



Ca-Color

AA

Direct colorimetric method for the determination of calcium in serum, heparinized 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, malabsorption, etc.

PRINCIPLE

Calcium reacts with o-Cresolphthalein complexone (o-CPC) at alkaline pH, yielding a magenta colored complex, which is photocolometrically measured at 570 nm.

PROVIDED REAGENTS

A. Reagent A: o-Cresolphthalein complexone solution and 8-Hydroxyquinoline.

B. Reagent B: aminomethyl propanol solution (AMP).

S. Standard: 10 mg/dl calcium solution.

Final concentrations

o-Cresolphthalein complexone	0.08 mmol/l
8-Hydroxyquinoline	4 mmol/l
AMP	3.5 mol/l

NON-PROVIDED REAGENTS

- Wiener lab.'s **Calibrador A plus**.
- Distilled or dionized 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.

Monoreagent (premixed): mix equal parts of Reagent A and Reagent B, according to the number of samples to be assayed.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Do not ingest. Avoid contact with skin and eyes. If spilt or splash, thoroughly wash affected area with water.

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: stable at room temperature (lower than 25°C) until the expiration date shown on the box.

Monoreagent (premixed): stable 4 days in refrigerator (2-10°C).

INSTABILITY OR DETERIORATION OF REAGENTS

High Blank absorbances indicate calcium contamination. Discard when they reach 0.600 O.D. or more.

SAMPLE

Serum, heparinized plasma or urine

a) Collection: obtain serum or plasma in the usual way. In case of 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 20 mg/dl (200 mg/l), triglycerides up to 680 mg/dl (6.8 g/l), hemoglobin up to 94 mg/dl and magnesium up to 9.9 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.

ASSAY CONDITIONS

- Wavelength: 570 nm in spectrophotometer or 560-590 nm in photocolorimeter with red filter.

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

- Reaction time: 5 minutes

- Sample volume: 50 ul

- Final reaction volume: 2.05 ml

PROCEDURE

I- SEPARATE REAGENTS TECHNIQUE

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

	B	S	U
Distilled water	50 ul	-	-
Standard	-	50 ul	-
Sample	-	-	50 ul
Reagent A	1.0 ml	1.0 ml	1.0 ml
Reagent B	1.0 ml	1.0 ml	1.0 ml

Mix, incubate 5 minutes at room temperature (15-25°C) and read absorbance in spectrophotometer at 570 nm or in photocolormeter with red filter (560-590 nm).

Microtechnique

Follow the procedure indicated in Technique I using 25 ul Sample, 0.5 ml Reagent A and 0.5 ml Reagent B.

II- MONOREAGENT TECHNIQUE (PREMIXED)

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

	B	S	U
Distilled water	50 ul	-	-
Standard	-	50 ul	-
Sample	-	-	50 ul
Monoreagent	2.0 ml	2.0 ml	2.0 ml

Mix, incubate 5 minutes at room temperature (15-25°C) and read absorbance in spectrophotometer at 570 nm or in photocolormeter with red filter (560-590 nm).

Microtechnique

Follow the procedure indicated in Technique II using 25 ul Sample, 1 ml Monoreagent.

For lipemic or hemolyzed samples it is necessary to process a Sample Blank as follows: mix 50 ul sample with 2 ml distilled water. Measure absorbance setting the instrument to zero O.D. with distilled water. Subtract absorbance from the initially obtained and use this difference for calculations.

STABILITY OF FINAL REACTION

Final reaction color is stable for 20 minutes, therefore absorbance should be read within that period.

CALCULATIONS

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

$$2) \text{ Urinary calcium (mg/24 hs)} = \frac{U}{S} \times S \text{ conc.} \times 10 \times V$$

where:

10 = factor to convert mg/dl to mg/l

V = diuresis volume in liters/24 hours

Example:

Absorbance of Standard = 1.050

Absorbance of Urine = 0.933

Concentration of Standard = 10 mg/dl

24 hr. Urine volume = 1.27 liters

$$\text{Urinary calcium} = \frac{0.933}{1.050} \times 10 \times 10 \times 1.27 = 113 \text{ mg/24 hours}$$

UNITS CONVERSION

$$\text{Ca (mg/dl)} = \text{Ca (mmol/l)} \times 4$$

$$\text{Ca (mmol/l)} = \text{Ca (mg/dl)} \times 0.25$$

$$\text{Ca (mg/dl)} = \text{Ca (mEq/l)} \times 2$$

$$\text{Ca (mEq/l)} = \text{Ca (mg/dl)} \times 0.5$$

QUALITY CONTROL METHOD

Each time the test is run, analyze two levels of a quality control material (**Standatrol S-E 2 niveles**) with known calcium concentration.

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 in the 95% of the results:

Urine: 60 - 200 mg/24 hs

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

PROCEDURE LIMITATIONS

See Known Interfering Substances under SAMPLE.

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.

PERFORMANCE

The assays were performed in an Express plus analyzer^(*). If using the kit with manual procedure, user must validate that similar performance to that stated below is obtained.

a) Reproducibility:

Intra-assay

Sample	Mean (mg/dl)	S.D. (mg/dl)	C.V.
Normal Level Serum	9.4	± 0.12	1.28 %
High Level Serum	12.0	± 0.16	1.30 %

Sample	Mean (mg/24 hs)	S.D. (mg/24 hs)	C.V.
Normal Level Urine	118	± 1.26	1.06 %
High Level Urine	349	± 2.38	0.68 %

Inter-assay

Sample	Mean (mg/dl)	S.D. (mg/dl)	C.V.
Normal Level Serum	9.8	± 0.17	1.74 %
High Level Serum	12.7	± 0.22	1.70 %

Sample	Mean (mg/24 hs)	S.D. (mg/24 hs)	C.V.
Normal Level Urine	121	± 3.01	2.50 %
High Level Urine	351	± 4.69	1.34 %

b) Linearity: reaction is linear up to 20 mg/dl. For higher values, repeat testing using 25 ul Sample or 1:2 diluted sample with saline multiplying final result by 2.

c) Correlation:

- Serum and plasma: calcium values of 143 specimens were determined using Wiener lab.'s **Ca-Color AA** kit and a commercial kit based on the same principle. The correlation coefficient was:

$r = 0.9963$, slope $b = 1.0185$ and intercept $a = -0.0362$

- Urine: calcium values of 69 specimens were determined using Wiener lab.'s **Ca-Color AA** kit and a commercial kit based on the same principle. The correlation coefficient was:

$r = 0.9943$, slope $b = 1.0207$ and intercept $a = 2.5116$

d) Sensitivity: based on an instrument resolution of $A = 0.001$, Wiener lab. calcium procedure has a sensitivity of 0.01 mg/dl.

PARAMETERS FOR AUTOANALYZERS

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

For calibration, it can be used a serum based calibrator (Wiener lab.'s **Calibrador A plus**).

WIENER LAB. PROVIDES

- 4 x 50 ml (Cat. 1152002).

REFERENCES

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SYMBOLS

The following symbols are used in 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

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