

Liquick Cor-CALCIUM ARSENAZO

DIAGNOSTIC KIT FOR DETERMINATION OF CALCIUM CONCENTRATION



Kit name	Cat. No
Liquick Cor-CALCIUM ARSENAZO 500	3-325
Liquick Cor-CALCIUM ARSENAZO "bulk"	3-294

INTRODUCTION

Calcium plays an essential role in many cell functions: intracellularly in muscle contraction and glycogen metabolism, extracellularly, in bone mineralization, in blood coagulation and in transmission of nerve impulses. Calcium is present in plasma in three forms: free, bound to proteins or complexed with anions as phosphate, citrate and bicarbonate. Decreased total calcium levels can be associated with diseases of the bone apparatus (especially osteoporosis), kidney diseases (especially under dialysis), defective intestinal absorption and hypoparathyroidism. Increased total calcium can be measured in hyperparathyroidism, malignant diseases with metastases and sarcoidosis. Calcium measurements also help in monitoring of calcium supplementation mainly in the prevention of osteoporosis.

METHOD PRINCIPLE

Photometric test using arsenazo III.

Calcium with arsenazo III at neutral pH yields a blue coloured complex, whose intensity is proportional to the calcium concentration. Interference by magnesium is eliminated by addition of 8-hydroxyquinoline-5-sulfonic acid.

REAGENTS

Package

	Liquick Cor-CALCIUM ARSENAZO 500	Liquick Cor-CALCIUM ARSENAZO "bulk"
1-CALCIUM ARSENAZO	4 x 500 ml	--*

*reagent volume is printed on the label.

The reagents when stored at 2-8°C are stable up to expiry date printed on the package. The reagents are stable for 11 weeks on board the analyser at 2-10°C. Do not freeze the reagent. Protect from light and avoid contamination!

Concentrations in the test

phosphate buffer (pH 7.5)	67 mmol/l
8-hydroxyquinoline-5-sulfonic acid	5 mmol/l
arsenazo III	100 µmol/l
detergents	

Warnings and notes

- Product for in vitro diagnostic use only.
- Contaminated glassware is the greatest source of error. The use of disposable plastic ware is recommended. Glassware should be soaked for a few hours in 2M HCl solution and then thoroughly rinsed with distilled water.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 650 nm (630-670 nm);
- thermostat at 37°C;
- general laboratory equipment;

SPECIMEN

Serum. Random or 24-hours urine.

Serum can be stored up to 7 days at 20-25°C or up to 3 weeks at 4-8°C. Samples frozen at -20°C can be stored up to 8 months. Discard contaminated specimens.

24-hours urine preparation: To prevent calcium salt precipitation specimens should be collected in 10 ml of 6M HCl. In case of presence of precipitants they can be solved by lowering pH of the urine to below 2.0.

Prior to determination dilute the sample with 0.9% NaCl in the ratio of 1 to 1. Multiply the result by the dilution factor. Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

This reagent may be used for manual assay and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	650 nm (630 – 670 nm)
temperature	37°C
cuvette	1 cm

Sample Start method

Pipette into the cuvettes:

	reagent blank (RB)	test (T)	calibrator (C)
1-CALCIUM ARSENAZO	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

calibrator	-	-	10 µl
sample	-	10 µl	-

Mix well, incubate for 2 min. Read the absorbance of calibrator sample A(C) and test sample A(T) against reagent blank (RB).

Calculation

$$\text{calcium concentration [mg/dl]} = \frac{A(T)}{A(C)} \times \text{calibrator concentration [mg/dl]}$$

REFERENCE VALUES ⁴

serum	mg/dl	mmol/l
adult	8.6 – 10.3	2.15 – 2.57
random urine	mg/dl	mmol/l
male	0.9 – 37.9	0.225 – 9.47
female	0.5 – 35.7	0.125 – 8.92
24-hours urine	mg/24h	mmol/24h
adult	100 – 300	2.5 – 7.5

It is recommended for each laboratory to establish its own reference ranges for local population.

Calcium concentration 24-hours urine – calculation

$$\text{calcium concentration in 24-hours urine [mg/24h]} = \frac{\text{calcium concentration in sample of 24-hours urine [mg/dl]} \times \text{urine volume of 24-hours urine [dl/24h]}}{\text{urine volume of 24-hours urine [dl/24h]}}$$

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY SERUM HN (Cat. No 5-172) and CORMAY SERUM HP (Cat. No 5-173) for determination in serum or CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) for determination in urine with each batch of samples.

For the calibration the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) is recommended.

The calibration curve should be prepared every 11 weeks, with change of reagent lot number or as required e.g. quality control findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using the automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.3 mg/dl (0.075 mmol/l).
- **Linearity:** up to 20 mg/dl (5 mmol/l).
For higher calcium concentrations dilute the sample with 0.9% NaCl in the ratio of 1 to 1 and reassay. Multiply the result by 2.
- **Specificity / Interferences**
Haemoglobin up to 1.88 g/dl, ascorbate up to 62 mg/l, bilirubin up to 40 mg/dl, triglycerides up to 1000 mg/dl, and magnesium up to 20 mg/dl do not interfere with the test.

▪ **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	9.38	0.10	1.03
level 2	12.43	0.07	0.58

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	9.18	0.12	1.33
level 2	12.32	0.12	0.94

▪ **Method comparison**

A comparison between calcium values determined at Biolis 24i Premium (y) and at ADVIA 1650 (x) using 25 samples gave following results:

$$y = 0.9581 x + 0.0146 \text{ mg/dl};$$

$$R = 0.980 \quad (R - \text{correlation coefficient})$$

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Endres DB, Rude RK. Mineral and bone metabolism. In: . Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 3rd ed. Philadelphia, PA: Moss D.W., Henderson A. R. (1999) p. 1395-1457.
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3. Bauer PJ. Affinity and stoichiometry of calcium binding by arsenazo III. Anal Biochem 1981; 110:61-72.
4. Alan H.B. Wu. editor. Tietz Clinical Guide to Laboratory Tests, 4th ed. St. Louis: W.B Saunders Company; 2006, p. 202-204.

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