



CALCIUM

Colorimetric determination of Calcium in biological fluids



ORDER INFORMATION

REF **Kit size**
GA4320 00 5x50 + 5x50 ml

INDICATION

Conditions such as parathyroid disorders, neoplasms with or without bone metastasis, myelomas or other bone diseases can cause alterations in calcium levels.

METHOD PRINCIPLE

Calcium in alkaline pH forms with methylthymol blue a blue-coloured complex stabilized by a reductant. The intensity of the colour is proportional to the calcium concentration present in the sample.

COMPOSITION

REAGENT A:

Methylthymol blue 80 µmol/l
Hydroxiquinoline 70 mmol/l
Acetic acid 25 mmol/l

REAGENT B:

Sodium sulfite 120 mmol/l
Monoethanolamine 1 mmol/l.

Irritant

R 31-36/37/38; S(2-)26

STANDARD:

1x5 ml
Calcium 10 mg/dl
Verified against NIST reference material.

PREPARATION OF REAGENTS

Bireagent procedure:

The reagents are liquids ready to use.

Monoreagent procedure:

Mix 1 part of Reagent A and 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

Storage and stability

Store at room temperature (15-25 °C). Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label, if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 3 days at 15-25 °C, even if developing a light blue colour.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- Temperature controlled water bath
- NaCl solution 9 g/l

SAMPLES

Serum, heparin plasma. Do not use haemolysed samples, separate immediately serum from cells. Do not use anticoagulants containing calcium salts or calcium chelating agents.

Urine 24h, diluted 1:3 with distilled water.

Stable 7 days at 15-25 °C.

Specimen collection / Preanalytical factors

It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Calcium concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Bireagent procedure

Pipette into disposable or well clean cuvettes :

	Blank	Standard	Sample
Reagent A	500 µl	500 µl	500 µl
Distilled H ₂ O	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix and incubate for **5 minutes** at **room temperature** (20-25 °C). Then add:

Reagent B	500 µl	500 µl	500 µl
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Mix and incubate for **5 minutes** at **room temperature** (20-25 °C). Read absorbance (A) of standard and samples at **615 (580-630) nm** against Blank. Colour is stable for 120 minutes.

Monoreagent procedure

Pipette into disposable or well clean cuvettes :

	Bianco	Standard	Campione
Working reagent	1000 µl	1000 µl	1000 µl
Distilled H ₂ O	10 µl	-	-
Standard	-	10 µl	-
Sample	-	-	10 µl

Mix and incubate for **5 minutes** at **room temperature** (20-25 °C). Read absorbance (A) of standard and samples at **615 (580-630) nm** against Blank. Colour is stable for 120 minutes.

CALCULATION OF RESULTS

Serum-plasma:

$$\text{Calcium, mg/dl} = \frac{A \text{ sample}}{A \text{ standard}} \times 10$$

Urine:

$$\text{Calcium, mg/24h} = \frac{A \text{ sample}}{A \text{ standard}} \times 300 \times 1/24\text{h}$$

Conversion factor

$$\text{Calcium [mg/dl]} \times 0.25 = \text{Calcium [mmol/l]}$$

$$\text{Calcium [mg/dl]} \times 0.50 = \text{Calcium [mEq/l]}$$

REFERENCE VALUES

Serum-plasma: 8 ÷ 10 mg/dl (adults)

Urine 24h: 100 ÷ 300 mg/24h (varying according to diet)

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

Precision

Within-run and between-run coefficients of variation have been calculated on replicates of two sera. The obtained results are reported in the following tables:

Within-run (n=30)

Sample	Mean (mg/dl)	SD	%CV
Serum #1	11.6	0.21	1.8
Serum #2	10.0	0.21	2.1

Tra-le-serie (n=30)

Sample	Mean (mg/dl)	SD	%CV
Serum #1	11.6	0.22	1.9
Serum #2	9.9	0.26	2.6

Linearity

The assay is linear up to 16 mg/dl.

Sensitivity

Test sensitivity, in terms of limit of detection, is 1 mg/dl.

Correlation

A correlation study comparing the present method and a commercial one gave the following results:

$$y = 1.04x + 1.10 \text{ mg/dl} \quad r = 0.98$$

Interferences

Hemoglobin > 250 mg/dl
 Bilirubin > 20 mg/dl
 Triglycerides > 1000 mg/dl

PRECAUTIONS IN USE

Reagent B is harmful (Irritant).

Refer to Safety Data Sheet.

Reagent A and Standard are not considered harmful according to 67/548/EEC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.

The use of laboratory reagents according to good laboratory practice is recommended.

As calcium is an ubiquitous ion, essential precaution must be taken against accidental contamination.

Only use disposable materials.

The use of laboratory reagents according to good laboratory practice is recommended.

Waste Management

Please refer to local legal requirements.

BIBLIOGRAPHY

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3. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC.
4. NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).

