Liquick Cor-CALCIUM

DIAGNOSTIC KIT FOR DETERMINATION OF CALCIUM CONCENTRATION

Kit name	Cat. No
Liquick Cor-CALCIUM 500	3-324
Liquick Cor-CALCIUM "bulk"	3-293

INTRODUCTION

Calcium and phosphorus as a hydroxyapatite constitute mineral portion of bone. Calcium occurs also as divalent cations (free or bound with negatively charged proteins) which participate in blood coagulation, neuromuscular excitability, skeletal and cardiac muscle contractility and in multiple cellular functions. Calcium flux in organism is controlled by action of parathyroid hormone (PTH), vitamin D and calcitonin. Calcium serum level abnormalities are caused usually by parathyroid or thyroid disease, disorders of vitamin D metabolism or acute pancreatitis.

METHOD PRINCIPLE

Calcium ions form a violet complex with o-cresolophthalein complexone in alkaline solution. The intensity of violet colour of this complex measured at 570-580 nm is proportional to the calcium concentration in the sample.

REAGENTS

Package

	Liquick Cor-CALCIUM 500	Liquick Cor-CALCIUM "bulk"
1-CALCIUM	3 x 400 ml	--*
2-CALCIUM	1 x 300 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 3 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination!

Working reagent preparation and stability

Assay can be performed with use of separate 1-CALCIUM and 2-CALCIUM reagents or with use of working reagent. For working reagent preparation mix gently 4 parts of 1-CALCIUM with 1 part of 2-CALCIUM. Avoid foaming.

Stability of working reagent: 7 days at 2-8°C
2 days at 15-25°C

The working reagent is slightly pink-coloured. This does not influence the results of the test. Avoid contamination!

Concentrations in the test

o-cresolophtalein complexone	0.06 mmol/l
8-quinolinol	8.6 mmol/l
hydrochloric acid	30 mmol/l
ethanolamine	377 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents are usable when the absorbance of the working reagent is less than 0.250 (read against distilled water, wavelength λ = 575 nm, cuvette l = 1 cm, at temp. 25°C).
- 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 2 M HCl solution and then thoroughly rinsed with distilled water.
- 2-CALCIUM meeting the criteria for classification in accordance with Regulation (EC) No 1272/2008.

Warning.

H335 May cause respiratory irritation.
P261 Avoid breathing spray.
P304+P340 IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P312 Call a POISON CENTER or doctor if you feel unwell.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 575 nm (570-580 nm);
- thermostat at 37ºC;
- general laboratory equipment;

SPECIMEN

Serum, heparinized plasma free from hemolysis, 24-hours urine.
Recommended anticoagulants: heparine lithium, sodium or ammonium salt.
Urine preparation: to prevent calcium salt precipitation in urine, specimens should be collected in HCl, 20-30 ml of 6 M for 24-h specimen. Any specimens collected without acid should be acidified using 20-30 ml of 6 M HCl, well mixed and allowed to stand for 1 h before aliquotting. Prior to determination dilute the sample with 0.9% NaCl in the ratio of 1 to 1. Multiply the result by the dilution factor.
Serum and plasma can be stored up to 8 hours at 15-25ºC or up to 1 day at 2-8ºC. Samples frozen at -20ºC can be stored up to1 year.
24-hours urine samples should be kept at 2-8ºC.
Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used both for manual assay (Sample Start and Reagent Start method) and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	575 nm (570-580 nm)
temperature	20-25ºC / 37°C
cuvette	1 cm

Sample Start method

Pipette into the cuvettes:

	reagent blank (RB)	test (T)	standard (S)
working reagent	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

standard / calibrator	-	-	10 µl
sample	-	10 µl	-

Mix well, incubate for 10 min. at 20-25°C or 5 min. at 37°C. Read the absorbance of the test A(T) and standard A(S) against reagent blank (RB). The intensity of colour is stable for 30 min.

Reagent Start method

The determination can be also performed with use of separate 1-CALCIUM and 2-CALCIUM reagents.

Pipette into the cuvettes:

	reagent blank (RB)	test (T)	standard (S)
1-CALCIUM	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination. Then add:

standard / calibrator	-	-	10 µl
sample	-	10 µl	-

Mix well, incubate for 5 min. Then add:

2-CALCIUM	250 µl	250 µl	250 µl

Mix well; perform measurement as described for Sample Start method.

Calculation

calcium concentration = A(T) / A(S) x standard / calibrator concentration

REFERENCE VALUES [8]

serum, plasma		mg/dl	mmol/l
premature		6.2 – 11.0	1.55 – 2.75
adults	18 – 60 yr	8.6 – 10.0	2.15 – 2.50
	60 – 90 yr	8.8 – 10.2	2.20 – 2.55
	> 90 yr	8.2 – 9.6	2.05 – 2.40
24-hours urine		mg/24h	mmol/24h
		100 – 300	2.5 – 7.5

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

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) or LEVEL 2 (Cat. No 5-162) for determination in urine with each batch of samples.

For the calibration of manual assay the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176), LEVEL 2 (Cat. No 5-175; 5-177) or CALCIUM STANDARD (Cat. No 5-132) is recommended.

For the calibration of automatic analysers systems the CORMAY MULTICALIBRATOR LEVEL 1 (Cat. No 5-174; 5-176) and/or LEVEL 2 (Cat. No 5-175; 5-177) is recommended.

The calibration curve should be prepared every 2 days, 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 an automatic analyser Biolis 24i Premium. Results may vary if a different instrument or a manual procedure is used.

- **Sensitivity:** 0.27 mg/dl (0.068 mmol/l).

- **Linearity:** up to 15 mg/dl (3.75 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 2.5 g/dl, bilirubin up to 20 mg/dl, ascorbate up to 62 mg/l and triglycerides up to 1000 mg/dl do not interfere with the test.

- **Precision**

Repeatability (run to run) n = 20	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	8.73	0.08	0.90
level 2	11.97	0.06	0.52

Reproducibility (day to day) n = 80	Mean [mg/dl]	SD [mg/dl]	CV [%]
level 1	8.40	0.25	2.98
level 2	11.46	0.36	3.16

- **Method comparison**

A comparison between calcium values determined at Biolis 24i Premium (y) and at COBAS INTEGRA 400 (x) using 60 samples gave following results:

y = 0.9946 x - 0.1547 mg/dl;

R = 0.9962 (R – correlation coefficient)

TRACEABILITY

CALCIUM STANDARD is traceable to the SRM 909B reference material.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

1. Connerty H.V., Briggs A.R.: Am. J. Clin. Path., 45, 290-296 (1966).
2. Gitelman H.J.: Anal. Biochem., 18, 521-531 (1967).
3. Baginski E.S., Marie S.S., Clark W.L., Zak B: Clin. Chim. Acta, 46, 49-54 (1973).
4. Faulkner W.R., Meites S.: Selected Methods of Clinical Chemistry, vol. 9, Washington DC, p. 125-129 (1982) .
5. Burtis C.A., Ashwood E.R., ed. Tietz Textbook of Clinical Chemistry, 2nd ed. Philadelphia, PA: WB Saunders, 1893, 1904, 2180, (1994).
6. Kaplan L.A., Pesce A.J., ed. Chemistry Theory, Analysis, and Correlation, 3rd ed. St Louis, MO: Mosby, 550 (1996).
7. Tietz N.W., ed. Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, PA: WB Saunders, 102, (1995).
8. Alan H.B. Wu: Tietz Clinical Guide to Laboratory Tests, 4th ed. WB Saunders, 202, (2006).

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