Use by


 Temperature limitation (store at)

 Do not freeze

 Biological risks

 Volume after reconstitution

 Contents


 Batch code


 Manufactured by:

 Harmful

 Corrosive / Caustic

 Irritant

 Consult instructions for use


 Calibrator

 Control

 Positive Control

 Negative Control

 Catalog number

 Wiener Laboratorios S.A.I.C.  
Riobamba 2944  
2000 - Rosario - Argentina  
<http://www.wiener-lab.com.ar>  
Dir. Téc.: Viviana E. Cétola  
Biochemist  
A.N.M.A.T. Registered product  
Cert. N°: 4209/01

 **Wiener lab.**

2000 Rosario - Argentina